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Herbal medicine for the treatment of chronic metabolic diseases

Edited by
Yongsheng Chen, Dongmei Li, Dan Tang and Raquel Abalo

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Herbal medicine for the treatment of chronic metabolic diseases

Topic editors

Yongsheng Chen — Jinan University, China
Dongmei Li — Georgetown University, United States
Dan Tang — Guangdong Pharmaceutical University, China
Raquel Abalo — Rey Juan Carlos University, Spain

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ORIGINAL RESEARCH
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Aqueous Extract of Guava (*Psidium guajava* L.) Leaf Ameliorates Hyperglycemia by Promoting Hepatic Glycogen Synthesis and Modulating Gut Microbiota

*Shuzhou Chu[1], Feng Zhang[1], Huiying Wang[1], Lijun Xie[1], Zhinan Chen[1], Weimin Zeng[2], Zhiguang Zhou[1] and Fang Hu[1]**

[1]National Clinical Research Center for Metabolic Diseases, Key Laboratory of Diabetes Immunology (Central South University), Ministry of Education, Metabolic Syndrome Research Center, Department of Metabolism and Endocrinology, The Second Xiangya Hospital of Central South University, Changsha, China, [2]Key Laboratory of Biometallurgy, School of Minerals Processing and Bioengineering, Ministry of Education, Central South University, Changsha, China

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Tongren University, China

***Correspondence:**
Fang Hu
hu_fang98@csu.edu.cn

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Type 2 diabetes mellitus (T2DM) is a major global health concern. *Psidium guajava* L. (guava) is widely used for food as well as a folk medicine. Previous studies have shown its anti-diabetic and anti-inflammatory properties. However, the underlying mechanisms remains to be elusive. In this study, we assessed the potential therapeutic effects of aqueous extract of guava leaves (GvAEx) on T2DM and explored their potential mechanisms *in vivo* and *in vitro*. GvAEx was gavage administered for 12 weeks in diabetic db/db mice. Our results have demonstrated that GvAEx significantly lowered fasting plasma glucose levels ($p < 0.01$) and improved glucose tolerance and insulin sensitivity ($p < 0.01$, $p < 0.05$, respectively). Additionally, GvAEx increased hepatic glycogen accumulation, glucose uptake and decreased the mRNA expression levels of gluconeogenic genes. Furthermore, GvAEx-treatment caused higher glucose transporter 2 (GLUT2) expression in the membrane in hepatocytes. Notably, for the first time, we have elaborated the possible mechanism of the hypoglycemic effect of GvAEx from the perspective of intestinal microbiota. GvAEx has significantly changed the composition of microbiota and increased short chain fatty acid (SCFA) -producing Lachnospiraceae family and *Akkermansia* genus in the gut. Taken together, GvAEx could alleviate hyperglycemia and insulin resistance of T2DM by regulating glucose metabolism in the liver and restoring the gut microbiota. Thus, GvAEx has the potential for drug development against T2DM.

Keywords: guava leaf, hyperglycemia, liver, glucose metabolism, gut microbiota

INTRODUCTION

Diabetes mellitus (DM) is one of the most prevalent metabolic diseases in the world. As of 2019, an estimated 463 million people worldwide have DM (8.8% of the adult population), with T2DM accounting for approximately 90% of cases (Cho et al., 2018). That number is expected to reach 643 million by 2030 and 783 million by 2045. Direct medical expenditures related to diabetes are already

approaching one trillion dollars and will exceed that figure by 2030 (IDF, 2019). DM is caused by either the pancreas not producing enough insulin, or the body's cells not responding appropriately to the effects of insulin (DeFronzo et al., 2015).

Insulin resistance is recognized as an essential risk factor for the development of T2DM. There is an impaired biological response to insulin stimulation in target tissues, mainly liver, muscle and adipose tissue (Wild et al., 2004). The liver plays a key role in glucose homeostasis by regulating different pathways of glucose metabolism, including glycolysis, gluconeogenesis, glycogenolysis sand glycogenesis (Han et al., 2016). Due to insulin resistant, hepatic glycogen synthesis is reduced while gluconeogenesis is increased, which eventually leads to the increased rate of hepatic glucose output and causes hyperglycemia (Petersen et al., 2017). Accordingly, it is important to identify new approaches to improve hepatic insulin sensitivity against T2DM. The therapeutic properties of some plants have long been recognized for hundreds and thousands of years. However, these herb medicines are used as mixtures or concentrated extracts without isolation of active compounds. Recent years, the development of advanced technologies have made it possible to extract the active compounds from these plants, which can be used in the pharmaceutical, food and chemical industries (Guo et al., 2021; Tan et al., 2022).

Psidium guajava L., also known as guava, is a member of the myrtle family (Myrtaceae), which is cultivated in many tropical and subtropical regions as fruit (Morton, 1987). Not only is guava widely used for food, but is also a folk medicine. Guava leaves are the most important part for medicinal purposes. Traditionally, the guava leaves are used as herbal medicine for the prevention and treatment of diseases. Nowadays, people also use extracts from its fruit, bark or roots for their anti-microbial, hepatoprotective, anti-diabetic and anti-inflammatory properties (Dakappa et al., 2013; Morais-Braga et al., 2016; Díaz-de-Cerio et al., 2017). The profile of secondary plant metabolites of guava includes several phytoconstituents such as various terpenoids, flavonoids, carotenoid and phenolic (Rojas-Garbanzo et al., 2017). Significantly, guava leaf has been reported to protect against T2DM. Guava-leaf aqueous extract (GvAEx) treatment for 6 weeks attenuated the progression of hyperglycemia and hyperlipidemia in diabetic mice (Jayachandran et al., 2020). Besides, GvAEx significantly reduced postprandial glucose level in human subject while had no hypoglycemia side effect (Deguchi and Miyazaki, 2010). It is reported that polysaccharides and flavonoid compounds purified from guava leaves synergistically inhibited α-glucosidase and α-amylase, which would delay the absorption of glucose in the small intestine to lower blood glucose levels (Zhang et al., 2016; Beidokhti et al., 2020). Other studies have found that guava leaf extract may exert its anti-diabetic effects by activating the PI3K/AKT signaling pathway in the liver and muscle of diabetic mice (Vinayagam et al., 2018; Jayachandran et al., 2020). Recently, many studies have implied that hypoglycemic drugs can work through the regulation of intestinal flora, and that changes in intestinal flora can, in turn, affect the efficacy of hypoglycemic drugs (Wu et al., 2017; Balaich et al., 2021). However, it remains to be determined on whether GvAEx exerts its anti-diabetic action by regulation of the intestinal flora.

In the present study, we investigated the hypoglycemic effects of GvAEx and explored its underlying mechanism *in vivo* and *in vitro*. This study provides new evidence about the anti-diabetic action of GvAEx. More importantly, for the first time, we elaborated the potential mechanism of the hypoglycemic effect of GvAEx from the perspective of intestinal microbiota.

RESEARCH DESIGN AND METHODS

Reagents

Guava leaf aqueous extracts were obtained from our previously reported methods (Bai et al., 2019). The primary antibodies for mouse β-actin, ATP1A1, phospho-AKT (Ser473), AKT, phospho-GSK3β (Ser9), GSK3β were obtained from Cell Signaling Technology Inc. (Beverly, MA, United States). Anti-GLUT2 antibody was purchased from Proteintech (Chicago, United States).

Animal Studies

Six-week-old male db/db mice were purchased from the National Resource Center for Mutant Mice (Nanjing, Jiangsu, China) and housed in a temperature-controlled environment with a 12:12 h light/dark cycle. The mice had free access to food and water *ad libitum*. After a 1-week adaptive period, the mice were randomly divided into two weight-matched groups (n = 8/group) and fed with chow diet (10% kcal; #12540B; Research Diets, Inc., New Brunswick, United States). The body weight and fasting plasma glucose level were monitored weekly. After 12 weeks of gavage administration with GvAEx (7.0 g/kg) or water, animals were sacrificed and their tissues were rapidly isolated, then immediately frozen in liquid nitrogen and stored at −80°C prior for further experiments. All animal experiments were conducted in accordance with the guidelines issued by the Institutional Animal Care and Use Committee (IACUC) of the Second Xiangya Hospital of Central South University, Changsha, Hunan, P. R. China (Approval #202022).

Glucose tolerance test (GTT) and insulin tolerance test (ITT) were performed according to previous reported methods (Kong et al., 2015). For GTT, mice were fasted overnight for about 16 h and glucose was injected intraperitoneally (i.p.) at a dose of 2 g/kg. For ITT, food was removed from mice 4 h prior to experiment and insulin was administered i.p. at 0.4 IU/kg. The caudal vein blood glucose levels were measured at 0, 30, 60, 90, and 120 min after the administration of glucose or insulin. Homeostatic model assessment for insulin resistance (HOMA-IR) index was calculated using the following equation [fasting blood glucose (mmol/L) × fasting insulin (μU/mL)]/22.5.

Metabolic and Biochemical Analyses

The content of glycogen in liver and muscle was measured according to manufacturer's protocols (Solarbio Science and Technology Co., Ltd. Beijing, China). Briefly, precipitates of liver and muscle were added 0.2% anthrone diluted with 98% concentrated sulfuric acid (H_2SO_4) and followed by boiling

water-bath heating for 20 min. After cooling on the ice, the OD values were measured at 620 nm by an ELISA reader. The serum insulin and c-peptide levels were determined using mouse insulin enzyme-linked immunosorbent assay (ELISA) kits (CUSABIO, Wuhan, China) according to manufacturer's instructions.

Serum lipids (total cholesterol, triglyceride) and functional parameters of liver [alanine aminotransferase (ALT), aspartate transaminase (AST)] and renal (creatinine, urea) were measured using a Modular Analytics analyzer (Roche Diagnostics GmbH, Mannheim, Germany) according to manufacturer's instructions.

Cell Culture and Treatment

HepIR mouse liver cells were cultured in MEMα (Gibco) culture medium supplemented with 4% fetal bovine serum (FBS; Gibco) 100 U/ml penicillin and 100 U/ml streptomycin (Gibco) at 37°C. Cells were pre-incubated in serum-free medium for 4 h and then treated with 200 uM PA to induce insulin resistance.

The glucose uptake of HepIR cells was evaluated using the fluorescent glucose 2-[N-(7-nitrobenz-2-oxa-1,3diazol-4-yl) amino]-2-deoxyglucose (2-NBDG) (Cayman Chemical, Ann Arbor, MI, United States). The effect of GvAEx on glucose uptake was assessed in PA induced insulin-resistant HepIR cells. Briefly, PA induced insulin-resistant HepIR cells were treated with GvAEx (0.1–2 mg/ml) or vehicle in glucose-free DMEM for 1 h. After aspirating the supernatant, the cells were incubated with insulin (10 nM) (Solarbio, Beijing, China) or saline for 10 min. The cells were then gently rinsed with HBSS and incubated with 100 ug/mL 2-NBDG at 37°C for 50 min. The cells were washed with HBSS and collected for further analysis. The fluorescence intensity of the cells was detected by a microplate reader (excitation/emission = 465/540 nm). Relative 2-NBDG (%) uptake was calculated using the following equation (intensity of treatment/intensity of normal control) × 100%.

Membrane and Cytosolic Protein Extraction

Proteins from the membrane and cytoplasm fractions of HepIR cells were isolated using the Membrane and Cytosol Protein Extraction Kit (P0033, Beyotime Biotechnology, China) according to the manufacturer's instructions. Briefly, about 5×10^6 cells in the culture medium were scrapped off the surface of the plate with a cell scraper. Cell suspension was centrifuged at 600 × g at 4°C for 5 min to precipitate cells. Membrane Protein Extraction Reagent A with Phenylmethanesulfonyl fluoride (PMSF) (ST506, Beyotime) was added in cells. The cells were gently and fully suspended and set in an ice bath for 10–15 min. The homogenate was placed on ice for 15 min, centrifuged at 700 × g for 10 min, then further centrifuged at 14,000 × g for 30 min. The supernatant (cell cytosol) was carefully collected, and the pellet was then re-suspended in Membrane Protein Extraction Reagent B and centrifuged at 14,000 × g for 5 min. Finally, the supernatant containing the cell membrane proteins was collected. Western blotting was performed for membrane and cell membrane proteins, and Na+/K + ATPase α1 and β-actin were used as internal controls for membrane and cytosolic proteins, respectively.

Cell Viability Analysis

The cytotoxicity of GvAEx on HepIR cells was assessed with Cell Counting Kit-8 (CCK-8, CK04, DOJINDO) according to the manufacturer's protocol. After cells were co-cultured with GvAEx (0.1–3 mg/ml) at 37°C for 24-h, CCK-8 reagent was added to the culture medium at a ratio of 1:10 for another 1 h. The absorbance value of each well was measured at 450 nm using a microplate reader to determine cell viability. Cell viability (%) = (absorbance of treatment - absorbance of blank well)/(absorbance of control-absorbance of blank well) × 100%.

Western Blotting

For protein extraction, HepIR cells were homogenized in 100 μL RIPA buffer (P0013B, Beyotime). Proteins were transferred to polyvinylidene difluoride membrane and incubated with a blocking buffer (5% BSA in 20 mM Tris-HCl, pH 7.5, 137 mM NaCl, and 0.1% Tween 20) for 1 h at room temperature. Membranes were incubated with primary antibodies for 16 h at 4°C, washed three times for 10 min each and then incubated with second antibodies for 1 h at room temperature. Signals were detected using the ChemiDoc™ XRS+ and the Image Lab™ system (BIO-RAD, United States).

Quantitative RT-PCR

Total RNAs were isolated from tissues or cells with the Trizol reagent (Invitrogen, Shanghai, China) following the manufacturer's protocol. Reverse transcription of mRNA was performed using a Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, United States). Real-time polymerase chain reaction was performed using the FastStart universal SYBR Green Master (Roche, United States) on a 7900HT Fast Real-Time PCR System (Applied Biosystems). The standard amplification program in polymerase chain reaction system was used, which consisted of 1cycle at 95°C for 10 mi, followed by 40 cycles of 95°Cdenatured for 30 s, 60°C annealed for 1 min, and72°C elongated for 1 min. Primers were listed in **Supplementary Table S1**.

Fecal DNA Extraction and 16s rRNA Gene Sequencing

Total genome DNA from samples was extracted using an Equal bit 1x dsDNA HS Assay Kit (Lot: 7E421E0) based on kit protocols. The specific experimental details used to amplify the 16S rRNA gene targeting the V3-V4 region by PCR were performed as previously described (Jia et al., 2021).

The DNA library concentration was determined using the Qubit 3.0 Fluorometer. DNA libraries were multiplexed and loaded on the Illumina MiSeq/NovaSeq (Illumina, San Diego, CA, United States) platform according to manufacturer's instructions. Sequencing was performed in pairs and image analysis and base calling were conducted by the control software embedded in the instrument. The 16S rRNA gene sequencing and preliminary data analysis were provided by GENEWIZ Inc. (Suzhou, China). A total of 705,692 reads were obtained, and 612,351 high-quality sequences were

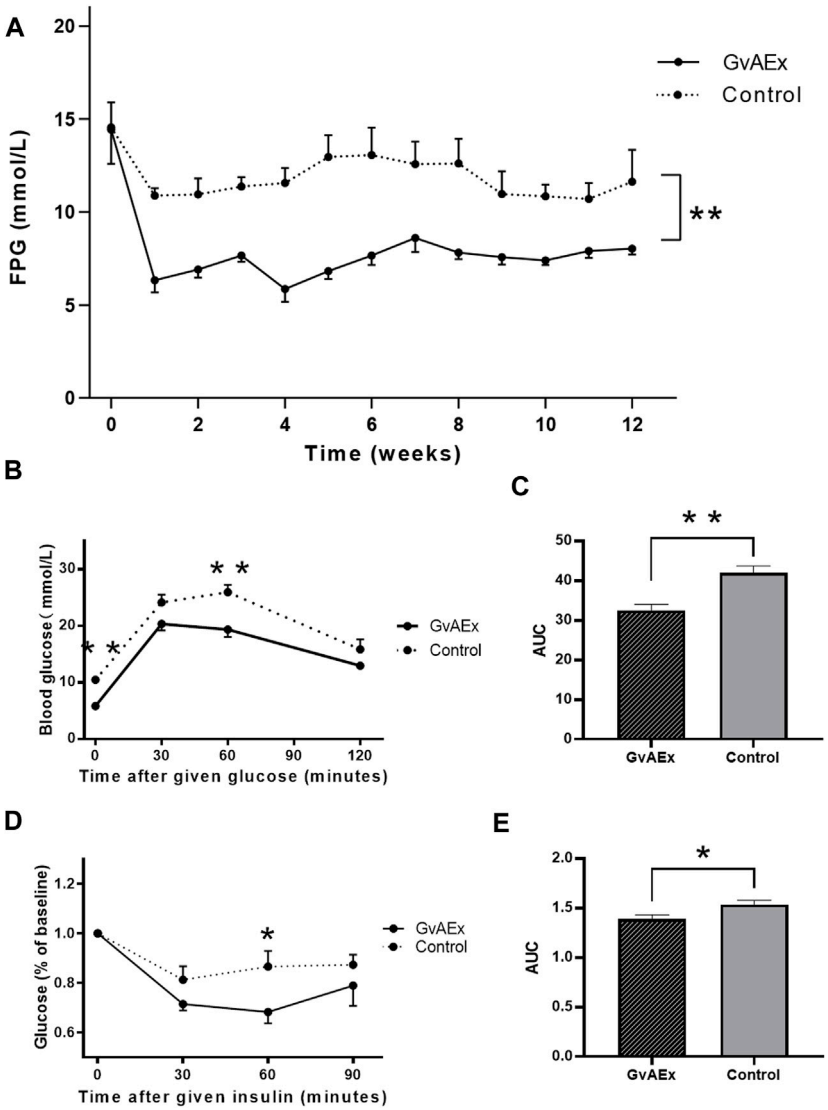

FIGURE 1 | GvAEx treatment lowers blood glucose in a dosage-dependent manner in db/db mice. **(A)** Fasting plasma glucose (FPG) levels of male db/db mice treated with GvAEx or vehicle for 12 weeks (n = 6/group). **(B)** Blood glucose levels in the db/db mice treated with GvAEx or vehicle as determined by IPGTT (n = 6/group). **(C)** Area under curve (AUC) from **(B)**. **(D)** Blood glucose levels as determined by ITT (n = 6/group). **(E)** AUC from **(D)**. *p < 0.05, **p < 0.01, ***p < 0.001.

obtained after optimizing the sequencing raw data. Among of them, 327,943 reads were obtained from GvAEx group, accounting for 53.6%; 284,408 reads were obtained from control group, accounting for 46.4%. The number of each sample reads ranged from 39,021 to 62,994, with an average length of 459 bp. The analysis of abundance, a and β diversity of OTUs were performed by Perl and R language software to obtain information on the species richness and evenness of the samples. Principal co-ordinates analyses (PCoA) were performed to determine the significant microbial community differences between the samples.

Data Analysis

All results were presented as means ± SEM. Student's t test analysis between two groups was performed with SPSS Statistics Software (version 19.0; IBM Corp., Armonk, New York). $p < 0.05$ was considered statistically significant.

RESULTS

GvAEx Treatment Lowers Blood Glucose in a Dosage-Dependent Manner in db/db Mice

To explore the effects of GvAEx on the blood glucose, we used three dosages (1.75 g/kg/d, 3.5 g/kg/d, 7.0 g/kg/d) in a preliminary experiment and found that the lowest dose (1.75 g/kg/d) had no effects on fasting plasma glucose (FPG) during 12 weeks of treatment (**Supplementary Figure S1A**). However, the median dose (3.5 g/kg/d) could reduce blood glucose in diabetic db/db mice after 10 weeks of

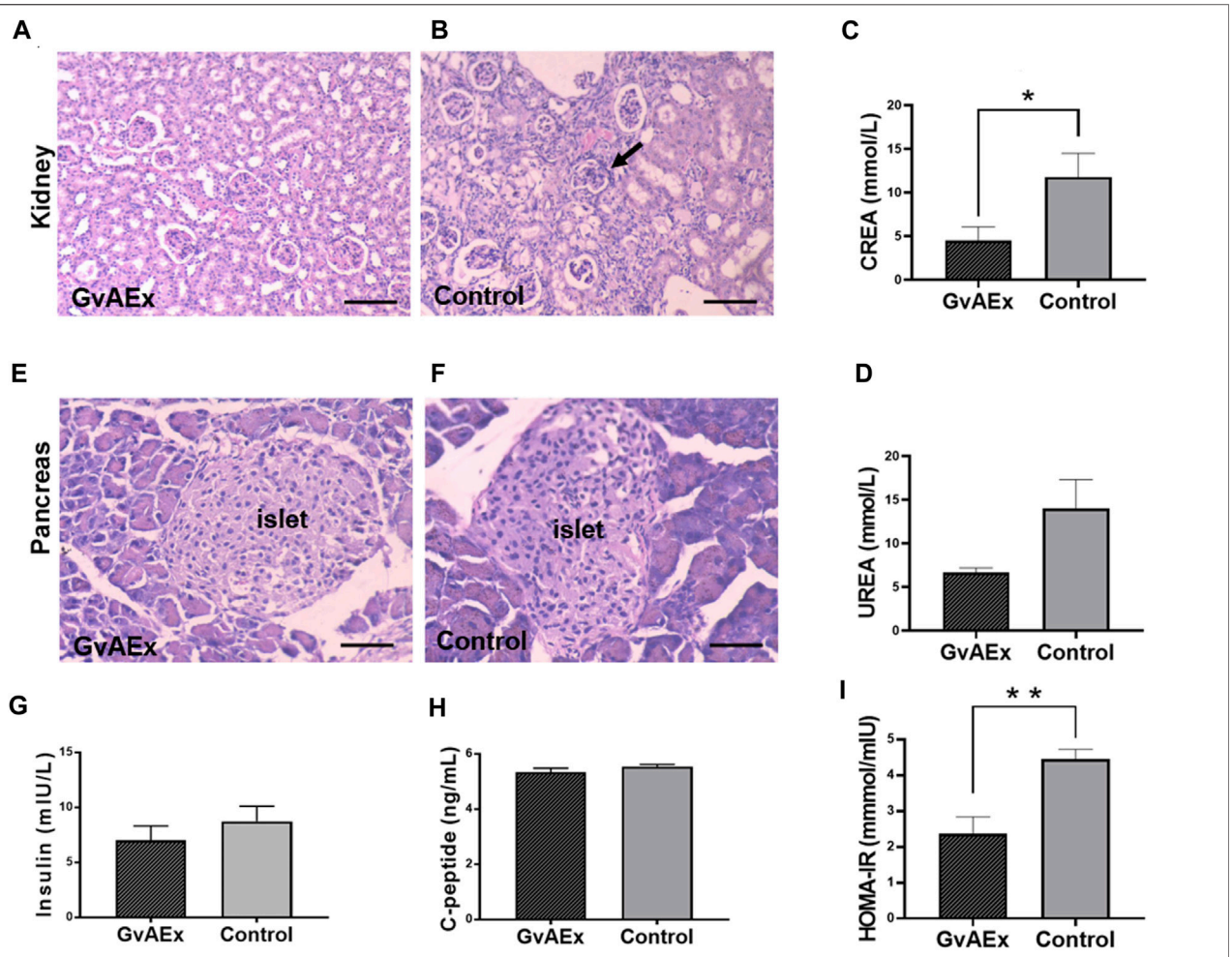

FIGURE 2 | Treatment of GvAEx improves kidney and islet function in diabetic mice. **(A,B)** Representative light microscopic images of kidney after 12 weeks of GvAEx **(A)** or vehicle **(B)** treatment (scale bar = 100 μm). **(C,D)** Plasma levels of Creatinine (CREA) **(C)** and urea **(D)** in the db/db mice treated with GvAEx or vehicle for 12 weeks (n = 6/group). **(E,F)** Representative light microscopic images of pancreatic islet in GvAEx **(E)** and control mice **(F)** (scale bar = 50 μm). **(G,H)** Serum levels of insulin **(G)** and c-peptide **(H)** (n = 6/group). **(I)** HOMA-IR index of GvAEx and control mice (n = 6/group). *p < 0.05, **p < 0.01, ***p < 0.001.

treatment (**Supplementary Figure S1A**), and the highest dose (7.0 g/kg/d) had obvious blood-lowering effects starting from the first week of treatment, which could be maintained throughout the 12 weeks treatment period (**Figure 1A**, p < 0.01), indicating a dosage-dependent blood glucose lowering effect of GvAEx in db/db mice. Therefore, we have selected 7.0 g/kg/d as the dosage of GvAEx treatment in the following studies. There were no differences in body weight between GvAEx and control groups (**Supplementary Figure S1B**).

To determine whether GvAEx had effects on glucose tolerance and insulin sensitivity, we performed GTT and ITT analyses after 12 weeks treatment and found that GvAEx treatment improved glucose tolerance and insulin sensitivity (**Figures 1B–E**, p < 0.01, p < 0.05, respectively) in db/db mice. Taken together, the GvAEx treatment had attenuated hyperglycemia and improved glucose tolerance and insulin sensitivity in db/db mice.

Treatment of GvAEx Improves Kidney and Islet Function in Diabetic Mice

To test the potential protective effects of GvAEx on diabetic nephropathy in db/db mice, we examined the histopathological features of kidney using hematoxylin-eosin (H-E) staining (**Figures 2A,B**). Diabetic kidney diseases are characterized by glomerular hyperfiltration, inflammation and fibrosis. Treatment with GvAEx showed less inflammatory cell infiltration and fibrosis in the kidney (**Figure 2A**) compared with control mice (**Figure 2B**). Consistent with less pathomorphologic features of diabetic nephropathy observed in the kidney, blood creatinine levels significantly decreased in the GvAEx-treated mice (**Figure 2C**; p < 0.05). There was also a trend of decrease in blood urea, but it did not reach statistical significance (**Figure 2D**).

In order to test whether glucose lowering effects of GvAEx were induced by improvement of islet function, we first detected

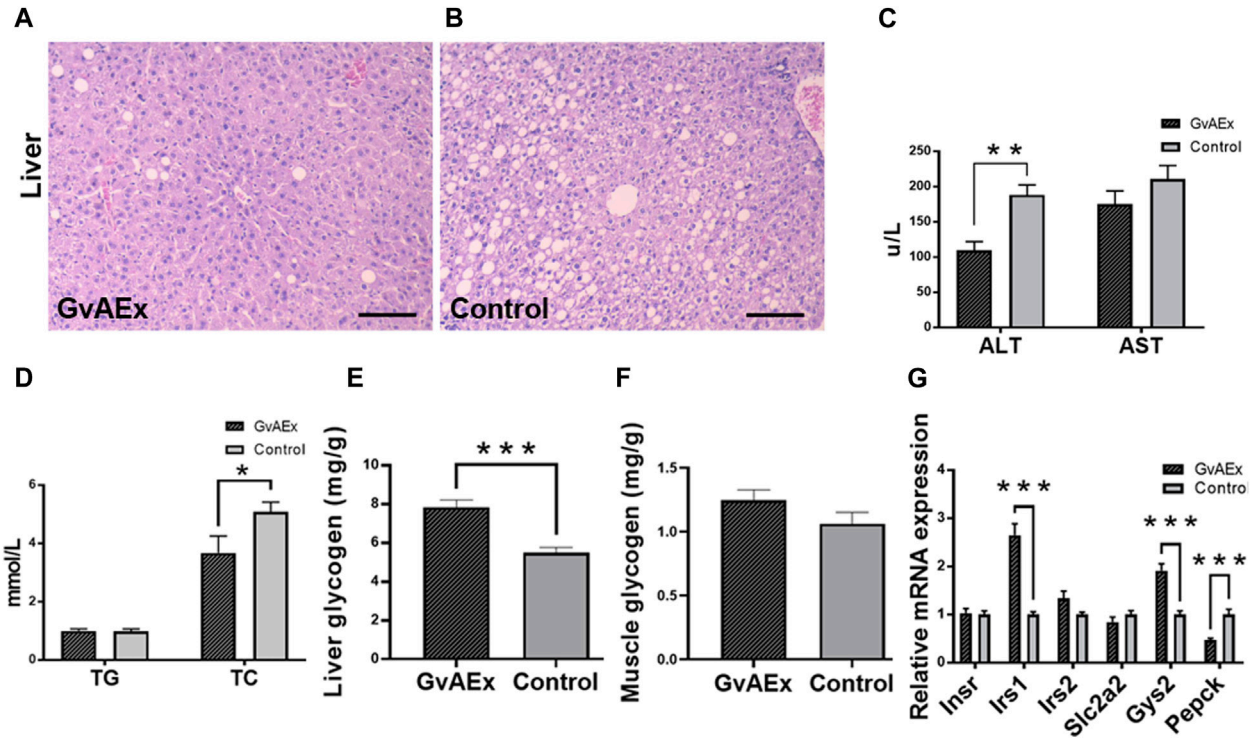

FIGURE 3 | GvAEx treatment promotes liver function and reduces gluconeogenesis. **(A,B)** Representative light microscopic images of liver in db/db mice treated with GvAEx **(A)** or vehicle **(B)** for 12 weeks (scale bar = 100 μm). **(C)** Plasma levels of alanine aminotransferase (ALT) and aspartate transaminase (AST) (n = 6/group). **(D)** Plasma levels of total cholesterol (TC) and triglyceride (TG). **(E)** Hepatic glycogen content. **(F)** Skeletal muscle glycogen content (n = 6/group). **(G)** Relative mRNA expression levels of genes involved in glycogen synthesis and gluconeogenesis in the liver. *p < 0.05, **p < 0.01, ***p < 0.001.

morphological changes of the pancreatic islets. Islets sizes were comparable between the GxAEx-treated and control animals (**Figures 2E,F**). Additionally, fasting serum insulin concentrations were also comparable between the GvAEx-treated and control groups after 3 months of treatment (**Figure 2G**). C-peptide is a widely used measurement of pancreatic beta cell function. It reflects the amount of endogenous insulin secreted and maintained at a more constant rate over a longer period of time (Leighton et al., 2017). Likewise, we found no difference of fasting serum c-peptide concentrations between the GvAEx-treated and control groups (**Figure 2H**). HOMA-IR index is a widely used parameter that can estimate insulin resistance. GvAEx-treated mice displayed lower HOMA-IR index compared to those of the controls (**Figure 2I** p < 0.01). These results indicate that the glucose lowering effect of GvAEx administration might not be caused by increased insulin secretion from the pancreas but by improved insulin sensitivity in peripheral tissues.

GvAEx Treatment Promotes Liver Function and Reduces Gluconeogenesis

The liver is one of the major peripheral targets for the action of insulin to regulate glucose homeostasis. The histopathological changes of liver obtained from gavage treatment of GvAEx or vehicle are shown in **Figures 3A,B**, respectively. Compared with the liver of vehicle control mice (**Figure 3B**) that showed foci of ballooning degeneration of hepatocytes, db/db mice treated with GvAEx displayed a relative normal liver histomorphology with fewer ballooning like structures (**Figure 3A**), indicating alleviated fatty liver conductions.

After 12 weeks of GvAEx treatment, there was a significantly decreased serum level of liver enzyme ALT (**Figure 3C**; p < 0.01) but not AST (**Figure 3C**). Plasma total cholesterol (TC) level significantly decreased in the GvAEx-treated mice compared to that of the vehicle controls (**Figure 3D**; p < 0.05), but triglyceride (TG) levels were comparable between GvAEx and control groups (**Figure 3D**).

Glycogen is primarily synthesized and stored in the liver and skeletal muscles (Prats et al., 2018). One of the key features of diabetes is dysregulation of liver glycogen metabolism, in which glycogen can be abnormally accumulated or depleted because of peripheral tissue insulin resistance. Therefore, we measured the proportion of glycogen in the liver to see if GvAEx had an impact on hepatic glycogen synthesis and storage. After 12 weeks of GvAEX gavage, glycogen levels in the liver dramatically increased compared to those of the control group (**Figure 3E**; p < 0.001). However, the skeletal muscle glycogen levels in db/db mice were comparable between the two groups (**Figure 3F**). Gene expression analyses have shown that in the case of Irs1 and Gys2, both

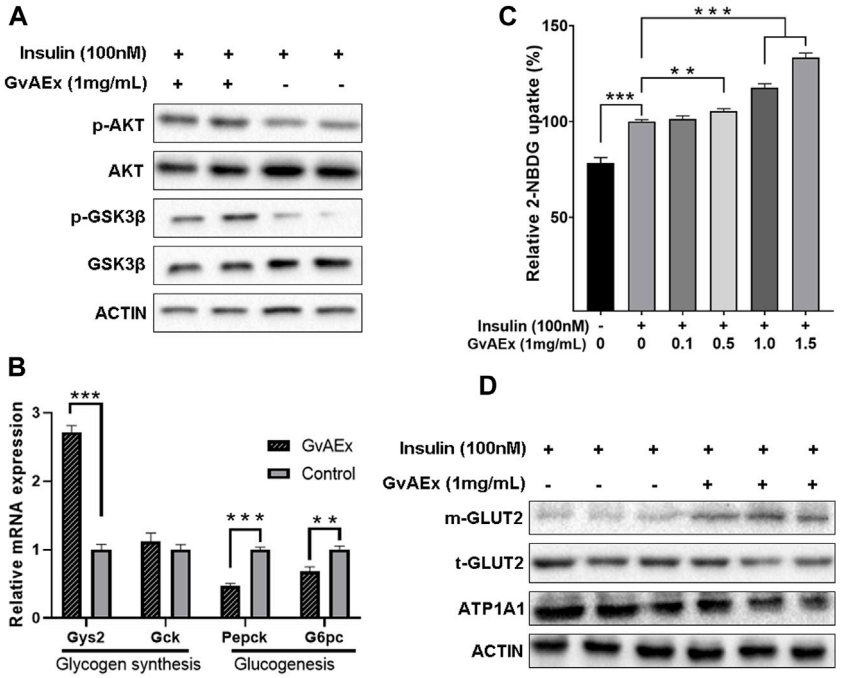

FIGURE 4 | GvAEx enhances insulin sensitivity and glucose uptake in hepatocytes. **(A)** Western blot analysis of insulin signaling-related molecules in HepIR cells with or without GvAEx treatment. **(B)** Relative mRNA expression levels of genes involved in glycogen synthesis and gluconeogenesis in HepIR cells treated with or without GvAEx ($n = 4$). **(C)** Effects of GvAEx on glucose (2-NBDG) uptake of insulin-resistant HepIR cells ($n = 6$). **(D)**: Western blot analysis of total and membrane GLUT2. p *<0.05, **$p < 0.01$, ***$p < 0.001$.

genes are involved in insulin-regulated glycogen synthesis, were elevated in the liver after GvAEx treatment (**Figure 3G**; $p < 0.001$). In mRNA levels of phosphoenolpyruvate carboxykinase (Pepck), a gene plays a key role in the gluconeogenesis (Méndez-Lucas et al., 2013) and was significantly decreased in the GvAEx group (**Figure 3G**; $p < 0.01$), suggesting that GvAEx treatment could increase glycogen synthesis but reduce hepatic gluconeogenesis.

Taken together, our results have demonstrated that GvAEx treatment could ameliorate hepatic fat deposition and attenuate liver injury. In the meantime, GvAEx could increase gene expressions of hepatic glycogen synthesis while decreasing glucogenesis genes, as a result leading to the hypoglycemic effect in db/db mice.

Effects of GvAEx on HepIR Cell Viability

To explore the effects of GvAEx *in vitro*, we treated mouse hepatic cell line HepIR with various doses of reagent. We first used CCK8 assay to measure cell viability and the cytotoxicity of GvAEx. The relative cell viabilities of HepIR treated with 0.1–3 mg/ml GvAEx for 24 h are shown in **Supplementary Figure S2**, and no significant inhibitions of cell viability were observed using GvAEx treatments with doses of up to 2 mg/ml when compared to vehicle controls. However, significant inhibitions of cell proliferation (29–41% inhibition) were observed starting at a dose of 2.2 mg/ml, and 3 mg/ml, the highest dosage used in the study (**Supplementary Figure S2A**). Therefore, we used 0.1–1.5 mg/ml GvAEx as safe dosage in the following *in vitro* studies.

GvAEx Enhances Insulin Sensitivity and Glucose Uptake in Hepatocytes

As insulin resistance is a key pathophysiologic factor of T2DM, we mimic the insulin resistance-like condition *in vivo* by treating HepIR live cells with palmitic acid. The **Supplementary Figure S2B** showed that 200 μM palmitic acid significantly reduced phosphorylation of AKT, which implied successfully induced insulin resistance in HepIR cells. All subsequent cell experiments were based on this experimental condition.

To clarify whether and how GvAEx improved insulin sensitivity in hepatocytes, the expression and phosphorylation levels of insulin signaling-related molecules with and without GvAEx treatment were assessed. In contrast to the control group, GvAEx significantly increased the tyrosine phosphorylation level of AKT in HepIR cells (**Figure 4A**). GSK-3, a ubiquitously expressed serine/threonine protein kinase, is a critical downstream element of the insulin/PI3K/AKT pathway, whose activity can be inhibited by Akt-mediated phosphorylation at Ser9 of GSK-3β. GSK-3 can phosphorylate and inactivate glycogen synthase that leads to less glycogen synthesis (Srivastava and Pandey, 1998). We found that co-treatment with insulin and GvAEx (1 mg/ml) significantly increased the phosphorylation levels of GSK-3β when compared to insulin treatment alone (**Figure 4A**). What's more, GvAEx treatment also up-regulated Gys2 mRNA expression levels but down-regulated

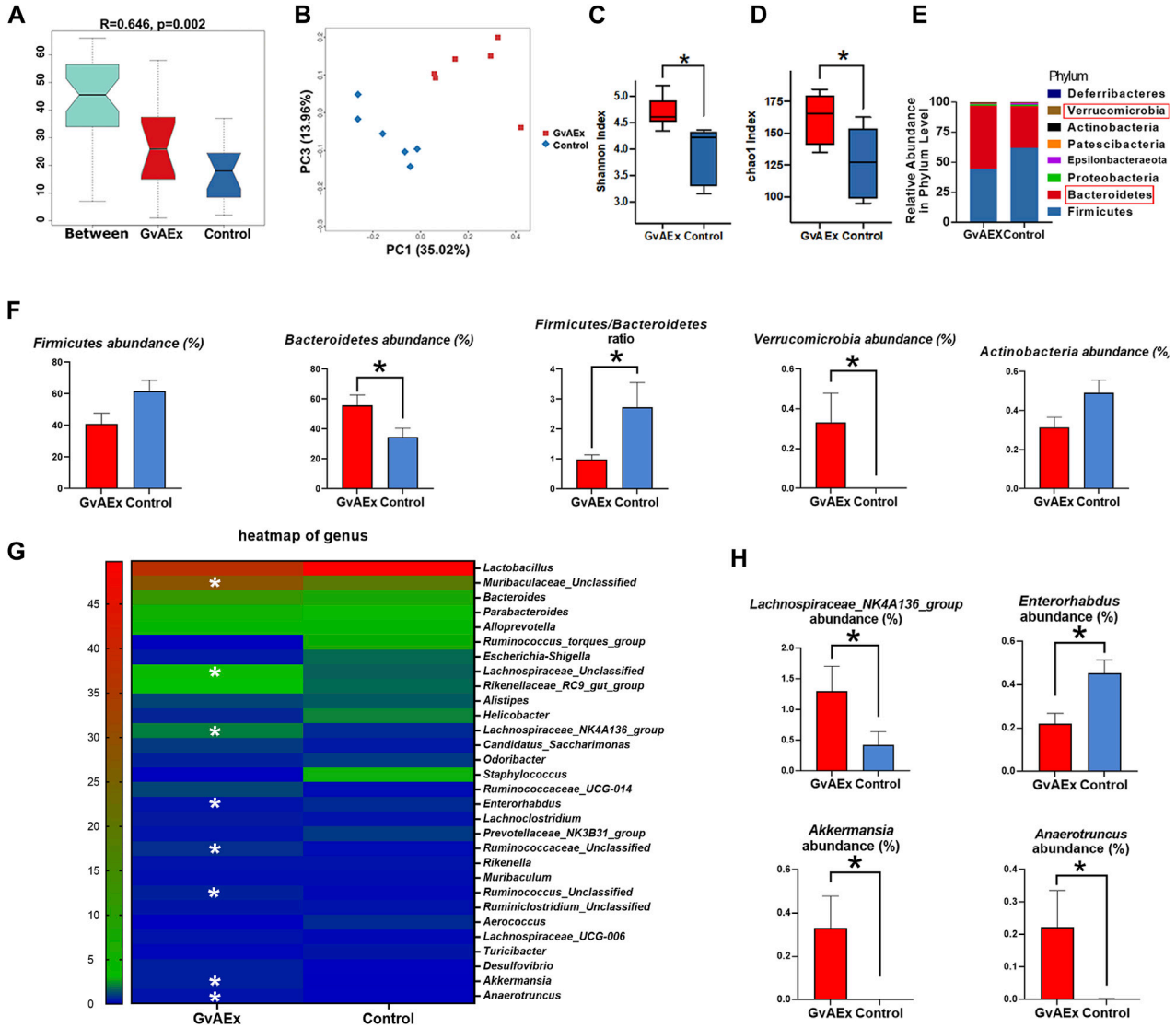

FIGURE 5 | GvAEx treatment alters the composition of gut microbiota in db/db mice. **(A)** Anosim analysis of gut microbiota in db/db mice treated with GvAEx or vehicle for 12 weeks ($R = 0.646$, $p = 0.002$) ($n = 6$/group). **(B)** PCOA of gut microbiota in mice based on weighted UniFrac. **(C)** Species diversity indices (Shannon). **(D)** Species richness estimates (Chao1). **(E)** Phylum level comparison of gut microbiota proportional abundance in feces of GvAEx and control. **(F)** Relative abundance of microbiota phylum between the groups. **(G)** Heatmap of gut microbiota at the genus level in mice. **(H)** Relative abundance of microbiota at the genus level between groups. $^*p < 0.05$.

Pepck level in HepIR cells (**Figure 4B**). Additionally, GvAEx decreased expression of G6pc, one of the glucose-6-phosphatase catalytic-subunit-encoding genes and a key enzyme in gluconeogenesis and glycogenolysis (**Figure 4B**). Our data has suggested that GvAEx treatment could activate insulin signaling, thus promoting glycogen synthesis while suppressing glucogenesis in the hepatocytes.

To determine changes in glucose uptake ability insulin-induced, we performed a fluorescent 2-NBDG (a fluorescence derivative of D-glucose) uptake assay. HepIR cells were treated with insulin (10 nM) alone or in the presence of GvAEx (0.1–1.5 mg/ml) and fluorescent 2-NBDG levels were monitored. The data has shown that insulin significantly increased 2-NBDG levels by up to 28.4% when compared to control cells (**Figure 4C**). In the presence of GvAEx there were significantly higher increased rates of 2-NBDG, whose effects were more pronounced with higher GvAEx concentrations, when compared to that of the insulin treatment alone (**Figure 4C**), suggesting that GvAEx could promote insulin-induced glucose uptake in a dose-dependent manner.

In the hepatocytes, GLUT2 is translocated from the cytoplasm to the plasma membrane in response to insulin and is the primary carrier for the transportation of extra-cellular glucose into the hepatocytes (Thorens, 2015). We have tested whether GvAEx had impacts on GLUT2 expression in hepatocytes. Serum-starved HepIR cells were treated with GvAEx (1 mg/ml) for 24 h. Serum-starvation and subsequent culture in the serum-free medium

ruled out the possible influence of serum factors on the membrane translocation of GLUT2. We then extracted the whole cells and cell membrane proteins, respectively. The levels of total GLUT2 (t-GLUT2) did not change markedly between the groups (**Figure 4D**). However, compared to the insulin alone treatment group, insulin combined with GvAEx significantly increased the content of membrane GLUT2 (m-GLUT2), indicating that GvAEx could promote insulin induced GLUT2 translocation in hepatocytes.

Taken together, GvAEx treatment enhanced the expression of genes involved in hepatic glycogen synthesis and inhibited gene expression in gluconeogenesis. Additionally, it promoted glucose uptake and GLUT2 translocation from cytosol to membrane *in vitro*.

GvAEx Treatment Alters the Composition of Microbiota in db/db Mice

In recent years, a large body of evidence has suggested that the gut microbiota may play an important role in the pathophysiology of obesity and diabetes (Qin et al., 2012; Wang et al., 2018; Gurung et al., 2020). Specifically, gut microbiota may mediate environmental factors related to glucose homeostasis by impacting major metabolic organs such as liver, muscle and fat (Gurung et al., 2020). Therefore, we have analyzed the composition of the intestinal flora of GvAEx oral gavage treated db/db mice using 16S rRNA sequencing. There were significant differences in colony distribution between the GvAEx treatment group and control group (**Figure 5A**, $R = 0.646$, $p < 0.01$). The PCoA analysis indicated that the gut microbiota structure in GvAEx-treated mice was distinctive from the untreated control group (**Figure 5B**). By estimating bacterial richness and calculated diversity, we have found that the GvAEx group had a significantly higher microbial diversity (**Figure 5C**, $p < 0.05$, Shannon) and number of microbial species (**Figure 5D**, $p < 0.05$, Chao1) than those of the control group.

The profound compositional changes of the gut microbiota were further analyzed in GvAEx-treated and vehicle group mice. At the phylum level, both *Firmicutes* and *Bacteroidetes* were the two most dominant phyla of the gut microbiota in GvAEx and control group (**Figure 5E**). However, GvAEx treatment significantly increased *Bacteroidetes* abundance when compared with that in the vehicle control (**Figure 5F**). Additionally, there were trends of decrease of *Firmicutes* abundance and *Actinobacteria* abundance in GvAEx group (**Figure 5F**). Meanwhile, the *Firmicutes* to *Bacteroidetes* ratio had also significantly decreased after GvAEx treatment (**Figure 5F**; $p < 0.05$). Furthermore, the phylum abundance of *Verrucomicrobia* increased when compared to that in vehicle control group (**Figure 5F**; $p < 0.05$).

At the genus level, we selected the top 30 abundant genera and generated a heatmap (**Figure 5G**). The data showed an increase in the abundance of Muribaculaceae, Lachnospiraceae, Lachnospiraceae_*NK4A136_group*, Ruminococcaceae, *Ruminococcus*, *Akkermansia* and *Anaerotruncus* after GvAEx treatment while the abundance of *Enterorhabdus* was reduced significantly (**Figures 5G,H**). Taken together, these data suggest that GvAEx treatment significantly reshaped the gut microbiota composition of db/db mice.

DISCUSSION

Psidium guajava L. leaves have traditionally been widely used to treat several diseases, including infectious diseases, neoplasm, metabolic diseases and digestive diseases (Díaz-de-Cerio et al., 2017). There are several studies that have focused on elucidating the anti-diabetic compounds present in guava leaves. In particular, quercetin in the aqueous extract of guava leaves has been found to promote glucose uptake in hepatocytes and alleviate hyperglycemia in diabetes (Cheng et al., 2009). However, the underlying mechanism by which quercetin regulates the glucose uptake of hepatocytes is still unclear. Besides, studies have reported that guava leaf extract might exert its anti-diabetic effects by activating the PI3K/AKT signaling pathway in the liver and muscle of diabetic mice (Vinayagam et al., 2018; Jayachandran et al., 2020). Polysaccharides and flavonoid compounds purified from guava leaves synergistically inhibited *a*-glucosidase and *a*-amylase which would delay the absorption of glucose in the small intestine to lower blood glucose levels (Zhang et al., 2016; Beidokhti et al., 2020). Glucagon-like peptide-1 (GLP-1) is a hormone that lowers blood glucose levels by stimulating insulin, inhibiting glucagon, suppressing gastric emptying and promoting islet cell regeneration. Its' degradation is controlled by dipeptidyl peptidase 4 (DPP4) (Smith et al., 2019). Ethanolic extract of guava leaves dose-dependently inhibited DPP4 due to individual flavonoid glycosides, such as quercetin, isoquercitrin, etc. (Eidenberger et al., 2013). The negative regulatory role of protein tyrosine phosphatase 1B (PTP-1B) in insulin signaling prevents insulin receptors from binding to insulin, which in turn causes insulin resistance and ultimately leads to T2DM. Methanol extract of *Psidium guajava* leaves was found to possess significant inhibitory effect on PTP1B (Oh et al., 2005). However, all of the above studies only confirmed the inhibitory effects of phytocompound of guava leaf on the related enzyme activities *in vitro*. We have previously reported that GvAEx is rich in flavonoids, which may partially contribute to its anti-diabetic effects (Bai et al., 2019); however, the underlying molecular mechanism is unclear. In the present study, we've provided evidence demonstrating that the total extract of guava leaves, GvAEx, could be used as an anti-diabetic agent by modulating hepatic glucose metabolism and altering the intestinal flora composition.

In our study, we have discovered that GvAEx significantly reduced FPG levels in T2DM mice in a dose-dependent manner. In a previous study, oral administration of guava leaf extract had beneficial anti-obesity effects on SHRSP. Z-Leprfa/IzmDmcr rats (Yoshitomi et al., 2012). However, inconsistent with this previous study, we did not observe reduced obesity after GvAEx administration in the db/db mice. This could be due to the different genetic background of the animal models used in the studies. Additionally, GvAEx could improve glucose tolerance and insulin sensitivity in db/db mice. These results suggested that GvAEx gavage have beneficial effects on glucose metabolism in diabetic mice.

It is well established that certain natural extracts such as ginseng could increase insulin secretion to lower plasma glucose in animals and humans (Lee et al., 2006). In our study we found no difference in serum insulin levels and c-peptide levels between the two groups, thus we proposed that the glucose lowering effects of GvAEx were

not due to promotion of insulin secretion, but to improve insulin sensitivity in the peripheral tissues.

The liver is the major site for glucose and lipid metabolism, and hepatic insulin resistance is thought to be one of the main causes of fasting hyperglycemia (Petersen and Shulman, 2018). In the current study, we've discovered that not only did GvAEx treatment alleviate fatty liver morphological conditions, it also improved liver insulin sensitivity *in vivo* and *in vitro*. Compared to the control group, GvAEx treatment significantly lowered HOMA-IR values, which is an indicator of improved liver insulin sensitivity. It is well established that insulin sensitivity can be regulated directly or indirectly by modulating the components of the insulin signaling pathway, such as insulin receptor (IR), insulin receptor substrates (IRS) and AKT (Yang et al., 2018). The insulin-bound IR promotes the binding and activation (Tyr phosphorylation) of IRS after insulin activation, which then activates the phosphoinositide 3-kinase (PI3K)/AKT pathway (Huang et al., 2018; Yang et al., 2018). AKT plays a pivotal role in the regulation of various biological processes, including apoptosis, proliferation and intermediary metabolism. We've discovered that GvAEx significantly increased AKT phosphorylation levels in HepIR cells compared with the control group, suggesting that improved insulin sensitivity might be caused by increased insulin signaling activity in hepatocytes.

Besides improvement of insulin sensitivity, the GvAEx treatment also regulated glycogen synthesis and gluconeogenesis in hepatocytes. Under physiological conditions, insulin lowers blood glucose levels by prompting the liver and muscles to take up glucose from the blood and store it as glycogen. GSK-3 could be inhibited upon phosphorylation *via* AKT, which results in an increase of glycogen synthesis (Sesti, 2006). Additionally, the liver contributes significantly to maintaining the balance of glucose metabolism by altering the levels of hepatic glucose release, controlling the processes of gluconeogenesis and glycogenolysis (Hatting et al., 2018). Our results have shown that the GvAEx treatment elevated levels of phosphorylation of GSK-3β in HepIR cells, which were consistent with increased hepatic glycogenesis *in vivo* and *in vitro*. In the meantime, the mRNA levels of Pepck and G6pc was significantly lower in the GvAEx group, suggesting a suppressive effect on gluconeogenesis in the hepatocytes.

Glut-2 is the principal transporter for transfer of glucose between liver and blood. It mediates the amount of glucose in and out of hepatocytes by altering the rate of glucose uptake in hepatocytes (Thorens, 2015). Insulin stimulation could promote the translocation of GLUT2 from the cytosol to the plasma membrane, which leads to increased glucose uptake in hepatocytes (Ding et al., 2016). In the present study, we've discovered that GvAEx administration could facilitate glucose uptake by promoting insulin-induced translocation of GLUT2 from the cytosol to the membrane, while not affecting overall GLUT2 level in HepIR cells, which might partly contribute to in the hypoglycemic effects of GvAEx treatment in db/db mice.

In previous studies, plant flavonoids and terpenoids were widely used in prevention of obesity and diabetes by ameliorating insulin resistance, specifically, by activating AMPK and PI3K/AKT signaling (Ding et al., 2012; Zhang et al., 2012; Paoli et al., 2013; Rodríguez-Rodríguez et al., 2015). Therefore, the pluripotent effects of GvAEx on hepatic glucose metabolism and glycogen might be due to its richness in terpenoids and flavonoids. Based on these previous studies, we've suggested that the multiple bioactive components present in GvAEx synergistically exerted anti-diabetic effects by improving insulin resistance, promoting glycogen synthesis, inhibiting hepatic gluconeogenesis and enhancing glucose uptake in the hepatocytes.

Numerous studies have noted the important role of gut microbiota in the development and treatment of T2DM (Xu et al., 2015; Bauer et al., 2018; Zhao et al., 2018). In particular, the gut microbiome can interact with dietary components and habits to influence host insulin sensitivity, intestinal permeability, and glucose and lipid metabolism (Gurung et al., 2020). Both first-line drug metformin and traditional Chinese medicine can significantly alter the composition of the gut microbiota, thus helping to ameliorate hyperglycemia (Xu et al., 2015; de la Cuesta-Zuluaga et al., 2017; Wang et al., 2017; Bauer et al., 2018). Several studies have shown that the gut microbiota of obese and diabetic animals and humans exhibit a higher *Firmicutes*/*Bacteroidetes* ratio compared with normal individuals, and suggested that this ratio could be used as a reliable biomarker of impaired glucose metabolism (Ley et al., 2006; Yue et al., 2019). However, no studies have explored the possible mechanisms of the hypoglycemic effects of GvAEx from the perspective of the gut microbiota. In the present study, for the first time, we reported that there were significant differences in microbiota composition between the GvAEx and control groups. GvAEx treatment significantly increased the abundance and diversity of intestinal flora in db/db mice. Besides, the GvAEx group exhibited less *Firmicutes* abundance but richer *Bacteroidetes* abundance. The *Firmicutes*/*Bacteroidetes* ratio also decreased significantly after GvAEx treatment, indicating an improvement of glucose homeostasis. Bacteroidetes mainly produce propionate, which may reach the colon and stimulate the secretion of GLP-1, thus producing a hypoglycemic effect (Chambers et al., 2015). However, we did not detect plasma GLP-1 level in our study, which would be an interesting investigation in the future. *Bacteroides* and *Alstipes* are positive outcome predictors for metabolic diseases, such as obesity and diabetes (Kuang et al., 2017; Zhang et al., 2021). In the present study, we've found that the relative abundance of *Bacteroides* and *Alstipes* was higher in GvAEx group, albeit not statistic significantly, probably due to the limited sample size. We've also found that GvAEx treatment significantly reduced relative abundance of *Enterorhabdus* compared to the control group. Inflammation plays a very important role in the development of insulin resistance. Currently, T2DM is considered to be a chronic inflammatory disease. In many studies related to intestinal inflammation, a significant increase in the abundance of *Enterorhabdus* has been observed (Tang et al., 2021; Wang et al., 2021). It was also found that the abundance of *Enterorhabdus* was significantly increased in non-alcoholic fatty liver disease (NAFLD) mice (Li et al., 2021). In addition, *Enterorhabdus* have been found to be associated with T2DM and enterotoxicity (Shibayama et al., 2019). Short chain fatty acid (SCFA) was reported to be beneficial in regulating the functions of adipose tissues, skeletal muscles and the liver, thus contributing to improved glucose homeostasis and insulin sensitivity (Canfora et al., 2015; Zhang et al., 2015; Ma et al., 2020; Xu et al., 2020). The

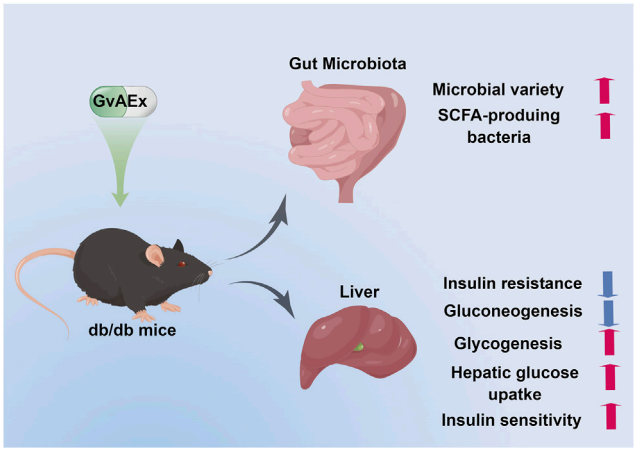

FIGURE 6 | Proposed working model of GvAEx induced anti-diabetic effect in db/db mice.

decrease in circulating SCFA may have an important role in the development of insulin resistant and diabetes (Saad et al., 2016). Importantly, SCFA plays a key role in glucose homeostasis due to its capacity to increase insulin sensitivity and resist inflammation (Säemann et al., 2000; Gao et al., 2009). Although we did not measure the intestinal SCFA level in the present study, the abundance of the Lachnospiraceae *NK4A136 group* (a SCFA-producing bacterium) and other beneficial bacteria *Akkermansia* and *Anaerotruncus* were significantly higher in the GvAEx-treated group. *Akkermansia* are thought to be associated with the amelioration of metabolic diseases and inflammation due to their anti-inflammatory and insulin-sensitive properties (Graessler et al., 2013). *Akkermansia* are able to degrade mucins and protect the intestinal mucosal layer (Zhang et al., 2019). These changes in these SCFA-producing-related bacteria may partially explain the ameliorated insulin resistance and improved lipid profiles in db/db mice treated with GvAEx.

To summarize, in this study, we have demonstrated the anti-diabetic functions and underlying mechanisms of GvAEx in db/db mice. As shown in **Figure 6**, GvAEx might improve insulin sensitivity in the liver by promoting hepatic glycogen synthesis and inhibiting hepatic gluconeogenesis *via* modulating insulin-related signaling pathways. We also found that GvAEx could elevate the expression of GLUT2 on the cell membrane of hepatocytes, which might promote the uptake of glucose by hepatocytes, leading to an improved glucose metabolism. More importantly, for the first time, we reported that GvAEx might also alter the composition of the gut microbiota and increase the enrichment of probiotics, thus exerting its sustained beneficial effects on glucose metabolism. GvAEx has the potential for drug development against T2DM.

Limitations and Future Investigations

In this study, GvAEx from Guava leaf aqueous extracts were used for their hypoglycemic effect as a compound formulation. The exact ingredients or class of ingredients that exerted their effects on the db/db mice were not clear and deserve further exploration in future studies. However, we revealed profound compositional differences in gut microbiota between GvAEx treatment and control group. Further metagenomic sequencing needs to be done to explore the functional diversity of microbial communities between the two groups. Besides, we only tested several indicators of hepatic and renal toxicity. The comprehensive toxicological studies of the exacts should be conducted in the future.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/, PRJNA820451.

ETHICS STATEMENT

The animal study was reviewed and approved by the University Committee on the Care and Use of Animals of the Central South University, China.

AUTHOR CONTRIBUTIONS

SC collected and assembled data and prepared the first draft of the manuscript. FZ, HW, LX, and ZC collected data. ZZ contributed to project initiation, data analysis and discussion, financial support and gave final approval of the manuscript. FH contributed to conceptualization and design, supervised the work, data analysis and interpretation, and manuscript writing; contributed to financial support; and gave final approval of the manuscript. All authors reviewed and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.907702/full#supplementary-material

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Sea Buckthorn Proanthocyanidins are the Protective Agent of Mitochondrial Function in Macrophages Under Oxidative Stress

Keshan Liu[1†], *Wenxia Li*[2†], *Michael Yuen*[2], *Tina Yuen*[2], *Hywel Yuen*[2], *Min Wang*[1] and *Qiang Peng*[1*]

[1]College of Food Science and Engineering, Northwest A&F University, Yangling, China, [2]Puredia Limited, Xining, China

Sea buckthorn proanthocyanidins (SBP) are the most important antioxidant components of sea buckthorn, which are widely used in functional foods and cosmetics. Studies have shown that SBP have significant protective effects on macrophages against oxidative stress induced by hydrogen peroxide (H_2O_2). However, the mechanism remains uncertain. In the present study, we explored the effects of SBP on mitochondrial function and the mechanism of their protective effects against oxidative stress in cells. Our results showed that SBP could increase mitochondrial membrane potential, inhibit mPTP opening, reduce mitochondrial swelling, and enhance mitochondrial synthesis and metabolism. Thus, they alleviated oxidative damage and protected the cells against mitochondrial function. Western blot analysis showed that SBP had a protective effect on RAW264.7 cells by activating the AMPK-PGC1α-Nrf2 pathway. These results showed that SBP alleviated mitochondrial damage and dysfunction caused by oxidative stress. This study revealed the mechanism of SBP in reducing oxidative damage and provided a theoretical basis for further research on natural bioactive compounds to exert antioxidant activity and prevent arteriosclerosis and other diseases.

Keywords: sea buckthorn, proanthocyanidins, antioxidant, mitochondrial function, RAW264.7 cells

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Raquel Abalo,
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Igor Piotr Turkiewicz,
Wroclaw University of Environmental and Life Sciences, Poland
Chengjiang Ruan,
Dalian Nationalities University, China

***Correspondence:**
Qiang Peng
pengqiang@nwsuaf.edu.cn

†These authors have contributed equally to this work and share first authorship

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INTRODUCTION

Sea buckthorn (Hippophae rhamnoides L.) belongs to the Solanaceae family and is widely grown in Asia, Europe, and North America. Sea buckthorn is a plant with strong vitality. It can resist drought, wind, and sand and grow in saline-alkali land. In China, Sea buckthorn is categorized as a "Medicine and Food Homology". It is a famous traditional Chinese herb used to treat indigestion, bronchitis, cardiovascular disease, irregular menstruation, and menopause. Sea buckthorn contains many nutritional and bioactive compounds such as proanthocyanidins, flavonoids, polyphenols, polyunsaturated fatty acids, carotenoids, sugar alcohols, and superoxide dismutase and phytosterols, which have cardiovascular, anti-inflammatory, immunological regulation and antioxidants effects (Wang et al., 2021). Sea buckthorn contains remarkably high amounts of antioxidants. Thus, it has an important nutritional value (Ciesarová et al., 2020). One of the most

Abbreviations: SBP, sea buckthorn proanthocyanidins; MMP, mitochondrial membrane potential; mPTP: mitochondrial permeability transition pore.

important antioxidants in sea buckthorn, proanthocyanidins, contributes to the nutritional benefits of sea buckthorn products.

Proanthocyanidins are polyphenols in plant foods that have many health benefits, including cancer prevention, cardiovascular protection, and diabetes prevention. The composition and degree of polymerization of proanthocyanidins determine their digestion, absorption, and biological activity. The absorption properties of oligomeric proanthocyanidins monomers and dimers are better in the small intestine than polymers (DP > 4) (Zhang et al., 2016). Our previous studies isolated sea buckthorn oligomeric proanthocyanidins (SBP) from the fruits of sea buckthorn produced in China's Qinghai Province. Chemical composition analysis showed that SBP was composed of epicatechin gallate, b-type proanthocyanidins, gallocatechin-catechin, and gallocatechin dimer (Zhu et al., 2021). Oxidative stress is closely related to cardiovascular problems (Shields et al., 2021). Mitochondria are intracellular energy factories associated with redox signal transduction, apoptosis regulation, and gene expression regulation. They are involved in developmental biology, genetics, aging, cardiovascular diseases, and other aspects of the organism.

Mitochondrial damage is one of the main factors causing atherosclerosis. Impaired mitochondrial respiratory function results in inhibition of electron transfer, low-density lipoprotein oxidation, loss of scavenging agents, and permanent dysfunction of cardiomyocytes (Chakrabarty et al., 2018). SBP also protect macrophages against oxidative stress induced by hydrogen peroxide (Zhu et al., 2021). However, the mechanisms by which SBP protect macrophages under oxidative stress conditions remain uncertain.

The present study investigated whether SBP treatment could alleviate mitochondria dysfunction induced by oxidative stress. In addition, the effect of SBP on mitochondrial morphology was investigated using transmission electron microscopy (TEM). Furthermore, the underlying mechanisms by which SBP alleviated H_2O_2-induced mitochondrial dysfunction in RAW 264.7 cells were studied. This study illuminated the role of SBP on mitochondria recovery after oxidative stress and provided a mechanistic link between SBP treatment, mitochondrial health, and antioxidative function.

MATERIALS AND METHODS

Materials and Reagents

Sea Buckthorn Proanthocyandins (SBP), were provided by Puredia Limited (Qinghai, China). SBP were extracted from the fruits of sea buckthorn by water extraction and macroporous resin column chromatography, trademarked as CyanthOx™. The purity of SBP was 91.5% comparing with the standard of proanthocyanidins. They were mainly composed of (-)-epicatechin gallate, b-type proanthocyanidins, (+)-gallocatechin-(+)-catechin, and (+)-gallocatechin dimer (Chakrabarty et al., 2018). Fetal bovine serum was purchased from PAN-Biotech GmbH (Aidenbach, Germany). RPMI1640 medium was purchased from BasalMedia Technologies (Shanghai, China). Mito-Tracker Green (C1048) and MitoTracker Red CMXRos (C1049B) were obtained from the Beyotime Institute of Bio-technology (Shanghai, China). Mitochondrial Permeability Transition Pore Assay Kit (C2009S), Cell Mitochondria Isolation Kit (C3601), and ATP Assay Kit (S0026) were purchased from Beyotime Biotechnology, Shanghai, China. The glucose quantification kit was purchased from Applygen, Beijing, China. The antibodies of HO-1, p-AMPK, and p-Nrf2 were purchased from Cell Signaling Technology (Shanghai, China). PGC-1α was purchased from Bioss Inc. (Woburn, America).

Cell Culture

RAW 264.7 cells, a macrophage cell line, were purchased from the American Type Culture Collection (ATCC, Manassas, VA, United States). The macrophages were cultured in RPMI1640 medium with 10% FBS, 100 U/mL penicillin, and 100 μg/ml streptomycin in a 5% CO_2 atmosphere at 37°C. Cells were passaged when they became 70–80% confluent.

Cell Treatment

The RAW 264.7 cells (1.0 cells/ml × 10^5 cells/ml) were incubated with different concentrations of SBP (0, 25, 50, and 100 μg/ml) for 4 h, followed by the addition of 800 μmol/L H_2O_2 and treated for another 4 h.

Measurement of Mitochondrial Membrane Potential

RAW264.7 cells (2.0 cells/ml × 10^5 cells/mL) were incubated in 6-well tissue culture plates at 37°C for 24 h, then removed from the medium and exposed to different concentrations (25, 50, 100 μg/ml) of SBP solution for 4 h. Subsequently, 800 μmol/L of H_2O_2 was added to sample groups and incubated for another 4 h. The parameters of mitochondrial membrane potential (MMP) and Malondialdehyde (MDA) were determined using the corresponding kits (Beyotime Institute of Biotechnology, Shanghai, China). The test cells were added with 100 μL of 5 μg/ml JC-1 for 30 min and then quantified by the fluorescence microscope and microplate reader.

Observation of Mitochondria Morphology

The distribution of mitochondria was analyzed using Mito-Tracker Red CMXRos (Mitochondrial red fluorescent probe). Cells were incubated in the medium containing 100 nm Mito-Tracker Red CMXRos for mitochondrial staining at 37°C for 40 min. After washes with cell culture medium, cells were observed by fluorescence microscopy (IX71-F22FL/PH, Olympus, Tokyo, Japan) (Maeda et al., 2004). Transmission electron microscopy was used to observe the microstructure of mitochondria. Cells were fixed in 2.5% glutaraldehyde for 2 h, then washed by PBS (phosphate-buffered saline) and fixed again in 1% osmium tetroxide for 1 h. Gradient dehydration was performed before immersing into epoxy resin overnight at 37°C and polymerizing at 60°C for 48 h. After that, the samples were sliced and stained with 2% uranium dioxide acetate dye and lead citrate, respectively. Finally, the samples were washed and dried before observation by Transmission electron microscopy (JEM-1230, JEOL, Tokyo, Japan).

Measurement of Mitochondrial Permeability Transition Pore and Swelling

Mitochondrial permeability was measured by monitoring the fluorescence of mitochondrial entrapped calcein (MPTP Assay Kit). Calcein AM staining solution and fluorescence quenching working fluid were added at 37°C followed by incubation in the dark for 30 min after PBS washing twice. Then, a fresh culture solution was added to incubate the cells for another 30 min in the dark at 37°C. Finally, the cells were washed with PBS twice before observing by fluorescence microscopy (IX71-F22FL/PH, Olympus, Tokyo, Japan) (Petronilli et al., 1999). Mitochondrial swelling was measured by a microplate reader at 540 nm. The mitochondria were isolated by a mitochondria isolation kit according to the manufacturer's instructions. The mitochondrial protein concentration was measured by bicinchoninic acid assay and 10 μL CaCl2 (20 mm) was mixed with cellular mitochondria (500 nmol mg^{-1} mitochondrial protein) to induce mitochondrial swelling (Luth et al., 2014; Han et al., 2021).

Determination of Mitochondrial Mass by Flow Cytometry Analysis

Mito-Tracker green was applied to characterize the mitochondrial morphology in RAW 264.7 cells. Briefly, Mito-Tracker green stock solution (1 mm) was prepared with anhydrous dimethylsulfoxide and diluted with Hank's Balanced Salt Solution. The cells were incubated with Mito-Tracker green staining solution (200 nm) at 37°C for 20 min. The staining solution was then removed, and fresh culture solution was added to the wells. The cells were determined by a flow cytometer (CyFlow Cube 6, Partec, Munich, Germany) at 488 nm of excitation wavelength and 516 nm of emission wavelength. Flowjo software was applied to the quantification of the number and individual volume of the mitochondria.

Measurement of Mitochondrial Adenosine Triphosphate Synthesis and Glucose Utilization

ATP levels were measured using the ATP Assay Kit. Briefly, the collected cells were lysed and centrifuged at 12,000 g for 5 min at 4°C. In 12-well plates, 100 μL of each supernatant was mixed with 100 μL ATP working dilution. Luminescence was detected using a microplate reader (PowerWave XS, Bio-Tek, Vermont, US). The ATP level was presented as nanomoles per milligram of protein. The glucose utilization of cells was determined by the glucose quantification kit. The glucose utilized over the 24 h period was calculated by subtracting the glucose concentration at 24 h from that at 0 h (Chen et al., 2018).

Western Blot Analysis

Briefly, RAW264.7 cells were washed with PBS (ice-cold) and collected after ABSP treatment. Then cell pellets were suspended in the Radioimmunoprecipitation assay (RIPA) lysis (Bioworld technology, Minnesota, US) for 30 min. After centrifugation (12,000 rpm) for 5 min, the supernatant was collected. A BCA protein quantitative kit (Biotopped, Beijing, China) was used to evaluate the total protein content in the sample. According to quantitative results, different groups were mixed with 5 times protein loading buffer by equal quality. After centrifugation, the samples were electrophoresed on SDS polyacrylamide gels and transferred to polyvinylidene fluoride membranes. After being incubated with the primary antibodies for 1 h (at room temperature), the membranes were rinsed with PBS (containing 0.2% Tween 20) and incubated with the corresponding secondary antibodies for another 1 h. With the chemiluminescent method, the proteins were finally detected after washing. The signals were detected and imaged using an ECL-Plus detection system (GE Healthcare, South Plainfield, United States).

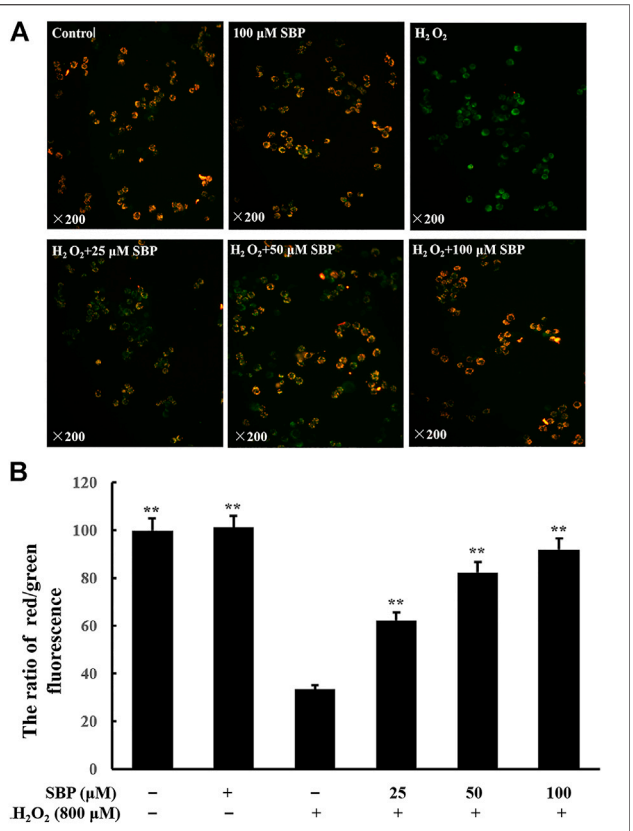

FIGURE 1 | Effects of SBP on mitochondrial membrane potential in H_2O_2-induced RAW 264.7 cells. RAW 264.7 cells were pretreated with SBP for 4 h, then treated with H_2O_2 (800 μM) for 12 h, and then dyed with 5 μM JC-1 for 1.5 h at 37°C in the dark. **(A)**: Qualitative analysis with an inverted fluorescence microscope (×200); **(B)**: Quantitative analysis with a multimode microplate reader at 485 nm excitation, 538 nm (green) and 585 nm (red) emissions for JC-1 staining. *$p < 0.05$, and **$p < 0.01$ versus H_2O_2-treated groups.

Statistical Analysis

A one-way fixed-effects analysis of variance (ANOVA) test was performed using statistical software (SPSS version 18.0, SPSS Inc.,

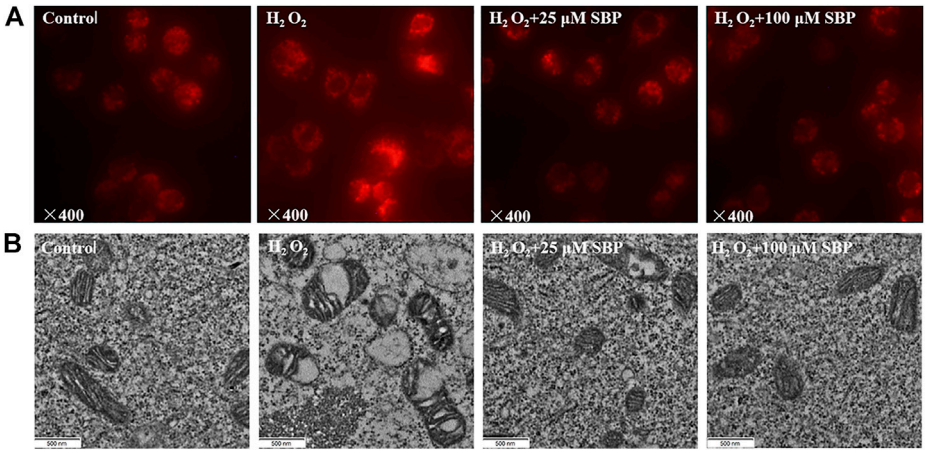

FIGURE 2 | Effects of SBP on morphology of mitochondrial of RAW 264.7 cells. **(A)**: Representative images show morphology of mitochondria. Mitochondrial morphology was visualized after staining the cells with MitoTracker Red and images were captured under fluorescence microscope; **(B)**: Transmission-electron-microscopy (TEM) images of mitochondria in RAW 264.7 cells (×21,000).

Chicago, IL, United States). All trials were done in triplicate, and the statistical means and standard deviations were calculated and shown.

RESULTS AND DISCUSSIONS

Protection of Sea Buckthorn Proanthocyanidins on the Mitochondrial Membrane Potential in H_2O_2-Induced Damage RAW 264.7 Cells

Mitochondria are responsible for redox homeostasis. They are cell organelles that are susceptible to oxidative damage. H_2O_2 is a known suppressing factor in mitochondrial dysfunction. The decrease in mitochondrial membrane potential indicates mitochondrial dysfunction and apoptosis (Kim, 2005). JC-1 can selectively enter mitochondria to form a monomer, which indicates the mitochondrial potential (MMP) in cells. If MMP collapses during apoptosis, the fluorescence of JC-1 changes from red to green. Thus, the green and red ratio of JC-1 indicates the variation of MMP (Taylor et al., 2003). As shown in **Figure 1A**, compared with the control group, the fluorescence of JC-1 of the H_2O_2 group turned green, which indicated that the MMP was significantly decreased. Meanwhile, the SBP groups showed less green emissions and more red emissions, which indicated that there is less reduction in the MMP.

To analyze this quantitatively, the ratio of red/green fluorescence was analyzed. Referring to **Figure 1B**, compared with the control group, there was a significant decrease in the ratio in the H_2O_2 group, which indicated a reduction in mitochondrial function. This ratio increased in the SBP groups in a concentration-dependent manner. These results indicated that SBP could protect the cells against H_2O_2-induced mitochondrial dysfunction.

Sea Buckthorn Proanthocyanidins Protects Mitochondria Microstructure From H_2O_2-Induced Damage in RAW 264.7 Cells

Since mitochondrial membrane potential is critical for maintaining mitochondrial bioenergetics and dynamics, we investigated the effects of SBP in mitochondrial morphology of RAW264.7 cells under disturbed redox homeostasis. Mitochondria are the major endogenous source of oxidative stress (Daverey and Agrawal, 2016). Hence, fluorescent probe Mito-Tracker Red dye was used to analyze the changes in mitochondria morphology induced by oxidative stress in RAW 264.7 cells. As shown in **Figure 2A**, the distribution of mitochondria changed under fluorescent microscopy. The mitochondria of normal cells were evenly distributed and had a clear tubular shape. Compared with the control group, the mitochondria morphology of the H_2O_2 group became swollen, fuzzy, uneven, and aggregated. A transmission electron microscope (TEM) was used to observe the morphological differences of mitochondria among different groups. Referring to **Figure 2B**, it showed that mitochondria transformed from the typical tubular shape into swollen and fragmented structures under oxidative stress. Furthermore, mitochondrial autophagy was also observed. Remarkably, the SBP treatment alleviated the H_2O_2-induced mitochondrial swelling in RAW 264.7 cells, indicating that SBP had protective effects on mitochondria. These results suggest that SBP prevents mitochondria damage induced by oxidative stress in RAW 264.7 cells.

Sea Buckthorn Proanthocyanidins Inhibited Mitochondrial Swelling via Permeability Transition Pore Induction in H_2O_2-Induced Damage RAW 264.7 Cells

Firstly, we measured the level of the mPTP opening at a cellular level by mPTP Assay Kit (C2009S, Beyotime Biotechnology,

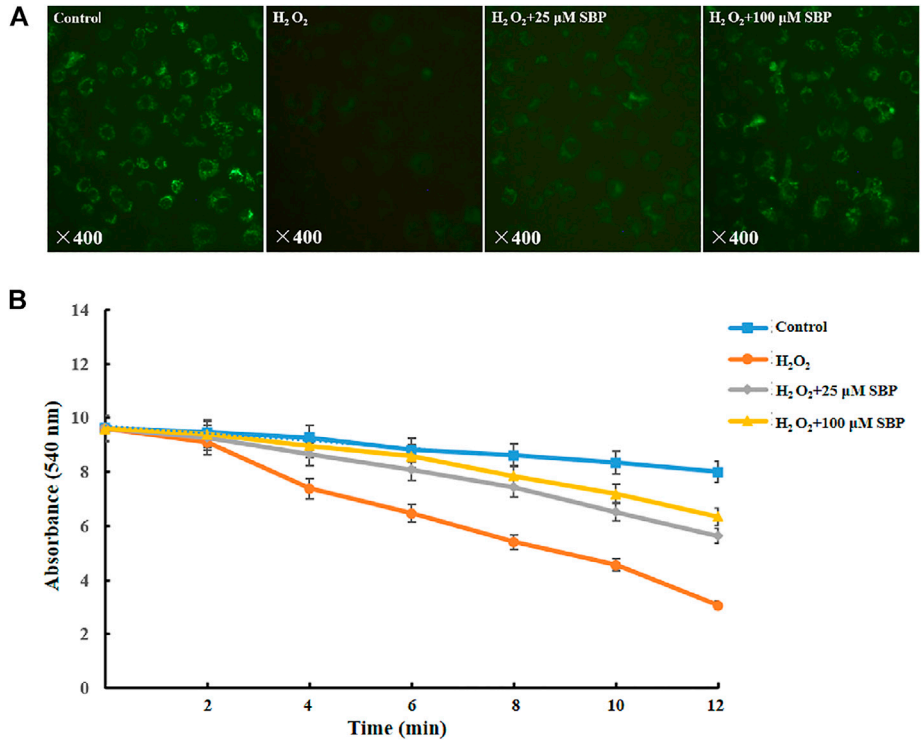

FIGURE 3 | Effects of SBP on morphology of mitochondrial permeability and swelling. **(A)** Mitochondrial permeability transition pore of RAW 264.7 cells. The green and red components of the images indicate calcein fluorescence. **(B)** Mitochondrial swelling of RAW 264.7 cells.

Shanghai, China). The degree of mPTP opening was judged according to the intensity of Calcein green fluorescence in mitochondria. If the green fluorescence was stronger, the opening of mPTP was lower; conversely, the weaker green fluorescence was, the opening of mPTP was higher. Referring to **Figure 3A**, compared with the control groups, the H_2O_2 group showed a significant reduction in green fluorescence intensity, indicating that the opening of mPTP was obviously enhanced. However, the SBP group significantly restored the green fluorescence intensity, indicating that SBP effectively inhibited the opening of mPTP. Under many pathological conditions, as the mitochondrial permeability transition pore (mPTP) opens, it leads to mitochondrial swelling, reduced oxidative phosphorylation capacity, and cell necrosis or apoptosis (Bernardi, 1999).

After that, we measured the mPTP opening at the mitochondrial level and mitochondrial swelling through *in vitro* separation. In the mitochondria-CA^{2+} system, referring to **Figure 3B**, compared with the control group, the H_2O_2 group showed a significant decrease in absorbance at 540 nm. This indicated that H_2O_2 led to mitochondrial swelling and damage of mitochondrial function. Besides, the SBP groups showed less reduction in the absorbance at 540 nm than the H_2O_2 group in a concentration-dependent manner. This suggested that SBP improved mitochondrial damage and dysfunction caused by oxidative stress. These results further supported the TEM observation.

Sea Buckthorn Proanthocyanidins Promoted the Biosynthesis of the Mitochondria in H_2O_2-Induced Damage RAW 264.7 Cells

Mitochondrial mass was measured by fluorescent probe Mito-Tracker Green through flow cytometry. As shown in **Figure 4**, compared with the control group, the H_2O_2 group showed a significant decrease in the mean fluorescence intensity. Conversely, the SBP groups showed less decrease in the mitochondrial mass than the H_2O_2 group in a concentration-dependent manner. Under oxidative stress, the mitochondria synthesis of cells was inhibited, which affected their energy metabolism and antioxidant capacity. This result showed that SBP improved mitochondrial synthesis, significantly protecting the mitochondrial density. Therefore, they suggested that SBP may affect mitochondrial energy production and enzyme activities related to energy metabolism.

Sea Buckthorn Proanthocyanidins Improved the Energy Metabolism of the Mitochondria in H_2O_2-Induced Damage RAW 264.7 Cells

As shown in **Figure 5**, compared with the control group, the H_2O_2 group showed a significant reduction in relative ATP levels. In contrast, the SBP groups showed a positive correlation between

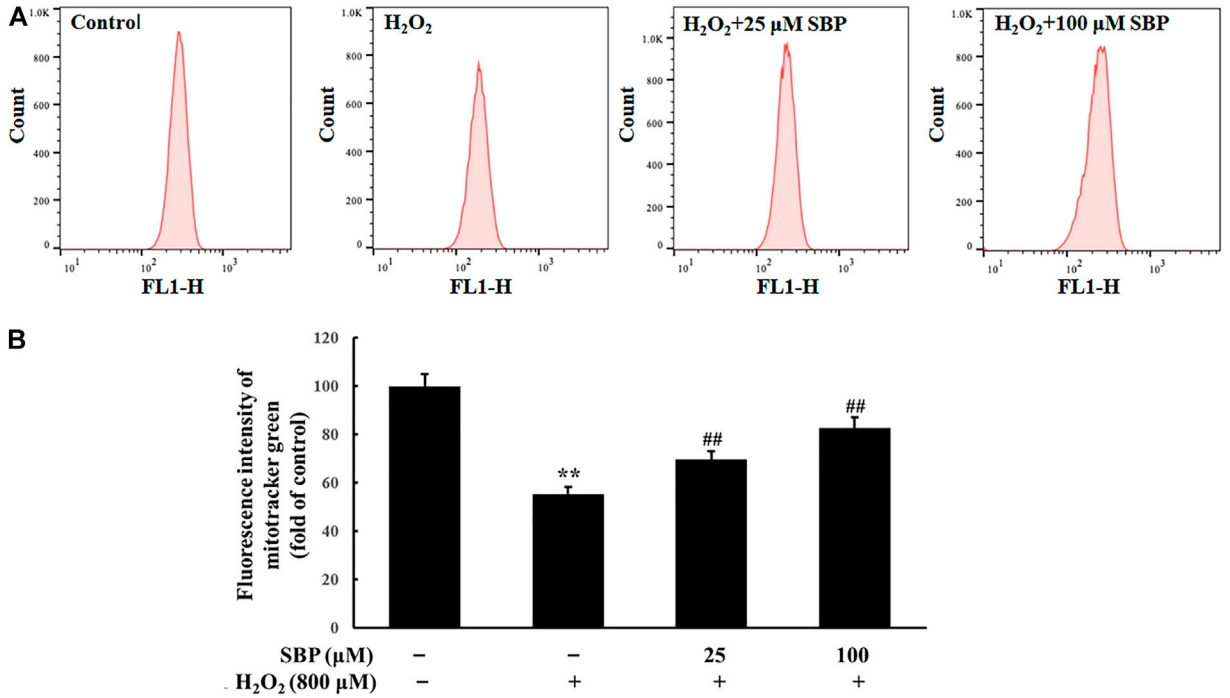

FIGURE 4 | Effect of SBP on the mitochondrial mass in RAW 264.7 cells. **(A)** Representative flow cytometric histograms of each group. **(B)** The relative fluorescence intensities of RAW 264.7 cells treated with Mito-Tracker green. $^{**}p < 0.01$ compared to the control group; $\#\#p < 0.01$ compared to the H_2O_2-treated group.

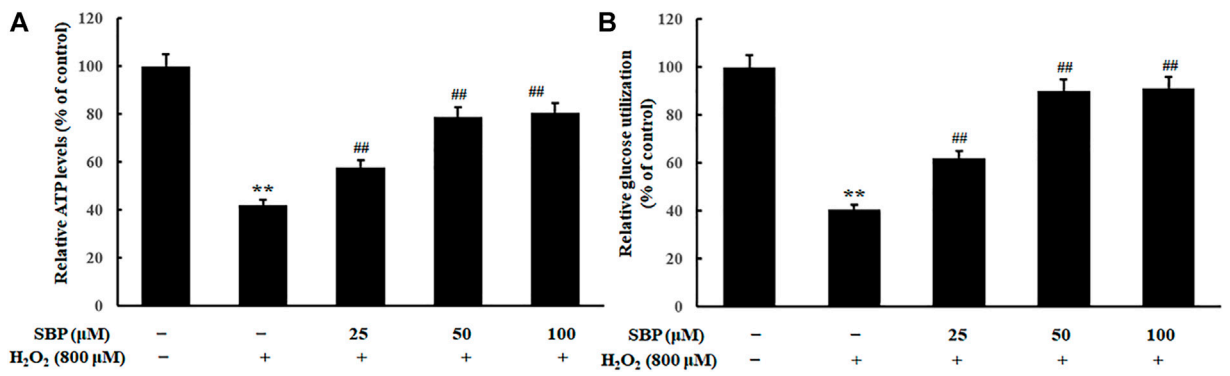

FIGURE 5 | Effect of SBP on the mitochondrial energy metabolism in RAW 264.7 cells. **(A)** Relative quantifying intracellular ATP levels in mitochondria after treatment with SBP. Representative flow cytometric histograms of each group. **(B)** Relative quantifying cellular glucose utilization in mitochondria after treatment with SBP. ATP content and glucose utilization were measured after 24 h treatment. $^{**}p < 0.01$ compared to the control group; $\#\#p < 0.01$ compared to the H_2O_2-treated group.

the relative ATP levels and the SBP concentration. ATP is produced in mitochondria to provide energy. Mitochondria are important organelles, which are responsible for cellular respiration. This is because they are responsible for synthesizing ATP required for daily life (Hong et al., 2020). Relative ATP levels were analyzed to determine whether SBP was effective in preventing the reduction of the mitochondrial enery metabolism in H_2O_2 treated RAW 264.7 cells.

Apart from that, relative glucose utilization is an important indicator of oxidative damage. Hence, we compared the relative glucose utilization of H_2O_2 treated Raw 264.7 cells with and without the presence of SBP. Compared with the control group, the H_2O_2 group showed a significant reduction in relative glucose utilization. Moreover, the SBP groups showed less reduction in the relative glucose utilization when compared with the H_2O_2 group in a concentration-dependent manner. This result

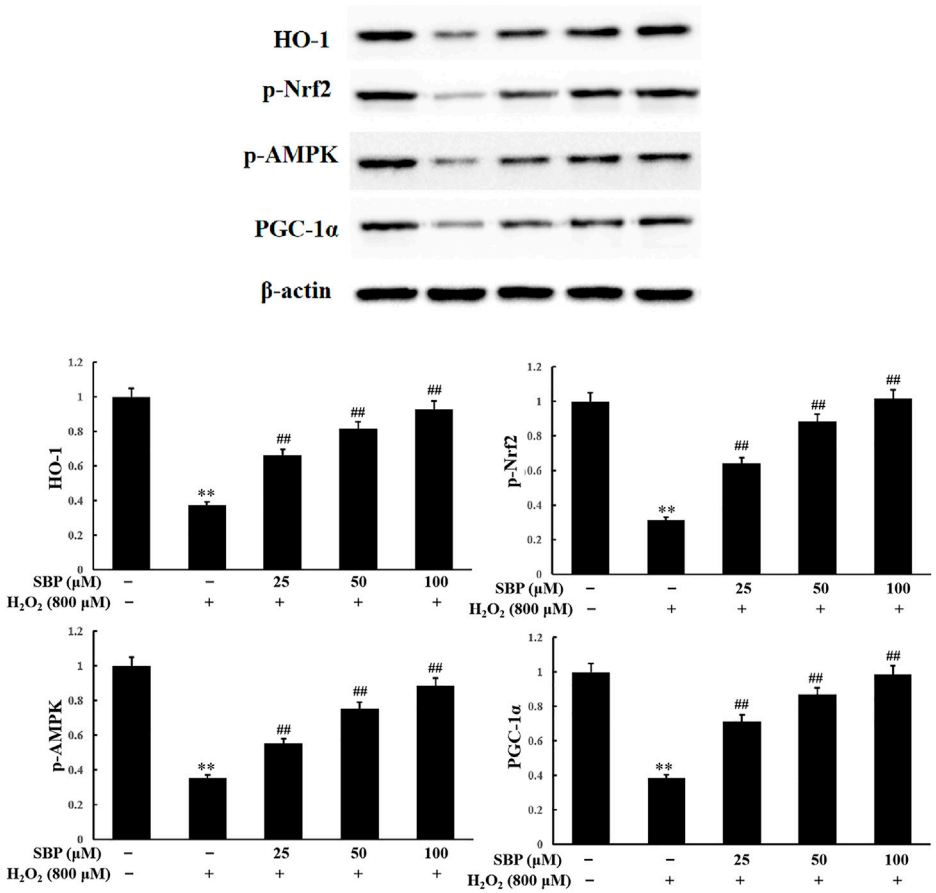

FIGURE 6 | Effect of SBP on the protein expression levels of HO-1, phosphorylated Nrf2, AMPK and PGC-1α. After that, cell lysates were collected and subjected to western blotting analyses. Data are calculated from three independently replicated experiments. $p < 0.01$ compared to the control group; $p < 0.01$ compared to the H_2O_2-treated group.

suggested that SBP helped protect Raw 264.7 cells from oxidative damages.

Sea Buckthorn Proanthocyanidins Regulate Nrf2-Regulated Signal Pathway in H_2O_2-Induced Damage RAW 264.7 Cells

To investigate the inhibitory effect of SBP on H_2O_2 oxidative stress and its relationship with the activation of the Nrf2/HO-1 signaling pathway, the level of Nrf2 and HO-1 expression was analyzed. As shown in **Figure 6**, the result of the Western Blot analysis showed that SBP significantly suppressed the reduction in HO-1 expression induced by H_2O_2 in RAW 264.7 cells. This suggested that SBP increased the activity of HO-1 under oxidative stress conditions. Since Nrf2 is needed to be phosphorylated and then transported to the nucleus to initiate the expression of antioxidant proteins, such as HO-1, we further investigated whether SBP could regulate the nuclear transport of p-Nrf2. Compared with the control group, SBP reduced the suppression of p-Nrf2 induced by H_2O_2 in a concentration-dependent manner. These results suggested that SBP mediated the induction of HO-1 through nuclear translocation of Nrf2 in RAW 264.7 cells.

HO-1 has various physiological functions, such as antioxidation, anti-apoptosis, anti-inflammation, and immune regulation. Nrf2 is the key transcription factor for intracellular resistance to oxidative stress, which can be activated by antioxidants. Nrf2 and PGC-1α cotranscript mitochondrial proteins and mtTFA, controlling mitochondrial DNA replication and transcription. This influences the generation of mitochondria (Staniek and Nohl, 2000). The activity of AMPK is closely related to the phosphorylation level of Nrf2. Both affect mitochondrial function. Pomegranate extract activated the AMPK/Nrf2 signaling pathway and alleviated oxidative stress and mitochondrial dysfunction in hypertensive rats (Sun et al., 2016). Moreover, butin also activated the AMPK/Nrf2 signaling pathway, reducing oxidative stress, improving mitochondrial function, and increasing HO-1 expression (Duan et al., 2017). As shown in **Figure 6**, compared to the control group, the H_2O_2 group showed a significant reduction in p-AMPK expression. The SBP groups showed an increase in

p-AMPK expressions when compared with the H_2O_2 group. There was a positive correlation between the SBP concentration and p-AMPK expressions. These results indicated that SBP significantly improved the phosphorylation of Nrf2 by activating p-AMPK expression.

PGC-1α plays an important role in the mitochondrial synthesis and redox homeostasis. SBP were shown to protect the number of mitochondria and AMPK. AMPK is an upstream signaling molecule of PGC-1α. Hence, we analyzed the expression level of PGC-1α. Referring to **Figure 6**, compared with the control group, the H_2O_2 group showed a significant reduction in PGC-1α expression. The SBP groups showed increase in PGC-1α expression in a concentration-dependent manner. These results suggested that SBP can activate PGC-1α expression, by promoting the generation of mitochondria in cells. This alleviated H_2O_2-induced cellular oxidative stress and damage. These results were consistent with the previous TEM and fluorescence results.

CONCLUSION

As to conclude, this study demonstrated that SBP played an important role in the protection of RAW264.7 cells under oxidative stress. The mechanism included the regulation of mitochondrial energy metabolism, generation, and morphological and functional properties. SBP increased mitochondrial membrane potential, inhibited mPTP opening, relieved mitochondrial swelling, and increased mitochondrial generation metabolism. Thereby, it improved oxidative damage and protected mitochondrial function. SBP were shown to activate the AMPK-PGC-1α-Nrf2 pathway. This study provided a deeper understanding of the mechanism behind the protective effect of SBP on mitochondria. These results will lay the foundation for the application of sea buckthorn proanthocyanidins as an antioxidant functional food.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

Conceptualization, QP; data curation, KL; formal analysis, WL; investigation, HY; methodology, TY; resources, MW; software, MY; writing—original draft, QP.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.914146/full#supplementary-material

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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A Review on the Antidiabetic Properties of *Moringa oleifera* Extracts: Focusing on Oxidative Stress and Inflammation as Main Therapeutic Targets

Fikile T. Mthiyane[1], *Phiwayinkosi V. Dludla*[2], *Khanyisani Ziqubu*[1], *Sinenhlanhla X. H. Mthembu*[1,2], *Ndivhuwo Muvhulawa*[1], *Nokulunga Hlengwa*[3], *Bongani B. Nkambule*[4] *and Sithandiwe E. Mazibuko-Mbeje*[1]*

[1]*Department of Biochemistry, North-West University, Mafikeng, South Africa,* [2]*Biomedical Research and Innovation Platform, South African Medical Research Council, Cape Town, South Africa,* [3]*Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa, South Africa,* [4]*School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa*

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Federal University of São Paulo, Brazil

***Correspondence:**
Sithandiwe E. Mazibuko-Mbeje
36588296@nwu.ac.za

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Moringa oleifera is one of the popular plants that have shown significant health benefits. Certainly, preclinical evidence (predominantly from animal models) summarized in the current review supports the beneficial effects of *Moringa oleifera* leaf extracts in combating the prominent characteristic features of diabetes mellitus. This includes effective control of blood glucose or insulin levels, enhancement of insulin tissue sensitivity, improvement of blood lipid profiles, and protecting against organ damage under sustained conditions of hyperglycemia. Interestingly, as major complications implicated in the progression of diabetes, including organ damage, *Moringa oleifera* leaf and seed extracts could efficiently block the detrimental effects of oxidative stress and inflammation in these preclinical models. Notably, these extracts (especially leaf extracts) showed enhanced effects in strengthening intracellular antioxidant defences like catalase, superoxide dismutase, and glutathione to lower lipid peroxidation products and reduce prominent pro-inflammatory markers such as tumor necrosis factor-α, interleukin (1L)-β, IL-6, monocyte chemoattractant protein-1 and nitric oxide synthase. From animal models of diabetes, the common and effective dose of leaf extracts of *Moringa oleifera* was 100–300 mg/kg, within the treatment duration of 2–8 weeks. Whereas supplementation with approximately 20 g leaf powder of *Moringa oleifera* for at least 2 weeks could improve postprandial blood glucose in subjects with prediabetes or diabetes. Although limited clinical studies have been conducted on the antidiabetic properties of *Moringa oleifera*, current findings provide an important platform for future research directed at developing this plant as a functional food to manage diabetic complications.

Keywords: diabetes complications, oxidative stress, inflammation, moringa (*Moringa oleifera*), therapeutic targets

INTRODUCTION

According to the World Health Organization, diabetes mellitus is amongst the top ten leading causes of mortality and morbidity around the world (World Health Organization, 2022). Diabetes is a metabolic disorder that is characterized by a state of hyperglycemia, that occurs alongside dysregulations in insulin levels and in some cases, it arises concurrently to overweight and obesity (International Diabetes Federation, 2021). Indeed, excessive body fat or obesity remains the major culprits in the development of type 2 diabetes (T2D), which is the predominant form of diabetes (International Diabetes Federation, 2021). The rapid increase in cases of diabetes mellitus, especially T2D, raises concerns, also highlighting an urgent need to investigate effective therapies to curb this disease (Ahmad et al., 2019). Accumulative research has focused on understanding the pathophysiological mechanisms implicated in the development of diabetes-associated complications, which is essential to discover effective therapies that can improve metabolic function and prevent multiple organ failure in those affected by this condition (King and Brownlee, 1996; Fowler, 2007; Wei et al., 2020).

As a prime example, inflammation and oxidative stress, which normally emerge as a result of an abnormal pro-inflammatory response, or an exacerbated production of free radical species are increasingly recognized as the key abnormalities implicated in the development and acceleration of diabetes-linked complications (King and Brownlee, 1996). Notably, oxidative stress is linked with the activation of protein kinase C (PKC), which is normally consistent with impaired insulin signaling and tissue damage in experimental models of diabetes (King and Brownlee, 1996). Importantly, this content supports the hypothesis by Randle and others (Randle et al., 1963) which stated that alteration in the uptake and metabolism of glucose and free fatty acids may be an instrumental process in the pathogenesis of insulin resistance, the major characteristic feature of T2D. Indeed, many diverse biochemical mechanisms, extending beyond inflammation and oxidative stress or activation of PKC, are implicated in the development of T2D (King and Brownlee, 1996).

Literature suggests that effective modulation of energy metabolism and insulin signaling through the regulation of AMP-activated protein kinase (AMPK) or phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways appears to reverse some devastating outcomes of diabetes (Long and Zierath, 2006; Huang et al., 2018; Mazibuko-Mbeje et al., 2018). In fact, plants and their related bioactive compounds are increasingly screened for their antidiabetic properties. Some natural plants have shown anti diabetic properties through lowering blood glucose and modulation of AMPK/PI3K/AKT pathways (Francini et al., 2019; Mazibuko-Mbeje et al., 2019; Costa et al., 2020). Consistently, our group continues to review literature on the impact of plants like *Camellia sinensis* and *Aspalathus linearis*, including prominent bioactive compounds from some of these plants such as gallic acid and isoorientin for their ameliorative effects against metabolic complications (Dludla et al., 2019; Ziqubu et al., 2020; Dludla et al., 2021).

Moringa oleifera is a medicinal plant that has gained a lot of interest for its diverse biological properties. Reviewed evidence indicates the biological capabilities of this plant expand to protecting against complications linked with heart disease, cancer, fatty liver, and diabetes mellitus (Paikra et al., 2017; Vergara-Jimenez et al., 2017; Abd Rani et al., 2018). For example, a previously published review supported the beneficial effects of the leaves of the *Moringa oleifera* in improving blood glucose control in experimental models of diabetes (Ahmad et al., 2019). Notably, this review indicated draw backs such as the limited number of studies that have reported on the potential beneficial effects of this plant, including the fact that summarized literature was mainly conducted in animals, through *in vitro* and *in vivo* preclinical models. Nevertheless, while such information already affirms the hypoglycaemic potential of this medicinal plant, data regarding the prominent biochemical mechanisms implicated in the antidiabetic effects of *Moringa oleifera* have not been critically reviewed. Recently, Louisa and others supported the potential benefits of *Moringa oleifera* in cardiovascular or metabolic disorders, mainly by ameliorating the undesired pro-inflammatory response and inhibiting oxidative stress by mediating molecular mechanisms such as hindering nuclear factor kappa B (NF-κB) translocation or enhancing the antioxidant response of nuclear factor-erythroid factor 2-related factor 2 (Nrf2) in different preclinical models (Louisa et al., 2022). Thus, there is a need to better understand such intracellular responses of *Moringa oleifera* within a setting of diabetes or in related metabolic complications. The current study provides a brief overview on *Moringa oleifera* as medicinal plant, followed by its therapeutic mechanisms in controlling diverse diabetic complications. Mostly focusing on understanding the modulatory effects of this medicinal plant in mechanisms of inflammation and oxidative stress in a diabetic state.

This current review includes evidence that was obtained from a search done (from inception until end of December 2021) on major search engines such as PubMed, Google Scholar and ScienceDirect. The systematic search was conducted using the following Medical Subject Heading (MeSH) terms "*Moringa oleifera*", "diabetes mellitus", "glucose metabolism", "insulin resistance", "oxidative stress", and "inflammation" as well as relevant synonyms. EndNote20 desktop software (Elsevier, Amsterdam, Netherlands) was used for references and identification of duplicated studies. Preclinical and clinical studies reporting on the mechanisms of *Moringa oleifera* in diabetes models and related metabolic syndrome was included in this review. However, review papers, and encyclopaedias were excluded but screened for primary findings. Notably, critical information related to the portion (part) of the plant that was assessed, as well as effective therapeutic dose and an experimental model used for the investigation is provided to better understand the potential benefits of *Moringa oleifera*.

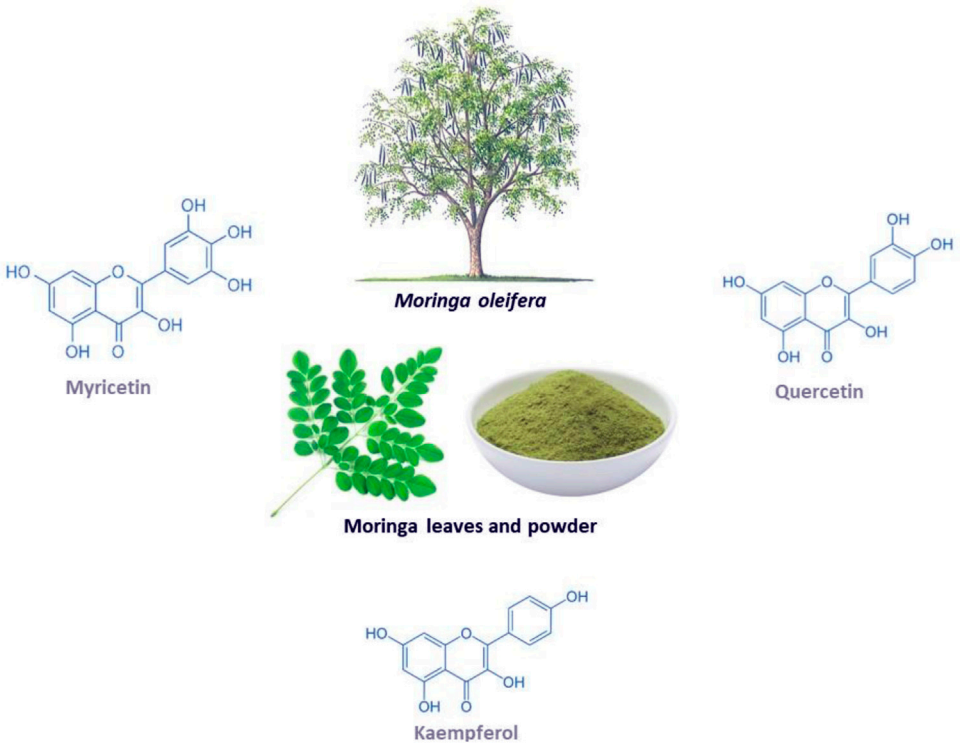

FIGURE 1 | The *Moringa oleifera* plant, including the chemical structures of some of its major flavonoids myricetin, quercetin and kaempferol.

AN OVERVIEW OF *MORINGA OLEIFERA* AND ITS DIVERSE BIOLOGICAL PROPERTIES

Moringa oleifera (shown in **Figure 1**) is a fast-growing tree that is classified as a vegetable that also serves as a medicinal plant (Gopalakrishnan et al., 2016; Trigo et al., 2020). This miracle tree originates from the sub-Himalayan parts of India, and it can be grown in both tropical and subtropical regions and is able to withstand droughts and mild frosty weather, hence it can be cultivated anywhere in the world (Gopalakrishnan et al., 2016). This plant has gained medical and socioeconomic popularity because it has shown great health benefit and it is easy to cultivate (Alegbeleye, 2018; Zhu et al., 2020). Traditionally, it is applied in diets to maintain healthy skin and it has also been used as a decoction to relieve stress and provide energy (Mishra et al., 2011; Kumar et al., 2018). All the parts of the plant can be utilized in a diet or as medicine since they are rich in minerals, proteins, vitamins, polyphenols, flavonoids, glucosinolates, isothiocyanates, alkaloids (Gopalakrishnan et al., 2016; Trigo et al., 2020). For example, the leaves can be eaten raw, dried or taken as an infusion of an aqueous extract, while the bark is boiled in water or soaked in alcohol to make drinks and infusions that help with toothaches, stomach aches, the same is done to the roots (Leone et al., 2015). Furthermore, the leaves are utilized the most for medicinal purposes and they are a great source of prominent anti-inflammatory and antioxidant flavonoids, namely myricetin, quercetin and kaempferol (Vergara-Jimenez et al., 2017). Interesting, these bioactive compounds are known to contain potential anticancer, hypolipidemic, hypotensive and antidiabetic properties, antioxidant and anti-inflammatory (Vergara-Jimenez et al., 2017). Other documented uses for this medicinal plant include its application as a diuretic, a testosterone stimulant, an antifungal and as an antibacterial (Mishra et al., 2011; Kumar et al., 2018). It can also be used to relieve a sore throat and symptoms of influenza, or as an anti-inflammatory agent (Mishra et al., 2011). Interestingly, evidence has grown that *Moringa oleifera* contains hypoglycemic effects in diabetic animal models, including its associated complications such as oxidative stress and inflammation (Balakrishnan et al., 2019; Chin et al., 2019; Bao et al., 2020).

OXIDATIVE STRESS AND INFLAMMATION AS PROMINENT MECHANISMS INVOLVED IN DIABETES-INDUCED COMPLICATIONS

Several pathophysiological mechanisms have been implicated in the aggravation of diabetes-related complications. For instance, individuals with T2D already present with the dyslipidemic feature which is normally characterised by the highly accumulation of lipids and these can easily be attached by free radicals to generate damaging oxidative products (Biswas et al., 2017:; Ito et al., 2019). This consequence is referred to as lipid peroxidation, and it remains as one of the vital parameters used to track the devastating outcomes of oxidative stress in conditions of

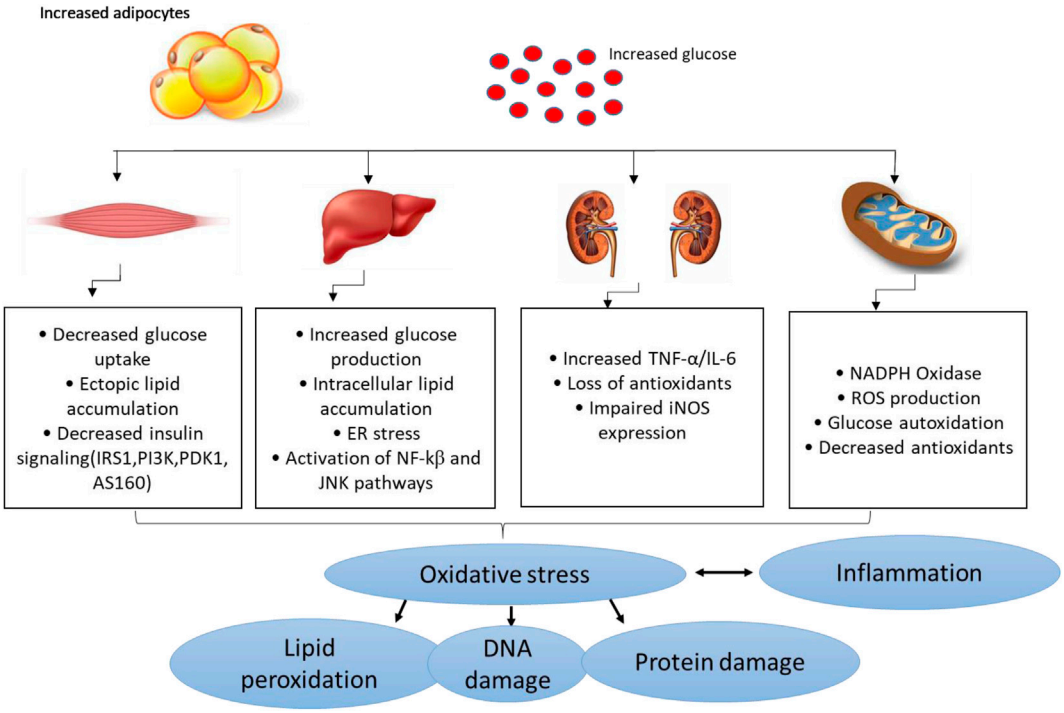

FIGURE 2 | An overview of pathological mechanisms implicated in the development of diabetes mellitus or related metabolic complications. Briefly, overnutrition (which may be characterized by increased adipocyte size) and consistent increased levels of glucose (a state of hyperglycemia) may induce detrimental effects in major organs of the body including the skeletal muscle, liver, and kidneys, and thus aggravate metabolic complications through enhanced oxidative stress and exacerbated inflammation. This consequence is predominantly characterized by impaired glucose homeostasis/insulin signaling, ectopic lipid accumulation, mitochondrial dysfunction, endoplasmic reticulum (ER) stress insufficient or decreased antioxidant responses/increased ROS (reactive oxygen species) production and altered actions of inducible nitric oxide synthase (iNOS) and lipid peroxidation/DNA damage. This may occur along with raised pro-inflammatory markers like tumor necrosis factor-alpha (TNF-α), like nuclear factor kappa β (NF-κβ), c-Jun N-terminal kinases (JNK) and interleukin-6 (IL-6).

diabetes or any related metabolic disease (Rahimi et al., 2005; Grotto et al., 2009; Augustine et al., 2021). Within the pathological state, free radicals can be generated through impaired mitochondrial function, or enhanced activities of some enzymes complexes such as NADPH oxidases, in a process like oxidative stress that is known to deplete intracellular antioxidant systems (Mittal et al., 2014; He et al., 2017). Generally, oxidative stress is generated as a disparity in the production of reactive oxygen species (ROS) or reactive nitrogen species, in comparison to counteractive activity of antioxidants in diabetes (Giacco and Brownlee, 2010). Some of the prominent free radical molecules include hydroxyl radical (•OH), superoxide anion ($O_2^{\bullet-}$), peroxynitrite ($ONOO^-$), and all these molecules are crucial for efficient metabolic process in a physiological state (Burgos-mor et al., 2019; Chandra et al., 2019). Also, individuals with T2D display classic signatures of oxidative stress by presenting significantly decreased levels of antioxidant mechanisms such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and heme oxygenawe-1 (HO-1) (Matough et al., 2012; Sharma et al., 2012; Dumanović et al., 2021). In diabetes or related metabolic complications, uncontrolled ROS can induce damage to the lipids, proteins, and nucleic acids which lead to impaired signaling mechanisms and activation of pro-inflammatory response (Burgos-mor et al., 2019; Kim et al., 2020). **Figure 2** gives an overview of some of the pathophysiological mechanisms implicating the detrimental effects of oxidative stress and inflammation in conditions of diabetes or related metabolic complications.

Currently, both oxidative stress and inflammation have been subject to ongoing research to improve metabolic function in conditions of syndrome (Furman et al., 2019; Monserrat-Mesquida et al., 2020; Oria et al., 2020). Also, accumulative research has evaluated the use of dietary compounds with antioxidant and anti-inflammatory effects such as salvianolic acid, aspalathin and resveratrol in combination with common drugs like metformin to lower glucose as well as attenuate the detrimental effects of oxidative stress and inflammation (Frendo-Cumbo et al., 2016; Wu et al., 2016; Dludla et al., 2018). This has been especially important aspect uncover to improve the long-term protective effects of metformin. The latter is the first line drug for diabetes which works by lowering blood glucose, body weight and lipid levels in the body it also mediates the activation of the AMPK pathway. On the other hand, other antidiabetic drugs like the thiozonidediones have been used to manage T2D, and function by activating peroxisome proliferator activated receptor gamma (PPARγ) and

mediate adipogenesis and the uptake of fatty acids in the adipocytes (Greenfield and Chisholm, 2004). This class of drugs improve insulin sensitivity by reducing the circulating fatty acids in the peripheral tissues and can control the production of hormones such as adiponectin to improve metabolic function (Greenfield and Chisholm, 2004). However, like metformin, thiozonidediones are known to present with various side effects (DeFronzo et al., 2016), and their long-term protects effects against deteriorated metabolic function is not proven. This fact, has created opportunities to evaluate alternative regimes, including medicinal plants like *Moringa oleifera* with antioxidant and anti-inflammatory for their capacity to improve metabolic function in conditions of T2D or metabolic syndrome (Rena et al., 2013; Yendapally et al., 2020). This is especially important since most medicinal plants and bioactive compounds are known to play a major role in cellular detoxifying mechanisms, especially in part activating Nrf2, the major antioxidant response element involved in the attenuation of oxidative stress and an undesired pro-inflammatory response in a disease state (Ma, 2013; Minhaj et al., 2016; Dludla et al., 2017b).

THE POTENTIAL *IN VITRO* ANTIOXIDANT PROPERTIES OF *MORINGA OLEIFERA*

Antioxidants are important substances that aid in eliminating oxidizing agents. Any imbalance of antioxidants caused by oxidative stress may lead to tissue damage (Kurutas, 2016). This may further prompt the disruption of lipids, membranes, nucleic acids and proteins which may further cause detrimental effect and metabolic complications (Phaniendra and Babu, 2015; Pizzino et al., 2017). For years, the first line of drugs for metabolic complications such as diabetes and related metabolic disorders have been metformin, thiazolidinediones and rosiglitazone but literature has proven that plant polyphenols and their bioactive compounds may potentially provide more efficacy in alleviating diabetes, especially through targeting oxidative stress and inflammation to promote human health (Marimoutou et al., 2015; Singh et al., 2016; Taïl et al., 2020; Do et al., 2021). For example, a study showed that *Moringa oleifera* has great scavenging activity, as measured through the DPPH (1,1-diphenyl-2-picrylhydrazyl (DPPH)-2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Pakade and Chimuka, 1996). However, these results were purely relevant to its potential antioxidant potential, meaning that additional studies, making use of established preclinical and clinical models of diabetes, were still required to confirm the efficacy of this plant. Congruently, the below preclinical and clinical evidence discusses the therapeutic potential of this plant to reduce limit pathological features of oxidative stress and inflammation to alleviate complications linked with diabetes and related metabolic complications, without causing any adverse complications.

MORINGA OLEIFERA EXTRACTS IMPROVE MARKERS OF OXIDATIVE STRESS AND INFLAMMATION IN PRECLINICAL MODELS OF T1D

Type 1 diabetes mellitus represents approximately 10% of all diagnosed cases of diabetes mellitus, with abnormally increased glucose levels "a state of hyperglycemia" being the major culprit implicated in the detrimental effects associated with this condition (Internationa Diabetes Federation, 2021). Generally accepted as an autoimmune disease that is categorized by immune-mediated damage to pancreatic β-cells, a persistent state of hyperglycemia is accredited for the damaging effects on major organs of the body in those with T1D (Roep et al., 2021). Accordingly, successful establishment of experimental models of T1D, characterized by chronic hyperglycemia, have been predominantly accomplished by employing chemicals that destroy the activity of pancreatic β-cells, triggering impaired insulin secretion (Kottaisamy et al., 2021). Persistent/sustained hyperglycemia is perhaps the main consequence responsible for major organ damage, especially through destructive mechanisms involving activation of oxidative stress and promoting a pro-inflammatory response (Giacco and Brownlee, 2010). Thus far, different animals, mostly rodents have been administering different chemicals such as streptozotocin and alloxan to generate preclinical models of T1D (Kottaisamy et al., 2021). Despite their usefulness in understanding the pathogenesis of T1D, these experimental models have become relevant for screening novel drugs for their potential antidiabetic properties (King, 2012). In fact, besides their potential capacity to reduce the abnormally elevated levels of glucose, increasing research has actively screened novel therapies for their ameliorative effects against oxidative stress and inflammation to alleviate organ damage within a state of T1D (Mima, 2013; Mokgalaboni et al., 2021a; Perreault et al., 2021). This has especially been relevant for plant sources like *Moringa oleifera*, with known antioxidant and anti-inflammatory properties (Xu et al., 2019).

Evidence summarized in **Table 1** reports on the impact of *Moringa oleifera* extracts on modulating markers of oxidative stress and inflammation in preclinical models of T1D. Importantly, most of these findings indicate that chemical exposure to STZ and alloxan, followed by a state of hyperglycemia, remains a principal method used to induce T1D in these animals. Consequently, most studies showed that *Moringa oleifera* extracts (at varied doses between 100 and 300 mg/kg) could effectively ameliorate hyperglycemia, when used for a period starting from 2 weeks (Tuorkey, 2016; Oboh et al., 2018), to an average time of 6-weeks (Omodanisi et al., 2017a; Omodanisi et al., 2017b), or even in treatments lasting 8-weeks (Yassa and Tohamy, 2014; Aju et al., 2020). Interestingly, treatment with *Moringa oleifera* leaf extracts proved effective in wound healing and tissue regeneration in animals exposed to sustained levels of hyperglycemia, when used for an estimated time of 3 weeks (Muhammad et al., 2016; Azevedo et al., 2018). In addition to wound healing properties, the extracts *Moringa oleifera* showed enhanced protective effects against damage to

TABLE 1 | Studies on the effect of *Moringa oleifera* extracts targeting markers of oxidative stress and inflammation in preclinical models of type 1 diabetes.

Author, year	Experimental model, effective dose and intervention period	Experimental outcome
Jaiswal et al. (2013)	Streptozotocin (STZ)-induced diabetes in Wistar rats treated with 200 mg/kg *Moringa oleifera* leaf extract for 3 weeks	Ameliorated oxidative stress by significantly increasing the antioxidant activity of superoxide dismutase (SOD), glutathione S-transferase (GST) and catalase (CAT) while decreasing the lipid peroxide levels
Yassa and Tohamy, (2014)	STZ-induced diabetes in Sprague Dawley rats treated with 200 mg/kg *Moringa oleifera* extract for 8 weeks	Lowered fasting plasma glucose (FPG) levels, reversed pancreatic damage, while also enhancing glutathione (GSH) and reducing malondialdehyde (MDA) pancreatic concentrations
Al-Malki El Rabey, (2015)	STZ-induced diabetes in albino rats were treated with 50 and 100 mg/kg with *Moringa oleifera* seed extract for 4 weeks	Decreased FPG, and increased the concentration of antioxidants like SOD, CAT and GSH in serum and kidney. Moreover, treatment decreased the concentration of interleukin (IL)-6 and lipid peroxides (MDA) in the serum and kidney tissue homogenate
Muhammad et al. (2016)	STZ-nicotinamide induced diabetes in Wistar rats treated with 0.5,1 and 2% w/w *Moringa oleifera* leaf aqueous fractions for 3 weeks	Decreased wound size under sustained hyperglycemic condition and improved wound contraction, and tissue regeneration. This was associated with reduced inflammatory mediator such as tumor necrosis factor alpha (TNF-α), IL-1β, IL-6, cyclooxygenase-2 (COX-2), nitric oxide synthase (iNOS) and upregulation of an angiogenic marker vascular endothelial growth factor in wound tissue
Tuorkey, (2016)	Alloxan-induced diabetes albino mice treated with 100 mg/kg of *Moringa oleifera* aqueous extract 2 weeks	Significantly decreased FPG and plasma insulin levels, concomitant to reversing insulin resistance. Total antioxidant capacity increased while the levels of creatinine and urea significantly declined. While cluster of differentiation (CD)44 was not changed, CD69 and interferon gamma I (NF-γ) were increased by treatment
Abd Eldaim et al. (2017)	Alloxan-induced diabetes in Wistar rats treated with 250 mg/kg *Moringa oleifera* leaf extract for 2.5 weeks	Prevented hepatic damage and normalized the reduced hepatic levels of glutathione (GSH), as well as the activities of SOD and CAT, while also reducing blood glucose levels, hepatic lipid peroxidation
Omodanisi et al. (2017a)	STZ-induced diabetes in Wistar rats treated with 250 mg/kg *Moringa oleifera* aqueous extract for 6 weeks	Reduced hepatic enzyme markers and normalized lipid profile parameters, while enhancing antioxidant capacity and alleviating inflammatory biomarkers of the liver. Specifically, reduced levels of MDA and increased endogenous antioxidants (SOD, CAT, GSH, GPx), while decreased inflammatory cytokines (IL-1α, IL-6, IL-12, IL-18, TNF-α) and (chemokine: MCP-1) in the serum, liver; kidney and erythrocytes
Omodanisi et al. (2017b)	STZ-induced diabetes in Wistar rats treated with 250 mg/kg *Moringa oleifera* leaf extract for 6 weeks	Reduced FPG and biomarkers of oxidative stress and inflammation. Specifically, reduced the level of lipid peroxidation (MDA), and improved antioxidant such as CAT, SOD, GSH, glutathione peroxidase (GPx), whilst decreasing pro-inflammatory makers such as tumor necrosis factor-alpha (TNF-α) and IL-6
Raafat and Hdaib, (2017)	Alloxan-induced diabetes albino rats treated with 250 mg/kg *Moringa oleifera* leaf extract for 2.5 weeks	Reduced FPG and hindered lipid peroxidation, whilst increasing hepatic GSH levels, as well as the activities of SOD and CAT, and the gene expression of glycogen synthase while reducing pyruvate carboxylase caspase 3 gene expression
	Alloxan-induced diabetes Swiss-Webster mice treated with 40,60 and 80 μg/ml *Moringa oleifera* seed extract for 8 weeks	Decreased FPG and inhibited alpha glucosidase activity. Serum insulin levels and serum CAT levels were significantly increased whilst lipid peroxidation and glycated hemoglobin (HbA1C) were decreased
Alejandra Sánchez-Muñoz et al. (2018)	STZ-induced diabetes in Wistar rats treated with 200 mg/kg *Moringa oleifera* leaf extract for 3 weeks	Ameliorated oxidative stress-induced modification in liver mitochondria, in part by improving mitochondrial respiration, as well as enhancing the antioxidant levels of GSH, glutathione reductase and heme oygenase-1 (HO-1) activity, which decreasing lipid peroxidation (MDA) and production of reactive oxygen species
Azevedo et al. (2018)	STZ-induced diabetes in Wistar adult rats were treated with 100 mg/kg of *Moringa oleifera* leaf extract for 3 weeks	Displayed wound healing properties and significantly reduced glycemia accompanied by a decreased in pro-inflammatory markers such as TNF-α, IL-□ and IL-6 in the serum
Oboh et al. (2018)	STZ-induced diabetes in Wistar rats treated with 2 and 4% *Moringa oleifera* leaf and seed extracts for 2 weeks	Both extracts reduced FPG, prevented cognitive dysfunction-induced by chronic hyperglycemia by reducing the activities of acetylcholinesterase, angiotensin-I converting enzyme and butyrylcholinesterase. This was concomitant to the increase in antioxidant molecules such as CAT, GST and GPx, as well as a decrease in lipid peroxidation (MDA) level in the brain
Aju et al. (2019)	STZ-induced diabetes in Sprague-Dawley rats treated with 300 mg/kg body weight leaf (methanol) extract for 8.5 weeks	Significantly decreased FPG and glycated hemoglobin but increased plasma insulin levels. The antioxidant enzymes like SOD, CAT, GPx and glutathione-reductase and non-enzymatic antioxidant GSH were increased causing a decrease in hydroperoxides, conjugated dienes and lipid peroxidation
Oguntibeju et al. (2020)		

(Continued on following page)

TABLE 1 | (*Continued*) Studies on the effect of *Moringa oleifera* extracts targeting markers of oxidative stress and inflammation in preclinical models of type 1 diabetes.

Author, year	Experimental model, effective dose and intervention period	Experimental outcome
	STZ-induced diabetes Wistar rats treated with 250 mg/kg leaf extract of *Moringa oleifera* for 6 weeks	Reduced nephrotoxic and hepatotoxic damage evident by a decrease in serum creatinine, albumin and bilirubin. Likewise, the inflammatory cytokines interleukin (IL)-1α, IL-12 and IL-18, and apoptotic markers caspase 3, caspase 9, B-cell lymphoma 2(BCL-2), NF-κβ, and p53 were decreased
Sierra-Campos et al. (2020)	Alloxan-induced diabetes in Wistar rats treated with 200 mg/kg leaf extract of *Moringa oleifera* for 3 weeks	Displayed antidiabetic effects by increasing the levels of serum paraoxonase 1 and liver cytosolic CAT
Oldoni et al. (2021)	STZ-induced diabetes in Wistar rats treated with 500 mg/kg crude leaf extract of *Moringa oleifera* (hydroalcoholic extract was produced by using ethanol: water (80:20 v/v)) for 6.5 weeks	Reduced FPG and protected against oxidative damage in liver and kidney by enhancing endogenous antioxidant defenses such as CAT, GST and non-protein thiol groups, while reducing lipid peroxidation in these tissues
Oyeleye et al. (2021)	STZ-induced diabetes in Wistar rats treated with 2 and 4% of *Moringa oleifera* leaf and seed extracts for 2 weeks	Both extracts reversed diabetes-induced erectile dysfunction by reducing FPG, as well as blocking lipid peroxidation by decreasing thiobarbituric acid reactive species (TBARS) levels. Treatments also attenuated the activity of phosphodiesterase type 5 (PDE-5) and arginase but improved the levels of nitric oxide

various organs, including the liver and kidneys, in these preclinical models of T1D (Abd Eldaim et al., 2017; Omodanisi et al., 2017a; Oguntibeju, 2019; Oldoni et al., 2021). The antidiabetic properties of these extracts extend to preventing cognitive or erectile dysfunction in rats, by mainly reducing the activities of enzymes like acetylcholinesterase, angiotensin-I converting enzyme and butyrylcholinesterase (Oboh et al., 2018; Oyeleye et al., 2021). Apparently, the ameliorative effects against oxidative stress or undesired pro-inflammatory response remain the predominant mechanisms by *Moringa oleifera* extracts protect against complications of T1D in preclinical (animal) models.

For instance, through blockade of lipid peroxidation as well as by reinforcing intracellular antioxidant capacity, as demonstrated by reduced levels of peroxidation products like MDA/thiobarbituric acid reactive species (TBARS) and elevated antioxidant defences such as SOD, GSH, GST and CAT, *Moringa oleifera* extracts showed enhanced effects in protecting against the detrimental effects of oxidative stress in preclinical models of T1D (Jaiswal et al., 2013; Yassa and Tohamy, 2014; Al-Malki and El Rabey, 2015; Raafat and Hdaib, 2017). In support of this mechanistic insight, it has long been established that induction of diabetes in rats with STZ or alloxan favors uncontrolled availability of lipid peroxidation products, while consequently suppressing the intracellular antioxidant defences (Maritim et al., 2003; Davì et al., 2005). This process prompts excess free radical production, as also observed in patients with T1D (Domínguez et al., 1998), while the accompanied hyperglycemic state may directly contribute to oxidative stress-induced organ damage (Maritim et al., 2003). Besides the harmful effects associated with lipid peroxidation, evidence summarized in **Table 1** indicates that treatment with *Moringa oleifera* extracts for 3 weeks remains effective in targeting other sources of oxidative stress like the mitochondria to ease complications linked with T1D. In actual fact, Alejandra Sánchez-Muñoz and others showed that a leaf extract of this plant improved mitochondrial respiration, while increasing levels of intracellular antioxidants like GSH, glutathione reductase and HO-1 activity, to reduce excess production of ROS liver cells of STZ-induced diabetic rats (Alejandra Sánchez-Muñoz et al., 2018). Generally, these results are of interest as many studies indicate that mitochondria remain one of the major therapeutic targets to ameliorate hyperglycemia-induced oxidative damage (Giacco and Brownlee, 2010; Dludla et al., 2017a; Teodoro et al., 2018).

Consistent with attenuating the destructive effects of oxidative stress, presented evidence showed that *Moringa oleifera* extracts could effectively reduce the elevated levels of pro-inflammatory mediators such as tumor necrosis factor alpha (TNF-α), IL-1β, IL-6, cyclooxygenase-2 (COX-2), nitric oxide synthase (iNOS), and chemokine (MCP-1) to protect against exacerbated inflammation, under sustained conditions of hyperglycemia (Muhammad et al., 2016; Omodanisi et al., 2017a; Azevedo et al., 2018). Significantly, such positive effects were associated with reduced nephrotoxic and hepatotoxic damage (Oguntibeju, 2019), including upregulation of an angiogenic marker vascular endothelial growth factor (VEGF) to protect against hyperglycemia-induced wound injury in a preclinical model of T1D (Muhammad et al., 2016). This is an essential result since tenacious hyperglycemia, seen in T1D, is already known to mediate iNOS induction, leading to the activation of protein kinase enzymes such as PKC/c-Jun N-terminal kinases (JNKs)/mitogen-activated protein kinase (MAPK) to propagate the detrimental effects of inflammation (Giacco and Brownlee, 2010). Notably, activation of such pro-inflammatory mechanisms can directly cause excess generation of oxidation products that precede the onset of atherosclerosis and endothelial dysfunction, which are major risk factors for the development of cardiovascular diseases (Rose et al., 2010; Mokgalaboni et al., 2020). Although there was limited information on its cardioprotective effects, much evidence suggests *Moringa*

oleifera extracts can significantly decrease pro-inflammatory and apoptotic markers such as TNF-α, IL-1β, IL-6, NF-κβ, caspase 3, caspase 9, and tumor protein (p53) to alleviate the detrimental effects of hyperglycemia in preclinical models of T1D (Muhammad et al., 2016; Omodanisi et al., 2017a; Azevedo et al., 2018; Oguntibeju, 2019). Overall, summarized evidence supports the beneficial effects of *Moringa oleifera* in lowering hyperglycemia in addition to ameliorating the detrimental effects of oxidative stress and inflammation in preclinical (animal) models of T1D. Some other takeaways from the current results indicate that most studies observed therapeutic effects when using doses between 100 and 300 mg/kg (Al-Malki and El Rabey, 2015; Tuorkey, 2016; Alejandra Sánchez-Muñoz et al., 2018; Azevedo et al., 2018), with an average dose of 250 mg/kg commonly exploited (Abd Eldaim et al., 2017; Omodanisi et al., 2017a; Omodanisi et al., 2017b; Raafat and Hdaib, 2017). Also, most studies reported on the use of leaf extracts over seed extracts of this plant (**Table 1**). This could be supported by available evidence already supporting the strong antioxidant properties of leaf extracts of over seed extracts (Ilyas et al., 2015; Saini et al., 2016).

MORINGA OLEIFERA EXTRACTS IMPROVE MARKERS OF OXIDATIVE STRESS AND INFLAMMATION IN PRECLINICAL MODELS OF T2D

Type 2 diabetes, remains the major form of diabetes, contributing to approximately 90% to all global cases of this condition, as regularly reported by the world leading health surveillance organizations (International Diabetes Federation, 2021). Modifiable risk factors, mostly involving sedentary lifestyle, taking place together with overnutrition are to blame for increased cases of T2D, as these factors cause overweight and obesity (Grundy, 2016). In such conditions, increased nutrient availability may drive excessive fat accumulation in key body areas such as the liver, skeletal muscle, blood circulation and heart muscle, leading to the development of pathological complications like non-alcoholic fatty liver disease, muscle wasting or sarcopenia and cardiovascular diseases (Grundy, 2016; Chait and den Hartigh, 2020). Just like in T1D, hyperglycemia remains the major pathological feature of T2D. Besides hyperglycemia, patients with T2D are known to present with insulin resistance and a cluster of other irregularities such as abnormal blood lipid profiles, as observed through aberrant levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol (Stanford and Goodyear, 2014; Oguntibeju, 2019). However, defects in insulin signaling or a state of insulin resistance has been seen as an early sign of T2D manifestation, which is likely to occur simultaneous to other metabolic dysregulations, including enhanced inflammatory signaling, generation of oxidative products and initiation of endoplasmic reticulum stress pathways (Muoio and Newgard, 2008). These are among the most explored pathological mechanisms in preclinical models of T2D. For example, rodents exposed to a high fat diet (HFD) or its combination with low dose STZ (Waterman et al., 2015; Jaja-Chimedza et al., 2018; Chin et al., 2019; Mohamed et al., 2019; El-Shehawi et al., 2021), as well as gene-defiant mice such as those considered leptin resistance (*db/db*) (Tang et al., 2017) are known to progressively develop T2D, including its complications involving oxidative stress and inflammation. This explains, the surge use of these preclinical models to test novel treatments against T2D.

Table 2 gives an overview of information on the antidiabetic properties of *Moringa oleifera* extracts, including its modulatory effects on markers of oxidative stress and inflammation in preclinical models of T2D. Most importantly, summarized evidence showed that these extracts were effective in reducing body weight, body fat mass and fasting plasma glucose levels, which are the major characteristic features of T2D (Tang et al., 2017; Jaja-Chimedza et al., 2018; El-Shehawi et al., 2021). Consistent with evidence summarized in **Table 1**, blocking hepatic lipid accumulation, in part through effective modulating the makers of oxidative stress and inflammation such as antioxidants like CAT, SOD, MDA content, uncoupling protein 2/3, TNF-α, 1L-β, IL-6, IL-2 and MCP-1 remains the major mechanism of action of these extracts (Joung et al., 2017; Mohamed et al., 2019). Some evidence showed these extracts could improve lipid profiles and reduce the expression of genes involved in energy metabolism or fat synthesis such as fatty-acid synthase, lipoprotein lipase, CCAAT-enhancer-binding protein homologous-α (C/EBPα), sterol regulatory element-binding protein 1c (SREBP1c), within the skeletal muscle (Joung et al., 2017; Tang et al., 2017). Partially indicating that *Moringa oleifera* extracts may be a potent remedy to decrease excess body fat or ameliorate complications linked with obesity, as reviewed elsewhere (Redha et al., 2021). Of further interest, some evidence indicated that *Moringa oleifera* extracts could target early pathological signs of T2D, such as improving glucose tolerance and insulin levels, while enhancing insulin sensitivity and glucose homeostasis in tissues of these preclinical models (Jaja-Chimedza et al., 2018; Mohamed et al., 2019). This hypothesis remains to be confirmed in other experimental models of T2D, however provides necessary information to guide future research.

SAFETY AND THE TOXICITY PROFILE OF *MORINGA OLEIFERA*

It is currently acknowledged that a large population of people depend on medicinal plants to treat different diseases, which mainly due to ancestral knowledge (Palhares et al., 2015; Muvhulawa et al., 2022). Thus, the general interest in the use of medicinal plants to cure various disease conditions has grown over the years (Rakotoarivelo et al., 2015; Michel et al., 2020). Therefore, it is important for plants to be evaluated for their toxicity to know how safe they are for human use. Accumulative research shows that *Moringa oleifera* exhibits a lot of important

TABLE 2 | An overview of studies on the effects of *Moringa oleifera* extracts targeting markers of oxidative stress and inflammation in preclinical models of type 2 diabetes.

Author, year	Experimental model, effective dose and intervention period	Experimental outcome
Waterman et al. (2015)	High fat diet (HFD)-fed C57BL/6L mice treated with 5% *Moringa oleifera* concentrate (delivering 66 mg/kg/d of moringa isothiocyanates)	Improved glucose tolerance and insulin signaling and did not develop fatty liver disease. Treatment also reduced plasma insulin, leptin, resistin, cholesterol, interleukin (IL)-1β, tumor necrosis factor alpha (TNF-α), and lowered hepatic glucose-6-phosphatase expression
Joung et al. (2017)	HFD-induced glucose intolerance C57BL/6 mice treated with 250 mg/kg *Moringa oleifera* leaf extract for 10 weeks	Did not affect body weights but reduced hepatic lipid accumulation. Also, reduced HFD-induced endoplasmic reticulum stress, oxidative stress, and lipotoxicity in quadriceps muscles. Reduced the expression of genes involved in energy metabolism such as fatty-acid synthase, lipoprotein lipase, CCAAT-enhancer-binding protein homologous-α (C/EBPα), sterol regulatory element-binding protein 1c (SREBP1c), within the skeletal muscle. Oxidative and inflammatory markers such as uncoupling protein 2/3, TNF-α, 1L-β, IL-6, IL-2 and monocyte chemoattractant protein-1 (MCP-1) were improved
Tang et al. (2017)	Type 2 diabetic (*db/db*) mice treated with 150 mg/kg *Moringa oleifera* leaf ethanolic extract for 5 weeks	Reduced fasting plasma glucose (FPG) and increased insulin levels, while improving lipid profiles by decreasing concentrations of triglycerides and low-density lipoprotein. Also, protected against renal damage by decreasing pro-inflammatory markers such as TNF-α, IL-1β, IL-6, cyclooxygenase-2 and inducible nitric oxide synthase (iNOS) in renal tissue
Jaja-Chimedza et al. (2018)	HFD-induced obese C57Bl/6 J mice treated with *Moringa oleifera* seed, containing 0.54 and 0.73% of extract supplemented in diet for 12 weeks	Reduced body weight, decreased adiposity, improved glucose tolerance, decreased inflammatory gene expression, and increased antioxidant gene expression. Specific, inflammatory genes that were decreased included IL-1β, IL-6 and TNF-α, while oxidative genes improved included iNOS and NADPH dehydrogenase [quinone] 1 (NQO1), in some of the tissues (liver, jejunum, ileum and colon)
Chin et al. (2019)	HFD and streptozotocin-induced diabetes in Sprague Dawley rats treated with 0.5% standardized aqueous *Moringa oleifera* leaf extract-loaded hydrocolloid film for 3 weeks	Significantly improved wound healing, and this was in part by effective modulation of pro-inflammatory markers and growth factors including TNF-α, IL-6, MCP-1, vascular endothelial growth factor, epidermal growth factor in the wound site
Mohamed et al. (2019)	HFD-induced insulin resistant in Sprague Dawley rats treated with 300 mg/kg *Moringa oleifera* aqueous extract for 4 weeks	Reversed hepatic insulin insensitivity and this was linked to up-regulation of genes involved in insulin receptors and glucose uptake such as insulin receptor, insulin receptor substrate-1 and glucose transporter (GLUT)4. Also improved hepatic antioxidants like catalase (CAT) and superoxide dismutase (SOD), while decreasing level of lipid peroxidation, the malonaldehyde (MDA) content
El-Shehawi et al. (2021)	HFD-induced obese Wistar rats treated with 300 mg/kg *Moringa oleifera* leaf extract for 14 weeks	Reduced body weight and body fat mass, while also decreasing FPG, insulin, and leptin levels, while increased adiponectin. Consistently, treatment improved lipid profiles like serum total cholesterol, triacylglycerol, and low-density lipoprotein, while enhancing hepatic antioxidant enzymes such as SOD, CAT. Lipid peroxidation (MDA) and some pro-inflammatory markers like nuclear factor kappa β (NF-κβ)- P65 were decreased

biological properties such as antioxidant, anti-inflammation, anti-hyperglycemic properties over the past years proving that it is a good plant to use as an alternative therapeutic for diabetes (Omodanisi et al., 2017b; Gothai et al., 2017; Paula et al., 2017; Abd et al., 2020; Xiong et al., 2021). *In vitro* and *in vivo* studies that have been conducted to show that this plant has no lethal dose and is safe to use. Indeed, work by Villarruel-Lòpez and others showed that the use of *Moringa oleifera,* at different doses ranging from 100 to 500 mg/kg, is not toxic in rats (Villarruel-López et al., 2018). Albrahim and Binobead also used rats to show that *Moringa oleifera* alleviates vetsin-induced cytotoxicity, as measured by alterations in liver functions, oxidative stress, DNA damage, and liver injury (Albrahim and Binobead, 2018a). Reviewed evidence from Asare and co-workers revealed that *Moringa oleifera* is genotoxic at supra-supplementation levels of 3,000 mg/kg body weight, with intake mostly considered is safe at levels ≤1,000 mg/kg (body weight) (Asare et al., 2012). However, other studies have indicated that although available literature is very promising (Awodele et al., 2012; Ajibade et al., 2013; Stohs and Hartman, 2015; Patriota et al., 2020; Siddiqui et al., 2021; Teshome et al., 2021), additional clinical (human) to accomplish standardized extracts of this plant.

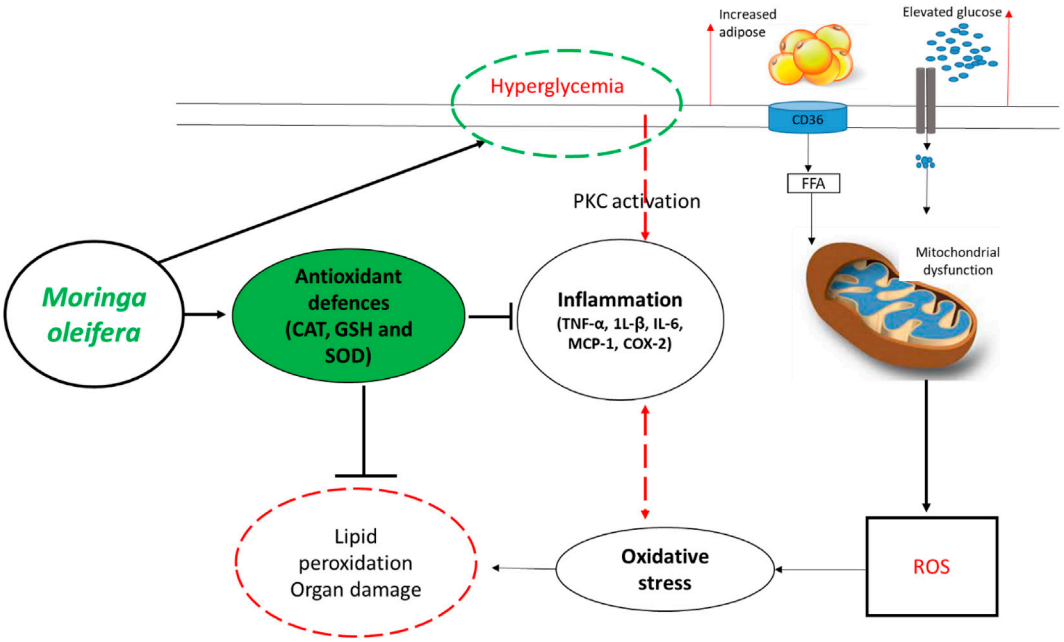

FIGURE 3 | An overview of therapeutic mechanisms associated with the ameliorative effects of *Moringa oleifera* extracts in preclinical (animal) models of diabetes. Briefly, overwhelming evidence supports the beneficial effects of this plant in enhancing intracellular antioxidants such as catalase (CAT), glutathione (GSH) and superoxide dismutase (SOD) to block the detrimental effects reactive oxygen species (ROS), lipid peroxidation and organ damage. This is in part by also improving glucose control (hyperglycemia) and reducing prominent pro-inflammatory markers like tumor necrosis factor-alpha (TNF-α), interleukin (1L)-β, IL-6, monocyte chemoattractant protein-1 (MCP-1) and COX-2-cyclooxygenase-2 (COX-2). Abbreviations: CD36-cluster of differentiation 36; FFA-free fatty acid; PKC-protein kinase C. Indicators: red lines-detrimental effects, bold lines/green lines-protective effects of *Moringa oleifera* extracts.

CLINICAL TRANSLATION

Preclinical studies are important to understand the pathophysiological mechanisms implicated in disease development, and this aspect remains essential to explore the therapeutic potential of plant extracts and their derivative compounds (Bonjour et al., 1999; Steinmetz and Spack, 2009). Whereas screening plant extracts and their biological compounds using preclinical models has become a routine procedure to determine effective doses, pharmacokinetic profile and evaluate relevant toxicological aspects before commencement of clinical trials (Bonjour et al., 1999; Steinmetz and Spack, 2009). Although accumulative literature supports the beneficial effects of plant extracts and naturally derived compounds against diabetes (Hung et al., 2012; Jugran et al., 2021), persistent setbacks have been the limited number of studies entering clinical trial phase, which is a vital component in drug development. Likewise, there has been limited number of studies on the antidiabetic properties of *Moringa oleifera* extracts. At present, only a few randomized controlled trials have been published on the antidiabetic potential (**Table 3**) of *Moringa oleifera*. In 2016, Anthanont and co-workers showed that capsules of *Moringa oleifera* leaf powder (at a dose of 4 g), taken after an overnight fast and every 2 weeks, could significantly increase insulin secretion in healthy subjects (Anthanont et al., 2016). Leone and others demonstrated that randomly giving Saharawi people with diabetes a traditional meal supplemented with 20 g leaf powder of *Moringa oleifera* on two different days could improve postprandial blood glucose response when compared to nondiabetic controls (Leone et al., 2018). Dixit and co-workers (Dixit et al., 2018) reported that intake of extracts of *Moringa oleifera* (LI85008F) at 900 mg/day (combined with modest calorie restriction and physical activity) for 16 weeks could reduce waist and hip circumferences, and improved lipid profiles in overweight participants. Also, this was relevant to reduced low-density lipoprotein (LDL) cholesterol decreased, while high-density lipoprotein (HDL) cholesterol increased, resulting in a significantly improved LDL/HDL ratio. While Gómez-Martínez and co-workers reported that giving subjects with prediabetes six daily capsules of *Moringa oleifera* leaf powder (2,400 mg/day) improved fasting blood glucose and glycated hemoglobin (HbA1c) when compared to the controls. Recently, Díaz-Prieto et al., demonstrated that consumption of 6 × 400 mg capsule/day of *Moringa oleifera* dry leaf powder for 12 weeks indicated that plasma tumor necrosis factor alpha (TNF-α) was a significant predictor of the subject's glycated hemoglobin (HbA1c) response in subjects with prediabetes (Díaz-Prieto et al., 2022). These results are consistent with some preclinical findings (Jaja-Chimedza et al., 2018; Mohamed et al., 2019), indicating that *Moringa oleifera* leaf extracts might be useful complications identified during the early development of T2D. Nonetheless, despite accumulative literature, as reviewed elsewhere (Ba et al., 2020; Watanabe et al., 2021), indicating that this plant might present with important antidiabetic properties, more needs to be done to confirm these in

TABLE 3 | An overview of clinical studies reporting on the antidiabetic properties of *Moringa oleifera*.

Author, year	Country	Study population	Dose and intervention period	Clinical outcome
Anthanont et al. (2016)	Thailand	Healthy subjects (*n* = 10)	Received an oral dose of *Moringa oleifera* at increasing dosages of 0, 1, 2, and 4 g, and monitored for 0.5, 1, 1.5, 2, 4, and 6 h	Improved baseline insulin levels, but did not affect blood glucose concentrations
Taweerutchana et al. (2017)	Thailand	Therapy-naïve type 2 diabetes patients (*n* = 32)	Received receive either 8 g per day of *Moringa oleifera* leaf capsules for 4 weeks	No effect on blood glucose levels, although non-significantly improved blood pressure
Dixit et al. (2018)	India	Overweight participants (*n* = 70)	Received combined extracts of *Moringa oleifera* (LI85008F) at 900 mg/day (combined with modest calorie restriction and physical activity) for 16 weeks	Reduced waist and hip circumferences, and improved lipid profiles. Also, reduced low-density lipoprotein (LDL) cholesterol decreased, while high-density lipoprotein (HDL) cholesterol increased, resulting in a significantly improved LDL/HDL ratio
Leone et al. (2018)	Italy	Subjects with type 2 diabetes (*n* = 17)	Received, on 2 different days, a traditional meal supplemented with 20 g of *Moringa oleifera* leaf powder	Improved blood glucose control
Sissoko et al. (2020)	Mali	Subjects with type 2 diabetes (*n* = 35)	Received n 1 and 2 g respectively, of *Moringa oleifera* leaf powder, 30 min after eating the white bread and were monitored for up to 180 min	Reduced post-prandial glycaemia in diabetic patients
Gómez-Martínez et al. (2021)	Spain	Subjects with prediabetes (*n* = 34)	Received six daily capsules of *Moringa oleifera* dry leaf powder (2,400 mg/day) for 12 weeks	Improved fasting blood glucose (FBG) and glycated hemoglobin (HbA1c). However, did not affect microbiota, hepatic and renal function markers or the appetite-controlling hormones measured
Díaz-Prieto et al. (2022)	Spain	Subjects with prediabetes (*n* = 31)	Received consumed 6 × 400 mg capsule/day of *Moringa oleifera* dry leaf powder for 12 weeks	Plasma tumor necrosis factor alpha (TNF-α) was a significant predictor of the subject's HbA1c response (improvement YES/NO; 77% correct classification) in the *Moringa oleifera* group

clinical settings. It remains crucial, to evaluate whether *Moringa oleifera* leaf extracts can prominent biomarkers of oxidative stress and inflammation, to verify preclinical findings.

SUMMARY AND FUTURE PERSPECTIVE

The swift prevalence of diabetes warrants urgent investigation into novel therapies to protect and better manage this chronic medical condition (International Diabetes Federation, 2021). Metformin and insulin, which are commonly used antidiabetic therapies, have certainly prolonged the lives of patients with diabetes (Joya-Galeana et al., 2011; Foretz et al., 2014; Bailey, 2017). Correspondingly, other effective interventions like physical exercise and caloric striction can be used to manage diabetes (Nyawo et al., 2021; Shakoor et al., 2021; Mthembu et al., 2022), however only a few individuals can constantly adhere to such strenuous interventions. Besides lowering glucose or improving insulin sensitivity, it has become imperative to uncover therapies that can target the amelioration of both oxidative stress and inflammation, as the prime dysregulations implicated in the pathogenesis of diabetes (Vikram et al., 2014; Mahlangu et al., 2020; Mokgalaboni et al., 2021b). This also explains the surge in research investigating the biological properties of nutritional plant sources like rooibos (*Aspalathus linearis*) and broccoli (*Brassica oleracea var. italica*) with abundant antioxidants properties in combating metabolic complications like oxidative stress and inflammation (Hwang and Lim, 2014; Dludla et al., 2017a; Orlando et al., 2022).

Plants have been studied for their therapeutic properties and they are also cheap, easily accessible and safer than synthetic conventional drugs (Ahmad et al., 2019). There is growing evidence that plants not only serve as a food source but as medicine, nutraceuticals and so forth (Alegbeleye, 2018). Also, they are a body of polyphenols, vitamins, flavonoids, alkaloids and other important phytochemicals. *Moringa oleifera* has been proven in a number of studies to alleviate insulin resistance by activating the insulin-independent pathway PI3K/AKT and also through AMPK pathway in the skeletal muscle and it can also improve skeletal muscle oxidative metabolism through the NAD-dependent deacetylase (SIRT1)-PPARα pathway and also through improving fatty acid peroxidation (Bao et al., 2020; Duranti et al., 2021).

In fact, overwhelming evidence summarized in this review supports the beneficial effects of *Moringa oleifera* in improving blood glucose levels, lipid profiles and insulin sensitivity, in addition to protecting against hepatic or nephrotic damage in preclinical (animal) models of T1D/T2D (**Table 1** and **2**). Interestingly, these extracts show enhanced effects in strengthening intracellular antioxidants like CAT, SOD, GSH, and GST to lower lipid peroxidation products MDA/TBARS, and reduce prominent pro-inflammatory markers like TNF-α, 1L-β,

IL-6, MCP-1, COX-2, and nitric oxide synthase in these animal models. **Figure 3** summarizes some therapeutic effects in protecting against oxidative stress and inflammation associated with *Moringa oleifera* extracts in preclinical models of diabetes. Furthermore, the current literature review indicates the common use of leaf extracts of *Moringa oleifera*, within a range 100–300 mg/kg, from initial treatment duration of 2 weeks up until 8 weeks (**Tables 1**, **2**). This further sets a platform for future research (which is currently limited) directed at developing *Moringa oleifera* as a functional food to manage diabetes mellitus. Importantly, additional clinical trials are necessary to evaluate whether *Moringa oleifera* leaf extracts can prominent biomarkers of oxidative stress and inflammation, to verify preclinical findings.

AUTHOR CONTRIBUTIONS

FM, PD, and SM-M concept and original draft. All authors, including FM, PD, KZ, SM, NM, NH, BN, and SM-M wrote and approved the final manuscript.

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GLOSSARY

AMPK-AMP activated protein kinase
AKT protein kinase B
ACOX1 peroxisomal acyl-CoA oxidase 1
BCL-2 B-cell lymphoma 2
CAT catalase
C/EBPα enhancer-binding protein
COX-2 cyclooxygenase-2
GPx glutathione reductase
GST glutathione-S-transferase
GLUT4 glucose transporter 4
GSH glutathione
HbA1C glycated hemoglobin
HDL high density lipoprotein
HO-1 heme oygenase-1
LDL low density lipoprotein
NJK-c-Jun N terminal kinases
MDA malonaldehyde
iNOS inducible nitric oxide synthase
INF-γ interferon gamma
IL interleukin
MAPK mitogen-activated protein kinase
MCP-1 monocyte chemoattractant protein-1
NF-κβ nuclear factor kappa β
T1D/T1D type 1/2 diabetes mellitus
TNF-α tumor necrosis factor alpha
TBARS thiobarbituric acid reactive species
PDE-5 phosphodiesterase type 5
PKC protein kinase C
PPARγ peroxisome proliferator-activated receptor gamma
SIRT1 NAD-dependent deacetylase
SOD superoxide dismutase
SREBP1c sterol regulatory element-binding protein 1c
WHO World Health Organization
VEGF vascular endothelial growth factor

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EDITED BY
Yongsheng Chen,
Jinan University, China

REVIEWED BY
Yong Zhou,
Shanghai General Hospital, China
Jiajia Song,
Southwest University, China

*CORRESPONDENCE
Xianglu Rong,
xl_rong@qq.com

†These authors have contributed equally to this work

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Astragalus mongholicus powder, a traditional Chinese medicine formula ameliorate type 2 diabetes by regulating adipoinsular axis in diabetic mice

Siyuan Xu[1†], Bixian Ye[2†], Jinlei Li[3†], Yonghui Dou[3], Yuying Yu[1], Yifan Feng[1], Lexun Wang[1], David Chi-Cheong Wan[4] and Xianglu Rong[1*]

[1]Key Laboratory of Glucolipid Metabolic Disorder, Guangdong TCM Key Laboratory for Metabolic Diseases, Guangdong Metabolic Diseases Research Center of Integrated Chinese and Western Medicine, Ministry of Education of China, Institute of Chinese Medicine, Guangdong Pharmaceutical University, Guangzhou, China, [2]Department of Nursing, Medical College of Jiaying University, Meizhou, China, [3]School of Chinese Meteria Medica, Guangzhou University of Chinese Medicine, Guangzhou, China, [4]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

The global morbidity of obesity and type 2 diabetes mellitus (T2DM) has dramatically increased. Insulin resistance is the most important pathogenesis and therapeutic target of T2DM. The traditional Chinese medicine formula Astragalus mongholicus powder (APF), consists of *Astragalus mongholicus* Bunge [Fabaceae], *Pueraria montana* (Lour.) Merr. [Fabaceae], and *Morus alba* L. [Moraceae] has a long history to be used to treat diabetes in ancient China. This work aims to investigate the effects of APF on diabetic mice and its underlying mechanism. Diabetic mice were induced by High-fat-diet (HFD) and streptozotocin (STZ). The body weight of mice and their plasma levels of glucose, insulin, leptin and lipids were examined. Reverse transcription-polymerase chain reaction, histology, and Western blot analysis were performed to validate the effects of APF on diabetic mice and investigate the underlying mechanism. APF reduced hyperglycemia, hyperinsulinemia, and hyerleptinemia and attenuate the progression of obesity and non-alcoholic fatty liver disease (NAFLD). However, these effects disappeared in leptin deficient ob/ob diabetic mice and STZ-induced insulin deficient type 1 diabetic mice. Destruction of either

Abbreviations: ACC, Acetyl-CoA carboxylase; APF, Astragalus mongholicus powder; FAS, fatty acid synthase; Glu, glucose; G6Pc, Glucose-6-phosphatase, catalytic subunit; HFD, high fat diet; HOMA-IR, homeostasis model of assessment for insulin resistance; HSL, hormone sensitive lipase; ITT, insulin tolerance test; LDL-C, low-density lipoprotein cholesterol; NAFLD, non-alcoholic fatty liver disease; NC, normal control; NEFA, non-esterified fatty acid; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; PEPCK, phosphoenolpyruvate carboxykinase; PPARα, peroxisome proliferator activated receptor α; PTP1B, protein tyrosine phosphatase 1B; SCD1, stearoyl-CoA desaturase-1; STZ, streptozotocin; TC, total cholesterol; TCM, traditional Chinese medicine; TCPTP, T-cell protein tyrosine phosphatase; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; TG, triglyceride.

these hormones would abolish the therapeutic effects of APF. In addition, APF inhibited the protein expression of PTP1B suppressing insulin–leptin sensitivity, the gluconeogenic gene PEPCK, and the adipogenic gene FAS. Therefore, insulin–leptin sensitivity was normalized, and the gluconeogenic and adipogenic genes were suppressed. In conclusion, APF attenuated obesity, NAFLD, and T2DM by regulating the balance of adipoinsular axis in STZ + HFD induced T2DM mice.

KEYWORDS

***Astragalus mongholicus* powder, type 2 diabetes mellitus, insulin resistance, adipoinsular axis, leptin**

Introduction

Nutritional excess increases the risk for obesity (Hale et al., 2015), which has developed in more than 370 million people suffering from type 2 diabetes mellitus (T2DM) (Kahn et al., 2014). T2DM has series of complications, such as dyslipidemia, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease (Cornier et al., 2008). All these metabolic disorders were introduced as glucolipid metabolic disease (GLMD) (Guo, 2017). Insulin resistance is an crucial etiology of GLMD. Leptin, a 16 kDa protein secreted by adipocyte and the key cytokine linked with obesity and T2DM (Halaas et al., 1995; Moon et al., 2013), acts as an anorectic hormone that restricts lipid storage and body weight gain (Lee et al., 2002), inhibits ectopic lipid accumulation, attenuates lipotoxicity, and improves insulin sensitivity (Minokoshi et al., 2002). This hormone has definite actions in insulin sensitivity and glucose homeostasis, although its anorectic effect contributing to insulin sensitivity is still controversial (Pelleymounter et al., 1995; Hedbacker et al., 2010).

The insulin resistance and hyperglycemia of leptin-deficient ob/ob mice can be reversed by exogenous leptin treatment (Schwartz et al., 1996; Murphy et al., 1997). But most patients with T2DM and obesity present hyperleptinemia because the leptin levels are proportional to the body mass (Moon et al., 2011). Leptin treatment is largely ineffective to improve insulin resistance and diabetic symptoms in these obese individuals (Mittendorfer et al., 2011). The poor biological function of endogenous leptin is also known as leptin resistance. Insulin stimulates the production and secretion of leptin, which in turn suppresses insulin secretion and enhance insulin sensitivity in peripheral tissues (Seufert et al., 1999; Kieffer and Habener, 2000; Alemzadeh and Tushaus., 2004; Covey et al., 2006). This leptin–insulin interaction is termed as “adipoinsular axis” (Kieffer and Habener, 2000). Dysfunction of adipoinsular axis could be a pivotal endocrine brake for insulin sensitivity and lipid oxidation.

Traditional Chinese medicine (TCM) and theory have specific merit on treating insulin resistance and GLMD (Xie and Du, 2011; Zhao et al., 2012; Guo, 2017; Gao et al., 2018). The TCM formula Astragalus mongholicus powder (APF) comprising *A. mongholicus* Bunge [Fabaceae], *P. montana* (Lour.) Merr. [Fabaceae], and *M. alba* L. [Moraceae] has been used to treat diabetes since ancient China. *A. mongholicus* and *P. montana* have potential hypoglycemic effects in diabetic animals (Hsu et al., 2003; Wang et al., 2009). In this study, the hypoglycemic effects and underlying mechanism of APF were investigated in a classic STZ + HFD induced T2DM mice, leptin deficient diabetic ob/ob mice and STZ induced T1DM mice (Kusakabe et al., 2009; Muzzin et al., 1996).

Materials and methods

Preparation of Astragalus mongholicus powder

Dried *A. mongholicus*, *P. montana* and *M. alba L.* were purchased from Guangzhou University of Chinese Medicine Pharmacy Co., Ltd. *A. mongholicus, P. montana*, and *Morus alba L.* were powdered and mixed in a ratio of 1:2:1. The mixture was extracted with 60% ethanol (1:8, wt/wt) for 2 h. Extraction of filtered residue was repeated for three times. The extracting solution was mixed, filtered, and concentrated to 0.12 g/ml. The concentrated solution was purified through PIPO-OO, HPD-500 macroporous resin and 732 ion exchange resin. The APF powder was obtained after freeze-drying. The ingredients of APF were determined by high-performance liquid chromatography (HPLC) and HPLC evaporation light scattering detection (HPLC-ELSD). Three representative compounds including Astragaloside IV, pueparin and calycosin-o-β-D-glucopyranoside were identified and determined as quality control. The specific content and

TABLE 1 Composition of APF.

Composition	Concentration
Astragaloside IV	5.71 mg/g
Pueparin	234.9 mg/g
Calycosin-o-β-D-glucopyranoside	0.903 mg/g

chromatographic condition were provided in previous Supplementary Data and Table 1.

Mice and nutrients

Normal chow, high-fat-diet (60% fat) and 8-week-old male C57BL/6J mice were purchased from Guangdong Medical Laboratory Animal Center (Foshan, China), and 8-week-old male B6.V-LepOb/LepOb (ob/ob) mice were obtained from Nanjing Biomedical Research Institution of Nanjing University (Nanjing, China). The mice were housed under pathogen-free conditions in a temperature-controlled room illuminated for 12 h every day and received humane care in accordance with the study guidelines established by the Guangzhou University of Chinese Medicine Laboratory Animal Holding Care. Following acclimation for 1 week, all C57BL/6J mice [except for 10 C57BL/6J mice as normal control (n = 10)] intraperitoneally received 120 mg/kg STZ once. After 3 weeks, the hyperglycemic mice were classified into four groups, namely, type 2 diabetic (n = 10), metformin (n = 10), 0.5 g/kg APF (n = 10), and 1.0 g/kg APF groups. The normal control group (NC) mice were fed with normal chow and treated with 5% acacia gum solution (p.o.). The T2DM, metformin, 0.5 g/kg APF, and 1.0 g/kg APF groups were fed with 60% HFD and treated with 5% acacia gum solution, 250 mg/kg metformin, 0.5 g/kg APF, and 1.0 g/kg APF (p.o.), respectively. After 12 weeks, all mice were sacrificed through cervical dislocation after anesthesia. Tissues were snap-frozen or fixed in formalin.

Type 1 diabetes (T1DM) was induced in mice by STZ. Except for 10 C57BL/6J mice as normal control (n = 10), all C57BL/6J mice intraperitoneally received five consecutive doses of 45 mg/kg STZ. After 2 weeks, the hyperglycemic mice were classified into T1DM (n = 6) and APF groups (n = 6) both fed with normal chow. Normal control (NC) and T1DM mice were treated with 5% acacia gum solution (p.o.). The APF group was treated with 1.0 g/kg APF (p.o.). After 6 weeks, all mice were sacrificed through cervical dislocation after anesthesia. Tissues were snap-frozen or fixed in formalin.

Ob/ob mice were designed into ob/ob (n = 8) and APF groups (n = 16) both fed with normal chow and individually treated with 5% acacia gum solution and 1.0 g/kg APF (p.o.), respectively. After 13 weeks, the APF mice were allocated to APF (n = 8) and APF + leptin groups (n = 8) that intraperitoneally received saline and 0.8 mg/kg recombinant rodent leptin, respectively, every 6: 00 p.m. After 3 weeks, all mice were sacrificed through cervical dislocation after anesthesia. Tissues were snap-frozen or fixed in formalin.

All animal experimental protocols were approved by the Institutional Animal Ethics Committee of Guangdong Pharmaceutical University (GDPULACSPF No. 2012062) in compliance with the revised Animals (Scientific Procedures) Act 1986 in the UK.

TABLE 2 Primer sequences for real-time PCR assays.

Gene	Primer
18s	F CGGCTACCACATCCAAGGA
	R CCAATTACAGGGCCTCGAAA
GAPDH	F TGTGTCCGTCGTGGATCTGA
	R TTGCTGTTGAAGTCGCAGGAG
FAS	F ACATGGACAAGAACCATTATGCTGA
	R CTGGTTTGCACTTGCACTTGGTA
ACC	F AGCGACATGAACACCGTACTGAA
	R TAGGGTCCCGGCCACATAAC
SCD1	F ATGTCTGACCTGAAAGCCGAGAA
	R GAGCACCAGAGTGTATCGCAAGAA
HSL	F TCCTGGAACTAAGTGGACGCAAG
	R CAGACACACTCCTGCGCATAGAC
PEPCK	F TCTTTGGTGGCCGTAGACCTG
	R GCCAGGTATTTGCCGAAGTTGTAG
G6Pc	F CAGCAACAGCTCCGTGCCTA
	R TCCCAACCACAAGATGACGTTC
PTP1B	F GAGCAGGAGGGTGTGAAGAG
	R CTAGAAGGTCGTGGGCAGAA
TCPTP	F CAAGGCTCAGGCTCATTGTG
	R CCGCCATAGTCAGTGAAGCA

Sequences: 5′ to 3′. Forward primers are designated by f and reverse primers by r.

Biochemical assays

Blood sample was collected from retinal vein plexus after the mice were fasted overnight or with feeding at 9:00 a.m. Mice were anesthetized by ether. Plasma was harvested after centrifugation. Plasma glucose (Glu), triglyceride (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) were determined using commercial kits from Rsbio (Shanghai, China). Non-esterified fatty acid (NEFA) was analyzed using NEFA assay kit from Wako (Osaka, Japan). Plasma insulin and leptin were examined using ELISA commercial kits from Cusabio (Wuhan, China). Hepatic TG and TC were extracted by isopropanol (1 mg tissue/20 μl isopropanol) allowed to stand at 4°C overnight after homogenization. Supernatants were harvested after centrifugation and determined using commercial kits.

Oral glucose tolerance test and insulin tolerance test

Glucose (2 g/kg) was intragastrically administered to mice that fasted overnight. Insulin (1 U/kg) was intraperitoneal

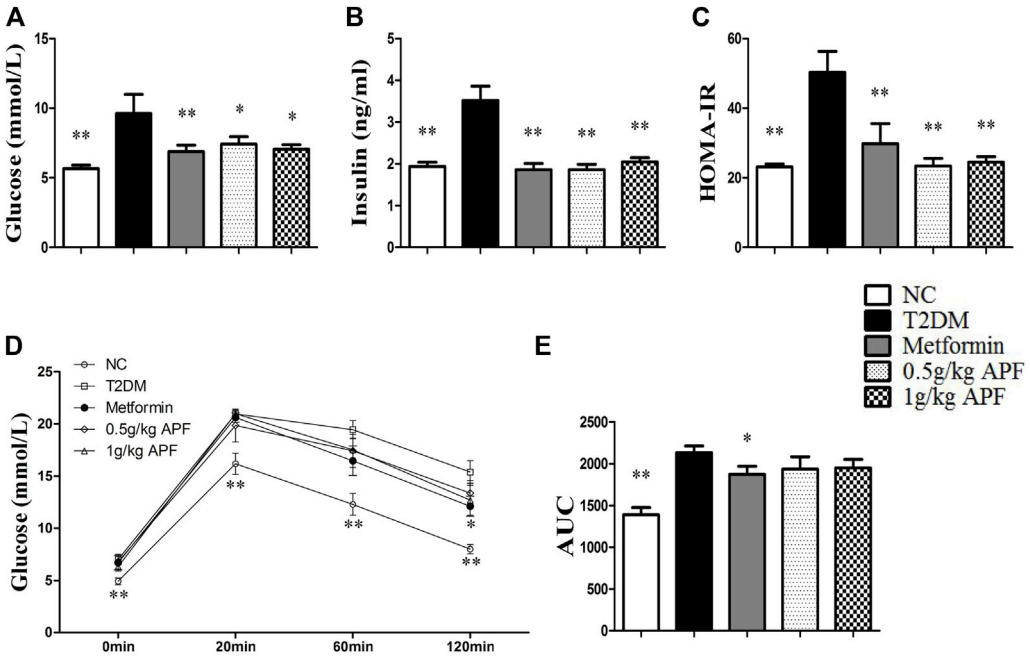

FIGURE 1
Effects of APF on insulin resistance in T2DM mice. Fasting plasma glucose **(A)**, insulin **(B)**, homeostatic model assessment of insulin resistance **(C)**, plasma glucose kinetics **(D)** and area under the curve of kinetics profiles **(E)** after 2 g/kg oral glucose administration of normal control (white bars), type 2 diabetes group (black bars), metformin group (gray bars), 0.5 g/kg APF group (spot bars) and 1 g/kg APF group (grid bars) after treatment with APF 10 weeks. Values are means ± SEMs, n = 8–10 per group. *$p < 0.05$, **$p < 0.01$ versus type 2 diabetes group.

injected to mice that fasted for 6 h. Insulin sensitivity was evaluated using the homeostatic model assessment of insulin resistance [HOMA-IR, fasting blood glucose (mmol/L) × fasting serum insulin [(mIU/L)/22.5].

Histology

Livers, pancreas, and adipose tissues were fixed in formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. For Oil Red O staining, hepatic tissues were embedded in optimal cutting temperature compound, sectioned, and stained with Oil Red O.

Real-time polymerase chain reaction

Total RNA was isolated by homogenizing tissues in Tiangen TRIzol reagent (Beijing, China), and single standard cDNA was synthesized by using Tiangen cDNA kit (Beijing, China). Quantitative real-time PCR was performed with Thermo Scientific PikoReal 96 Real-Time polymerase chain reaction (PCR) System (Waltham, MA, United States). Primer sequences are listed in Table 2.

Western blot

Total protein extracts were fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. The membranes were then blocked with 5% nonfat milk in Tris-buffered saline with Tween-20 for 2 h at room temperature and incubated with anti-PTP1B (Abcam, United States), anti-TCPTP, and anti-GAPDH (Cell Signaling Technology, United States) at 4°C overnight, rinsed three times with TBST, and incubated with respective secondary antibodies for 2 h at room temperature. Protein bands were visualized with Thermo Fisher Scientific SuperSignal West Femto Maximum Sensitivity Substrate (Rockford, United States) and captured using an Image Quant LAS4000 imaging system (Shanghai, China).

Data analysis

All results were expressed as means ± standard error of the mean. Data were analyzed by Kolmogorov-Smirnov and Mann-Whitney U test for normal distribution analysis. Data from more than two groups were analyzed by one-way ANOVA. Student's

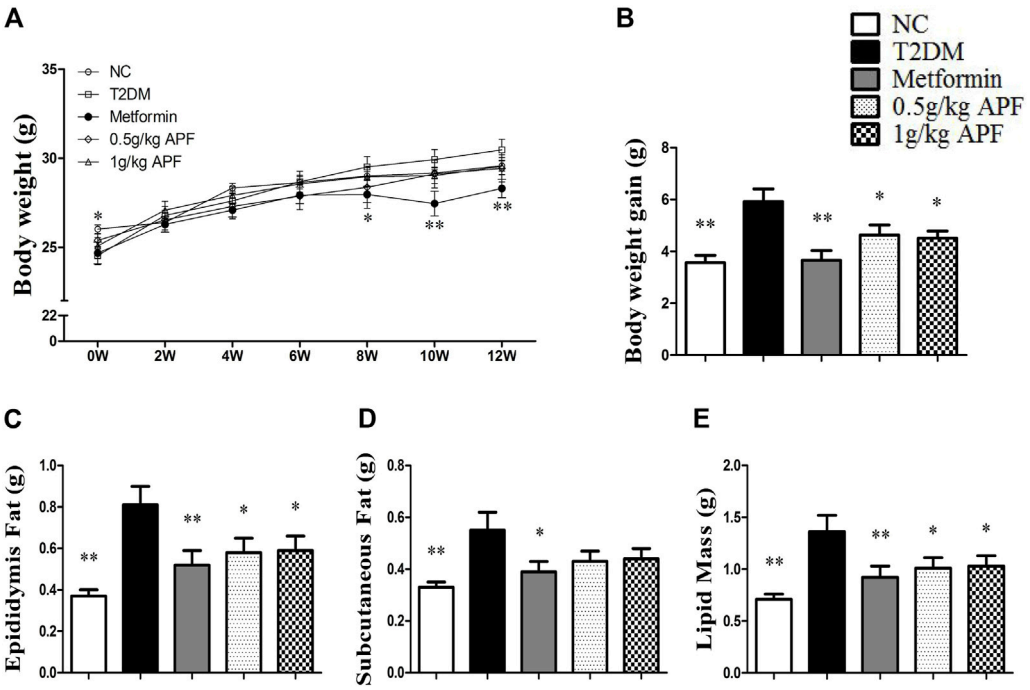

FIGURE 2
Effects of APF on obesity in T2DM mice. Body weight changes **(A)**, body weight gain **(B)**, epididymal fat mass **(C)**, subcutaneous fat mass **(D)** and total fat mass **(E)** of normal control (white bars), type 2 diabetes group (black bars), metformin group (gray bars), 0.5 g/kg APF group (spot bars) and 1 g/kg APF group (grid bars) after treatment with APF 12 weeks. Values are means ± SEMs, n = 8–10 per group. *$p < 0.05$, **$p < 0.01$ versus type 2 diabetes group.

t test was performed to identify differences between two groups. $p < 0.05$ was considered significant.

Results

Astragalus mongholicus powder improved insulin sensitivity in STZ + HFD induced type 2 diabetic mice

After feeding with HFD 10 weeks, the T2DM group exhibited hyperglycemia, hyperlipidemia, hyperinsulinemia, impaired glucose tolerance, and elevated HOMA-IR index (Figures 1A–E; Supplementary Figures S1A,B), indicating the strong development of T2DM. However, the levels of fasting glucose, insulin, and HOMA-IR were normalized by treating with 0.5 g/kg APF, 1 g/kg APF, and metformin, respectively (Figures 1A–C). Meanwhile, STZ administration elicited the injury of morphology of pancreas in T2DM group (Supplementary Figure S1C), which was attenuated by 0.5 and 1 g/kg APF treatment (Supplemental Figure S1C). These data suggested that APF reshapes the STZ-injured pancreas and reduces the compensatory secretion of insulin. Therefore, the HFD-induced insulin resistance and hyperinsulinemia are alleviated by APF treatment.

Astragalus mongholicus powder ameliorated obesity and non-alcoholic fatty liver disease development

STZ administration significantly reduced the body weight of mice (Figure 2A). After feeding with HFD, the T2DM group had higher body weight gain than the NC group (Figure 2B). Fat mass weight was also monitored, and the results showed that epididymal fat, subcutaneous fat, and total lipid mass were significantly enhanced after HFD feeding (Figures 2C–E). Plasma leptin levels proportional to the fat mass also increased with HFD feeding (Figure 3A). In addition to body mass, liver weight, and hepatic TC, the TG content was significantly higher than that in the NC group (Figures 3B–D). Histological staining also displayed substantial amounts of hepatic macrovesicular steatosis in the T2DM group (Supplementary Figure S2). These results showed that the T2DM group turned obese and developed NAFLD accompanied with insulin resistance. With regard to the

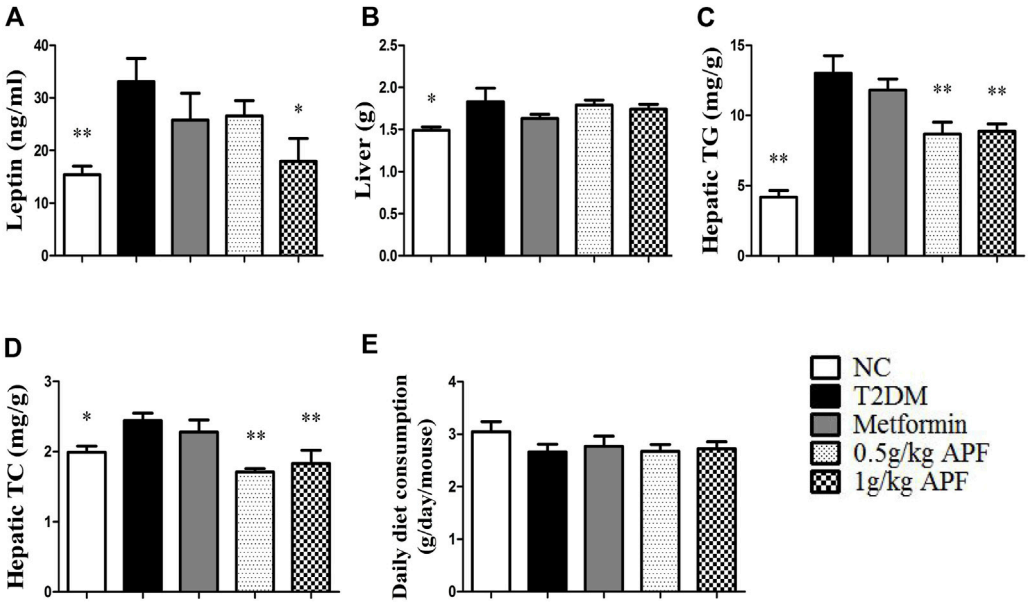

FIGURE 3
Effects of APF on hepatic steatosis in T2DM mice. Plasma leptin **(A)**, liver weight **(B)**, hepatic TG **(C)**, hepatic TC **(D)** and daily diet consumption **(E)** of normal control (white bars), type 2 diabetes group (black bars), metformin group (gray bars), 0.5 g/kg APF group (spot bars) and 1 g/kg APF group (grid bars) after treatment with APF 12 weeks. Values are means ± SEMs, n = 8–10 per group. *p < 0.05, **p < 0.01 versus type 2 diabetes group.

biological function of leptin in anti-ectopic lipid accumulation, the T2DM group showed insulin and leptin resistance.

APF treatment significantly inhibited the increase in body weight gain (Figure 2B). Epididymal fat mass and total lipid mass also deceased with APF intervention (Figures 2C,E). Moreover, 1 g/kg APF treatment diminished the diameter of the adipocyte and might have contributed to improving the endocrine function of adipose tissues (Supplementary Figure S3). Thus, the hyperleptinemia of T2DM mice was normalized by 1 g/kg APF treatment (Figure 3A). Finally, APF administration reduced the hepatic TC and TG contents and remarkably diminished the hepatic macrovesicular steatosis without effects in daily diet consumption (Figures 3C–E; Supplementary Figure S2). The results indicated that APF attenuates obesity, hyperleptinemia and NAFLD. Based on the effects of APF in insulin sensitivity. We considered APF reversed the insulin-leptin resistance though regulating the adipoinsular axis.

Astragalus mongholicus powder failed to ameliorate insulin resistance, obesity and non-alcoholic fatty liver disease in leptin-deficient ob/ob mice

Leptin-deficient ob/ob mice were selected to validate whether leptin is essential for APF improve insulin sensitivity by regulating adipoinsular axis (Supplementary Figure S4C). In the status of absent of leptin, APF could not normalize the hyperglycemia and hyperinsulinemia of ob/ob mice (Figures 4A,B). APF even increased the levels of plasma insulin and HOMA-IR index (Figures 4B,C). We considered that APF may still influence the endocrine function of pancreas, but the effects are unpredictable without leptin. Monitoring revealed that insulin resistance was robust in ob/ob mice with APF treatment (Figures 4D,E).

Based on the physiological effects of adipoinsular axis, the pathological features of obesity and NAFLD in ob/ob mice with APF administration were examined. Different from that in STZ + HFD induced T2DM mice, the weight-loss effects of APF were not observed in ob/ob mice (Figures 5B,D). Meanwhile, the hepatic macrovesicular steatosis of ob/ob mice was not reversible by APF intervention (Supplementary Figure S5). Although APF reduced the hepatic weight, APF failed to decrease the levels of hepatic TG and TC (Figures 6A–C). On the basis of body weight, the fat mass of ob/ob mice was examined. APF could not reduce the subcutaneous fat mass and even enhanced the epididymal fat mass in ob/ob mice (Figures 6D,E). As implied by these data, the destruction of adipoinsular axis in the leptin side would abolish the effects of APF. APF lost its effects of anti-insulin resistance, obesity, and NAFLD without leptin regulation but still had minimal influence on pancreas and adipose tissues.

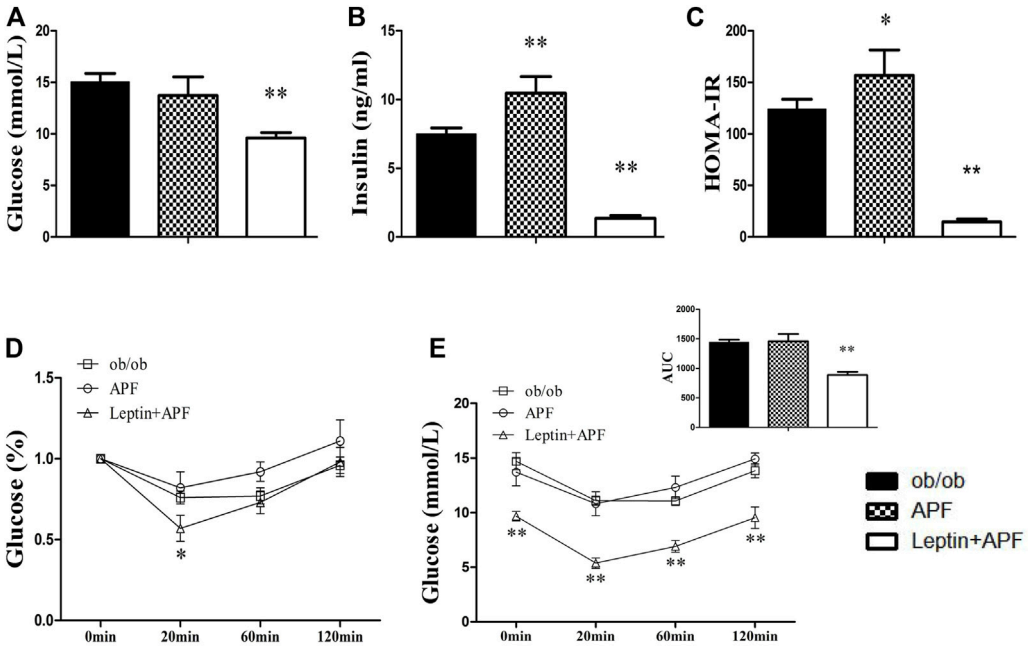

FIGURE 4
Effects of APF and supplementary Leptin on insulin resistance in ob/ob mice. Fasting plasma glucose **(A)**, insulin **(B)**, homeostatic model assessment of insulin resistance **(C)**, intraperitoneal insulin tolerance test **(D)** and area under the curve of kinetics profiles **(E)** after 1 U/kg intraperitoneal injected insulin administration of ob/ob group (black bars), APF group (grid bars) and APF + leptin group (white bars) after treatment with APF 15 weeks or APF + lepin 2 weeks. Values are means ± SEMs, n = 6-8 per group. *$p < 0.05$, **$p < 0.01$ versus ob/ob group.

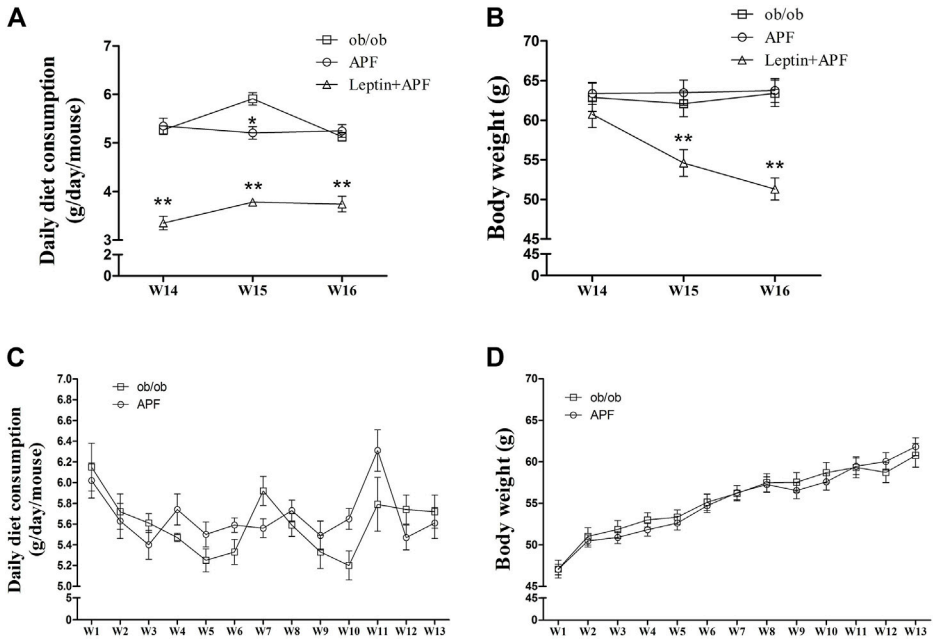

FIGURE 5
Effects of APF and supplementary Leptin on ob/ob mice. Daily diet consumption **(A,C)**, body weight changes **(B,D)** of ob/ob group (white squares), APF group (white cycles) and APF + leptin group (white trigons) after treatment with APF 16 weeks or APF + letpin 3 weeks. Values are means ± SEMs, n = 7–8 per group. *$p < 0.05$, **$p < 0.01$ versus ob/ob group.

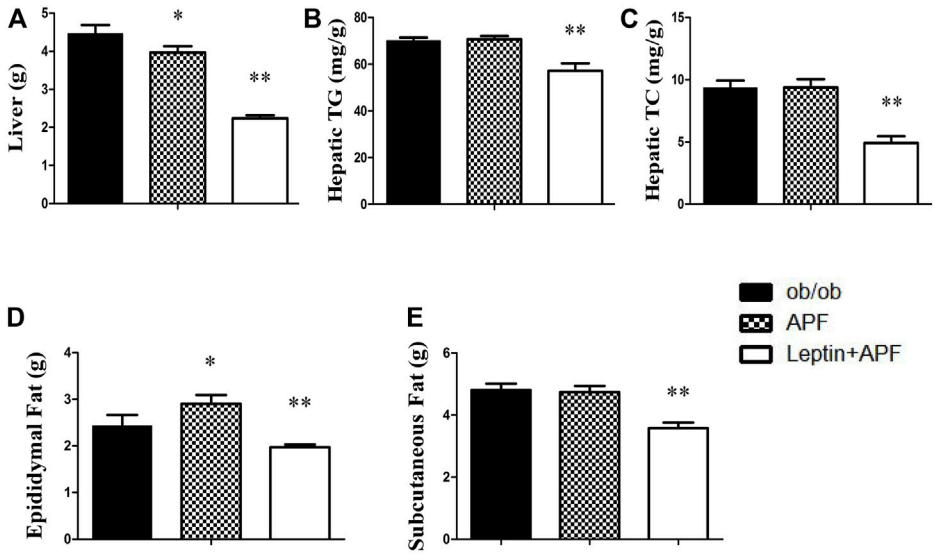

FIGURE 6
Effects of APF and supplementary Leptin on abdominal obesity in ob/ob mice. Liver weight **(A)**, hepatic TG **(B)**, hepatic TC **(C)**, epididymal fat mass **(D)** and subcutaneous fat mass **(E)** of ob/ob group (black bars), APF group (grid bars) and APF + leptin group (white bars) after treatment with APF 16 weeks or APF + lepin 3 weeks. Values are means ± SEMs, n = 7–8 per group. *$p < 0.05$, **$p < 0.01$ versus ob/ob group.

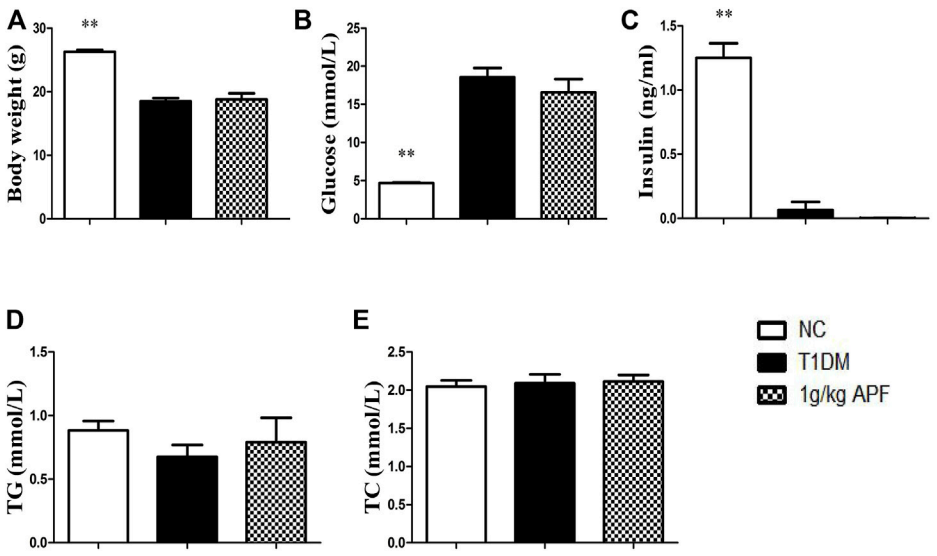

FIGURE 7
Effects of APF in T1DM mice. Body weigt **(A)**, plasma glucose **(B)**, insulin **(C)**, TG **(D)** and TC **(E)** of normal control (white bars), type 1 diabetes group (black bars) and APF group (grid bars) after treatment with APF 6 weeks. Values are means ± SEMs, n = 6–10 per group. *$p < 0.05$, **$p < 0.01$ versus T1DM group.

Leptin supplementary treatment reversed the metabolic syndrome of ob/ob mice

Ob/ob mice were administered with recombinant rodent leptin (*i.p.*) after APF treatment for 13 weeks (Supplementary Figure S4C). Hyperglycemia, hyperinsulinemia, HOMA-IR, and insulin tolerance were normalized after leptin treatment (Figures 4A–E). Leptin supplementary treatment significantly inhibited their daily diet consumption and body weight (Figures 5A–D). For NAFLD and fat mass, leptin administration attenuated hepatic steatosis and reduced hepatic TC and TG levels (Supplementary Figure S5; Figures 6A–C). Therefore, fat mass,

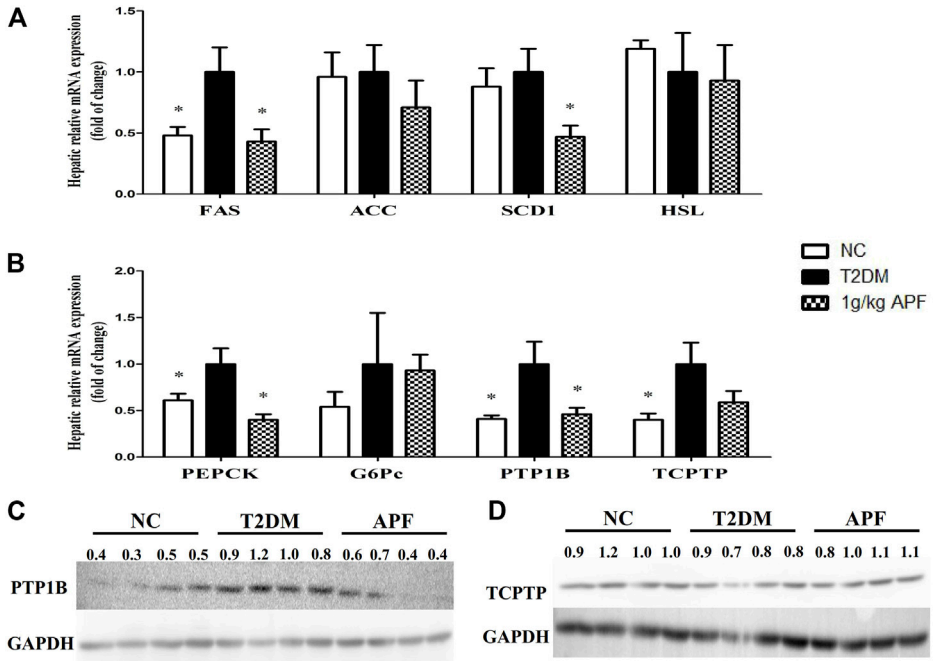

FIGURE 8
Effects of APF on liver in T2DM mice. Hepatic FAS, ACC, SCD1, HSL **(A)**, PEPCK, G6Pc, PTP1B, TCPTP **(B)** relative mRNA expression and protein expression of P1P1B **(C)** and TCPTP **(D)** of normal control (white bars), type 2 diabetes group (black bars) and 1 g/kg APF group (grid bars) after treatment with APF 12 weeks. Values are means ± SEMs, n = 4–5 per group. *$p < 0.05$, **$p < 0.01$ versus type 2 diabetes group.

including epididymal and subcutaneous fat, was decreased by leptin supplement (Figures 6D,E). Leptin also diminished the diameter of ob/ob mouse adipocytes, which was the same effect as APF in STZ + HFD-induced T2DM mice (Supplementary Figures S3, S6). All these data were confirmed by previous studies of leptin and validated the effects of adipoinsular axis from another point of view.

Astragalus mongholicus powder lost its hypoglycemic effects in insulin-deficient type 1 diabetes mice

We hypothesized that APF ameliorate diabetes by regulating adipoinsular axis. Except for the leptin, insulin also must be essential for the hypoglycemic effects of APF. As expected, APF could not ameliorate diabetes and metabolic syndromes without leptin presence in ob/ob mice. Then, a classic T1DM mice model was established to investigate the anti-diabetic effects of APF in absence of inuslin. After STZ administration, the mice showed body loss, hyperglycemia, and insulin deficiency (Figures 7A–C). However, the hypoglycemic effects of APF in T2DM mice was not observed in T1DM mice (Figure 7B). In addition, the levels of plasma insulin was hardly detected with APF treatment (Figure 7C). Meanwhile, APF also had no effects in TC and TG in T1DM mice (Figure 7D, E). Therefore, both hormones (leptin and insulin) are indispensable in regulating the adipoinsular axis by APF. Abolishment of each hormone would disrupt the effects of APF for anti-diabetes, obesity, and NAFLD.

Potential mechanism of astragalus mongholicus powder in adipoinsular axis

APF ameliorated insulin resistance and NAFLD by improving the adipoinsular axis. The underlying mechanism of these effects was then investigated. Hepatic lipid metabolic gene expression was determined based on the decreased hepatic lipid accumulation by APF. APF treatment significantly suppressed the expression of lipogenic gene, fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD1) (Figure 8A). Both genes contribute to hepatic *de novo* lipogenesis (Wang et al., 2015). On the contrary, APF alleviated the hepatic expression of gluconeogenesis gene phosphoenolpyruvate carboxykinase (PEPCK), a target gene for leptin to improve insulin sensitivity (Burcelin et al., 1999). These data suggested that APF improves the hepatic biological effects of insulin–leptin and suppresses the expression of hepatic lipogenic and gluconeogenic genes. Protein tyrosine phosphatase

1B (PTP1B) and T-cell protein tyrosine phosphatase (TCPTP) are negative regulators of insulin and leptin reaction (Thareja et al., 2012; Zhang et al., 2015). Thus, their expression levels were determined by real-time PCR and Western blot. The genetic and protein expression levels of PTP1B in STZ + HFD-induced T2DM mice were significantly enhanced (Figures 8B,C), and this finding was consistent with the phenotype of dysfunction of adipoinsular axis in mice. However, APF treatment significantly reversed the overexpression of PTP1B but not TCPTP (Figures 8B–D), implying that APF improves the adipoinsular axis balance by suppressing PTP1B and consequently contributes to the attenuation of obesity, NAFLD, and T2DM.

Discussion

Overnutrition triggers lipid excess in adipose tissues and increases lipolysis and circulating lipids. The excess circulating lipid causes ectopic lipid accumulation in peripheral tissues (liver, skeletal muscles, and β cells); induces lipotoxicity, chronic inflammation, and endoplasmic reticulum stress; and contribute to obesity, NAFLD, and insulin resistance development, which means modulation the interplay between liver and adipose tissue are critical in GLMD development (Guilherme et al., 2008; Samuel and Shulman, 2012; Ye et al., 2017). Leptin plays a pivotal role in energy homeostasis and controls body weight as an anorexigenic hormone in hypothalamic area (Cowley et al., 2001; Balthasar et al., 2004). This hormone also prevents ectopic lipid accumulation and lipotoxicity by promoting lipid oxidation (Minokoshi et al., 2002; Zeng et al., 2015) and even directly inhibits the secretion of insulin and improves insulin sensitivity (Liu et al., 2003; Laubner et al., 2005). In turn, the lipogenic hormone insulin promotes adiposity and increases leptin production that is proportional to fat mass (Kim et al., 1998; Aas et al., 2009). The balance of feedback (adipoinsular axis) contributes to global energy homeostasis, and its dysfunction is a primary pathogenesis linked to obesity, NAFLD, and T2DM (Vickers et al., 2001; Simmons and Breier, 2002).

In this research, well-recognized HFD + STZ induced obese T2DM mice were established to investigated the effects of APF. After STZ broke the islet form, HFD induced hyperglycemia, hyperinsulinemia, and insulin resistance. Meanwhile, HFD promoted obesity, hepatic steatosis, and hyperleptinemia in mice. These data indicated that the mice lost adipoinsular axis balance and developed insulin–leptin resistance, which strongly contributes to T2DM and NAFLD deterioration. However, APF treatment reversed hyperleptinemia and suppressed NAFLD and obesity development. Histological staining displayed that APF could diminish the diameter of adipocytes. Hypertrophic adipocytes prompt the endocrine dysfunction of adipose tissues (Gustafson et al., 2015). Thus, APF may have potential effects on the endocrine function of adipose tissues and leptin metabolism. APF also normalizes the levels of plasma glucose, insulin, and HOMA-IR index. Moreover, the injured islets were restored with APF treatment. Hence, we considered that APF could improve the endocrine function of islet and attenuate insulin compensatory secretion and insulin resistance. A previous study reported that leptin administration enhances islet transplant performance in diabetic mice (Denroche et al., 2013). Thus, we suggested that both effects of APF on reversing hyperleptinemia and hyperinsulinemia were based on balancing the adipoinsular axis. Insulin and leptin resistance was consequently ameliorated, and obesity, T2DM, and NAFLD were repressed in mice.

Several compounds, such as peroxisome proliferators-activated receptor alpha (PPARα) agonist, can ameliorate insulin resistance and obesity and normalize hyperleptinemia and hyperinsulinemia independent of adipoinsular axis (Ye et al., 2001; Jeong and Yoon, 2009). The leptin-deficient ob/ob mice and insulin-deficient T1DM mice were established to confirm that insulin and leptin are essential for APF anti-metabolic syndromes by regulating adipoinsular axis. In ob/ob mice, obesity and hepatic steatosis were refractory to APF treatment without leptin. In addition to obesity, insulin resistance was still robust with APF treatment. APF treatment enhanced the levels of plasma insulin and epididymal fat mass in ob/ob mice. These effects were contrary to APF influences on T2DM mice. We considered that APF still had potential effects on tissues of adipoinsular axis (pancreas and adipose tissues), but these actions became unpredictable when the adipoinsular axis was abolished. The effects of APF on anti-obesity, NAFLD and T2DM were not observed in ob/ob mice. These data indicated that APF improves insulin resistance and metabolic syndrome in a leptin-dependent manner. APF had no significant effects on the daily diet consumption of ob/ob mice and T2DM mice. We considered that the effects of APF on adipoinsular axis were independent of leptin's action on the central nervous system. The anorectic function of leptin in the central nervous system is independent of insulin sensitivity and NAFLD (Hedbacker et al., 2010). Meanwhile, the hypoglycemic effects of APF was lost in STZ induced T1DM mice that were deficient in insulin, which means the hypoglycemic effects of APF rely on insulin existing. On the basis of these data, APF ameliorates obesity, NAFLD, and diabetes by regulating adipoinsular axis. Destruction of each side of adipoinsular axis would diminish the effects of APF.

Although the balance of adipoinsular axis is crucial for energy homeostasis, leptin and insulin sensitivity may be suppressed by some endogenetic signailing pathways (Taniguchi et al., 2006; Balland and Cowley, 2015). PTP1B and TCPTP contribute to insulin resistance (Galic et al., 2003; Delibegovic et al., 2009). However, deletion of PTP1B could not improve insulin resistance in leptin receptor mutant db/db mice (Ali et al., 2009; Tsou et al., 2014). These data revealed that the

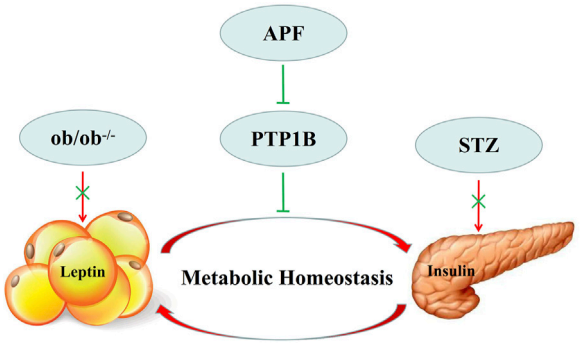

FIGURE 9
Model describing of APF in adipoinsular axis. APF suppress the inhibition effects of PTP1B in adipoinsular axis, which contribute to the balance of adipoinsular axis in metabolic homeostasis. This effect can be abolished by blocking the insulin-leptin feedback.

suppression of PTP1B improves insulin sensitivity in a leptin-dependent manner. Hence, PTP1B could be a candidate therapeutic target for balancing adipoinsular axis. In this research, APF significantly decreased the hepatic mRNA and protein expression of PTP1B in T2DM mice. Therefore, the expression levels of gluconeogenesis and lipogenesis (PEPCK, FAS, and SCD1) hepatic genes were inhibited. These results were supported by leptin agonist attenuating insulin resistance and reducing the expression of gluconeogenic and lipogenic genes (Burcelin et al., 1999; Tsuchiya et al., 2012). Therefore, APF diminishes the suppressive effects of PTP1B on adipoinsular axis and consequently improves leptin and insulin sensitivity. The gluconeogenic and lipogenic genes were then repressed. The role of adipoinsular axis in energy homeostasis has been reported, and the mechanism of leptin in insulin sensitivity has been revealed (Buettner et al., 2006; Berglund et al., 2012; Könner and Brüning, 2012; Luan et al., 2014). However, the feedback mechanism of adipoinsular axis required further study. This research illustrated that APF attenuates metabolic diseases by balancing the adipoinsular axis due to its suppressive effects on PTP1B expression (Figure 9). However, the specific mechanism of APF in adipoinsular axis is still unclear. In addition to the adipoinsular axis, APF decreased the levels of plasma low density lipoprotein (LDL) in ob/ob mice (Supplementary Figure S4B). This effect may contribute to other diseases. Further works are required to investigate the mechanism of APF in the adipoinsular axis.

Conclusion

APF regulated the balance of adipoinsular axis in STZ + HFD induced T2DM mice due to its suppressive effects on PTP1B expression. Hyperleptinemia/leptin resistance and hyperinsulinemia/insulin resistance were ameliorated. As a result, the hepatic genes of gluconeogenesis and lipogenesis were inhibited, and hyperglycemia, hepatic steatosis, and fat mass excess were attenuated. Finally, GLMD (obesity, NAFLD, and T2DM) development were repressed by APF treatment.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Ethics Committee of Guangdong Pharmaceutical University (GDPULACSPF No. 2012062). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

XR and SX designed the research protocol. SX, BY, JL, YD, and YY implemented the research protocol. SX analyzed data. SX, DW, LW, and XR wrote the manuscript. All authors read and approved the final study.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.973927/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Fasting plasma TG , **(A)** TC **(B)** and representative hematoxylin and eosin staining of pancreas of normal control (white bars), type 2 diabetes group (black bars), metformin group (gray bars), 0.5 g/kg APF group (spot bars) and 1 g/kg APF group (grid bars) after treatment with APF 12 weeks. Values are means ± SEMs, n = 8–10 per group. *$p < 0.05$, **$p < 0.01$ versus type 2 diabetes group.

SUPPLEMENTARY FIGURE S2
Representative hematoxylin and eosin staining of livers from each group after treatment with APF 12 weeks.

SUPPLEMENTARY FIGURE S3
Representative hematoxylin and eosin staining of epididymal fat from each group after treatment with APF 12 weeks.

SUPPLEMENTARY FIGURE S4
Fasting plasma TG, NEFA **(A)** TC, LDL-C **(B)** and research protocol **(C)** of ob/ob group (black bars) and APF group (grid bars) after treatment with APF 13 weeks. Values are means ± SEMs, n = 8–16 per group. *$p < 0.05$, **$p < 0.01$ versus ob/ob group.

SUPPLEMENTARY FIGURE S5
Representative hematoxylin, eosin staining or Oil red O staining of pancreas and livers from each group after treatment with APF 16 weeks or APF+lepin 3 weeks.

SUPPLEMENTARY FIGURE S6
Representative hematoxylin and eosin staining of epididymal fat and subcutanoues fat after treatment with APF 16 weeks or APF+lepin 3 weeks.

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Dubai Health Authority, United Arab Emirates

*CORRESPONDENCE
Freeborn Rwere,
frwere@stanford.edu

†These authors have contributed equally to this work

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Natural products for the treatment and management of diabetes mellitus in Zimbabwe-a review

Remigio Usai[1†], Stephen Majoni[2†] and Freeborn Rwere[3,4*†]

[1]Department of Chemistry, Marquette University, Milwaukee, WI, United States, [2]Department of Chemical and Forensic Sciences, Botswana International University of Science and Technology, Palapye, Botswana, [3]Department Anesthesiology, Perioperative, and Pain Medicine, School of Medicine, Stanford University, Stanford, CA, United States, [4]Department of Chemistry, School of Natural Sciences and Mathematics, Chinhoyi University of Technology, Chinhoyi, Zimbabwe

Use of medicinal plants and herbs in the treatment and management of diseases, including diabetes mellitus and its complications remains an integral part of African tradition. In Zimbabwe, nearly one million people are living with diabetes mellitus. The prevalence of diabetes mellitus in Zimbabwe is increasing every year due to lifestyle changes, and has accelerated the use of traditional medicines for its treatment and management in urban areas. In addition, the high cost of modern medicine has led many people in rural parts of Zimbabwe to rely on herbal plant medicine for the treatment of diabetes mellitus and its complications. This review highlights a number of studies carried out to evaluate the antidiabetic properties of indigenous plants found in Zimbabwe with the goal of treating diabetes mellitus. Further, we discuss the mechanism of action of various plant extracts in the treatment and management of diabetes mellitus. Together, this review article can open pathways leading to discovery of new plant derived medicines and regularization of use of crude plant remedies to treat diabetes mellitus by the Zimbabwean government and others across Africa.

KEYWORDS

phytochemicals, diabetes mellitus, hyperglycemia, antidiabetic, streptozotocin

Introduction

Diabetes mellitus or diabetes is a chronic disease in which blood glucose, also referred to as blood sugar, becomes too high (Saeedi et al., 2019; Mohammed and Tajuddeen, 2022; Sun et al., 2022). Initially considered a disease of the Western world, diabetes mellitus is now a global pandemic that affects approximately 536.6 million people worldwide, and is predicted to rise to 643 million people by 2030 and 783.2 million people by 2045 (Saeedi et al., 2019; Lin et al., 2020; Sun et al., 2022). In 2021 the International Diabetes Federation (IDF) estimated that Africa had a diabetes mellitus prevalence of 23.6 million people (4.5%) and projected an increase of up to 5.2% (54.9 million people) in 2045 (Sun et al., 2022). Diabetes mellitus is characterized by hyperglycemia resulting from defects in

insulin secretion, insulin action, or both (Tran et al., 2020; Mohammed and Tajuddeen, 2022). Hyperglycemia caused by a deficiency in insulin production by the β-cells of the pancreas is known as Type 1 diabetes mellitus (T1DM) and due to insufficiency of insulin production in the face of insulin resistance or β-cells dysfunction is Type 2 diabetes mellitus (T2DM) (Care, 2013; Tran et al., 2020). High blood glucose can lead to pathophysiologies including, heart disease, stroke, kidney disease, eye problems, dental disease, nerve damage and feet problems (Policardo et al., 2015; Bragg et al., 2017; Yang et al., 2019). Insulin is the most common type of medication prescribed in type 1 diabetes mellitus treatment and can also be used in some type 2 diabetes mellitus cases. Nearly 65 drugs are clinically approved to lower blood sugar in order to limit the occurrence of Type 2 diabetes-related pathophysiologies. However, anti-diabetic drugs have also shown a vast array of side effects including diarrhea, nausea, vomiting, heartburn and lactic acidosis (Ashraf et al., 2022).

Approximately four billion people in developing countries depend on herbal traditional medicine for the treatment of metabolic diseases such as diabetes mellitus because of the presence of a wide range of bioactive phytochemical compounds in plants (Ekor, 2014; Choudhury et al., 2018). In addition, plant-derived drugs such as metformin from *Galega officinalis* plant are commercially marketed for diabetes mellitus treatment (Bailey, 2017). Plant-based traditional medicines are considered to be cheap and readily available to the majority of the rural population in Africa (Mugumbate et al., 2018). In Zimbabwe, it was estimated that ~850,000 people (about 5.7% of the population) are living with diabetes mellitus (Mutowo et al., 2015) and the average cost of conventional treatment of diabetes mellitus was a little over $1,300 United States Dollars (USD) (Mutowo et al., 2016). A study at a major hospital in Harare, the capital city of Zimbabwe showed that only 41.8% (76 of 182 diabetic patients) had good glycemic control using conventional medication (Chirombe et al., 2018). This has resulted in the majority of patients using medicinal plant-based treatment in combination with their conventional treatment. In addition, for over two decades, Zimbabwe has experienced an economic downturn that has led many people to rely on plant-derived medicines for treatment of many ailments, including diabetes mellitus due to high cost of modern medicine, and lack of foreign currency to procure drugs (Mutowo et al., 2016). Hence, much attention is needed to understand medicinal plants and their potential bioactive phytochemicals. Furthermore, there is an increased concern for safety and drug resistance from continued use of modern drugs. Despite their widespread use, natural plant products have some drawbacks including the presence of potential carcinogenic agents in some of these plants and complexity of the intrinsic metabolites making them unsuitable for therapeutic applications (Fennell et al., 2004). Also, traditional healers and herbalists do not possess adequate knowledge to understand the active components of the plant extracts and their mechanism of action. As such, it is important to understand and document the role of plant-derived bioactive phytochemicals in regulating blood glucose levels and diabetes mellitus treatment in Zimbabwe.

For many years, traditional medicines from plant extracts have proven to be clinically effective in the treatment of chronic ailments (Cock et al., 2019; Cock and Van Vuuren, 2020; Mohammed and Tajuddeen, 2022). In several cultures across Africa, there is widespread traditional use of decoctions prepared from medicinal plants in the treatment of diabetes mellitus (Mohammed and Tajuddeen, 2022). Use of decoctions in the treatment of complex diseases such as diabetes mellitus is important because plants contain many bioactive phytochemical compounds with various beneficial biological effects, thus potentially creating an effective and affordable multi-targeted treatment strategy. Diabetes mellitus has been linked to oxidative stress which arises from the excessive production of free radicals in the mitochondrial electron transport chain (Giacco and Brownlee, 2010; Asmat et al., 2016). Phytochemicals, including polyphenols and flavonoids have antioxidant properties and can scavenge free radicals and reduce oxidative stress, leading to treatment of diabetes mellitus (Johansen et al., 2005; Lv et al., 2021). They exert anti-hyperglycemic effects by binding to glucose transporters and competitively inhibiting the digestive enzymes (α-amylase and α-glucosidase). Other secondary plant metabolites such as terpenes, alkaloids, and saponins may enhance insulin secretion and regulate glucose uptake and glucose utilization. Bioactive phytochemicals can also exert antidiabetic effects by improving the performance of pancreatic tissue, often done by increasing insulin secretions or reducing the intestinal absorption of glucose by inhibiting key enzymes involved in glucose production (Kooti et al., 2016). Therefore, it is important to document plants with bioactive phytochemicals with antioxidant properties and are capable of inhibiting key enzymes (including α-amylase and α-glucosidase) to treat diabetes mellitus in traditional systems.

In this review, we discuss important medicinal plants found in Zimbabwe with the goal of treating type 2 diabetes mellitus and its management in rural communities. In addition, we will discuss how different plant extracts regulates blood glucose and their mechanism of action. Selection of plants for this study was based on indigenous knowledge related to plants used in Zimbabwe for the treatment of diabetes mellitus and its complications. However, there is limited research in Zimbabwean literature to justify the pharmacological use of some of these indigenous plants. Here, we summarize research findings for plants indigenous to Zimbabwe but investigated in Zimbabwe or other parts of the world. The summarized research was obtained from exhaustive search of plants on international databases, including Wiley library, Google Scholar, PubMed, SciFinder, Science Direct, Scopus and Springer Link using

TABLE 1 Medicinal plants in Zimbabwe with antidiabetic properties and their pharmacological outcomes.

Botanical name	Family	Image	Medicinal part/s used	Type of extract	Animal model	Pharmacological outcome/s
Cassia abbreviata Oliv.	Caesalpiniaceae		Stem bark	Aqueous, ethanol, acetone	Diabetic rats	1. Doses of *Cassia abbreviate* Oliv. normalized glucose level, and helped to maintain normal body weights in T2DM (Bati et al., 2017) 2. The aqueous extracts enhanced glucose uptake and induced a two-fold increase in glucose transporter 4 (GLUT3) translocation in C2C12 mouse skeletal muscle cells (Kamga-Simo et al., 2021).
Artemisia afra Jacq. ex Willd	Asteraceae		Leaf, Herbal tea	Aqueous, methanolic	Rats, Diabetic mice	1.Doses of *Artemisia afra* Jacq. ex Willd. extracts increased body weight, and decreased blood glucose level (Sunmonu and Afolayan, 2013). 2. Blood glucose level in alloxan induced diabetic mice was decreased by 24.0% and 56.9%, in groups that received aqueous extracts. Methanolic extracts lowered glucose level by 49.8% (Issa and Bule, 2015). 3. The blood glucose level was significantly reduced and insulin levels increased in diabetic rats fed with 100 mg/kg body weight aqueous extracts (Afolayan and Sunmonu, 2011). 4. Aqueous extracts increased the levels of glutathione reductase, glutathione peroxidase, superoxide dismutase and glutathione in the liver and kidney of diabetic rats to normal levels and reduced the levels of lipid-peroxidation products (Afolayan and Sunmonu, 2013).
Moringa oleifera Lam.	Moringaceae		Stem bark, dried fruit powder, leaf, pods, seed powder	Ethanolic, methanol, aqueous	Alloxan Albino rats, STZ-induced diabetic rats, humans	1.The blood and urine glucose levels were significantly reduced by a single dose ethanolic extract (Kar et al., 2003) 2. The pancreatic islets were rejuvenated after treatment of streptozotocin induced diabetic rats with methanol extracts of *Moringa oleifera* Lam. pods (Gupta et al., 2012; Al-Malki and El Rabey, 2015). 3. Aqueous extracts of *Moringa oleifera* Lam. inhibited α-amylase and α-glucosidase with IC_{50} values of 52.5 and 33.4 mg/ml (Khan et al., 2017). 4. *Moringa oleifera* Lam. powdered extracts and formulated cookies significantly reduced postprandial glucose in diabetic patients (Ahmad et al., 2018; Sissoko et al., 2020).

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TABLE 1 (*Continued*) Medicinal plants in Zimbabwe with antidiabetic properties and their pharmacological outcomes.

Botanical name	Family	Image	Medicinal part/s used	Type of extract	Animal model	Pharmacological outcome/s
Aloe vera (L.) Burm.f	Asphodelaceae		Gel, leaf	Methanol	STZ-induced diabetic rats, obesogenic WNIN/GR-Ob rats, Wistar rats, humans	1. A significant decrease in blood glucose level was observed with a dosage of 500 mg/kg body weight *Aloe vera* leaf pulp extract (Okyar et al., 2001). 2. Blood glucose and insulin levels were restored in diabetic rats. The pancreatic islets of diabetic rats were improved (Najafian et al., 2019). 3. The carbohydrate fraction of *Aloe vera* extract rejuvenated the pancreatic β-cells and improved insulin production. In addition, the fraction lowered fasting plasma glucose, glucagon and glucose-6-phosphatase levels in diabetic rats (Govindarajan et al., 2021). 4. The methanolic extract significantly decreased the formation of advanced glycation end products (AGEs) and reduced the activities of α-amylase and α-glucosidase. The extract also increased the content of thiol groups (Muñiz-Ramirez et al., 2020). 5. *Aloe vera* (L.) Burm.f extracts significantly reduced fasting blood glucose in diabetic and pre-diabetic patients (Devaraj et al., 2013; Alinejad-Mofrad et al., 2015).
Psidium guajava L.	Myrtaceae		leaf	Aqueous, ethanol	Rats	1. Significant decrease in blood sugar levels was observed in diabetic rats treated with extract compared to control group (Shen et al., 2008; Musdja et al., 2017). Long term administration resulted in increased plasma insulin level and glucose utilization in diabetic rats (Shen et al., 2008). 2 Aqueous extracts significantly lowered fasting plasma glucose levels and improved glucose tolerance and insulin sensitivity of diabetic mice. The extracts also altered the composition of the gut microbiota and increased the enrichment of probiotics (Chu et al., 2022). 3.The fasting blood glucose and hemoglobin A1c (HbA1c) of the diabetic rats were decreased (Khan et al., 2013; Li et al., 2021). 4. Leaf and bark extracts significantly inhibited α-glucosidase with IC_{50} values of 1.0 ± 0.3 and 0.5 ± 0.01 μg/ml, and α-amylase with IC_{50} values of

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TABLE 1 (*Continued*) Medicinal plants in Zimbabwe with antidiabetic properties and their pharmacological outcomes.

Botanical name	Family	Image	Medicinal part/s used	Type of extract	Animal model	Pharmacological outcome/s
						10.6 ± 0.4 μg/ml (Beidokhti et al., 2020). 5. Bread fortified with *Psidium guajava* L. leaves attenuated diabetic and acute renal failure symptoms in diabetic rats (El-khalik, 2017).
Persia Americana Mill.	Lauraceae		Leaf, seeds, fruit	Hydroethanolic, hydroalcoholic, aqueous	Streptozotocin (STZ)-induced diabetic Rats	1. Blood glucose levels and metabolic rates of animals were significantly improved. Activation of protein kinase B (PKB) was observed in the liver and skeletal muscle of treated rats compared with untreated rats. Possibly act to regulate glucose uptake in liver by PKB/Akt activation (Lima et al., 2012). 2. The seed extracts promoted the activation of the PI3K/AkT pathway and inhibited β-cell death in diabetic rats (Ojo et al., 2022).
Lippia javanica (Burm.f.) Spreng.	Verbenaceae		Herbal tea, leaf	Aqueous	Alloxan-induced diabetic mice	1. Aqueous extracts significantly lowered blood glucose levels in alloxan-induced diabetic mice (Arika et al., 2015).
Parinari curatellifolia Planch. ex Benth.	Chrysobalanaceae		Seeds, stem bark, peel flour	Ethanolic	Alloxan-induced diabetic rats, *Drosophila melanogaster* flies	1. Plasma glucose and low-density lipoprotein (LPL)-cholesterol levels were significantly reduced in diabetic rats treated with ethanolic extracts relative to control group. Significant increase in high-density lipoprotein (HDL)-cholesterol was also observed in the treated group compared to control group (Ogbonnia et al., 2008, 2011). 2. The ethanolic extract significantly reduced blood glucose, total thiol and nitric oxide levels of diabetic-induced *Drosophila melanogaster* flies. The extracts also increased glutathione-S-transferase and catalase activities of the diabetic treated flies (Omale et al., 2020). 3. The *Parinari curatellifolia* Planch. ex Benth peel flour formulated biscuits have improved total phenolic and flavonoid content as well as antioxidant activity (Ramashia et al., 2021).
Mangifera indica L.	Anacardiaceae		Leaf, fruit peel	aqueous	STZ-induced diabetic rats, Alloxan-induced diabetic mice	1. *Mangifera indica* L. supplemented diet reduced lipid peroxidation products in the cerebellum and cortex of diabetic rats (Cázares-Camacho et al., 2021).

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TABLE 1 (*Continued*) Medicinal plants in Zimbabwe with antidiabetic properties and their pharmacological outcomes.

Botanical name	Family	Image	Medicinal part/s used	Type of extract	Animal model	Pharmacological outcome/s
						2. Significant decrease in glucose and leptin levels coupled with elevation in insulin levels and C-peptide were observed in diabetic rats (El-Sheikh, 2012). 3. Remarkable decrease in postprandial blood glucose level was observed in diabetic mice. In addition, glucose tolerance and body weight and lipid profiles improved in diabetic treated mice. The extracts also decreased the damage to β-cells (Saleem et al., 2019). 4. The extracts significantly inhibited α-glucosidase and α-amylase (Gondi and Prasada Rao, 2015; Ojo et al., 2018).
Momordica charantia L.	Cucurbitaceae		Fruit juice, skin, flesh, whole fruit	Aqueous	STZ-induced diabetic rats, Sprague Dawley rats, humans	1. Increased levels of serum insulin, HDL-cholesterol, total antioxidant capacity levels, β-cell function, and pancreatic reduced glutathione (GSH) content was observed with fruit juice administration (Mahmoud et al., 2017). 2. The fruit juice reduced glycated hemoglobin A1c, blood glucose, body weight, BMI, fat percentage, and waist circumference in humans. The fruit juice also caused an increment of insulin area under curve (AUC), first phase and total insulin secretion (Cortez-Navarrete et al., 2018). 3. A whole fruit juice resulted in 31.6% lowering of blood glucose level and 27.4% increase in insulin level in hyperglycemic rats (Mahwish et al., 2021) 4. *Momordica charantia* L. fruit juice significantly reduced fasting blood glucose, body weight, HbA1c and blood serum glucose, and increase in insulin secretion of diabetic and prediabetic subjects (Cortez-Navarrete et al., 2018; Krawinkel et al., 2018; Majeed et al., 2021).
Annona Stenophylla Engl. and Diels	Annonaceae		Roots, root bark	Ethanolic, Aqueous	Alloxan induced diabetic rats	1. Dose dependent decrease in plasma glucose levels of alloxan induced diabetic rats (Verengai et al., 2017) and increase in glucose uptake was observed in C2C12 myocytes treated with ethanolic extracts (Taderera et al., 2019). 2. *Annona Stenophylla* Engl. and Diels extracts resulted in an increase in transcription of GLUT4 mRNA when compared with untreated control (Taderera et al., 2019). 3. The extracts significantly inhibited α-glucosidase and α-amylase (Taderera et al., 2016).

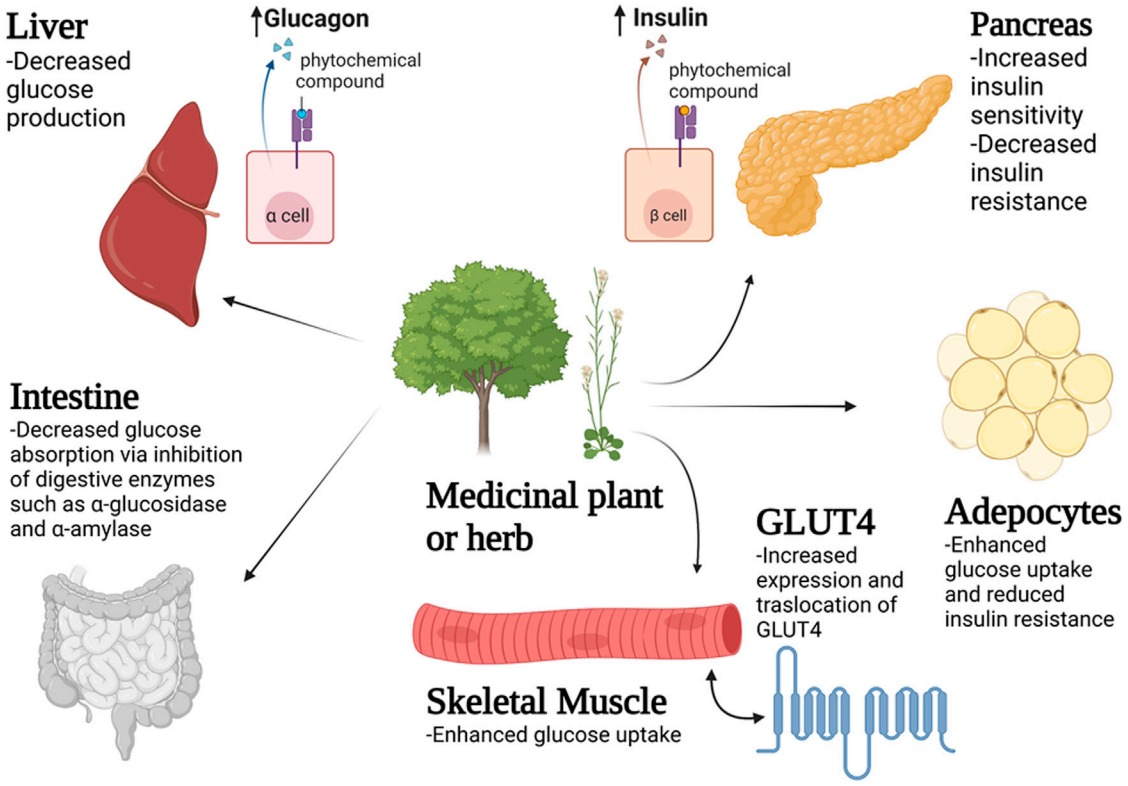

FIGURE 1
The mechanism of decreasing blood glucose levels by medicinal plants and herbs on different tissues and organs such as the liver, intestine, pancreas, skeletal muscle and adipose tissues.

plant names and key words such as antidiabetic and hyperglycemia. Taken together with the present findings, research focusing on medicinal plants could ultimately lead to discovery of novel antidiabetic compounds to treat high blood sugar for low-income countries, and ultimately improve the healthcare of marginalized rural people with diabetic complications.

Medicinal plants in Zimbabwe with antidiabetic properties

Over the years, there has been an increase in the emergence of multidrug, extreme and total drug resistant pathogens. Resistance to current drugs, insufficient/incompatible therapies and negative side effects associated with some of the currently used drugs have severely affected the management of diabetes mellitus in many parts of Africa (Care, 2013). Various categories of antidiabetic drugs available on the market for treatment and management of diabetes mellitus include insulin analogues, sulphonylureas, biguanides, dipeptidyl peptidase-4 inhibitors, thiazolidinediones and α-glucosidase inhibitors. However, the cost of these modern drugs has been on an upward trend worldwide. In addition, conventional diabetic therapies using modern medicines have resulted in increased side effects for many patients. As a result of these issues, there is a growing interest in the use of herbal remedies for the treatment and management of diabetes mellitus as they are perceived to be cheaper and with fewer side effects when compared to modern medicines. Table 1 summarizes the medicinal plants in Zimbabwe with antidiabetic properties used in this review, while Figure 1 presents the mode of action exerted by these plants in decreasing blood glucose levels.

Cassia abbreviata oliv.

Cassia abbreviata Oliv. From the *Fabaceae* family is widely distributed across many parts of Southern Africa, including Zimbabwe. Although *Cassia abbreviata* Oliv. has been used in African traditional medicine for the treatment of diabetes mellitus, there are few pharmacological findings to justify its use as an antidiabetic medicinal plant. Here we summarize some of the recent studies that show its potential to act as an

antidiabetic medicinal plant. (Kamga-Simo et al., 2021) assessed the potential of *Cassia abbreviata* Oliv. aqueous extracts to function as an antidiabetic agent on skeletal muscle cells and showed that the aqueous concoction enhanced glucose uptake and induced a two-fold increase in glucose transporter 4 (GLUT4) translocation in C2C12 mouse skeletal muscle cells. Real-time (RT) qPCR showed an increased expression of GLUT4, IRS1 (insulin receptor substrate 1) and PI3K (phosphoinositide 3-kinase) in cells treated with bark extract (Kamga-Simo et al., 2021). A study by (Shai et al., 2010) showed that acetone extracts of *Cassia abbreviata* Oliv. resulted in an 88% inhibition of yeast α-glucosidase activity at the study's highest concentration with an IC_{50} of 0.6 mg/ml. Enzyme kinetic studies suggested non-competitive inhibition by the active components in the extracts. *Cassia abbreviata* Oliv. extracts also showed pronounced antioxidant activity (Shai et al., 2010).

(Bati et al., 2017) evaluated the antidiabetic effects of ethanolic bark extract of *Cassia abbreviata* Oliv. on diabetic albino rats and showed that two doses of the ethanolic bark extract normalized blood glucose levels and helped to maintain normal body weights of the rats. Additionally, it was observed that the ethanolic doses increased hexokinase and decreased glucose-6-phosphate activities in the liver and kidneys. The extracts also inhibited the activity of α-glucosidase and enhanced glucose uptake (Bati et al., 2017). Taken together, these studies indicate that bioactive phytochemical compounds and other secondary plant metabolites in *Cassia abbreviata* Oliv. crude extracts can be used in the lowering and management of diabetes mellitus with maximal effects and minimal side effects.

Artemisia afra jacq. ex willd

Artemisia afra Jacq. ex Willd. or African wormwood is one of the most widely used medicinal plants in many parts of southern Africa because of its acclaimed healing properties against many ailments including diabetes mellitus (Dabe and Kefale, 2017). Concoctions of *Artemisia afra* Jacq. ex Willd. have also been used to treat the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or Covid-19 disease (Orege et al., 2021). For over a decade, studies have been carried out in order to evaluate the antidiabetic properties of *Artemisia afra* Jacq. ex Willd. based on indigenous knowledge which showed that concoctions of the plant can alleviate diabetes mellitus and its complications (Afolayan and Sunmonu, 2011; Dabe and Kefale, 2017; Lutgen et al., 2020).

The hypoglycemic activity and toxicity effect of aqueous leaf extract of Artemisia afra Jacq. ex Willd. was evaluated in streptozotocin-induced diabetic rats (Sunmonu and Afolayan, 2013). Administration of the leaf extract to the streptozotocin-induced diabetic rats significantly increased their body weight, decreased blood glucose levels, increased glucose tolerance, and improved imbalance in lipid metabolism compared to the control group (Sunmonu and Afolayan, 2013). In addition, it was shown that a 200 mg/kg body weight of the extract produced the optimal hypoglycemic action when compared to a standard drug, glibenclamide, demonstrating the potential of *Artemisia afra* Jacq. ex Willd. to act as an antidiabetic medicinal plant (Sunmonu and Afolayan, 2013). Aqueous extracts of *Artemisia afra* Jacq. ex Willd. increased the levels of glutathione reductase, glutathione peroxidase, superoxide dismutase and glutathione in the liver and kidneys of diabetic rats to normal levels and reduced the levels of lipid-peroxidation products (Afolayan and Sunmonu, 2013). Another study by (Afolayan and Sunmonu, 2011) showed that administration of aqueous extracts of *Artemisia afra* Jacq. ex Willd. significantly reduced glucose levels and increased insulin levels in diabetic rats. The aqueous plant extracts also led to regeneration of pancreatic β-cells as indicated by the restoration of the pancreas/body weight ratio (from 0.13 to 0.23%) to near the normal value of 0.27%. *Artemisia afra* Jacq. ex Willd. extracts also exhibited antioxidant activity as seen by the reduction in the levels of lipid peroxidation products such as malondialdehyde (MDA) (Afolayan and Sunmonu, 2011). The enzymatic activities of glutathione peroxidase, glutathione reductase and superoxide dismutase were significantly improved after treatment with the aqueous extract (Afolayan and Sunmonu, 2011). These findings suggest that *Artemisia afra* Jacq. ex Willd. may play a protective role on tissues by reducing oxidative stress.

A case study in the province of Maniema, Democratic Republic of Congo (DRC) showed that *Artemisia afra* Jacq. ex Willd. and *Artemisia annua* L. herbal tea can treat diabetes mellitus (Lutgen et al., 2020). In this study, it was observed during initial trials that several patients were suffering from diabetes mellitus with fasting blood sugar levels ranging from 180 to 280 mg/ml. Upon treatment with *Artemisia annua* and *Artemisia afra* Jacq. ex Willd. aqueous infusions, their blood sugar decreased significantly to 100–130 mg/ml and were comparable to those of the controls. (Issa and Bule, 2015) orally administered aqueous and methanolic extracts of *Artemisia afra* Jacq. ex Willd. collected from Goba town, southeast of Ababa Addis to alloxan induced diabetic mice and evaluated their antidiabetic effect. Their results showed that blood glucose level was significantly decreased by up to 57% in groups that received doses of 750 mg/kg of aqueous extracts of *Artemisia afra* Jacq. ex Willd. relative to untreated group. In addition, the methanolic extracts of *Artemisia afra* Jacq. ex Willd. significantly lowered blood glucose by 49.8% compared to the control group. Overall, the research articles presented here demonstrate that *Artemisia afra* Jacq. ex Willd. contain important bioactive phytochemicals with antidiabetic properties and can be used in traditional medicine systems to treat type 2 diabetes mellitus.

Moringa oleifera lam.

Moringa oleifera Lam. of the *Moringaceae* family is native to northern India, but widely used in many regions of the world including Zimbabwe to treat metabolic diseases because of the presence of bioactive phytochemical compounds and minerals in its leaves, barks and roots (Gopalakrishnan et al., 2016; Hassan et al., 2021). In particular, the leaves are rich in minerals (calcium, potassium, zinc, magnesium, iron and copper), vitamins (including vitamins A, B, C, D, and E) and other secondary plant metabolites such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones and alkaloids (Hassan et al., 2021). The leaves are also rich in amino acids, including aspartic acid, glutamic acid, serine, glycine, threonine, α-alanine, valine, leucine, isoleucine, histidine, lysine, cysteine, methionine, arginine, and tryptophan (Gopalakrishnan et al., 2016). Other parts of the plant including barks and roots contain important bioactive phytochemicals with antioxidant, anticancer, anti-inflammatory, antidiabetic and antimicrobial properties. *Moringa oleifera* Lam. seeds have anticoagulant properties and are also extensively used in water treatment (Shan et al., 2017). Studies have shown that *Moringa oleifera* Lam. has antidiabetic properties and can help cure type 2 diabetes mellitus (Nova et al., 2020; Watanabe et al., 2021). The *in vitro* and docking studies conducted recently suggest that the natural polyphenols and flavonoids in *Moringa oleifera* Lam. ethanolic leaf extracts have antioxidant and antidiabetic properties (Chigurupati et al., 2022).

Human and animal studies have shown evidence to support *Moringa oleifera* Lam.'s antidiabetic properties and have been extensively reviewed (Vargas-Sánchez et al., 2019; Nova et al., 2020). (Kar et al., 2003) evaluated the hypoglycemic activities of the organic parts of *Moringa oleifera* Lam. using alloxan-diabetic albino rats. Application of a single dose of *Moringa oleifera* Lam. stem bark ethanolic extract on alloxan induced diabetic rats resulted in significant lowering of blood glucose and urine sugar to undetectable levels. Bioassay-directed isolation and purification of the methanolic extracts of *Moringa oleifera* Lam. identified N-benzyl thiocarbamates, N-benzyl carbamates, benzyl nitriles and a benzyl ester which were all shown to significantly trigger the release of insulin in rodent pancreatic β-cells, and have cyclooxygenase enzyme and lipid peroxidation inhibitory effects (Francis et al., 2004). The leaves of *Moringa oleifera* Lam. exerted hypoglycemic and anti-hyperglycemic effects probably because of the presence of terpenoids, which appeared to be linked with the stimulation of β-cells and subsequent secretion of insulin (Tende et al., 2011). The antihyperglycemic activity of *Moringa oleifera* Lam. tea was investigated on Wistar albino rats and humans by (Fombang and Saa, 2016). *Moringa oleifera* Lam. tea suppressed glucose elevation in both rats and humans, and lower doses were more effective in reducing hyperglycemia possibility due to inhibition of glucose uptake in the intestinal walls by the phytochemical compounds in the tea extract.

A pilot clinical study on thirty-five diabetic and non-diabetic healthy volunteers in Mali demonstrated that *Moringa oleifera* Lam. powder significantly reduced post-prandial blood glucose in diabetic patients but had no effect on blood glucose of the healthy volunteers (Sissoko et al., 2020). The hypoglycemic effect of *Moringa oleifera* Lam. leaf powder supplemented to the local diet of Saharawi diabetic and healthy subjects was investigated in order to determine its hypoglycemic potential in humans (Leone et al., 2018). Seventeen Saharawi diabetic and ten healthy subjects were randomly administered with a traditional meal supplemented with 20 g of *Moringa oleifera* Lam. leaf powder on two different days. Although the healthy subjects did no show differences in mean glycemic meal response, in diabetic subjects, the mean glycemic meal response with *Moringa oleifera* Lam. leaf powder was lower than with control meal. In addition, the postprandial glucose response of diabetic subjects peaked earlier and at lower concentrations with *Moringa oleifera* Lam. leaf powder than with control meal. Next (Anthanont et al., 2016), investigated the effect of *Moringa oleifera* Lam. powdered capsules on plasma glucose and insulin secretion of ten healthy Thai volunteers, and showed that the powdered capsules had no effect on plasma glucose, but significantly increased insulin section. These results suggest that the powder capsules contain important phytochemical nutrients to treat type 2 diabetes mellitus. Formulating bakery cookies with natural product extracts could be a means to treat chronic diseases because they are popular snacks eaten by many people worldwide. However, cookies contain wheat flour that contributes to hyperglycemic and hyperinsulinimic responses. In this regard (Ahmad et al., 2018), conducted a randomized clinical trial to investigate the effect of incorporating *Stevia rebaudiana* and *Moringa oleifera* Lam. powder in cookies on postprandial glycemia, appetite, palatability, and gastrointestinal well-being in humans. Compared to the control cookies and *Stevia rebaudiana* enriched cookies, the *Moringa oleifera* Lam. enriched cookies significantly improved postprandial glycemia and reduced hunger in subjects (Ahmad et al., 2018). Interestingly, a randomized placebo-controlled study by (Taweerutchana et al., 2017) on thirty-two type 2 diabetic patients fed with *Moringa oleifera* Lam. leaf capsules or placebo for 4 weeks demonstrated that *Moringa oleifera* Lam. leaf capsules had no effect on glycemic control and adverse effects in type 2 diabetes mellitus subjects. Together, these studies showed that *Moringa oleifera* Lam. leaves enriched with local foods could be a hypoglycemic herbal drug for people living with diabetes mellitus and more clinical trials are needed because of conflicting results from different research groups.

Gupta et al. (2012) and Al-Malki and El Rabey (2015) observed a rejuvenation of pancreatic islets after treatment of streptozotocin induced diabetic rats with methanol extracts of

Moringa oleifera Lam. pods. Further, normalization of markers of oxidative stress which include reduction in the plasma levels of glutathione and increased levels of MDA, and reduced activities of the antioxidant enzymes SOD and catalase were observed in diabetic rats treated with moringa seed powder (Al-Malki and El Rabey, 2015). These observations were attributed to the presence of potent free radical scavengers (glucomoringin, quercetin, kaempferol, ursolic acid, myricetin and chlorogenic acid) that have been shown to reduce oxidative stress in diabetic rats treated with Moringa (Gupta et al., 2012; Ampofo-yeboah et al., 2013; Ali et al., 2015). In addition, there was a reduction in the levels of cytokines (Interleukin-6, Interleukin -1β and tumor necrosis factor α), an indication of normalization of metabolic processes in treated diabetic rats (Al-Malki and El Rabey, 2015). Aqueous extracts of *Moringa oleifera* Lam. inhibited α-amylase and α-glucosidase with IC_{50} values of 52.5 and 33.4 mg/ml, respectively (Khan et al., 2017). The aqueous extracts also resulted in increased glucose uptake by yeast cells, and skeletal muscles in rats, while a decrease in intestinal glucose absorption was observed (Khan et al., 2017). Overall, *Moringa oleifera* Lam. extracts contain bioactive phytochemical compounds that act synergistically to lower blood glucose via different mechanisms making Moringa one of the ideal plant-based solutions to management of diabetic conditions.

Aloe vera (L.) Burm.f.

Aloe vera (L.) Burm.f. of the *Asphodelaceae* family is a perennial succulent plant commonly found in many parts of Africa and has been used in traditional medicine for more than 2,000 years because of its various medicinal properties (Surjushe et al., 2008). It exhibits antioxidant, antiulcer, anticancer, anti-inflammatory, anti-atherosclerosis and wound-healing effects (Noor et al., 2017). In addition, *Aloe vera* (L.) Burm.f. lowers blood glucose in diabetic patients, and improves the responsiveness of body tissues towards insulin, thus making insulin more effective (Yongchaiyudha et al., 1996). *Aloe vera* (L.) Burm.f. contain a number of anthraquinones, C-glycosides and resins, its gel has several organic acids and biostimulators with topical healing properties (Iwu, 2014). The plant also contains several flavonoids (aloesin, aloenin and barbaloin), polysaccharides, vitamins, lignins, minerals, glycoproteins, phytosterols, and saponins (Iwu, 2014). As such, *Aloe vera* (L.) Burm.f. has many applications in the health and cosmetic industry. The antidiabetic effect of *Aloe vera* (L.) Burm.f. has been extensively reviewed (Radha and Laxmipriya, 2015; Suksomboon et al., 2016; Haghani et al., 2022). A systematic review of the evaluation of biological properties and clinical effectiveness of *Aloe vera* (L.) Burm.f. suggested that the gel can help people achieve better fasting blood glucose levels, as well as reduce body fat and weight (Radha and Laxmipriya, 2015).

A double-blind randomized clinical trial to evaluate the efficacy of *Aloe vera* (L.) Burm.f. in healing of diabetic foot ulcer (DFU) showed that the gel significantly reduced the ulcer surface compared with the control group which showed no significant difference in terms of the ulcer depth (Najafian et al., 2019). Another double blind randomized controlled trial of seventy-two subjects with pre-diabetes mellitus symptoms showed that *Aloe vera* (L.) Burm.f extracts significantly reduced fasting blood glucose, HbA1C, triglyceride, total cholesterol and LDL-C levels with a concomitant increase in HDL-C level compared to placebo (Alinejad-Mofrad et al., 2015). In an effort to understand the metabolic effect of an *Aloe vera* (L.) Burm.f gel complex on obese individuals with prediabetes or early untreated diabetes mellitus, one-hundred and thirty-six subjects were randomly given the gel complex, while the control group were given a soft capsule composed of natural pigments and excipients (Choi et al., 2013). The authors showed that fasting blood glucose and body weight of the subjects fed with *Aloe vera* (L.) Burm.f. gel complex were significantly lower than those of the control group. A clinical trial of *Aloe vera* (L.) Burm.f. products (UP780 and AC952) in patients with prediabetes/metabolic syndrome showed that the formulations can reverse the abnormalities in fasting glucose and improve glucose tolerance of the subjects (Devaraj et al., 2013). These clinical studies provide significant evidence that show the potential of *Aloe vera* (L.) Burm.f. gel and its products to effectively improve the parameters associated with diabetes mellitus in prediabetes and early non-treated diabetic patients.

(Noor et al., 2017) investigated the role of *Aloe vera* (L.) Burm.f. extract on improvement of insulin secretion and pancreatic β-cell function by morphometric analysis of pancreatic islets in STZ-induced diabetic Wistar rats and showed that oral administration of 300 mg/kg body weight *Aloe vera* (L.) Burm.f. extract to diabetic rats for 3 weeks restored blood glucose levels to normal levels and increased their insulin levels. In addition, morphometric analysis of pancreatic sections showed quantitative gain in number, diameter, volume and area of the pancreatic islets of treated diabetic rats compared to the untreated diabetic rats. This study showed that *Aloe vera* (L.) Burm.f. extract exerts antidiabetic effects by improving insulin secretion and pancreatic β-cell function by restoring pancreatic islet mass in STZ-induced diabetic Wistar rats. (Govindarajan et al., 2021) investigated the antidiabetic effect of *Aloe vera* (L.) Burm.f. carbohydrate fraction on insulin secretion, cell proliferation and inflammation using streptozotocin-induced oxidative stress on RIN-m5F cells of diabetic rats. The *Aloe vera*-treated RIN-m5F cells significantly increased bromodeoxyuridine levels and insulin secretion with a concomitant decrease of tumor necrosis factor α (TNF-α), interleukin 6 (IL-6) and nitric oxide levels. Increase in bromodeoxyuridine levels after *Aloe vera* (L.) Burm.f. treatment is associated with rejuvenation of pancreatic β-cells and this leads to improved insulin production. Additionally, the

Aloe vera (L.)-treated streptozotocin-induced diabetic rats had lower fasting plasma glucose, glucagon and glucose-6-phosphatase levels. The insulin, hexokinase, and glycogen synthase levels and, glycogen content were improved with doses of the *Aloe vera* (L.) Burm.f. carbohydrate extract. The extracts also inhibited α-amylase and α-glucosidase in a dose-dependent manner with IC_{50} values of 60.44 ± 1.02 and 82.85 ± 1.05 μg/ml, respectively. Together, these results suggest that *Aloe vera* (L.) Burm.f. extract regulates glucose metabolism by activation of glycogenesis and down-regulation of gluconeogenesis. Thus, *Aloe vera* (L.) Burm.f. extracts can be used as an alternative medicine in the alleviation of type 2 diabetes mellitus.

Protein glycation is considered one of the main causes of diabetic complications such as vasculopathy, retinopathy, nephropathy and neuropathy, cataracts and chronic kidney disease because it brings about the formation of advanced glycation end products (AGEs), which modify the structure of proteins and alter enzymatic activity. To prevent the formation of AGEs, (Muñiz-Ramirez et al., 2020), evaluated the inhibitory effect of methanolic *Aloe vera* (L.) Burm.f. extract *in vitro* and showed that the extract can significantly decrease the formation of AGEs, fructosamine, Nε-carboxymethyl-Lysine and carbonyl protein. The antiglycation activity of the extract is possibly due to the presence of aloin and aloe-emodin compounds acting synergistically to decrease the formation of AGEs (Froldi et al., 2019). In addition, the methanolic extract significantly reduced the activities of *α*-amylase and *α*-glucosidase and increased the content of thiol groups. These findings suggest that a decrease in formation of AGEs brought about by the *Aloe vera* (L.) Burm.f. plant extract may lead to reduced postprandial glucose and prevent diabetes mellitus complications associated with AGE.

In a clinical trial to determine the potential of *Aloe vera* (L.) Burm.f. in lowering blood glucose, ninety non-insulin dependent diabetic subjects from Punjab Agricultural University and Civil hospitals of Ludhiana, India were subjected to *Aloe vera* (L.) Burm.f. gel powder for 3 months, and further supplemented with gel powder and nutrition counselling for another 3 months (Choudhary et al., 2014). A significant decrease in fasting blood glucose level (by 11.4% and 15.4%) and post prandial glucose level (by 18.5% and 27.8%) was observed in the subjects supplemented with gel powder and counselling after the study. The reduction in blood glucose and lipid profiles of the non-insulin dependent diabetic patients was ascribed to the presence of phytochemicals in *Aloe vera* (L.) Burm.f. powder and nutrition counselling. Another study on effects of *Aloe vera* (L.) Burm.f. gel on behavioral functions, oxidative status, and neuronal viability in the hippocampus of streptozotocin (STZ)-induced diabetic rats showed that the hypoglycemic and antioxidative properties of *Aloe vera* (L.) Burm.f. gel are possible mechanisms that improve behavioral deficits and protect hippocampal neurons in diabetic animals (Tabatabaei et al., 2017). In addition, (Deora et al., 2021), showed that *Aloe vera* (L.) Burm.f. extracts are capable of alleviating diabetes mellitus-related complications by lowering lipid profile, and restoration of β-cell function in diabetic rats. In summary, we showed that *Aloe vera* (L.) Burm.f. extracts have antidiabetic effects that are comparable to conventional drugs and can significantly improve the healthcare of patients with type 2 diabetes mellitus.

Psidium guajava L.

Psidium guajava L., commonly known as guava, is a native plant of many tropical regions of the world including many southern parts of Africa and South America. Various parts of *Psidium guajava* L. have long been used in folk-lore as a medicinal herb to cure infectious diseases, neoplasm, metabolic diseases, digestive diseases and diabetes mellitus (Chu et al., 2022), and various other ailments such as wounds, cough, ulcers, bronchitis, eyesores and diarrhea (Daswani et al., 2017). *In vitro* and *in vivo* animal studies showed that guava leaf extracts improved blood sugar levels, long-term blood sugar control, and insulin resistance. A recent study by (Chu et al., 2022) showed that the aqueous extracts of *Psidium guajava* L. significantly lowered fasting plasma glucose levels and improved glucose tolerance and insulin sensitivity of diabetic mice. Further, the aqueous extracts of *Psidium guajava* L. increased hepatic glycogen accumulation, glucose uptake and decreased mRNA expression levels of gluconeogenic genes, in addition to increasing the expression of glucose transporter 2 (GLUT2) on the cell membrane of hepatocytes. The extracts also altered the composition of the gut microbiota and increased the enrichment of probiotics. These results indicate that aqueous extracts can alleviate hyperglycemia and insulin resistance of T2DM by regulating metabolism of glucose in the liver and restoring gut microbiota.

(Li et al., 2021) evaluated the *in vivo* hypoglycemic and hepatoprotective effects of dried- and rice-fried *Psidium guajava* L. leaf decoctions in diabetic rats and showed a decrease in fasting blood glucose and hemoglobin A1c (HbA1c) of the diabetic rats. Further, upregulation expression of glucokinase (GK), glucose transporter 2 (GLUT2), insulin growth factor-1 (IGF-1), insulin receptor substrate-1 (IRS-1), and insulin receptor substrate-2 (IRS-2) was observed in both dried- and rice-fried *Psidium guajava* L. leaf-treated groups. High-performance liquid chromatography and ultra-performance liquid chromatography-tandem mass spectrometry analysis of the *Psidium guajava* L. leaf extracts revealed the presence of a high content of ellagic acid, hyperoside, isoquercitroside, reynoutrin, guaijaverin, and quercetin in the rice-fried extract compared to the dry extract. This finding suggests that the rice-fried extract, unlike the dry, has higher antidiabetic effect because of the processing method. Treatment with *Psidium guajava* L. extract showed a significant reduction in

blood glucose and HbA1c levels and a significant increase in plasma insulin levels. (Khan et al., 2013) also showed that treatment of STZ-induced diabetic rats with ethanolic *Psidium guajava* L. leaf extract significantly reduced blood glucose and HbA1c levels and increased plasma insulin levels. Additionally, the activities of carbohydrate metabolizing enzymes were restored by treatment with the extract. The antidiabetic effect of the ethanolic extract of *Psidium guajava* L. leaves is likely due to the presence of flavonoids and other phenolic components present in the extract. Furthermore, (Zhu et al., 2020), showed that *Psidium guajava* L. leaf flavonoids, guaijaverin and avicularin have antidiabetic and liver protective effects in diabetic mice.

In another study, (Beidokhti et al., 2020), investigated the inhibitory activities of *Psidium guajava L.* leaf and bark extracts against yeast α-glucosidase and porcine α-amylase and showed that the leaf and bark extracts significantly inhibited α-glucosidase with IC_{50} values of 1.0 ± 0.3 and 0.50 ± 0.01 μg/ml, respectively and α-amylase with IC_{50} values of 10.6 ± 0.4 μg/ml. The extracts had no effect on glucose-6-phosphatase activity in rat hepatoma H4IIE cells but significantly increased 2-deoxy-D-[1–^{3}H]-glucose uptake in C2C12 muscle cells and enhanced triglyceride accumulation in 3T3-L1 cells compared to vehicle (DMSO) and positive control (rosiglitazone). These biological activities can likely contribute to improved glycemic control *in vivo*, indicating that *Psidium guajava* L. may have a beneficial role in the management of type 2 diabetes mellitus.

(Musdja et al., 2017) studied the antidiabetic potential of ethanolic *Psidium guajava* L. extracts on diabetic induced male albino rats and showed that the extracts lowered blood glucose levels of diabetic rats by 28–32% compared to the control. (Luo et al., 2019) isolated polysaccharides from guava leaves and evaluated their antidiabetic effects on diabetic mice induced by streptozotocin combined with high-fat diet. When treated with low-dose or high-dose polysaccharides for 2 weeks, the body weight of diabetic mice was partly recovered compared to the control group. The results indicate that guava leaves could significantly ameliorate body weight loss in diabetic mice. Further, fasting blood glucose of the diabetic mice treated with polysaccharides from guava leaves and acarbose (positive control) were significantly reduced compared with the control. Their findings suggested that polysaccharides from guava leaves could provide health benefits for diabetic patients. (Choi et al., 2021) investigated the effects of guava leaf extract on adipogenesis, glucose uptake, and lipolysis of adipocytes and demonstrated that the extracts inhibits adipogenesis and improves adipocyte function by reducing basal lipolysis and increased insulin-stimulated glucose uptake in adipocytes, indicating the antidiabetic effects of guava leaves. These findings show that *Psidium guajava* L. can lower blood glucose and ameliorate body weight loss in diabetic mice.

Studies have shown that the fruit of *Psidium guajava* L. exert antihyperglycemic and antioxidative effects in streptozotocin (STZ)-induced diabetic rat via activation of key effector molecules of the PI3K/Akt pathway, and phosphorylation of AMPK pathway in liver of diabetic rats (Jayachandran et al., 2018; Vinayagam et al., 2018; Tella et al., 2019). In an effort to understand how *Psidium guajava* L. exerts its antidiabetic and anti-hyperlipidemic effects on STZ-induced diabetic rats (Tella et al., 2019), assessed the activities of glycogen synthase (GS), glycogen phosphorylase (GP) and hormone sensitive lipase enzyme (HSL) using radio-chemical methods. The leaf extract significantly decreased HSL activity in adipose tissue and liver of diabetic rats, and increased glycogen storage and HDL-cholesterol levels. In addition, the leaf extract lowered serum triglycerides, total cholesterol and LDL-cholesterol. In addition (Tella et al., 2022), also showed that the *Psidium guajava* L. extracts ameliorated damage to the pancreatic islets and lowered blood glucose in male Sprague-Dawley diabetic rats due to the presence of phenolic compounds and triterpenes in the extracts. (Shen et al., 2008) investigated the effect of aqueous and ethanol extracts of *Psidium guajava* L. leaves on hypoglycemia and glucose metabolism in type 2 diabetic rats. Diabetic rats were fed with aqueous and ethanol extracts of *Psidium guajava L.* over 6 weeks, and the oral glucose tolerance test (OGTT) and other biochemical properties were conducted after sacrificing the rats. The authors showed that acute and long-term feeding of diabetic rats with *Psidium guajava* L. extracts significantly reduced blood sugar levels compared to the control group. Further, long-term administration of guava leaf extracts also increased the plasma insulin level and glucose utilization in diabetic rats. (El-khalik, 2017) studied the effects of *Psidium guajava* L. leaf extracts and bread fortified with *Psidium guajava* L. leaves, and showed that the fortified bread and leaf extracts significantly attenuated diabetic and acute renal failure symptoms in diabetic rats. Together, these experiments provide evidence to support the antihyperglycemic effect and antioxidant properties of guava extracts and their health function against type 2 diabetes mellitus.

Persea americana mill.

Persea americana Mill. or avocado belongs to the flowering plant of the *Lauraceae* family. Avocado fruit, seed and leaves are rich in phytochemicals, vitamins, and micronutrients. Studies on antidiabetic properties of indigenous plants have demonstrated that the leaves and seeds of *Persea americana* Mill. are used in the treatment of diabetes mellitus in many parts of Latin America and Africa (Gondwe et al., 2008; Lima et al., 2012; Kouamé et al., 2019). The antidiabetic activity of *Persea americana* Mill. Is believed to be due to the presence of a number of bioactive phytochemical compounds. Liquid-chromatography electrospray ionization mass spectroscopic (LC-ESI-MS) analysis of ethanolic extracts of avocado fruit and leaves revealed the presence of twenty-six bioactive phytogenic compounds (categorized into fatty acids, sterols, triterpenes,

phenolic acids, and flavonoids) (Abd Elkader et al., 2022). (Lima et al., 2012) evaluated the antidiabetic activity of hydroethanolic extracts from *Persea americana* Mill. leaves and the mechanism of action via activation of protein kinase B (PKB/Akt) in streptozotocin-induced diabetic rats. By supplying STZ-diabetic rats with hydroalcoholic extracts of the leaves of *Persea americana* Mill., vehicle and metformin, the blood glucose levels, and metabolic state of the animals were significantly improved. In addition, activation of protein kinase B (PKB) was observed in the liver and skeletal muscle of treated rats when compared with untreated rats. These results indicated that hydroalcoholic extracts of *Persea americana* Mill have antidiabetic properties, and possibly act to regulate glucose uptake in liver and muscles by way of PKB/Akt activation.

In another study, (Ojo et al., 2022), examined the role of *Persea americana* Mill. seeds in attenuating alloxan-induced diabetes mellitus by suppressing oxidative stress, inflammation, and β-cell apoptotic death, and by upregulating glucose uptake by stimulating the PI3K/AKT signaling pathway. Their results showed that administration of aqueous *Persea americana* Mill. seed extracts can promote the activation of the PI3K/AkT pathway and inhibit β-cell death, which may be the primary mechanism by which *Persea americana* Mill. seed extracts promotes insulin sensitivity and regulates glycolipid metabolism (Ojo et al., 2022). A study by (Abd Elkader et al., 2022) showed that both fruit and leaf extracts of *Persea americana* Mill. have high content of polyphenols and exhibited high α-amylase inhibitory activities. The fruit and leaf extracts inhibited α-amylase by 92.13% and 88.95% respectively. These results indicate that the antioxidant properties of *Persea americana* Mill. coupled with its ability to activate the pathways and key enzymes involved in glucose metabolism can help in management and treatment of diabetes mellitus.

Lippia javanica (Burm.f.) spreng.

Lippia javanica (Burm.f.) Spreng. of the *Verbenaceae* family has long been used in tropical Africa as indigenous herbal tea, refreshing beverage and food additive based on its perceived health and medicinal properties. (Arika et al., 2015) studied the effect of oral and intraperitoneal administration of aqueous leaf extracts of *Lippia javanica* (Burm.f.) Spreng. on blood glucose levels in alloxan induced diabetic mice and demonstrated that the aqueous leaf extracts significantly lowered blood glucose levels in diabetic mice compared to controls. The *Lippia javanica* (Burm.f.) Spreng. aqueous extracts showed hypoglycemic activity in a dose independent manner. The antidiabetic effect of *Lippia javanica* (Burm.f.) Spreng. in diabetic mice was attributed to the presence of a number of secondary metabolites (particularly flavonoids and saponins) acting synergistically as hypoglycemic agents to lower blood glucose. Despite its widespread use as herbal tea to lower blood glucose, there are limited studies in literature to support its antidiabetic action and future work focused on elucidating the antidiabetic compounds and the mode of action of *Lippia javanica* (Burm.f.) Spreng. can help understand its therapeutic benefits.

Parinari curatellifolia planch. ex benth.

Parinari curatellifolia Planch. ex Benth. is an evergreen tropical tree of Africa. In Zimbabwe, it is used for faith healing by some indigenous groups and for traditional ceremonies. The seeds of *Parinari curatellifolia* Planch. ex Benth. are commonly used in folk medicine for the treatment of diabetes mellitus and other diseases. (Ogbonnia et al., 2008) evaluated the biochemical safety and hypoglycemic effects of ethanolic extracts of *Parinari curatellifolia* Planch. ex Benth. seeds in alloxan-induced diabetes mellitus in rats. Their results showed a significant reduction in the plasma glucose and low-density lipoprotein (LDL)-cholesterol levels along with a significant increase in high density lipoprotein (HDL)-cholesterol in the treated diabetic groups compared to the control. These results indicate that *Parinari curatellifolia* Planch. ex Benth. seed concoction can be used as a hypoglycemic agent. In a separate study (Ogbonnia et al., 2011), studied the anti-diabetic effect of a 50:50% mixture of ethanolic extract of *Parinari curatellifolia* Planch. ex Benth. seed and *Anthocleista vogelii* Planch. root extract in alloxan-induced diabetic albino rats, and showed that a combination of the two plant extracts was effective in reducing plasma glucose levels in the diabetic rats compared to control group. In addition, significant reductions in LDL-cholesterol, AST and ALT levels and increased HDL-cholesterol were observed in the treated diabetic groups (Ogbonnia et al., 2011). The pancreatic tissue of diabetic rats treated with the extract mixture also showed marked necrotic changes while that of diabetic untreated animals showed more severe β-cell necrosis. These results indicate that the extract mixtures have good hypoglycemic activity and potentially beneficial effects on cardiovascular risk factors.

In another study, the antidiabetic activities of ethanolic stem-bark extract of *Parinari curatellifolia* Planch. ex Benth. was evaluated in *Drosophila melanogaster* fed with high sucrose diet to induce insulin resistance diabetes mellitus (Omale et al., 2020). The high sucrose diet resulted in decreased body size, decreased locomotor activities and delayed emergence of the larva (L3) in flies, all of which are known symptoms of type 2 diabetes mellitus in flies. When administered to the diabetic flies, the extracts of *Parinari curatellifolia* Planch. ex Benth. and standard drugs significantly reduced their glucose levels. Additionally, there was a substantial decrease in the total thiol content, nitric oxide levels, acetylcholinesterase activity and a

significant increase in glutathione-S-transferase and catalase activities of the diabetic treated flies.

(Ramashia et al., 2021) investigated the impact of *Parinari curatellifolia* Planch. ex Benth. peel flour on the nutritional, physical, and antioxidant properties of formulated biscuits. Their studies showed that inclusion of *Parinari curatellifolia* Planch. ex Benth. peel flour to biscuit formulations greatly improved the total phenolic and flavonoid content as well as antioxidant activity of the biscuits. The total energy of the biscuits was lowered by ~8% when the biscuits were enriched with various amounts of *Parinari curatellifolia* Planch. ex Benth. peel flour. Therefore, the formulations of *Parinari curatellifolia* Planch. ex Benth. peel flour could help in reducing weight for overweight/obese persons or in controlling diabetes mellitus. These studies demonstrate the potential of *Parinari curatellifolia* Planch. ex Benth. peel flour as a food additive in reducing diabetes mellitus. Overall, *Parinari curatellifolia* Planch. ex Benth. peel flour has antidiabetic properties because of the presence of polyphenolic compounds and other phytochemical compounds and can be a useful raw material for manufacturing of functional bakery products such as biscuits.

Mangifera indica L.

Mangifera indica L. (mango) is tropical plant native to India and Southeast Asia but also widely grown in tropical regions of Africa and Central America (Lauricella et al., 2017). *Mangifera indica* L. contains the polyphenol, mangiferin, a phytochemical compound with hypoglycemic and antioxidant activity (Imran et al., 2017). Oxidative stress has been implicated in the pathogenesis and progression of diabetes mellitus (Giacco and Brownlee, 2010). *Mangifera indica* L. fruit is rich in bioactive phytochemical compounds with antioxidant properties including mangiferin that potentially exerts potent neuroprotective properties against diabetes mellitus-induced oxidative stress. (Cázares-Camacho et al., 2021) investigated effects of *Mangifera indica* L. supplemented diet (peel and pulp) on oxidative stress markers in two brain regions (cerebral cortex and cerebellum) of the STZ-induced diabetic rats and observed a decrease in lipid peroxidation in diabetic rats supplemented with *Mangifera indica* L. diet. In addition, the mango supplemented diet reduced polyphagia and weight loss, and maintained a stable glycemia in diabetic rats. These results indicate that *Mangifera indica L.* may exert neuroprotective properties against diabetes mellitus-induced oxidative stress and can be an alternative to prevent and treat diabetes mellitus. A study by (Gu et al., 2019) showed that norathyriol from *Mangifera indica* L. extract significantly inhibited α-glucosidase with an IC_{50} of 4.22 μg/ml. Further, *in vitro* activity assay showed that mangiferin also inhibited α-glucosidase with IC_{50} of 36.84 μg/ml, comparable to acarbose standard (21.33 μg/ml), whereas the IC_{50} value for the whole *Mangifera indica* L. fruit juice, was 112.8 μg/ml, demonstrating the potential of mangiferin and norathyriol to reduce post-prandial glucose level in diabetic patients (Sekar et al., 2019).

Using leaf aqueous extracts on streptozotocin diabetic rats, (El-Sheikh, 2012), observed a significant reduction in serum glucose (~37.7%) and leptin levels (~24.3%) accompanied by significant elevation in insulin and C-peptide levels of 28.1 and 24.0%, respectively. Administration of the leaf extract also ameliorated the diabetic effects on asymmetric dimethylarginine (inhibitor of endothelial nitric oxide synthase), Endothelin-1, and serum nitric oxide values. (Saleem et al., 2019) evaluated the potential of *Mangifera indica* L. leaves on postprandial blood glucose, oral glucose tolerance and body weight of alloxan-induced diabetic mice and showed that treated diabetic mice had a remarkable decrease in postprandial blood glucose level compared to untreated diabetic mice. Further, the plant extracts increased glucose tolerance, and body weight, improved lipid profile and decreased the damage to β-cells. The α-amylase and α-glucosidase inhibitory activities of *Mangifera indica* L. leaves was investigated in Nigeria, by (Ojo et al., 2018). The leaf extracts had a considerably high inhibitory effect on α-glucosidase (IC_{50} = 25.11 ± 0.01 μg ml^{-1}) and α-amylase (IC_{50} = 24.04 ± 0.12 μg ml^{-1}). Electrospray ionization mass spectroscopy (ESI-MS) and high-performance liquid chromatography (HPLC) chemical evaluation of the leaf extract revealed the presence of mangiferin, chlorogenic acid, myricetin, quercetin, rhamnetin, catechin, epicatechin, iriflophenone 3-C-β-D-glucoside and gallic acid (Saleem et al., 2019; Sarkar et al., 2022). Therefore, the inhibitory activities observed in extracts are likely due to the presence of these bioactive antidiabetic agents, and thus *Mangifera indica* L. leaves can be formulated to generate plant-derived nutraceutical drugs to improve human health.

Studies have shown that whole peel powder of *Mangifera indica* L. can help protect from both type I and type II diabetes mellitus (Gondi et al., 2015; Gondi and Prasada Rao, 2015; Irondi et al., 2016). In this regard, (Gondi et al., 2015), studied the effects of mango peel on diabetic rats and showed that urine sugar, urine volume, fasting blood glucose, total cholesterol and triglycerides were significantly reduced in diabetic rats fed with a diet supplemented with mango peel at 5% and 10% levels in the basal diet. In addition, the antioxidant enzyme activities of diabetic rats treated with mango peel increased and the lipid peroxidation in plasma, kidneys and liver decreased compared to untreated diabetic rats (Gondi et al., 2015). Glomerular filtration rate and microalbuminuria were ameliorated in mango peel-treated diabetic group. The peel extract also inhibited α-amylase and α-glucosidase activities, with IC_{50} values of 4.0 and 3.5 μg/ml respectively (Gondi and Prasada Rao, 2015). Another study showed that *Mangifera indica* L. kernel flour improved fasting

blood glucose, hepatic glycogen, glycosylated hemoglobin, lipid profile, plasma electrolytes, hepatic and pancreatic malonaldehyde, and the liver function markers of the diabetic rats compared with the diabetic control rats (Irondi et al., 2016). Together, these results indicate that mango peel powder has the potential to function as a therapeutic food component for treatment of diabetes mellitus and management of its complications.

Momordica charantia L.

Momordica charantia L (also known as bitter melon, karela, balsam pear, or bitter gourd) is a member of the *Cucurbitaceae* Juss. Family with a wide array of beneficial bioactive phytochemical compounds. It originated in Africa and is a widely grown and consumed vegetable in Asia, East and Southern Africa, India, and South America. The bioactive phytochemicals present in *Momordica charantia* L. include flavonoids, alkaloids and polyphenols in addition to vitamins and minerals which all contribute to its remarkable versatility in treating a wide range of ailments. The potential of *Momordica charantia* L. to modulate blood glucose has received greater attention from researchers studying natural foods or compounds that are useful in the treatment of diabetes mellitus and have extensively been reviewed (Lucas et al., 2010; Joseph and Jini, 2013; Peter et al., 2019; Liu et al., 2021; Oyelere et al., 2022; Çiçek, 2022). Various parts of the plant have been shown to possess hypoglycemic properties in many animal models and cell-based assays, while a limited number of human clinical trials have been conducted.

In a study investigating the antidiabetic activities of *Momordica charantia* L. on streptozotocin-induced type 2 diabetes mellitus in rats, it was shown that the fruit juice induced a significant increase of serum insulin, HDL-cholesterol, total antioxidant capacity levels, β cell function percent, and pancreatic reduced glutathione (GSH) content and improved histopathological changes of the pancreas (Mahmoud et al., 2017). In addition, *Momordica charantia* L. fruit juice increased glucose uptake by diaphragms of normal and diabetic rats in the absence and presence of insulin (Mahmoud et al., 2017). *Momordica charantia* L. is thought to exert its anti-glycemic property via direct action on β cells of the pancreas and on the intestinal absorption of dietary glucose and amino acids. In a recent study, normal and hyperglycemic Sprague Dawley rats were fed on skin, flesh and whole fruit of *Momordica charantia* L. and assessed for diabetes mellitus prophylaxis and treatment. It was shown that a whole fruit juice resulted in ~31.6% lowering of blood glucose level and ~27.4% increase in insulin level in hyperglycemic rats (Mahwish et al., 2021). In another study, (Mahwish et al., 2017), conclusively showed that skin, flesh and whole fruit of *Momordica charantia* L. significantly increased insulin production and high-density lipoprotein levels with a subsequent decrease in blood glucose, low density lipoprotein and triglycerides in male Sprague Dawley rats. Accordingly, *Momordica charantia* L. possesses hypoglycemic properties and can be a useful additive in the food industry to alleviate medical conditions resulting from complications in blood glucose metabolism.

A double-blind study to compare the antihyperglycemic potential of GlycaCare-II (a herbal formulation of *Momordica charantia* L. and other plants) to metformin drug showed that GlycaCare-II and metformin significantly reduced glycosylated hemoglobin (HbA1c) in prediabetic and newly diagnosed diabetic patients (Majeed et al., 2021). Furthermore, GlycaCare-II showed better results for postprandial blood sugar compared to metformin, while a comparable reduction in fasting blood sugar was observed. These clinical trial results suggested that GlycaCare-II may be effective in managing type 2 diabetes mellitus in prediabetic and diabetic subjects. In clinical trials of twenty-four patients who received *Momordica charantia* L. or placebo for 3 months, it was shown that *Momordica charantia* L. administration reduced glycated hemoglobin A1c, blood glucose, body weight, BMI, fat percentage, and waist circumference, with an increment of insulin AUC, first phase and total insulin secretion (Cortez-Navarrete et al., 2018). Further, a double-blinded, placebo-controlled, clinical trial of twenty-four type 2 diabetic subjects supplied with *Momordica charantia* L. juice or placebo for 3 months showed a reduction in body weight, HbA1c and blood serum glucose, and increase in insulin secretion of the experimental subjects compared to placebo (Cortez-Navarrete et al., 2018). In addition, it was shown in another randomized clinical study that the hypoglycemic effect of *Momordica charantia* L. was weaker than that of glibenclamide but ameliorates the diabetes mellitus associated cardiovascular risk factors more favorably than glibenclamide (Rahman et al., 2015). (Boone et al., 2017) investigated the effect of acute ingestion of a beverage containing *Momordica charantia* L. on blood glucose regulation during an oral glucose tolerance test of ten prediabetic subjects. Interesting, acute ingestion of bitter melon beverage led to the reduction of postprandial glucose in only 50% of the subjects. However, the beverage did not affect insulin response of all subjects. Next, a randomized placebo-controlled single blinded clinical trial of fifty-two prediabetic subjects in Tanzania fed with *Momordica charantia* L. powder led to a decrease in fasting plasma glucose of prediabetic subjects compared to placebo (Krawinkel et al., 2018). A randomized placebo-controlled study to determine the efficacy and safety of *Momordica charantia* L. as an adjuvant treatment in ninety-six Korean subjects with type 2 diabetes mellitus conclusively demonstrated that the HbA1c levels of patients treated with *Momordica charantia* L. and placebo were unchanged, while the fasting glucose levels were

significantly reduced in *Momordica charantia* L. treated group (Kim et al., 2020). The extracts had no adverse effects on the patients. These clinical studies indicate that *Momordica charantia* L. contain bioactive phytochemical compounds with antidiabetic properties capable of ameliorating diabetes mellitus-induced complications in humans.

Annona stenophylla engl. & diels

Annona stenophylla Engl. & Diels is a low-growing perennial plant with woody rhizomes that spread underground with shoots that can rise up to 1 m tall. It is a member of the Annonaceae, custard apple, or soursop family. *Annona stenophylla* Engl. and Diels has been identified in the southern Africa regions including Angola, Botswana, the Democratic Republic of Congo (DRC), Mozambique, Namibia, Zambia, and Zimbabwe. It has been documented to have wide medicinal uses including treatment of abdominal and muscle pains, anemia, malaria, and a range of sexually transmitted diseases among other ailments (Maroyi, 2019). The roots have been used as antidotes and snake repellents (Maroyi, 2019). (Verengai et al., 2017) studied the effects of ethanolic extracts of *Annona stenophylla* Engl. and Diels on alloxan-induced diabetic rats, and observed a dose dependent decrease of plasma glucose levels of the diabetic rats. The most reduction of plasma glucose was achieved by a combination of *Annona stenophylla* Engl. and Diels, *Zingiber officinale* Roscoe, and *Citrus limon* (L.) Osbeck. (Taderera et al., 2019) used muscle cell lines C2Cl2 myocytes to investigate the antidiabetic activity of aqueous root extract of *Annona stenophylla Engl. and Diels* and its mechanism of action. They observed a general dose dependent increase in glucose uptake that was comparable with the positive control (insulin administration). In addition, *Annona stenophylla* Engl. and Diels and insulin resulted in an increase in the transcription of GLUT4 mRNA (an insulin-regulated glucose transporter protein that regulates glucose uptake into fat and muscle cells), when compared to the untreated control (Taderera et al., 2019).

Another study by (Taderera et al., 2016) showed that the root extract of *Annona stenophylla* Engl. and Diels had antidiabetic activity that was comparable with the positive controls (glibenclamide and insulin) with glucose reductions of 45%, 46% and 63% for plant extracts, glibenclamide and insulin respectively. (Taderera et al., 2015) also showed that *Annona stenophylla* Engl. and Diels aqueous root bark extracts inhibited both α-glucosidase and α-amylase enzymes with the inhibition not statistically different from acarbose, the positive control. These results showed that *Annona stenophylla* Engl. and Diels plant extracts had comparable effects on the inhibition of both α-glucosidase and α-amylase enzymes as commercial antidiabetic formulations suggesting their potential as antidiabetic agents.

Why do we need to use crude plant extracts in Africa?

Many valuable African natural plant products have been used for centuries in traditional medicines for the management and treatment of diseases, improvement of human and animal health and nutrition. Africa's wealth of biodiversity and knowledge of indigenous plants and their products represents an area that remains largely unstudied and undocumented. Even though 80% of the population in the developing countries is estimated to use traditional medicines, the knowledge is undocumented and only a few individuals in communities understand the health benefits of the natural products. These include the elderly and traditional healers and the knowledge is usually passed from one generation to the next through oral means and usually depending on the closeness and interest in use of traditional medicines. Poverty in the majority of the populations living in rural areas is the major driving factor for using natural products to prevent and treat human and animal diseases.

The increased interest and trade in natural products are becoming more important to rural African communities as part of income generating activities. Therefore, natural products can serve as a driver in economic development, providing communities with sustainable harvesting and judicious use of the natural resources. A crude (unfractionated) plant extract contains a range of structurally diverse and often novel chemical compounds. This is important in that crude plant products from a single plant may potentially treat or prevent a number of diseases. Biological activity is often detected and attributed to a single compound or a set of related compounds produced by the plant. Natural products play a vital role in improving human health and have been drugs of choice despite tough competition from modern medicines due to their safety and efficacy.

Nowadays, there is a growing number of companies that engage in packaging and commercialization of crude plant extracts. These products are relatively cheaper compared to modern drugs and have been extensively marketed in Africa including herbal teas such as zumbani (*Lippia javanica* (Burm.f.) Spreng.) tea in Zimbabwe (Mfengu et al., 2021). COFSOL is a cough medicine made from *Lippia javanica* (Burm.f), produced and marketed in Zimbabwe. Imbiza (a mixture of *Cyrtanthus obliquus* (L.f.) Aiton and *Lippia javanica* (Burm.f.) Spreng.) is a herbal tonic commonly used in South Africa to boost the immune system, and for the treatment of chronic diseases (Mahlangeni et al., 2018). In addition, herbal products such as diabecon have been formulated with natural herbs that help reduce excessive blood sugar and decrease hepatic glucose production and prevent hyperglycemia. Thus, diabecon formulation is relatively cheaper than modern drugs and is very effective in the management of type II diabetes mellitus. Accordingly, making herbal formulations from a combination of crude plant extracts could provide a means to prevent and cure

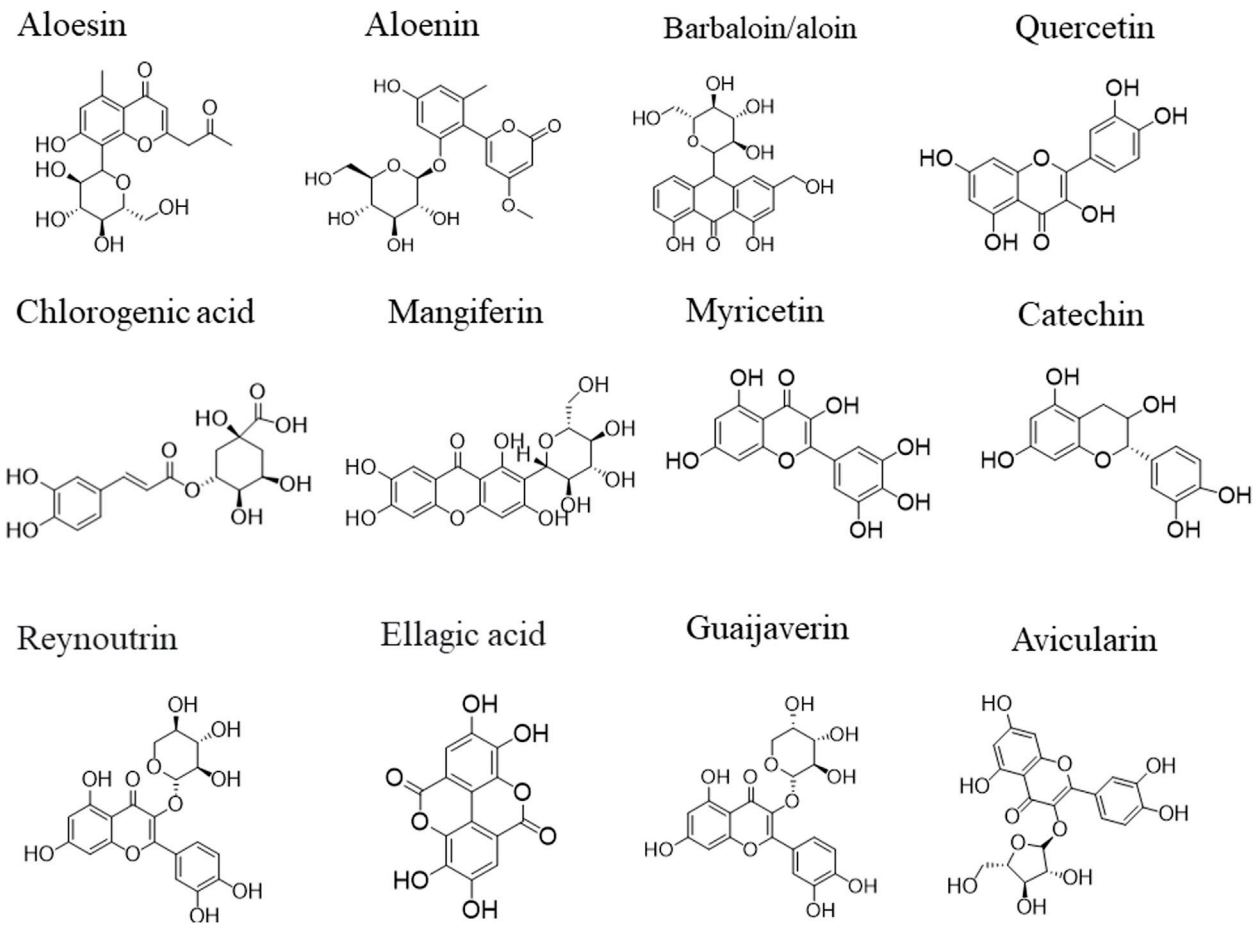

FIGURE 2
Selected structures of isolated phytochemical compounds with antioxidant and antidiabetic properties found in medicinal plants of Zimbabwe.

diabetes mellitus in many parts of the world where modern drugs are expensive.

Future directions

Although a number of techniques are available to characterize phytochemical compounds, their precise identification in crude plant extracts is generally complex because they contain a wide variety of structures. In view of this, many people in developing nations have limited capacity to study medicinal plants before using them for medical purposes. Therefore, conducting research covering the toxicity of crude plant extracts may provide a way to inform people of the health risks associated with use of certain medicinal plants. Thus, before packaging crude extracts and selling them on streets (usually common in many African markets), it is important for governments in developing countries to implement rules and strategies that assess the toxicity of these medicinal plants.

A number of phytochemical compounds with antidiabetic properties have been identified using structural tools such as gas chromatography mass spectroscopy (GC MS), liquid chromatography mass spectroscopy (LC MS) or tandem mass spectroscopy (LC MS/MS) and are becoming available in many natural product databases/libraries (Figure 2). In many cases, these compounds have not been fully screened or evaluated for antidiabetic properties. Thus, systematic *in vitro* and *in vivo* studies are needed to evaluate the antidiabetic potential of these compounds guided by computational tools. A variety of powerful computational techniques including protein-ligand docking and virtual drug screening with Autodock tools or Schrödinger Maestro tools have successfully been employed to screen for novel drug leads in many libraries. Thus, future screening of antidiabetic compounds in natural product libraries/databases with computational tools such as Schrödinger Maestro tools and Autodock tools and evaluating their bioactive synergistic characteristics *in vitro* on digestive enzymes (α-amylase or α-glucosidase), GLUT4, GLUT2 and other enzymes involved in

regulation of blood glucose can unveil novel compounds for diabetes mellitus treatment. In addition, *in vivo* experiments with novel compounds on humans or animal models can lead to discovery of novel plant-based drug leads for treatment and management of diabetes mellitus. These novel drugs can also be used as prototypes to develop more effective and less toxic drugs for diabetes mellitus treatment by medicinal chemists.

Additionally, there is a growing list of undocumented medicinal plants such as *Xeroderris stuhlmannii* (Taub.) Mendonça & E.P. Sousa that are used frequently to control or treat diabetes mellitus (Selemani et al., 2021). In view of this, it is important for governments to provide funds for research that promotes the use of medicinal herbs to combat many emerging pathogens and diabetes mellitus complications. Finally, even though a number of modern drugs show good hypoglycemic activities, they are often associated with several complications such as nephrological disorders, fatigue, upset stomach and diarrhea. Thus, future work on promoting and regulating research on pharmacological importance of medicinal plants with antihyperglycemic properties and allowing for proper administration of correct doses for crude extracts can be beneficial for ameliorating the various complications associated with diabetes mellitus.

Conclusion

Awareness about the role of medicinal plants in treatment and prevention of diabetes mellitus will improve health care of many rural populations that rely on herbal remedies for disease control. The use of plants for diabetes mellitus treatment provides an alternative to synthetic drugs as they can be sourced easily and cheaply. Plants contain complex bioactive phytochemical compounds such as polyphenols and flavonoids with many natural bioactive principles and fewer side effects. Thus, determining how these bioactive phytochemicals interact in the human body is important in diabetes mellitus treatment. Therefore, research on this topic may open pathways leading to discovery of new plant derived-drugs, and regularization of use of plant remedies to treat diabetes mellitus by governments across Africa and the world at large.

Author contributions

FR, RU, and SM were involved in concept development. All authors contributed equally in writing the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Yongsheng Chen,
Jinan University, China

REVIEWED BY
Liu Tang,
Renmin Hospital of Wuhan University, China
Yuba Raj Pokharel,
South Asian University, India

*CORRESPONDENCE
Zhengqiang Yuan,
yuanzq@gdut.edu.cn
Zhiyun Du,
zhiyundu@gdut.edu.cn

†These authors have contributed equally to this work

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Tremella fuciformis polysaccharides alleviate induced atopic dermatitis in mice by regulating immune response and gut microbiota

Lingna Xie[1,2†], Kaiye Yang[3†], Yiheng Liang[1,2], Zhenyuan Zhu[4,5], Zhengqiang Yuan[1*] and Zhiyun Du[1*]

[1]School of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou, China, [2]Guangdong Provincial Key Laboratory of Plant Resources Biorefinery, Guangdong University of Technology, Guangzhou, China, [3]Infinitus Company Ltd., Guangzhou, China, [4]State Key Laboratory of Food Nutrition and Safety, Tianjin University of Science and Technology, Tianjin, China, [5]College of Food Science and Engineering, Tianjin University of Science and Technology, Tianjin, China

Atopic dermatitis (AD), characterized by severe pruritus, immune imbalance, and skin barrier dysfunction, has a high incidence worldwide. Recent evidence has shown that the modulation of gut microbiota is crucial for alleviating clinical symptoms of AD. Tremella fuciformis polysaccharides (TFPS) have been demonstrated to have a variety of biological activities such as immunomodulatory, anti-tumor, antioxidant, anti-inflammatory, neuroprotective, hypoglycemic and hypolipidemic effects. However, their effects on AD treatment have never been investigated. In this study, we compared the therapeutic effects of topical or oral administration of TFPS on AD in dinitrofluorobenzene (DNFB)-induced AD mice. Both topical application and oral administration of TFPS led to improvement on transdermal water loss, epidermal thickening, and ear edema in AD mice, but the oral administration showed significantly better efficacy than the topical application. The TFPS treatment increased the proportion of CD4 (+) CD25 (+) Foxp3 (+) regulatory T cells in mesenteric lymph nodes. Additionally, the non-targeted metabolomics and sequencing of 16S rDNA amplicons were performed, revealing metabolite modulation in feces and changed composition of gut microbiota in mice, which were induced for AD-like disorder and treated by oral administration of TFPS. Collectively, these data suggest that the oral administration of TFPS may constitute a novel effective therapy for AD, with underlying mechanisms associated with the regulation of immune response, and improvement of both metabolism and the composition of intestinal microbiota.

KEYWORDS

Tremella fuciformis **polysaccharides, atopic dermatitis, immuno-modulatory, microbiota, metabolism**

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease, which is characterized by recurrent pruritus, dryness, eczematous skin lesions, and increasing incidence worldwide (Jin et al., 2020; Langan et al., 2020; Li et al., 2021). The pathogenesis of AD is associated with a variety of causes, such as an imbalance of Th1/Th2 responses (Stone et al., 1976), function defects of keratinocytes, elevated serum immunoglobulin E (Ig E), disturbed metabolism, and abnormal apoptosis of eosinophils. Topical application of corticosteroid creams is the common treatment for AD, and the systemic administration of immunosuppressant drugs or phototherapy (UV light) may be needed for reducing severe condition of the disease (Thomsen, 2014). However, these therapies cannot cure the disease, currently only aiming to alleviate its severity and duration. More effective therapeutic agents or treatment strategies are thus needed.

Normal skin can prevent antigens from entering the body and causing an inflammatory reaction because of the skin barrier function (Yang et al., 2020). However, skin barrier dysfunction in AD patients leads to enhanced penetration of allergens and transdermal infection of microorganisms, and damages to the stratum corneum of skin often leads to increased transepidermal water loss (TEWL) (Elias, 2005; Elias et al., 2008). Pro-inflammatory cytokines such as thymic stromal lipoprotein (TSLP), interleukin (IL)-25, and IL-33 can activate Th2 cells, which dominate early in the disease process and express cytokines such as IL-4 and IL-13 at high levels, leading to increase of IgE (Gandhi et al., 2016; Weidinger et al., 2018). In contrast, activation of Th1- and TH17-mediated responses have been reported in the development of chronic lesions in AD (Brunner et al., 2017).

The gut microbiota and its biochemical responses have an impact on many aspects of host health, including metabolism, immunity, development, and behavior (Sommer and Bäckhed, 2013; Belkaid and Hand, 2014; Collins, 2014; Behera et al., 2020). Gut microbes may play a role in the development of AD by regulating the immune system through interactions between the microbes and the host, and in particular, alterations in the gut microbiota affect immune system homeostasis by altering the production of metabolites. Several studies have demonstrated that the development of AD can be prevented by modulating the intestinal flora and metabolites (Fang et al., 2020; Kim et al., 2020; Chun et al., 2021).

Tremella fuciformis is a widely cultivated edible fungus in China. *T. fuciformis* polysaccharides (TFPS) has been identified as its main active ingredient. TFPS has a wide range of biological activities, including anticancer, antioxidant, anti-aging, and regulation on blood glucose and lipid. Recently, *T. fuciformis* polysaccharides (mainly 1 → 3-α-D-mannans) have been shown to alleviate dextran sodium sulfate (DSS)-induced colitis in mice through immunomodulation and restoration of intestinal microbiota and metabolites (Xu et al., 2021a). A recent study has revealed that TFPS was stable under simulated digestive conditions and could be utilized by the human fecal gut microbiota to increase short-chain fatty acid production (Wu et al., 2022). However, the TFPS potential for AD treatment has never been examined. It thus would be interesting to find out if TFPS can be used to alleviate AD or even cure the disease.

In this study, we assessed the therapeutic effect of TFPS on AD in a mouse AD model, and explored the possible action mechanisms by examining the effects of TFPS on Treg cells, gut microbiota, and gut microbes-derived metabolite composition in mice.

Materials and methods

Preparation of *Tremella fuciformis* polysaccharides

The TFPS used in this study was extracted from *T. fuciformis* by hot water extraction and ethanol precipitation. Briefly, *T. fuciformis* was ground into powder, mixed with 40 times of distilled water (W/W), and extracted in a 96°C water bath for 4 h. The supernatant was concentrated and precipitated with 75% ethanol. The crude polysaccharide extract was deproteinated by the Sevag method, and subsequently freezing-dried for further analysis.

Composition analysis of *Tremella fuciformis* polysaccharides

The TFPS samples were hydrolyzed by trifluoroacetic acid to monosaccharide solution, and the composition was determined by using a Bruker Scion TQ triple quadruple mass spectrometer (Bruker, Fremont, California). Xylose, mannose, fucose, rhamnose, glucose, arabinose, and galactose were purchased (Sigma Aldrich, United States)and used as monosaccharide standards and identified according to the characteristic retention times.

Establishment of dinitrofluorobenzene-induced allergic dermatitis in Babl/c mice

Six-week-old female Babl/c mice were purchased from the Experimental Animal Center in Sun Yat-sen University and fed libitum for 1 week before the experiment. After shaving hair on dorsal skin of mice, 0.25% (w/v) dinitrofluorobenzene (DNFB) in acetone and olive oil (3:1) was applied to both dorsal skin and ears of mice on day 1 and day4 at 100 μl/mouse and 25 μl/mouse,

respectively. On day 7 and 10, animal dorsal and ear skins were further treated with 100 and 25 μl of 0.2% (w/v) DNFB, respectively. Balb/c mice were randomly divided into 8 experimental groups, including normal group (control), DNFB treatment group, 50 mg/kg prednidolone (PD) treatment group (including topical or oral administration of PD), 50 mg/kg TFPS treatment group (topical or oral administration of TFPS) and 200 mg/kg TFPS treatment group (topical or oral administration of TFPS). For the topical administration study, 100 μl of 12.5 mg/ml PD (50 mg/kg PD treatment group), 12.5 mg/ml TFPS (50 mg/kg TFPS treatment group) or 50 mg/ml TFPS (200 mg/kg TFPS treatment group) were applied to the animal dorsal skin, respectively. Neither normal group (control) nor the DNFB induction group was given any therapeutic treatments. Drug treatment was performed once a day and the epidermal recovery was examined. Treatment was carried out from day 5 to day 14. Animal feces were collected on day 15, and stored at −80°C; the dorsal skin was excised and stored at −80°C for further analysis.

Evaluation of the severity of dermatitis in mice

The contact between the DNFB and the mouse ear skin stimulated itches making animals scratch. The intenser the itches, the more frequent scratching is observed. Skin symptoms were recorded on day 15. The severities of skin lesions were evaluated macroscopically as 4 levels: 0, no symptoms; 1, mild; 2, moderate; and 3, severe using the previously described scoring standards of atopic/eczema dermatitis syndrome concerning dryness/scaling, erythema/hemorrhage, and excoriation/erosion (Kang et al., 2017).

Measurement of transepidermal water loss rates in dermatitis model mice

Transepidermal water loss (TEWL) rates on the dorsal skin of Babl/c mice were measured with the use of Multi Probe Adapter MCP 2 (CK, Germany), and the TEWL was recorded on day 5, day 8, day 11, and day 14, respectively.

H&E staining

The skin tissue samples from different groups were immobilized in 4% formalin and embedded in paraffin. Each sample was deparaffined using xylene, rehydrated with a series of gradient alcohols, and stained with hematoxylin and eosin. Images were collected using a Nikon optical microscope (Japan) equipped with an eyepiece micrometer. All procedures are in accordance with the manufacturer's guidelines.

Immunohistochemistry

For immunohistochemical (IHC) staining, the fixed and paraffin-embedded skin samples were deparaffined, autoclaved, and heat-treated to recover antigens in citrate saline buffer. Tumor necrosis factor (TNF)-α and interferon (IFN)-γ were examined for expression in skin tissues by IHC. Stained sections were examined and imaged by using a Nikon optical microscope (Japan) equipped with an eyepiece micrometer under the ×200 magnification.

Enzyme-linked immunosorbent assay

The concentration of IgE in serum was determined by the ELISA assay. A mouse IgE ELISA assay kit was purchased (Jiangsu Meibiao Biological Technology Co. Ltd., Jiangsu, China) and used to detect the serum IgE in each test group following the manufacturer's instructions.

Flow cytometry analysis

Mesenteric lymph nodes were isolated from each group of mice, and lymphocytes were isolated by a mouse lymphocyte isolation kit. Lymphocyte suspensions were prepared and adjusted to 2.0×10^6 cells per vial in RPMI 1640 medium containing 10% fetal bovine serum (FBS). Then prepared cells were labelled for 20 min in ice bath using the fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD4 antibody (ab), phycoerythrin (PE)-conjugated anti-mouse CD25 ab, and allophycocyanin (APC)-conjugated anti-mouse Fork head box (Fox) P3 ab. The labeled samples were analyzed by flow cytometry (FlowSight, Merck Millipore), and data was analyzed using FlowJo software (Tree Star Inc. Ashland, OR, United States).

16S rDNA amplicon sequencing

Microbial community genomic DNA was extracted from feces samples using the E. Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The DNA extract was checked on 1% agarose gel, and DNA concentration and purity were determined with NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, United States). The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, United States). The PCR amplification of the 16S rRNA gene was performed as follows:

initial denaturation at 95°C for 3 min, followed by 27 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72 °Cfor 45 s, and single extension at 72°C for 10 min, and end at 4°C. The PCR mixtures contain 5 × TransStart FastPfu buffer 4 μl, 2.5 mM dNTPs 2 μl, forward primer (5 μM) 0.8 μl, reverse primer (5 μM) 0.8 μl, TransStart FastPfu DNA Polymerase 0.4 μl, template DNA 10 ng, and finally ddH2O up to 20 μl. PCR reactions were performed in triplicate. The PCR product was extracted from 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, United States) according to the manufacturer's instructions and quantified using Quantus™ Fluorometer (Promega, United States).

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, United States) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database.

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by fastp version 0.20.0 (Chen et al., 2018), and merged by FLASH version 1.2.7 (Chen et al., 2018) with the following criteria: 1) the 300 bp reads were truncated at any site receiving an average quality score of <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded; 2) only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region is 0.2. Reads that could not be assembled were discarded; 3) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatches in primer matching.

Operational taxonomic units (OTUs) with a 97% similarity cutoff (Stackebrandt and GOEBEL, 1994; Edgar, 2013)were clustered using UPARSE version 7.1, and chimeric sequences were identified and removed. The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 (Wang et al., 2007) against the 16S rRNA database (eg., Silva v138) using a confidence threshold of 0.7.

Metabolomics profiling of feces samples

Before analysis, the feces samples were thawed at 4°C, diluted (80 mg of feces in 200 μl of purified water), vortex-shocked for 20 s, and then centrifuged at 13,000 g for 10 min at 4°C, and the resulting supernatant was transferred to an EP tube. To the precipitate obtained by centrifugation, 200 μl of methanol was added, shaken and mixed, and centrifuged at 13,000 g for 10 min at 4°C, and the resulting supernatant was again added to the EP tube. To the precipitate obtained by centrifugation, 200 μl of acetonitrile was added, shaken, and mixed, and centrifuged at 13,000 g for 10 min at 4°C. The supernatant was again added to the EP tube described above. The mixture in the EP tube was shaken well, centrifuged at 13,000 g for 10 min at 4°C, and passed through a 0.22 microporous membrane in preparation for the HRAM LC-MS/MS analysis. A 20 μl volume from each sample supernatant was mixed to prepare quality control samples.

A Thermo Scientific Ultimate 3000 RSLC and a Q Exactive Orbitrap desktop high-resolution mass spectrometer was used to carry out the HRAM LC-MS/MS analysis. Thermo Scientific Xcalibur was used for data collection, and Compound Discoverer 2.1, mzCloud database (Thermo Scientific, HTTP://www.mzcloud.org) was used for data analysis. The data was analyzed using Compound Discoverer 2.1 to measure the statistical differences of metabolites between each group. MetaboAnalyst (https://www.metaboanalyst.ca/) was used to enrich the metabolic pathways of significantly different metabolites.

Analysis of short-chain fatty acids

Two hundred mg of mice fecal sample was extracted with 5 ml of 70% isopropanol and then subjected to 3-NPH derivatization. Standard short-chain fatty acid (SCFA) solutions (acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, and hexanoic acid) were run at 6 different concentrations. The data were analyzed using Multiquant (SCIEX). Concentrations of SCFA were normalized to the weight of fecal sample (mg).

A Thermo Scientific Ultimate 3000 RSLC that is coupled to a 4000 QTRAP triple quadrupole mass spectrometer (AB Sciex, Concord, ON, Canada) equipped with an ESI source and operating in negative ion mode was used. Chromatographic separations were performed on a BEH C_{18} (2.1 × 100 mm, 1.7 μm, Waters) UPLC column using water: formic acid (100: 0.01, v/v; solvent A) and methanol: formic acid (100:0.01, v/v; solvent B) as the mobile phases for gradient elution. The column flow rate was 0.35 ml/min; the column temperature was set at 50°C and the autosampler was kept at 5°C.

Statistical analysis

Quantitation data were presented as means ± SD, and differences between the three groups were analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test. A correlation analysis was used to analyze the correlation between changes of intestinal flora composition and metabolite levels, and the Pearson correlation coefficient was selected. A p-value < 0.05 was considered statistically significant.

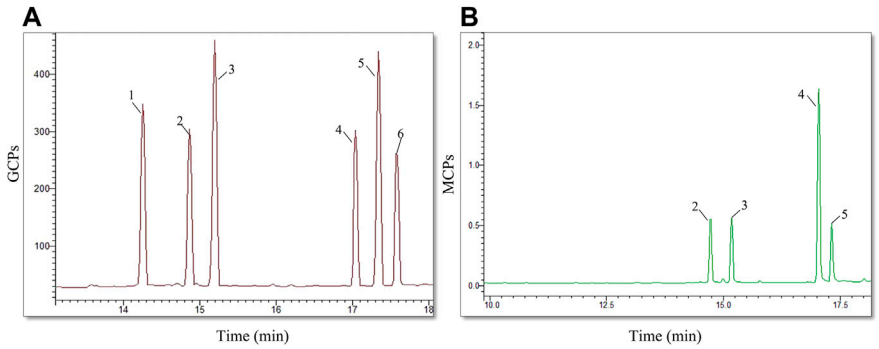

FIGURE 1
The monosaccharide composition analysis. **(A)**The chromatogram of mixed monosaccharide standards, showing a composition of rhamnose (1), arabia sugar (2), xylose (3), mannose (4), glucose (5), galactose (6). **(B)** The chromatogram of TFPS hydrolysate shows the monosaccharide composition of arabia sugar (2), xylose (3), mannose (4), and glucose (5).

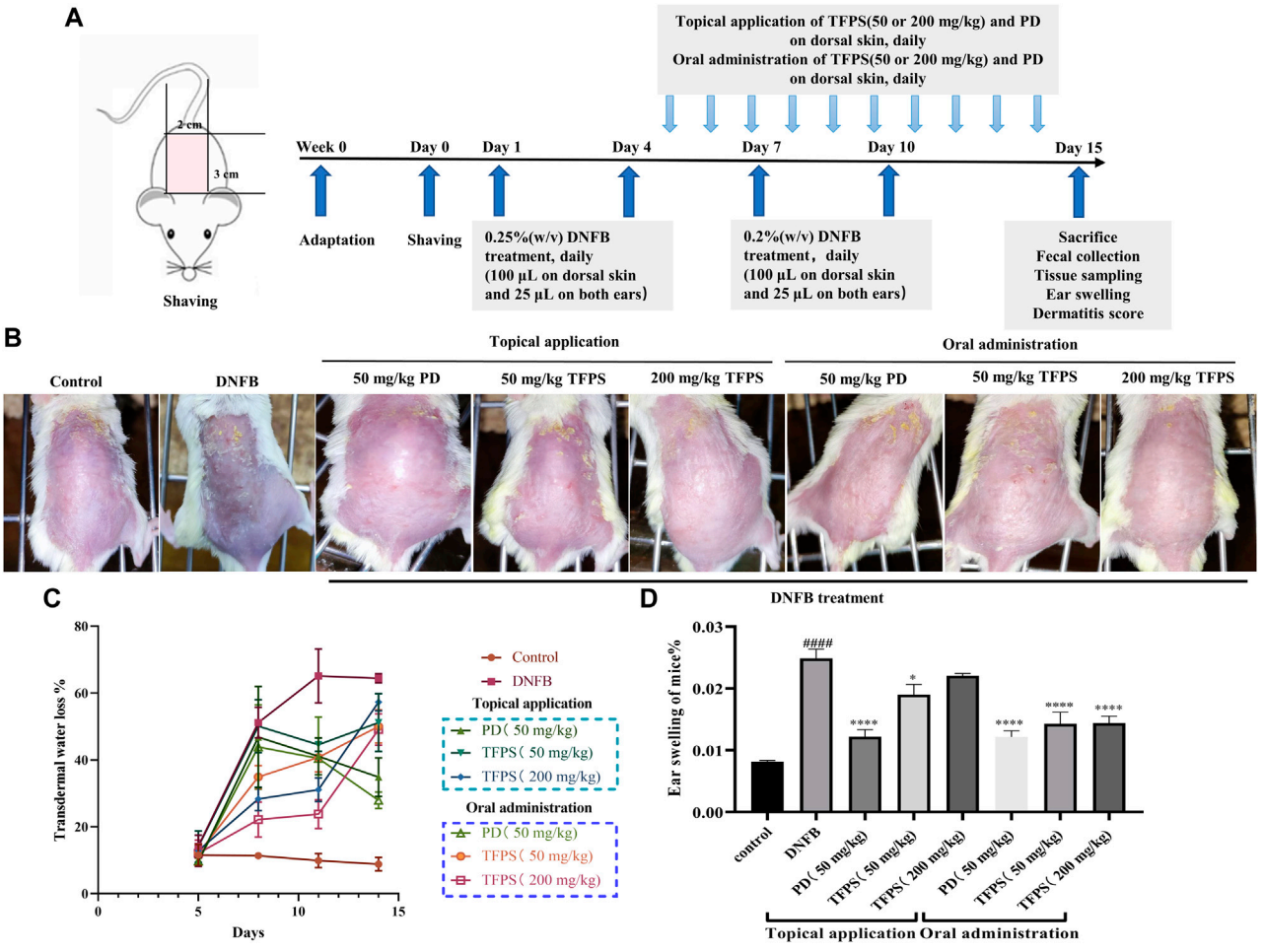

FIGURE 2
Improvement of AD-like symptoms by TFPS treatment in a murine AD model. **(A)***In vivo* experimental schedule is shown. Babl/c mice were divided into eight groups: normal control (Control group), DNFB + PBS (DNFB group), 50 mg/kg PD group (including topical or oral administration of PD), 50 mg/kg TFPS treatment group (including topical or oral administration of TFPS) and 200 mg/kg TFPS treatment group (including topical or oral administration of TFPS). To study the effect of TFPS on atopic dermatitis, mice were treated with DNFB on day 1, day 4, day 7, and day 10, then topical or oral administration of TFPS for 10 days after the DNFB treatment. **(B)** The mouse taken on the 14th day is rephotographed. **(C)**The TEWL was tested by electrolyzed water analysis on day 5, day 8, day 11, and day 14. **(D)** Animals were culled and animal ears were removed for punch weighing on day 15. All values are presented as means ± S.E.M (n = 3). ###$p < 0.001$, compared with Control group. *$p < 0.05$, ***$p < 0.0001$, compared with DNFB group.

Results

Preparation and characterization of *Tremella fuciformis* polysaccharides

The Tremella fuciformis mushroom was ground and extracted for polysaccharide preparation in hot distilled water. After deproteinization, the extracted TFPS fraction was measured by high-performance gel permeation chromatography (HPGPC), revealing molecular weights ranging from 30 to 1,230 kDa. This indicates that the prepared product is not a single polysaccharide, but a mixture of different polysaccharides. Subsequently, the extracted TFPS was hydrolyzed in trifluoroacetic acid and analyzed for monosaccharide composition by mass spectrometry. As shown in Figure 1, the prepared TFPS is composed of xylose, mannose, arabia sugar and glucose at a molar ratio of 1.3:3.4:1.0:1.0.

Tremella fuciformis polysaccharides is effective for attenuating the development of dinitrofluorobenzene-induced Atopic dermatitis-like symptoms in mice

The therapeutic efficacy of TFPS was evaluated in a DNFB-induced murine AD model. As scheduled in Figure 2A, DNFB was applied on mice dorsal and ear skins to induce AD-like symptom. As shown in Figure 2B, DNFB successfully stimulated AD-like symptoms, including erythema/bleeding, edema, shedding/erosive, and dry/desquamation. One previous study had used 40 mg/kg of prednisolone for the treatment of contact dermatitis, which showed effective and no significant toxic side effects (Takeuchi et al., 2010). Thus, a similar dose of prednisolone (50 mg/kg) was tested in this study. Previously, Xu et al. have demonstrated the good tolerance of oral administration of 200–400 mg/kg TFPS (Xu et al., 2021a), thus 50 mg/kg and 200 mg/kg of TFPS treatment were tested for AD treatment, respectively, in this study.

Of note, both TFPS and positive drug (PD) prednidolone treatments attenuated the development of AD condition on mice. The measurement of transepidermal water loss (TEWL) was performed in dorsal skin of mice to assess therapeutic efficacies. The obtained results showed that the DNFB stimulation caused significant increase of TEWL (Figure 2C). However, both topical application and oral administration of TFPS successfully attenuated the TEWL of dorsal skin. Moreover, mice were sacrificed on day 15, and animal ears were removed, punched and weighed to examine the effect of TFPS treatment on reducing the severity of ear swelling induced by DNFB (Figure 2D). The significant increase of ear weight in DNFB group suggested that the severe ear swelling was induced in mice by the DNFB irritation. Interestingly, both topical application and oral administration of TFPS were effective for reducing the ear swelling. However, the oral administration of TFPS appeared more effective than topical application. Collectively, these data demonstrated that TFPS treatment was effective for improving the DNFB-induced AD-like symptoms in mice.

Tremella fuciformis polysaccharides treatment suppressed dinitrofluorobenzene-induced epidermal thickening and mast cell infiltration in mice

Next the effects of TFPS on skin barrier function were examined. The H&E staining of animal ear and dorsal skins revealed evident epidermal hyperkeratosis, thickened stratum corneum, and severe AD-like lesions induced by DNFB irritation in mice (Figures 3A,C). Notably, both topical application and oral administration of TFPS significantly reduced the DNFB-induced epidermal thickening, with comparable effectiveness with the positive control drug PD (Figures 3A–D). Importantly, the oral administration of TFPS appeared better reduction of epidermal thickening than the topical application of TFPS at the dose of 200 mg/kg. Additionally, mast cell infiltration was investigated by toluidine blue staining, revealing that the DNFB irritation induced significant mast cell infiltration compared with the normal control (Figure 3E). Interestingly, like the PD drug, the TFPS treatment by either topical application or oral administration showed significant suppression on the mast cell infiltration (Figures 3E,F). These observations indicate that TFPS can improve the skin barrier function and thus is beneficial for AD treatment.

Tremella fuciformis polysaccharides treatment reduced the inflammatory response in dinitrofluorobenzene-induced mice

To detect cytokine levels in dorsal skin, we performed immunohistochemistry assessment on TNF-α and IFN-γ (Figure 4A), respectively, and the mean integral optical density (IOD) of TNF-α and IFN-γ IHC were shown in Figures 4B,C. The obtained results showed that TFPS treatment significantly reduced TNF-α and IFN-γ expression in DNFB-induced AD mice. In addition, animal serum IgE expression was found to be significantly upregulated in AD mice by ELISA comparing with that in control group (Figure 4D). However, the IgE level was significantly reduced after TFPS treatment in the AD mice, indicating that the TFPS treatment could alleviate DNFB-induced inflammatory responses.

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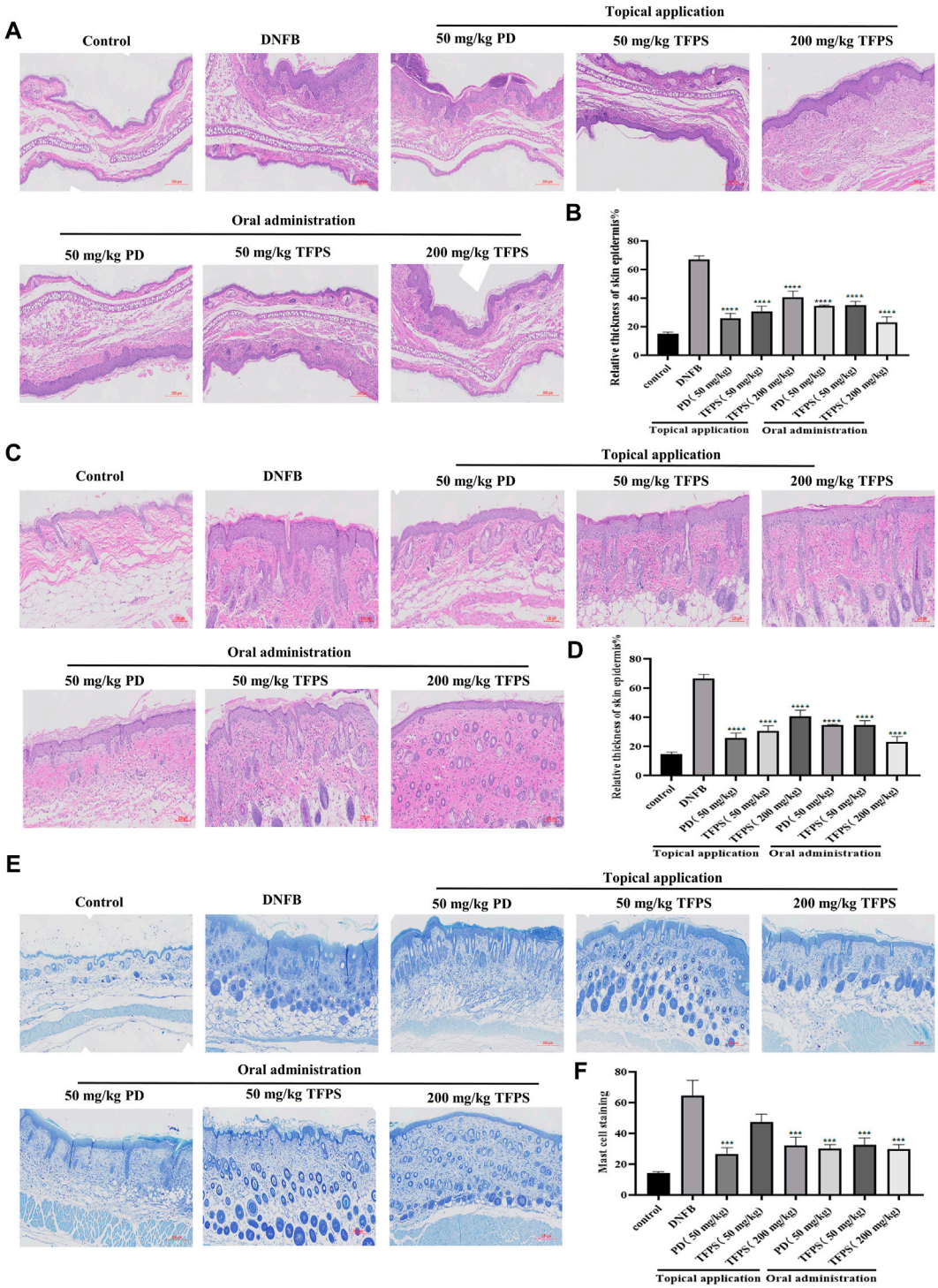

FIGURE 3
Effects of TFPS on the histomorphological changes in mice with DNFB induced AD-like symptoms. **(A)** HE stained section of ear tissue (magnification ×300) **(B)** The measurement of ear skin epidermal thickness in different experimental groups is presented as relative to that of control group, for which the value is set as 100%. **(C)** HE staining of dorsal skin tissues (magnification ×400) **(D)** The measurement of dorsal skin epidermal thickness in experimental groups is presented as relative to that of the control group, for which the value is set as 100%. **(E)** Staining of mast cells in the dorsal skin tissue (magnification ×300) **(F)** Calculate the number of mast cells at random locations. All values are represented as means ± S.E.M (n = 3). ###p < 0.001, compared with the control group. *p < 0.05, **p < 0.01, ***p < 0.001, compared with the DNFB group.

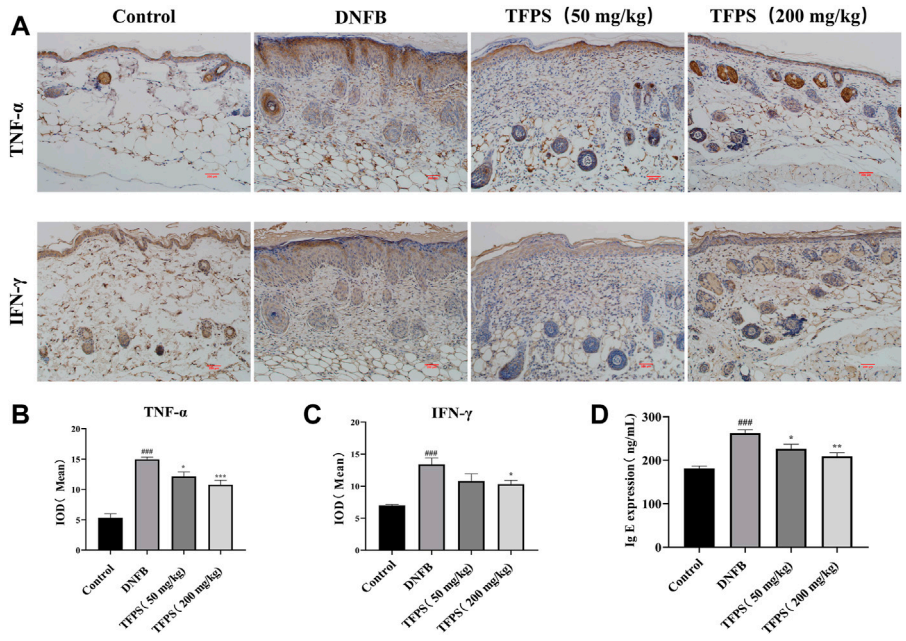

FIGURE 4
Effect of oral administration of TFPS on cytokine expression in the dorsal skin of AD mice. **(A)** The IHC results of TNF-α and IFN-γ in kidney of Control, DNFB and TFPS(50 or 200 mg/kg) groups (magnification ×200) **(B)** Mean IOD of TNF-α group. **(C)** Mean IOD of IFN-γ IHC in each group. **(D)** Determination of serum IgE level in each test group. All values are represented as means ± S.E.M (n = 3). ###p < 0.001, compared with Control group. *p < 0.05, **p < 0.01, ***p < 0.001, compared with DNFB group.

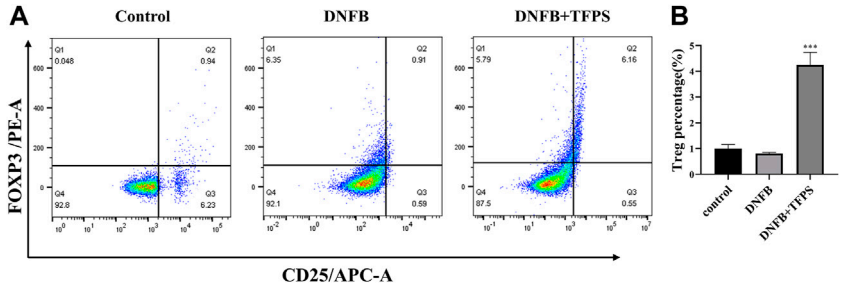

FIGURE 5
Effects of oral administration of TFPS on Treg number in mesenteric lymph nodes (MLNs) of AD mice. **(A)** The MLN cells were isolated from babl/c mice treated by vehicle control, DNFB, or DNFB plus 200 mg/kg TFPS. **(B)** The number of CD4+ CD25+ Foxp3+ cells was calculated based on the percentage of total MLN cell counts. All values are represented as means ± S.E.M (n = 3). ***p < 0.001, compared with the DNFB group.

Tremella fuciformis polysaccharides treatment increases the treg cell population in the mesenteric lymph nodes

The CD4 (+) CD25 (+) FOXP3 (+) regulatory T (Treg) cells stem from the further differentiation of CD4 (+) T cells, and play essential roles in the regulation of excessive immunity and maintenance of immune tolerance. The pathogenesis of AD is not fully understood yet and it is not known if Treg cells participate in the immune modulation of AD. To address this issue, lymphocytes were isolated from animal mesenteric lymph nodes, immuno labelled and analyzed for Treg ratio to CD4 (+) T lymphocytes by flowcytometry.

As shown in Figures 5A,B, the DNFB stimulation slightly downregulated the Treg ratio in mice compared with normal control. However, oral administration of TFPS (200 mg/kg) significantly upregulated the Treg ratio in DNFB-induced AD

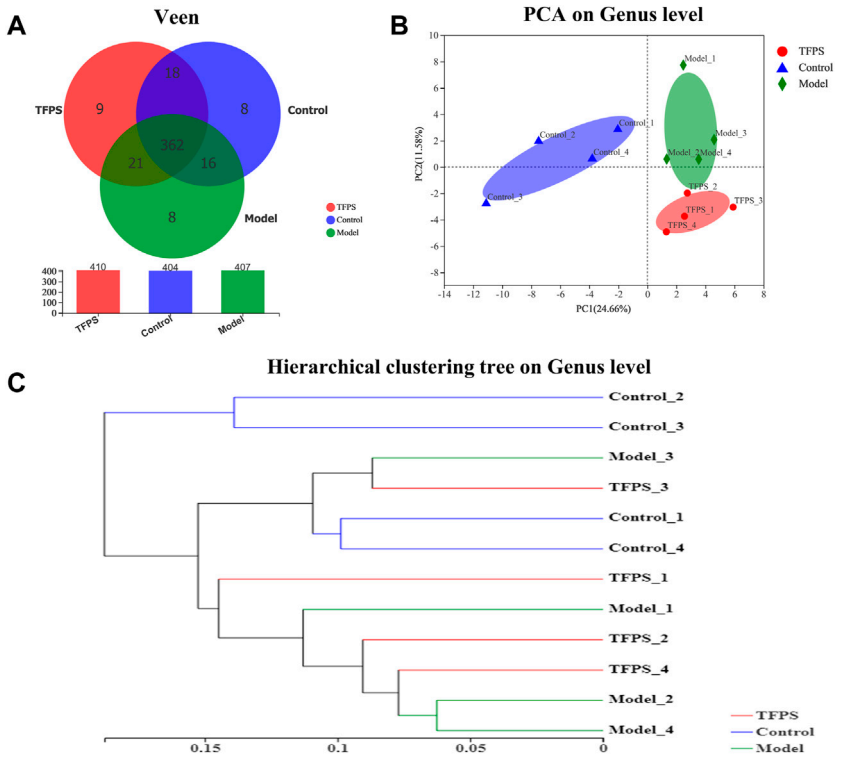

FIGURE 6
TFPS relieves DNFB-induced microbiota changes in AD-like mice. **(A)**Venn diagrams of OTUs. **(B)** PCA plot with unweighted UniFrac. **(C)** UPGMA clustering results based on unweighted UniFrac distance of microbial 16S rRNA sequences from animal feces samples.

mice. This observation suggests that TFPS may attenuate AD development via increasing the number of Treg cells to modulate excessive immune responses.

The influence of *Tremella fuciformis* polysaccharides on the taxonomic composition of the gut microbiota

The dynamic interaction of the gut microbiota with other microorganisms and the host is vital to the health of the host. We conducted 16s rRNA sequencing analysis on mouse feces to study the effect of TFPS on the composition of the intestinal microbiota in DNFB-induced AD mice. We obtained a total of 495,630 high-quality 16s rRNA reads. After diluting the samples to the same sequencing depth, the minimum sequence number is 26,008. In three groups of samples, the bacteria belonged to 9 phyla, 14 classes, 31 orders, 48 families, 107 genera, 175 species and 442 OTUs were identified. A Venn diagram was constructed to examine the existence of OTUs with a relative abundance >0.1% in each group (Figure 6A). Most OTUs (362 in all) were shared by all three groups. However, a total of 18 OTUs were specifically shared by the control and the TFPS treatment groups. Additionally, a total of 21 OTUs were shared by only the control and the model groups, and 16 OTUs were shared by only the model and the TFPS treatment groups. In total, the unique OUTs of the control group, model group, and TFPS group were 8, 8, and 9, respectively.

TABLE 1 Effects of TFPS on the *a*-diversity index of gut microbiota.

	Control	Model	TFPS
Shannon	2.872 ± 0.090	2.602 ± 0.018	2.533 ± 0.053
Simpson	0.105 ± 0.011	0.130 ± 0.004	0.166 ± 0.017
Ace	91.513 ± 3.081	92.001 ± 1.273	89.299 ± 0.765
Chao 1	88.900 ± 2.456	89.433 ± 1.402	87.621 ± 1.218

The intestinal microbiota diversity of the control group, the model group, and the TFPS group is shown in Table 1. We observed no significant difference in the chao1 index or the Simpson index among the three groups. In order to evaluate the changes in intestinal flora *ß* diversity between different groups, principal coordinate analysis based on unweighted uniform

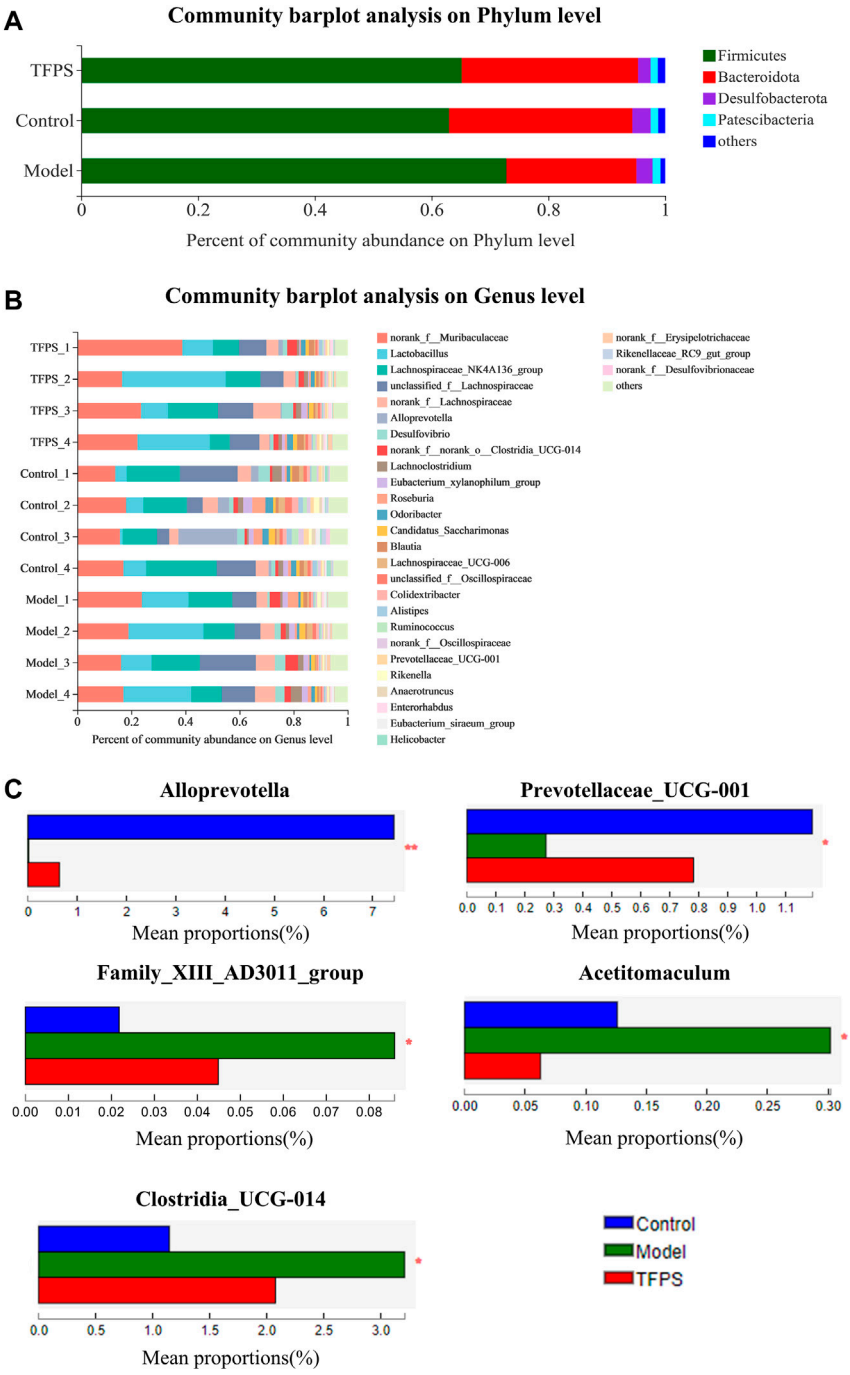

FIGURE 7
(A) Effects of TFPS on the relative abundance of gut microbiota at the phylum level. **(B)** Relative abundance of gut microbiota at the genus level. **(C)** Comparison of the relative abundance of significantly different microbial groups at the genus level. The Mann-Whitney *U* test was used for statistical analysis, and the false discovery rate (FDR)-corrected, compared with Model group (N = 4 mice per group). *, $p < 0.05$, **, $p < 0.01$.

distance matrix analysis was performed (Figure 6B). The PCA results showed a significant separation of bacterial composition in the control group, model group, and TFPS group, and there were significant differences in bacterial communities in the different groups. These findings suggested that TFPS can improve the intestinal flora composition of AD-like mice.

The UPGMA cluster analysis was performed using the unweighted UniFrac method, and the obtained results were

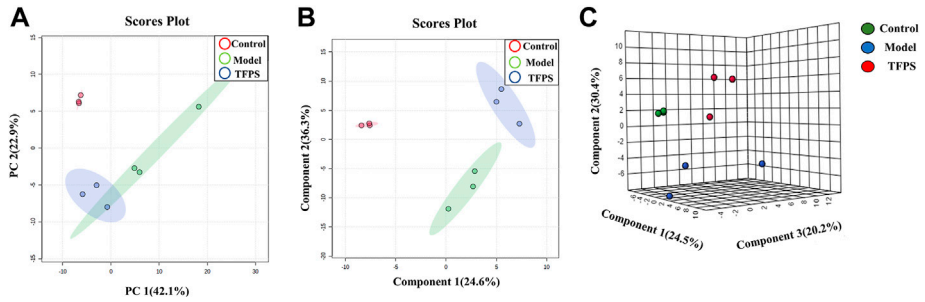

FIGURE 8
PCA and partial least squares discriminant analysis (PLS-DA). **(A)** The PCA scores plot of PC1 (first principal component) vs. PC2 (second principal component) showing the separation between control group (red), model group (green) and TFPS group (blue); **(B)** The PLS-DA score plot of control group (red), model group (green) and TFPS group (blue); **(C)** PLS-DA (3D) spots of control group (green), model group (blue) and TFPS group (red).

shown as a dendrogram (Figure 6C), which revealed that the species composition of animal gut microbiota was significantly changed in DNFB-induced AD mice, however the TFPS treatment can revert the changes.

According to taxonomic analysis, the relative abundance of each phylum was calculated, revealing that *Firmicutes*, *Bacteroides*, *Desulfobacteria*, *Proteobacteria*, and *Actinomycetes* are the mainly detected bacterial phyla in the feces microbiota (Figure 7A). Our study found that Firmicutes and Bacteroidetes accounted for more than 90.0% of the total gut microbial composition in all analyzed samples. The phyla F/B ratio of the gut microbiota was higher in DNFB-induced mice (3.72 ± 0.18) than in the control group (1.72 ± 0.98). Notably, the oral administration of TFPS stimulated the expansion of Bacteroidetes populations but reduced the Firmicute populations, leading to the restoration of F/B ratio to 1.83 ± 0.50. Taxonomic-based analysis suggested that Alloprevotella and Prevotellaceae-UCG-001 increased significantly after TFPS treatment, while Acetitomaculum, Family XIII AD3011_group and Clostridia_UCG-014 decreased significantly in mouse gut microbiota, indicating that the composition of gut microbiota was significantly modulated by TFPS treatment in mice (Figures 7B,C).

Metabolomics analysis

Having established that oral administration of TFPS is superior to topical application for suppressing the development of AD, we further investigated whether the therapeutic effect of oral TFPS on DNFB-induced AD is associated with the regulation of metabolism in mice. Animal feces were analyzed for metabolites by using the principal component analysis (PCA), which is an unsupervised pattern recognition method used to reduce the dimensionality of UPLC-MS data and reveal the inherent clustering of samples. Additionally, the PCA was used to summarize the feces metabolic phenotypes and compare composition of metabolites between the control group, model group, and TFPS group. The PCA score chart shows that the metabolite clusters of the control group and the model group are evidently separated, and the trend of separation between the TFPS group and the model group is also obvious (Figure 8A). In addition, the partial least squares-discriminant analysis (PLS-DA) was used to perform classification and regression on the high-dimensional feces metabolomics data.

As shown in Figures 8B–C, the PLS-DA score shows an evident separation among the control, model and TFPS treatment groups, indicating that DNFB irritation has induced significant changes in animal feces metabolites, and TFPS therapy restored the normal metabolite composition in mice feces.

Screening and identification of potential biomarkers

The multivariate analysis was carried out to explore specific differential metabolites. Differential metabolites were successfully identified among the normal control, modelling and TFPS treatment groups by using the OPLS-DA software package. The obtained scores showed complete separation of metabolic profiles between the control and model groups (Figure 9A), or between the model and TFPS treatment groups (Figure 9E), indicating the existence of potential metabolic biomarkers for DNFB-induced AD-like condition. The OPLS-DA analysis also obtained a good model explanation and prediction by the 2000X permutation test, with $R^2 = 0.998$ and $Q^2 = 0.925$ for control and model groups, and $R^2 = 1$, $Q^2 = 0.83$ for model and TFPS groups (Figures 8B,F).

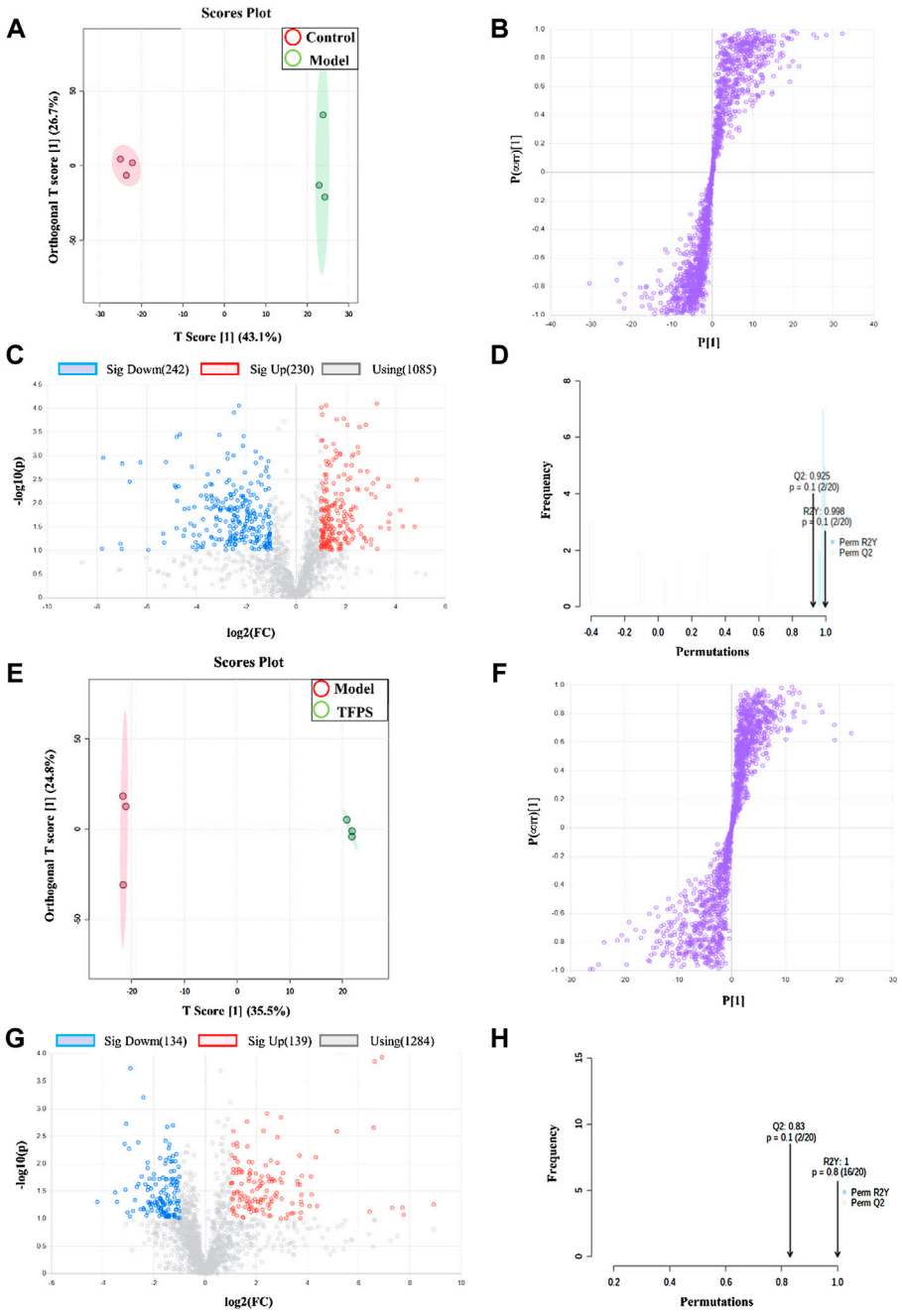

FIGURE 9
OPLS-DA analysis on experimental groups. **(A)** OPLS-DA score chart for control and model groups. **(B)** Statistically verified scatter plot was obtained through 2000X replacement test for control and model groups. **(C)** OPLS-DA S-plot of metabolic profiling for control and model groups. **(D)** Volcano chart by the OPLS-DA analysis for control and model groups. **(E)** OPLS-DA score plot for model and TFPS groups. **(F)** Statistically verified scatter plot was obtained by 2000X permutation test for model and TFPS groups. **(G)** OPLS-DA S-plot of metabolic profiling for model and TFPS groups. **(H)** Volcano plot for model and TFPS groups.

In S-plots and volcano curves (Figures 9C,D,G,H), differential metabolites were visualized and filtered through the OPLS-DA mode, and 12 potential biomarkers that were significantly regulated by high-dose of oral TFPS were screened and identified (Table 2; Figure 10). The TFPS modulation biomarkers from the feces include travoprost,

TABLE 2 Identified potential biomarkers regulated by TFPS treatment.

	RT [min]	Calc. MW	Formula	HMDB ID
Travoprost	7.644	500.23775	C26 H35 F3 O6	0014432
Paraldehyde	0.984	132.07892	C6 H12 O3	0032456
Guanine	1.990	151.04915	C5 H5 N5 O	0000132
Leu-Gly-Pro	8.474	285.16825	C13 H23 N3 O4	
Adenine	2.690	135.05435	C5 H5 N5	0000034
2,4,6-triaminotoluene	0.757	137.09516	C7 H11 N3	0247627
Ala-Pro	6.645	186.10013	C8 H14 N2 O3	
Stearoylglycine	13.205	341.2922	C20 H39 N O3	0013308
Triphenylphosphine oxide	11.726	278.08545	C18H15OP	0259265
trans-2-Dodecenoylcarnitine	12.930	341.25597	C19 H35 N O4	0013326
Stearamide	13.371	283.28686	C18 H37 N O	0034146
Leu-Gln	6.844	259.15267	C11 H21 N3 O4	0028927

paraldehyde, guanine, Leu-Gly-Pro, adenine, 2,4,6-triaminotoluene, Ala-Pro, stearoylglycine, triphenylphosphine oxide, trans-2-dodecenoylcarnitine, stearamide and Leu-Gln.

Changes in short-chain fatty acidss after *Tremella fuciformis* polysaccharides-treatment

Gut microbiota-derived metabolites, including short-chain fatty acids (SCFAs), are involved in maintaining epithelial barrier homeostasis and modulating inflammatory responses. As shown in Figure 11, the concentrations of acetate, propionate, butyrate, isobutyrate, valerate and isovalerate were all significantly decreased in DNFB-induced AD mice comparing with the control group (Figures 11A–F). However, the TFPS treatment restored the reduced levels of acetate, propionate, butyrate, isobutyrate, valerate and isovalerate in DNFB-induced AD groups. These findings suggest that the modulation of gut microbial-derived SCFAs by TFPS may contribute to the alleviation of AD.

Correlation between Atopic dermatitis symptoms, relative abundance of gut microbiota and metabolite changes in Atopic dermatitis mice

To explain the relationship between AD severity, relative abundance of gut microbiota, and metabolite alterations, we performed the Spearman rank correlation analysis on AD-related conditions like ear swelling, ear thickness, skin thickness, mast cell counting, TEWL, expression levels of TNF-α, IFN-γ and IgE, relative abundance of gut microbiota, metabolites and SCFAs (Figure 12). At the genus level, the abundance of *Prevotellaceae-UCG-001* that belongs to the phylum *Bacteroides* is significantly and negatively correlated with the ear swelling, ear thickness, skin thickness, mast cell counting, TEWL, TNF-α and IFN-γ expressions ($p < 0.05$). It was also observed that the abundance of *Family XIII AD3011_group* is significantly and positively correlated with the AD-like conditions including ear thickness, IgE level ($p < 0.05$) and IFN-γ secretion ($p < 0.01$). Similarly, the abundance of *Clostridia_UCG-014* is significantly and positively correlated with ear swelling, ear thickness, TEWL, expression levels of TNF-α ($p < 0.05$) and IFN-γ ($p < 0.001$). Moreover, at the genus level, the beneficial bacteria *Prevotellaceae-UCG-001* were significantly and positively correlated with metabolites paraldehyde, 2,4,6-triaminotoluene, Ala-Pro, stearoyl glycine ($p < 0.05$) and trans-2-dodecenoylcarnitine ($p < 0.01$), and negatively correlated with travoprost and Leu-Gln ($p < 0.05$). Additionally, SCFAs were found to be significantly and negatively correlated with the severity of AD symptoms, levels of TNF-α, IFN-γ and IgE ($p < 0.01$), while the abundance of prevotellaceae-UCG-001 appeared positively correlated with SCFA levels. The above results suggest that the TFPS treatment is beneficial to ameliorate DNFB-induced AD-like symptoms through modulation of gut microbiota in mice.

Discussion

AD is a common chronic inflammatory skin disease characterized by an impaired Th2 immune response. In recent years, the associations between gut flora disturbances and atopic dermatitis have been identified (Kukkonen et al., 2007; Behera et al., 2020). Our study demonstrated that oral administration of TFPS was able to alleviate the pathological severity of DNFB-induced AD-like

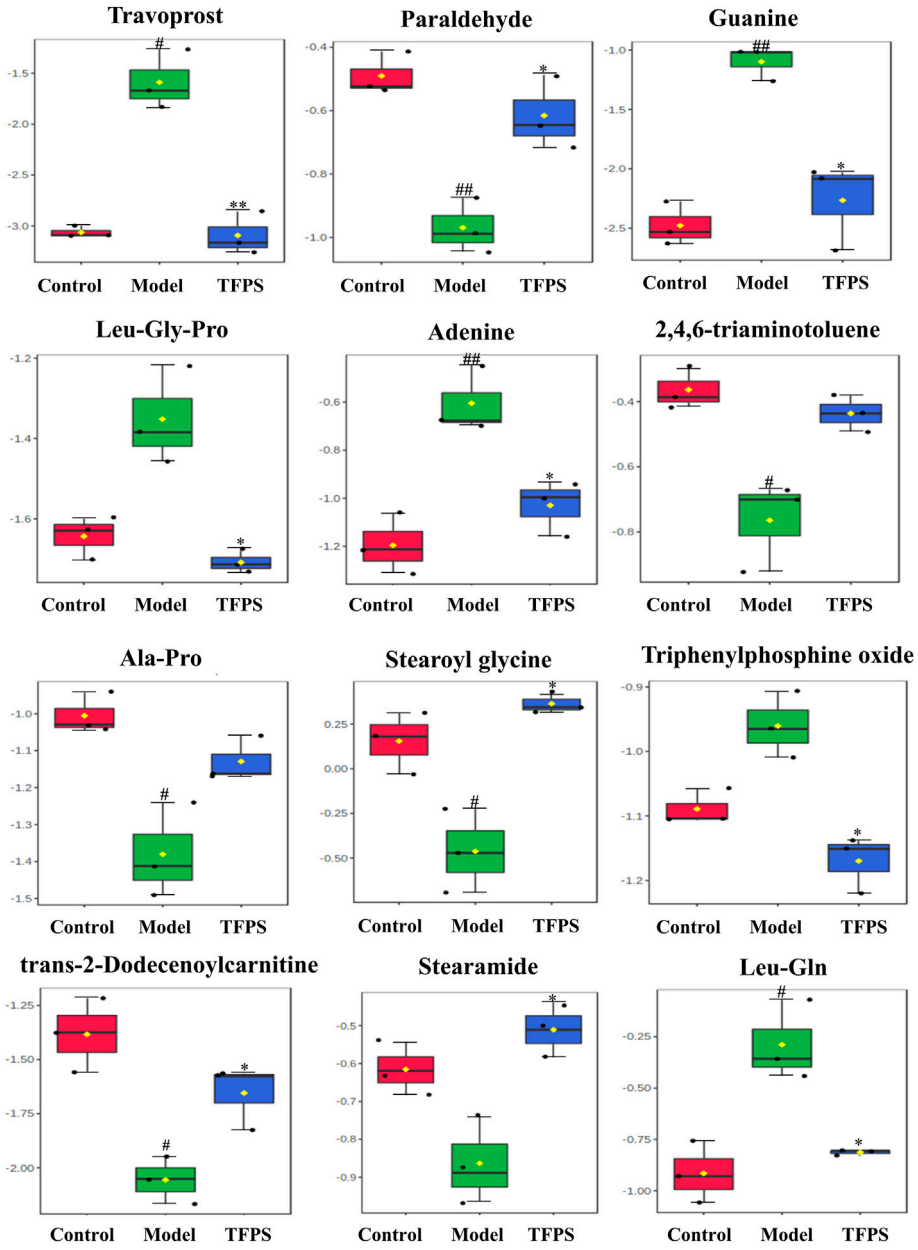

FIGURE 10
Changes of potential metabolite biomarkers by TFPS treatment in DNFB-induced AD-like mice. All values are presented as means ± S.E.M (n = 3). $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the control group; $*p < 0.05$, $**p < 0.01$compared with the model group.

condition in mice. The underlying mechanisms appear to be related to the increase of Treg cells and the regulation of intestinal microbiota and their metabolites.

In this study, we examined AD treatment by topical application or oral administration of TFPS. Previously, Essendoubi et al., revealed that the hyaluronic acid (HA) with 20–300 kDa of low molecular weights could pass through the stratum corneum, by contrast, the higher molecular weight of HA fractions (1,000–1,400 kDa) showed the evident impermeability of skin (Essendoubi et al., 2016). The TFPS that we have extracted range from 30 to 1,230 kDa in sizes, partially distributing in the range of 20–300kDa, so at least partially and potentially penetrate the skin and function to alleviate DNFB-induced AD-like conditions. And this may provide one reason for explaining the observed improvement of AD condition through topical application of the TFPS preparations in this study.

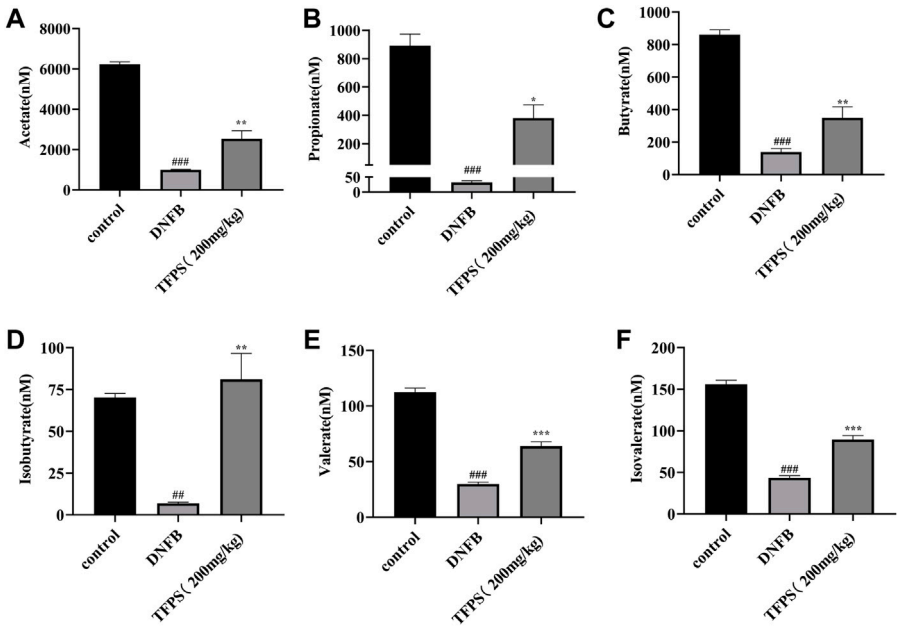

FIGURE 11
Changes of SCFAs by TFPS treatment in DNFB-induced AD mice. **(A)** Measurement of concentrations of acetate **(A)**, propionate **(B)**, butyrate **(C)**, isobutyrate **(D)**, valerate **(E)** and isovalerate **(F)** in control-, DNFB induction- and TFPS treatment groups, respectively. All values are presented as means ± S.E.M ($n = 3$). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the control group; $*p < 0.05$, $**p < 0.01$, $***p < 0.01$compared with the model group.

We have to point out that the previous HA penetration was examined on normal skin. And in our study, the TFPS was applied on DNFB irritation damaged skin, which may allow the higher molecular weight of TFPS (>300 kDa) to penetrate the skin epithelium. Additionally, it is possible that the topically applied larger fraction of TFPS (>300 kDa) may constitute a moisturizing polysaccharide coating/hydrogel on the damaged animal skin, which possibly improves the re-epithelialization of AD skin.

Blocking receptors for IL-4 and IL-13 with dupilumab has been described to lead to improvement of AD well before IgE levels change.

The pathogenesis of AD is involved in both genetic and environmental causes (Kwon et al., 2018). Th2 cells may be triggered to secrete excessive interleukin (IL) 4 and IL-13 and increase specific IgE production by polycyclic aromatic hydrocarbons in polluted air, which induced mast cells and basophils to release inflammatory factors leading to the onset of AD (Werfel et al., 2016). Meanwhile, IFN-γ and other pro-inflammatory cytokines, such as TNF-α, are abundant in the chronic phase of AD (Suárez-Fariñas et al., 2013). TNF-α and IFN-γ are able to induce an increase in pro-inflammatory mediators such as IL-6 and COX-2 in HaCaT cells (Lee et al., 2020). Therefore, the inhibitory effects of TFPS on the production of proinflammatory cytokines can be one of the mechanisms by which TFPS treatment attenuate the development of AD symptoms such as epidermal thickening and mast cell infiltration.

Increasing evidences have demonstrated that the development and progression of AD are closely correlated to the dysregulation in the composition and diversity of intestinal flora (Stokholm et al., 2018; Fang et al., 2021; Lopez-Santamarina et al., 2021). Metabolites from intestinal microbiota may stimulate activation of immune cells in host, such as short-chain fatty acid (SCFA), vitamins and amino acids (O'Hara and Shanahan, 2006). This observation indicates that the gut flora can be targeted to modulate host immunity to alleviate the clinical symptoms of AD. Actually, the correlation of AD with gut microbiota was well documented via sequencing analysis and the "gut-skin" axis has been recognized as an important target for developing novel AD therapies.

The diversity of intestinal flora is decreased in patients with AD compared to healthy populations. It was shown that the intestinal flora of AD patients had a significantly higher proportion of *Clostridia*, *Clostridium difficile*, *Escherichia coli*, and *Staphylococcus aureus* (*S. aureus*), than healthy controls, while the proportion of *Bifidobacteria*, *Bacteroidetes*, and *Bacteroides* was reduced (Lee et al., 2018). Kalliomäki et al. demonstrated that *Lactobacillus* can effectively prevent early atopic dermatitis in high-risk children through modulation of intestinal flora by giving *Lactobacillus* GG to mothers who had at least one first-degree relative (or partner) with atopic eczema, allergic rhinitis, or asthma prenatally, and to their

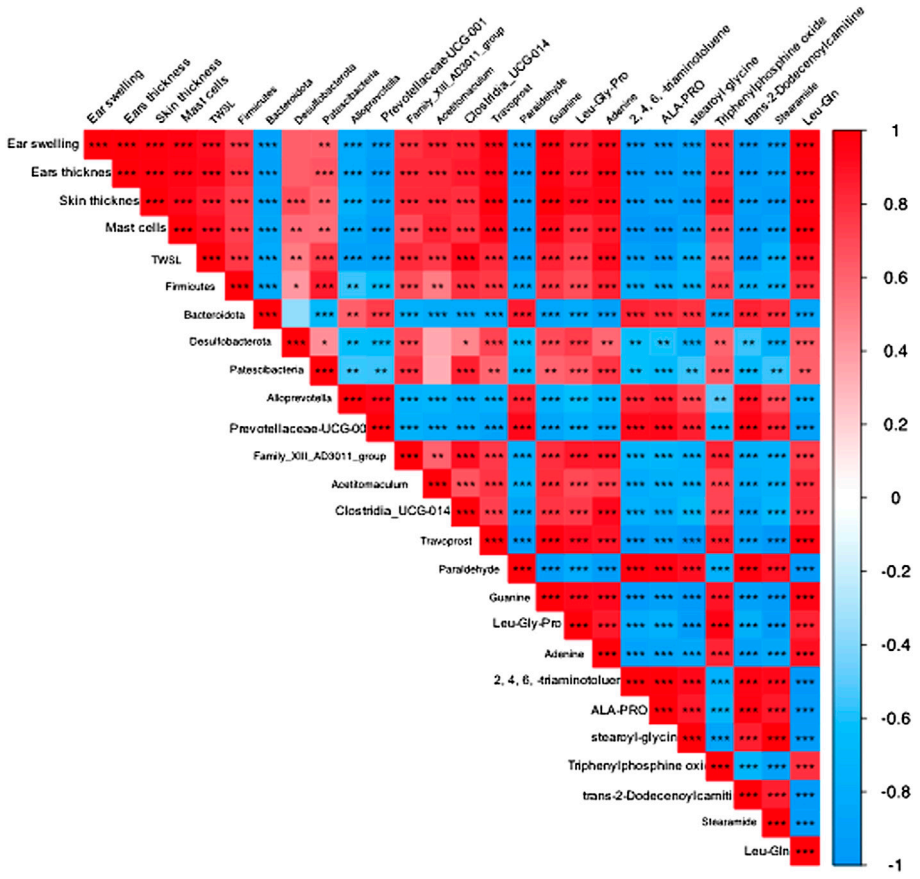

FIGURE 12
Correlation among AD symptoms, relative abundance of gut bacterial and metabolite changes. The correlation was determined using the Spearman's rank correlation analysis. The red color indicates positive correlation, and the blue color indicates negative correlation. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

infants for 6 months postnatally (Kukkonen et al., 2007). Thus, modulating the diversity and composition of the intestinal flora may reduce the occurrence and progression of AD as an alternative approach to reduce adverse drug reactions of AD treatment.

A recent study has shown that high dose of *T. fuciformis* polysaccharides (HTPs) can alleviate dextran sodium sulfate (DSS)-induced colitis in mice through modulation of immune response and intestinal flora (Xu et al., 2021b). However, TFPS has never been examined for their effects on alleviation of AD through modulation of intestinal flora. Our study found that Oral administration of TFPS reduced the F/B ratio of intestinal flora in DNFB-induced mice compared to the model group. This suggests that the TFPS therapy could rescue the intestinal microbial dysbiosis induced by DNFB and bring it back to normal condition. To our best knowledge, this is the first study revealing TFPS therapeutic efficacy on AD via regulating the gut-skin axis.

The increase in *Firmicutes/Bacteroidetes* (F/B) ratio was also associated with the maintenance of normal intestinal homeostasis and consequently increased the concentration of SCFAs in the gut (Dei-Cas et al., 2020). SCFAs, as the most abundant microbial metabolites in the colonic lumen, are the main energy source for epithelial cells and affect the expression of genes required to maintain epithelial barrier and defense functions. They regulate the activity of innate immune cells such as macrophages, neutrophils and dendritic cells, and antigen-specific adaptive immunity mediated by T and B cells (Ellis et al., 2019). Among SCFAs, butyrate is the preferred fuel for the colonic epithelial cells, which stimulates the differentiation of regulatory T cells (Tregs) to maintain the balance between Th1 and Th2 (Tanoue et al., 2016).

Alloprevotella, *Prevotellaceae-UCG-001*, *Acetitomaculum*, *Family XIII AD3011_group* and *Clostridia_UCG-014* were the most affected microbial genera in the TFPS treatment group. *Alloprevotella* belonging to the phylum *Bacteroidetes* has shown anti-inflammatory effects (Ning et al., 2020). Moreover, Prevotellaceae-UCG-001 that belongs to the Prevotellaceae family, a Gram-negative anaerobic bacteria, produces SCFAs in the gut microbiota (Liu et al., 2021). Also, DNFB-induced AD mice showed reduction in the

number of bacteria, which have anti-inflammatory and SCFAs-producing capacities, compared with the control animals. However, the TFPS treatment effectively regulated the DNFB disturbed intestinal microbiome, and recovered the composition of gut microbes almost back to normal level in control animals. These observations thus suggest that the gut microbiome and its derived metabolites like SCFAs, are essential for the maintenance of skin health. Of note, among the 12 identified potential biomarkers, the change in purine metabolism is most pronounced. Purines have important biosynthetic functions, such as the formation of nucleic acids, DNA and nucleic acid monomer precursors. Mycophenolic acid (MPA), an inhibitor of purine synthesis, has been clinically used to treat severe AD (Knol et al., 2012). Interestingly, our results indicated that the TFPS treatment significantly inhibited DNFB-induced purine elevation in AD-like mice. This may be a potential mechanism by which TFPS intervenes in AD. Overall, this study provides a potential strategy to use natural food products for the prevention and treatment of AD via modulation of gut microbial metabolism and immune responses.

This study demonstrated the potential of using oral TFPS for AD treatment. However, some issues need to be elucidated for the clinical application of TFPS. First, the animal model effectiveness cannot guarantee the similar therapeutic efficacy in human diseases, and thus next clinical trials must be performed before putting forward the product to medical practice. Second, the animal model dosage may not work for human AD treatment and the therapeutic safety has to be determined in human cases. Third, the oral TFPS formulation needs to be optimized. Fourth, the patient selection criteria must be established for the precision medicinal application of oral TFPS.

Conclusion

T. fuciformis polysaccharides (TFPS) were successfully extracted by hot distilled water. Both topical application and oral administration of TFPS are effective for treating DNFB-induced AD-like conditions in mice, however the latter demonstrates significantly better efficacy. The AD-attenuation effects of TFPS are associated with the upregulation of Treg cell number and restoration of gut microbiota and metabolites, such as SCFAs. Moreover, 12 metabolites were found being significantly modulated by TFPS treatment and thus identified as potential AD biomarkers. These observations suggest that the oral administration of TFPS potentially constitutes a novel AD therapy.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by Laboratory Animal Center of Wuyi University (SCXK/2016-0041).

Author contributions

LX: investigation, data curation, writing-original draft. KY: investigation, writing-review and editing. YL: investigation. ZZ: formal analysis. ZY: conceptualization, methodology, supervision, writing-review and editing. ZD: conceptualization, Supervision, resources, and funding acquisition.

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Conflict of interest

Author KY is employed by Infinitus Company Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.944801/full#supplementary-material

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EDITED BY
Dan Tang,
Guangdong Pharmaceutical University, China

REVIEWED BY
Suresh Kumar,
Punjabi University, India
Gui-Sheng Zhou,
Nanjing University of Chinese Medicine, China

*CORRESPONDENCE
Mingquan Guo,
guomq@wbgcas.cn

†These authors have contributed equally to this work

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Exploring potential antidiabetic and anti-inflammatory flavonoids from *Euphorbia humifusa* with an integrated strategy

Tojofaniry Fabien Rakotondrabe[1,2,3†], Minxia Fan[1,3,4†] and Mingquan Guo[1,2,3,4*]

[1]CAS Key Laboratory of Plant Germplasm Enhancement and Specialty Agriculture, Wuhan Botanical Garden, Chinese Academy of Sciences, Wuhan, China, [2]University of Chinese Academy of Sciences, Beijing, China, [3]Sino-Africa Joint Research Center, Chinese Academy of Sciences, Wuhan, China, [4]Innovation Academy for Drug Discovery and Development, Chinese Academy of Sciences, Shanghai, China

E. humifusa Willd, a monoecious annual plant, native to Eastern Asia, has been traditionally attributed to the treatment and prevention of miscellaneous diseases, including diabetes mellitus and its associated complications. Earlier studies have supported this species' pharmacological efficacies including its antibacterial, antidiabetic, and anti-inflammatory properties. Even so, the underlying bioactive components with their mechanisms of action associated with its antidiabetic and anti-inflammatory effects remain elusive. The preamble *in vitro* assessments of the crude extract and its different fractions revealed that the *n*-butanol fraction (EHNB) exhibited the best activity, which was subsequently subjected to a rapid screening of candidate ligands through bio-affinity ultrafiltration with the two enzyme targets: α-glucosidase (α-Glu) and cycloxygenase-2 (COX-2) combined with UPLC/QTOF-MS. As a result, 7 compounds were identified from EHNB, among them, vitexin and astragalin were screened out as the most active ligand compounds. Vitexin showed great specific binding (SB) affinity values of 1.26 toward α-Glu and 1.32 toward COX-2, while astragalin showed 1.32 and 1.36, respectively. The docking simulation results exhibited strong interactions of vitexin and astragalin with the key residues of the enzyme targets, suggesting their possible mechanisms of action. The *in vitro* antidiabetic validation revealed noticeable half-maximal inhibitory effects (IC_{50}) of 36.38 ± 3.06 μM for vitexin and 42.47 ± 4.13 μM for astragalin, much better than that of the positive drug acarbose (109.54 ± 14.23 μM). Similarly, these two compounds showed the inhibitory activity against COX-2 with the half-maximal inhibitory effects (IC_{50}) at 27.91 ± 1.74 μM and 49.05 ± 1.49 μM, respectively. Therefore, these two flavonoid compounds (vitexin and astragalin) were speculated as potential antidiabetic and anti-inflammatory compounds from *E. humifusa*. Taken together, the integrated strategy applied to *E. humifusa* led to the fast identification of two potential double-acting flavonoids and enlightened its antidiabetic and anti-inflammatory uses. Besides these findings, the integrated strategy in this study could also be used to facilitate the rapid discovery and development of active candidates from other traditional herbal medicines against multi-drug

targets and to aid in revealing their mechanisms of action for their traditional uses.

KEYWORDS

Euphorbia humifusa, antidiabetic, anti-inflammatory, bio-affinity, flavonoid, ligand

1 Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by reduced insulin sensitivity or insulin deficiency, causing blood hyperglycemia in the postprandial and fasting state (Kazeem et al., 2021). By 2045, it is projected that around 800 million of the global population will be hurt by this pandemic metabolic disorder with 6.7 million recorded deaths in 2021 (Li et al., 2017; Ni et al., 2022). Persistent hyperglycemia encompasses multiple complications leading to nephropathy, retinopathy, neuropathy and vascular damage (Yen et al., 2021). Inflammation, oxidative stress, and obesity are among the related symptoms that are crucial in the pathophysiology of DM (Ma et al., 2018; Paun et al., 2020; Zhang et al., 2021). Therefore, researchers thrive on the quest and development of safe and cost-effective drugs to address DM issues and especially to limit its related complications.

From ancient times, herbal medicines have played significant roles in managing diverse health disorders including DM. The available pharmacotherapy enables the treatment of DM from different approaches, but in many cases there has been an increase of side effects such as gastrointestinal discomfort, hypoglycemia, and dizziness. These factors encourage researchers to seek new and safer alternatives from natural resources. Recent discovery evolving the uses of the powerful screening tool, based on bio-affinity ultrafiltration, has helped researchers to bypass the labor-intensive and time-consuming drug discovery process (Ye et al., 2022). This innovative approach consists of promoting the ligand-macromolecule complex formation between a mixture and a drug target, then fishing out the ligands candidates *via* centrifugal ultrafiltration, followed by their identification on analytical instruments like UPLC/QTOF-MS (Feng et al., 2021).

E. humifusa Willd, commonly called creeping euphorbia, is a monoecious annual plant native to eastern Asia (Kim et al., 2016). Growing up to 20 cm in height and generally glabrous, the plant presents a fine slender multiple branched stems and opposite leaves (Pahlevani and Riina, 2011). According to the Chinese pharmacopeia (2020 edition), this species has been attributed to the treatment of multifarious ailments such as dysentery, enteritis, and traumatic bleeding. In addition, previous research has supported the pharmacological potencies of this plant including its antibacterial, antidiabetic, and anti-inflammatory activities (Kang et al., 2012; Tian et al., 2019). However, this species bioactive components together with their associated mechanisms of action in terms of its antidiabetic and anti-inflammatory uses need to be explored. Hence, the purpose of this present study was to rapidly investigate the potential bioactive components of *E. humifusa* and elicit their possible modes of action. A preamble *in vitro* assessment of different *E. humifusa* extracts led us to depict the powerful active *n*-butanol fraction (EHNB) which was subsequently subjected to bio-affinity ultrafiltration combined with UPLC/QTOF-MS. Thereafter, docking simulations were used to investigate the interactions between the compounds with high affinity and the active pocket of the target enzymes α-Glu and COX-2. The lead compounds vitexin and astragalin's capacities to mitigate the two target enzymes functions were confirmed *in vitro* with noticeable IC_{50} values as compared to the positive drugs. Despite the attempt to generate scientific support for the empirical virtues of *E humifusa*, this work offers also a valuable strategy for discovery and development of bioactive components from traditional herbal medicinal herbs.

2 Materials and methods

2.1 Plant materials preparation and extraction

Whole plant materials of *E. humifusa* were harvested around Wuhan Botanical Garden (Wuhan, China) in September 2020. A herbarium voucher specimen (No: 20200923) was prepared and authenticated by a senior taxonomist, Professor Guangwan Hu of the Key Laboratory of Plant Germplasm Enhancement and Agriculture Specialty (Wuhan Botanical Garden, Chinese Academy of Sciences). The samples were cleansed, shade-dried, and milled into powder before storage in an airtight recipient.

The extraction procedure of *E. humifusa* powder (400 g) was performed through an ultrasonic bath with 70% ethanol (ratio 1:8, w:v) for three consecutive times. The combined filtrates were evaporated under low pressure and lyophilized to afford the crude extract (EHCE: 93.22 g). Thereafter, from 20 g of EHCE were conducted sequential liquid-liquid separation yielding to petroleum ether fraction (EHPE: 2.20 g), ethyl acetate fraction (EHEA: 4.40 g), *n*-butanol fraction (EHNB: 3.74 g), and water fraction (EHWA: 5.02 g) after evaporation.

2.2 Chemicals and reagents

The extraction solvents ethanol, petroleum ether, ethyl acetate *n*-butanol and the dimethyl sulfoxide (DMSO) were supplied by Shanghai Chemical Reagent Corp. (Shanghai, China). The different standards used for the assays comprising gallic acid, rutin, ascorbic acid (Vit C), butylated hydroxytoluene (BHT), Trolox, 2,2-diphenyl-1-picrylhdrazyl (DPPH), 2,2′-azinobis-(3-ethyl-benzthiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-Tri (2-pyridyl)-1,3,5-triazine (TPTZ), and indomethacin with a purity ≥99.5% were purchased from Sigma-Aldrich Corp (Shanghai, China). The HPLC grade solvents acetonitrile (ACN), methanol, and formic acid (FA) were obtained from TEDIA Company Inc. (Fairfield, OH, United States). The *p*-nitrophenyl α-D-glucopyranoside (*p*-NPG) substrate was acquired from Aladdin Bio-Chem Technology Corp. The COX-2 inhibitor screening assay kit (No: S0168) was purchased from Beyotime Biotechnology (Shanghai, China). The α-glucosidase enzyme was bought from Sigma Aldrich (St Louis, MO, United States) whereas Wuhan Antgene Biotechnology Co., Ltd. (Wuhan, China) supplied the COX-2 enzyme. The centrifugal ultrafiltration membranes 10 kDa Cut-off (YM-10) were purchased from Millipore Co. Ltd. (Bedford, MA, United States). Finally, Shanghai Macklin Biochemical Co. (Shanghai, China) supplied the phytochemical standards vitexin and astragalin (≥98%).

2.3 Total phenolic content and total flavonoid content

The phenolic contents of *E. humifusa* extract and its fractions were evaluated by Folin-Ciocalteu method, slightly modified from a previous report by Fan and coworkers (Fan et al., 2020). In brief, the sample was incubated with Folin-Ciocalteu reagent (10%, v/v) and sodium carbonate (7.5%, w/v) before 60 min incubation in the dark. The absorbance of the mixture was then read on a UV spectrophotometer set at a wavelength of 760 nm (UV 1100, MAPADA Shanghai, China). Gallic acid was used as the calibration standard, and the results were presented as its equivalent per gram of dried sample (mg GAE/g). The Aluminium chelating colorimetric method was performed for the estimation of total flavonoid content using rutin for the standard curve (Xu et al., 2019). An equal volume of the sample and sodium nitrite were mixed with 10% (w/v) $AlCl_3.6H_2O$. Thereafter, the mixture was supplemented with sodium carbonate (4%, w/v) and then incubated in the dark for 15 min. The absorbance of the mixture was finally read on a UV spectrophotometer set at a wavelength of 510 nm. The obtained results were denoted by milligram rutin equivalent per gram of dried sample (mg RE/g).

2.4 Antioxidant activity assay

The antioxidant activity of our samples was ascertained by evaluating their capacity to scavenge free radicals and reduce ferric ions. The DPPH and ABTS free radical scavenging assays were performed as per the method of Xu and his colleagues (Xu et al., 2019). Vit C, Trolox, and BHT were utilized as positive controls whereas pure methanol was for the blank control. The scavenging rate was calculated using the equation:

$$\text{Scavenging rate } (\%) = \frac{(A_0 - A_S)}{A_0} \times 100\%$$

With A_0 and A*s* are ascribed to the absorbance value of the blank control, and the tested sample or positive control respectively. The results were expressed in IC_{50} values ($n = 3$, mean ± standard deviation).

The ferric chelating behavior using the ferric reducing antioxidant power (FRAP) assay was conducted based on a priorly reported method by our research group (Rakotondrabe et al., 2022). The calibration curve was plotted from a range dilution of $FeSO_4{\cdot}7H_2O$ (0.06–1.50 mM) standard and the results were expressed in Millimolar Fe^{2+} per gram (mM Fe^{2+}/g) of three replicate assays.

2.5 *In Vitro* α-Glu inhibition assay

The α-Glu inhibition activity of *E. humifusa* was determined through a minor modified method from a previous report (Liu et al., 2020). In brief, 150 µL phosphate buffer (pH 6.8) was mixed in a 96 well plate transparent with 25 µL of α-Glu enzyme solution (0.26 units/mL) and 25 µL of the diluted sample (DMSO: 5%, v/v). Then, the mixture was incubated for 15 min at 37°C before supplementation with 50 µL *p*-NPG (0.3125 mM) substrate to initiate the reaction. An incubation for another 15 min was monitored prior to the reaction termination with an addition of 50 µL sodium carbonate 200 mM. In the end, the absorbance was immediately read at 405 nm in a microplate reader (Tecan infinite M200 Pro). The α-Glu inhibition rate was figured using the following equation:

$$\text{Inhibition rate } (\%) = 100 - \left[\frac{(A_S - A_{Sc})}{(A_C - A_{Bc})} \times 100\right]$$

With A_S and A_{Sc} representing the absorbance of the sample test and the sample control while A_C and A_{Bc} for the absorbance of the control and the blank control accordingly.

2.6 *In Vitro* COX-2 inhibition assay

The *E. humifusa* anti-inflammatory activity was ascertained by a COX-2 inhibitor screening kit (Beyotime S0168). In brief, the working solutions including COX-2 cofactor, COX-2 enzyme

solution, COX-2 substrate, and COX-2 probe were first prepared in accordance with the supplier manual, which were subsequently diluted ten times with the buffer kit assay. Thereafter, 150 μL Tris-HCl (pH 7.8), 10 μL of COX-2 cofactor, and 10 μL of work solution excluding the blank group were mixed in a 96 well black plate. The mixture was supplemented with 10 μL of samples or positive control (indomethacin) and instead, DMSO was used for the blank control and the 100% enzyme groups and mixed vigorously. After a 10 min incubation at 37°C, the reaction was initiated with 10 μL of COX-2 probe and 10 μL of COX-2 substrate. Finally, their relative fluorescence unit (RFU) was read from an excitation wavelength of 560 nm and an emission wavelength of 590 nm, after 20 min of incubation (37°C). The COX-2 inhibition rate was calculated from the equation:

$$\text{COX} - 2 \text{ inhibition rate } (\%) = \frac{\left(\text{RFU}_{100\% \text{ enzyme}} - \text{RFU}_{sample}\right)}{\left(\text{RFU}_{100\% \text{ enzyme}} - \text{RFU}_{blank\ control}\right)} \times 100\%$$

With $\text{RFU}_{100\%\ enzyme}$, RFU_{sample}, and $\text{RFU}_{blank\ control}$ represent the relative fluorescence value of the intact enzyme, samples and blank control respectively. The experiment was conducted in triplicates and the IC_{50} values were expressed into mean ± standard deviation.

2.7 Screening and identification of potential inhibitors through UF-UPLC/QTOF-MS

2.7.1 UPLC/QTOF-MS identification

The ultra-performance liquid chromatography (UPLC) system was monitored with CDS OpenLAB 2.0 (Agilent Technologies, Santa Clara, United states). The chromatographic separation was carried out using a Sunniest C18 HT (100 mm × 2.1 mm, 2 μm thickness, Waters, Manchester) column. The injection volume was 5 μL with a flow rate kept at 0.2 ml/min while the column temperature was maintained at 30°C. The mobile phases were composed of eluent A (aqueous formic acid, 0.1%) and B (ACN). The elution followed a gradient program of 5%–10% B in 0–5 min, 10% B in 5–15 min, 10%–15% B in 15–30 min, 15%–17% B in 30–35 min, held at 17% B in 35–45 min, finally raised to 70% B from 45–55 min. The components were identified through an Agilent 6530 iFunnel QTOF-MS mass spectrometer equipped with an electrospray ionization (ESI) system. Mass-to-charges and spectra were obtained in negative electrospray ionization mode at a mass range from 50–1,500 m/z. The drying, nebulizing, and collision gas were nitrogen. The drying gas flow rate was 8 L/min with an ESI spray voltage of 3.5 kV. The heated capillary temperature was set to 350°C and nebulizer pressure at 35 psi. The data acquisition and analysis were processed by Mass Hunter Workstation software (version B.08.01.00, Agilent Technology, United States).

2.7.2 Screening for potential inhibitors via bio-affinity ultrafiltration

The bio-affinity screening of potential ligands from the EHNB fraction was performed using a slender modified method by Li and his coworkers (Li et al., 2022). Concisely, 100 μL volume of EHNB sample was incubated with 40 μL COX-2 (8 units), or 100 μL α-Glu (6 units) active enzymes. Afterward, the mix solutions were incubated for 45 min and subsequently transferred to a centrifugal ultrafiltration vial 10 kDa cut-off filter membrane. They were initially centrifuged at 10,000 rpm for 10 min (25°C) to withdraw the extra solution followed by three successive centrifugations with 200 μL phosphate-buffered saline (PBS) to wash away the non-binding compounds. Finally, the fixed ligand candidates were fished out from the complex throughout enzyme denaturation with 200 μL of 70% (v/v) aqueous methanol before centrifugation (10,000 rpm, 10 min). The filtrates from three denaturation processes were gathered and dried on nitrogen blowing before reconstitution in 50 μL of aqueous methanol (50%, v/v) for further analytical analysis.

2.8 Molecular docking simulations

The docking simulations were carried out using the AutoDock 4.2 software. Ligand molecules were drawn with ChemBioDraw Ultra 14.0 and converted to 3-Dimensional (3D) structures by ChemBio3D Ultra 12.0. MM2 option was used for energy minimization of the 3D structures, which were further subjected to Gasteiger charges. The crystal receptor structures of α-Glu (PDB id: 3A4A) and COX-2 (PDB id: 1CX2) PDB files were downloaded from the protein data bank (www.rcsb.org). Water molecules and initial ligands were removed before adding polar hydrogens and Kolliman charges. Lamarckian genetic algorithm was used for docking calculation of 50 independent genetic algorithm runs within 250 population size and 25 × 10^5 energy evaluation. The standard docking procedure for a rigid protein and flexible ligands was conducted within a grid map (60 × 60 × 60), centered at the accredited catalytic site. The default settings were used for all other parameters. At the end of docking, the best poses were analyzed for hydrogen bonding, hydrophobic interaction, and binding energy (BE) values using Discovery Studio Visualizer 2021 program (Dassault Systems BIOVIA, San Diego, CA, United States).

2.9 Statistical analysis

All experimental tests were performed in triplicate and results were expressed in mean ± standard deviation (SD). The half-maximal inhibitory concentration (IC_{50}) values were calculated from the non-linear regression of the dose-response

TABLE 1 The antioxidant activities, total phenolic content (TPC), and total flavonoid content (TFC) of *E. humifusa* samples.

Samples	IC_{50} DPPH (μg/ml)	IC_{50} ABTS (μg/ml)	FRAP (mM Fe^{2+}/g)	TPC (mg GAE/g)	TFC (mg RE/g)
EHCE	10.46 ± 1.14^{c}	9.10 ± 0.03^{c}	0.79 ± 0.01^{e}	45.02 ± 0.19^{c}	21.71 ± 0.56^{c}
EHPE	59.54 ± 0.78^{a}	59.67 ± 0.54^{a}	0.18 ± 0.01^{g}	1.64 ± 0.58^{e}	2.06 ± 0.22^{e}
EHEA	6.32 ± 0.60^{d}	3.78 ± 0.93^{e}	1.02 ± 0.01^{d}	94.40 ± 0.11^{b}	31.45 ± 0.11^{b}
EHNB	2.45 ± 0.05^{f}	2.04 ± 0.42^{f}	1.45 ± 0.02^{c}	119.45 ± 1.42^{a}	62.28 ± 0.67^{a}
EHWA	19.95 ± 0.69^{b}	15.87 ± 0.21^{b}	0.45 ± 0.02^{f}	20.36 ± 0.19^{d}	10.06 ± 0.40^{d}
BHT	10.82 ± 0.37^{c}	Nt	Nt	Nt	Nt
Vit C	3.35 ± 0.12^{f}	4.18 ± 0.06^{e}	2.04 ± 0.01^{a}	Nt	Nt
Trolox	Nt	6.15 ± 0.98^{d}	1.79 ± 0.01^{b}	Nt	Nt

Note: The values are presented as the mean ± SD, of three replicates; the different superscript letters (a-g) in the same column represent the statistical difference using one way ANOVA DMRT, at p-value < 0.05 and Nt means not tested.

curve analysis function in GraphPad Prism 8.0.1.2 (GraphPad Software, San Diego, California United States). The significance of the results was compared through one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) on SPSS statistic 22.0.0 (SPSS Inc. Chicago, IL, United States). For all analyses, a difference at p-value < 0.05 was regarded as statistically significant.

3 Results

3.1 Antioxidant activity of *E. humifusa*

Oxidative stress plays key function in the pathogenesis of metabolic disorders, whereas exogenous antioxidants from natural sources enhance the organism's defense and its redox homeostasis (Abdel-Daim et al., 2019). The antioxidant activities evaluation of the different *E. humifusa* samples showed noticeable potentialities of the *n*-butanol fraction (EHNB) as compared to the others. EHNB exerted the highest scavenging of DPPH and ABTS with an IC_{50} value of 2.45 ± 0.05 μg/ml and 2.04 ± 0.42 μg/ml respectively. Its scavenging abilities were assessed significantly more active than the positive control BHT and Vit C at a p-value < 0.05, as illustrated in Table 1. Meanwhile, the FRAP reducing activity of this EHNB fraction was slightly lower than the Vit C and Trolox positive controls having values of 1.45 ± 0.02 mM Fe^{2+}/g, 2.04 ± 0.01 mM Fe^{2+}/g, and 1.79 ± 0.01 mM Fe^{2+}/g, respectively. Similarly, Table 1 also reveals the richness of EHNB fraction in terms of phenolic content (119.45 ± 1.42 mg GAE/g) and flavonoid content (62.28 ± 0.67 mg RE/g) when compared to the others.

3.2 α-Glu inhibition activity of *E. humifusa*

The hypoglycemic activities of *E. humifusa* extract and its fractions were assessed through *in vitro* α-Glu inhibition assay. The α-Glu enzyme has been reported to play a key role in the catalysis of carbohydrate digestion, therefore, its inhibition leads to a diminution of postprandial glucose absorption. This present study was initially designed to determine the most effective hypoglycemic fraction among the different types of *E. humifusa* samples. The result in Figure 1 exerted that all samples possessed great inhibition of α-Glu in comparison with the well-known antidiabetic drug acarbose. EHNB, possessing a low IC_{50} value of 0.54 ± 0.12 μg/ml, was determined significantly more potent than acarbose (IC_{50}: 112.53 ± 12.87 μg/ml), followed by the ethyl acetate fraction (EHEA) and the crude extract (EHCE).

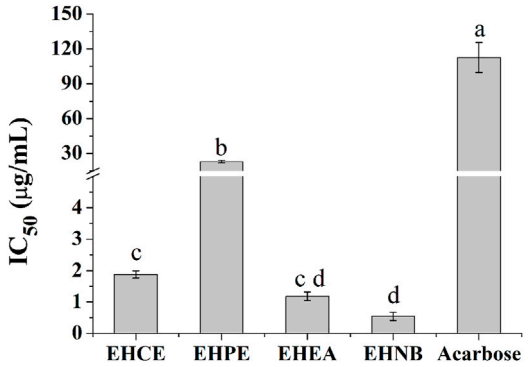

FIGURE 1
α-Glu inhibitory effects of *E. humifusa* crude extract (EHCE) and the different fractions. Petroleum ether (EHPE), ethyl acetate (EHEA) and *n*-butanol (EHNB). The label letters (a–d) represent the significant difference at p-value < 0.05 by ANOVA DMRT.

3.3 COX-2 inhibition activity of *E. humifusa*

COX-2 enzyme has been recognized to possess a pivotal role in mediating inflammation response by converting the arachidonic acid to prostaglandin G2 and H2. It was

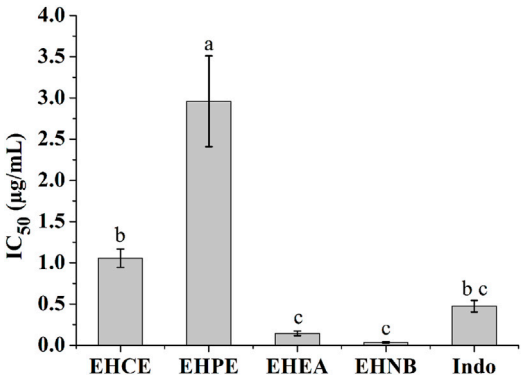

FIGURE 2
COX-2 inhibitory effects of the *E. humifusa* crude extract (EHCE) and its different fractions. Petroleum ether (EHPE), ethyl-acetate (EHEA) and *n*-butanol (EHNB). The label letters (a–c) represent the significant difference at *p*-value < 0.05 by ANOVA DMRT.

discovered that EHNB and EHEA had equipotent COX-2 inhibition activity together with the non-steroidal anti-inflammatory drug (NSAID) indomethacin showing IC_{50} values of 0.03 ± 0.00 μg/ml, 0.14 ± 0.02 μg/ml and 0.47 ± 0.07 μg/ml correspondingly (Figure 2).

As a whole, the *n*-butanol fraction (EHNB) exerted the best activities both as hypoglycemic and anti-inflammatory which were in line with the antioxidant results. Hence, the bioactive components of this potent fraction were further screened out *via* the high throughput bio-affinity ultrafiltration combined with UPLC/QTOF-MS targeting α-Glu and COX-2 enzymes.

3.4 Ultrafiltration UPLC/QTOF-MS analysis

3.4.1 Characterization and identification of chemical constituents of EHNB

The chemical components characterization of this potent fraction EHNB was determined by a high-resolution UPLC/QTOF-MS instrument. The tentative identification of all components was performed based on the peak retention time, the parent ions *m/z* with their respective fragmentation cascade as well as the mass error between the observed and theoretical mass. Among the 8 obtained peaks, 7 compounds were identified including tannins, phenolic acid and flavonoid glycosides. The features and characteristics of each identified compounds are displayed in Table 2 and their respective structures illustrated in Figure 3. Two tannins compounds (1, 2) showing precursor ions mass of *m/z* 633.0766 $[M-H]^-$ and 800.0822 $[M-H]^-$ in the negative mode were respectively assigned as, galloyl HHDP-glucose and gallotanin (Anand et al., 2021). Compound 4, with a precursor ion mass of m/z 300.9990 $[M-H]^-$, consists of phenolic acid and was tentatively identified as ellagic acid (Camargo et al., 2014). The compounds 3, 5, 6 and 8 belong to flavonoid glycosides, which were respectively deprotonated at *m/z* 595.1299 $[M-H]^-$, *m/z* 463.0885 $[M-H]^-$, *m/z* 447.0927 $[M-H]^-$, and *m/z* 431.0967 $[M-H]^-$. According to their features, they were ascribed as quercetin-3-*O*-apiosyl-(1–2) galactoside, quercetin 3-*O*-glucoside, astragalin, and vitexin (Chen et al., 2019; Lu et al., 2022; Rakotondrabe et al., 2022).

3.4.2 Discovery of potential α-Glu and COX-2 ligand candidates

Within medicinal plants, one or more bioactive components are attributed to be responsible for their biological activities. In this study, the high throughput ligand screening bio-affinity ultrafiltration UPLC/QTOF-MS targeting α-Glu and COX-2 has been utilized to fish out these key hypoglycemic and anti-inflammatory chemicals. This method has been testified efficient and reliable by several researchers to depict potential ligands from a complex mixture. Based on the comparison of peak areas of the chromatograms obtained from the sample incubated with the active enzyme and that of the denatured group, the relative binding of each candidate was assessed via the specific binding value calculated from the equation:

$$\text{Specific binding (SB)} = A_{active}/A_{inactive}$$

With A_{active} and $A_{inactive}$ corresponding to the area of the peaks within the chromatogram of those incubated with active and inactive enzymes. From our experiment, it was discovered that three compounds showed an affinity with the two targets, notably ellagic acid, astragalin, and vitexin (Figure 4). Astragalin showed the highest specific binding (SB) value of 1.32 with α-Glu followed by ellagic acid (SB: 1.28) and vitexin (SB: 1.26). However, vitexin took the second rank when targeting the COX-2 enzyme with the respective SB values of 1.36 (astragalin), 1.32 (vitexin), and 1.05 (ellagic acid). These rates suggested that these three compounds could be speculated as ligand candidates for our targets. Additionally, their interactions with the target's active site were simulated *in silico* to in-depth their affinity and appended to the *in vitro* validation activities assessment.

3.5 Molecular docking analysis

For a better understanding of the binding mechanism and interaction of these ligand candidates with the target receptor, *in silico* molecular docking simulations were conducted. Simultaneous docking of the respective positive drugs acarbose and indomethacin into the active site of α-Glu and COX-2 were executed to validate the docking method. The root mean square deviation value (RMSD) obtained from the docking validation of the complex acarbose-α-Glu was 1.010 ± 0.265 Å when 0.643 ± 0.038 Å for indomethacin-COX-2. The ligand receptor complex binding stability was assigned based on the BE values of the best docking pose;

TABLE 2 The identification of the potential ligands from EHNB and their specific binding values with molecular docking analysis.

Peak No	RT (min)	Observed *m/z*[M-H]$^-$	Theoretical *m/z* (Δ ppm)	Characteristic fragments (abundance %)	Tentative identification	α-Glu			COX-2		
						SB	BE (kcal/mol)	IC_{50} (μM)	SB	BE (kcal/mol)	IC_{50} (μM)
1	23.035	633.0766	633.0806 (−6.31)	465.0017 (18.08), 300.9412 (100), 275.0033 (23.96), 168.9701 (64.5)	Galloyl HHDP-glucose	—	—	—	—	—	—
2	28.410	801.0822	801.0856 (−4.24)	632.9739 (100), 464.9822 (18.08), 300.9429 (3.17), 169.5332 (2.54)	Gallotanin	—	—	—	—	—	—
3	30.523	595.1299	595.1305 (−1.00)	300.9827 (13.51), 270.9843 (2.61), 178.9503 (1.50), 150.9559 (3.1)	Quercetin-3-*O*-apiosyl-(1–2) galactoside	—	—	—	—	—	—
4	32.988	300.9981	300.9990 (−2.99)	284.2479 (1.25), 256.9575 (3.55), 228.9680 (3.58)	Ellagic acid	1.28	−7.67	Nt	1.05	−6.65	Nt
5	34.423	463.0885	463.0882 (0.65)	300.9767 (69.05), 270.9786. (4.65), 178.9593 (5.97), 150.9683 (19.26)	Quercetin 3-*O*-glucoside	—	—	—	—	—	—
6	34.815	447.0929	447.0933 (−1.34)	284.9838 (100), 255.1797 (1.6), 226.9957 (15.12), 151.0157 (3.54)	Astragalin (Kaempferol -3-*O*-glucoside)	1.32	−8.61	42.47 ± 4.13	1.36	−8.94	49.05 ± 1.49
7	41.285	279.0373		127.9924 (11.3), 96.9934 (100)	Unknown	—	—	—	—	—	—
8	44.256	431.0967	431.0984 (3.94)	311.0053 (1.73), 268.9930 (34.89)	Vitexin (Apigenin 8-*C*-glucoside)	1.26	−8.65	36.38 ± 3.06	1.32	−9.19	27.91 ± 1.74

Peak No. And retention time (RT) correspond to the UPLC-UV chromatogram of Figure 4; SB represents the specific binding; BE represents the binding energy; Nt means not determined, and - stands for nothing.

from which the lower the value, the stronger the interaction. Toward α-Glu, the highest stability was found with vitexin exhibiting a BE value of −8.65 kcal/mol. Its interaction was explained by the occurrence of nine hydrogen bonds toward the key residues Ser241, Gln279, Arg315, Glu411, His280, Asn415, and Asp242 (Figure 5A). In addition, hydrophobic binding was determined with the pocket constituted by Tyr158, Arg315, and Lys156. The astragalin exerted BE value of −8.61 kcal/mol formed of eight hydrogen bonds with Ser241, Arg315, Asp307, Ser311, Lys156, Pro312, and Leu313 key residues. The astragalin-α-Glu complex was stabilized by hydrophobic interactions between the ligand and the pocket established with Tyr158 and Lys156 (Figure 5C). The weakest complex stability was identified with ellagic acid, showing a BE value of −7.67 kcal/mol. Toward COX-2, vitexin remained the greatest bound ligand with a BE value of −9.19 kcal/mol. The interactions with the catalytic site were composed of six hydrogen bonds to the key residues Arg120, Tyr355, Ser530, Ala527, Met522, and Gln192 (Figure 5B). Electrostatic and hydrophobic interactivity between vitexin and the pocket were also present and formed of Arg513, Val349, Ala527, Leu531, Val523, and Leu352. However, the astragalin-COX-2 BE value was −8.94 kcal/mol, made by seven hydrogen bonds with the residues Arg120, Tyr355, Ala527, Ser530, Gln192, and Gly526 (Figure 5D). The same electrostatic interactivity with Arg513 was identified and supplemented by hydrophobic bonds with Ala516, Val523, and Leu352. Likewise, the lowest stability was found on the ellagic acid-COX-2 complex with a BE value of −6.45 kcal/mol. Due to the low relative binding values along with the poor stabilities established between the ellagic-acid and targets, only vitexin and astragalin were speculated as potential ligands toward α-Glu and COX-2. The *in-vitro* confirmation of their activities was then investigated.

3.6 Potential ligands activities validations

To validate the hypoglycemic and anti-inflammatory activities of the two screened-out potential ligands, the same colorimetric *in vitro* inhibition assays as for the preliminary *E. humifusa* crude extract and its fractions activities assessment were conducted. As illustrated in Figure 6A, astragalin and vitexin exerted interesting equipotent activities against α-Glu with a noticeable IC_{50} of 42.47 ± 4.13 μM and 36.38 ± 3.06 μM accordingly. They were statistically more active than the positive drug acarbose showing an IC_{50} of 109.54 ± 14.23 μM. On the other hand, the two-screened ligands showed moderate COX-2 inhibition capacity in comparison to the positive NSAID indomethacin as displayed in Figure 6B. Vitexin exhibited a greater half-maximal inhibitory concentration of 27 0.91 ± 1.74 μM than astragalin 49.05 ± 1.49 μM whereas indomethacin has an IC_{50} of 13.24 ± 2.20 μM.

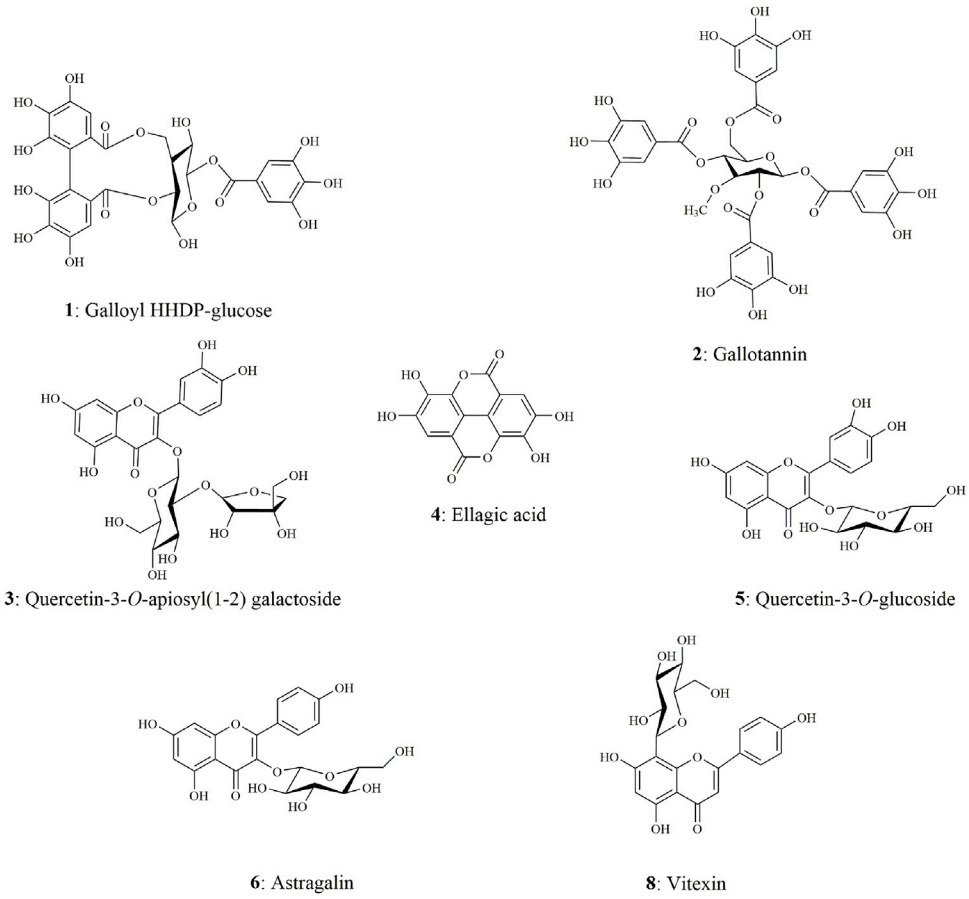

FIGURE 3
Chemical structures of the seven identified compounds from EHNB.

4 Discussion

4.1 Antidiabetic and anti-inflammatory activities of *E. humifusa*

DM is a ubiquitous health disorder resulting from insulin deficiency or malfunction, which leads to a high rate of glucose in the blood (Kazeem et al., 2021). Research revealed that a high level of blood glucose enhances oxidative stress at the cell level, which boosts the pro-inflammatory cytokines activities, such as interleukins, tumor necrosis factors as well as mediators like nitric oxide. The aforementioned cytokines induce the expression of COX-2 and increase prostaglandin synthesis, implying that diabetes plays a crucial role in the chronic inflammatory state (Burgos-Morón et al., 2019; Kaur and Singh, 2022). The side effect and toxicity of most actual existent drugs urges researchers to seek for safe and effective nature-based agents to manage this prevalence and debilitating complication of DM. Reducing postprandial hyperglycemia is one of the relevant therapeutic approaches for DM, which can be achieved by inhibiting digestive enzymes such as α-Glu. In the same way, blocking the COX-2 enzyme function is among the principal strategy to regulate inflammation (Karim et al., 2019). Although *E. humifusa* has been used to cure a variety of health disorders including hyperglycemia and inflammation-based diseases, the scientific reports describing the key bioactive compounds in this species remain elusive. Therefore, in this present study, we have determined primarily the biological potential of *E. humifusa* crude extract and its fractions followed by the characterization and screening of the bioactive ligands through bio-affinity UPLC/QTOF- MS.

The preliminary *in vitro* evaluation of all *E. humifusa* samples showed good antidiabetic activity, which is in line to the investigation of Kang and his coworkers, demonstrating the noticeable capacity of *E. humifusa* methanol extract to inhibit α-Glu (Kang et al., 2012). Among these samples, the *n*-butanol fraction (EHNB) was discovered to be the most active part with an IC_{50} value of 0.54 ± 0.12 μg/ml and significantly more potent than the common hypoglycemic acarbose drug. Similarly, the anti-inflammatory activity of EHNB was assessed the best, even

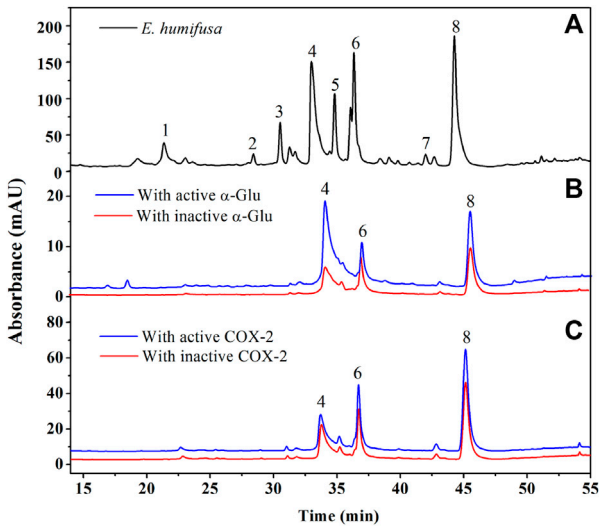

FIGURE 4
UPLC-UV chromatograms comparison of the EHNB sample (black) EHNB incubated with active enzyme (blue), and inactive enzyme (red) obtained at 360 nm. **(A)** Represents the chromatogram obtained from EHNB sample, **(B)** from α-Glu target and **(C)** from COX-2 target.

when compared to the NSAID indomethacin. Therefore, our finding enhances prior discoveries demonstrating the anti-inflammatory of this species through inhibition of soluble epoxide hydrolase (SEH), lipopolysaccharide (LPS)-induced nitric oxide and the tumor necrosis factors (TNF) productions (Luyen et al., 2014). Interestingly, the antioxidant evaluations via DPPH, ABTS and FRAP assays showed the highest potentialities of the EHNB fraction. The close correlations between these EHNB favorable pharmacological effects are consistent with previous findings describing the relevance of Euphorbiaceae-based antioxidants in controlling the initiation or propagation of other chronic diseases (Majid et al., 2015; Mustafa et al., 2021). Furthermore, the richness of this EHNB fraction with phenolic and flavonoids (Table 1) may support the multiple drug activities of this plant due to the abilities of plant polyphenols to modulate various enzymes and immune cells in human, in spite their antioxidant potentials (Sekhon-Loodu and Rupasinghe, 2019; Sun et al., 2020).

4.2 Potential inhibitors in EHNB and their docking poses

The chemical characterization of EHNB allowed us to identify seven phytoconstituents of which one phenolic acid named ellagic acid and two flavonoid glycosides astragalin and vitexin were fished out as ligand candidates through bio-affinity ultrafiltration UPLC/QTOF-MS. Astragalin and vitexin were selected as potential inhibitors due to their relative binding strength values together with the *in silico* BE values, whose activities were further verified (Xu et al., 2022; Ye et al., 2022; Zhang et al., 2022). As illustrated in Table 2, those two flavonoid glycosides showed better SB values towards the enzyme targets α-Glu and COX-2. This binding strength diversity was speculated to be caused by the competitive interaction of the ligand candidates with the target enzymes. Additionally, these two ligands were docked well in the catalytic site of the targets with a BE values lower than the respective drugs acarbose and indomethacin. The network interaction diagram (Figure 7) highlights the discovery of the potential ligands vitexin and astragalin as well as their double acting actions. Both vitexin and astragalin interacted with the recognized key amino acid residues of α-Glu catalytic pocket composed of Arg315, Glu411, His280, Asp242, Asp307, Ser311, Pro312, Leu313, Tyr158, and Lys156 (Cai et al., 2020; Nokhala et al., 2020). The lower BE value of vitexin (−8.65 kcal/mol) was supported by the presence of one extra H-bond with GLU 411 which was absent for astragalin (−8.61 kcal/mol). It is emphasized that α-Glu complexes formed with those potent ligands were far more stable than those made with the antidiabetic drug acarbose, which had just a BE value of −5.25 kcal/mol. In the meantime, vitexin was found more stable within the COX-2 catalytic site than astragalin due to H-bonds number differences and hydrophobic interactions. Nonetheless, both of them revealed better BE value than the NSAID indomethacin which were ranked as follows vitexin (−9.19 kcal/mol)> astragalin (−8.94 kcal/mol)> indomethacin (−6.64 kcal/mol). The interactions of those potential ligands with the reported important key residues Arg120, Arg513, Tyr355, and Val523 inside the active pocket are consistent with the intercalation of selective COX-2 inhibitors docking (Baek et al., 2021).

4.3 Vitexin and astragalin activities validations

The antidiabetic *in vitro* validation results synthesized in Figure 6 and Table 2 demonstrated that vitexin had better activity than astragalin with an IC_{50} of 36.38 ± 3.06 μM and 42.47 ± 4.13 μM, respectively. Both candidates exerted a significant inhibition potentiality than the well-known hypoglycemic drug acarbose possessing an IC_{50} of 109.54 ± 14.23 μM. Recent studies have highlighted the relevance of vitexin in glucose homeostasis. It helps in the protection of pancreatic tissues from damage, enhances the uptake of glucose, and modulates the catalytic function of the two main digestive enzymes α-amylase and α-Glu (He et al., 2016; Seyedan et al., 2019; Abdulai et al., 2021). In the

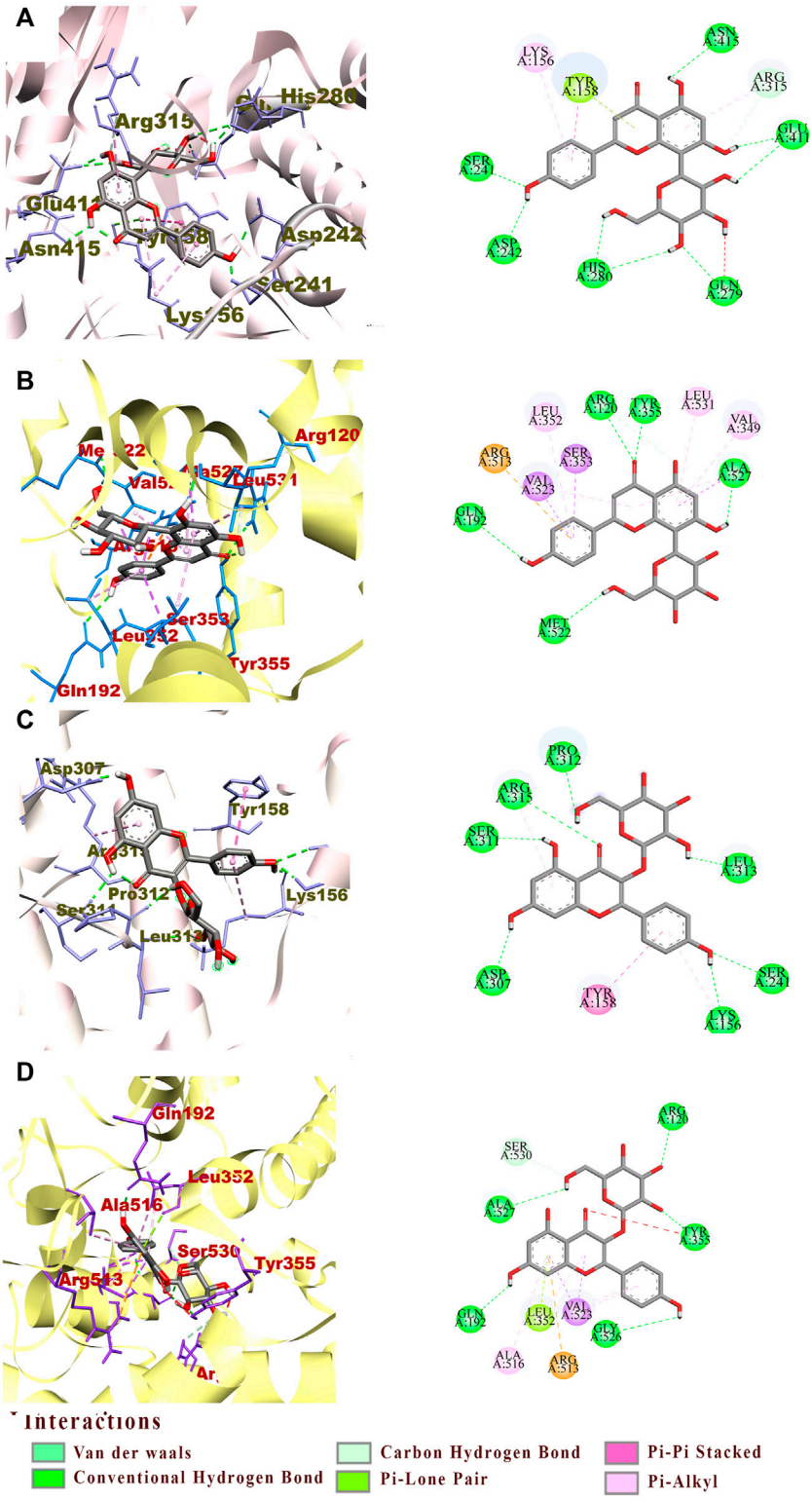

FIGURE 5
The interactions between active pocket of α-Glu (grey) or COX-2 (yellow) with vitexin **(A,B)** and astragalin **(C,D)** by molecular docking analysis. The key interacting amino acid residues are: Serine (Ser), Glutamine (Gln), Arginine (Arg), Glutamic acid (Glu), Tyrosine (Tyr), Lysine (Lys), Aspartic acid (Asp), Proline (Pro), Leucine (Leu), Valine (Val), and Glycine (Gly).

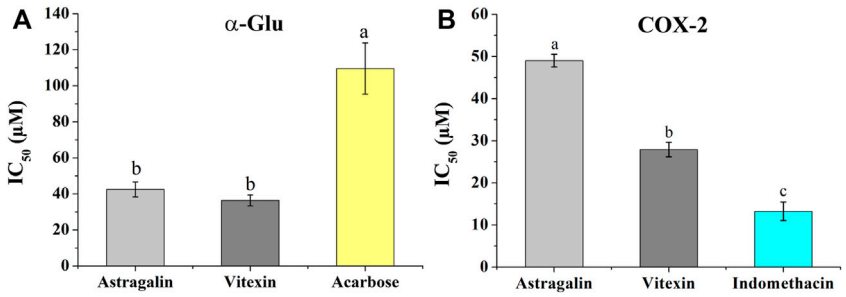

FIGURE 6
Inhibition effects of astragalin and vitexin compared to the respective positive controls on α-Glu **(A)** and COX-2 **(B)**. The label letters **(a,b,c)** represent the significant difference at *p*-value < 0.05 by ANOVA DMRT.

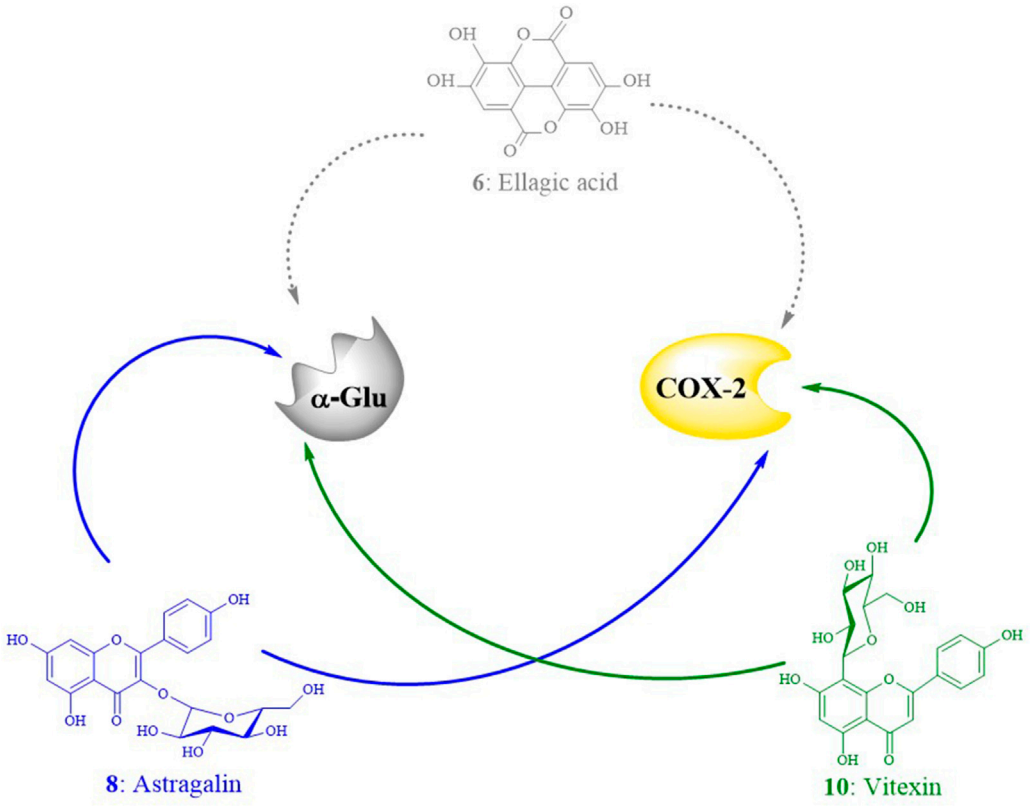

FIGURE 7
Interaction networks diagram of the ligand candidates from *E. humifusa* toward the enzyme targets α-Glu and COX-2. The dotted arrows reflect weak ligand affinities toward the targets active site, whereas the plain ones represent strong affinities.

same way, previous investigations have highlighted the hypoglycemic effectivities of astragalin through inhibition of key enzymes like α-Glu, α-amylase, and tyrosine phosphatase (PTP 1B) (Cen et al., 2017; Lima et al., 2018). The *in vitro* anti-inflammatory validation exhibited good COX-2 inhibition activity of vitexin with an IC_{50} value of 27.91 ± 1.74 μM. This result was in support of prior studies, which demonstrated the anti-inflammatory activity of vitexin via downregulation of pro-inflammatory cytokines releases, as well as reducing the actions of inflammatory mediators like COX-1 and COX-2 (Babaei et al., 2020; Abdulai et al., 2021; Peng et al., 2021). Likewise, the moderate COX-2 inhibition of

astragalin ($IC_{50:}$ 49.05 ± 1.49 μM) contributed to the explanation of its multifaceted activity to mitigate inflammation. Therefore, besides astragalin's capacities to block mitogen-activated protein kinases (MAPK) signaling pathways and to inhibit the production of inflammatory mediators like prostaglandin E2 (PGE2) (Zhang et al., 2017; Alblihed, 2020), here it is supported to regulate COX-2 function.

Taken together, the finding from this study helps to understand the principal bioactive compounds playing pivotal roles in the multifarious empirical uses of *E. humifusa*. The two screened-out active ligands (vitexin and astragalin) were confirmed to exert potential antidiabetic and anti-inflammatory activities. Therefore, the development of natural therapeutic drug candidates for diabetes mellitus and its associated complications based on those two flavonoids could be promising.

5 Conclusion

DM and its associated complications have devastated human beings in the last 2 decades. So far, *E. humifusa* has been reported to possess multiple virtues including its antidiabetic and anti-inflammatory activities. The present investigation was designed to screen out the potential double-acting bioactive compounds and their mechanism of action toward hyperglycemia and inflammation. Integrated method comprising the high throughput bio-affinity ultrafiltration combined with UPLC/QTOF-MS targeting α-Glu and COX-2 enzymes, *in vitro* assays, and *in silico* study were adopted throughout the research. The *n*-butanol (EHNB) fraction was revealed to be the best active fraction, from which two flavonoid glycosides vitexin and astragalin were fished out as potential ligands. The *in silico* simulation showed their great stability within the active site of the two target enzymes. Meanwhile, the validation activity assays confirmed their capacities to inhibit concomitantly α-Glu and COX-2. To sum up, this present investigation offered additional scientific support for the antidiabetic and anti-inflammatory use of *E. humifusa* and facilitated eliciting the modes of action of its potential active compounds.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

TR performed the experiments, analyzed the data, and wrote the original manuscript. MF co-supervised and revised the manuscript for submission. MG conceived of, designed, administrated the project and supervised the whole study. All authors have read and agreed to the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Dongmei Li,
Georgetown University, United States

REVIEWED BY
Md. Moklesur Rahman Sarker,
State University of Bangladesh, Bangladesh
Rohit Sharma,
Banaras Hindu University, India

*CORRESPONDENCE
Guirong Liu,
645173627@qq.com

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Systematic review and meta-analysis of *Coptis chinensis* Franch.-containing traditional Chinese medicine as an adjunct therapy to metformin in the treatment of type 2 diabetes mellitus

Linlin Pan[1], Xin Zhai[2], Zhanhui Duan[2], Kun Xu[2] and Guirong Liu[1]*

[1]Department of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China, [2]Department of Chinese Medicine Literature and Culture, Shandong University of Traditional Chinese Medicine, Jinan, China

Background: In China, *Coptis chinensis* Franch. (Chinese name: Huanglian) prescriptions (HLPs) are prominent hypoglycemic agents used in glycemic control. However, the curative effect of HLPs as adjunctive therapies for type 2 diabetes mellitus (T2DM) has not been evaluated. Based on a systematic review and a meta-analysis, this study was conducted to assess the effects of HLPs combined with metformin as a reinforcing agent for T2DM.

Materials and methods: A total of 33 randomized controlled trials (RCTs) reporting on 2,846 cases concerning the use of HLPs in the treatment of T2DM were identified from the China National Knowledge Infrastructure (CNKI), Weipu (VIP), Wanfang, PubMed, Cochrane Library, and EMBASE databases. Primary outcomes included fasting blood glucose (FBG), 2-h postprandial blood glucose (2hPG), glycosylated hemoglobin, type A1c (HbA1c), fasting serum insulin (FINS), and homeostasis model assessment of insulin resistance (HOMA-IR). Secondary outcomes included total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and gastrointestinal dysfunction (GD). Continuous data were expressed as mean differences (MDs) with 95% confidence intervals (CIs). The methodological quality of the included RCTs was assessed by Cochrane evidence-based medicine systematic evaluation.

Abbreviations: 2hPG, 2-h postprandial blood glucose; AIS, accrued information size; CI, confidence intervals; CNKI, China National Knowledge Infrastructure; DHHL, Dahuang huanglian xiexin; FBG, fasting blood glucose; FINS, fasting serum insulin; GD, gastrointestinal dysfunction; GGQL, Gege qinlian; GRADE, Grades of Recommendation Assessment Development and Evaluation; HbA1c, glycosylated hemoglobin type A1c; HDL-c, high density lipoprotein cholesterol; HLEJ, Huanglian ejiao; HLJD, Huanglian jiedu; HLPs, Huanglian prescriptions; HLWD, Huanglian wendan; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-c, low density lipoprotein cholesterol; MD, mean difference; RCTs, randomized controlled trials; RIS, required information size; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TCM, traditional Chinese medicine; TG, triglyceride; TSA, trial sequential analysis.

Statistical analysis was performed using the Review Manager and Stata software. The required information size and treatment benefits were evaluated by trial sequential analysis (TSA). The quality of evidence was rated using the Grades of Recommendation Assessment, Development, and Evaluation (GRADE) approach.

Results: The results revealed that HLPs are beneficial to improve the following: FBG (MD = −1.16%, 95% CI: −1.24 to −1.07), 2hPG (MD = −1.64%, 95% CI: −1.84 to −1.43), HbA1c (MD = −0.78%, 95% CI:−0.96 to −0.60), FINS (MD = −1.94%, 95% CI: −2.68 to −1.20), HOMA-IR (MD = −0.77%, 95% CI: −1.28 to −0.27), TC (MD = −0.70%, 95% CI: −1.00 to −0.39), TG (MD = −0.57%, 95% CI: −0.74 to −0.40), LDL-c (MD = −0.70%, 95% CI: −0.97 to −0.43), and HDL-c (MD = −0.21%, 95% CI: −0.32 to −0.10) for patients with T2DM. The funnel plot, Egger's test, and trim-and-fill method indicated a moderate publication bias in the results. The TSA showed that the required sample size of HLPs in improving FBG, 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c could sufficiently draw reliable conclusions. GRADE assessment revealed that the quality of the evidence for the effectiveness of HLPs in improving FBG was moderate, but the quality of evidence for 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c was low, and for GD was very low.

Conclusion: The systematic review and meta-analysis suggested that HLPs were beneficial for achieving glycemic control. However, HLPs recommended for T2DM patients have yet to be confirmed because of the poor methodological quality of some trials. Therefore, more RCTs with multicenter and double-blind designs are needed to assess the efficacy of HLPs for patients with T2DM.

KEYWORDS

coptis chinensis franch, type 2 diabetes mellitus, systematic review, meta-analysis, curative effect

1 Introduction

Diabetes mellitus, which seriously endangers human health, is mainly caused by defects in insulin secretion and insulin action and is characterized by disorders of glucose metabolism. (Lin and Sun, 2010). An International Diabetes Federation survey predicted that patients with diabetes mellitus will exceed 645 million by 2045 (Carracher et al., 2018). Generally, more than 90% of diabetes mellitus patients have type 2 diabetes mellitus (T2DM). In addition to following diet and lifestyle guidelines, due to the significant hypoglycemic effect of metformin, it is often recommended to intervene with metformin in patients with T2DM (Sharma et al., 2015; Sanchez-Rangel and Inzucchi, 2017). However, due to the certain limitations of metformin in long term use, options from natural products are being searched to meet the need (Sharma and Prajapati, 2017). In recent decades, traditional Chinese medicine (TCM) and its active ingredients have become increasingly popular in Asian countries, and combined with metformin, is widely used as a reinforcing agent in glycemic control (Pang et al., 2018; Tian et al., 2019; Wu et al., 2019).

Ancient TCM theories effectively study a disease as a whole and propose that the pathogenesis of diabetes mellitus lies in damp-heat accumulation in the spleen and stomach (Tong et al., 2009). In classic TCM books, *Explanation of Materia Medica* (Chinese name: *Bencaojing Jizhu*) and *Tang Materia Medica* (Chinese name: *Tang Bencao*) clarified that the prescriptions containing *Coptis chinensis* Franch. (Chinese name: Huanglian) can effectively alleviate the symptoms of polydipsia, polyphagia, and polyuria (Tong, 2013). *Coptis chinensis* Franch. As a treatment for diabetes mellitus and related complications, also has a long history in Japan, Korea, Malaysia, Singapore, and India (Li et al., 2013; Sharma et al., 2021). Modern pharmacological investigations have indicated that some ingredients in *Coptis chinensis* Franch. such as berberine, jatrorrhizine, coptisine, palmatine, epiberbeine, and polysaccharides, exert significant therapeutic effects on multiple targets to improve islet function and regulate glucose metabolism (Fu et al., 2005; Chen et al., 2012; Wang et al., 2019). For example, alkaloids can help alleviate hyperglycemia by promoting glucose uptake (Yang et al., 2014), polysaccharides can produce antidiabetic activity via its antioxidative effect (Jiang et al., 2015), and

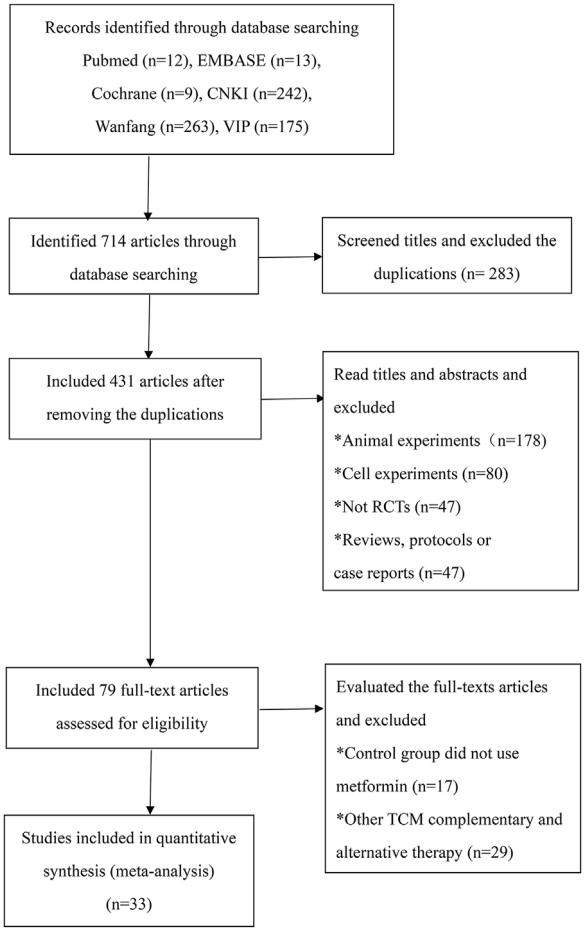

FIGURE 1
Flow diagram of the literature search process.

berberine can improve insulin resistance by inhibiting the expression of tumor necrosis factor-α and free fatty acids (Huang et al., 2018).

Recent studies have indicated that Huanglian prescriptions (HLPs) contribute to enhancing insulin sensitivity, stimulating insulin secretion, protecting β-cells, and regulating glycometabolism disorders (Liu et al., 2010; Yu et al., 2012; Li et al., 2019). Therefore, either as monotherapy or adjunct therapy, HLPs are recognized as the most effective TCM antidiabetic prescriptions for T2DM in China. HLPs, such as Dahuang huanglian xiexin (DHHL) decoction, Gegen qinlian (GGQL) decoction, Huanglian ejiao (HLEJ) decoction, Huanglian jiedu (HLJD) decoction, and Huanglian wendan (HLWD) decoction, have been widely used as adjuvant therapies to metformin for glycemic control (Fan et al., 2017; Li et al., 2017; Song et al., 2022; Wang 2020; Zhou et al., 2022). However, to date, there is no large scale clinical evidence on the inhibitory effects of HLPs on T2DM. Also, no published reports can comprehensively evaluate the intervention and side effects of HLPs on glycolipids. Therefore, we included clinical randomized controlled trials (RCTs) for systematic review and meta-analysis to evaluate the effectiveness of HLPs as adjuvant therapies to metformin for patients with T2DM.

2 Materials and methods

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, obtaining data from published trials.

2.1 Search strategies

All articles were searched using medical subject headings terms and free words in the China National Knowledge Infrastructure (CNKI), Wanfang, Weipu (VIP), PubMed, Cochrane Library, and EMBASE databases. The search period for the encompassed articles from the established time to 30 July 2022. Two authors (Xin Zhai and Linlin Pan) independently searched the related articles regardless of type and language. The following terms were used in English databases: ["Type 2 diabetes" or "Type 2 diabetes mellitus" or "T2DM" or "Non insulin dependent diabetes mellitus" or "Impaired fasting glucose" or "Impaired glucose tolerance" or "Xiaoke"] and ["Random allocation" or "Randomized controlled trial" or "Random" or "Randomized" or "Placebo" or "RCT"] and ["Huanglian"or "*Coptis chinensis* Franch." or "Coptidis Rhizoma" or "Coptis chinensis" or "Rhizoma coptidis"]. The following terms were used in Chinese databases: ["Erxing Tangniaobing" or "Xiaoke" (T2DM)] and ["Suiji duizhao shiyan" or "Mangfa" or "Anweiji" (RCT)] and ["Huanglian"]. The search strategies are presented in detail in Supplementary Table S1.

2.2 Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) *Participants*. Diagnosed with T2DM; 2) *Interventions*. Control group treated with metformin and experimental group treated using metformin incorporated with HLPs; 3) *Type of trials*. RCT; 4) *Outcomes*. Fasting blood glucose (FBG), 2-h postprandial blood glucose (2hPG), glycosylated hemoglobin, type A1c (HbA1c), fasting serum insulin (FINS), homeostasis model assessment of insulin resistance (HOMA-IR), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and gastrointestinal dysfunction (GD). The exclusion criteria were as follows: 1) Non-clinical intervention trials (animal research, cell research, review, protocol); 2) Patients diagnosed with other diseases; 3) Patients with other TCM medications, acupuncture, massage or moxibustion.

TABLE 1 Characteristics of the included studies.

References	Trial types	Sample size (E/C)	Sex (M/F)	Age (years) (E/C)	Course of disease (year) (E/C)	Interventions		Course of treatment
			EC			E	C	
Li et al. (2017)	RCT	76 (43/33)	Unknown	39-67 (Mean 53.2 ± 7.7)	Mean 5.3 ± 3.4	DHHL	M (50 mg ET, Tid)	12 weeks
Wu et al. (2019)	RCT*	86 (43/43)	(28/15)/(29/14)	Mean 52.6 ± 10.2/52.3 ± 9.7	Mean 2.6 ± 1.7/2.6 ± 1.6	DHHL	M (0.5 g ET, Tid)	Unknown
Zou et al. (2016)	RCT*#	106 (53/53)	(29/24)/(27/26)	Mean 53.69 ± 10.14/52.38 ± 10.03	Median 5-10	DHHL	M (50 mg ET, Tid)	24 weeks
Fan et al. (2017)	RCT	70 (35/35)	(21/14)/(19/16)	18-60 (Mean 36.4 ± 7.1/38.0 ± 6.5)	Mean 3.1 ± 1.7/3.4 ± 1.5	GGQL	M (0.85 g ET, Bid)	8 weeks
Fu (2017)	RCT*	66 (33/33)	(17/13)/(16/14)	18-60 (Mean 56.07 ± 8.25/57.50 ± 8.19	Mean 5.20 ± 2.09/5.60 ± 2.01	GGQL	M (0.5 g ET,Tid)	12 weeks
Jin et al. (2019)	RCT*	60 (30/30)	(16/14)/(18/12)	25-83(Mean 58.06 ± 3.14)/22-85 (Mean 57.98 ± 3.72)	Mean 3.52 ± 0.86/3.47 ± 0.91	GGQL	M (0.5 g ET, Qd)	8 weeks
Pang et al. (2018)	RCT#	90 (45/45)	(23/22)/(24/21)	41-72(Mean53.5 ± 8.2)/42-71(Mean54.1 ± 8.3)	Mean 5.5 ± 1.3/5.4 ± 1.1	GGQL	M (0.25 g ET, Tid)	8 weeks
Xiong (2019)	RCT*	100 (50/50)	(29/21)/(30/20)	40-70(Mean53.7 ± 7.7)/40-70(Mean53.5 ± 7.8)	Mean 4.85 ± 1.05/4.75 ± 1.10	GGQL	M (0.25 g ET, Tid)	8 weeks
Zhang (2019)	RCT	70 (35/35)	Unknown	35-70/36-71	Unknown	GGQL	M (0.25 g ET, Bid)	8 weeks
Zhang et al. (2018)	RCT*	95 (48/47)	(26/22)/(25/22)	Mean 51.3 ± 6.8/51.2 ± 7.3	Mean 5.4 ± 2.3/5.6 ± 2.1	GGQL	M (0.5 g ET, Tid)	8 weeks
Liu (2006)	RCT	76 (47/29)	(29/18)/(17/12)	45-63/41-65	1.5-16/1-16	HLEJ	M (0.5 g ET, Tid)	4 weeks
Liu et al. (2017)	RCT*	86 (43/43)	(30/13)/(28/15)	45-76 (Mean 65.4 ± 4.7)/45-75 (Mean65.1 ± 4.8)	Mean 6.8 ± 1.7/6.4 ± 1.5		M	
Gao (2020)	Unknown	66 (33/33)	(18/15)/(19/14)	58.62 ± 6.13/58.54 ± 5.49	Mean 6.02 ± 1.68/6.08 ± 1.70	HLEJ	M (0.5 g ET, Bid)	4 weeks
Wang (2020)	RCT*	90 (45/45)	(27/18)/(23/22)	Mean 51.4 ± 3.4/52.3 ± 5.1	Unknown	HLEJ	M (0.5 g ET, Tid)	15 days
Zhou et al. (2022)	RCT	120 (60/60)	(29/31)/(33/27)	22-75 (Mean 53.25 ± 11.47)/24-75(Mean 54.25 ± 10.85)	Mean6.45 ± 2.51/6.51 ± 2.44	HLEJ	M (0.5 g ET, Tid)	8 weeks
Ding (2018)	RCT	104 (52/52)	(22/30)/(23/29)	57-86 (Mean 69.0 ± 4.6)/57-85(Mean 69.2 ± 4.7)	Mean 4.3 ± 2.4/4.1 ± 2.5	HLJD	M (0.25 g ET, Tid)	12 weeks
Feng (2019)	RCT*	90 (45/45)	(25/20)/(26/19)	53-80(Mean 64.2 ± 7.5)/54-81(Mean 64.7 ± 7.3)	Mean 4.48 ± 1.59/4.55 ± 1.67	HLJD	M (0.25 g ET, Tid)	12 weeks
Xing et al. (2017)	RCT	106 (51/55)	(27/24)/(28/27)	35-61(Mean 58.3 ± 12.6)/38-62(Mean 56.6 ± 11.7)	Mean 3.5 ± 1.8/3.6 ± 1.7	HLJD	M (0.5 g ET, Tid)	2 weeks
Yang and Wang. (2013)	RCT	66 (33/33)	(22/11)/(20/13)	25-65 (Mean 42 ± 16/40 ± 15)	Unknown	HLJD	M	24 weeks
Zhang (2014)	RCT	260 (130/130)	140/120	42 -65 (Mean 51.9 ± 5.8)	Unknown	HLJD	M (0.25 g ET, Tid)	12 weeks
Chen and Wang. (2021)	RCT*	99 (50/49)	(28/22)/(25/24)	38-70(Mean54.37 ± 2.56)/39-71(Mean54.41 ± 2.24)	Mean10.27 ± 3.96/10.25 ± 3.94	HLJD	M (0.5 g ET, Tid)	12 weeks
Wei et al. (2021)	RCT	60 (30/30)	Unknown	40-65(Mean53.68 ± 5.02)/39-68(Mean55.05 ± 4.82)	Mean 5.1 ± 0.5/5.00 ± 1.01	HLJD	M (0.5 g ET, Tid)	Unknown
Song et al. (2022)	RCT*	100 (50/50)	(34/16)/(32/18)	29-70(Mean 52.45 ± 7.12)/30-70(Mean 52.48 ± 7.15)	4 weeks-6 years/3 weeks-6 years	HLJD	M (0.25 g ET, Tid)	8 weeks
Chen (2018)	RCT	60 (30/30)	(12/18)/(12/18)	Mean 58.57/58.9	Unknown	HLWD	M (0.5 g ET, Tid)	8 weeks
Dong (2017)	RCT*	70 (35/35)	(20/15)/(19/16)	40-65 (Mean 51.3 ± 5.1/52.8 ± 4.7)	Unknown	HLWD	M (0.5 g ET, Tid)	12 weeks
Ji (2017)	RCT	60 (30/30)	(14/16)/(12/18)	20-79	Mean 4.12 ± 3.45/4.66 ± 2.87	HLWD	M (0.5 g ET, Tid)	12 weeks

(Continued on following page)

TABLE 1 (*Continued*) Characteristics of the included studies.

References	Trial types	Sample size (E/C)	Sex (M/F)	Age (years) (E/C)	Course of disease (year) (E/C)	Interventions		Course of treatment
			EC			E	C	
Zhang (2019)	RCT	60 (30/30)	(17/13)/(13/17)	30-65 (Mean47.5 ± 7.7/48.53 ± 8.59)	Mean 3.64 ± 2.63/3.78 ± 3.42	HLWD	M (0.5 g ET, Tid)	12 weeks
Fu (2021)	RCT*	120 (60/60)	(30/30)/(31/29)	20-70(Mean55.72 ± 1.62)/19-71(Mean56.59 ± 1.71)	Mean 7.21 ± 2.62/7.35 ± 2.23	HLWD	M (0.85 g ET, Tid)	
Liu et al. (2021)	RCT*	68 (34/34)	(19/15)/(11/23)	Mean 55 ± 11/55 ± 7	Unknown	HLWD	M (0.5 g ET, Tid)	8 weeks
Pan et al. (2021)	RCT	80 (41/39)	(20/21)/(18/21)	40-60(Mean 50.1 ± 5.5)/42-62(Mean51.2 ± 5.4))	Mean (4.32 ± 0.19/4.12 ± 0.23	HLWD	M (0.5 g once a day)	16 weeks
Wang et al. (2021)	RCT*	60 (30/30)	(15/15)/(13/17)	Mean 60.07 ± 7.1/58.70 ± 6.97	Unknown	HLWD	M (0.5 g ET, Tid)	8 weeks
Wang Y 2022	RCT*	50 (25/25)	(16/9)/(15/10)	50-70	1-6	HLWD	M (0.5 g once a day)	16
Zhang (2022)	RCT	76 (38/38)	(18/20)/(19/19)	Mean 69.42 ± 12.4/68.92 ± 11.89	1-6 (month)	HLWD	M (0.25 g ET, Bid)	8

Notes: E, experimental group; C, control group; M, metformin; *, Random number table method; #, Double-blind; Qd, One time a day; Bid, Two times a day; Tid, Three times a day; ET, each time.

2.3 Literature selection and data extraction

Two authors (Linlin Pan and Xin Zhai) independently evaluated the title, abstract, and full texts of the articles. The articles that met the inclusion criteria were then selected. Inconsistencies were settled by discussion. Finally, important information from the included articles was extracted for analysis, including the name of the first author, year of publication, trial types, sample size, sex, age, course of the disease, interventions, and course of treatment.

2.4 Risk of bias

Linlin Pan and Xin Zhai independently evaluated the methodological quality of each trial by using the Cochrane risk-of-bias tool (Higgins et al., 2011). Disagreements were discussed and resolved by Guirong Liu. The criteria assessed were as follows: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other biases. The risk of bias was rated as high, unclear, or low.

2.5 Data synthesis and analysis

RevMan (version 5.3) was used to perform statistical analysis. Continuous data were expressed as the mean difference (MD) with a 95% confidence interval (CI), and $p < 0.05$ was considered statistically significant. Heterogeneity was evaluated using the chi^2 and I^2 tests, and $p < 0.10\%$ or $I^2 > 50\%$ was considered to have marked heterogeneity. The low-heterogeneity data ($p > 0.10\%$ or $I^2 < 50\%$) used the fixed-effect model, and the high-heterogeneity data ($p < 0.10\%$ or $I^2 > 50\%$) used the random-effects model. Sensitivity analysis was evaluated using various statistical methods. Publication bias was assessed by visual observation of the symmetry of funnel plots, Egger's test ($p < 0.05$ indicates publication bias), and the trim-and-fill method.

Trial sequential analysis (TSA) was conducted to calculate the required information size (RIS) for meta-analysis and evaluate the intervention benefits on the basis of the accrued information size (AIS). The risk of a type I error was set at 5% with a power of 80%. The variance was calculated based on the data included in the trials, and the relative risk reduction was set at 20% (Wetterslev et al., 2017). The evidence for the intervention was considered reliable when cumulative Z-curves crossed sequential monitoring boundaries. The Grades of Recommendation Assessment, Development, and Evaluation (GRADE) approach was used to rate the quality of the evidence as high, moderate, low, or very low (Guyatt et al., 2008).

3 Results

3.1 Search results

A total of 714 articles were identified in the initial database search (Figure 1). First, we used Endnote to exclude 283 duplicates, and the articles were decreased to 431. Second, we read the titles and abstracts and excluded animal experiment

TABLE 2 Details of the HLPs for each study.

Interventions	References	Prescription
DHHL decoction	Li et al. (2017)	*Coptis chinensis* Franch. 5 g, *Rheum palmatum* L. 10 g, Scutellaria baicalensis Georgi 5 g
	Wu et al. (2019)	*Coptis chinensis* Franch. 3 g, *Rheum palmatum* L. 6 g, Scutellaria baicalensis Georgi 10 g
	Zou and lao, (2016)	*Coptis chinensis* Franch. 5 g, *Rheum palmatum* L. 10 g, Scutellaria baicalensis Georgi 5 g
GGQL decoction	Fan et al. (2017)	*Coptis chinensis* Franch. 10 g, *Scutellaria baicalensis* Georg 15 g, *Pueraria lobata* (Willd.) Ohwi 30 g, *Glycyrrhiza uralensis* Fisch. 6 g
	Fu, (2017)	*Coptis chinensis* Franch. 30 g, *Scutellaria baicalensis* Georg 20 g, *Pueraria lobata* (Willd.) Ohwi 50 g, *Glycyrrhiza uralensis* Fisch. 6 g
	Jin et al. (2019)	*Coptis chinensis* Franch. 10 g, *Scutellaria baicalensis* Georg 15 g, *Pueraria lobata* (Willd.) Ohwi 30 g, *Glycyrrhiza uralensis* Fisch. 6 g
	Pang et al. (2018)	*Coptis chinensis* Franch. 22.5 g, *Scutellaria baicalensis* Georg 22.5 g, *Pueraria lobata* (Willd.) Ohwi 60 g, *Glycyrrhiza uralensis* Fisch. 15 g, *Zingiber officinale* Roscoe 3.5 g
	Xiong, (2019)	*Coptis chinensis* Franch. 22.5 g, *Scutellaria baicalensis* Georg 22.5 g, *Pueraria lobata* (Willd.) Ohwi 60 g, *Glycyrrhiza uralensis* Fisch. 15 g, *Zingiber officinale* Roscoe 3.5 g
	Zhang et al. (2019)	*Coptis chinensis* Franch. 5 g, *Scutellaria baicalensis* Georg 20 g, *Pueraria lobata* (Willd.) Ohwi 20 g, *Glycyrrhiza uralensis* Fisch. 5 g
	Zhang et al. (2018)	*Coptis chinensis* Franch. 20 g, *Scutellaria baicalensis* Georg 20 g, *Pueraria lobata* (Willd.) Ohwi 30 g, *Glycyrrhiza uralensis* Fisch. 9 g
HLEJ decoction	Liu, 2006	*Coptis chinensis* Franch. 10 g, *Scutellaria baicalensis* Georgi 15 g, *Paeonia anomala* L. 15 g, Asparagus acutifolius L 20 g, Colla corii asini 15 g
	Liu, (2017)	*Coptis chinensis* Franch. 10 g, *Scutellaria baicalensis* Georgi 15 g, *Paeonia anomala* L. 15 g, Asparagus acutifolius L 20 g, Colla corii asini 15 g
	Gao, (2020)	*Coptis chinensis* Franch. 8 g, *Rheum palmatum* L. 10 g, *Paeonia anomala* L. 15 g, Colla corii asini 10 g, Semen Ziziphi Spinosae 25 g, *Rehmannia glutinosa* (Gaertn.) DC. 20 g, *Polygonum multiflorum* Thunb. 15 g, *Anemarrhena asphodeloides* Bunge 10 g, fresh egg yolk 1
	Wang, (2020)	*Coptis chinensis* Franch. 10 g, Scutellaria baicalensis Georgi 15 g, *Paeonia anomala* L. 15 g, Asparagus acutifolius L 20 g, Colla corii asini 15 g)
	Zhou et al. (2022)	*Coptis chinensis* Franch. 10 g, Scutellaria baicalensis Georgi 6 g, *Paeonia anomala* L. 10 g, fresh egg yolk 1, Colla corii asini 10 g
HLJD decoction	Ding, (2018)	*Coptis chinensis* Franch. 12 g, *Scutellaria baicalensis* Georgi 9 g, *Phellodendron amurense* Rupr. 9 g, *Gardenia jasminoides* J.Ellis 12 g
	Feng, (2019)	*Coptis chinensis* Franch. 12 g, *Scutellaria baicalensis* Georgi 9 g, *Phellodendron amurense* Rupr. 9 g, *Gardenia jasminoides* J.Ellis 12 g
	Xing et al. (2017)	*Coptis chinensis* Franch. 12 g, *Scutellaria baicalensis* Georgi 12 g, *Phellodendron amurense* Rupr. 9 g, *Gardenia jasminoides* J.Ellis 12 g
	Yang and Wang, (2013)	*Coptis chinensis* Franch. 15 g, *Scutellaria baicalensis* Georgi 10 g, *Phellodendron amurense* Rupr. 6 g, *Gardenia jasminoides* J.Ellis 10 g
	Zhang, (2014)	*Coptis chinensis* Franch. 9 g, *Scutellaria baicalensis* Georgi 6 g, *Phellodendron amurense* Rupr. 6 g, *Gardenia jasminoides* J.Ellis 9 g
	Chen and Wang, (2021)	*Coptis chinensis* Franch. 10 g, *Scutellaria baicalensis* Georgi 10 g, *Phellodendron amurense* Rupr. 10 g, *Gardenia jasminoides* J.Ellis 10 g, *Ophiopogon japonicus* (Thunb.) Ker Gawl. 12 g, *Scrophularia ningpoensis* Hemsl. 12 g, *Rehmannia glutinosa* (Gaertn.) DC. 12 g, *Forsythia suspensa* (Thunb.) Vahl 15 g, *Taraxacum mongolicum* Hand.-Mazz. 15 g, *Lonicera japonica* Thunb. 20 g
	Wei et al. (2021)	*Coptis chinensis* Franch. 10 g, *Scutellaria baicalensis* Georgi 15 g, *Phellodendron amurense* Rupr. 15 g, *Gardenia jasminoides* J.Ellis 10 g
	Song et al., 2022	*Coptis chinensis* Franch. 12 g, *Scutellaria baicalensis* Georgi 12 g, *Phellodendron amurense* Rupr. 9 g, *Gardenia jasminoides* J.Ellis 12 g
HlLWD decoction	Chen, (2018)	*Coptis chinensis* Franch. 9 g, *Scutellaria baicalensis* Georgi 9 g, *Pueraria lobata* (Willd.) Ohw 30 g, *Trichosanthes kirilowii Maxim.* 30 g, *Citrus reticulata* Blanco 15 g, *Pinellia ternata* (Thunb.) Makino 9 g, *Bambusa tuldoides* Munro 9 g, *Curcuma phaeocaulis* Valeton 9 g, *Fritillaria thunbergii* Miq. 15 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Atractylodes macrocephala* Koidz. 15 g, *Salvia miltiorrhiza* Bunge 30 g, *Bupleurum chinense* DC. 15 g

(Continued on following page)

TABLE 2 (*Continued*) Details of the HLPs for each study.

Interventions	References	Prescription
	Dong, (2017)	*Coptis chinensis* Franch. 9 g, *Citrus reticulata* Blanco 12 g, *Pinellia ternata* (Thunb.) Makino 9 g, *Bambusa tuldoides* Munro 6 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Atractylodes macrocephala* Koidz. 15 g, *Salvia miltiorrhiza* Bunge 15 g, *Citrus aurantium L.* 12 g, *Astragalus propinquus* Schischkin 20 g, *Glycyrrhiza uralensis* Fisch. 6 g, Trigonellafoenum-graeeum 15 g
	Ji, (2017)	*Coptis chinensis* Franch. 9 g, *Scutellaria baicalensis* Georgi 12 g, *Trichosanthes kirilowii Maxim* 30 g, *Citrus reticulata* Blanco 15 g, *Pinellia ternata* (Thunb.) Makino 9 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Atractylodes macrocephala* Koidz. 15 g, *Pueraria lobata* (Willd.) Ohw 15 g, *Salvia miltiorrhiza* Bunge 30 g, *Citrus aurantium L.* 6 g, *Rheum palmatum* L. 6 g
	Zhang, (2019)	*Coptis chinensis* Franch. 6 g, *Citrus reticulata* Blanco 10 g, *Citrus reticulata* Blanco 10 g, *Pinellia ternata* (Thunb.) Makino 6 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Atractylodes macrocephala* Koidz. 15 g, *Pueraria lobata* (Willd.) Ohw 15 g, *Salvia miltiorrhiza* Bunge 15 g, *Agastache rugosa* (Fisch. and C.A.Mey.) Kuntze 10 g, *Magnolia officinalis* Rehder and E.H.Wilson 6 g, *Coix lacryma-jobi* L. 15 g, *Glycyrrhiza uralensis* Fisch. 3 g
	Fu, (2021)	*Coptis chinensis* Franch. 10 g, *Poria Cocos* (Schw.) Wolf. 20 g, *Citrus aurantium L.* 10 g, *Pinellia ternata* (Thunb.) Makino 15 g, *Bambusa tuldoides* Munro 10 g, *Citrus reticulata* Blanco 15 g, *Pueraria lobata* (Willd.) Ohw 15 g, *Eupatorium fortunei* Turcz. 10 g, *Glycyrrhiza uralensis* Fisch. 10 g
	Liu et al. (2021)	*Coptis chinensis* Franch. 10 g, *Poria Cocos* (Schw.) Wolf. 20 g, *Citrus aurantium L.* 10 g, *Pinellia ternata* (Thunb.) Makino 15 g, *Bambusa tuldoides* Munro 10 g, *Citrus reticulata* Blanco 15 g, *Pueraria lobata* (Willd.) Ohw 15 g, *Eupatorium fortunei* Turcz. 10 g, *Glycyrrhiza uralensis* Fisch. 10 g
	Pan et al. (2021)	*Coptis chinensis* Franch. 15 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Citrus aurantium L.* 12 g, *Pinellia ternata* (Thunb.) Makino 6 g, *Bambusa tuldoides* Munro 15 g, *Citrus reticulata* Blanco 12 g, *Glycyrrhiza uralensis* Fisch. 15 g, *Zingiber officinale* Roscoe 10 g, *Atractylodes macrocephala* Koidz. 15 g
	Wang et al. (2021)	*Coptis chinensis* Franch. 15 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Citrus aurantium L.* 12 g, *Pinellia ternata* (Thunb.) Makino 6 g, *Bambusa tuldoides* Munro 15 g, *Citrus reticulata* Blanco 12 g, *Glycyrrhiza uralensis* Fisch. 15 g, *Zingiber officinale* Roscoe 10 g, *Atractylodes macrocephala* Koidz. 15 g
	Wang, (2020)	*Coptis chinensis* Franch. 15 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Citrus aurantium L.* 12 g, *Pinellia ternata* (Thunb.) Makino 6 g, *Bambusa tuldoides* Munro 15 g, *Citrus reticulata* Blanco 12 g, *Glycyrrhiza uralensis* Fisch. 15 g, *Zingiber officinale* Roscoe 10 g, *Atractylodes macrocephala* Koidz. 15 g
	Zhang, 2022	*Coptis chinensis* Franch. 15 g, *Poria Cocos* (Schw.) Wolf. 15 g, *Citrus aurantium L.* 12 g, *Pinellia ternata* (Thunb.) Makino 6 g, *Bambusa tuldoides* Munro 15 g, *Citrus reticulata* Blanco 12 g, *Glycyrrhiza uralensis* Fisch. 15 g, *Zingiber officinale* Roscoe 10 g, *Atractylodes macrocephala* Koidz. 15 g

articles (n = 178), cell experiment articles (n = 80), reviews (n = 24), protocols (n = 8), case reports (n = 15), and non-RCT experimental trials (n = 47). Third, the trials using other TCM therapies (n = 29) and without metformin in the control group (n = 17) were excluded after reading the full text. Ultimately, 33 RCTs satisfying the inclusion criteria were identified (Yang and Wang, 2013; Zhang, 2014; Zhang et al., 2013; Zhou et al., 2022; Zou and Lao, 2016; Dong, 2017; Fan et al., 2017; Fu, 2017; Ji, 2017; Li et al., 2017; Liu, 2017; Liu, 2017; Xing et al., 2017; Chen et al., 2018; Ding, 2018; Pang et al., 2018; Song et al., 2022; Zhang et al., 2018; Feng, 2019; Jin et al., 2019; Wu et al., 2019; Xiong, 2019; Zhang, 2019; Zhang et al., 2019; Gao, 2020; Wang, 2020; Chen and Wang, 2021; Fu, 2021; Liu et al., 2021; Pan et al., 2021; Wang et al., 2021; Wang Y 2022; Wei et al., 2021).

3.2 Study characteristics

A total of 33 RCTs published from 2006 to 2022 were included in this study. The RCTs consisted of 2,846 patients with T2DM between 18 and 86 years of age (Table 1). All trials were single-center trials, and the detection time ranged from 2 to 24 weeks. A total of 1,437 patients in the experimental group underwent treatment using HLPs plus metformin, and 1,409 patients in the control group underwent metformin treatment. Among the 33 trials, three trials with 268 patients used DHHL decoction, seven trials with 551 patients used GGQL decoction, five trials with 438 patients used HLEJ decoction, eight trials with 885 patients used HLJD decoction, and ten trials with 704 patients used HLWD decoction (Table 2).

3.3 Quality assessment

A total of 33 RCTs were identified in this study (Figure 2), of which 16 used the random number table method to generate random sequences (Chen et al., 2012; Zou and Lao, 2016; Dong, 2017; Fu, 2017; Liu, 2017; Zhang et al., 2018; Feng, 2019; Jin et al., 2019; Wu et al., 2019; Xiong, 2019; Wang 2020; Wang, 2020; Fu, 2021; Liu et al., 2021; Song et al., 2022; Wang et al., 2021), and others only mentioned randomly assigned participants. Three trials used the double-blind method for participants and personnel (Zou and Lao, 2016; Pang et al., 2018; Song et al., 2022), and others provided no detailed information. The risk of detection bias was low in all trials, because FBG, 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, HDL-c, LDL-c, and GD levels were evaluated based on objective criteria. In the study

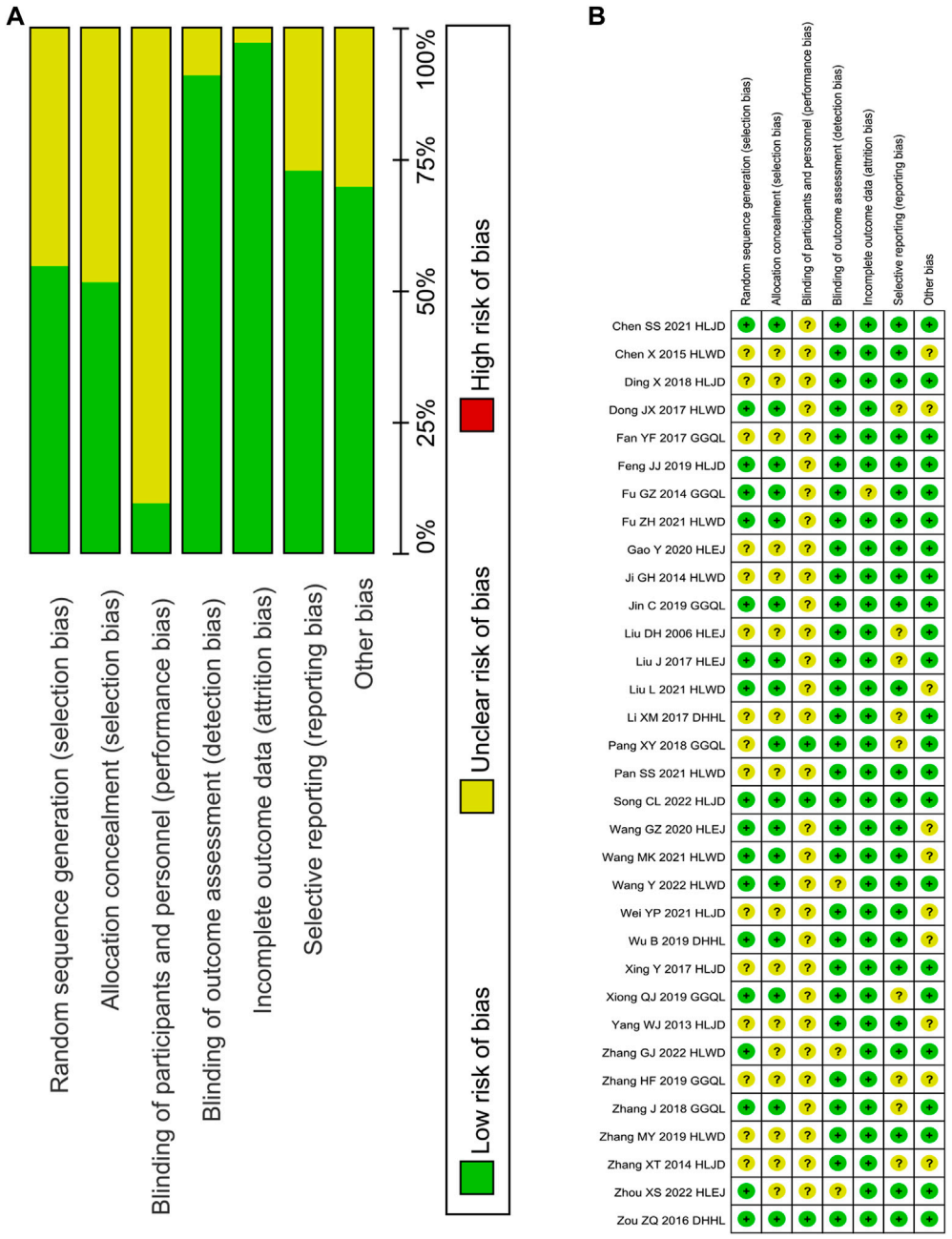

FIGURE 2
Risk of bias graph. Note: **(A)**, judgements about each risk of bias item presented as percentages across all included studies; **(B)**, judgements about each risk of bias item for each included study.

conducted by Fu (2017), three patients in the experimental group and the control group withdrew from the trial (9% exit rate). The remaining trials without the loss of follow-up patients or with the loss of follow-up rate <5% were described as having a low-attrition bias. For the reporting bias, nine trials with only positive results were determined as unclear (Zhang, 2014; Dong, 2017; Li et al., 2017; Liu, 2006; Liu, 2017; Pang et al., 2018; Zhang et al., 2018; Xiong, 2019; Zhang, 2019). For other bias, ten trials were unclear in the sex of the patient, course of the disease, and course of treatment (Chen et al., 2012; Dong, 2017; Liu et al., 2021; Wang, 2020; Wang et al., 2021; Wei et al., 2021; Wu et al., 2019; Yang and

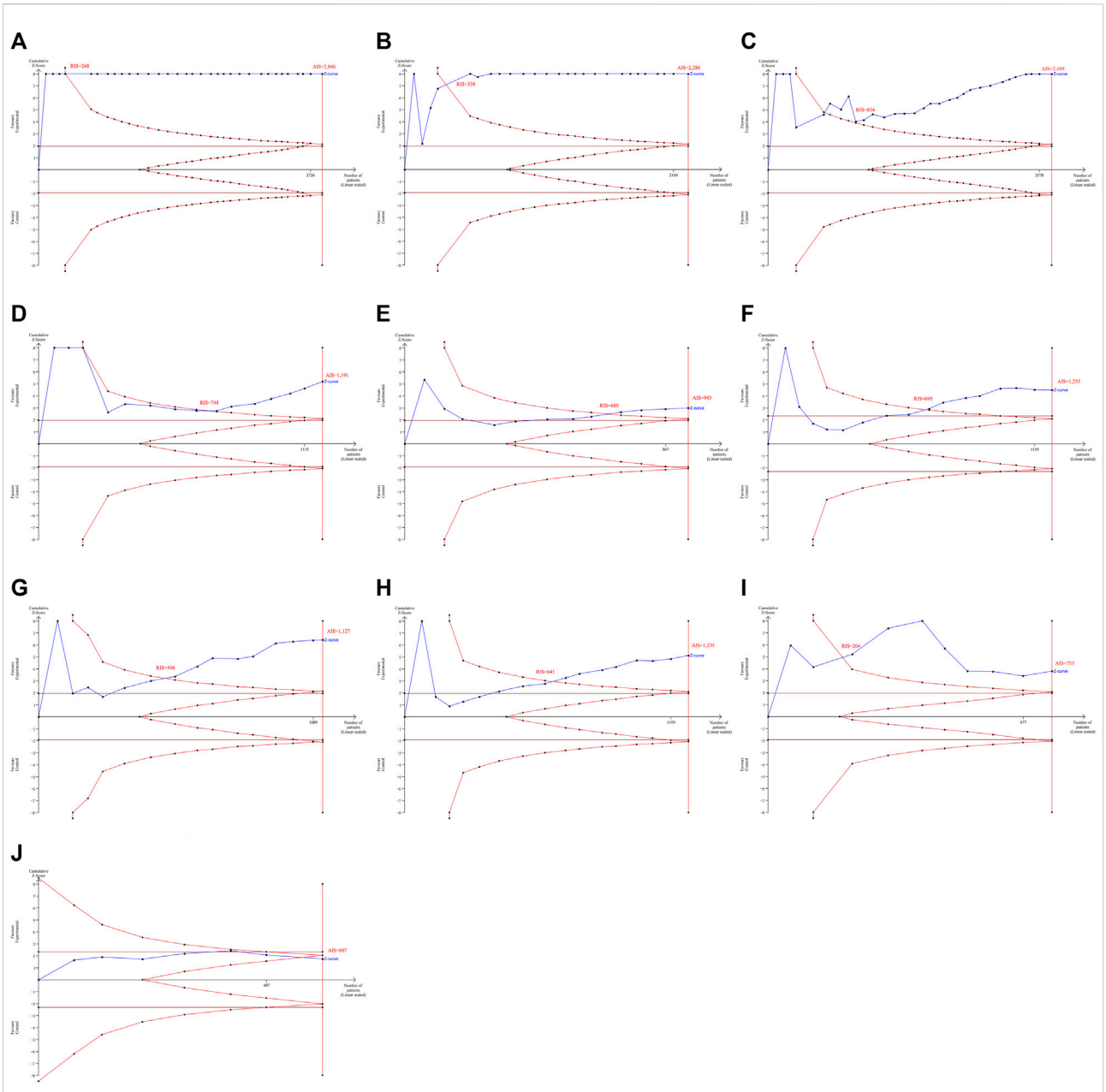

FIGURE 3
TSA analysis for the effectiveness of HLPs in improving T2DM. Note: **(A)** Represents FBG; **(B)** Represents 2hPG; **(C)** Represents HbA1c; **(D)** Represents FINS; **(E)** Represents HOMA-IR; **(F)** Represents TC; **(G)** Represents TG; **(H)** Represents LDL-c; **(I)** Represents HDL-c; **(J)** Represents GD.

Wang, 2013; Zhang, 2014; Zhang, 2019). Meanwhile, others with detailed information presented a low risk.

TSA results revealed that the AIS exceeded the RIS for the effectiveness of HLPs in improving FBG (AIS 2,846 was larger than RIS 268), 2hPG (AIS 2,280 was larger than RIS 338), HbA1c (AIS 2,698 was larger than RIS 834), FINS (AIS 1,191 was larger than RIS 748), HOMA-IR (AIS 943 was larger than RIS 680), TC (AIS 1,235 was larger than RIS 695), TG (AIS 1,127 was larger than RIS 506), LDL-c (AIS 1,235 was larger than RIS 641), and HDL-c (AIS 753 was larger than RIS 204), and their cumulative Z-curves crossed the trial sequential monitoring boundary (Figures 3A–I), indicating that their current evidence was sufficient to draw a reliable conclusion. However, the AIS didn't exceed the RIS for the effectiveness of HLPs in

TABLE 3 GRADE evidence profile of clinical efficacy.

Quality assessment					Effect	Quality	Importance
Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
FBG							
Serious[a]	No serious inconsistency	No serious indirectness	No serious imprecision	None	MD 1.16 lower (1.24–1.07 lower)	ÅÅÅO MODERATE	CRITICAL
2hPG							
Serious[b]	Serious[b]	No serious indirectness	No serious imprecision	None[c]	MD 1.64 lower (1.84–1.43 lower)	ÅÅOO LOW	CRITICAL
HbA1c							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None[c]	MD 0.78 lower (0.96–0.60 lower)	ÅÅOO LOW	CRITICAL
FINS							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None	MD 1.94 lower (2.68–1.2 lower)	ÅÅOO LOW	CRITICAL
HOMA-IR							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None	MD 0.77 lower (1.28–0.27 lower)	ÅÅOO LOW	CRITICAL
TC							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None	MD 0.70 lower (1.00–0.39 lower)	ÅÅOO LOW	IMPORTANT
TG							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None	MD 0.59 lower (0.76–0.41 lower)	ÅÅOO LOW	IMPORTANT
HDL-c							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None	MD 0.21 lower (0.32–0.1 lower)	ÅÅOO LOW	IMPORTANT
LDL-c							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	None	MD 0.70 lower (0.97–0.43 lower)	ÅÅOO LOW	IMPORTANT
GD							
Serious[a]	Serious[b]	No serious indirectness	No serious imprecision	Reporting bias[c]	32 fewer per 1,000 31 fewer per 1,000	ÅOOO VERY LOW	IMPORTANT

[a]Note: Most domain had unclear methodological bias risk.
[b]The trials included had obvious heterogeneity.
[c]The number of included studies is insufficient.

improving GD (Figure 3J), indicating that the current evidence was't sufficient to draw a reliable conclusion. GRADE assessment suggested that the quality of evidence was moderate for the effectiveness of HLPs in improving FBG, but the quality of evidence was low for 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c, even very low for GD (Table 3).

3.4 Effectiveness of HLPs for T2DM

3.4.1 HLPs for FBG

As shown in Figure 4A, a total of 33 trials comprising 1,437 subjects in the experimental group and 1,409 subjects in the control group evaluated the effectiveness of HLPs in improving FBG. Subgroups were divided depending on the type of HLPs for FBG. The results indicated that T2DM patients who received metformin in combination with DHHL decoction (MD = −0.99%, 95% CI: −1.35 to −0.63, and $p < 0.00001$), GGQL decoction (MD = −0.96%, 95% CI: −1.14 to −0.79, and $p < 0.00001$), HLEJ decoction (MD = −1.43%, 95% CI: −1.57 to −1.29, and $p < 0.00001$), HLJD decoction (MD = −0.97%, 95% CI: −1.13 to −0.81, and $p < 0.00001$), and HLWD decoction (MD = −1.21%, 95% CI: −1.44 to −0.98, and $p < 0.00001$) respectively were more likely to have reduced FBG relative to those with metformin alone. No significant heterogeneity was indicated in DHHL decoction ($I^2 = 0\%$), GGQL decoction ($I^2 = 8\%$), HLEJ decoction ($I^2 = 38\%$), HLJD decoction ($I^2 = 0\%$), and HLWD decoction ($I^2 = 15\%$) for FBG. Overall analysis showed that compared with metformin alone, HLPs

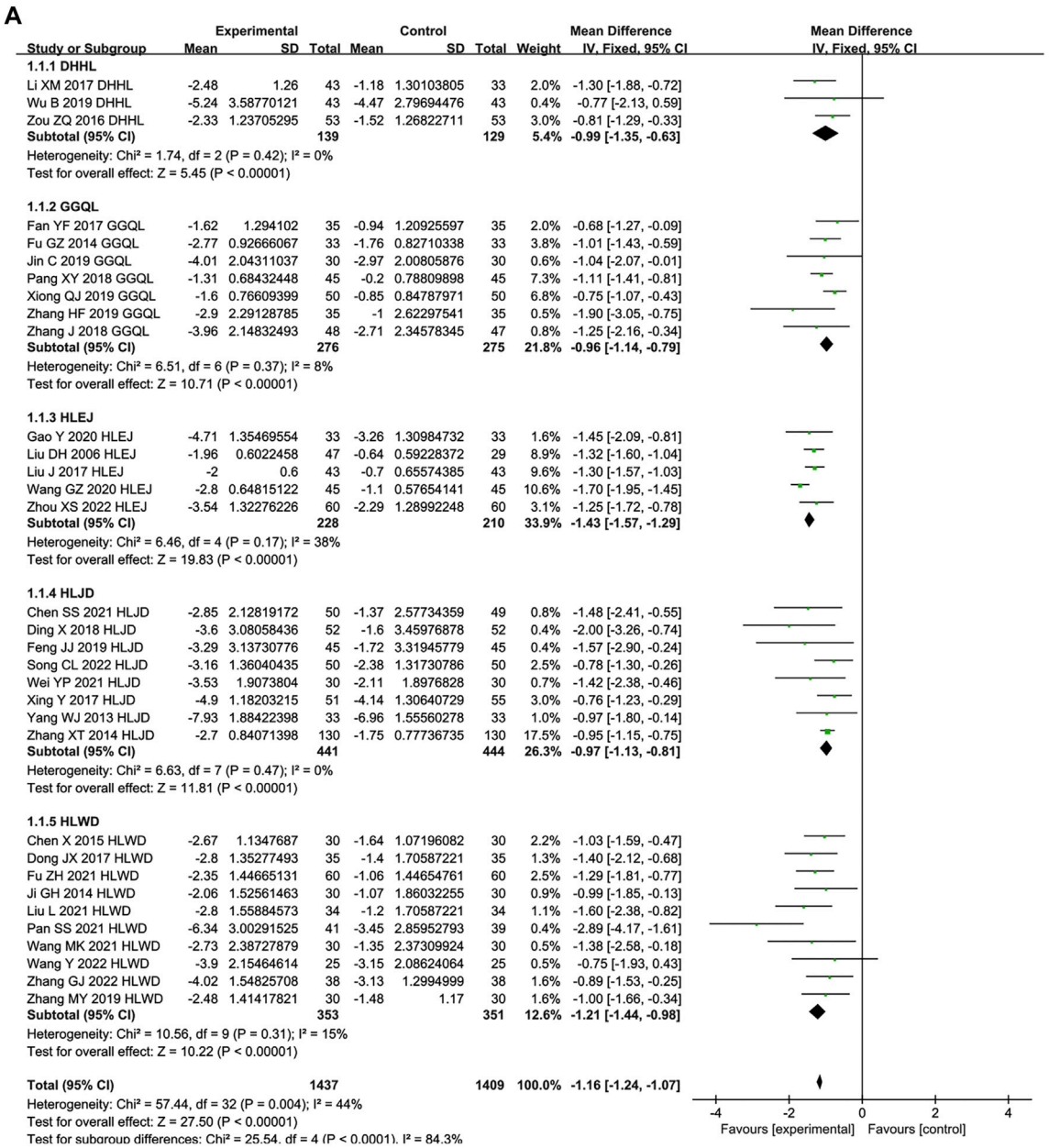

FIGURE 4
(Continued).

combined with metformin improved FBG more (I^2 = 44%, MD = −1.16%, 95% CI: −1.24 to −1.07, and p < 0.00001).

3.4.2 HLPs for 2hPG

As shown in Figures 4A,B total of 27 trials comprising 1,191 subjects in the experimental group and 1,165 subjects in the control group compared the 2hPG among patients with T2DM. Subgroup analysis was performed based on the type of HLPs for 2hPG. Patients who received the following decoctions, combined with metformin, were more likely to exhibit reduced 2hPG relative to the controls: DHHL decoction (MD = −2.38, 95% CI: −3.40 to −1.35, and p < 0.00001), GGQL decoction (MD = −1.18%, 95% CI: −1.46 to −0.91, and p < 0.00001), HLEJ decoction (MD = −1.84%, 95% CI: −2.01 to −1.67, and p < 0.00001), HLJD decoction (MD = −1.49%, 95% CI: −2.12 to −0.87, and p < 0.00001), and HLWD decoction (MD = −1.54%, 95% CI: −1.90 to −1.18, and p < 0.00001). No significant heterogeneity was found in the following: GGQL

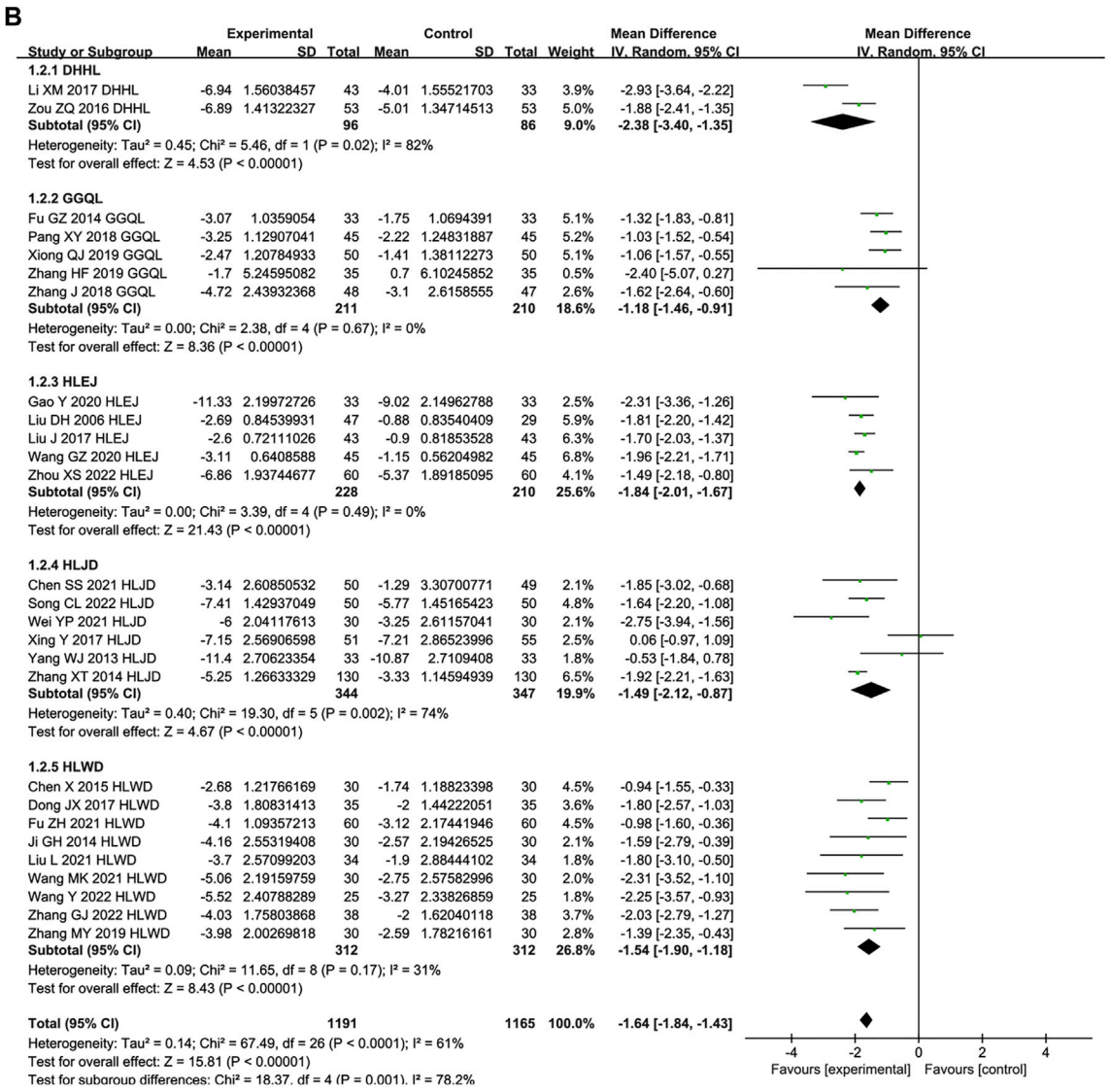

FIGURE 4
(Continued).

decoction (0%), HLEJ decoction (I^2 = 0%), and HLWD decoction (I^2 = 31%) for 2hPG. By contrast, significant heterogeneity was found in DHHL decoction (I^2 = 82%) and HLJD decoction (I^2 = 74%) for 2hPG. Overall analysis indicated that decreases in 2hPG were greater in groups treated using HLPs combined with metformin (I^2 = 61%, MD = −1.64%, 95% CI: −1.84 to −1.43, and $p < 0.00001$).

3.4.3 HLPs for HbA1c

A total of 31 trials comprising 1,362 subjects in the experimental group and 1,336 subjects in the control group assessed changes in HbA1c levels (Figure 4C). Subgroup analysis was used in different types of HLPs for HbA1c. Patients who received the following decoctions in combination with metformin were more likely to exhibit reduced HbA1c relative to that with metformin alone: DHHL decoction (MD = −0.25%, 95% CI: −0.41 to −0.09, and $p = 0.003$), GGQL decoction (MD = −0.74%, 95% CI: −1.17 to −0.32, and $p = 0.0006$), HLEJ decoction (MD = −1.13%, 95% CI: −1.47 to −0.78, and $p < 0.00001$), HLJD decoction (MD = −0.72%, 95% CI: −1.05 to −0.38, and $p < 0.00001$), and HLWD decoction (MD = −0.86%, 95% CI: −1.13 to −0.59, and $p < 0.00001$). No significant heterogeneity in DHHL decoction for HbA1c was found (I^2 = 0%). However, GGQL decoction (I^2 = 95%), HLEJ decoction (I^2 = 83%), HLJD decoction (I^2 = 88%), and

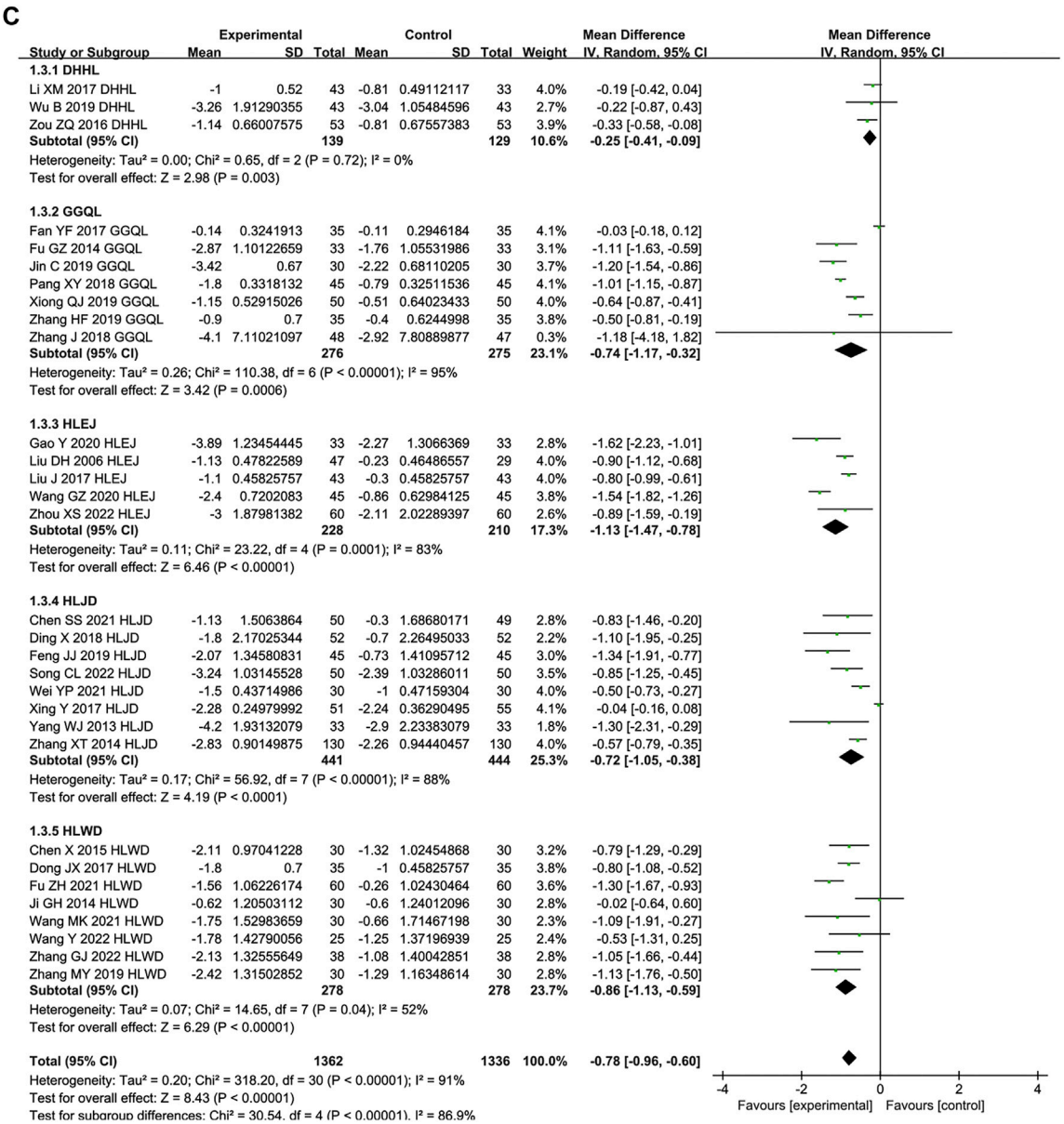

FIGURE 4
(Continued).

HLWD decoction (I^2 = 52%) for HbA1c exhibited significant heterogeneity. Overall analysis indicated that HLPs combined metformin provided additional benefits to reduce HbA1c (I^2 = 91%, MD = −0.78%, 95% CI:−0.96 to −0.60, and $p < 0.00001$).

3.4.4 HLPs for FINS

A total of 15 trials comprising 595 subjects in the experimental group and 596 subjects in the control group assessed changes in FINS levels (Figure 4D). Subgroup analysis was used in different types of HLPs for FINS. Patients who received the following decoctions in combination with metformin were more likely to exhibit reduced FINS relative to that with metformin alone: DHHL decoction (MD = −0.67%, 95% CI: −1.04 to −0.30, and p = 0.0004), GGQL decoction (MD = −3%.18%, 95% CI: −3.92 to −2.45, and $p < 0.00001$), and HLWD decoction (MD = −2.26%, 95% CI:−3.00 to −1.51, and $p < 0.00001$). No significant heterogeneity in DHHL decoction (I^2 = 0%) and

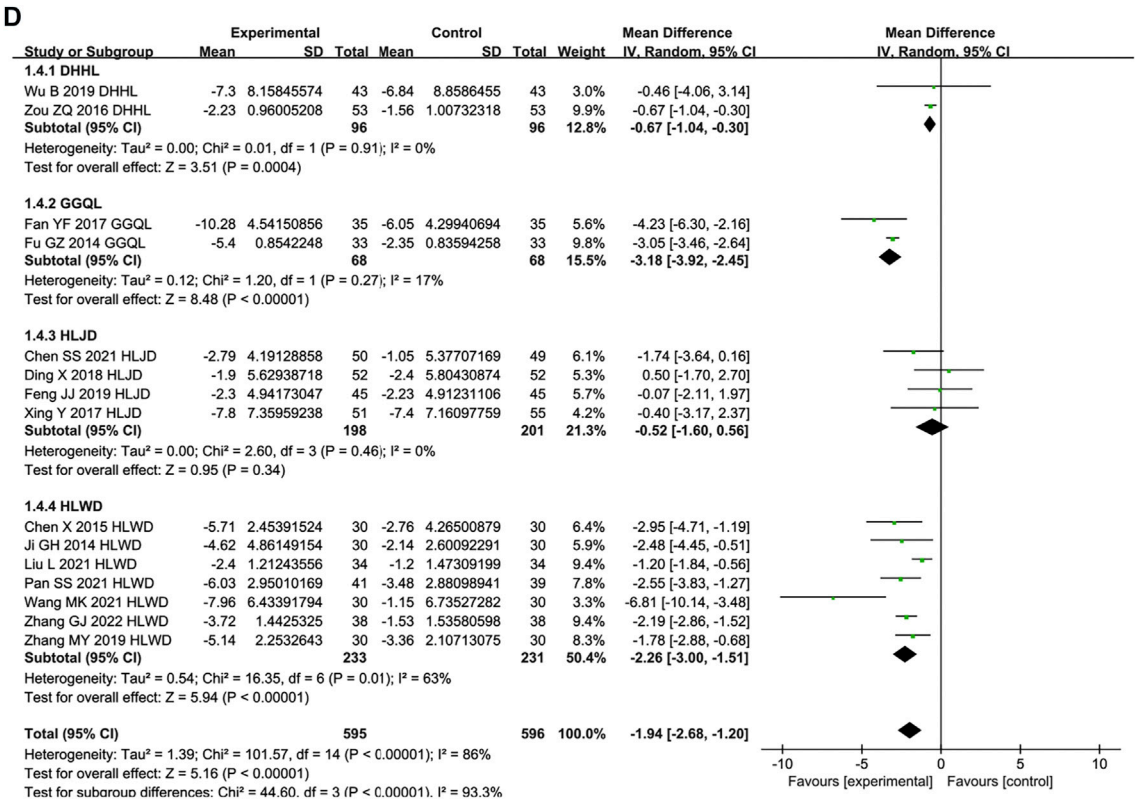

FIGURE 4
(Continued).

GGQL decoction (I^2 = 17%) for FINS was found, while HLWD decoction for FINS had significant heterogeneity (I^2 = 63%). In addition, HLJD decoction for FINS was not statistically significant (MD = -0.52, 95% CI: -1.60 to 0.56, and p = 0.34). Overall analysis indicated that patients treated with HLPs in combination with metformin were more likely to reduce FINS (I^2 = 86%, MD = −1.94%, 95% CI: −2.68 to −1.20, and p < 0.00001).

3.4.5 HLPs for HOMA-IR

A total of 12 trials comprising 471 subjects in the experimental group and 472 subjects in the control group reported HOMA-IR as an outcome (Figure 4E). Subgroup analysis was used in different types of HLPs for HOMA-IR. The results showed that patients who received metformin in combination with GGQL decoction (MD = −1.56%, 95% CI: −1.63 to −1.49, and p < 0.00001), HLJD decoction (MD = −0.82%, 95% CI: −1.08 to −0.56, and p < 0.00001), and HLWD decoction (MD = −0.58%, 95% CI: −0.80 to −0.36, and p < 0.00001) respectively were more likely to have reduced HOMA-IR relative to those with metformin alone. No significant heterogeneity was indicated in GGQL decoction (I^2 = 0%), HLJD decoction (I^2 = 0%), and HLWD decoction (I^2 = 0%) for HOMA-IR. However, DHHL decoction for HOMA-IR was not statistically significant (MD = −0.08%, 95% CI: −0.22 to 0.06, and p = 0.26). Overall analysis showed that HLPs combined with metformin were more likely to reduce HOMA-IR compared with metformin alone (I^2 = 97%, MD = −0.77%, 95% CI: −1.28 to −0.27, and p = 0.003).

3.4.6 HLPs for blood lipids

A total of 16 trials comprising 628 subjects in the experimental group and 607 subjects in the control group evaluated the effectiveness of HLPs in improving TC (Figure 4F). Subgroups were divided depending on the type of HLPs for TC. The results revealed that patients who received metformin in combination with GGQLdecoction (MD = −0.57%, 95% CI:−0.99 to −0.15, and p = 0.008), HLEJ decoction (MD = −1.38%, 95% CI:−1.62 to −1.14, and p < 0.00001), and HLJD decoction (MD = −1.53%, 95% CI:−1.87 to −1.19, and p < 0.00001) respectively were more likely to have reduced TC relative to those with metformin alone. No significant heterogeneity was indicated in HLJD decoction for TC (I^2 =

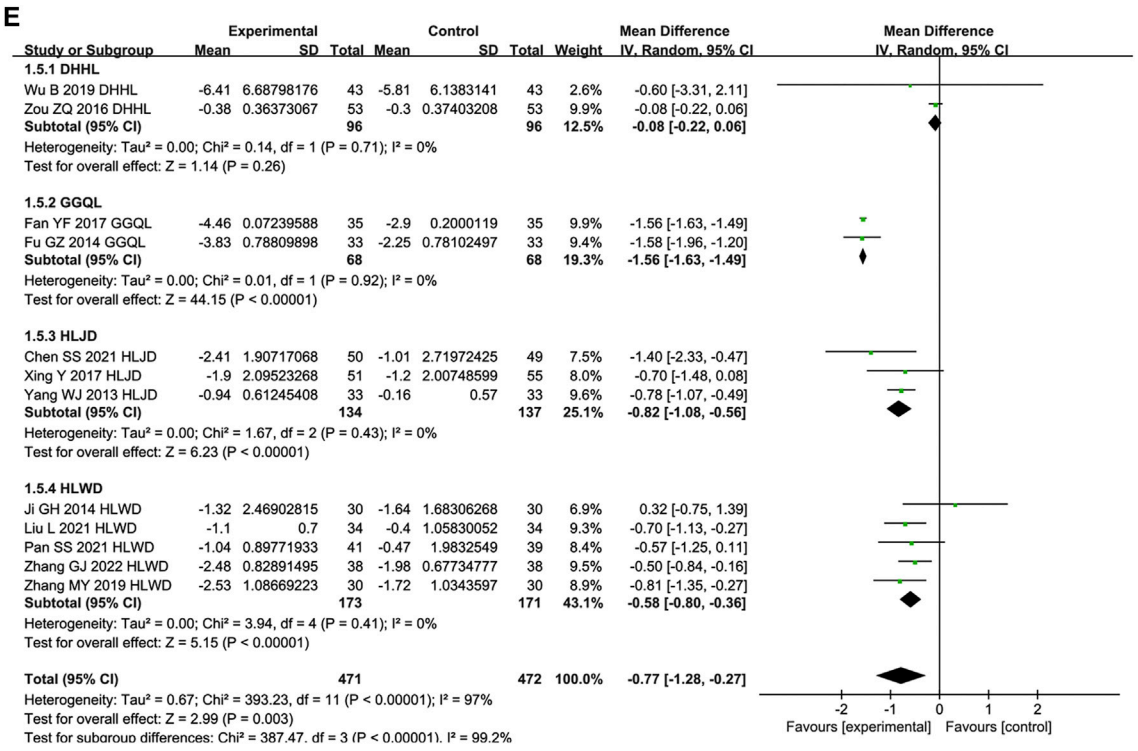

FIGURE 4
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0%), while significant heterogeneity was found in GGQL decoction (I^2 = 88%) and HLEJ decoction (I^2 = 70%) for TC. In addition, HLWD decoction for TC was not statistically significant (MD = −0.17%, 95% CI: −0.39 to 0.05, and p = 0.13). Overall analysis indicated that decreases in TC were greater in groups treated using HLPs combined with metformin (I^2 = 93%, MD = −0.70%, 95% CI: −1.00 to −0.39, and p < 0.00001).

A total of 15 trials comprising 593 subjects in the experimental group and 572 subjects in the control group evaluated the curative effect of HLPs in improving TG (Figure 4G). Subgroups were divided depending on the type of HLPs for TG. Patients who received the following decoctions, combined with metformin, were more likely to exhibit reduced TG relative to the controls: GGQL decoction (MD = −0.46%, 95% CI:−0.78 to −0.13, and p = 0.006), HLEJ decoction (MD = -1.19%, 95% CI:−1.84 to −0.55, and p = 0.0003), HLJD decoction (MD = −0.48%, 95% CI:−0.58 to −0.38, and p < 0.00001), and HLWD decoction (MD = −0.32%, 95% CI:−0.49 to −0.14, and p = 0.0003). No significant heterogeneity was found in HLJD decoction (I^2 = 0%) and HLWD decoction (I^2 = 18%) for TG, while significant heterogeneity was found in GGQL decoction (I^2 = 89%) and HLEJ decoction (I^2 = 96%) for TG. Overall analysis indicated that decreases in TG were greater in groups treated using HLPs combined with metformin (I^2 = 89%, MD = −0.57%, 95% CI: −0.74 to −0.40, and p < 0.00001).

A total of 16 trials comprising 628 subjects in the experimental group and 607 subjects in the control group evaluated the curative effect of HLPs in improving LDL-c (Figure 4H). Subgroups were divided depending on the type of HLPs for LDL-c. Patients who received the following decoctions, combined with metformin, were more likely to exhibit reduced LDL-c relative to the controls: GGQLdecoction (MD = −0.41%, 95% CI:−0.54 to −0.29, and p < 0.00001), HLEJ decoction (MD = −1.17%, 95% CI: −1.77 to −0.57, and p = 0.0001), and HLJD decoction (MD = −1.62%, 95% CI:−1.85 to −1.40, and p < 0.00001). No significant heterogeneity was found in GGQL decoction (I^2 = 11%) and HLJD decoction (I^2 = 0%) for LDL-c, while significant heterogeneity was found in HLEJ decoction for LDL-c (I^2 = 94%). In addition, HLWD decoction for LDL-c was not statistically significant (MD = −0.36%, 95% CI: −0.71 to 0.00, and p = 0.05). Overall analysis indicated that HLPs combined metformin provided additional benefits to reduce LDL-c (I^2 = 94%, MD = −0.70%, 95% CI: −0.97 to −0.43, and p < 0.00001).

A total of 10 trials comprising 378 subjects in the experimental group and 375 subjects in the control group evaluated the curative effect of HLPs in improving HDL-c

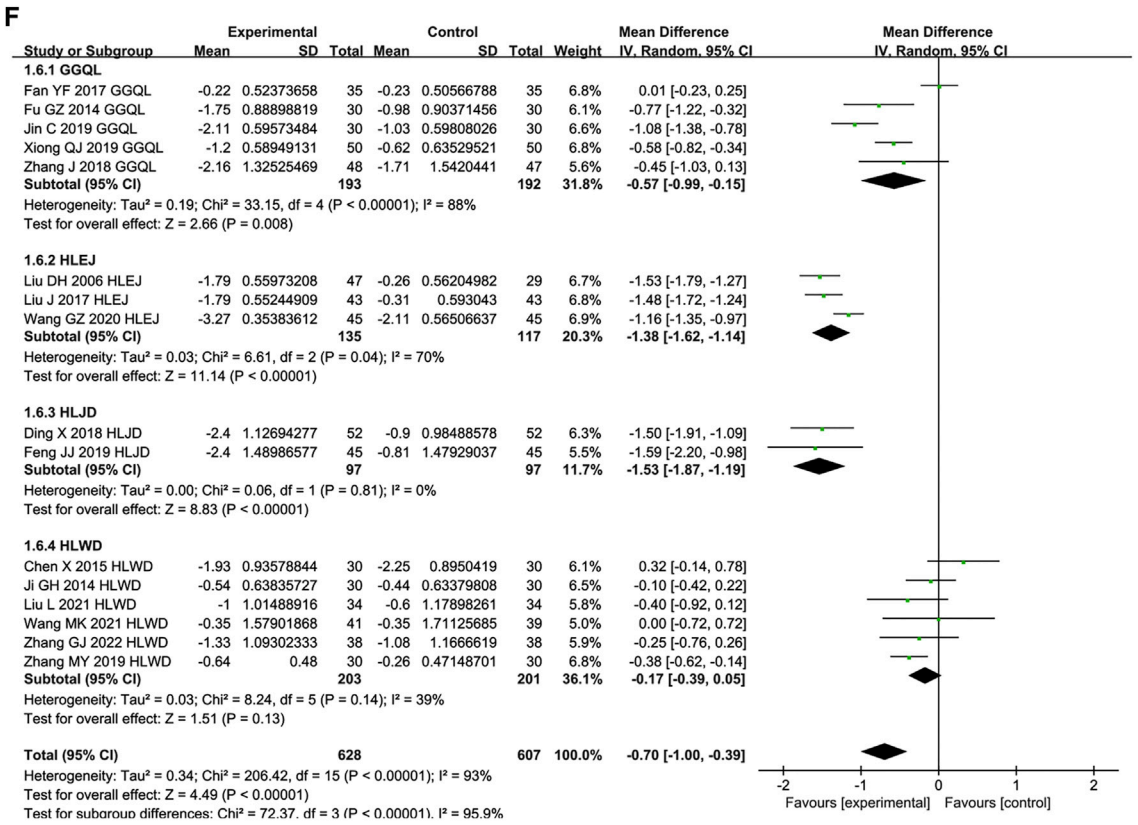

FIGURE 4
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(Figure 4I). Subgroups were divided depending on the type of HLPs for HDL-c. The results revealed that patients who received metformin in combination with GGQL decoction (MD = −0.32%, 95% CI:−0.54 to −0.10, and p = 0.005) and HLJD decoction (MD = −0.32%, 95% CI:−0.41 to −0.23, and p < 0.00001) respectively were more likely to have reduced HDL-c relative to those with metformin alone. No significant heterogeneity was indicated in HLJD decoction for HDL-c (I^2 = 0%), while significant heterogeneity was found in GGQL decoction for HDL-c (I^2 = 81%). In addition, HLWD decoction for HDL-c was not statistically significant (MD = −0.07%, 95% CI: −0.17 to 0.03, and p = 0.19). Overall analysis showed that compared with metformin alone, HLPs combined with metformin improved HDL-c more (I^2 = 84%, MD = −0.21%, 95% CI: −0.32 to −0.10, and p = 0.0002).

3.4.7 HLPs for GD

A total of seven trials comprising 313 subjects in the experimental group and 294 subjects in the control group conducted analysis of HLPs for GD (Figure 4J). Patients who received HLPs can't reduce GD relative to those with metformin alone (OR = 0.54%, 95% CI:0.26 to 1.10, and p = 0.09).

3.5 Sensitivity analysis

The results in Table 4 suggest that patients with T2DM in the experimental group show improved FBG, 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c relative to those in the control group. However, changes in the effectiveness of HLPs in improving 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c showed significant heterogeneity. With regard to the subgroup sensitivity analysis, after excluding some underestimated or overestimated trials, the heterogeneity of the majority of studies was significantly reduced, including the following: HLJD for 2hPG, HLWD for FINS; GGQL, HLEJ, HLJD, and HLWD for HbA1c; GGQL and HLEJ for TC; GGQL and HLEJ for TG; HLEJ and HLWD for LDL-c; and DHHL for HDL-c. However, no statistically significant difference was found in DHHL for 2hPG, HLJD for FINS, and DHHL for HOMA-IR.

3.6 Publication bias

As shown in Figure 5, the funnel plots used to evaluate the effectiveness of HLPs in improving FBG are nearly

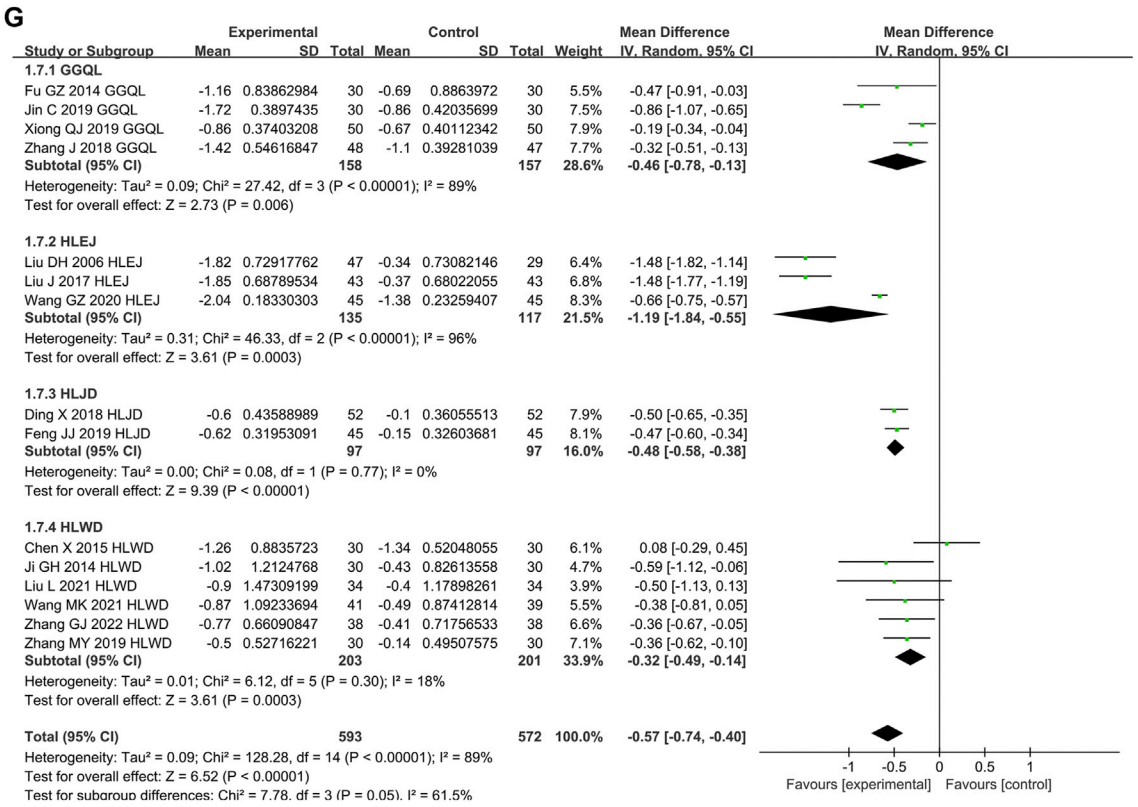

FIGURE 4
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symmetrical, whereas those used to assess the effects of HLPs on 2hPG, HbA1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c are asymmetrical. Therefore, Egger's test (Stata version 13.0) was also performed to evaluate their publication bias. The Egger's test used to assess publication bias suggested that $p > 0.05$ in FBG, 2hPG, TC, TG, LDL-c, and HDL-c, whereas $p < 0.05$ in HbA1c, FINS, and HOMA-IR (Table 5). Finally, the trim-and fill-method (Stata version 13.0) was used to evaluate the publication bias of HbA1c and HOMA-IR. In Figure 6A, theoretically missing studies show an adjusted improvement in HbA1c, corresponding to −1.083 MD [95% CI, −1.346 to −0.853], relative to -0.932 MD [95% CI, −1.182 to −0.791]. As shown in Figure 6B, five theoretically missing studies show corrected improvement in FINS, corresponding to 0.275 MD [95% CI, 0.069 to 0.412], compared with -1.144 MD [95% CI, −1.792 to −0.645]. As shown in Figure 6C, five theoretically missing studies show corrected improvement in HOMA-IR, corresponding to 0.141 MD [95% CI, 0.061 to 0.371], compared with −1.142 MD [95% CI, −1.787 to −0.582].

4 Discussion

The potential of HLPs to prevent and treat T2DM has been investigated in several studies, and its hypoglycemic mechanism is becoming increasingly apparent. DHHL decoction can regulate the glucose level by activating AMPKα and upregulating the expression of PGC-1α and GLUT4 (Hao et al., 2019). GGQL decoction can enhance glucose metabolism by regulating tryptophan, pantothenic acid, and adenine in IR-HepG2 cells (Chen et al., 2018), as well as improve liver insulin resistance by upregulating SIRT1 expression and reducing FoxO1 acetylation (Sui et al., 2018). HLEJ decoction can exert glucose-lowering and lipid-lowering effects by resisting inflammation and improving insulin resistance (Feng, 2015). HLJD decoction can exert hypolipidemic effects by inhibiting the increased activity of intestinal pancreatic lipase (Zhang et al., 2013) and increasing GLUT4 and PI3K p85 mRNA expression in adipose and skeletal muscle tissues (Chen et al., 2007; Jin et al., 2007). HLWD decoction can effectively treat glycometabolism disorder by repairing the insulin signaling pathway and inhibiting the release of inflammatory cytokines (Li et al., 2016; Chen et al., 2019).

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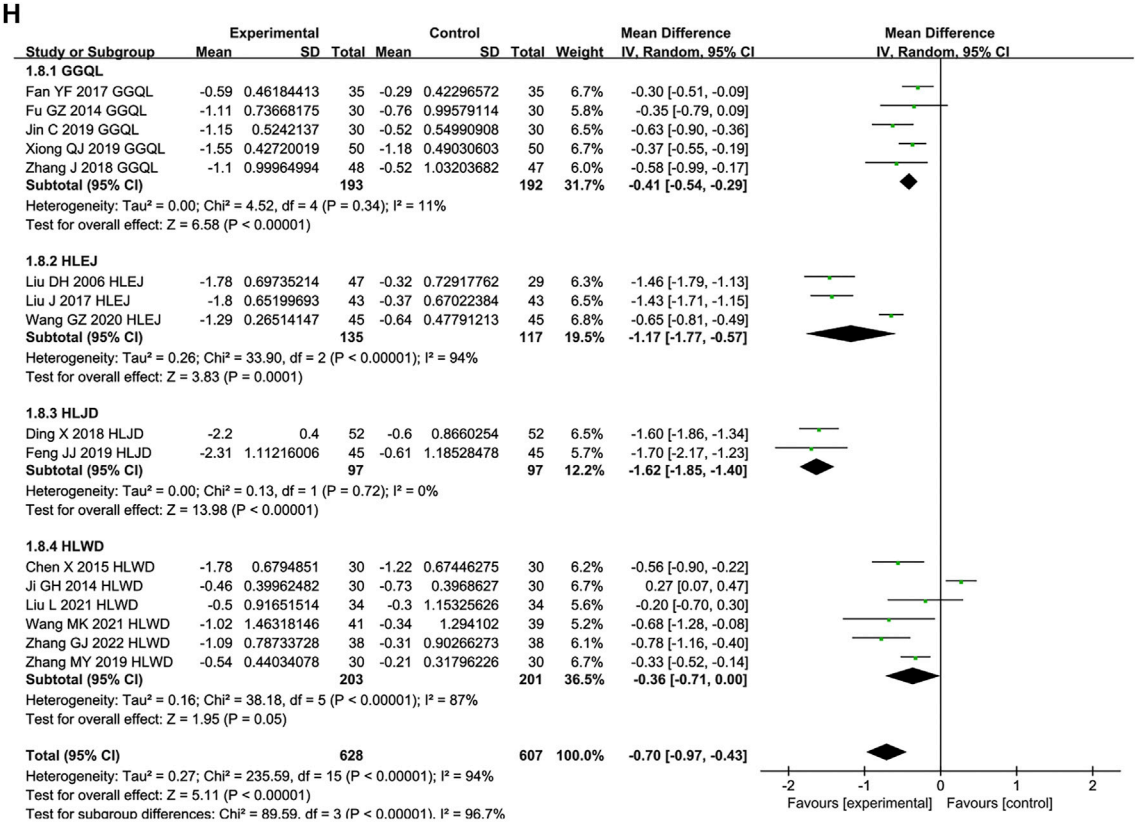

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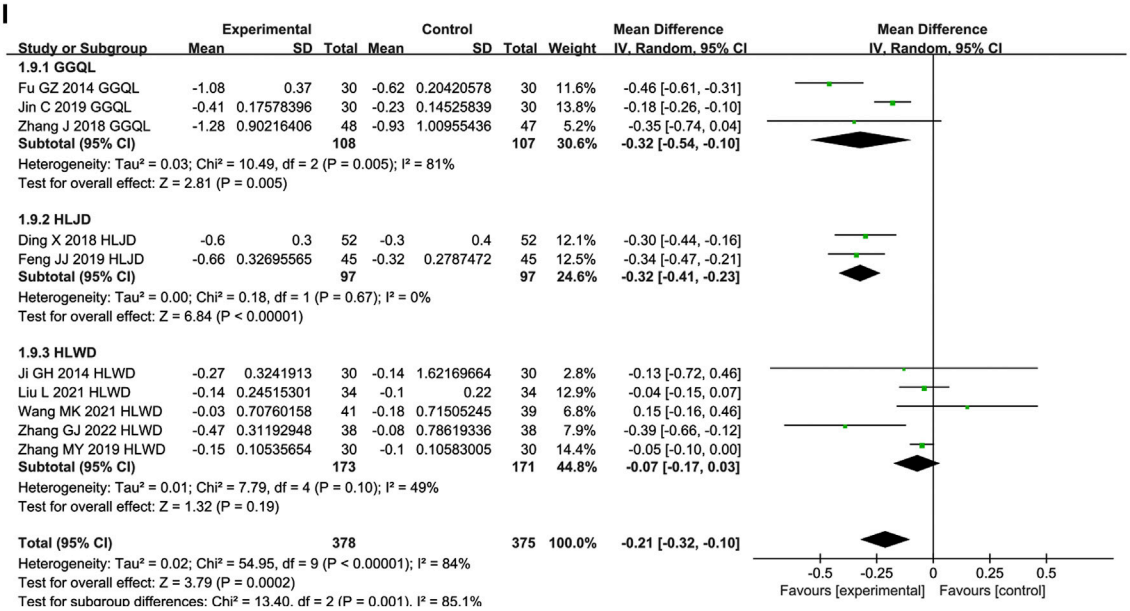

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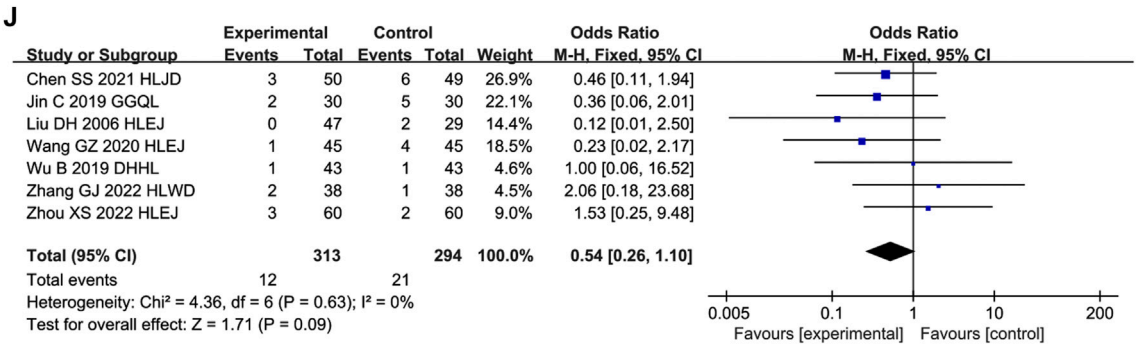

FIGURE 4
(Continued). Forest plot for evidence that compared HLPs plus metformin with metformin. Note: **(A)**, HLPs plus metformin vs. metformin for FBG; **(B)**, HLPs plus metformin vs. metformin for 2hPG; **(C)**, HLPs plus metformin vs. metformin for HbA1c; **(D)**, HLPs plus metformin vs. metformin for FINS; **(E)** HLPs plus metformin vs. metformin for HOMA-IR; **(F)**, HLPs plus metformin vs. metformin for TC; **(G)**, HLPs plus metformin vs. metformin for TG; **(H)**, HLPs plus metformin vs. metformin for LDL-c; **(I)**, HLPs plus metformin vs. metformin for HDL-c; **(J)**, HLPs plus metformin vs. metformin for GD.

This systematic review and meta-analysis included 33 RCTs involving 2,846 participants. In this study, the included RCTs were rigorously screened and controlled. With regard to quality, the risks of detection bias (33 trials had low risks), attrition bias (32 trials had low risks), reporting bias (24 trials had low risks), and other bias (23 trials had low risks) were generally low, but the risks of selection bias (18 trials had low risks) and performance bias (3 trials had low risks) were generally unclear. Therefore, the methodological quality was considerably moderate. Findings from this study indicate that compared with metformin alone, HLPs combined with metformin is more beneficial for FBG, 2hPG, HAb1c, FINS, HOMA-IR, TC, TG, LDL-c, and, HDL-c, but the improvement of HLPs on GD was not statistically significant.

TABLE 4 Sensitivity analysis via excluding the under or over estimated trials.

Analysis	MD (95% CI)	I2 (%)	*p* (Z test)	Excluded studies [reference]	MD (95% CI)	I^2 (%)	*p* (Z test)
2hPG-DHHL	−2.38 [−3.40,−1.35]	82%	$p < 0.00001$		Not applicable		
2hPG-HLJD	−1.49 [−2.12,−0.87]	74%	$p < 0.00001$	Xing et al. (2017)	−1.80 [−2.23, −1.37]	42	$p < 0.00001$
HbA1c-GGQL	−0.74 [−1.17,−0.32]	95%	$p = 0.0006$	Fan et al. (2017)	−1.04 [−1.16,−0.92]	0	$p < 0.00001$
				Xiong, (2019)			
				Zhang, (2019)			
HbA1c-HLEJ	−1.13 [−1.47, −0.78]	83%	$p < 0.00001$	Gao, (2020)	−0.85 [−0.99, −0.70]	0	$p < 0.00001$
				Wang, (2020)			
HbA1c-HLJD	−0.72 [−1.05, −0.38]	88%	$p < 0.0001$	Feng, (2019)	−0.64 [−0.81, −0.48]	15	$p < 0.00001$
				Xing et al. (2017)			
HbA1c-HLWD	−0.86 [−1.13, −0.59]	52%	$p < 0.00001$	Ji, (2017)	−0.96 [−1.15, −0.77]	8	$p < 0.00001$
FINS-HLJD	−0.52 [−1.60, 0.56]	0%	$p = 0.34$	Not applicable			
FINS-HLWD	−2.26 [−3.00, −1.51]	63%	$p < 0.00001$	Wang et al. (2021)	−1.97 [−2.51, −1.42]	37	$p < 0.00001$
HOMA-IR-DHHL	−0.08 [−0.22,0.06]	0%	$p = 0.26$	Not applicable			
TC-GGQL	−0.57 [−0.99, −0.15]	88%	$p = 0.008$	Fan et al. (2017)	−0.60 [−0.80, −0.40]	0	$p < 0.00001$
				Jin et al. (2019)			
TC-HLEJ	−1.38 [−1.62, −1.14]	70%	$p < 0.00001$	Wang, (2020)	−1.50 [−1.68, −1.33]	0	$p < 0.00001$
TG-GGQL	−0.46 [−0.78, −0.13]	89%	$p = 0.006$	Jin et al. (2019)	−0.26 [−0.38, −0.14]	4	$p < 0.0001$
TG-HLEJ	−1.19 [−1.84, −0.55]	96%	$p = 0.0003$	Wang, (2020)	−1.48 [−1.70, −1.26]	0	$p < 0.00001$
LDL-c-HLEJ	−1.17 [−1.77, −0.57]	94%	$p = 0.0001$	Wang, (2020)	−1.44 [−1.66, −1.23]	0	$p < 0.00001$
LDL-c-HLWD	−0.36 [−0.71, 0.00]	87%	$p = 0.05$	Ji, (2017)	−0.48 [−0.68, −0.28]	36	$p < 0.00001$
HDL-c-DHHL	−0.32 [−0.54, −0.10]	81%	$p = 0.005$	Jin et al. (2019)	−0.45 [−0.59, −0.30]	0	$p < 0.00001$

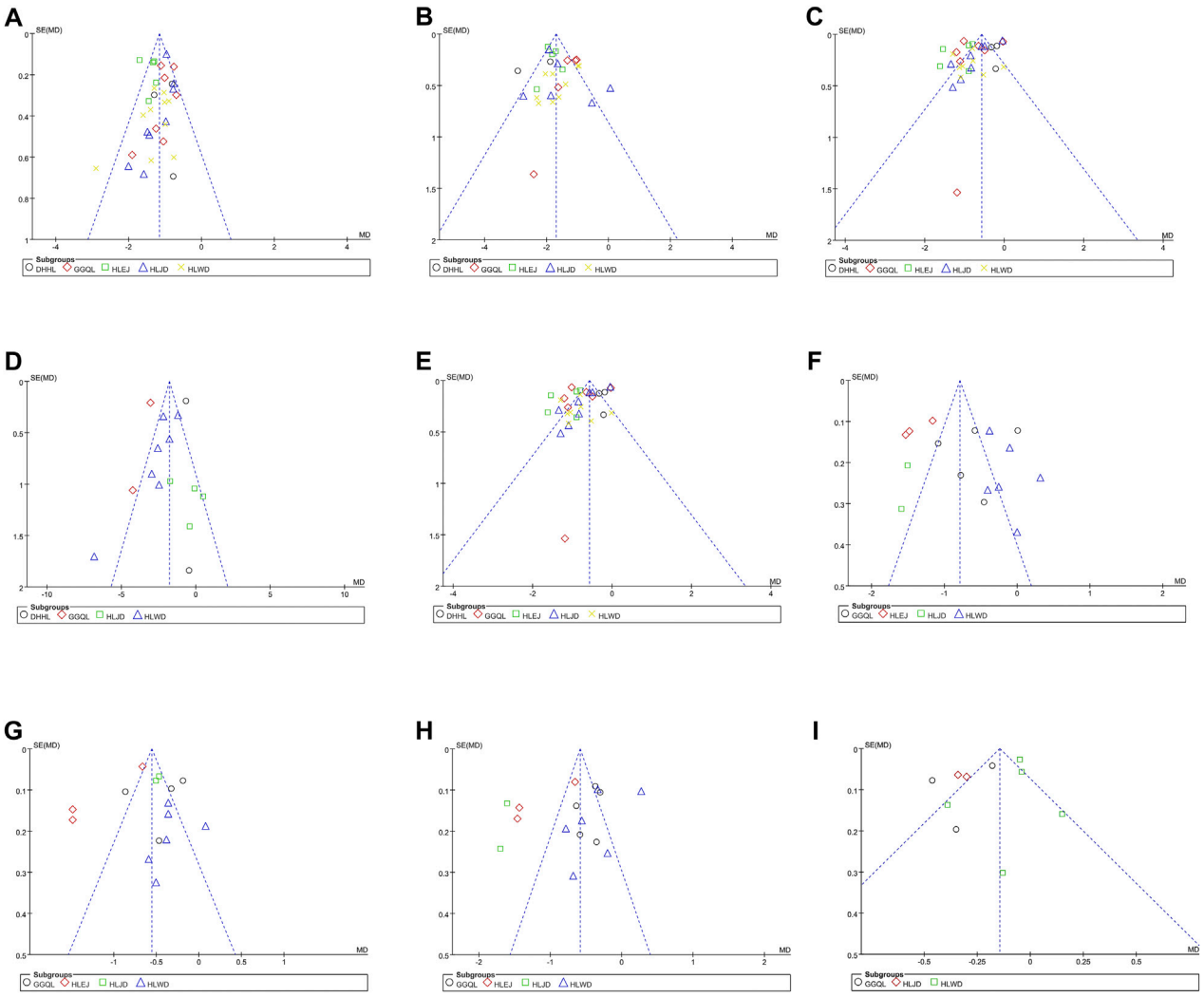

FIGURE 5
Funnel plots of the trials that compared HLPs plus metformin with metformin. Note: **(A)** Represents FBG; **(B)** Represents 2hPG; **(C)** Represents HbA1c; **(D)** Represents FINS; **(E)** Represents HOMA-IR; **(F)** Represents TC; **(G)** Represents TG; **(H)** Represents LDL-c; **(I)** Represents HDL-c.

TABLE 5 Egger's publication test of the trials that compared HLPs plus metformin vs. metformin.

Detection indicators	*p* Value
FBG	$p = 0.325$
2hPG	$p = 0.233$
HbA1c	$p = 0.007$
FINS	$p < 0.001$
HOMA-IR	$p < 0.001$
TC	$p = 0.063$
TG	$p = 0.058$
LDL-c	$p = 0.286$
HDL-c	$p = 0.370$

In this study, treatment with different HLPs exhibited different hypoglycemic and lipid-lowering effects, suggesting that metformin combined with different HLPs may cause variations in medicinal metabolism. This study found that DHHL decoction can improve FBG, 2hPG, HbA1c, and FINS, but does not affect HOMA-IR. In addition, no well-established data are available to analyze the effect of DHHL decoction on TC, TG, LDL-c, and HDL-c. GGQL decoction can improve all blood glucose and blood lipid indicators. HLEJ decoction can improve FBG, 2hPG, HbA1c, TC, TG, and LDL-c, but its role in FINS, HOMA-IR, and HDL-c has not been reported. HLJD decoction can improve FBG, 2hPG, HbA1c, HOMA-IR, TC, TG, LDL-c, and HDL-c, but exerts no effect on FINS. HLWD decoction can improve FBG, 2hPG, HbA1c, FINS, HOMA-IR, and TG, but the

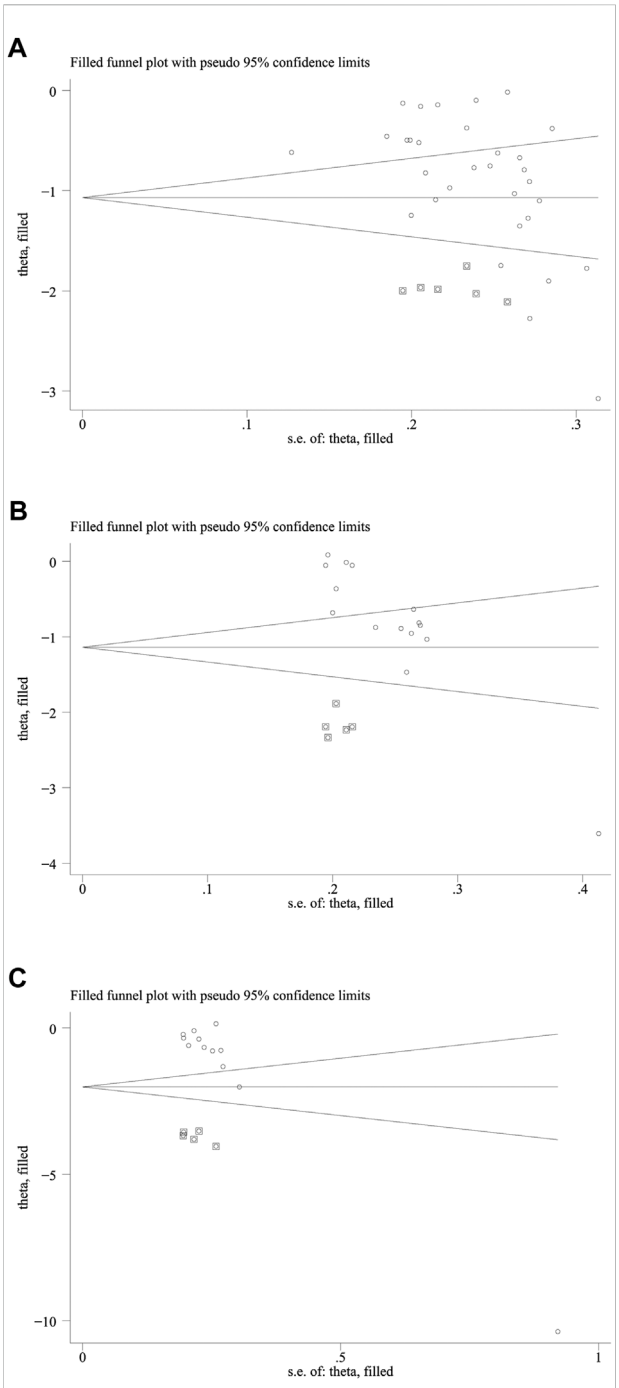

FIGURE 6
The trim and fill analysis of HbA1c and HOMA-IR. Note: **(A)** Represents the trim and fill analysis of HbA1c; **(B)** Represents the trim and fill analysis of FINS; **(C)** Represents the trim and fill analysis of HOMA-IR. The circle represents the actual estimate and the square represents the theoretical estimate when publication bias does not exist.

improvement in TC, LDL-c, and HDL-c was not statistically significant. Therefore, among all HLPs, GGQL decoction is potentially the most effective prescription for improving T2DM.

The advantages of this study are as follows: 1) In the sensitivity analysis, the difference in prescriptions may be the important source of heterogeneity, so we performed a subgroup analysis in different HLPs. Meanwhile, the overall results exhibited heterogeneity in this study, so we excluded the individual trials that caused heterogeneity, and the heterogeneity was significantly reduced. 2) With regard to publication bias, we used funnel plot, Egger's test, and trim-and-fill method to evaluate the publication bias. The results of funnel plot and Egger's test suggest that no publication bias was found in the enhancing effect of HLPs on FBG, 2hPG, TC, TG, LDL-c, and HDL-c. Then the trim-and-fill method was used to further evaluate the publication bias of HbA1c, FINS, and HOMA-IR, which still has important reference significance for the improvement of HbA1c, FINS, and HOMA-IR with HLPs. 3) This study also applied TSA analysis to assess the sample size required and thereby draw reliable conclusions. The sample size of all but one (HLPs for GD) were found sufficient to support this study and thereby draw reliable conclusions. Therefore, the results of this study present high reliability.

The present study also has several limitations: 1) All RCTs included in this study were Chinese, which likely led to geographical bias. Thus, an international collaboration should be conducted to ensure the generalizability of the findings. 2) The methodological quality of the RCTs was low, only half of the RCTs described the allocation concealment and blinding method, which might have led to a nonnegligible risk of bias. Thus, more scientific RCTs with specific randomize allocation details are needed. 3) Different kinds of HLPs vary in their hypoglycemic mechanism of action. Thus, high heterogeneity was observed among different HLPs, limiting the confirmation of the efficacy of HLPs in the treatment of T2DM. 4) Variations in dose in the same prescription are a concern in TCM. Variations in dose may also lead to differences in efficacy, leading to heterogeneity in research. 5) Current evidence shows that GGQL decoction can be potentially used as the optimal complementary approach to regulate glucose and lipid levels, but this finding has yet to be proved. Therefore, more rigorously designed and large-scale RCTs are required to confirm our findings.

5 Conclusion

Current evidence from this meta-analysis and systematic review suggests that compared with metformin alone, HLPs provide more benefits for the treatment of T2DM, particularly in FBG, 2hPG, HAb1c, FINS, HOMA-IR, TC, TG, LDL-c, and HDL-c. Due to insufficient data from the included RCTs, the therapeutic effect of HLPs on GD has not been demonstrated, and the findings should be elucidated with caution because of the limitations. Therefore, larger-scale and well-designed RCTs are essential to verify HLPs as a promising candidate treatment for patients with T2DM.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

Conception and design, development of methodology by LP and ZD. Literature search, article selection and data extraction by LP and XZ. The assessment of methodological bias risk and statistical analysis by GL and LP. Preparing the manuscript draft by LP and GL. Study supervision, review and revision of the manuscript by KX and ZD. All authors read and approved the final version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.956313/full#supplementary-material

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EDITED BY
Dan Tang,
Guangdong Pharmaceutical University, China

REVIEWED BY
Zehuai Wen,
Guangdong Provincial Hospital of Chinese Medicine, China
Fengmei Lian,
China Academy of Chinese Medical Sciences, China

*CORRESPONDENCE
Tsung-Hsien Yang,
yangth1012@gmail.com

†These authors have contributed equally to this work

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Chinese herbal medicine is associated with higher body weight reduction than liraglutide among the obese population: A real-world comparative cohort study

Yu-Ning Liao[1†], Hsing-Yu Chen[1,2,3†], Ching-Wei Yang[1,2], Pai-Wei Lee[4], Chiu-Yi Hsu[4], Yu-Tung Huang[4] and Tsung-Hsien Yang[1,5*]

[1]Division of Chinese Internal Medicine, Center for Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan, [2]School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan, [3]Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan, [4]Center for Big Data Analytics and Statistics, Chang Gung Memorial Hospital, Linkou Medical Center, Taoyuan, Taiwan, [5]Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan

Introduction: In Taiwan, many people receive Chinese herbal medicine (CHM) as an alternative choice to help control body weight. However, the clinical effectiveness of CHM on weight control has not been well studied, while potential risks and adverse effects are still unknown. The aim of our study is to find out a safe and efficient treatment model of CHM for weight control compared to liraglutide in a real-world setting.

Methods: we retrospectively analyzed obese subjects [body mass index (BMI)≧ 25 kg/m^2] from Chang Gung Research Database (2013–2018). We evaluated the effect on body weight and BMI changes in obese groups receiving CHM or western medicine (WM, represented liraglutide) within 180 days. The proportion of subjects who achieved 5 and 10% weight reduction was calculated as well. Furthermore, the potential adverse events were analyzed during the study period. Overlap weighting was used to balance the baseline differences between CHM and WM groups.

Results: The full cohort comprised 1,360 participants: 701 in the CHM group and 659 in the WM group. At baseline, the CHM group was younger (42.75 ± 12.12 years old in CHM vs. 52.31 ± 11.7 years old in WM, p-value <0.001) and has more female subjects (77.6% in CHM vs. 53.0% in WM, p-value <0.001). On the other hand, CHM users had lower body weight (79.83 ± 15.66 kg vs. 84.68 ± 17.14 kg, p-value <0.001) and BMI (30.58 ± 5.20 vs. 32.84 ± 6.95, p-value <0.001). At day 180, CHM users lost more body weight (−4.5 ± 4.07 kg vs. −2.15 ± 4.05 kg, p-value <0.001) and higher reduction in BMI (−1.77 ± 1.73 vs. −0.9 ± 2.14, p-value <0.001). A total of 53.21% (n = 373) CHM users lost at least 5% of body weight (22.46% for WM users, p-value <0.001), and 18.97% (n = 132) lost at least 10% of body weight

(4.55% for WM users, p-value <0.001). The benefit remained consistent with and without overlap weighting. For adverse events, 18 cases of hypertension occurred in 659 subjects in the WM group (2.7%) in comparison to 1 of 701 subjects in the CHM group (0.1%).

Conclusion: CHM led to clinically meaningful weight loss without serious adverse events in a real-world setting. Further clinical trials are warranted to validate this result.

KEYWORDS

traditional Chinese medicine, obesity, overweight, weight loss, body mass index, liraglutide, weight control

Introduction

Obesity, a worldwide epidemic issue, causes a global public health problem for both individuals and society (Stefan et al., 2013). According to "2013–2016 Nutrition and Health Survey in Taiwan", the prevalence of being overweight and obesity (BMI≧24) in people above 19 years old is 45.4% (Chang et al., 2017). Obesity is correlated with the formation of hypertension, diabetes mellitus, hyperlipidemia, and other cardiovascular diseases. More seriously, being overweight and obese increases the risk of death from all causes, cardiovascular disease, cancer, or other diseases for both men and women in all age groups (Calle et al., 1999). For this reason, weight loss is a crucial pathway to health improvement for patients with obesity-associated risk factors and comorbidities (Ryan and Yockey, 2017). It would be useful for overweight patients who have been unsuccessful with lifestyle modification, diet, and exercise alone to combine their weight reduction methods with medications approved for chronic weight management. To date, as we know, the gradual progression of body weight loss is more likely to be maintained over a longer period of time (Mertens and Van Gaal 2000). Therefore, the novel anti-obesity strategy has focused on modest weight loss, defined as a weight loss of 5%–10% of an individual's baseline weight, which has been demonstrated to reduce complications related to obesity and improve quality of life (Mertens and Van Gaal 2000; Warkentin et al., 2014; American College of Cardiology/American Heart Association Task Force on Practice Guidelines, Obesity Expert Panel, 2013, 2014).

The Food and Drug Administration (FDA) has approved five major weight loss medications available for weight management, which are orlistat, phentermine, phentermine/topiramate extended-release, naltrexone/bupropion sustained-release, and liraglutide (Müller et al., 2022). These anti-obesity drugs have been reported to have a statistically average mean weight loss of 3%–7% from the baseline in clinical trials (Srivastava and Apovian 2018; Müller et al., 2022). However, the safety or significant tolerability issues of the currently available anti-obesity medications arise in the long term use (Lee and Dixon 2017). Phentermine, a sympathomimetic agent, poses side effects, such as tachycardia, insomnia, constipation, and agitation. Commonly, phentermine is a short acting medication and short-term use is suggested since the suppression on appetite may wear off in several weeks (Apovian et al., 2015b). Orlistat, inhibiting pancreatic and gastric lipases, has adverse effects, including steatorrhea, oily spotting, and fecal incontinence, which is poorly tolerated (Apovian et al., 2015a). Topiramate, an anticonvulsant and also a centrally acting drug, has been reported with dose-related cognitive side effects, including psychomotor slowing, decreased concentration and attention, memory impairment, and an unexpected surge in suicidal thoughts (Shin and Gadde 2013). Liraglutide, a glucagon-like peptide-1 (GLP-1) agonist with, presently, the most promising weight-lowering effect, increases incidence of symptomatic gallstones and may elevate the risk of pancreatitis apart from gastrointestinal symptoms (Pi-Sunyer et al., 2015). Hence, safety, tolerability, and efficacy are three impartible core issues surrounding the commencement of weight-loss medications. As the long-term benefits are likely to be outweighed by the risks and costs of treatment, especially cardiovascular and mental health safety issues, new medications or even dietary supplements for obesity are still needed (Müller et al., 2022).

In accord with this approach, numerous complementary and alternative therapies have been used in the Eastern part of the globe for a long time, however, recently, these unconventional therapies are increasingly applied worldwide including Chinese herbal medicine (CHM) (Hasani-Ranjbar et al., 2009). CHM, being documented for thousands of years, has been applied as a form of health promotion and disease treatment throughout the world as a result of being a natural compound, which is regarded as a safer option than synthetic chemical agents (Ignjatovic et al., 2000). In Taiwan, there are many people receiving CHM to help control body weight. Nevertheless, the evidence base for therapeutic efficacy of CHM has not been widely verified by using international standards yet in spite of their extensive use in current clinical practices (Sui et al., 2012). Owing to little information about the efficacy of CHM on weight control, potential risks and adverse effects are confirmed. Thus, we investigated one of CHMs which is used as a therapeutic

TABLE 1 The botanical and mineral drugs contained in Ma-Xing-Gan-Shi-Tang (every 3 g of the water extract are derived from 20 g of the raw materials).

The name of botanical and mineral drugs	Ratio (%w/W)
Ephedra sinica Stapf [Ephedraceae; Ephrdrae herba]	20.0
Prunus armeniaca L. var. *ansu* Maxim [Rosaceae; Armeniacae semen amarum]	20.0
Glycyrrhiza uralensis Fisch [Leguminosae; Glycyrrhizae radix et rhizoma]	10.0
Gypsum fibrosum	50.0

option to promote weight loss in our present clinical specialty. Ma-Xing-Gan-Shi-Tang (MXGST), a Chinese medicine formula, has been traditionally used to adjust the lung qi stagnation, clear the pathological heat, and reduce phlegm. In clinical practice, traditional Chinese medicine (TCM) doctors always judge the clinical symptoms and signs of individuals to adjust the prescription to achieve holistic effects. Based on TCM doctor's clinical experience, MXGST may play a major role in the prescription to treat obesity, combating the overweight dilemma in several different ways in our clinical observations and speculations, such as improving the phlegm-dampness constitution by adjusting the lung qi stagnation. However, it lacks sufficient evidence-based studies or clinical trials to evaluate the promising weight-reducing effects of MXGST herbal formulation in present research.

The aim of this cohort study is to explore the safety and effectiveness of CHM treatment on weight control in comparison with liraglutide, and further attestation of its possible therapeutic values in this overwhelming pandemic of obesity.

Materials and methods

Preparation and composition of MXGST

MXGST used in our clinical practice is the dry powder derived from the water extract of a mixture of four botanical and mineral drugs, which contains *Ephedra sinica* Stapf, *Prunus armeniaca* L. var. *ansu* Maxim, *Glycyrrhiza uralensis* Fisch, and Gypsum fibrosum. Every 3 g of the water extract are derived from 20 g of the raw materials. The ratio of each botanical and mineral drugs is according to the authority of TCM in Taiwan and is presented in Table 1 (Ministry of Health and Welfare).

Data source and study protocol

The Chang Gung Research Database (CGRD) was used as the data source of this study. The CGRD was composed of daily clinical practices, including all procedures and medications of outpatient, inpatient, and emergency visits in the Chang Gung Memorial Hospital (CGMH). The CGMHs provide CHM and Western medicine (WM) for patient care, comprising eight medical institutes with different hospital levels, and serve as the largest medical system in Taiwan (Ku et al., 2021). According to the statistics, CGMHs own 10,070 beds and admit more than 280,000 patients each year, supporting over 8,500,000 outpatient visits and 500,000 emergency department visits in 2015 (Shao et al., 2021). The huge amount of clinical data has made the CGRD become a great resource for clinical studies (Shao et al., 2021).

We retrieved the electronic medical records (EMR) from eight CGMHs to provide real-world evidence, which involved patient information demographics, clinical parameters, diagnostic information, prescription information, and other health care facility information (Shao et al., 2021). The selection period for treatment initiation was from 1 January 2013 to 31 December 2018, with baseline demographic data based on the 6 months period prior to initiation, including baseline weight, biochemical and physiological profiles, and co-morbidities. We selected MXGST as the CHM prescription to help weight-loss in the CHM group and chose liraglutide as the anti-obesity medication in the WM group. Index date was the date when MXGST or liraglutide treatment was initiated during the selection period. The end date of the follow-up was the last return appointment after a 6 months therapeutic period. Patients were evaluated every month until day 180. All patients received standardized counseling on CHM or WM weight-loss prescription by a clinical physician on an approximately monthly basis with their weight reduction as well as any documentation of adverse events. To reveal the trend of body weight reduction in the 6-months follow-up duration, the subjects' body weight changes at day 30, 60, 90, 120, 150, and 180 after the start of treatment were retrieved for this study. In this observational real-world study, body weight was compared retrospectively with the measured value at the start of treatment. The study design and protocol were reviewed and approved by the Institutional Review Board of the Chang Gung Memorial Foundation (IRB No: 201801526B0). The need for informed consent was waived because the identification number of each subject was well-

encrypted and therefore it was impossible to recognize the real identity of each subject.

Study population and covariates

The International Classification of Diseases, 10th Revision, Clinical Modification (ICD-10-CM) and Ninth Revision, Clinical Modification (ICD-9-CM) were used to determine obese population. We included patients who were properly diagnosed with obesity or metabolic syndrome between 2013/01 and 2018/12 from the CGRD (ICD-9-CM codes: 278.0, 278.00, 278.01, 278.03, 649.10–14, 793.91, V85.30–39, V85.41–45, V85.54, and ICD-10-CM codes: E65, E668, E669). The highest body weight around 1 month before or after the index date and the following body weight check-up dates were recognized as the body weight of each time point. Subjects were included in this study if they fulfilled all of the following criteria: 1) subjects were ≥18 years old and ≤75 years old at the index date; 2) they had a body mass index (BMI; the weight in kilograms divided by the square of the height in meters) ≥25 kg/m^2 with at least one weight-related disease such as prediabetes, diabetes mellitus (DM), hypertension (HTN), dyslipidemia, or hepatic steatosis during the 360 days before the index date; 3) they had at least one prescription for MXGST or liraglutide at the discretion of the physician; and 4) they had at least one reported baseline body weight measurement within 1 month before the index date; and those who continued to visit the clinic after 1–2 months were treated with MXGST or liraglutide after the index date. The exclusion criteria were listed as follows: 1) missing weight or height record from EMR at baseline and around the end date of follow-up; and 2) taking other anti-obesity medications or a history of bariatric surgery. We collected data of people who met the inclusion criteria as afore mentioned at first visit to outpatient department from the CGRD, including the visit date, age, gender, height, weight, blood pressure, heart rate, underlying comorbidities, information about the use of CHM and WM, laboratory data, and examinations.

Outcome assessment

Our retrospective study assessed the weight-loss efficacy of MXGST in the CHM group and liraglutide in the WM group as well as anti-obesity therapy-related adverse effects in this cohort according to the 6 months of treatment of compliance with these prescriptions. The primary outcome was the body weight change from baseline to the 180th day. The secondary outcome was the proportion of subjects who lost at least 5% of their baseline body weight, and the proportion of subjects who lost more than 10% of their baseline body weight. To compute BMI, we used the average value to impute subjects' body height if not recorded; however, to disclose the real-world status, the missing value of body weight on 30-, 60-, 90-, 120- and 150-days body weight was not imputed in the final analysis. Besides, we also evaluated whether there are potential adverse effects, such as cerebrovascular disease and cardiovascular system impairment. We scrutinized adverse events that occurred during the 6 months therapeutic period, including the medical records of emergency room or hospitalization with onset on or after the first day of treatment and no later than 30 days after the last day of treatment.

Statistical analysis

For baseline demographic features, descriptive statistics with X^2 statistics and independent t-tests were used to examine the differences between CHM and WM users. Moreover, independent t-tests were used to examine the weight changes between two groups, while X^2 statistics were used to examine the differences of the proportion of 5%- and 10%-weight reduction and adverse events between two groups at the end of study. Also, overlap weighting was used to minimize the potential confounding bias caused by the different baseline features of CHM and WM users. Overlap weighting is a propensity score (PS)-based statistical method widely used in observational studies to mimic randomized clinical trials, especially when considering case imbalance or potential biases caused by prominently different initial status (Li, Thomas, and Li 2019; Thomas, Li, and Pencina 2020). In this study, age, gender, Charlson Comorbidity Index (CCI), mean arterial pressure (MAP), and body weight were used to generate the PS. PS was assigned as the weight of WM users while 1-PS was assigned to CHM users before effect estimation. On the other hand, we also conducted sensitivity tests by using other PS-related models, including 1:1 propensity score matching and Inverse probability of treatment weighting (IPTW) to examine the effect of the CHM treatment. All statistical calculations were carried out by SAS and results with a p-value <0.05 was considered to be statistically significant.

Results

Baseline characteristics of study patients

During 2013 and 2018, there were 701 CHM users and 659 WM users entering the final analysis stage who completed 180 days of treatment (Figure 1). The baseline clinical characteristics of subjects with CHM and WM treatment are shown in Table 2. Overall, CHM users tended to be younger than WM users, 42.75 and 52.31 years old, respectively

TABLE 2 Baseline demographic features of study subjects.

	CHM user (n = 701)	WM user (n = 659)	*p*-value
Demographics			
Age—years	42.75 ± 12.12	52.31 ± 11.7	<0.001
Gender—no. (%)			
Male	157 (22.4)	310 (47.0)	<0.001
Female	544 (77.6)	349 (53.0)	<0.001
Smoker	5 (0.71)	73 (11.08)	<0.001
Alcohol drinker	3 (0.43)	39 (5.92)	<0.001
Betel nut user	0 (0.00)	17 (2.58)	<0.001
Comorbidities (%)			
Hypertension	82 (11.7)	407 (61.8)	<0.001
Dyslipidemia	64 (9.1)	442 (67.1)	<0.001
Ischaemic heart diseases	6 (0.8)	81 (12.3)	<0.001
Diabetes mellitus	39 (5.6)	510 (77.4)	<0.001
Chronic hepatitis	16 (2.3)	83 (12.6)	<0.001
Fatty liver	4 (0.6)	9 (1.4)	0.22
Nonalcoholic steatohepatitis	1 (0.1)	12 (1.8)	0.004
CCI	0.3 ± 0.7	2.33 ± 1.59	<0.001
Biochemical and physiological profiles (mean ± SD)			
MAP—mmHg	131.7 ± 15.1	128.8 ± 15.28	0.001
Weight—kg	79.38 ± 15.66	84.68 ± 17.14	<0.001
BMI—kg/m^2	30.58 ± 5.2	32.84 ± 6.95	<0.001
AST—mg/dL	32.18 ± 15.94	49.44 ± 79.4	0.003
ALT—mg/dL	35.84 ± 32.2	48.46 ± 46.98	<0.001
BUN—mg/dL	13.1 ± 4.79	25.72 ± 19.02	<0.001
Creatinine—mg/dL	0.71 ± 0.2	1.10 ± 0.98	<0.001
HbA1C—%	6.51 ± 1.25	9.82 ± 1.7	<0.001
Fasting glucose—mg/dL	106.1 ± 33.82	213.49 ± 73.45	<0.001
Lipid profile (mean ± SD)			
Total cholesterol—mg/dL	196.31 ± 29.78	186.12 ± 44.45	0.004
Triglyceride—mg/dL	152.83 ± 76.84	230.06 ± 222.2	<0.001
LDL cholesterol—mg/dL	121.65 ± 28.86	104.83 ± 32.63	<0.001
HDL cholesterol—mg/dL	45.34 ± 10.01	40.12 ± 10.1	<0.001

*Abbreviations: CCI, charlson comorbidity index.
*Continuous covariates are presented as the median (interquartile range, IQR) while categorical covariates are presented as a number (percentage).
*Body mass index (BMI) is the weight in kilograms divided by the square of the height in meters.
*The *p*-value was calculated by means of Fisher's exact test, Pearson's chi-squared test, and Student's t-test on the basis of the number of participants.

(*p*-value <0.001), and a higher proportion of female subjects was found among CHM users, 77.6 vs. 53.0% WM users, respectively (*p*-value <0.001). Additionally, a higher proportion of WM users had hypertension, dyslipidemia, and diabetes mellitus (DM) (CHM vs. WM users, 11.7 vs. 61.8% for hypertension, *p*-value <0.001; 9.1 vs. 67.1% for dyslipidemia, *p*-value <0.001; and 5.6 vs. 77.4% for DM, respectively, *p*-value <0.001), and a higher CCI (0.30 for CHM vs. 2.33 for WM users, respectively, *p*-value <0.001). As to biochemical and physiological profiles, there were prominent differences in MAP, body weight, BMI, and lipid profiles, which were higher in the WM group (all *p*-values <0.05). However, WM users seemed to have a higher HbA1C and fasting glucose level than CHM users, 9.82 vs. 6.51% for HbA1C and 213.49 ± 73.45 mg/dl vs. 106.1 ± 33.82 mg/dl for fasting glucose level, respectively (*p*-value <0.001). The use of overlap weighting properly eliminated the differences in baseline features among CHM and WM users in age, gender, CCI index, MAP, and body weight covariates (Table 3).

Body weight reduction

After 180 days, the use of CHM led to a significantly greater reduction in body weight than WM. CHM users had lost 4.57 ± 4.51 kg of their body weight, whereas WM users had lost 2.3 ± 5.48 kg of their body weight at the end of study (Table 4). Weight loss from the baseline during the study is shown in Figure 2. The trend of estimated mean weight loss in this cohort study was significantly greater with CHM than with WM from stem to stern. A greater reduction in the body weight of CHM users than WM users was observed (at day 30, −2.9 ± 3.36 kg vs. −1.28 ± 2.71 kg; at day 60, −3.48 ± 3.45 kg vs. −1.66 ± 3.45 kg; at day 90, −4.2 ± 3.82 kg vs. −1.63 ± 3.44 kg; at day 120, −4.46 ± 3.82 kg vs. −1.85 ± 3.61 kg; at day 150, −4.64 ± 3.98 kg vs. −2.28 ± 3.96 kg; and at day 180, −4.5 ± 4.07 kg vs. −2.15 ± 4.05 kg) (Table 5). After overlap weighting, the data also revealed the significant weight loss difference between CHM users and WM users (Table 5). Body weight loss was the most significant within the first month and gradually plateaued since day 150 in the CHM group as well as in the WM group with a smaller slope (Figure 2).

After the 6-months treatment, 53.21% (n = 373) of the subjects in the CHM group lost more than 5% weight from baseline, which was significantly more than that in the WM group (22.46%, n = 148, *p*-value <0.001). The proportion of people losing more than 10% of their baseline weight was greater with CHM treatment (18.97%, n = 132) than with WM treatment (4.55%, n = 30, *p*-value <0.001). Furthermore, more individuals (3.57%, n = 25) treated with CHM lost more than 15% of baseline weight than those treated with WM (1.37%, n = 9, *p*-value <0.001) (Figure 3).

Overall, approximately 84.45% of the subjects in the CHM group vs. 67.37% of the subjects in the WM group lost weight after treatment in this trial. The majority of subjects had lost more than 5%–10% of body weight in the CHM group and the maximum weight loss didn't exceed 20% of body weight after 180 days of treatment. Instead of losing weight, weight gain wasn't beyond 5% of body weight in both groups (Figure 4). The CHM group also had a greater reduction than the WM group in mean BMI (1.77 ± 1.73 vs. 0.9 ± 2.14, *p*-value<0.001) (Table 4). Adjusted with overlap weighting, the trend of body weight loss in

TABLE 3 Baseline characteristics of subjects were balanced by using overlap weighting.

	Without overlap weighting			With overlap weighting		
	CHM user	WM user	SMD	CHM user	WM user	SMD
Demographics						
Age—years	42.75 ± 12.12	52.31 ± 11.7	0.803	47.84 ± 5.53	47.84 ± 5.54	0.000
Gender—no. (%)			0.535			0.000
Male	157 (22.4)	310 (47)		33.3	33.3	
Female	544 (77.6)	349 (53)		66.8	66.8	
Comorbidities						
CCI	0.3 ± 0.7	2.33 ± 1.59	1.653	0.97 ± 0.51	0.97 ± 0.31	0.000
Biochemical and physiological profiles						
MAP—mmHg	131.7 ± 15.1	128.8 ± 15.28	0.191	132.1 ± 6.81	132.1 ± 7.68	0.000
Weight—kg	79.38 ± 15.66	84.68 ± 17.14	0.323	82.27 ± 16.95	82.77 ± 16.28	0.000

*Abbreviations: CCI, charlson comorbidity index.
*Continuous covariates are presented as the median (interquartile range, IQR) while categorical covariates are presented as a number (percentage).
*The balance of covariates using the standardized mean difference (SMD).
*An SMD ≤0.1 indicates a negligible difference in potential confounders between the two study groups.
*Weighted covariates were age, gender, CCI, index, and body weight.

both groups was similar to the original data (Table 4 and Figure 2). Several sensitivity analyses confirmed the superiority of CHM over WM with respect to the primary end point as well. In the IPTW model, CHM users lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.0001). In the PSM model, CHM users also lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.05). These two models showed the consistent result that CHM users had significant weight loss than WM users during the whole treatment course (Supplementary Tables S1, S2 in the Supplementary Appendix SA1).

The subgroups analyses reveal that both in the groups older or younger than the median age, 48 years old, CHM users lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.0001). As to comorbidity subgroups, in the subgroup with diabetes mellitus, there is no significant difference in body weight loss between CHM users and WM users. In the subgroup without diabetes mellitus, CHM users lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.01). In the subgroup with hypertension, CHM users lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.05). In the subgroup without hypertension, CHM users lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.001). In addition, among 25≦BMI<30, 30≦BMI<35 and 35≦BMI subgroups, CHM users also lost more body weight than WM users on treatment course Day 30, 60, 90, 120, 150, and 180 (p-value <0.001) (Supplementary Table S3 in the Supplementary Appendix SA1).

Side effects and adverse events

At the end of study, 18 cases of hypertension occurred in 659 patients in the WM group (2.7%) in comparison to 1 case in 701 patients in the CHM group (0.1%). Furthermore, the incidence of ischaemic heart disease events was higher in WM users than in CHM users (2 cases occurred in 659 subjects in the WM group (0.3%) in comparison to 0 cases in 701 subjects in the CHM group, p-value = 0.055), however, there was no statistical difference observed (Table 6). There were also no reported cases of cerebrovascular disease or brain disease occurring in both group treatments. No adverse effects with respect to hypertensive encephalopathy were observed in both groups by the end of the trial (Table 6). The diagnosis codes used in the study is presented in Supplementary Table S4 in the Supplementary Appendix SA1.

Discussion

Overall findings

The main findings of this study are as follows: First, CHM users led to a significantly greater reduction in body weight (primary end point) than WM users. The mean change in body weight in the CHM group was −5.74% (−4.57 ± 4.51 kg), which was shown to be superior to the WM group with respect to the primary end point after 180 days of treatment. The treatment effect was similar across the baseline BMI categories. Second, subjects with the CHM only reported an adverse event once, which was related to hypertension. Unlike currently prescribed

TABLE 4 Changes in primary end point between the baseline and at day 180.

	Without overlap weighting			With overlap weighting		
	CHM user	WM user	*p*-value	CHM user	WM user	*p*-value
Changes in Body Weight	−4.57 ± 4.51	−2.3 ± 5.48	<0.001	−4.34 ± 2.54	−1.93 ± 2.02	<0.001
Kilograms of body weight						
% of body weight	−5.74	−2.62	<0.001	−5.15	−2.29	<0.001
Loss of >5% body weight—no. (%)	373 (53.21)	148 (22.46)	<0.001	45.9	19.51	<0.001
Loss of >10% body weight—no. (%)	132 (18.97)	30 (4.55)	<0.001	15.64	4.5	0.001
Changes in BMI	−1.77 ± 1.73	−0.9 ± 2.14	<0.001	−1.67 ± 0.98	−0.77 ± 0.94	<0.001

*The *p*-value was calculated by Pearson's chi-squared test and Student's t-test.

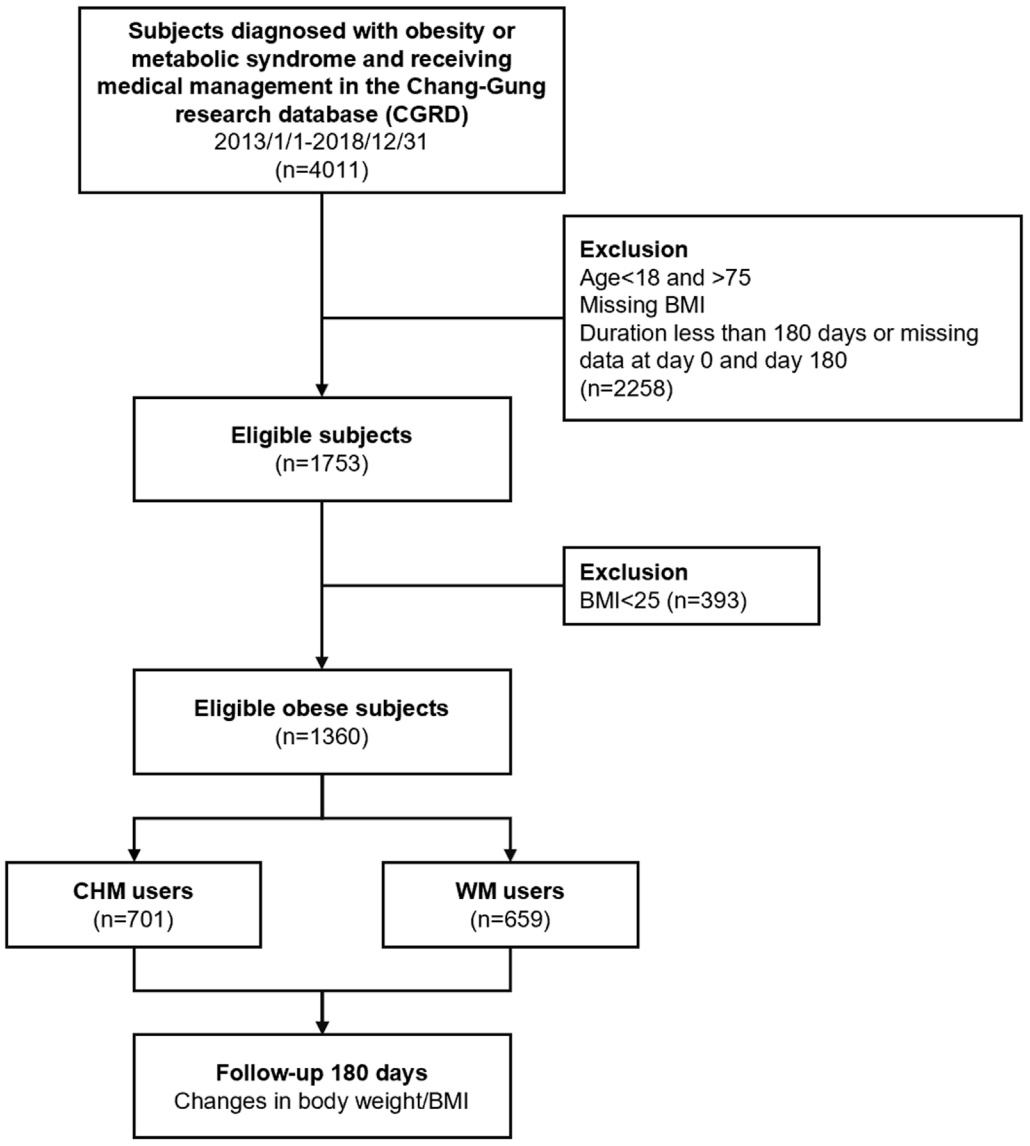

FIGURE 1
A flowchart of the collection of subjects from the CGMH hospital outpatient database from 2013 to 2018 in Taiwan.

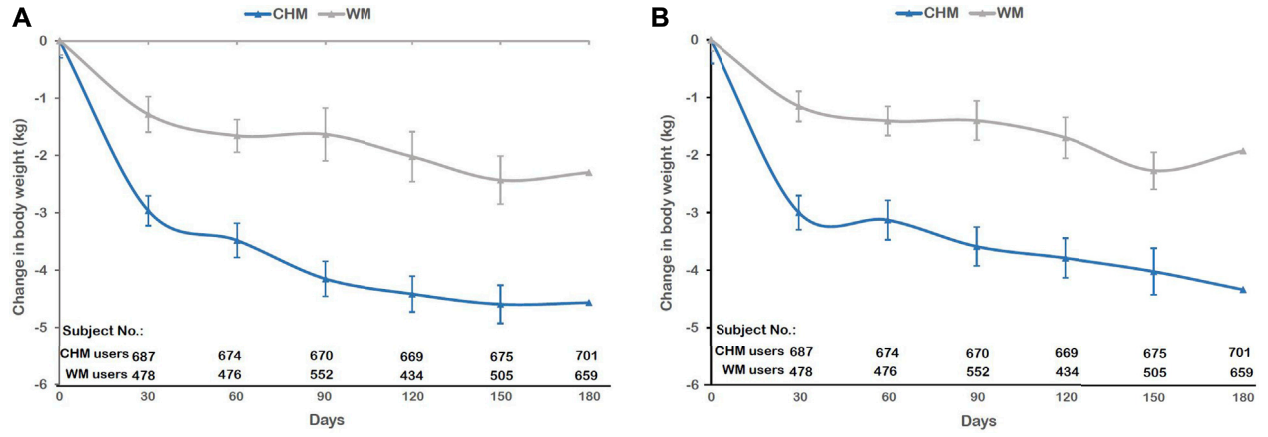

FIGURE 2
Changes in body weight after 180 days of CHM and WM treatment. **(A)**Without overlap weighting and **(B)** with overlap weighting.

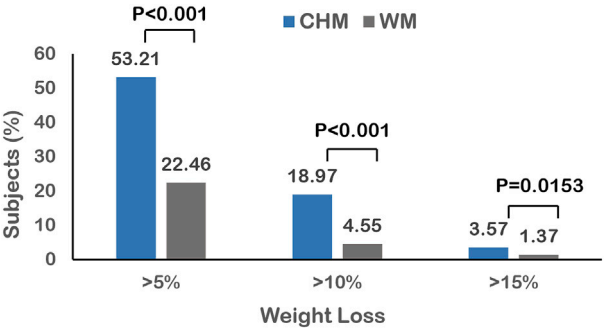

FIGURE 3
The differences in proportion of obese subjects achieving 5, 10, and 15% at day 180 between CHM and WM users.

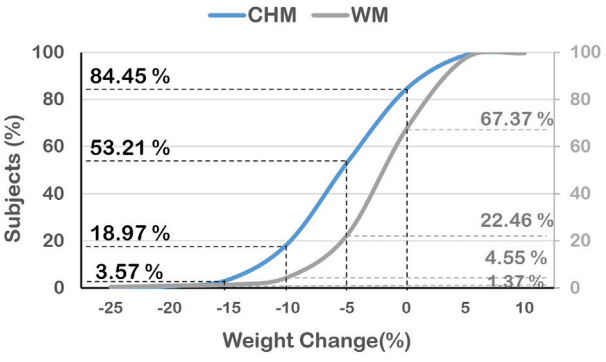

FIGURE 4
The differences in distribution of the percentage of reduced body weight among obese subjects at day 180 between CHM and WM users.

anti-obesity drugs, the use of the CHM had the distinct advantage of significantly reducing adverse events compared with WM use.

The clinical significance of CHM for weight loss

In terms of results, the CHM group had greater weight loss than the WM group and it seems that the CHM could afford substantial help for achieving modest weight loss, a weight loss of 5–10% from the baseline weight, that has been verified to decrease comorbidities associated with obesity and promote better quality of life in previous studies (Mertens and Van Gaal 2000; Warkentin et al., 2014). As far as we know, overweight or obesity is associated with higher risk for mortality and cardiometabolic diseases, and many studies have demonstrated that a modest 5% of weight loss can produce clinically meaningful improvement in various risk factors, comorbid diseases, and mortality that are associated with obesity (Ryan and Yockey, 2017). It has been reported that 5% of weight loss is capable of decreasing the plasma concentration of biomarkers related to cardiometabolic diseases, improving liver and adipose tissue insulin sensitivity, and lowering fasting glucose and hemoglobin A1c. (Magkos et al., 2016). As to risk factors of cardiovascular disease (CVD), 2%–5% of weight loss can improve hypertriglyceridemia and systolic blood pressure, 5% of weight loss can decrease intra-hepatic triglyceride by 13%, and 5%–10% of weight loss can improve diastolic blood pressure and high-density lipoprotein cholesterol level (Magkos et al., 2016). Besides, obesity is one of important risk factors of knee osteoarthritis (Raud et al., 2020). Patients who achieved more than 5% of weight loss would produce significant improvements in knee functionality, speed, walking distance, and pain (Messier et al., 2004). Research also indicated that each

TABLE 5 Body weight reduction at day 30, 60, 90, 120, 150 and 180 after the start of treatment.

Day	CHM user		WM user			Overlap weighting		
	Subjects	△Body weight	Subjects	△Body weight		CHM user	WM user	
	(n)	(kg)	(n)	(kg)	*p*-value	△Body weight (kg)	△Body weight (kg)	*p*-value
30	687	−2.9 ± 3.36	478	−1.28 ± 2.71	<0.0001	−2.76 ± 1.81	−1.15 ± 1.03	<0.0001
60	674	−3.48 ± 3.45	476	−1.66 ± 3.45	<0.0001	−3.13 ± 1.81	−1.41 ± 1.4	<0.0001
90	670	−4.2 ± 3.82	552	−1.63 ± 3.44	<0.0001	−3.73 ± 1.88	−1.4 ± 1.46	<0.0001
120	669	−4.46 ± 3.82	434	−1.85 ± 3.61	<0.0001	−3.94 ± 1.82	−1.68 ± 1.67	<0.0001
150	675	−4.64 ± 3.98	505	−2.28 ± 3.96	<0.0001	−4.17 ± 1.87	−2.26 ± 1.89	<0.0001
180	701	−4.5 ± 4.07	659	−2.15 ± 4.05	<0.0001	−4.11 ± 1.93	−1.92 ± 1.97	<0.0001

TABLE 6 Adverse events during the treatment period.

	CHM user	WM user	*p*-value
	(n = 701)	(n = 659)	
The Incidence of Adverse Events—no. (%)			
Hypertension	1 (0.1)	18 (2.7)	<0.001
Ischaemic heart disease	0	2 (0.3)	0.055
Cerebrovascular disease	0	0	—
Brain disease	0	0	—
Hypertensive encephalopathy	0	0	—

*The *p*-value was calculated by Pearson's chi-squared test and Student's t-test.

pound of weight loss would result in a 4-fold reduction in the load exerted on the knee per step during daily activities (Messier et al., 2005). Moreover, even a minimal weight loss of only 2%–5% of total body weight can improve ovulatory function by reducing ovarian volume and micro-follicle number, and is more likely to result in spontaneous pregnancy (Kiddy et al., 1992; Crosignani et al., 2003). Our cohort study demonstrated that more than half of CHM users could lose >5% of baseline body, although the therapeutic window is only limited to 180 days, which may be too short to confirm the influence of Chinese herbal medicine on diseases for the long term. This result inspired us that targeted health outcome goals may be reached by an individual with more than 5%–10% weight loss. The anti-obesity CHM has the benefits of not only achieving a weight loss goal, but also reaching targeted health outcome goals. What is meaningful about our study is that patients don't have to reach the level of BMI <25 kg/m^2 in all instances, and they can be healthier at any weight as long as they have a moderate weight loss.

When it comes to adverse events in taking medications, the herbal formulae used in the CHM group showed better tolerability than in the WM group (). The reasons to why CHM group had better weight-loss effects than the WM group in our cohort study are as following: First, CHM users had a larger proportion of female subjects (77.6 vs. 53.0% among WM users, *p*-value <0.001) and their average age was younger than WM users (42.75 years old for CHM vs. 52.31 years old for WM users, *p*-value <0.001). Previous studies reported that women and younger participants more easily achieved higher acceptable weight loss percentages due to powerful motivation concerning the increasing social desirability of weight loss which is associated with body image dissatisfaction and awareness of illness prevention (Czeglédi 2017; Nguyen et al., 2021). Second, WM users consisted of a greater proportion of seniors who may face challenges complicated by the progressive loss of skeletal muscle and accumulation of excess adipose tissue, which has been commonly referred to as sarcopenic obesity (Coker and Wolfe 2018). Given the lower level of aerobic fitness, a higher proportion of lean body mass loss, and a progressively decreasing metabolic rate, sarcopenic obesity may become a part of clinical conundrum in older individuals with obesity in the WM group (Coker and Wolfe 2018). Third, a higher proportion of WM users had hypertension, dyslipidemia, and DM. Chronic health problems like hypertension, DM, and endocrine problems may lead to a lower metabolic rate and make it harder to lose weight (Ganguly et al., 2018). The complexity of conditions in patients with more comorbidities may lead to less body weight loss in participants with metabolic syndrome than in those without such comorbidities (Zhou et al., 2012).

As to weight reduction concepts in TCM, the imbalance of the physiological state in energy (yang) deficiency, materials (yin) deficiency, or phlegm-stasis constitution may cause a high tendency of obesity (Hou et al., 2021). The TCM doctors' prescriptions should adhere to the philosophy of CHM emphasizing on "personalized therapy" and TCM doctors would judge the clinical symptoms and signs of individuals to adjust CHM treatments for different people, being effective

in reducing the side effects promptly during treatment (Sui et al., 2012). It is in contrast to Western medical doctors who conform to the uniform therapeutic guidelines and prescribe similar medications to patients with the same clinical diagnosis. This difference in therapeutic approach may partly explain the higher efficacy and lower incidence of adverse effects in the CHM group than in the WM group in the real-world (Sui et al., 2012). Given the health burden of obesity and metabolic syndrome, complementary use of the CHM is a possible option to address this unmet therapeutic aspect. Ma-Xing-Gan-Shi-Tang (MXGST) is an oriental herbal formula that has traditionally been used in patients with phlegmatic asthma and productive cough. MXGST is composed of *Ephedra sinica* Stapf (Ma-huang), *Glycyrrhiza uralensis* Fisch (Gan-cao), Gypsum fibrosum (Shi-gao), and *Prunus armeniaca* L. var. *ansu* Maxim (Xing-ren). The exact anti-obesity mechanisms of MXGST have not been validated yet, so we review experimental and clinical studies to infer the possible mechanisms. To our knowledge, MXGST might solve the body fat dilemma in several ways. Ma-huang has been applied to treat asthma, common cold, edema, arthralgia, and other symptoms in Asia for over 5,000 years (Ding 2009). Ma-huang is enriched with ephedrine-type alkaloids, of which ephedrine is the most abundant and active isomer. Ephedrine is a potential slimming drug that mediates thermogenic effects, primarily by the enhancement of sympathetic neuronal release of norepinephrine (NE) and epinephrine in both humans and laboratory animals (Bukowiecki, Jahjah, and Follea 1982; Dulloo, Seydoux, and Girardier 1992; Dulloo 1993). Given that brown adipocyte is an important site of catecholamine-induced thermogenesis in homeotherms (Bukowiecki, Jahjah, and Follea 1982), previous *in vitro* research revealed that ephedrine mimics the calorigenic action of norepinephrine by stimulating brown adipocyte respiration directly via beta-adrenoceptors (Ramsey et al., 1998). Gan-cao, has long been used as a traditional herbal medicine for stomach-invigorate and coordinating the drug actions of a prescription. Previous animal research had demonstrated that the extract of licorice root has beneficial influences on preventing atherosclerotic lesion development which is associated with inhibition of low-density lipoprotein (LDL) oxidation in hypercholesterolemic rats (El-Beshbishy et al., 2006). Some studies further provided evidence on anti-obesity properties of licorice root which can lower total cholesterol (TC) and low-density lipoprotein (LDL) in patients with hypercholesterolemia, being effective in reducing abdominal fat deposition and improving lipid profiles (Fuhrman et al., 2002; Bell, Canale, and Bloomer 2011; Mirtaheri et al., 2015). It seems that supplementation with Gan-cao may efficiently improve the lipid profile in overweight and obese subjects. Shi-gao, which is mainly composed of CaSO4, has been used as a treatment for reducing fevers and alleviating thirst in various TCM (Yuan et al., 2002; Ikarashi et al., 2013). It has been reported that the Shi-gao plays an important role in promoting urination and draining dampness in Chinese indigenous medicine and pharmacology. More recently, previous research proved that a combined administration of Ma-huang and Shi-gao would increase urine excretion (Huo et al., 2015). We conclude that Shi-gao may be a useful herbal medicine for changing the distribution of body fluid and increasing urine excretion with the highest potential to eliminate edema. Xing-ren, of which Amygdalin is a significant component (Jaswal et al., 2018), and is rich in oil, can promote intestinal peristalsis and prevent constipation. It has been used as a traditional herbal medicine for relieving constipation (Aritomi, Kumori, and Kawasaki 1985; Du et al., 2005; Chang et al., 2006; Erdogan-Orhan and Kartal 2011). We suggest that administering Xing-ren to improve bowel movements may be helpful for cleaning the accumulation of waste and toxins in the body. Based on our knowledge, it seems that the anti-obesity mechanisms of MXGST are remarkably different from liraglutide, which induces weight loss by delaying gastric emptying as well as suppressing appetite and energy intake (van Can et al., 2014; Knudsen and Lau 2019). In brief, MXGST has the potential for promoting metabolism, moistening intestines to relieve constipation, and regulating blood viscosity, making it has an obvious curative effect for losing weight. Thus, our retrospective cohort study demonstrates the efficacy and safety of MXGST in the management of obesity. Our results suggest that the MXGST treatment has a favorable benefit-risk ratio in obese groups. Careful observations and sophisticated surveillance are needed to investigate further the safety and long-term effects of this medication.

The differences between real-world settings and RCTs

Our cohort study had several differences compared with other randomized controlled trial (RCT) studies. It may reveal that the weight loss effect of liraglutide in our real-world study in Asian populations was significantly less than that reported in RCT studies in Western populations. Liraglutide, a glucagonlike peptide-1 analogue, has been assessed by several randomized controlled trials indicating that it has anti-obesity effects possessing beneficial effects on glycemic control and could lower the risk of cardiovascular death in obese individuals (Howell, Wright, and Clements 2019). In our study, there are 22.46% (n = 148) and 4.55% (n = 50) of WM users who showed ≥5 and ≥10% body weight reduction, respectively, and the mean change in body weight of WM users was −2.62% (−2.3 ± 5.48 kg) at the end of the 180-days treatment. Previous research reported that patients who are overweight or obese in liraglutide group, the mean

weight loss with liraglutide (administered subcutaneously in daily doses: 1.2–3.0 mg daily) was 4.8–7.2 kg in a 20-weeks randomized trial (Astrup et al., 2009). Additionally, in a 56-weeks trial, patients receiving once-daily subcutaneous injections of liraglutide, 3.0 mg, had lost a mean of 8.4 ± 7.3 kg of body weight, and approximately 63.2% of liraglutide users had lost at least 5% of their initial body weight (Pi-Sunyer et al., 2015). Another 56-weeks liraglutide 3.0 mg randomized trial had demonstrated that 81.4% of participants had maintained above 5% weight loss, and they had lost a mean of 6.2% of their initial body weight from randomization to the 56th week (Wadden et al., 2013). It seems that the body weight reduction effect of liraglutide observed in our study was slightly lower than that reported in RCTs. The reasons we presumed are as following: First, the baseline BMI of the subjects enrolled in our cohort study was 32.84 ± 6.95 kg/m^2, which was lower than the average BMI >35 kg/m^2 reported in previous studies of mostly Western populations (Astrup et al., 2012; Wadden et al., 2013; Davies et al., 2015; Pi-Sunyer et al., 2015). The mean body weight of our subjects showed a similar situation, which was much lower than the average body weight in the majority of previous studies of Western populations (84.68 ± 17.14 kg in our WM group vs. a body weight >105 kg in previous Western trials) (Pi-Sunyer et al., 2015; Davies et al., 2015; Wadden et al., 2013; le Roux et al., 2017). A previous study had found that absolute and relative reductions in body weight were dependent on the baseline BMI, which means that those populations with higher baseline BMI would lose more relative body weight than those with a lower baseline BMI (Ponzani 2013; Chitnis et al., 2014). It is consistent with the findings of our study that Asian ethnic groups, which do not usually have a very high BMI, are associated with less weight loss with liraglutide treatment. Second, liraglutide has been prescribed as a therapeutic agent to provide glycemic control for type 2 diabetes mellitus (T2DM), which would not be applied with the maximal dose daily for weight control purposes (Nauck et al., 2009). The retrospective nature of our study showed a higher proportion of T2DM individuals in the WM group under submaximal dosage of liraglutide for glycemic control. It may infer that the maximal medication benefits of reducing appetite and energy intake were not achieved in the WM group. Additionally, there were a wider range of subjects with T2DM comorbidity in the WM group. Some studies found that the complexity of conditions in patients with T2DM may lead to less body weight loss in participants with T2DM than in those without T2DM (Apovian et al., 2015a; Thomas et al., 2020). Third, chances are high that subjects' motivation would de-escalate as time goes by, and it probably would result in low adherence after 180 days of treatment. In view of considerable variability in the general population and fluctuating compliance to anti-obesity treatment, the results of RCTs cannot always reflect the responses in real-world clinical practices (Lin and Schneeweiss 2016). The results obtained from RCTs are through precise controls and close monitoring of those specific participants, including diet control and exercise education. Although RCTs demonstrate the complete evaluation of drug efficacy and safety, they cannot completely reflect the real-world while patients in the real-world actually live in an uncontrolled environment and usually have a variety of comorbidities as well as inconsistent adherence to treatment (Park et al., 2021). As stated before, those reasons may have contributed to the relatively low weight loss extent in WM users compared with previous RCTs.

Limitation

Our study had several limitations. First, it is hard to reflect on patients' adherence to lifestyle modification and actual compliance between CHM users and WM users during the course of treatment in our retrospective observational study. Second, the prescription of the CHM may combine different formulas to achieve more holistic effects and it does not always exactly follow the consistent dosage or formula as well as WM. Third, since most subjects were Asian, the generalizability of these results for other ethnicities may be concerned. Forth, there were some baseline demographic differences between both groups. Despite the baseline differences in weight loss were adjusted in propensity score models, we still need further large randomized controlled trials to confirm the real causality of anti-obesity prescriptions and to collect comprehensive side effects.

Conclusion

In conclusion, the results of our retrospective cohort study demonstrated that the CHM may provide a significant new dimension in our pursuit of weight control and prevention of metabolic syndrome by means of achieving body weight loss that was almost on a par with WM users in the real-world. Although the pharmacological mechanism of anti-obesity action is still vague and remains to be investigated, it is essential to conduct large-scale clinical trials and more longitudinal real-world studies for the potential of CHM in future in order to deliberate on the effectiveness and benefits of CHM in clinical practices along with assessing comorbidities and adverse events during follow-up. It is promising to develop a patient-centered long-term approach to weight management by providing the combined use of Western principles and Chinese medications therapeutic strategies.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by The Institutional Review Board of the Chang Gung Memorial Foundation (IRB No: 201801526B0). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

The work presented here was carried out in collaboration between all of the authors. Y-NL performed the data collection and manuscript writing. P-WL and C-YH were responsible for the statistical analysis and data interpretation. Y-TH commented on the study design and collected patients. C-WY provided TCM's viewpoint. H-YC and T-HY designed the methods and revised the article. All authors approved the final version. Y-NL and H-YC equally contributed to this work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Dan Tang,
Guangdong Pharmaceutical University, China

REVIEWED BY
Helda Tutunchi,
Tabriz University of Medical Sciences, Iran
Asra Iftikhar,
University of Faisalabad, Pakistan

*CORRESPONDENCE
Beihui He,
graf303@sina.com

†These authors contributed equally to this work

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Natural flavonoids: Potential therapeutic strategies for non-alcoholic fatty liver disease

Panli Tan[1†], Li Jin[2†], Xiang Qin[1] and Beihui He[1*]

[1]The First Affiliated Hospital of Zhejiang Chinese Medical University (Zhejiang Provincial Hospital of Traditional Chinese Medicine), Hangzhou, China, [2]School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou, China

The incidence of non-alcoholic fatty liver disease (NAFLD) is increasing rapidly worldwide; however, there are currently limited treatments for NAFLD. The disease spectrum includes simple fatty liver, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and progression to hepatocellular carcinoma (NASH-HCC). The therapeutic effects of NAFLD remain controversial. Although researchers have conducted studies on the pathogenesis of NAFLD, its pathogenesis and anti-NAFLD mechanisms have not been fully elucidated. Previous studies have found that flavonoids, as natural substances with extensive pharmacological activity and good therapeutic effects, have excellent antioxidant, anti-inflammatory, metabolic disease improvement, anti-tumor, and other properties and can significantly alleviate NAFLD. Flavonoids could be further developed as therapeutic drugs for NAFLD. In this paper, the pathogenesis of NAFLD and the mechanisms of flavonoids against NAFLD are summarized to provide a theoretical basis for screening flavonoids against non-alcoholic liver injury.

KEYWORDS

non-alcoholic fatty liver disease, natural flavonoids, antioxidant, anti-inflammatory, intestinal flora, oxidat ive stress, inflammtion

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver disease characterized by excessive fat deposition in hepatocytes, which is not caused by alcohol or other clear liver-damaging factors (Cobbina and Akhlaghi, 2017). The global incidence rate of NAFLD is approximately 25%, particularly in patients with diabetes and obesity (Mundi et al., 2020). NAFLD is the most common chronic liver disease worldwide and is expected to be the main cause of liver transplantation in the future (Younossi et al., 2016b). NAFLD encompasses a wide range of liver disorders, including simple fat accumulation in the liver cells, non-alcoholic steatohepatitis (NASH), fibrosis through the final stages of cirrhosis, and NASH-HCC (Cobbina and Akhlaghi, 2017). The incidence of NAFLD and NASH is related to sedentary lifestyle and excess dietary energy (Farrell et al., 2013). To date, the Food and Drug Administration has not approved any drugs for the treatment of NASH (Eduardo et al., 2015). Currently, NAFLD can be effectively alleviated only through non-drug management approaches, such as healthy lifestyle, diet, and moderate physical

activity (Guillaume et al., 2015). Given the limited clinical treatment for NAFLD, the development of drugs that can effectively alleviate NAFLD is of great significance.

Pathogenesis of non-alcoholic fatty liver disease

The pathogenesis of NAFLD remains unclear so far. However, recent studies have suggested a bidirectional association between NAFLD and metabolic syndrome, with type 2 diabetes increasing the risk of cirrhosis and related complications (Powell et al., 2021). Insulin resistance, diabetes mellitus, and genetic variations in transmembrane 6 superfamily member 2 (TM6SF2) and patatin-like phospholipase domain containing 3 (PNPLA3) play important roles in NAFLD progression (Cobbina and Akhlaghi, 2017). NAFLD is characterized by excessive fatty accumulation in the liver, while simple steatosis is considered pathologically benign. NASH generally indicates liver damage that can progress to severe pathology (Zhang et al., 2018).

The "two-hit" pathogenesis of NAFLD/NASH was widely accepted in the early stage (Chi, 2017). The "first hit" is characterized by an increase in hepatic fat, especially accumulation of hepatic triglycereides and insulin resistance. Once the accumulation of hepatic fat exceeds 5%, it corresponds to hepatic steatosis (Fang et al., 2018). The most direct cause of NAFLD is abnormal liver lipid metabolism, and a large quantity of free fatty acids and triglycerides that accumulate in liver cells (Xiaxia et al., 2019). The "second hit" is that reactive oxygen species (ROS) triggers an inflammatory cascade of liver parenchymal cells and fibrosis (Xiaxia et al., 2019). These effects include high levels of inflammatory cytokines, mitochondrial dysfunction, and oxidative stress. Necrotizing inflammation and fibrosis can progress and eventually lead to cirrhosis (Chi, 2017). However, the widely accepted theory is the "multiple-hit" pathogenesis (Ayonrinde et al., 2015). Changes due to the interaction of genetic and environmental factors, as well as the interactions between different organs and tissues, pancreas, gut, and liver, and broader metabolic dysfunction, are involved (Berardis and Sokal, 2014; Chi, 2017; Vlad et al., 2018). Moreover, scholars believe that environmental and genetic factors and the change in gut microbes in the induction of NAFLD in genetic predisposition, as well as intestinal flora changes lead to intestinal fatty acid, further activate the inflammatory pathways and release proinflammatory factors. Inflammatory cytokines increase liver inflammation and lipid accumulation, and the formation of gut-liver axis to a vicious cycle (Buzzetti et al., 2016; Xiaxia et al., 2019).

In recent years, the functional activity of key genes that synthesize proteins has been decisive in NAFLD. The PNPLA3 variant has been identified as the main genetic determinant of NAFLD. Variants with moderate effect sizes in TM6SF2, membrane bound O-acyltransferase domain containing 7 (MBOAT7), and glucokinase regulator (GCKR) were also shown to contribute significantly (Bellentani et al., 2004). PNPLA3, an enzyme that encodes I148M, is involved in the hydrolysis of triglycerides in adipocytes (Romeo et al., 2008). The lipid TM6SF2 is located in the endoplasmic reticulum and encodes E167K (rs58542926C/T), resulting in the loss of protein function, which in turn increases triglyceride deposition in the liver (Dongiovanni et al., 2015). Natural candidate genes are significantly involved in glucose and lipid metabolism during NAFLD development. Among the single nucleotide polymorphisms (SNPs) that lead to coding region mutations, such as PNPLA3 and TM6SF2, it is reasonable to infer that these defective proteins may be involved. For example, TM6SF2 mutants reduce liver production of very low-density lipoprotein (VLDL), thereby increasing the triglyceride (TG) content in the liver (Bonora et al., 2010).

Some studies have suggested that NAFLD progression follows the process of steatosis, lipotoxicity, and inflammation (Jou et al., 2008). The development of steatosis involves the interaction of many factors, such as dietary habits, gut flora, and genetic factors (Romeo et al., 2008; Jiang et al., 2015; Kirpich et al., 2015). Fat regenesis occurs through upregulation of adipogenic transcription factors, including sterol regulatory binding protein-1c (SREBP1c), carbohydrate-responsive element-binding protein (chREBP), and peroxisome proliferator-activated receptor gamma (PPAR-γ) (Anderson and Borlak, 2008). Fatty acids are mainly stored in the adipose tissue in the form of triacylglycerol. A previousstudy found that fatty acids in obese volunteers seemed to migrate from normal storage organs to the bone and liver tissue. Notably, FAT/CD36 (fatty acid translocation enzymes) promote fatty acid uptake by bone and liver tissues, which are significantly elevated in patients with obesity and NAFLD (Greco et al., 2008; Fabbrini et al., 2009). The accumulation of fat in the liver can lead to lipotoxicity and dysfunction of organelles, such as the mitochondria and endoplasmic reticulum (Browning and Horton, 2004; Bell et al., 2008). Steatosis further leads to the activation of IKKβ, which leads to increased signaling of the transcription factor nuclear factor kappa β (NF-κβ). Activation of NF-κβ induces the production of pro-inflammatory factors. These include tumour necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), and interleukin-1beta (IL-1β) levels. These inflammatory factors can promote aggregation and activation of resident hepatic macrophages to further promote NASH inflammation (Ramadori and Armbrust, 2001; Fabbrini et al., 2009).

Oxidative stress may play an important role in NAFLD progression, and under normal physiological conditions, mitochondrial oxidation is the main oxidation pathway of fatty acid deposition. When ROS are overproduced during fatty acid oxidation, hydrogen polyunsaturated fatty acids are extracted from the liver, resulting in mass production of

TABLE 1 Flavonoids from several different sources and their anti-NAFLD mechanisms.

Class	Source of plant	Example	Mechanisms of anti-NAFLD	References
Flavone	Leaves, fruits, trunks of Asteraceae, Labiatae plants	Luteolin	Sirt1-AMPK signal pathway/Restoration of intestinal mucosal barrier damage and microbiota imbalance/ Targeting the pro-inflammatory IL-1 and Il-18 pathways/ Abolish lipid accumulation induced by LXR-SREBP-1c activation	Zhu et al. (2020) Xia et al. (2021) Abu-Elsaad and El-Karef, (2019) (Yin et al., 2017)
		Apigenin	Regulating hepatocyte lipid metabolism and oxidative stress/ XO/NLRP3 pathways/PI3K/AKT-Dependent Activation/ PPARγ/PGC-1α-Nrf2 pathway	Zhang et al. (2018b) Fan et al. (2017) Lv et al. (2019) Feng et al. (2017)
		Baicalein	Inhibited DNL and improved glucose tolerance, oxidative stress, liver histology, and hepatokine secretion/Via maintaining V-ATPase assembly/Reduce hepatic fat accumulation and to ameliorate NAFLD-related biochemical abnormalities	Sun et al. (2020) Zhu et al. (2019) Xing et al. (2021)
Flavonones	Citrus, Fabaceae, Moraceae, Myrtaceae	Eriodictyol	Induced a persistent increase in autophagic flux	Lascala et al. (2018) Geng et al. (2020)
		Hesperetin	PI3K/AKT-Nrf2-ARE pathway/Induction of GRP78 in hepatocytes	Li et al. (2021)
		Naringenin	down-regulating the NLRP3/NF-κB pathway	Ke et al. (2015)
			Enhancing Energy Expenditure and Regulating Autophagy via AMPK decreases adipose tissue mass and attenuates ovariectomy-associated metabolic disturbances	Yang et al. (2021) Chen et al. (2019b)
Flavonol	Leaves of various plants	Quercetin/ Kaempferol	Ameliorating inflammation, oxidative stress, and lipid metabolism/Modulating intestinal microbiota imbalance and related gut-liver axis activation/IRE1a/XBP1s pathway	(Yin et al., 2017; Zhu et al., 2018) Yang et al. (2019)
		Galangin	Promoting Autophagy	Zhang et al. (2020)
		Myricetin	Regulating the expression of transcription factors of hepatic lipid metabolism, the antioxidant system, and pro-inflammatory cytokines	Choi et al. (2021)
			Modulating gut microbiota	Sun et al. (2021)
Isoflavone	Legumes	Daidzein	Direct regulation of hepatic *de novo* lipogenesis/Indirect control of adiposity and adipocytokines	Kim et al. (2011)
		Genistein	Directly targeted cyclooxygenase-1 activity as well as its downstream TXA2 biosynthesis/AMPK Activation	Zhong et al. (2017) Wang et al. (2018)
Anthocyanidin	Petals	Delphinidin	Induced endotoxemia and associated liver inflammation	Cremonini et al. (2022)
	Leaves Rhizomes	Malvidin	Nrf2/ARE Signaling Pathway/Hyperglycemia, insulin resistance, hyperlipidemia, and NAFLD in diabetic rats were alleviated	Zou et al. (2021) Xu et al. (2021)
Flavan-3OLS	Woody plants containing tannins	Catechin	GTE limitedly alters the hepatic metabolome/Reduce the contents of TG, TC, MDA, ALT and AST, increase the content of SOD	Gan et al. (2021) Sasaki et al. (2021)
		Galocatechin	Up-regulated mRNA and protein expressions of LPL, PPAR-α, CYP7A1 and CPT1, down-regulated PPAR-γ and C/EBP-α in liver of NAFLD mice	Liu et al. (2019)
		Theaflavin	Activating an AMPK Signaling Pathway by targeting Plasma Kallikrein/Anti-oxidant, anti-inflammatory, and anti-apoptotic mechanisms	Luo et al. (2012) Wenji Zhang et al. (2020)

malondialdehyde (MDA) (Esterbauer et al., 1991). MDA can spread from its original site to other cells both inside and outside the cell, causing damage (Esterbauer et al., 1991). Catalase and glutathione levels decrease when ROS levels are elevated, and oxidative stress is exacerbated (Hongming et al., 2018). Lipid peroxidation increases collagen synthesis and cell death, which promotes steatosis and fibrosis (Huang et al., 2018).

Fatty acid outflow from the diet increases, and new fat formation releases free fatty acids from adipose tissue, contributing to TG accumulation in the liver, although to varying degrees (Yeh and Brunt, 2014). However, TG accumulation in the liver itself is not pathological, and may be protective in some cases. Hepatic diacylglycerol acyltransferase 2 (DGAT2) inactivation catalyzes TG synthase and reduces hepatic

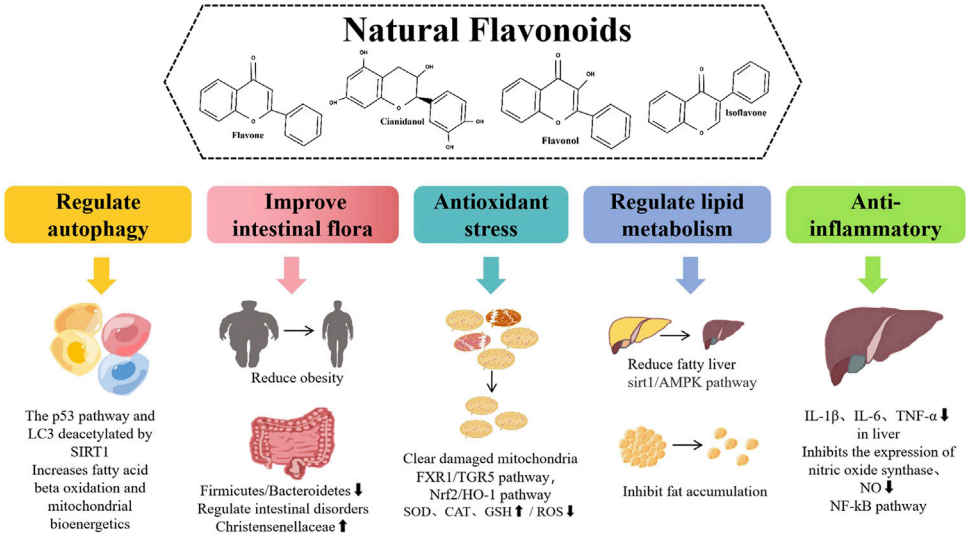

FIGURE 1
Different pharmacological effects and different mechanisms of natural flavonoids for alleviating NAFLD.

TG content but increases hepatitis and balloon-like changes (Brunt et al., 1999). This may seem paradoxical, but highlights the importance of liver fat in metabolic function. One possible mechanism for NASH-associated dysfunction involves a shift from minimal to substantial edema. This increase can be achieved by reducing the phosphatidylcholine (PC) levels (Machado et al., 2006) or lipid droplets coated with proteins (Soderberg et al., 2010; Angulo et al., 2013). Total PC levels were reduced in patients with both NAFLD and NASH (Ekstedt et al., 2006), which may be attributable to choline intake associated with NASH rather than choline deficiency (Richardson et al., 2007). In summary, NAFLD is a multifactorial disease with a complex pathogenesis. The prevention and treatment of NAFLD require further clinical and basic research.

Classification of flavonoids

Some studies have confirmed that flavonoid intake is inversely related to the risk of NAFLD (Mm et al., 2019). The mechanisms by which flavonoids exert anti-NAFLD effects are mainly through ameliorating inflammation, oxidative stress, and lipid metabolism, and regulating intestinal microbiota imbalance and the related gut liver axis. Flavonoids are natural polyphenol compounds that exist widely in all types of natural plants. Now, more than 9,000 kinds of flavonoids have been identified with a structure of a two phenolic hydroxyl benzene ring (A- and B-loops) interconnected through the central three carbon atoms. The basic parent nucleus is called a 2-phenylchromone (Tsuji et al.), biosynthesis from acetic acid and phenylalanine in plants (Weston and Mathesius, 2013). Flavonoids can be divided into flavonoids, flavonols, orange ketones, isoflavones, anthocyanins, chalcones, and dihydrogen derivatives according to the difference in the three-carbon atomic structure of the linked A and B rings, such as whether the ring is formed, oxidized, or replaced (Tsuchiya, 2010). The types of flavonoids from different sources and their anti-NAFLD mechanisms of action are listed in Table 1.

The main targets of flavonoids

Flavonoids have a variety of pharmacological effects, including antitumor, antioxidant, antibacterial, antiviral, anti-inflammatory, and analgesic effects (Maleki et al., 2019; Makunga, 2020). Interestingly, flavonoids have positive effects on various NAFLD pathways, such as regulating lipid metabolism, insulin resistance, inflammation, and oxidative stress (Wier et al., 2015). Based on the above advantages, finding new anti-NAFLD drugs derived from plant flavonoids is a hot topic in current research (Figure 1).

Improve the intestinal flora

Intestinal microbiota is involved in the pathogenesis of obesity, NAFLD, and metabolic syndrome (Abu-Shanab and Quigley, 2010). In NAFLD, changes in the gut microbiome and increased intestinal permeability lead to exposure of the liver to bacterial products from the gut, leading to chronic endotoxemia (Aron-Wisnewsky et al., 2013). Porras D found that quercetin could regulate intestinal microflora dysregulation

in high fat diet (HFD)-induced NAFLD mice and reverse HFD-induced inhibition of short-chain fatty acids (SCFAs) production and related intestinal barrier dysfunction (Yin et al., 2017). Some scholars have pointed out through animal experiments that the use of flavonols can make mice intestinal *Firmicutes*/*Bacteroidetes* (F/B) ratio significantly reduced (Li, 2018). The F/B ratio is an indicator of intestinal health, and lowering it can reduce the risk of diabetes and obesity (Vebø et al., 2016). This suggests that flavonol protection of the intestinal flora can be achieved by reducing the F/B ratio. In addition, flavonol protection of the intestinal flora can also improve intestinal barrier function by increasing the expression of butyric acid receptors and conjunction in the intestinal mucosa (Chen et al., 2019). Anthocyanins can be digested by various intestinal structures to form metabolites that are transmitted throughout the body and exert positive biological effects (Aedín and Anne-Marie, 2017). Some studies have confirmed the results of *in vitro* microbial experiments. Anthocyanins can increase the growth rate of probiotics, such as *Lactobacillus acidophilus*or *Bifidobacterium*, and inhibit the growth of harmful bacteria, such as *Staphylococcus aureus* and *Salmonella typhimurium* (Hanju et al., 2018). Lima et al. (2019) confirmed through experimental studies that long-term supplementation of hesperidin and citra can effectively protect intestinal flora because the number and reproduction rate of *Bifidobacteria* and *Lactobacillus* in the intestinal tract are regulated by their influence, thus increasing the content of SCFAs to protect intestinal flora. Researchers studied the effects of flavonoids on intestinal microbes and found that when the dosage reached a certain concentration, it could significantly inhibit the reproduction of *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus*, and *Bacillus* (Madheshwar and Perumal, 2017). Pure total flavonoids from citrus can regulate intestinal flora disorders, particularly Christensenellaceae, to attenuate NAFLD (He et al., 2021). Raw bowel tea polyphenols can reduce the level of *Firmicutes* in the feces of NAFLD mice, increase the minimum levels of *Bacteroidetes* and *Akkermansia*, and reduce the F/B ratio, acting as a regulator of the gut microbiome (Liu et al., 2019). Vine tea polyphenol reduced the F/B ratio and increased the relative abundance of *Akkermansia* in NAFLD mice (Xie et al., 2020).

Interactions between flavonoids and the microbiome contribute significantly to human health. The ability of flavonoids to regulate microbes also holds promise for dietary therapies that can be used to treat a variety of diseases associated with microbial disorders.

Regulate lipid metabolism

Quercetin is widely distributed in photosynthetic plants, such as cereals, vegetables, fruit, tea leaves, and Chinese medicinal materials, and is the most abundant foodborne natural flavonoid (Martinon et al., 2002). Yang et al. (2019) established Type 2 diabetes mellitus (T2DM)-induced NAFLD and quercetin treatment models *in vivo* and *in vitro*, and found that quercetin reduced serum transaminase levels and significantly reduced liver histological changes. Wang (2021) found that mice fed a high-fat diet exhibited severe fat accumulation in their livers, and a large number of red fat droplets appeared in their visual field. After total flavonoids of Broussonetia papyrifera (TFBP) treatment, the fat content in the liver cells of mice decreased significantly and finally reached the levels observed in normal liver. These results indicated that TFBP had the ability toreduce fat accumulation in hepatocytes. Chian-jiunliou *et al.* staining with the fluorescent dye BODIPY 493/503 showed that incubating HepG2 cells with oleic acid-induced lipid accumulation and licorice chalcone significantly inhibited the aggregation of lipid droplets and confirmed that licorice chalcone promoted the Sirtuin1/AMP-activated protein kinase (Sirt1/AMPK) pathway in the liver *in vivo* and *in vitro*. It effectively inhibited adipogenesis and increased lipid decomposition and fatty acid β-oxidation in NAFLD mice (Liou et al., 2019). Luteolin, lycopene, and their combinations indirectly activate the SIRT1/AMPK pathway *in vivo* and *in vitro*, which in turn inhibits lipogenesis and increases β-oxidation, defending against the “two-hit” in NAFLD (Zhu et al., 2020).

Antioxidant stress

Flavonoids may inhibit oxidative stress by regulating malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT). Wang (2021) found that total flavonoids from the leaves of *Broussonetia papyrifera* (TFBP) effectively inhibited the production of ROS, reduced the content of myeloperoxidase, improved the activity of SOD, and reduced injury to the body by oxidative stress. Western blot results showed that TFBP could regulate oxidative stress depending on the nuclear factor erythroid 2-related factor 2/heme oxygenase 1 (Nrf2/HO-1) signaling pathway, and promote Nrf2 entry into the nucleus of mouse liver cells and HO-1 production, thus improving the body’s ability to resist oxidative stress. Other researchers have concluded that theaflavins significantly reduce ROS production in steatotic hepatocytes and TNF-α production in LPS-stimulated RAW264.7 cells (Luo et al., 2012).

Cyanidin-3-O-glucoside is the most abundant anthocyanidin in the flavonoid family. Li *et al.* found that centaulin-3-O-glucoside eliminated damaged mitochondria to maintain mitochondrial homeostasis and alleviate oxidative stress (Yin et al., 2017). These results suggest that cybernin-3-O-glucoside alleviates NAFLD by activating PTEN-induced kinase 1 (PINK1)-mediated mitochondrial phagocytosis. In a NASH cell model, the levels of MDA and ROS were significantly increased significantly, while the levels of SOD, CAT, and GSH were significantly decreased. After stimulation

with different concentrations of alpha-naphthoflavone (ANF), the level of SOD in the cells was decreased, but the level of SOD was significantly increased. Furthermore, MDA and ROS levels in the liver tissues of HFD-fed mice with different concentrations of ANF were significantly lower than those inthe model group (Xia et al., 2019). Yang et al. (2019) found that quercetin restored the levels of superoxide dismutase, catalase, and glutathione in the liver of NAFLD mice. By activating the farnesoid X receptor 1 (FXR1)/TGR5 signaling pathway, quercetin eliminated lipid droplets and restored total cholesterol and triglyceride levels in HepG2 cells co-cultured with high D-glucose and free fatty acids. Wang et al. (2021) found that hyperoside can regulate bile acids (BAs) in the liver, reduce unconjugated BAs, and increase liver-conjugated BA levels. The expression of FXR in the liver is increased, leading to the promotion of free fatty acid β-oxidation.

Regulate autophagy

Autophagy is a conserved self-digestion process that brings unnecessary or potentially dangerous cytoplasmic materials, such as damaged organelles and misfolded or unfolded proteins, to lysosomes for degradation. Lipid oxidation mainly occurs in the mitochondria, and oxidative stress produces a large amount of ROS, which leads to mitochondrial dysfunction and may inhibit autophagy because autophagy is generated in the mitochondria (Tang et al., 2017). Studies have shown that epigallocatechin-3-gallate (a flavonoid 3-alcohol phenolic compound) can increase the proliferation and autophagy of the liver in HFD-fed mice but reduce apoptosis. This may alleviate HFD-induced NAFLD by inhibiting apoptosis and promoting autophagy (Wu et al., 2021). Galangin is a flavonol and a curcumin derivative. Recent studies confirmed that galangin induces autophagy. Previous studies have reported that galangin mediates autophagy through the p53 pathway, and SIRT1 deacetylates LC3 in HepG2 cells (Zhang et al., 2021). Similarly, apigenin has been found to improve liver lipid deposition by activating mitochondrial autophagyto increase fatty acid β-oxidation and mitochondrial bioenergetics (Hsu et al., 2021).

Anti-inflammatory effect

Oxidative stress-mediated inflammatory responses are an important pathological mechanism of NAFLD. When the level of oxidative stress increases, it can promote IL-6, IL-1β, and TNF-α expressionand induce liver injury (Xiao et al., 2018). The anti-inflammatory effect of flavonoids occurs mainly through the inhibition of the NF-κβ pathway (González et al., 2011). Flavonoids inhibit the phosphorylation of inhibitor of nuclear factor kappaB (IKB) and the inhibitor of nuclear factor kappaB kinase (IKK) complex (Kim et al., 2005) and the activity of regulatory enzymes, such asphospholipid oxygenase and protein tyrosine kinase (Manthey, 2009). Wang *et al.* found that the levels of IL-1β, IL-6, and TNF-α in the liver tissue of rats in the NAFLD model group were significantly increased, and total flavonoids of *Scutellaria baicalensis* could reduce these inflammatory factors, suggesting that total flavonoids in *Scutellaria baicalensis* could reduce the inflammatory response in the liver of rats in the NAFLD model group (Mengmeng et al., 2022). NO leads to highly destructive formation of peroxynitrite under oxidative stress conditions. Flavonoids inhibit inducible nitric oxide synthase (iNOS) expression and NO production (González-Gallego et al., 2010). In addition, flavonoids prevent the degeneration of the anti-inflammatory effects of the glucocorticoid cortisol. Oxidative stress worsens the anti-inflammatory effects of cortisol by eliminating these effects and creating cortisol resistance (Ruijters et al., 2014). Luteolin can significantly reduce a variety of inflammatory factors in NAFLD rats, which indicates that, in addition to its antioxidant effect, luteolin has also a very good anti-inflammatory effect (Abu-Elsaad and El-Karef, 2019). This suggests that NAFLD progression is often accompanied by inflammation and oxidative stress.

Summary and prospect

The incidence of NAFLD increases each year, similar to clinical stress. Currently, NAFLD has an estimated annual medical and social cost of $292 billion (Younossi et al., 2016a). The different manifestations of NAFLD complicate the diagnosis, which ignores the true condition. The medical system is facing a severe challenge incombating this growing liver disease. Flavonoids have been proven to have very strong pharmacological activity and have excellent alleviating effects on NAFLD and NASH. Flavonoids may ameliorate NAFLD by regulating lipid metabolism, intestinal flora, and autophagy. Therefore, natural flavonoids have huge potential for the clinical development of NAFLD drugs in the future.

Author contributions

PT, LJ, XQ, and BH participated in drafting the manuscript. All of the authors read and approved the final manuscript. Author XQ contributed equally to this work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Glosarry

ALT alanine aminotransferase

AMPK AMP-activated protein kinase

ANF alpha-naphthoflavone

ARE antioxidant response element

AST aspartate aminotransferase

BP broussonetiapyrifera

C/EBP-α CCAAT/enhancer binding protein alpha

ChREBP carbohydrate-responsive element-binding protein

CPT1 carnitine palmitoyltransferase 1A

CYP7A1 cytochrome P450 7A1

DGAT2 diacylglycerol acyltransferase 2

DNL lysosomal DNA-ase

E167K residue 167

FA fatty acids

FXR1 farnesoid X receptor 1

GCKR glucokinase regulator

HO-1heme hemeoxygenase 1

IKB inhibitor of nuclear factor kappaB

IKK inhibitor of nuclear factor kappaB kinase

IKKβ inhibitor of nuclear factor kappaB kinase beta

IL-1β interleukin-1β

IL-6 interleukin 6

iNOS inducible nitric oxide synthase

IRE1α inositol-requiring enzyme 1 Alpha

LPL lipoProtein lipase

MBOAT7 membrane bound o-acyltransferase domain-containing 7

MDA malondialdehyde

NAFLD non-alcoholic fatty liver disease

NASH non-alcoholic steatohepatitis

NASH-HCC non-alcoholic steatohepatitis-hepatocellular carcinoma

NF-κβ nuclear factor kappa β

NLRP3 nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain-containing 3

Nrf2 nuclear factor erythroid 2-related factor 2

PC phosphatidylcholine

PGC-1α peroxisome proliferator-activated receptor-gamma coactivator 1 alpha

PI3K phosphatidylinositol-3-kinase

PINK1 putative kinase 1-mediated

PNPLA3 patatin-like phospholipase domain containing 3

PPAR-α peroxisome proliferator-activated receptor alpha

PPAR-γ peroxisome proliferator-activated receptor gamma

ROS reactive oxygen species

Sirt1 sirtuin 1

SOD speroxide Dismutase

SREBP1c sterol regulatory binding protein-1c

T2DM type 2 diabetes mellitus

TAG triacylglycerol

TC total cholesterol

TFBP total flavonoids of broussonetia papyrifera

TG triglyceride

TM6SF2 transmembrane 6 superfamily member 2

TNF-α tumor necrosis factor-α

TXA2 thromboxane A2

V-ATPase vacuolar proton ATPase

VLDL very low density lipoprotein

XBP1s X-box binding protein 1 spliced

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Yongsheng Chen,
Jinan University, China

REVIEWED BY
Yao Wang,
University of California, San Francisco, United States
Liang Zeng,
Southwest University, China

*CORRESPONDENCE
Qingwei Li,
dsliqingwei@163.com
Linhua Zhao,
melonzhao@163.com

†These authors have contributed equally to this work

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Herbal tea, a novel adjuvant therapy for treating type 2 diabetes mellitus: A review

Xiangyuan Zhang[1,2†], Lili Zhang[1†], Boxun Zhang[1†], Ke Liu[1], Jun Sun[3], Qingwei Li[1*] and Linhua Zhao[1*]

[1]Department of Institute of Metabolic Diseases, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2]Graduate College, Beijing University of Traditional Chinese Medicine, Beijing, China, [3]Graduate College, Changchun University of Traditional Chinese Medicine, Jilin, China

Type 2 diabetes mellitus (T2DM) is a metabolic, endocrine disease characterized by persistent hyperglycemia. Several studies have shown that herbal tea improves glucose metabolism disorders in patients with T2DM. This study summarizes the published randomized controlled trials (RCTs) on herbal tea as a adjuvant therapy for treating T2DM and found that herbal teas have potential add-on effects in lowering blood glucose levels. In addition, we discussed the polyphenol contents in common herbal teas and their possible adverse effects. To better guide the application of herbal teas, we further summarized the hypoglycemic mechanisms of common herbal teas, which mainly involve: 1) improving insulin resistance, 2) protecting islet β-cells, 3) anti-inflammation and anti-oxidation, 4) inhibition of glucose absorption, and 5) suppression of gluconeogenesis. In conclusion, herbal tea, as a novel adjuvant therapy for treating T2DM, has the potential for further in-depth research and product development.

KEYWORDS

herbal tea, type 2 diabetes, randomized controlled trial, review, mechanism

1 Introduction

Diabetes mellitus (DM) poses a major threat to global health. According to the latest report of International Diabetes, by 2045, approximately 783 million people worldwide will suffer from DM, of which more than 90% will have type 2 diabetes mellitus (T2DM) (Zheng et al., 2018a; International Diabetes Federation, 2021). In the early stages of T2DM, only a simple increase in blood sugar levels was observed. As the disease progresses, it can lead to serious complications such as diabetic retinopathy, diabetic kidney disease, and diabetic foot ulcers, which can cause great pain and a heavy economic burden to the patient (Harding et al., 2019). Clinical trials have shown that improved blood glucose control can slow the progression of T2DM and its complications (Taylor et al., 2021). Researchers have developed insulin, metformin, and other drugs that function through different mechanisms to lower blood sugar levels. However, patients with T2DM experience difficulties controlling their blood glucose levels, such as high blood glucose fluctuations,

susceptibility to hypoglycemia, and a high incidence of adverse gastrointestinal reactions (Wilson and Castle, 2020; Guo et al., 2021). Therefore, it is necessary to develop a safe and effective auxiliary treatment for T2DM.

For thousands of years, herbs have been widely used to prevent and treat various diseases owing to their remarkable efficacy and minor side effects (Jiang et al., 2013; Huang et al., 2018a; Jeon et al., 2019). With the popularization of tea drinking and the gradual accumulation of medical practice experience, some herbs with remarkable curative effects are processed and directly brewed for drinking, forming a new dosage form called herbal tea (HT). HT is a combination of herbs and traditional teas. Generally, HT is defined as water infusions/decoctions prepared with herbal ingredients other than *Camellia sinensis* (L.) Kuntze (Fu et al., 2018). It is prepared from different herbs' seeds, fruits, flowers, leaves, stems, and roots. HT is widely used as a traditional medical treatment for T2DM. In the Tang Dynasty of China, doctors created an HT called "Xi Thirst Tea" to improve symptoms such as urine sugar, polydipsia, and polyphagia. In South Africa, Rooibos Herbal Tea and Honeybush Herbal tea are considered attractive strategies for managing T2DM and have been successfully commercialized (Ajuwon et al., 2018). While some clinical trials have confirmed the beneficial effects of HTs in controlling blood glucose levels in recent years, the quality of these studies remains unclear. In addition, the intake and habits of HT consumption vary among individuals, and the bioavailability of compounds in HTs, such as polyphenols, polysaccharides, and alkaloids, is susceptible to various chemical and biological factors. Therefore, a more comprehensive understanding of HTs' hypoglycemic mechanisms should help prevent diabetes and its related complications. While reviews on the mechanisms by which HTs or herbs improve blood glucose levels have been published in recent years, these reviews either fail to include HTs commonly used worldwide or comprehensively discuss the hypoglycemic mechanisms of important herbs in HTs, or fail to summarize the latest findings. To address these limitations, we compiled the evidence and level of evidence for the clinical efficacy of HTs in treating T2DM and provided a comprehensive discussion of the glucose-lowering mechanisms of HTs and their bioavailability.

2 Clinical research

We searched electronic databases including PubMed, Cochrane Library, Embase, WOS, CNKI, CBM, CSPD, and VIP for variants of the terms "herbal tea," "herbal medicine," and "beverage," "type 2 diabetes mellitus," or "non-insulin-dependent diabetes." In addition, clinical trials were searched to identify relevant unpublished data. We included clinical studies that met the following criteria: study participants were diagnosed with T2DM and randomly assigned to receive HTs or placebo treatment. We also evaluated the quality of the RCTs according to the modified Jadad rating scale (Table 1).

There are many types of HTs. Based on the number of medicinal herbs, we divided them into two categories. The first category consists of a single herb. HTs that use one herb as the main ingredient to lower blood sugar levels and are supplemented with other herbs fall into this category. The second category consists of two or more herbs. In this category, it is impossible to determine which herb is the main ingredient responsible for lowering sugar levels. In addition, we summarized the composition, dose, and mode of administration of HTs (Tables 2 and 3).

2.1 Herbal teas consisting of a single herb as the main hypoglycemic effect

2.1.1 Mulberry leaf tea

Mulberry leaf (ML) tea consists of dried leaves of *Morus alba* L. and is popular in East Asian countries, such as China and Japan. In traditional Chinese medicine, mulberry leaves are an essential herb for treating diabetes. A 3-months RCT showed that combining ML tea (6 g/d) with metformin could decrease the blood glucose indices; however, there was no significant difference between the two groups (Xie et al., 2020). In comparison, other RCTs using a daily dose of 10 g and 6 months of intervention showed that ML tea could produce more obvious benefits in improving fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), and 2-h postprandial blood glucose (2hPBG) values in patients with T2DM and pre-diabetes, and the incidence of adverse reactions in the intervention group (6.00%) was lower than that in the control group (8.00%) (Yang and Fang, 2019).

2.1.2 Chamomile tea

Chamomile tea is made from dried flowers of *Chamaemelum nobile* L. It is one of the most widely used HTs worldwide, originating from Europe and West Asia (Khan et al., 2014). In a 4-weeks clinical trial, T2DM patients were administered chamomile tea (10 g/100 ml) twice daily before meals. At the end of the experiment, FPG and 2hPBG of T2DM patients were significantly reduced (Bracesco et al., 2011). In another 8-weeks clinical trial, T2DM patients were treated with chamomile tea (3 g/150 ml) after three daily meals. Chamomile tea not only significantly reduced HbA1c concentration, serum insulin level, and homeostatic model evaluation of insulin resistance in T2DM patients but also significantly increased total antioxidant capacity and the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in the patients. Therefore, the hypoglycemic effect of chamomile tea may be associated with an improvement in oxidative stress (Zemestani et al., 2016). In addition, the results of both studies suggest that short- or long-term use of chamomile tea can improve blood sugar levels in patients with diabetes. However, the time taken for

TABLE 1 Effectiveness of HTs in the management of T2DM.

Trials	Herbal Tea name	No of patients		Sex(male/female)		Age(years)		Treatment		Treament time		
		text group	Control group	text group	Control group	text group	Control group	text group	Control group			
Xie et al. (2020)	Mulberry leaf tea	40	40	26/14	24/16	53.88 ± 14.31	54.08 ± 12.74	Conventional treatment + Metformin sustained-release tablets 0.5 g/Bid + Mulberry tea	Conventional treatment + Metformin sustained-release tablets 0.5 g/Bid	3 months	12 weeks	
Yang et al., (2019)	Mulberry leaf tea	50	50	24/26	27/23	43.20 ± 10.72	43.58 ± 10.12	Conventional treatment + Mulberry leaf Black Tea	Conventional treatment	6 months	24 weeks	
Rafraf M et al., (2015)	Chamomile tea	32	32	6/26	6/26	50.19 ± 7.08	51.97 ± 6.42	Chamomile Tea	Warm water	2 months	8 weeks	
Kaseb et al., (2018)	Chamomile tea	25	25	-	-	55.33 ± 7.85	55.22 ± 6.72	Chamomile Tea	Conventional treatment	1 month	4 weeks	
Huyen et al., (2011)	Gynostemma tea	12	12	8/4	9/3	63.5 ± 6.5	57.2 ± 8.2	Gynostemma pentaphyllum Tea	Placebo	3 months	12 weeks	
Klein et al., (2011)	Yerba mate tea	11	9	1/10	4/5	54.3 ± 6.9	60 ± 6.7	Mate tea	Dietary intervention	2 months	60 days	
Jayawardena et al. (2005)	Kothala himbutu tea	28	23	16/12	12/11	53.2 ± 7.5	54.3 ± 6.9	Kothala Himbutu Tea	placebo	3 months	12 weeks	
Jia et al. (2002)	Puda tea	38	32	12/26	9/23	53.77 ± 9.18	58.97 ± 9.07	Puda Tea	Placebo	1 month	4 weeks	
CampbellTofte et al. (2011)	Rauvolfia-Citrus tea	12	11	6/5	7/5	63.4 ± 8.8	63.9 ± 5.2	Rauvolfia-Citrus Tea	Placebo	4 months	16 weeks	
Yue et al., 2018	Tuckwheat yuzhu tea	53	52	-	-	76.63 ± 7.01	76.63 ± 7.01	Conventional treatment + Tartary Buckwheat Polygonatum Tea	Conventional treatment	6 months	24 weeks	
Liu et al., (2018)	Mulberry black tea	20	20	-	-	-	-	Metformin tablets +Compound Danshen Dripping Pills +Mulberry Black Tea	Metformin tablets +Compound Danshen Dripping Pills	3 months	12 weeks	
Fang (2001)	Shuning tea	18	15	8/10	6/9	58.3	57.4	Metformin tablets + Shu Ning Tea	Metformin tablets	1 month	4 weeks	
Han et al., (2015)	Zhongyue jiangsangao tea	30	30	17/13	16/14	44.8 ± 8.4	45.1 ± 7.9	Metformin tablets + Yuejiang Sangao Herbal Tea	Metformin tablets	3 months	12 weeks	
Hu et al., (2018)	Tianhan diabetes tea	52	52	13/39	15/37	56.71 ± 7.52	54.20 ± 6.00	Tianhan diabetes Tea	Blank control	2 months	8 weeks	
Mahmoud F et al., 2016	Diabetes tea	30	20	-	-	51.5	54.2	Diabete Tea extract	Placebo	3 months	12 weeks	
Wen et al., (2018)	Self-developed herbal tea	50	50	12/38	16/34	67.26 ± 7.10	65.81 ± 6.58	Acupoint self-massage + Herbal Tea	Acupoint self-massage	6 months	24 weeks	
Xie et al. (2020)	Mulberry leaf tea	6.74 ± 1.22	6.93 ± 1.60	6.86 ± 0.96	6.87 ± 1.11	9.49 ± 3.06	9.79 ± 2.95	7.83 ± 3.74	9.76 ± 3.72	2.45 ± 1.43	3.19 ± 1.66	3
Yang et al., (2019)	Mulberry leaf tea	5.73 ± 1.15	6.52 ± 1.34	6.05 ± 0.67	6.31 ± 0.58	7.29 ± 1.16	8.96 ± 1.25	-	-	-	-	4

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TABLE 1 (*Continued*) Effectiveness of HTs in the management of T2DM.

Trials	Herbal Tea name	No of patients		Sex(male/female)		Age(years)		Treatment		Treament time		
		text group	Control group	text group	Control group	text group	Control group	text group	Control group			
Rafraf M et al., (2015)	Chamomile tea	8.89 ± 3.7	8.74 ± 1.56	7.48 ± 1.59	7.50 ± 0.92	-	-	-	-	4.24 ± 1.95	5.55 ± 1.12	4
Kaseb et al., (2018)	Chamomile tea	8.16 ± 0.37	9.05 ± 0.37	-	-	12.35 ± 0.61	13.20 ± 0.61	-	-	-	-	4
Huyen et al., (2011)	Gynostemma tea	7.0 ± 1.4	8.7 ± 2.2	7.4 ± 1.0	8.1 ± 1.3	-	-	-	-	4.82 ± 2.64	6.89 ± 3.8	7
Klein et al., (2011)	Yerba mate tea	-	-	-	-	-	-	-	-	-	-	2
Jayawardena et al. (2005)	Kothala himbutu tea	-	-	6.29 ± 1.02	6.65 ± 1.04	-	-	-	-	-	-	6
Jia et al. (2002)	Puda tea	9.16 ± 3.66	12.14 ± 3.23	-	-	14.4 ± 13.41	20.01 ± 4.52	8.70 ± 5.09	8.50 ± 4.60	-	-	3
CampbellTofte et al. (2011)	Rauvolfia-Citrus tea	7.7 ± 2.2	8.1 ± 2.1	6.1 ± 1.2	6.7 ± 1.1	14.5 ± 5.5	15.8 ± 3.8	11 ± 4	7 ± 3	-	-	7
Yue et al., 2018	Tuckwheat yuzhu tea	7.71 ± 2.01	8.52 ± 2.32	8.12 ± 1.71	8.63 ± 1.62	-	-	-	-	-	-	4
Liu et al., (2018)	Mulberry black tea	6.47 ± 1.09	7.31 ± 1.42	6.01 ± 0.58	6.42 ± 0.65	8.21 ± 1.34	9.13 ± 1.50	-	-	-	-	3
Fang 2001	Shuning tea	8.0 ± 1.7	9.5 ± 1.9	-	-	9.3 ± 2.6	10.1 ± 2.3	-	-	-	-	2
Han et al., (2015)	Zhongyue jiangsangao tea	7.2 ± 2.3	9.8 ± 2.9	-	-	9.8 ± 2.1	12.4 ± 2.7	7.2 ± 1.4	9.2 ± 1.4	3.2 ± 1.0	4.3 ± 1.5	1
Hu et al., (2018)	Tianhan diabetes tea	6.38 ± 2.25	8.12 ± 3.25	6.21 ± 0.93	6.08 ± 1.14	9.96 ± 2.55	11.25 ± 3.08	-	-	-	-	1
Mahmoud F et al., 2016	Diabetes tea	8.7 ± 0.38	8.12 ± 57	8.26 ± 0.21	7.70 ± 0.37	-	-	-	-	-	-	3
Wen et al., (2018)	Self-developed herbal tea	7.12 ± 0.35	7.02 ± 1.02	6.12 ± 0.35	7.02 ± 1.02	10.12 ± 0.35	11.02 ± 1.02	-	-	-	-	1

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chamomile tea consumption should not be limited to before or after meals.

2.1.3 Gynostemma tea

Gynostemma tea is made from dried leaves of *Gynostemma pentaphyllum* (Thunb.) Makino. Gynostemma tea has been regarded as an important health medicine in Asian countries, such as China. Based on its remarkable hypoglycemic and antihypertensive properties, GP was also listed as the first batch of "precious traditional Chinese medicine" developed by the "Spark Plan" developed by China's Ministry of Science and Technology (Su et al., 2021). In an RCT, Gynostemma tea improved blood sugar levels in patients with T2DM. All T2DM patients were randomly assigned to either Gynostemma tea or placebo tea (6 g/day) groups. After 12 weeks of treatment, the FPG level and homeostatic model assessment of insulin resistance (HOMA-IR) were significantly lower in the treatment group than in the control group (Huyen et al., 2010). This study is the first to confirm that Gynostemma tea has a significant hypoglycemic effect and can improve insulin sensitivity. In addition, in this study, Gynostemma tea had no adverse effects, such as liver and renal toxicity. Therefore, it is considered a safe HT.

2.1.4 Yerba mate tea

Yerba mate (YM) tea is made from dried leaves of *Ilex paraguariensis* A. St.-Hil. It is a widely consumed HT in southern Latin American countries, including southern Brazil, Argentina, Paraguay, and Uruguay. In addition, it is rapidly seeping into the global market, including the United States (Heck and de Mejia, 2007). The latest RCT compared the differences between HT and dietary interventions in improving blood sugar levels. A specific measure of dietary intervention is to reduce calorie intake. In this study, all patients were randomly divided into three groups: YM tea, diet intervention, and YM tea plus diet intervention. Specifically, each person had to drink YM tea (330 ml) three times a day and/or receive dietary nutrition counseling for more than 60 days. At the end of treatment, compared with baseline values, only FPG and HbA1c levels in the experimental group decreased significantly (Klein et al., 2011). Therefore, YM can be considered a dietary alternative to control T2DM and pre-diabetes blood sugar levels.

2.1.5 Kothala himbutu tea

Kothala himbutu (KH) tea is a mixture of herbs, and the main ingredients are the root and stem of *Salacia renetulata* Wight (Kishino et al., 2009). In traditional Ayurvedic medicine in India and Sri Lanka, KH is considered a specific treatment for early diabetes (Im et al., 2009). An RCT showed that KH tea significantly reduced HbA1C levels in patients in the intervention group. Furthermore, there were no serious adverse reactions or abnormalities in liver or kidney function (Jayawardena et al., 2005). Therefore, KH tea can be considered effective and safe for reducing blood sugar levels.

2.1.6 Puda tea

Puda tea contains the fruit of *Momordica charantia* L., which is the main ingredient. It is also supplemented with other medicinal plants and traditional tea leaves. Bitter gourd is one of the most widely studied traditional medical drugs (Ooi et al., 2012). In traditional Chinese medicine, Ayurvedic and other traditional medicines are often used in diabetes management. The results of an RCT showed that Puda tea could significantly reduce FPG in patients with T2DM and effectively improve impaired glucose tolerance (Jia et al., 2002). In addition, it can improve symptoms such as polydipsia and polyphagia.

2.2 Herbal teas that contain various herbs with a hypoglycemic role

2.2.1 *Rauvolfia-Citrus* tea

Rauvolfia-Citrus tea is made with the foliage of *Rauvolfia vomitoria* Wennberg and the fruits of *Citrus aurantium* L. In northern Nigeria, it is believed that taking this HT while giving up alcohol and following a calorie-restricted diet can improve diabetes. In a 4-months RCT, compared with the baseline values before treatment, treatment with RC tea reduced HbA1b and FPG in T2DM patients by 6% and 10%, respectively. In addition, no adverse reactions occurred in any patient (Campbell-Tofte et al., 2011).

2.2.2 Tuckwheat yuzhu tea

Tuckwheat yuzhu tea is composed of *Fagopyrum tataricum* (L.) Gaertn. seeds and dried rhizomes of *Polygonatum odoratum* (Mill.) Druce. It is commonly used as a hypoglycemic drug in Traditional Chinese Medicine (TCM). In an RCT, the FPG level decreased significantly in the intervention group of middle-aged T2DM patients; however, the HbA1c level did not change significantly. This may be due to the short observation period (Yue et al., 2018).

2.2.3 Mulberry leaf black tea

With the gradual confirmation of the hypoglycemic function of mulberry leaves, various HTs containing mulberry leaves have been developed and used. In China, many people know HTs, such as Mulberry leaf black tea, Mulberry leaf Sophora japonica tea, and Mulberry leaf oolong tea. However, the hypoglycemic effect of these HTs has rarely been confirmed in clinical studies. Currently, only one RCT has confirmed the hypoglycemic effect of mulberry leaf black tea in T2DM patients. However, after 12 weeks of treatment, FPG, HbA1C, and OGTT2h levels decreased more in the

TABLE 2 Herbs in HTs for the treatment of T2DM.

	Herbal tea name	Composition	Parts
Single Herbal Tea	Mulberry leaf tea	Morus alba L.	leaf
	Chamomile tea	*Chamaemelum nobile L.*	flower
	Gynostemma pentaphyllum tea	Gynostemma pentaphyllum (Thunb.) Makino.	leaf
	Yerba mate tea	Ilex paraguariensis A.St.-Hil.	leaf
	Kothala himbutu tea	Salacia renetulata Wight.	root and stem
	Puda tea	Momordica charantia L.	fruit
Compound Herbal Tea	Rauvolfia-Citrus tea	Rauvolfia vomitoria Wennberg	foliage
		Citrus × aurantium L.	fruit
	Tuckwheat yuzhu tea	Fagopyrum tataricum (L.) Gaertn.	seed
		Polygonatum odoratum (Mill.) Druce.	rhizome
	Mulberry leaf black tea	Morus alba L.	leaf
		Camellia sinensis (L.) Kuntze	leaf and bud
	Shunning tea	Morus alba L.	leaf
		Other composition unknown	-
	Zhongyue Jiangsangao herbal tea	Crataegus pinnatifida Bunge	fruit
		Cassia obtusifolia L.	seed
		Sophora japonica L.	flower and alabastrum
		Lycium barbarum L.	fruit
		Polygonatum odoratum (Mill.) Druce.	rhizome
		Pueraria lobata (Willd.) Ohwi	root
		Ziziphus jujuba Mill. var. spinosa (Bunge) Hu ex H. F. Chou	seed
		Dioscorea opposita Thunb.	rhizome
		Prunus mume (Sieb.) Sieb.et Zucc.	fruit
		Glycyrrhiza uralensis Fisch.	root and rhizome
	Tianhan Xiaoke tea	*Astragalus* membranaceus (Fisch.) Bge.	root
		Cornus officinalis Sieb. et Zucc.	fruit
		Ophiopogon japonicus (Thunb.) Ker Gawl.	rhizome
		Rehmannia glutinosa (Gaertn.) DC.	rhizome
		Dioscorea opposita Thunb.	rhizome
		Poria cocos (Schw.) Wolf	sclerotium
	Diabetea tea	*Cassia* tora L.	leaf and seed
		Ficus racemosa L.	bark and fruit
		Syzygium cumini (L.) Skeels	bark
		Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	bark
		Terminalia chebula Retz.	fruit
		Terminalia bellirica (Gaertn.) Roxb.	fruit
		Phyllanthus emblica L.	fruit
		Tribulus terrestris L.	fruit
		Trigonella foenum- graecum L.	seed
		Cardiospermum halicacabum L.	leaf
		Cinnamomum zeylanicum Blume	bark
		Camellia sinensis (L.) Kuntze	leaf
		Azadirachta indica A.Juss.	leafs and seed
		Ficus benghalensis L.	bark and fruit
	Self-developed herbal tea	Morus alba L.	leaf
		Crataegus pinnatifida f. major (N.E.Br.) W.Lee	fruit
		Nelumbo nucifera Gaertn.	leaf
		Camellia sinensis (L.) Kuntze	leaf and bud

TABLE 3 Dosage and usage of HTs.

Trials	Herbal tea	Single dose (g)/ time	Water for making herbal tea	Brewing time	Daily times	Total dosage (g)/day	Treament time	Others
Xie et al. (2020)	Mulberry leaf tea	6	Hot water		2	12	3 months	
Yang et al., (2019)	Chamomile tea	5	Hot water (85–95°F)	-	2	10	6 months	-
Rafraf M et al., (2015)	Chamomile tea	10	100 ml Boiling water	10 min	2	20	2 months	Before lunch and dinner
Kaseb et al., (2018)	Gynostemma tea	9	150 ml Hot water	10 min	3	27	1 month	Drink immediately after meals
Huyen et al., (2011)	Mate tea	3	-	-	2	6	3 months	-
Klein et al., (2011)	Kothala himbutu tea	6.6	330 ml Boiling water	10 min	3	19.8	2 months	Before or during meals
Jayawardena et al. (2005)	Puda tea	-	-	-	3	-	3 months	During meals
Jia et al. (2002)	Rauvolfia-Citrus tea	-	-	-	-	4	1 month	Daily way of drinking tea
Yue et al. (2018)	Tuckwheat yuzhu tea	8	300 ml Hot water		2	-	6 months	After meals
Liu et al., (2018)		30	500 ml Water		at least 3	-	3 months	-
Fang 2001	Shuning tea	-	-	-	3	-	1 month	-
Han et al., (2015)	Zhongyue jiangsangao tea	5–10	Boiling water	Until the tea broth is tasteless		-	3 months	-
Hu et al., (2018)	Tianhan diabetes tea	1.8	-	-	2	-	2 months	-
Mahmoud F et al., 2016	Diabetes tea	2.72	250 ml Boiling water	5 min	at least 4	-	3 months	-
Wen et al., (2018)	Self-developed herbal tea	60	Boiling water			-	6 months	

treatment group than in the control group (Liu Zhan-Liang et al., 2018).

2.2.4 Shuning tea

Shuning tea, native to China, is a mixture of various herbs. Mulberry leaves are the main ingredients. In a 1-month RCT, the improvement in blood glucose levels was better in the Shu Ning tea combined with the metformin group than in the metformin-only group (Fang, 2001).

2.2.5 Zhongyue Jiangsangao tea

Zhongyue Jiangsangao tea consists of 10 kinds of Chinese medicines including hawthorn. After 3 months of treatment with this HT, Han found that FPG, 2hPBG, fasting insulin, and HOMA-IR of patients with T2DM diagnosed for the first time were significantly lower than those in the control group (Chao-yang, 2015).

2.2.6 Tianhan Xiaoke tea

Tianhan Xiaoke (THXK) tea contains various traditional Chinese medicines and green tea, developed by the Shaanxi Tea Research Institute in China. An RCT showed that after taking THXK tea for 2 months, the decreased range of FPG and HbA1C in the observation group was significantly different from that in the control group (Hu et al., 2018).

2.2.7 Diabetes tea

The main ingredient in Diabetes tea is black tea derived from *Camellia sinensis* (L.) Kuntze. It is supplemented by 12 other medicinal plants. Patients were administered three cups (600 ml) of diabetes tea extract or placebo extract daily for 12 weeks. At the end of the study period, a significant decrease in HbA1c levels was observed in the diabetes tea group. In addition, a study also showed that diabetes tea has anti-inflammatory and anti-hyperlipidemic effects (Mahmoud et al., 2016).

2.2.8 Self-made Chinese herbal tea

Wen et al. developed an HT consisting of 15 g of mulberry leaves, 15 g of hawthorn, 15 g of lotus leaves, and 15 g of green tea. In a 6-months RCT, the control group used acupressure intervention, and the experimental group used HT. At the end of the experiment, the decrease in HbA1c, FPG, and oral glucose tolerance test (OGTT) levels were significantly greater in the

experimental group than in the control group (Wen and Xuejun, 2018).

In conclusion, 16 RCTs confirmed the benefits of HTs in improving blood sugar in T2DM patients (Table 1). Among them, six kinds of medicinal tea, including mulberry leaf tea, produce the main hypoglycemic effect as a single herbal medicine. In addition, these seven kinds of HTs, including Rauvolfia-Citrus tea, play a role in lowering blood sugar levels through various herbs. We have listed HTs' doses and administration methods in Table 3 to improve their clinical use further. RC tea was prepared by boiling Rauvolfia vomitoria foliage and Citrus aurantium fruits (400 g: 2 kg for 10 L). It was administered in 250 ml portions taken three times daily with the three main meals of the day (Campbell-Tofte et al., 2011). The remaining HTs were brewed by the patients. In addition, we evaluated the quality of the included RCTs using the modified Jadad rating scale. Nine articles scored 1-3. Seven articles scored 4-7. Overall, the quality of the articles was low.

3 Polyphenolic compounds

Polyphenols have long been considered the main reason for tea's wide range of health benefits (Khan and Mukhtar, 2018). Polyphenols typically contain at least one or more aromatic rings linked to hydroxyl groups. There is growing evidence that a moderate long-term intake of this substance can benefit the incidence of cancer and chronic diseases (Cao et al., 2019; Fraga et al., 2019). Epidemiological findings have shown that plant polyphenols can manage and prevent T2DM. Table 4 shows herbs' total polyphenol and polyphenol content in the first category of HTs (Kubola and Siriamornpun, 2008; Mateos et al., 2018; Juan, 2019; Catani et al., 2021; Zhen et al., 2021). The polyphenol content in herbs of different origins differs. The table shows the content of other phenolic compounds in the herbs in the category with the highest total polyphenol content. Currently, studies on the polyphenol content of Puda tea are lacking; therefore, only the polyphenol content of its main component, bitter melon, is shown. Because of the complexity of the composition of the second group of HTs and the synergistic effects between various compounds, their polyphenol contents are not discussed for now.

4 Safety assessment

Currently, HT is widely used worldwide; however, owing to the lack of professional guidance, its security remains the focus of attention. Li et al. evaluated the toxicological profile of mulberry leaf extract (MLE) using acute, subacute toxicity, and genotoxicity tests. They considered MLE safe, supporting its application as a novel food ingredient or product (Li et al., 2018). The safety of KT root water extract was evaluated using DNA microarray, and the results showed that KT tea had no acute liver toxicity (Im et al., 2008). In addition, reverse mutation, chromosome aberration, and mouse micronucleus assays were used to evaluate the potential genotoxicity of KT root extract. The study results indicated that the plant is safe (Flammang et al., 2006).

Notably, some HTs have certain contraindications. There have been two cases of hypoglycemia and convulsion in children after taking balsam pear tea (Raman and Lau, 1996). The use of balsam pear tea in children should be avoided because of the lack of dose advice. In addition, couples preparing for pregnancy should carefully consider the intake of bitter gourd because it has shown significant characteristics of inhibiting sperm motility and promoting abortion (Basch et al., 2003). Individuals with G6P dehydrogenase deficiency and renal insufficiency should also avoid large doses or prolonged use of bitter melon. Significant adverse reactions have been reported after taking Momordica charantia in these two groups of patients (Raman and Lau, 1996). Patients with severe allergic reactions to chamomile and a history of contact dermatitis should avoid chamomile tea consumption. In addition, coumarin in chamomile can enhance the therapeutic effect of warfarin and provide additional hemodilution of aspirin and other drugs (Heck et al., 2000; Abebe, 2002). Therefore, chamomile tea should be avoided with the above two drugs. Because chamomile has a mild sedative effect, it can also increase the effect of other sedative drugs, such as apioid analgesics, **benzodiazepines**, or alcohol, on the central nervous system (McKay and Blumberg, 2006; Nguyen et al., 2021). Therefore, the author suggests that Chamomile tea use should be minimized after consuming these drugs and drinking much alcohol. In a recent toxicity study, GP showed good safety. However, Gynostemma can increase the toxicity of other drugs. Therefore, when used in combination with other drugs, drinkers' liver and kidney function should be watched. A study on YM tea showed that drinking beverages had been linked to an increased risk of cancer (Bracesco et al., 2011). The polycyclic aromatic hydrocarbon content of YM tea may cause an increased incidence of cancer (Kamangar et al., 2008; Bracesco et al., 2011). In addition, hot water brewing may also be the reason for an increased incidence of cancer, particularly esophageal cancer (Humans IWGotEoCRt, 2018). Furthermore, a clinical study conducted in Uruguay between 1990 and 2004 supplemented the carcinogenic characteristics of hot YM teas. The study's findings showed that drinking hot YM tea was strongly associated with cancers of the esophagus, lung, and bladder and was significantly associated with cancers of the cervix, prostate, and kidney. However, no correlation was observed for gastric, colon, rectal, and breast cancers (Stefani et al., 2011).

TABLE 4 Polyphenol content in herbs.

HTs	Total polyphenols (mg/g)	Content of polyphenolic compounds (mg/g)							References
Mulberry leaf tea	13.36	Astragalin	Chlorogenic acid	Kaempferol-glycoside	Quercetin-glycoside	Quercetin	Rutinum	-	Deng et al. (2021)
		0.09	1.106	0.434	0.502	1.099	0.814	-	
Chamomile tea	100.5	Apigenin	Apigenin-7-O-glucoside	Chlorogenic acid	Caffeic acid	Ferulic acid	Luteolin	Luteolin-7-O-glucoside	Catani MV et al. (2021)
		1.22	9.93	13.25	0.77	2.85	2.6	6.51	
		p-coumaric acid	Quercetin	Rutinum	-	-	-	-	
		10.05	1.97	1.58	-	-	-	-	
Gynostemma tea	75	Isoquercitrin	Kaempferol	Para-hydroxybenzoic acid	Protocatechuic acid	Quercetin	Rutinum	-	Deng et al. (2019)
		0.36	0.82	0.04	0.15	1.36	10.07	-	
Yerba mate tea	84.887	Caffeic acid	Caffeoyl-glycosides	Caffeoylquinic acids	Caffeoyl-feruloylquinic acids	Caffeoyl-p-coumaroylquinic acids	Caffeoyl-sinapoylquinic acids	Dicaffeoylquinic acids	Mateos R et al. (2018)
		0.231	2.9	54.134	0.548	0.053	0.091	16.608	
		Feruloylquinic acids	Kaempferol-glycoside	Kaempferol-rhamnoglucoside	p-Coumaroylquinic acids	Quercetin-glycoside	Rutinum	-	
		2.033	7.604	0.922	0.358	0.581	0.581	-	
Bitter melon	224	Catechin	Gallic acid	p-Coumaric	Tannic acid	-	-	-	Kubola and Siriamornpun (2008)
		4.54*	202*	0.16*	1.41*	-	-	-	

In this table, the total polyphenol content was determined by Folin-Ciocalteu colorimetric method and the phenolic compounds were analyzed by high performance liquid chromatography.
*The units of these values are mg/l.

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In conclusion, these HTs should be consumed daily under the guidance of medical professionals. If necessary, the number of hypoglycemic drugs and insulin should be adjusted over time. In addition, because a doctor's permission is not required to obtain HTs, we should be wary of their abuse.

5 Mechanism research

In RCTs, 14 HTs have been shown to have antidiabetic activity. However, the hypoglycemic mechanism of HTs composed of multiple herbs is complex, and the interactions between drugs are unclear. Therefore, this section only comprehensively discusses the mechanism of action of HTs composed of single herbs (Figures 1–6, Table 5).

5.1 Improve insulin resistance

Insulin resistance (IR) refers to the decreased sensitivity of surrounding target tissues to insulin, such as the liver, muscle, and adipose tissue. IR, the main pathogenesis of T2DM, is a difficult problem treating diabetes. Therefore, improving insulin resistance during diabetes treatment is important (Petersen and Shulman, 2018).

IR is involved in a complex insulin signal transduction network. The insulin receptor (IRS) and insulin receptor substrates (IRSs) are key nodes in insulin signaling (Taniguchi et al., 2006). Recent studies have shown that the water extract of mulberry leaves can upregulate the expression of IRS and insulin receptor in the adipose tissue of diabetic mice (Tian et al., 2019a). Suppressor cytokine signaling-3 (SOCS3) is a cytokine signal transduction inhibitor family member that can significantly inhibit the activation of major signaling molecules in the insulin signaling pathway. Momordica charantia polysaccharide can improve insulin sensitivity by downregulating the expression of SOCS3 (Ma et al., 2017).

The skeletal muscle, considered the major driver of systemic insulin resistance, is responsible for approximately 80% of postprandial glucose clearance (Merz and Thurmond, 2020). In skeletal muscle, two pathways are responsible for glucose transport: the PI3K/Akt and AMPK pathways (Saltiel and Kahn, 2001). Studies have shown that MLE can improve glucose utilization in skeletal muscles using the PI3K/AKT and AMPK pathways (Bae et al., 2018).

The liver, an important organ for human energy metabolism, is a key target organ of IR in T2DM. In the liver, the PI3K/Akt pathway is responsible for most of the metabolic activity of insulin. It is activated by tyrosine phosphorylation of IRS upon insulin stimulation (Huang et al., 2018b). Tyrosine dephosphorylation is regulated by tyrosine phosphatases (PTPs). A study showed that mulberry polysaccharides (MLPII) could restore the blood glucose level in diabetic rats. This may be related to the expression of PI3K and AKT2, reducing the expression of protein tyrosine phosphatase 1B (PTP1B) (Ren et al., 2015). Similar to MLPII, dammarane-type triterpenes in GP also showed inhibitory activity against PTP1B (Hamid et al., 2015). In addition, YM tea can restore the phosphorylation of IRS1 and Akt in the liver and muscle and reduce blood glucose levels in obese mice (Arcari et al., 2011).

Dysfunctional adipose tissue plays a key role in IR (Ahmed et al., 2021). Adiponectin, an adipokine, is specifically expressed in adipose tissue and can directly reverse IR. *In vitro* studies have shown that MLE can promote the secretion of adiponectin in 3T3-L1 preadipocytes in mice (Naowaboot et al., 2012). *In vivo* studies have shown that mulberry leaves' anthocyanin extract (MAE) can significantly increase adiponectin levels in db/db mice (Yan et al., 2016). An important factor regulating insulin sensitivity is increased free fatty acid (NEFAs) concentration, which can lead to IR through multiple mechanisms (Shulman, 2000). Sheng et al. suggested that mulberry leaves might improve IR by inhibiting NEFA signal transduction (Sheng et al., 2017). Dysfunctional obese adipose tissue can also develop IR by inducing lipotoxicity (Ahmed et al., 2021). Akase reported that Salacia reticulata extract could improve IR in mice by inhibiting visceral fat accumulation (Akase et al., 2011).

The intestinal tract is considered an important tissue for blood glucose control. Bitter gourd extract can interact with intestinal epithelial cells before circulating to other tissues. Bitter gourd ethanol extract can improve IR in intestinal cells by acting as an insulin sensitizer and replacement. Specifically, the insulin-sensitizing effect of bitter gourd may be associated with restoring insulin-induced activation of Akt signaling. Since AMPK protein mediates the insulin-independent increase in glucose, the insulin replacement function may be associated with the activation of AMPK protein (Chang et al., 2021). In addition, changes in the gut microbiota composition may be linked to the development of hyperglycemia and diabetes (Thingholm et al., 2019). Studies have shown that the structure of bitter gourd polysaccharides can be altered by fermentation with *Lactobacillus* Plantarum. This change can increase the production of single-chain fatty acids, enhancing the antidiabetic effect of balsam pear polysaccharides in rats (Gao et al., 2018). In addition, mulberry leaves may improve IR by restoring Bacteroides, Proteus, and Clostridium species in diabetic rats (Sheng et al., 2017). GP can also regulate intestinal microflora to improve IR (74).

5.2 Protect islet beta cells and promote insulin secretion

Autophagy is important for normal homeostasis and the survival of islet cells. Therefore, induction of islet cell-protective autophagy using drugs or other techniques is considered a promising approach for treating or preventing

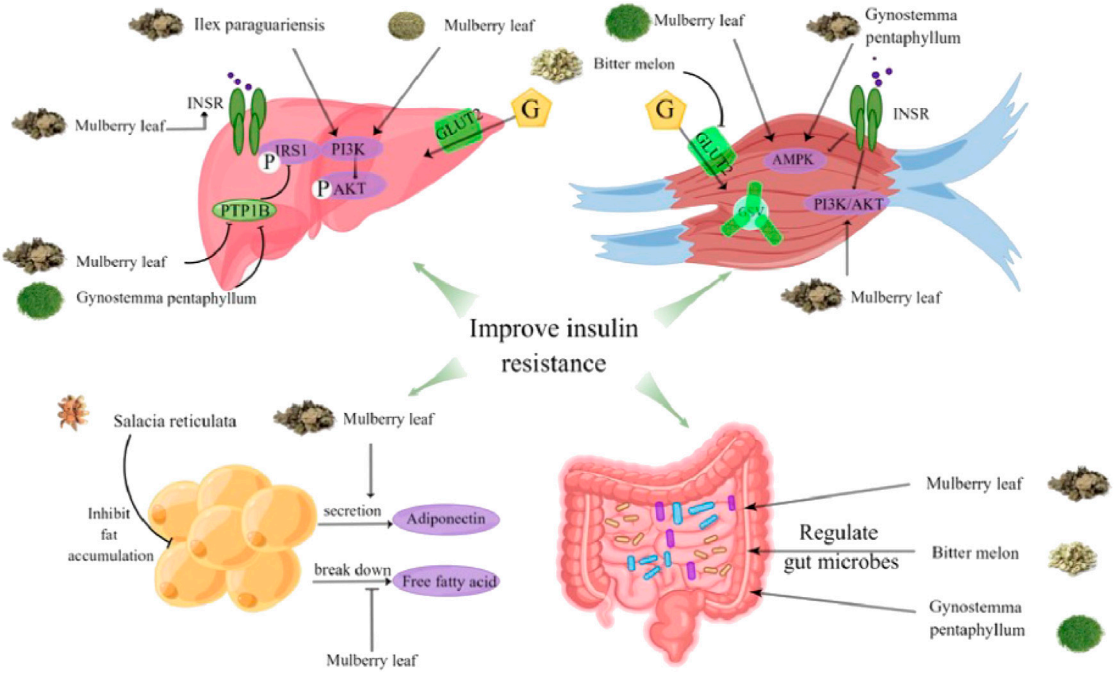

FIGURE 1
The effects of herbal teas in improving insulin resistance.

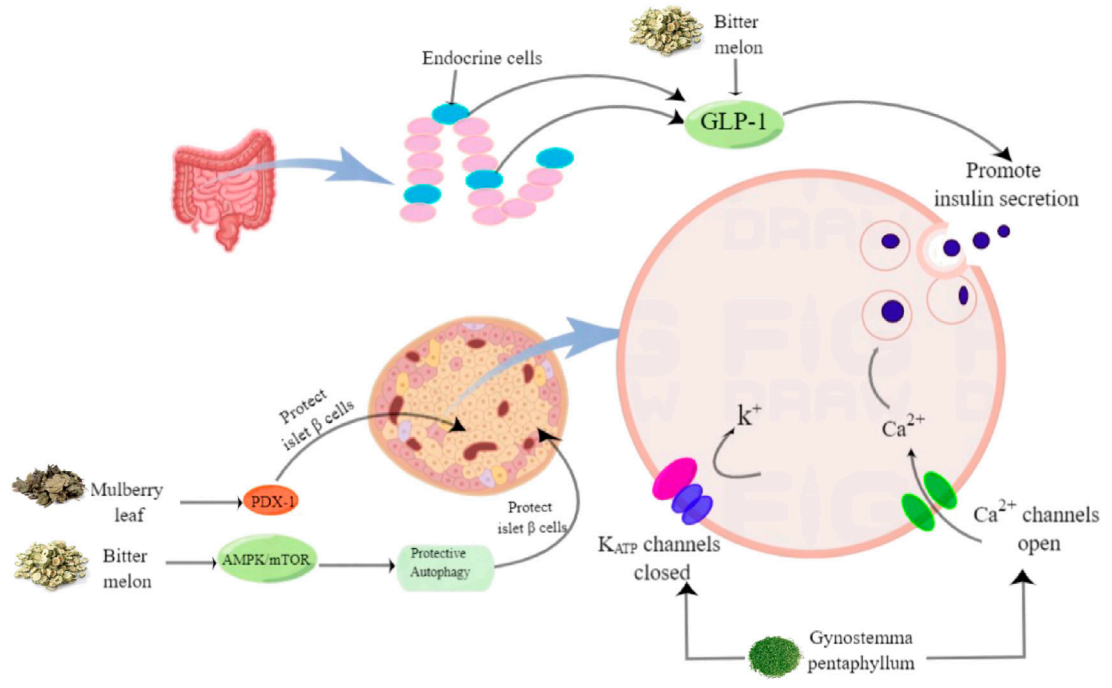

FIGURE 2
The effects of herbal teas in protecting islet beta cells and promoting insulin secretion.

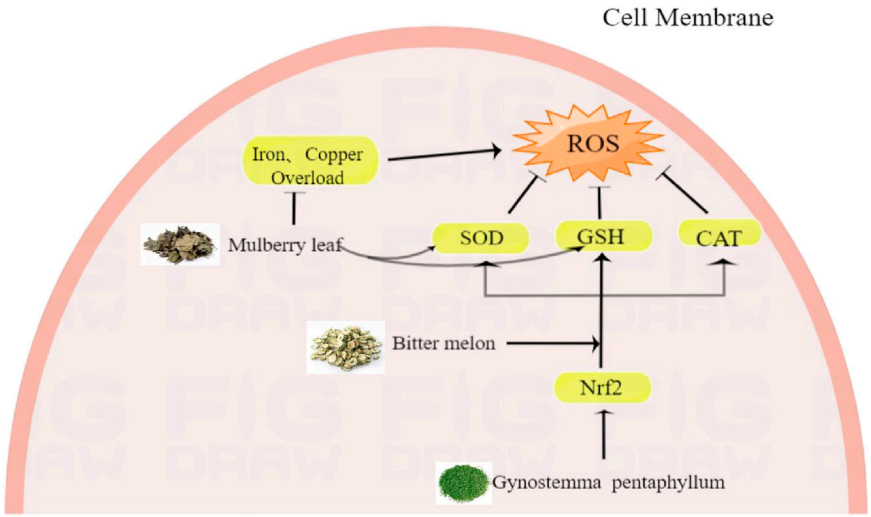

FIGURE 3
The Anti-inflammatory and anti-oxidant effect of herbal teas in Type 2 diabetes mellitus.

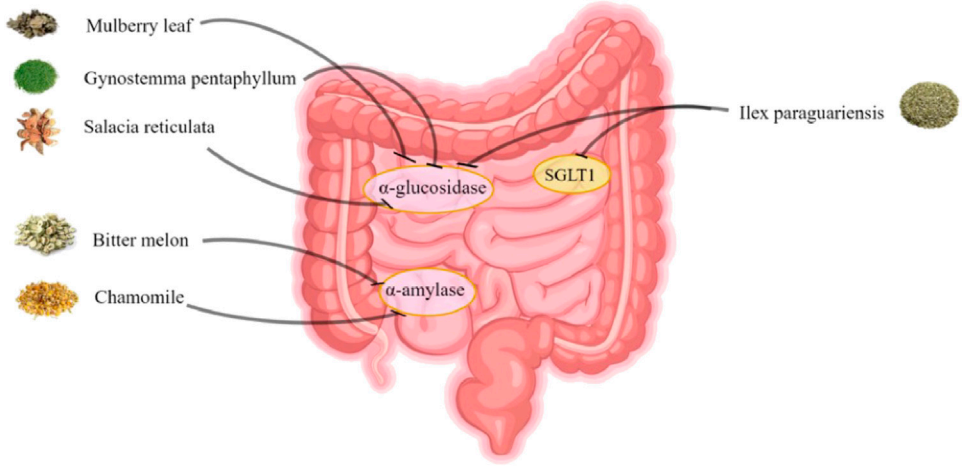

FIGURE 4
Inhibitory effect of herbal teas on glucose absorption.

T2DM (Marasco and Linnemann, 2018). By inducing AMPK/mTOR pathway-mediated autophagy, the mulberry leaf ethanolic extract can protect islet cells (Liu et al., 2017). Pancreatic duodenal homeobox-1 (PDX-1), a "master regulator" of pancreatic development, is the most critical intracellular factor in beta cells. If the gene encoding this protein is missing, the human pancreas becomes underdeveloped. Malekshahi suggested that bitter gourd might have a protective effect on pancreatic beta cells by upregulating the expression of PDX-1 (Malekshahi et al., 2019). In addition, bitter gourd ethanolic extract stimulates insulin secretion by activating TAS2Rs as GLP-1 secretagogues in enteroendocrine cells (Müller et al., 2019). *In vivo*, GP extract significantly improved glucose tolerance and increased plasma insulin levels in Goto-Kakizaki rats (Hoa et al., 2007). *In vitro*, GP extract stimulated insulin release from isolated rat islets under high glucose conditions. This may be mediated by adenosine triphosphate-dependent potassium channels and L-type calcium channels (Lokman et al., 2015). In

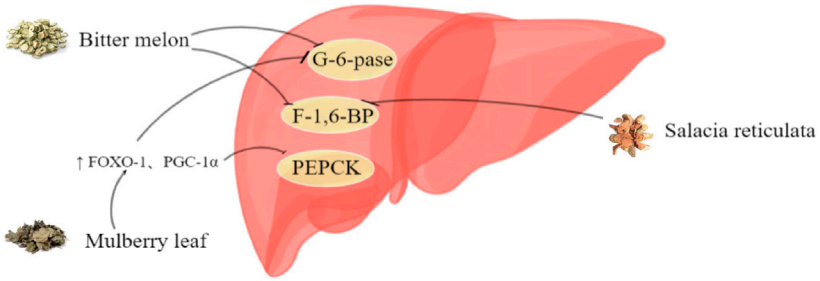

FIGURE 5
Inhibitory effect of herbal teas on gluconeogenesis.

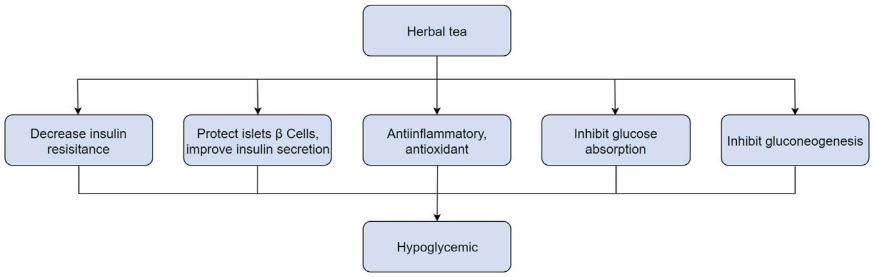

FIGURE 6
The effect of herbal teas in type 2 diabetes mellitus.

addition, the research by Pereira DF showed that the ethyl acetate and n-butanol components of YM tea also have significant insulin secretion-stimulating effects (Pereira et al., 2012).

5.3 Anti-inflammatory and antioxidant

Low-grade inflammation is a typical manifestation of T2DM. High glucose levels increase the production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) (Hameed et al., 2015). Chronic and low-level administration of TNF-α in rodents results in systemic IR, whereas neutralization of TNF-α significantly increases insulin uptake to peripheral glucose (Lang et al., 1992; Hotamisligil et al., 1993). Wang et al. found that GP significantly reduces TNF-α levels (Wang et al., 2020a). Blocking the NF-KB signaling pathway is also an effective way to reduce the production of pro-inflammatory cytokines. In 2006, Bai et al. discovered that bitter gourd could inhibit the activation of NF-kB by inhibiting the degradation of IkBα(Yang et al., 2015).

Redox processes are important in the human body (Sies et al., 2017). Physiologically, the body is in a redox homeostasis state. When the human body is subjected to various harmful stimuli, homeostasis is disrupted, eventually damaging the body. Because the activity of various antioxidant enzymes in pancreatic islets is lower than that in other tissues, oxidative stress is most harmful to human pancreatic cells (Tiedge et al., 1997). Oxidative stress is not only considered to be responsible for the progressive dysfunction of beta cells but also a possible causative factor in the development of IR (Robertson et al., 2003; Bloch-Damti and Bashan, 2005).

Wang et al. found that bitter gourd polysaccharides can significantly improve the body's antioxidant capacity and alleviate pancreatic damage caused by streptozotocin. The protein extract of bitter gourd can significantly increase the SOD, CAT, and GSH-Px levels, reduce glutathione (GSH) levels and improve IR (Wang et al., 2019a). Antioxidant experiments have shown that the polysaccharide components of GP can also increase the activities of SOD, CAT, and GSH-Px, reduce malondialdehyde (MDA) activity, and improve antioxidant capacity (Wang et al., 2020a). Furthermore, total Gynostemma saponins can stimulate insulin secretion by stimulating the Nrf2 antioxidant pathway (Gao et al., 2016). Studies have shown that the oral intake of mulberry leaves

maintains beta-cell function and reduces pancreatic endoplasmic reticulum stress in db/db mice. MLE can effectively restore the SOD/ CAT balance in alloxan-induced diabetic rats and increase the reduced GSH/oxidized glutathione (GSSG) ratio, thereby minimizing the damage to islet beta cells caused by oxidative stress (Araujo et al., 2015). In addition, essential trace elements, especially iron, zinc, copper, and manganese, play key roles as catalytic lefts for various enzymes in various redox reactions. Both the deficiencies and excess of these micronutrients perturb the antioxidant balance, increasing free radical formation and oxidative stress in cells and tissues (Dubey et al., 2020). MLEs can correct iron and copper overload to alleviate oxidative events in diabetes (Krol et al., 2016).

5.4 Inhibition of glucose absorption

Starch in food needs to be hydrolyzed into dextrins and oligosaccharides under the action of α-amylase and then decomposed into glucose under the action of α-glucosidase, which is finally absorbed and utilized by small intestinal cells. This reaction process causes postprandial blood glucose to rise. Therefore, in treating T2DM, alpha-amylase and alpha-glucosidase inhibitors are effective remedies for postprandial hyperglycemia (Hossain et al., 2020).

The polyhydroxyalkaloid compound found in mulberry leaves, 1-deoxywildomycin (DNJ), is a promising α-glucosidase inhibitor (Ji et al., 2016). RCTs have demonstrated that long-term intake of DNJ-enriched MLE can improve postprandial glycemic control in individuals with impaired glucose metabolism (Asai et al., 2011). The flavonoids and alkaloids in the water extract of mulberry leaves can also significantly inhibit the activity of α-glucosidase, with inhibition rates of 86.12 ± 1.79% and 87.29 ± 1.32%, respectively (Han et al., 2020). Shivanagoudra et al. isolated seven compounds from acetone and methanol extracts of bitter gourd, all exhibiting different α-amylase and α-glucosidase inhibitory activities (Perera et al., 2021). Four classes of compounds were isolated from the water-soluble fraction of Salacia reticulata: kotalanol, salacinol, 13-membered ring thiocyclitol, and 13-membered sulfoxide. These four substances are natural and effective α-glucosidase inhibitors (Liu et al., 2006; Han et al., 2020). Morikawa et al. found that the methanol fraction of Salacia reticulata Wight has an inhibitory effect on α-glucosidase (Morikawa et al., 2021). Recent research suggests that α-glucosidase inhibitors reversibly bind to maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI), thereby inhibiting carbohydrate hydrolysis in the small intestine. ntMGAM is the catalytic domain of MGAM. Sim et al. believe that deoxy-sulfomatrine, an extract of Salacia reticulata, is the most potent ntMGAM inhibitor reported to date. Its inhibitory effect is approximately 2,000 times stronger than that of acarbose (Sim et al., 2010). Chamomile showed a strong inhibitory effect on MGAM and SI in brush border membranes prepared from the small intestine of rats (Kato et al., 2008). Villa-Rodriguez et al. believed that the presence of apigenin and apigenin 7-O-glucoside is one of the reasons why chamomile inhibits alpha-amylase. Unlike acarbose, this combination of polyphenols gently impairs carbohydrate digestion and sugar absorption, thereby avoiding gastrointestinal side effects (Villa-Rodriguez et al., 2018). GP's polysaccharide components and total saponins can effectively inhibit α-glucosidase activity (Wang et al., 2019b). In addition, studies have confirmed that YM significantly inhibits disaccharidase *in vitro*, and the inhibitory effect is acute (Pereira et al., 2012).

SGLT1 plays an important role in intestinal glucose absorption. Inhibition of SGLT1 in the intestine delays and attenuates intestinal glucose absorption and prevents a rapid increase in postprandial glucose levels (Tsimihodimos et al., 2018). In a study by Oliveira, Yerba Mate significantly reduced SGLT1 gene expression in mice's upper and middle intestines. This suggests that the bioactive compounds in Yerba mate may interfere with glucose absorption by reducing SGLT1 expression (Oliveira et al., 2008).

5.5 Inhibition of gluconeogenesis

It is well known that gluconeogenesis, an important part of glucose metabolism, is the main cause of increased fasting blood glucose in patients with T2DM (Petersen et al., 2017). Most HTs in this study may inhibit gluconeogenesis by inhibiting glucose-6-phosphatase (G6Pase) and fructose 1,6-bisphosphatase (FBPase) (Méndez-Lucas et al., 2013). In HepG2 cells with IR, mulberry leaf anthocyanin extract promotes the upregulation of FOXO-1 and PGC-1α, thereby reducing the activities of phosphoenolpyruvate carboxykinase (PEPCK) and G6Pase (Yan et al., 2016). Mangiferin, an aqueous extract of Salacia reticulata, acts directly on hepatocytes and inhibits FBPase expression (Im et al., 2009). A study showed that the liver glycogen content was significantly increased by approximately 75% after oral administration of the n-butanol component of YM for 3 h compared with the control group (Pereira et al., 2012). In an STZ-induced diabetic rat model, quercetin, luteolin, and apigenin in chamomile showed good inhibitory activity on glycogen phosphorylase (Kim and Jobin, 2005). In addition, genistein in mulberry leaves may ameliorate hepatic gluconeogenesis dysfunction by modulating AMPK-CRTC2 and MEK/ERK signaling pathways (Bae et al., 2018).

6 Discussion

T2DM is a chronic metabolic disease requiring long-term medication. The World Health Organization's traditional

medicine strategy promotes using herbal medicines to treat chronic diseases (Kishino et al., 2009). HT is an invaluable resource for in-depth studies as a special method of consuming herbs. It has the advantage of regulating multiple targets and affecting multiple biological processes. By summarizing RCTs, we found that HTs have a certain clinical efficacy in improving blood sugar levels, which can be considered a promising auxiliary hypoglycemic method for T2DM.

Compared with traditional teas, HTs are more advantageous in improving blood sugar in T2DM patients. Whether herbal medicines and teas have adjunctive therapeutic effects on T2DM is a topic of widespread concern. Liu et al. conducted a meta-analysis on the hypoglycemic effect of herbal medicines in 2004 and 2011, respectively. The results suggest that herbal medicines can improve blood sugar levels in T2DM patients (Liu et al., 2004a; Liu et al., 2004b). In 2019, Tian et al. selected high-quality RCTs published in the past 10 years for research and confirmed the efficacy of herbal medicine in improving blood sugar (Tian et al., 2019b). In addition, studies on various herbal extracts such as ginsenosides, resveratrol, and guava have shown that herbal medicines have a positive therapeutic effect on T2DM (Luo et al., 2019; Zhou et al., 2019; Huang et al., 2020). As the second most popular beverage globally, tea consumption is inversely linearly associated with the incidence of T2DM. Multiple epidemiological studies suggest that drinking four or more cups of tea daily may reduce the incidence of T2DM (Jing et al., 2009; van Woudenbergh et al., 2012). However, there is no direct evidence that tea can control blood sugar levels in T2DM patients (Li et al., 2016; Yu et al., 2017). Therefore, further exploration of whether HTs can combine the advantages of herbs and teas to play an ideal role in blood sugar control in T2DM is required.

Compared with traditional herbal decoctions, HTs are more convenient. Traditional Chinese medicine decoctions must be continuously boiled for at least 30 min, whereas HT requires only hot or cold water to brew. In addition, Chinese scholars have compared the efficacy and dissolution quality of HTs and traditional herbal decoctions. In 2003, Liu compared the differences in dissolution quality between tea preparations and traditional decoctions of four herbs. The four herbs were peony bark, rhubarb, honeysuckle, and pale bamboo leaves. The experimental results showed that the differences in dissolution quality between HT preparations and traditional decoctions of these four herbs were not statistically significant (Guangxi, 2003). Subsequently, Li et al. expanded their study to 18 herbs. They believed that herbs containing tannins and acids or fruits and leaves should be made into teas, while herbs containing volatile oils should be made into decoctions (LI Jian-kai et al., 2004). These studies suggest that HTs composed of single-flavored plants may be no less effective than traditional decoctions, except those containing volatile oils.

Whether HTs have significant sensory drawbacks is critical for consumers to remain enthusiastic about products. The ideal product should be sweet, mild, fruity, not too bitter, astringent, pungent, strong, or unfermented in flavor (Yang and Lee, 2020). Among these, astringency is the most troublesome attribute. Fresh tea leaves often contain varying amounts of astringent and bitter compounds that emit little to no aromas (Chao-hua, 2014). However, processing leads to the development of rich flavor compounds. Similarly, when mulberry leaf tea is fried, green astringency is significantly reduced, and the aroma is strong and mellow. In addition, consumers who do not tolerate the bitter taste of meta-tea and bitter melon often add sugar, juice, or honey to increase their appreciation (Samoggia et al., 2021). However, this approach does not apply to patients with diabetes. Furthermore, not all HTs have unacceptable tastes. For example, the citrus aroma in Rauvolfia-Citrus tea can somewhat offset bitterness discomfort (Campbell-Tofte et al., 2011). A systematic review of the literature on Rauvolfia-Citrus tea reveals that the HT has “fruity,” “floral,” “green leaf,” and “fatty " aromatic notes (Wang et al., 2020b). In addition, chamomile tea has been widely consumed for centuries owing to its pleasant flavor (Guzelmeric et al., 2015).

The molecular mechanism of HT in improving blood sugar involves improving IR, protecting islet β cells, anti-inflammatory, and antioxidant effects, inhibiting glucose absorption, and inhibiting gluconeogenesis. Among these, the most significant hypoglycemic mechanism of HT is improving IR. The pathogenesis of IR is a complex process. According to the different stages of pathogenesis, the pathological mechanisms can be divided into three stages: pre-receptor, receptor, and post-receptor. Abnormalities in the post-receptor link and disturbances in signal transduction are the main causes of IR (Chen et al., 2019). Similarly, improving post-receptor link abnormalities is the main method for improving IR in medicinal tea. The signaling pathways regulated by medicinal tea mainly include the PI3K/AKT, AMPK, and GLUT4 signaling pathways. In addition, based on the upregulation of insulin receptor expression by mulberry leaves and bitter gourd, we believe that the receptor link may e a way medicinal tea can improve IR. The protective effect of HTs on islet β-cells is mainly by promoting the proliferation of islet β-cells, increasing the level of GLP-1, and regulating the level of autophagy. In addition, some researchers have suggested that islet microcirculation is involved in islet β-cell dysfunction. In pre-diabetes, the islet microcirculation is in a state of hyperperfusion. This state leads to high pressure in islet microvessels and damage to endothelial cells, eventually leading to insufficient perfusion of the islet microcirculation and aggravating islet dysfunction. However, no studies have confirmed the effects of HT on islet microcirculation (xin, 2008). Notably, HTs still have anti-inflammatory and antioxidant properties similar to traditional teas. These properties are an important reason for HTs’ widespread use in managing diabetes. It is well known that traditional teas’ anti-inflammatory and antioxidant properties

TABLE 5 Hypoglycemic mechanism of herbal tea based on single herbal tea.

Herbal Tea Name	Extract	Animal model/ Cell	Duration	Effect	In vivo/ vitro	References
Mulberry leaf tea	Mulberry leaf polysaccharide (MLPII)	Male Wistar rats; High-fat diet; injection of low-dose STZ	6 weeks	Inhibiting the expression of PTP1B, activating the PI3K–AKT pathway and mitigating oxidative stress	*in vivo*	Ren et al. (2015)
	Mulberry leaf	Male Sprague-Dawley rats; High-fat diet; injection of low-dose STZ	13 weeks	inhibiting NEFA signaling pathway, improving the community structure of the intestinal microbiota	*in vivo*	Sheng et al. (2017)
	Water extracts of mulberry leaf (WEM)	Sprague-Dawley male rats; high-fat and high-sugar diet; injection of low-dose STZ	10 weeks	Inhibiting TLR2 signalling pathway, Stimulation of insulin signal pathway, Inhibit the production of TNF- α in serum	*in vivo*	Tian et al. (2019a)
	mulberry leaf extract (MLE)	Male db/db mice	8 weeks	stimulating glucose disposal in skeletal muscle cells via the PI3K/Akt and AMPK pathways	*in vivo*	Ui-Jin Bae, et al. (2018)
	Mulberry leaf flavonoids (MLF)	Male db/db mice and db/m mice	7 weeks	Ameliorating muscle glucose uptake and mitochondrial function in L6 muscle cells via AMPK/PGC/1α signaling pathway	*in vivo*	Qinghai Meng, et al. (2020)
	Folium Mori extract (FME): contained the flavonoid and polyphenol components	Male Sprague-Dawley rats; high fat and high sugar diet; injection of STZ	4 weeks	Activating the IRS-1/PI3K/Glut4 signalling pathway in skeletal muscles	*in vivo*	Shengyu Cai, et al. (2016)
	Mulberry leaf extract (MA)	Mouse 3T3-L1 preadipocytes	8 days	Stimulating adipogenesis and adiponectin secretion in 3T3-L1 cells	*in vitro*	Naowaboot et al. (2012)
	Mulberry anthocyanin extract (MAE)	Male db/db mice and their nondiabetic lean littermates	8 weeks	Activating of PI3K/AKT pathways, activating AKT phosphorylation and its downstream targets in insulin-sensitive tissues	*in vivo*	Fujie Yan, et al. (2016)
	Mulberry leaf	Male Sprague-Dawley rats; High-fat diet; injection of low-dose STZ	13 weks	Inhibition of NEFA signalling pathway transduction, restored the phyla Bacteroidetes and Proteobacteria and class Clostridia in the intestinal tract	*in vivo*	Sheng et al. (2017)
	Mulberry leaf	Male db/db mice	20 weeks	Maintain insulin levels and pancreatic β-cell mass by suppressing endoplasmic reticulum stress	*in vivo*	Suthamwong P, et al. (2020)
	Mulberry leaf	Female Fischer rats; injection of alloxan monohydrate	30 days	Decreasing MMP-2 levels and SOD/CAT ratio	*in vivo*	Araujo, et al. (2015)
	Mulberry leaf extracts (acetone-water (AE) and ethanol-water (EE))	Male Wistar rats; High-fat diet; injection of STZ	4 weeks	Reducing the uptake of Fe and Cu ions and mitigating oxidative events	*in vivo*	Król et al. (2016)
	Mulberry leaf polysaccharide (MLPII)	Male Wistar rats; High-fat diet; injection of STZ	5 weeks	Inhibiting pancreatic islet apoptosis via elevation of Bcl-2/Bax ratio, ameliorating insulin secretory capacity via restoration of PDX-1 nuclear localization and expression levels	*in vivo*	Zhang et al. (2014)
	Morus alba leaves ethanol extract (MLE)	Male Sprague-Dawley (SD) rats; High-fat diet; injection of STZ	8 weeks	Protect islet cells against dysfunction and death by inducing AMPK/mTOR-mediated autophagy	*in vivo* and *in vitro*	Ji et al. (2021)
	Cryptochlorogenic acid (CCA)	Sprague-Dawley (SD) rats; injection of STZ	2 weeks	Inhibition of ferroptosis via activation of xc-/GPX4/Nrf2 and inhibition of NCOA4	*in vivo* and *in vitro*	Zhou Y. (2020)
	Mulberry leaf	High-fat mice	14 weeks	Inhibiting α-glucosidase activity; reducing the serum-free fatty acid (FFA), tumor necrosis factor-α (TNF-α), insulin, and glycated serum protein content and improving intestinal microbiota	*in vivo* and *in vitro*	Han X, et al. (2020)
	Mulberry anthocyanin extract (MAE)	HepG2 cells; male C57BL6/J genetic background (db/db) mice	8 weeks	Activating PI3K/AKT pathways	*in vivo* and *in vitro*	Yan et al. (2016)

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TABLE 5 (*Continued*) Hypoglycemic mechanism of herbal tea based on single herbal tea.

Herbal Tea Name	Extract	Animal model/ Cell	Duration	Effect	In vivo/ vitro	References
Puda tea	Bitter melon	Male OLETF rats	6 weeks	Inhibiting NF-κB and JNK pathways	*in vivo*	Yang et al. (2015)
	M. charantia ethanol extracts (MCE)	SPF-grade male SD rats; High-fat diet; injection of STZ	8 weeks	Increase in hepatic glycogen, peripheral tissue's GLUT-4 expression, and higher insulin sensitivity by down-regulating the expression of SOCS-3 and JNK.	*in vivo*	Ma et al. (2017)
	Bitter melon extract (BME)	NCI-H716 cells and IEC-18 cells	6 h	Activating the TAS2R-signaling pathway in enteroendocrine cells and leading to GLP-1 secretion	*in vitro*	Chang et al. (2021)
	ethyl acetate (EtOAc)-soluble fraction	Male Lepob/ob (ob/ob) and Lep+/+ (wild-type) mice	7 days	Increasing the levels of both insulin receptor mRNA and protein, decreasing the interleukin-1β mRNA and hepatic lipid accumulation in hepatocytes	*in vivo*	Dwijayanti et al. (2020)
	Bitter melon protein extract	Male Wistar rats; High-fat diet; injection of low-dose STZ	30 days	Anti-lipidemic and antioxidant	*in vivo*	Poovitha et al. (2020)
	Saponins of Momordica charantia L. (SMC)	Male Kunming mice; High-fat diet; injection of low-dose STZ	35 days	Activating AMPK/NF-κB signal pathway	*in vivo*	Wang et al. (2019b)
	polysaccharides of Momordica charantia L. (PMC)	Male Kunming mice; High-fat diet; injection of low-dose STZ	35 days	Repairing the damaged pancreatic-β cells and promoting antioxidantcapacity	*in vivo*	Wang et al. (2019a)
	Momordica charantia	Rats; injection of STZ	6 weeks	Activating pancreatic beta cells and protecting liver tissue	*in vivo*	Malekshahi et al. (2019)
	Momordica charantia fruit pulp ethanolic extract	Wistar rats, injection of STZ	28 days	Improved serum insulin and β-cell function	*in vivo*	Hafizur et al. (2011)
	Momordica charantia extracts	Male albino rats	90 min	Inhibiting of glucose-6-phosphatase and fructose-1, 6-bisphosphatase, enhancing principal enzyme G6PDH	*in vivo*	Shibib et al. (1993)
	Momordica charantia seeds	Male Wistar rats, injection of Alloxan	3 days	Contain an effective anti-hyperglycemic protein(s)	*in vivo*	Choudhary et al. (2012)
Chamomile Tea	Chamomile flowers extract (CFE)	Male C57BL/6 mice; Primary subcutaneous preadipocytes	20 weeks; 6 weeks	Activing PPARs and other factors	*in vivo* and *in vitro*	Weidner et al. (2013)
	luteolin	3T3-L1 adipocytes	24 h	Activating the PPARγ pathway and by acting at insulin signaling cascade	*in vivo*	Ding et al. (2010)
	Chamomile flowers	Male Wistar rats, injection of STZ	21 days	Inhibition of hepatic GP, inhibition of ALR2	*in vivo*	Kato et al. (2008)
	Maltodextrin-Free Chamomile	The Caco-2 cell line	4 days	Acute inhibition of GLUT2 and GLUT5	*in vitro*	Villa-Rodriguez, et al. (2018)
Gynostemma pentaphyllum Tea	polysaccharide (GPP) extracted from Gynostemma pentaphyllum herb	Kunming mice, injection STZ	4 weeks	Enhancing the SOD, CAT, and GSH-Px activities, decreasing the MDA activity, improving the levels of IL-4 and IL-10, and decreasing the levels of TNF-α and IL-6	*in vivo* and *in vitro*	Wang et al. (2020a)
	Gynostemma pentaphyllum saponins (GPs)	Male Wistar rats; injection STZ	40 days	Antioxidant effect	*in vivo*	Gao et al. (2016)
	Gynostemma pentaphyllum	Male C57 BL/6J mice, high-fat diet	12 weeks	Improve glycolipid metabolism, and stimulate BAT activity, WAT browning and lipid β-oxidation, while decreasing the ratio of Firmicutes to Bacteroidetes and enhancing the abundance of Akkermansia muciniphila	*in vivo*	Liu et al. (2017)

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TABLE 5 (*Continued*) Hypoglycemic mechanism of herbal tea based on single herbal tea.

Herbal Tea Name	Extract	Animal model/ Cell	Duration	Effect	In vivo/ vitro	References
	Damulin A and damulin B	L6 cells		Activating AMPK, increasing β-oxidation and glucose uptake, increasing GluT4 translocation to the plasma membrane	*in vitro*	Nguyen et al. (2011)
	gypenoside	Normal Wistar rats and Diabetic Goto-Kakizaki rats		Stimulating insulin secretion	*in vivo*	Hoa et al. (2007)
	a polysaccharide (GPP)	Specific-pathogen-free mice;		Inhibiting α-glucosidase activity, inhibiting the glucose absorption, affecting the protein expression of GLUT2	*in vivo*	Wang et al. (2019a)
	Gynostemma pentaphyllum Extract	Male spontaneous type 2 diabetic Goto-Kakizaki rats		Stimulation of insulin release via K-ATP and L-type Ca2+ channels	*in vivo*	Lokman et al. (2015)
	Gynostemma pentaphyllum	Male Zucker fatty rats	5 weeks	Inhibiting alpha-glucosidase activity, improving insulin receptor sensitivity	*in vivo*	Megalli S, et al. (2006)
	G. pentaphyllum ethanol extract (GPE)	Male C57BL/KsJ-db/db mice	5 weeks	Enhancing insulin secretion and its sensitivity, enhancing hepatic glucose utilization	*in vivo*	Yeo et al. (2008)
Salacia reticulata Tea	Salacia extract	Male TSOD mice and male TSNO mice	8 weeks	Preventing obesity and associated metabolic disorders including the development of metabolic syndrome	*in vivo*	Akase et al. (2011)
	Mangiferin	Male KK-Ay mice	4 weeks	Down-regulating the gluconeogenic pathway through regulation of FBP expression	*in vivo*	Im et al. (2009)
Yerba Mate Tea	Yerba Mate	Male Wistar rats; injection of alloxan	28 days	Decreasing the intestinal SGLT1 gene expression	*in vivo*	Oliveira, et al. (2008)
	Ilex paraguariensis tea	Male Wistar rats	180 min	Inducing-insulin secretion; inhibiting *in vitro* disaccharidases activities	*in vivo* and *in vitro*	Pereira, et al. (2012)
	Yerba Mate Tea	Male Swiss strain mice; high fat diets	8 weeks	Inhibiting hepatic and muscle TNF-α and restoring hepatic insulin signalling	*in vivo*	Arçari et al. (2011)
	Gallic acid	3T3-L1 cells	30 min	Increasing GLUT4 translocation and glucose uptake activity	*in vitro*	Prasad CN, et al. (2010)

are attributed to their rich polyphenol content (Hussain et al., 2016). The polyphenols in tea include EGCG, quercetin, and theaflavin (Winiarska-Mieczan et al., 2021). In this study, polyphenolic compounds were the main material basis for HTs' anti-inflammatory and antioxidant properties. Unique flavonoids in Rooibos tea, Aspalathin and Xanthone mangiferin in Honeybush tea (Ajuwon et al., 2018), and chlorogenic and gallic acids in YM tea (Oliveira et al., 2008) are all anti-inflammatory and antioxidant. In addition, herbs' polysaccharides, saponins, and alkaloids are also the main components responsible for their anti-inflammatory and antioxidant effects (Sun et al., 2014; Chen et al., 2017; Zhang et al., 2019; Zhou et al., 2019; Song et al., 2020). In addition, HTs affect glucose metabolism by inhibiting glucose absorption and gluconeogenesis. Intestinal glucose absorption is dependent on α-glucosidase activity (Van de Laar et al., 2005). The only marketed α-glucosidase inhibitor, acarbose, have adverse gastrointestinal side effects (Chiasson et al., 2002). HTs not only inhibit α-glucosidase but also do not cause adverse reactions in the gastrointestinal tract. Therefore, it is a reliable hydrolase inhibitor Table 5.

Whether these mechanisms are linked to the actions of HTs *in vivo* requires further investigation. The HTs in this study exerted hypoglycemic effects mainly through polyphenols, polysaccharides, saponins, and alkaloids. Polyphenols are poorly absorbed in the body's circulation either because of their low intrinsic activity or poor absorption or rapid elimination in the intestine (Manach et al., 2004). Polysaccharides originating from TCM are mostly

heteropolysaccharides composed of different monosaccharides, which is why they are less stable and less absorbed (Wang et al., 2022). Saponins and alkaloids have undesirable physicochemical properties and poor pharmacokinetic characteristics (Zheng et al., 2018b; Kim et al., 2018). This study showed that the bioavailability of HTs was unclear or low. This differs from the performance of HTs reported in clinical studies. Therefore, using bioavailability to explain the beneficial hypoglycemic effects of HTs seems inappropriate. The concept of bioavailability integrates numerous variables, such as intestinal absorption, microbiota metabolism, intestinal and hepatic metabolism, and plasma kinetics (Bhattaram et al., 2002). Few studies have been conducted on HTs to integrate sufficient information and link variables to organ-level health effects. The relative weight of each bioavailability variable may depend on the specific ingredients under consideration. For example, some polyphenols may be absorbed at lower rates than others but still achieve comparable plasma concentrations due to their lower secretion into the intestinal lumen and lower metabolism and elimination (Manach et al., 2004). Therefore, there is a need to develop an accurate method to detect the specific content of hypoglycemic components in HTs and to invest more effort in determining the correlation between hypoglycemic components and their biochemical endpoints.

In this study, we suggest for the first time that HTs may be an adjunctive treatment modality to improve blood sugar levels in patients with T2DM. This may be related to the potential pharmacological effects of compounds such as polyphenols in HTS to improve blood glucose levels. The antidiabetic effects of HTs appear to be mediated by mechanisms such as improving IR, protecting islet beta cells, anti-inflammatory, and antioxidant effects, inhibiting glucose absorption, and inhibiting gluconeogenesis. While HTs have attracted the attention of researchers in recent years for their health-promoting effects, their mechanisms of action remain unclear because of the complex compounds typically present in each HT. In the future, more research is needed to evaluate the antidiabetic benefits of multiple classes of compounds in HTs to elucidate their exact mechanisms. Understanding the dosage and administration of HTs and the bioavailability of their active compounds is a prerequisite for understanding their pharmacological mechanisms of action in diabetes treatment. In addition, studies on the metabolism of bioactive compounds in HTs are needed to make effective recommendations on HT intake. However, most clinical studies have been of low quality. Therefore, there is an urgent need to design more rigorous RCTs with larger sample sizes. In conclusion, this review provides useful data and information for further research and applications of HTs in the treatment of T2DM.

Author contributions

Overall design and manuscript revision: QL and LZ. Manuscript draft preparation: XZ, LZ, and BZ. Publication retrieval: KL and JS. XZ, LZ, and BZ contributed equally to the study. All the authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Yongsheng Chen,
Jinan University, China

REVIEWED BY
Da Pan,
Southeast University, China
Xinye Li,
Beijing University of Chinese Medicine, China

*CORRESPONDENCE
Yi Shan Huang,
bucmyishan@163.com
Lin Hua Zhao,
melonzhao@163.com

†These authors have contributed equally to this work and share the first authorship

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Efficacy and safety of traditional chinese medicine treatment for overweight and obese individuals: A systematic review and meta-analysis

Zhi Ge Wen[1†], Qi Qi Zhang[1†], Li Li Zhang[1†], Meng Fei Shen[1,2], Yi Shan Huang[1*] and Lin Hua Zhao[1*]

[1]Institute of Metabolic Diseases, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2]Graduate School, Beijing University of Chinese Medicine, Beijing, China

Background: The prevalence of obesity is increasing worldwide, causing a global health issue. Traditional Chinese medicine (TCM) used in treating overweight/obesity has been widely implemented in clinical practice, but its overall efficacy and safety remain unclear. This review aims to evaluate the effectiveness and safety of TCM based on randomized controlled trials (RCTs).

Methods: A systematic review was conducted by searching PubMed, Cochrane Library, Web of Science, Embase, and Clinical Trails from their inception to March 2021. Two reviewers screened studies, extracted the data, and assessed the risk of bias independently. The data were pooled for meta-analysis or presented narratively.

Results: Twenty-five RCTs involving 1,947 participants were included. Compared with placebo or blank control, TCM preparations reduced Body Mass Index (BMI) [MD = −1.16; 95% confidence interval (CI) = −1.44, −0.89; I^2 = 34%], reduced weight (MD = −2.53; 95% CI = −3.08, −1.99; I^2 = 34%), reduced waist circumference (MD = −2.64; 95% CI = −3.42, −1.87; I^2 = 0%), reduced hip circumference (MD = −3.48; 95% CI = −4.13, −2.83; I^2 = 0%), reduced total cholesterol (TCHO) (MD = −10.45; 95% CI = −18.92, −1.98; I^2 = 63%), reduced triglycerides (TG) (MD = −4.19; 95% CI = −6.35, −2.03; I^2 = 25%), increased high-density lipoprotein (HDL) (MD = −3.60; 95% CI = −6.73, −0.47; I^2 = 81%), reduced fasting blood glucose (FBG) (MD = −0.77; 95% CI = −1.24, −0.29; I^2 = 91%). Glycated hemoglobin (HbA1c)、body fat rate、low-density lipoprotein (LDL) were not statistically significant. For people with hypertension, decreased systolic blood pressure (SBP) (MD = −5.27; 95% CI = −8.35, −2.19; I^2 = 58%), decreased diastolic blood pressure (DBP) (MD = −4.30; 95% CI = −5.90, −2.69;

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; DHS, damp-heat syndrome; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; IGT, Impaird glucose tolerance; LDL, low-density lipoprotein; SBP, systolic blood pressure; TCHO, Total cholesterol; TCM, Traditional Chinese Medicine; TG, Triglycerides.

I^2 = 0%). For people with normal blood pressure, there was no significant change. There was no significant difference in liver function.

Conclusion: It has been demonstrated that TCM preparations have good clinical efficacy and safety for overweight/obesity. TCM may be suitable for overweight/obesity in adult populations for its efficacy and safety of long-term treatment.

KEYWORDS

traditional Chinese medicine, obesity, overweight, systematic review, meta-analysis

1 Introduction

Over the past 50 years or so, the prevalence of overweight and obesity has increased globally, reaching pandemic levels (Bluher, 2019). The number of cases of obesity and related diseases has significantly increased globally. By 2019, there were more than 1.9 billion overweight adults and 650 million obese adults (Yao et al., 2017). Obesity is one of the leading preventable threats to global health. Excessive obesity is defined as the excessive accumulation of fat in adipose tissue due to an imbalance between energy intake and energy expenditure (Yao et al., 2017). Obesity is a major health challenge and a negative factor affecting the health and longevity of community residents. Obesity is associated with many diseases, including cardiovascular diseases, diabetes mellitus, hypertension, hyperlipidemia, and fatty liver (Ogden et al., 2014).

Weight control can alleviate these problems (Monteiro and Azevedo, 2010). Current research has proved that lifestyle interventions for obese adults were effective in reducing weight (5% or more of initial weight) and the incidence rate of diabetes (Lee and Cha, 2016; Ryan and Yockey, 2017). For other related health problems, such as cardiometabolic risk factors, the benefits are unclear. Drug therapy is used as an adjunct to lifestyle, especially when lifestyle changes fail to produce an ideal weight loss effect, and the choice of drugs depends on the presence of comorbidities (Fisher et al., 2018). The guidelines suggested that patients with body mass index (BMI) > 27 kg/m^2 and obesity-related complications or BMI >30 kg/m^2 can take orally five kinds of weight loss drugs approved by the United States Food and Drug Administration (Jackson et al., 2015): orlistat, liraglutide, clokaserin, naltrexone/amphetamine compound, and fentamine/topiramate compound. At present, surgery has also been proven to be effective for severely obese people (Apovian et al., 2015; LeBlanc et al., 2018). Bariatric surgery can reduce the risk of obesity-related complications, but its significant costs and risks severely limit its widespread use (Waters et al., 2013). Up to no, obesity prevention and treatment strategies have failed to achieve long-term success either at the individual or group level.

In China, the obese population is also growing, and health problems are becoming more prominent. TCM has a long history of thousands of years. It is widely used to treat a variety of chronic diseases, including overweight, obesity, and these complications. TCM can play an important role in the treatment of obesity (Kazemipoor et al., 2015; Sahebkar-Khorasani et al., 2019) by inhibiting appetite (Pories, 2008), stimulating thermogenic metabolism promoters, inhibiting pancreatic lipase activity, reducing fat absorption, increasing fat decomposition and reducing fat production (Hong et al., 2017; Liu et al., 2019). However, conflicting opinions still exist due to the lack of sufficient evidence to support the efficacy and safety of TCM for the treatment of overweight and obesity. For these reasons, RCTs have also been conducted to evaluate the scientific evidence on the effectiveness of TCM. This paper systematically reviews the potential role of TCM in the treatment of overweight and obesity and summarizes the scientific evidence.

The previous systematic review evaluated the efficacy of TCM and its products in treating obesity and metabolic syndrome (Payab et al., 2020). In recent years, with the increasingly serious health risks brought by obesity, it is necessary to systematically evaluate the effectiveness and safety of only using TCM in overweight and obese people. Although not enough RCTs of TCM for overweight and obesity have been retrieved, and the population base of clinical trials is small, we try to provide the most authentic and reliable evidence on the effectiveness and safety of TCM for treating overweight and obesity.

2 Materials and methods

2.1 Search strategy

We comprehensively searched five English language databases, PubMed, Embase, Cochrane Library, Web of Science, and Clinical Trails, from inception to 15 March 2021. We used traditional Chinese medicine, Chinese medicine treatment of overweight or obesity, randomized controlled retrieval overweight or obesity, randomized controlled clinical trials, and meta-analyses as the keywords. Additional studies were searched in the reference lists of all identified publications, including relevant meta-analysis and systematic reviews. Finally, we identified 25 published randomized controlled clinical trials that met the inclusion criteria.

2.2 Inclusion criteria

We included all published RCTs and met the following criteria: 1) Participants were overweight or obese adults (age ≥ 18 years, BMI ≥ 24 kg/m^2). 2) With or without other obesity-related metabolic diseases. 3) RCTs. 4) Control was placebo or blank. 5) The treatment group used herbal preparations including a single botanical drug, poly-herb, or herbal extracts.

2.3 Exclusion criteria

We excluded the following characteristics of clinical studies: 1) Patients with drug-induced obesity, i.e., drug-induced obesity. 2) Patients taking hormones were excluded.

2.4 Data extraction

Two students independently extracted data from 25 original test reports using standardized forms. The extracted data include the characteristics of 25 clinical trials (first author, year of publication, sample size, intervention and control, treatment cycle, and follow-up time), characteristics of 1,947 patients (inclusion criteria, average age, male proportion, intervention and control measures, baseline weight, waist circumference, BMI, waist circumference, hip circumference, FBG, blood pressure, blood lipid level and safety index level), outcome BMI, weight, waist circumference, waist circumference, hip circumference, and FBG, blood pressure, blood lipid levels, safety indicators, and adverse events) and methodological information. When we needed additional information that was not available in online publications or supplementary materials, we contacted the study authors.

2.5 Quality assessment

We used the Cochrane bias risk tool to assess the bias risk of RCTs (Higgins et al., 2019). Two investigators independently completed the assessments, and discrepancies were discussed with a third person and resolved by consensus. Additionally, the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) framework was used to assess the quality of evidence contributing to each network estimate. This framework characterizes the quality of the body of evidence based on study limitations, imprecision, inconsistency, indirectness, and publication bias of the main results. (Guyatt et al., 2008).

2.6 Statistical analyses

The data entry and analysis were conducted using Microsoft Excel 2016 and Review Manager software version 5.3, respectively. The risk ratio and standard mean difference with a 95% confidence interval (CI) of the outcomes were calculated as the effective measures. We calculated the heterogeneity of the I^2 statistic as a measure of the proportion of overall variation attributable to inter-study heterogeneity. The fixed-effects (FE) model was used if $I^2 < 50\%$; otherwise, the random-effects model was used. Additionally, sensitivity analyses were performed before combining RCTs in the meta-analyses to determine possible additional sources of heterogeneity and changes in effect sizes. Publication bias was tested by visual inspection of the funnel plots. When few studies are included in the analysis, the power of the tests is too low. Therefore, publication bias was only examined if > 10 study comparisons were included in the analysis (Bluher, 2019).

3 Results

3.1 Study characteristics

The search identified 1,143 papers, of which 165 were duplicates. Then, 71 articles remained after screening titles and abstracts, and 47 articles remained after the full-text screening. Finally, 25 eligible manuscripts (1,947 participants) (Boozer et al., 2001; Coffey et al., 2004; Hioki et al., 2004; Wang et al., 2006; Kim et al., 2008; Kamiya et al., 2011; Kamali et al., 2012; Ke et al., 2012; Lenon et al., 2012; Sengupta et al., 2012; Kazemipoor et al., 2013; Ofner et al., 2013; Park et al., 2013; Tong et al., 2013; Park et al., 2014; Azushima et al., 2015; Mirtaheri et al., 2015; Chung et al., 2016; Kudiganti et al., 2016; Satapathy et al., 2016; Cho et al., 2017; Zuniga et al., 2017; Daneshi-Maskooni et al., 2018; Gholaman and Gholami, 2018; Cheon et al., 2020) assessed the effect of TCM intervention on weight loss in overweight and obese people. The subjects were all overweight and obese people, of which 14 were simple overweight or obesity and the remaining 11 trials were overweight and obesity combined with one or more metabolic risk factors (e.g., abnormal blood lipids and blood pressure, or varying degrees of blood glucose abnormalities: impaired glucose tolerance or newly diagnosed patients with type 2 diabetes mellitus). Figure 1 shows the screening process. Table 1 shows the characteristics of included studies. Supplementary materials show the ingredients for each study herbal treatment group. According to the composition, TCM groups are divided into two subgroups, preparations based on a single botanical drug group and polyherbal preparations group.

3.2 Evaluation of the risk of bias in the selected studies

We used the Cochrane Bias Risk Tool to assess the bias risk of 25 RCTs included. RCTs had a low overall risk of bias. Most RCTs are unclear about the risk of bias in sequence generation, allocation concealment, and reporting other biases because no

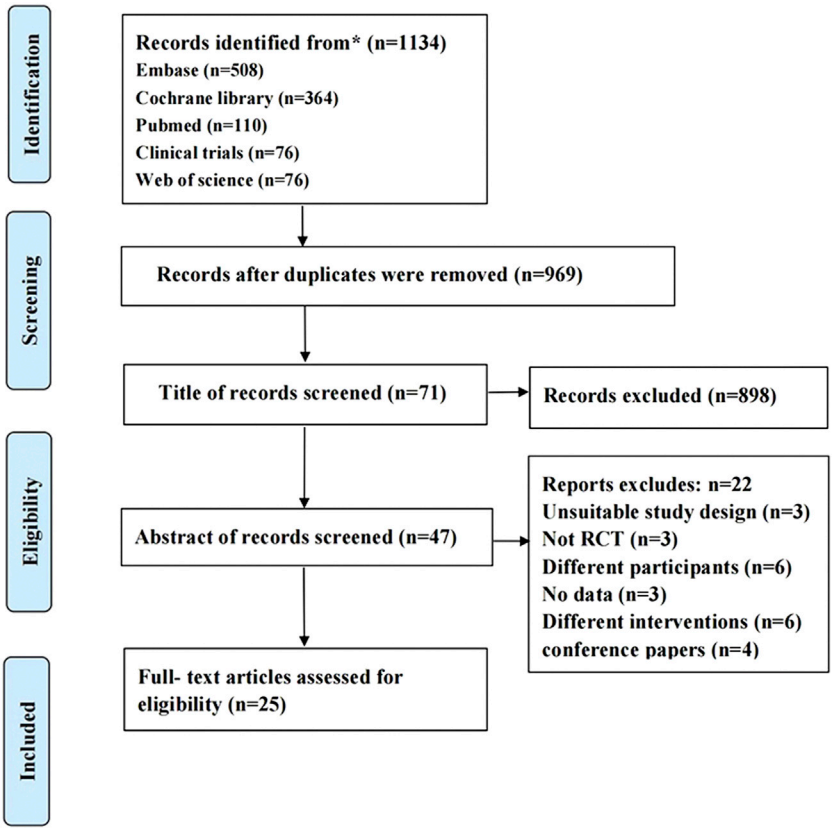

FIGURE 1
Prisivia flow chart of literature searching.

detailed information is provided. However, six studies had a high risk of bias in the integrity of outcome data for participants, one study had a hidden high risk of bias in allocation concealment, one study had a high risk of bias in implementation and measurement, and the results of one study were evaluated by the blind method. One study had a high risk of bias in allocation concealment because it could not be conducted. In addition, most studies had a low risk of bias and incomplete outcome data. The risk of bias assessment is shown in Figure 2.

3.3 Meta-analysis results

3.3.1 Main efficacy indicators—body mass index

Twenty-three studies analyzed BMI index changes between TCM treatment (n = 1,036) and control (n = 823). The TCM groups include 7 preparations based on a single botanical drug groups and 16 polyherbal preparations groups. The decline of BMI in the polyherbal preparations groups was significantly higher than control groups (MD = −1.16, 95% CI = −1.44, −0.89; p < 0.00001; I^2 = 34%). There was no significant difference in the preparations based on a single botanical drug groups (MD = 0.08, 95% CI = −0.61, 0.78; p = 0.81; I^2 = 0%) (Figure 3)

3.3.2 Secondary efficacy index

3.3.2.1 Weight

Twenty studies analyzed weight changes between TCM treatment (n = 962) and control (n = 755). The TCM groups include 7 preparations based on a single botanical drug groups and 13 polyherbal preparations groups. The decline of weight in the polyherbal preparations groups was significantly higher than control groups (MD = −2.53, 95% CI = −3.08, −1.99; p < 0.00001; I^2 = 34%). There was no significant difference in the preparations based on a single botanical drug groups (MD = −0.13, 95% CI = −1.90, 1.64; p = 0.89; I^2 = 13%) (Figure 4)

3.3.2.2 Waist circumference

Seventeen studies analyzed waist circumference changes between TCM treatment (n = 869) and control (n = 658). The TCM groups include 7 preparations based on a single botanical drug groups and 13 polyherbal preparations groups. The decline of Weight in the polyherbal preparations groups was significantly higher than control groups (MD = −2.64, 95% CI = −3.42, −1.87;

TABLE 1 Characteristics of included studies.

Study ID	Sample size	Age (years old) (m ± sd)		Number (male/ Total)		Base line BMI (m±sd)		Study population	intervention		Lifestyle intervention		Study duration
		TCM	Control	TCM	Contral	TCM	Contral		TCM	Contral	Diet	Sports	
Boozer et al., (2001)	67	40.0 ± 9.4	42.2 ± 8.1	4/32	6/35	32.6 ± 2.9	32.7 ± 2.7	simple obesity	Ma Huang-Guarana combination, 2 tablets, three times daily	placebo, 2 tablets, three times daily	limit intake of dietary fat to 30% of calories	walking 30 min a day, three times a week	8 weeks
Coffey et al., (2004)	102	44.9 ± 9.1	42.1 ± 10.9	5/52	9/50	35.1 ± 2.9	34.0 ± 2.9	simple obesity	125 mg Ma huang, 250 mg Kola nut, and 100 mg White willow bark, two caplets, three times daily	Placebo, two caplets, three times daily	a pamphlets about lifestyle modifications but no additional counseling		12 weeks
Hioki et al., (2004)	81	52.6 ± 14.0	54.8 ± 12.5	0/41	0/40	36.7 ± 6.80	36.1 ± 3.30	with Impaird glucose tolerance (IGT)	Bofu-tsusho-san, three times daily	placebo, three times daily	1,200 kcal a day for the two months before the start of the study	5,000 steps a day for the two months before the start of the study	24 weeks
Wang et al., (2006)	60	50.97 ± 11.10	49.24 ± 10.07	19/31	18/29	28.02 ± 2.17	28.72 ± 2.23	with hypertension	Pinggan Yishen Ditan Yin, take one dose a day, twice; lotensin, 10 mg a day	lotensin, 10 mg a day	reasonable diet	moderate exercise	8 weeks
Kim et al., (2008)	37	33.8 ± 7.9	30.8 ± 7.4	0/21	0/16	27.4 ± 2.3	27.9 ± 2.0	simple overweight/ obesity	2 g of ephedra and 1 g corn starch	placebo, 3 g corn starch	low-calorie diet of 1,200 kcal a day	40 min walk, five times in a week	8 weeks
Kamiya et al., (2011)	36	39.3 ± 12.4	36.7 ± 9.4	15/18	15/18	25.5 ± 2.9	26.5 ± 2.5	simple overweight/ obesity	300 mg PFE	300 mg placebo	males: 2,650 kcal a day females: 2,300 kcal a day	NA	8 weeks
Kamali et al., (2012)	60	39.16 ± 9. 59	36.36 ± 9. 9	6/30	8/30	37.14 ± 5.40	36.29 ± 4.66	simple obesity	Itrifal Saghir, 5 g, twice daily	placebo, 5 g, twice daily	keep existing diet and life style during the study period		12 weeks
Ke et al., (2012)	85	46.5 ± 7.3	45.7 ± 7.5	23/45	20/40	28.7 ± 3.4	28.5 ± 3.7	with IGT	Linggui Zhugan Decoction, twice a day for a month, Stop the medicine for one month, three consecutive cycles	blank contral	carbohydrates (50%-60%), protein ≤ 30%, high-fiber; proportions of three meals, 2:2:1	60 min each time, three times a week, six months.	6 months
Sengupta et al., (2012)	41	41.6 ± 1.37	37.2 ± 1.52	7/21	5/20	34.41 ± 0.74	33.0 ± 0.73	simple obesity	LI85008F, 900 mg a day	placebo, 900 mg a day	2,000 kcal standard diet	Walk 5 days a week, 30 minutes each time	8 weeks

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TABLE 1 (*Continued*) Characteristics of included studies.

Study ID	Sample size	Age (years old) (m ± sd)		Number (male/ Total)		Base line BMI (m±sd)		Study population	intervention		Lifestyle intervention		Study duration
		TCM	Control	TCM	Contral	TCM	Contral		TCM	Contral	Diet	Sports	
Lenon et al.,(2012)	117	39.3 ± 13.2	40.4 ± 10.2	10/59	10/58	35.3 ± 4.8	36.0 ± 5.5	simple obesity	RCM-104, 4 capsules per time, three times per day	placebo, 4 capsules per time, three times per day	keep existing diet and life style during the study period		12 weeks
Tong et al., (2013)	399	54.4 ± 7.7	54.5 ± 7.6	149/292	47/107	26.3 ± 2.1	26.4 ± 2.4	with Type 2 diabetes mellitus (T2DM)	Tang-Min-Ling-Wan, 6 g, three times daily	placebo, 6 g, three times daily	NA	NA	12 weeks
Kazemipoor et al., (2013)	70	37.23 ± 9.34	37.00 ± 7.90	0/35	0/35	29.24 ± 3.36	30.39 ± 4.69	simple overweight/ obesity	caraway seed extract, 30 ml, once daily	placebo, 30 ml, once daily	NA	aerobics training for 180 minutes a week	12 weeks
Park et al., (2013)	112	39.2 ± 9.5	38.8 ± 10.1	7/57	10/55	31.8 ± 2.6	31.9 ± 3.8	controlled hypertension or T2DM or treated hyperlipidemia	Taeeumjowi-tang, 7 g, three times daily	placebo, 7 g, three times daily	1,500 kcal a day for men and 1,200 kcal a day for women	NA	12 weeks
Ofner et al., (2013)	40	48.1 ± 9.6	46.8 ± 9.4	4/20	4/20	31.2 ± 4.7	30.0 ± 5.3	simple overweight/ obesity	Salacia reticulata and vitamin D3 (SRD), three times daily	blank contral	guideline for lifestyle	twice-weekly training programs (each session 45 minutes)	4 weeks
Park et al., (2014)	111	41.56 ± 8.62	39.21 ± 10.12	unclear/ 55	unclear/ 56	29.72 ± 6.17	29.28 ± 3.11	simple overweight/ obesity	Bofutsushosan, 12 capsules a day	placebo, 12 capsules a day	20-25 kcal a day per kg body weight	NA	8 weeks
Mirtaheri et al., (2015)	64	36.0 ± 11.97	33.6 ± 4.8	unclear/ 32	unclear/ 32	33.6 ± 4.8	32.7 ± 3.7	simple obesity	licorice extract, 0.5 g, three times daily	placebo, 0.5 g, three times daily	reduce energy intake by 500 kcal	NA	8 weeks
Azushima et al., (2015)	106	59.2 ± 14.5	60.0 ± 12.9	28/54	29/52	31.3 ± 5.0	30.6 ± 4.9	with hypertension	Bofu-tsusho-san, 2.5 g, once daily	conventional control therapy group, 2.5 g, once daily	25-30 kcal/kg-standard body weight a day	exercise therapies	24 weeks
Kudiganti et al., (2016)	60	36.63 ± 1.64	39.47 ± 1.73	10/30	14/30	28.48 ± 0.25	28.20 ± 0.24	simple obesity	Meratrim: one capsule (400 mg), two times daily	placebo, one capsule (400 mg), two times daily	2,000 kcal a day	30 min walk for five days per week	16 weeks
Satapathy et al., (2016)	30	21.06 ± 1.39	20.86 ± 0.66	unclear/ 16	unclear/ 14	25.21 (24.03–28.35) *	26.25 (24.49–27.70) *	simple overweight/ obesity	Tulsi (Ocimum sanctum) extract: 250 mg, twice daily	blank contral	keep their diet and sports		8 weeks

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TABLE 1 (*Continued*) Characteristics of included studies.

Study ID	Sample size	Age (years old) (m ± sd)		Number (male/ Total)		Base line BMI (m±sd)		Study population	intervention		Lifestyle intervention		Study duration
		TCM	Control	TCM	Contral	TCM	Contral		TCM	Contral	Diet	Sports	
Cho et al., (2017)	60	39.5 ± 11.2	41.7 ± 11.1	10/30	8/30	27.1 ± 1.5	27.2 ± 1.2	simple overweight/ obesity	YY-312, 800 mg, three times daily	placebo, 800 mg, three times daily	reduce their energy intake by 500 kcal a day	maintain their usual level of physical activity	12 weeks
Chung et al., (2016)	20	50.00 ± 5.85	45.20 ± 9.52	6/10	6/10	29.50 ± 3.63	28.89 ± 2.96	more than 2 metabolic risk factors	Qingxue Dan, 900 mg a day	placebo, 900 mg a day	write a self-reporting diet and exercise diary everyday		8 weeks
Zuniga et al., (2017)	24	43 ± 1	41 ± 4	2/12	2/12	31.2 ± 2.5	30.3 ± 3.2	2 metabolic risk factors	Gymnema sylvestre, 300 mg, twice daily	placebo, 300 mg, twice daily	maintain their normal diet and physical activity levels		12 weeks
Daneshi-Maskooni et al., (2018)	20	unclear	unclear	0/10	0/10	33.11 ± 1.01	32.86 ± 1.88	with T2DM	Fenugreek, 5 g per serving were consumed 30 min before meals	placebo, 100 g yoghurt with flavors	NA	NA	8 weeks
Gholaman and Gholami, (2018)	87	45.5 ± 8.9	45.0 ± 7.7	27/43	27/44	30.5 ± 2.4	30.7 ± 3.2	with nonalcoholic fatty liver disease (NAFLD)	green cardamom, 3 g a day	placebo, 3 g a day	24 h food recall	aerobic physical activity at least 3 times a week for 30-45 minutes	3 months
Cheon et al., (2020)	149	43.7 (41.6-45.8)*	42.5 (40.0-44.9)*	0/76	0/73	32.4 (31.6-33.3)*	33.3 (32.3-34.3)*	Depends on BMI. None or more than one of metabolic risk factors	Euiiyin-tang, 3 g, three times daily	placebo, 3 g, three times daily	low-calorie diet during the study	NA	12 weeks

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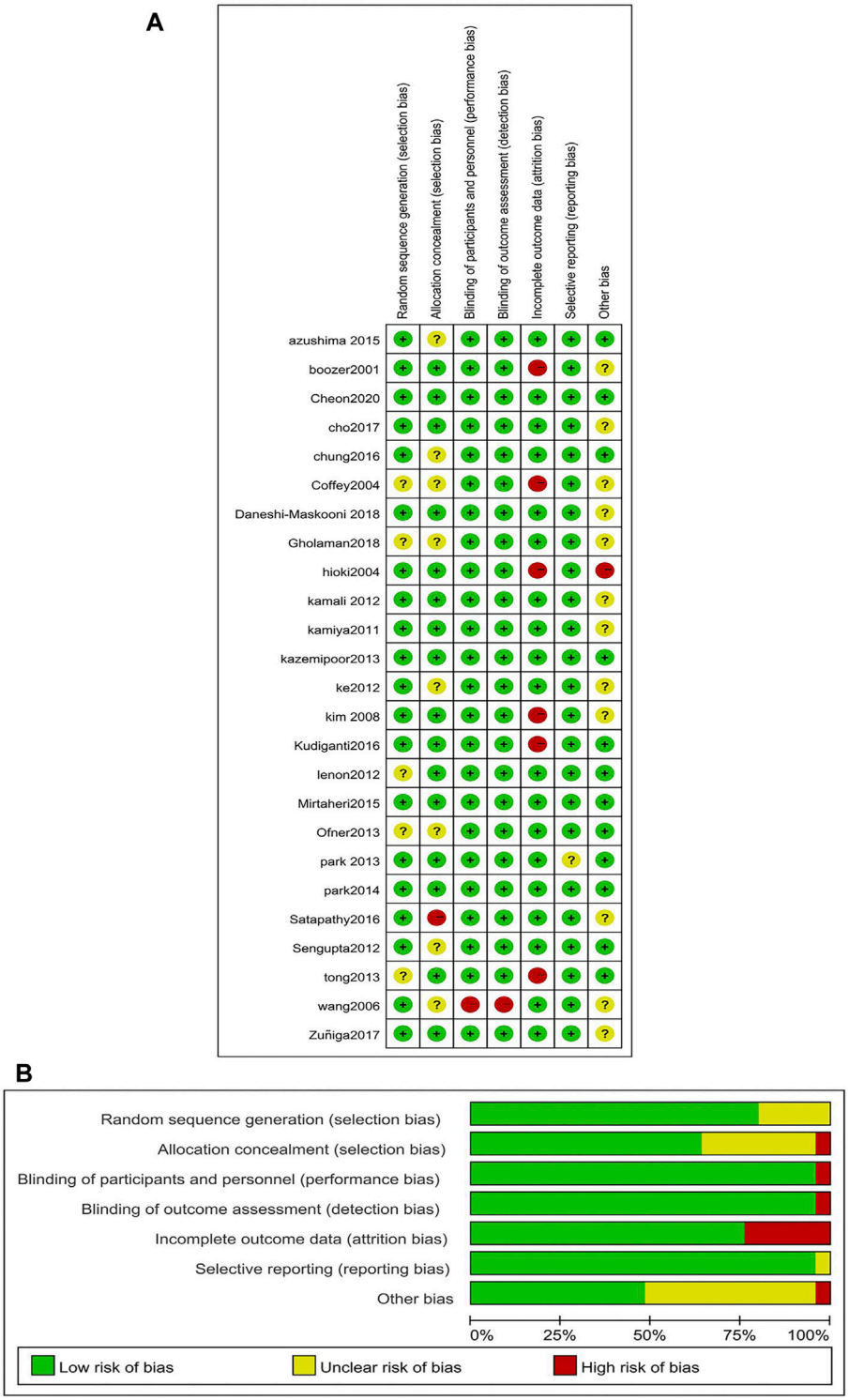

FIGURE 2
Risk of assessment for eligible studies. **(A)** Risk of bias summary; **(B)** Risk of bias graph.

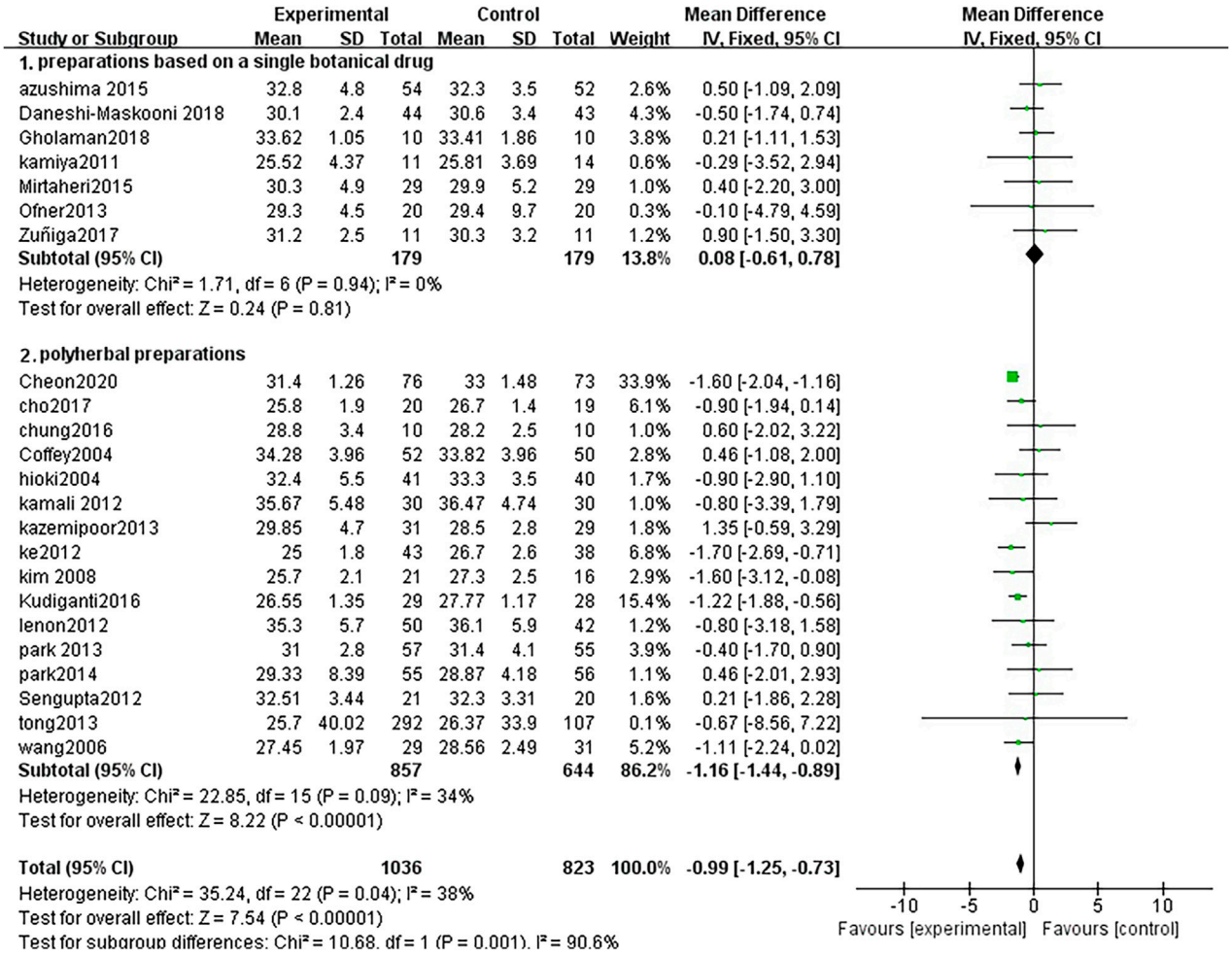

FIGURE 3
Forest plot of BMI. Comparison: TCM treatment (1.preparations based on a single botanical drug 2.polyherbal perparations). vs. placeo or blank control.

$p < 0.00001$; $I^2 = 0\%$). There was no significant difference in the preparations based on a single botanical drug groups (MD = −1.69, 95% CI = −5.12, 1.73; $p = 0.33$; $I^2 = 0\%$) (Figure 5)

3.3.2.3 Hip circumference

Eight studies analyzed the changes in Hip circumference between TCM treatment ($n = 317$) and control ($n = 304$). The hip circumference reduction in the TCM group was significantly more than control groups (MD = −3.48, 95% CI = −4.13, −2.83; $p < 0.00001$; $I^2 = 0\%$) (Figure 6).

3.3.2.4 Body fat rate

Ten studies analyzed the changes in the body fat rate between TCM treatment ($n = 50$) and control ($n = 339$). The TCM groups include 3 preparations based on a single botanical drug groups and 7 polyherbal preparations groups. There was no significant difference in the polyherbal preparations groups (MD = 0.47, 95% CI = −0.80, 1.75; $p = 0.47$; $I^2 = 15\%$). There was no significant difference in the preparations based on a single botanical drug groups (MD = −0.98, 95% CI = −2.05, 0.09; $p = 0.07$; $I^2 = 41\%$) (Figure 7)

3.3.2.5 Triglycerides

Nine studies analyzed the changes in TG levels between TCM treatment ($n = 297$) and control ($n = 283$). The TCM treatment groups were polyherbal preparations. The decrease of TG in the polyherbal preparations groups was significantly higher than control groups (MD = −4.19, 95% CI = −6.35, −2.03; $p = 0.0001$; $I^2 = 25\%$) (Figure 8).

3.3.2.6 Total cholesterol

Eight studies analyzed the changes in TCHO levels between TCM treatment ($n = 276$) and control ($n = 263$). The TCM treatment groups were all polyherbal preparations. The decrease

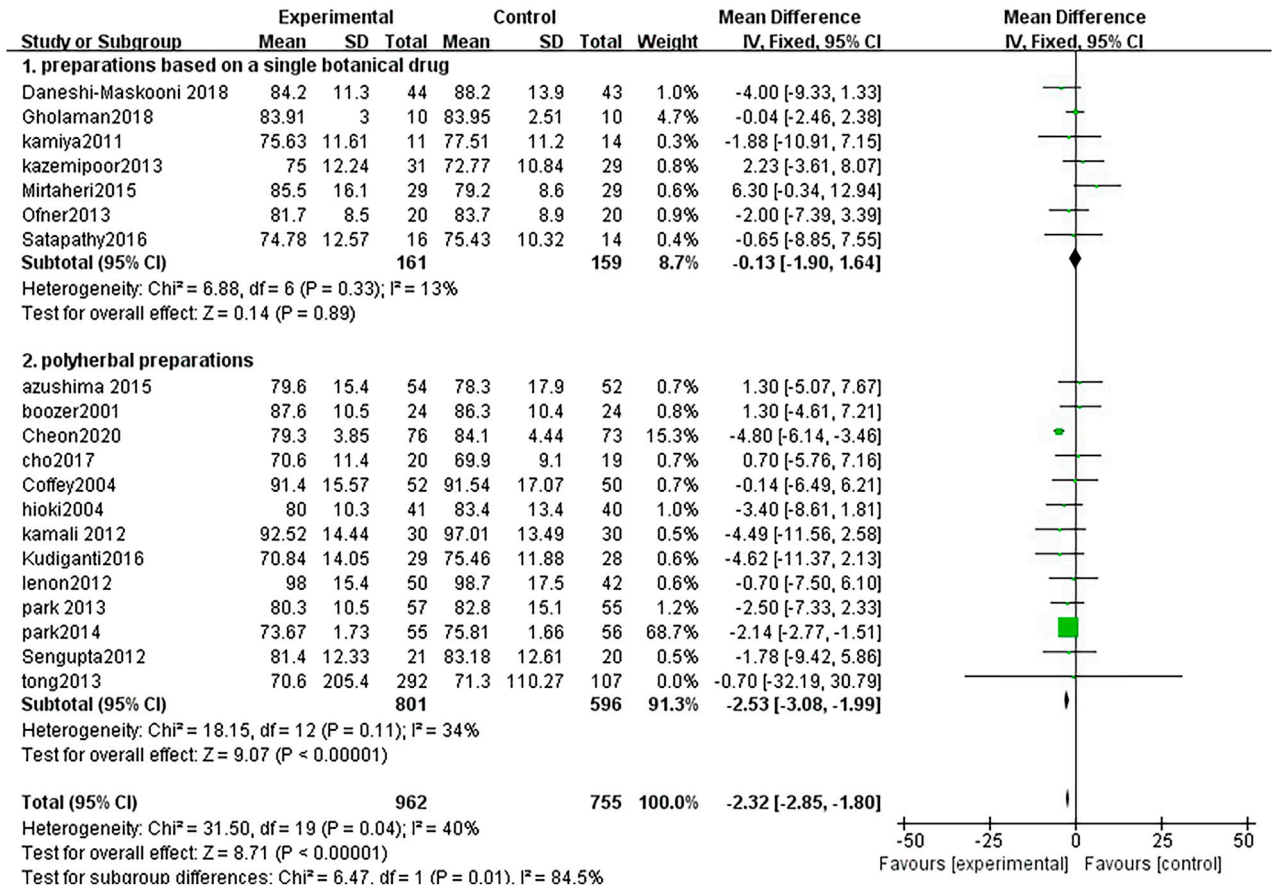

FIGURE 4
Forest plot of weight. Comparison: TCM treatment (1.preparations based on a single botanical drug 2.polyherbal perparations). vs. placeo or blank control.

of TCHO in the TCM intervention group was significantly higher than control group (MD = −10.45, 95% CI = −18.92, −1.98; p = 0.02; I^2 = 63%) (Figure 9).

3.3.2.6 Low-density lipoprotein

Seven studies analyzed the changes in LDL between TCM treatment (n = 219) and control (n = 212). The TCM treatment groups were all polyherbal preparations. There was no significant difference in LDL between TCM intervention group and control group (MD = −7.10, 95% CI = −16.43, 2.23; p = 0.14; I^2 = 68%) (Figure 10).

3.3.2.7 High-density lipoprotein

Eight studies analyzed HDL changes between TCM treatment (n = 276) and control (n = 267). The TCM treatment groups were all polyherbal preparations. The decrease of HDL in the TCM group was significantly higher than control group (MD = −3.60, 95% CI = −6.73, −0.47; p = 0.02; I^2 = 81%) (Figure 11).

3.3.2.8 Fasting blood glucose

Four studies analyzed FBG levels in TCM treatment group (n = 386) compared with control group (n = 195). The FBG level in the TCM group was lower than control group (MD = −0.77, 95% CI = −1.24, −0.29; p = 0.001; I^2 = 91%) (Figure 12).

3.3.2.9 Glycated hemoglobin

Three studies analyzed HbA1c levels in the TCM treatment group (n = 376) compared with the control group (n = 185). There was no significant difference between TCM group and control group (MD = −0.04, 95% CI = −0.21, 0.14; p = 0.69; I^2 = 0%) (Figure 13).

3.3.2.10 Blood pressure

Ten studies analyzed blood pressure in overweight and obese people with TCM treatment (n = 359) and control (n = 343), of which two studies were focusing on overweight and obesity combined hypertension and the remaining eight

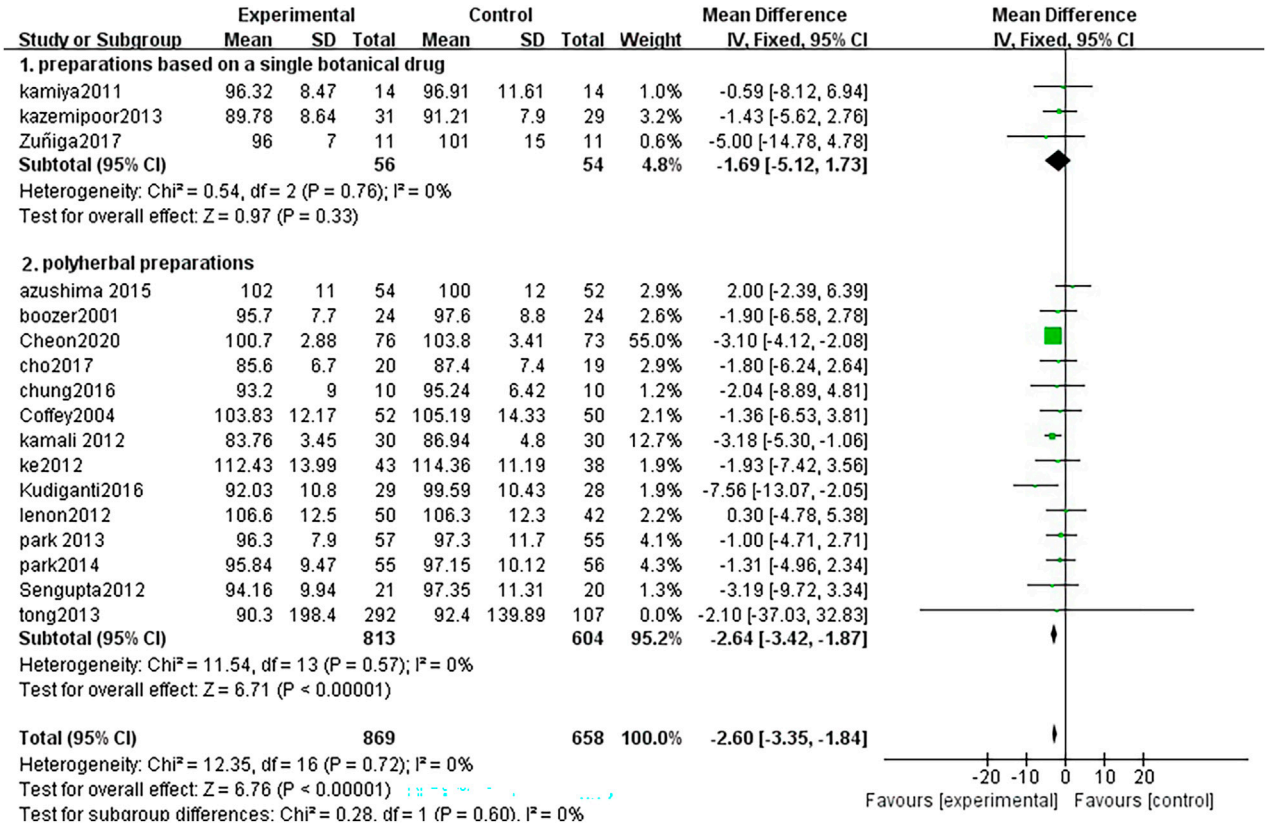

FIGURE 5
Forest plot of waist circumference. Comparison: TCM treatment (1.preparations based on a single botanical drug 2.polyherbal perparations). vs. placeo or blank control.

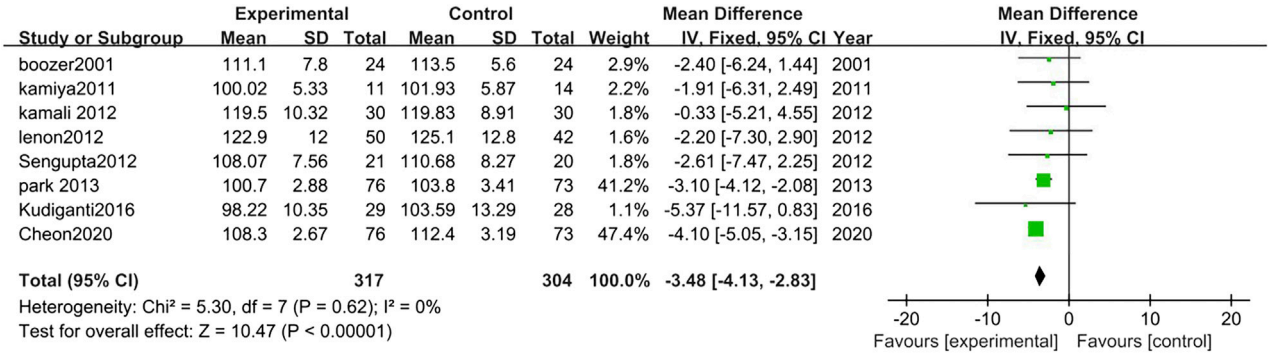

FIGURE 6
Forest plot of hip circumference. Comparison: TCM treatment vs. placeo or blank control.

studies were on overweight and obesity with normal blood pressure. The results of two study populations with hypertension are as follows: SBP: MD = −5.27, 95% CI = −8.35, −2.19; p = 0.0008; I^2 = 58%, DBP: MD = −4.30, 95% CI = −5.90, −2.69; p < 0.00001; I^2 = 0%. The results of the other eight studies: SBP: MD = −5.27, 95%

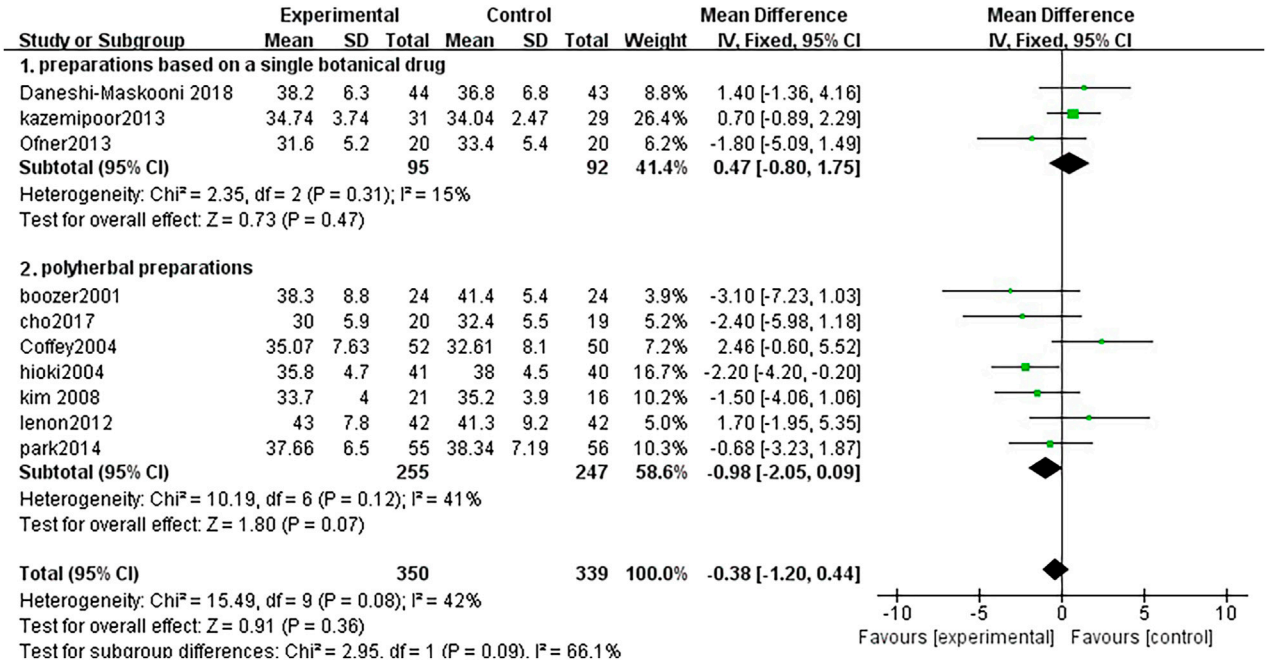

FIGURE 7
Forest plot of body fat rate. Comparison: TCM treatment (l.preparations based on a single botanical drug 2.polyherbal perparations). vs. placeo or blank control.

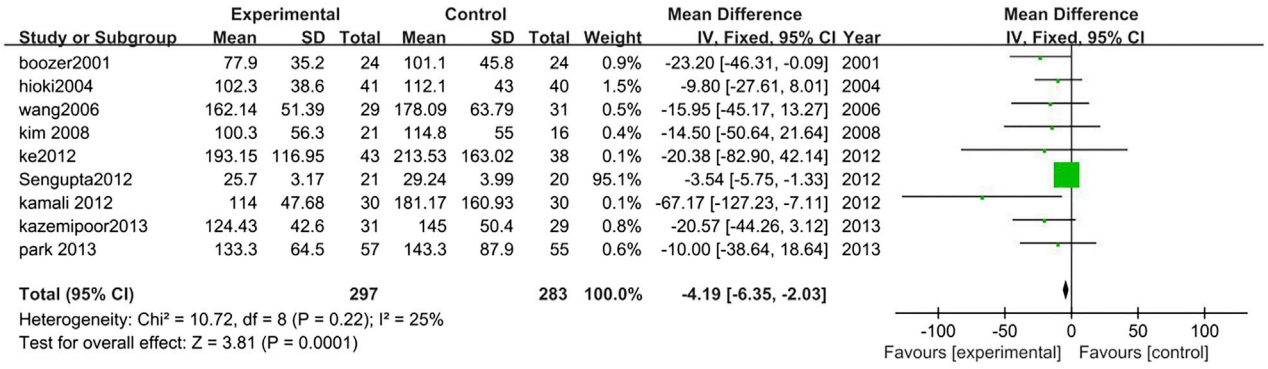

FIGURE 8
Forest plot of TG. Comparison: TCM treatment vs. placeo or blank control.

CI = −8.35, −2.19; p = 0.0008; I^2 = 58%, DBP: MD = −4.30, 95% CI = −5.90, −2.69; p < 0.00001; I^2 = 0%. According to the results of the current analysis, there was no significant difference in blood pressure before and after TCM intervention in overweight and obese people with normal blood pressure. For overweight and obese people with hypertension, TCM has a certain antihypertensive effect (Figure 14).

3.3.2.11 Safety

Six studies analyzed the safety between TCM treatment (n = 282) and control (n = 276).The results suggested that

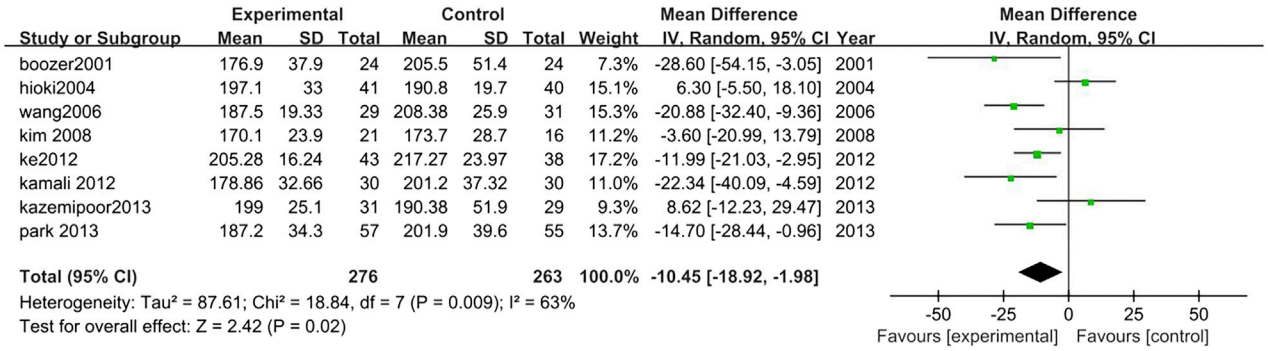

FIGURE 9
Forest plot of TCHO. Comparison: TCM treatment vs. placeo or blank control.

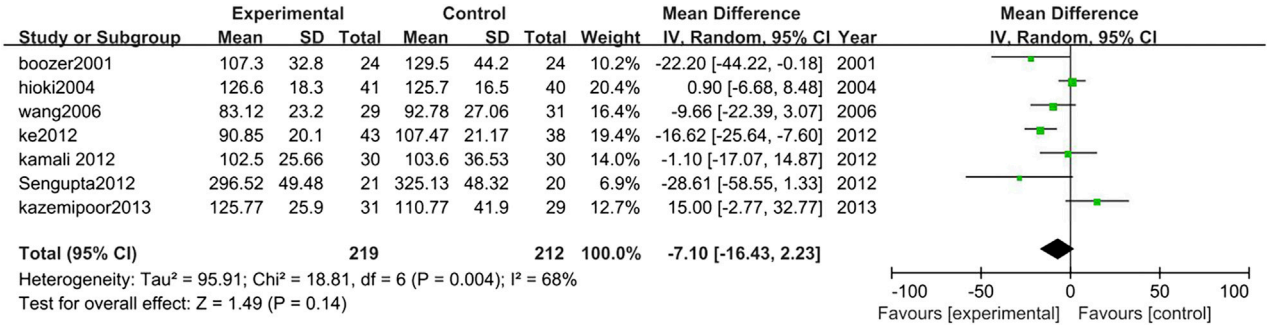

FIGURE 10
Forest plot of LDL. Comparison: TCM treatment vs. placeo or blank control.

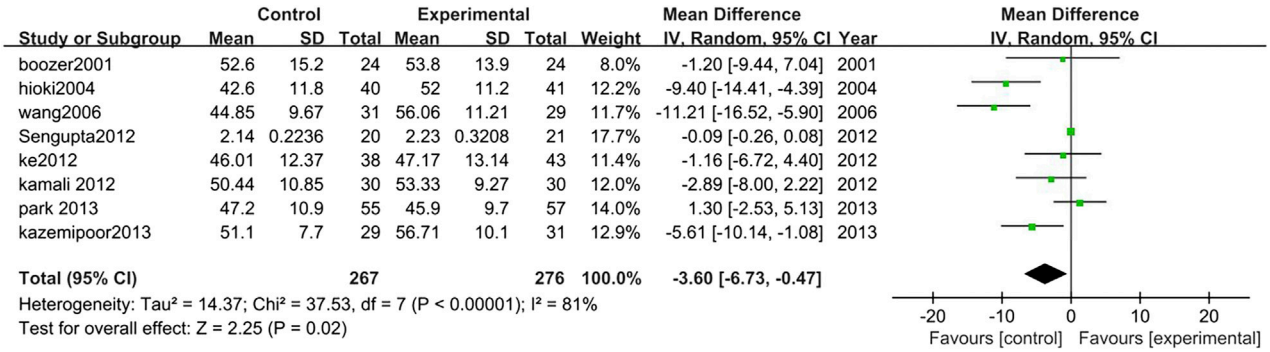

FIGURE 11
Forest plot of HDL. Comparison: TCM treatment vs. placeo or blank control.

there was no difference of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) before and after the TCM treatment (AST: MD = −0.19, 95% CI = −1.02, 0.64; $p = 0.65$; $I^2 = 0\%$, ALT: MD = −4.42, 95% CI = −9.52, 0.68; $p = 0.09$; $I^2 = 84\%$). However, in the study of Cheon et al. (2020) one patient presented with elevated ALT and AST after TCM

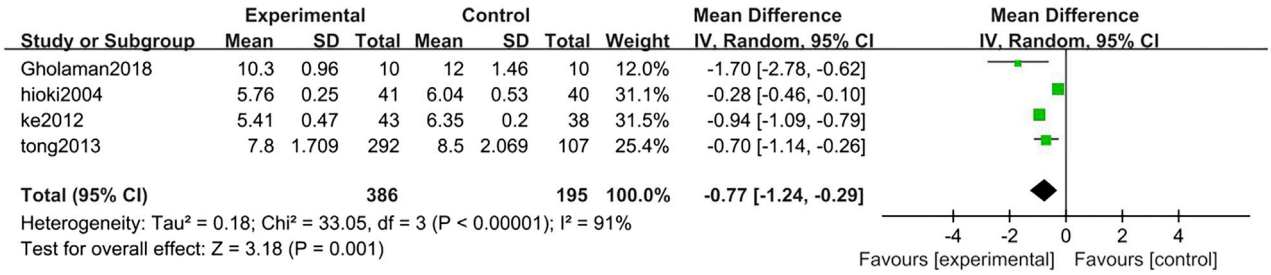

FIGURE 12
Forest plot of FBG. Comparison: TCM treatment vs. placeo or blank control.

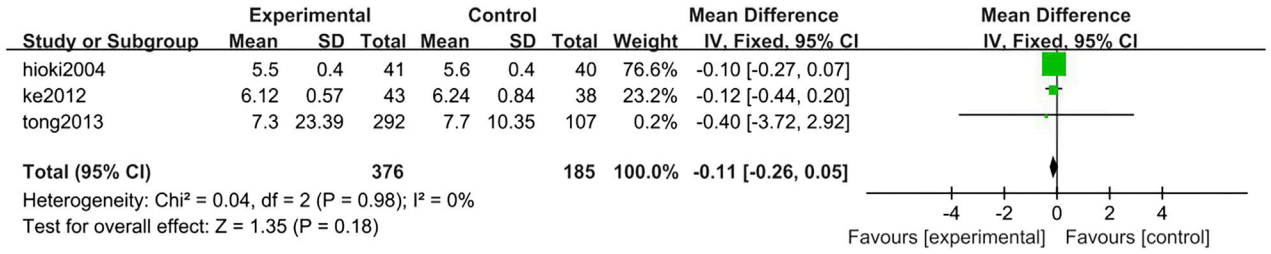

FIGURE 13
Forest plot of HbAlc. Comparison: TCM treatment vs. placeo or blank control.

treatment. Tong et al. (2013) reported that two of these patients had transient ALT and AST elevation (Figure 15).

3.3.2.12 Adverse effects

Adverse effects were reported in 19 of the 25 studies. Mirtaheri et al. (2015) reported no adverse effects, other five studies (Wang et al., 2006, Kamiya et al., 2011, Ofner et al., 2013; Satapathy et al., 2016; Gholaman and Gholami, 2018) did not mention the occurrence of adverse effects. Most of the reported adverse reactions were mild, mainly in the digestive system, such as dry mouth, epigastric pain, nausea and indigestion, constipation, or diarrhea. As well as neurological symptoms such as dizziness or headache, insomnia, etc., and mood changes. See Table 2 for details.

3.3.2.13 Publication bias

The software was used to analyze the publication bias of 20 studies on the main outcome - BMI and 23 studies on the outcome—weight (Figure 16).

3.3.2.14 GRADE of the outcomes

We used GRADE Profiler 3.6 to evaluate all outcome indicators in the following respects: 1) downgrading the quality of evidence, risk of bias, inconsistencies, indirectness, inaccuracy, and publication bias. 2) upgrading the quality of evidence, large effect, possible confounding change effect, and dose-response gradient. After a comprehensive analysis, the evidentiary body was formed and found that all outcome indicators had low quality or extremely low quality. See Table 3 for details.

3.3.2.15 Sensitivity analysis

We used stata15 SE software to conduct sensitivity analysis on its main outcome-weight and BMI (Figure 17).

4 Discussion

4.1 Research results

We conducted a systematic evaluation based on 25 current RCTs including 1,947 subjects. We compared the efficacy of weight reduction and the effects on blood glucose, blood lipids, and blood pressure in overweight and obese patients in the TCM group and control group. TCM treatment is divided into preparations based on a single botanical drug and polyherbal preparations. From the analysis results, TCM preparations for overweight and obese people can effectively reduce weight, BMI, waist circumference, and hip circumference after certain periods. There was no significant difference in the preparations based on a single botanical drug group compared to the control group. At the same time, the obesity-related risk factors were analyzed.

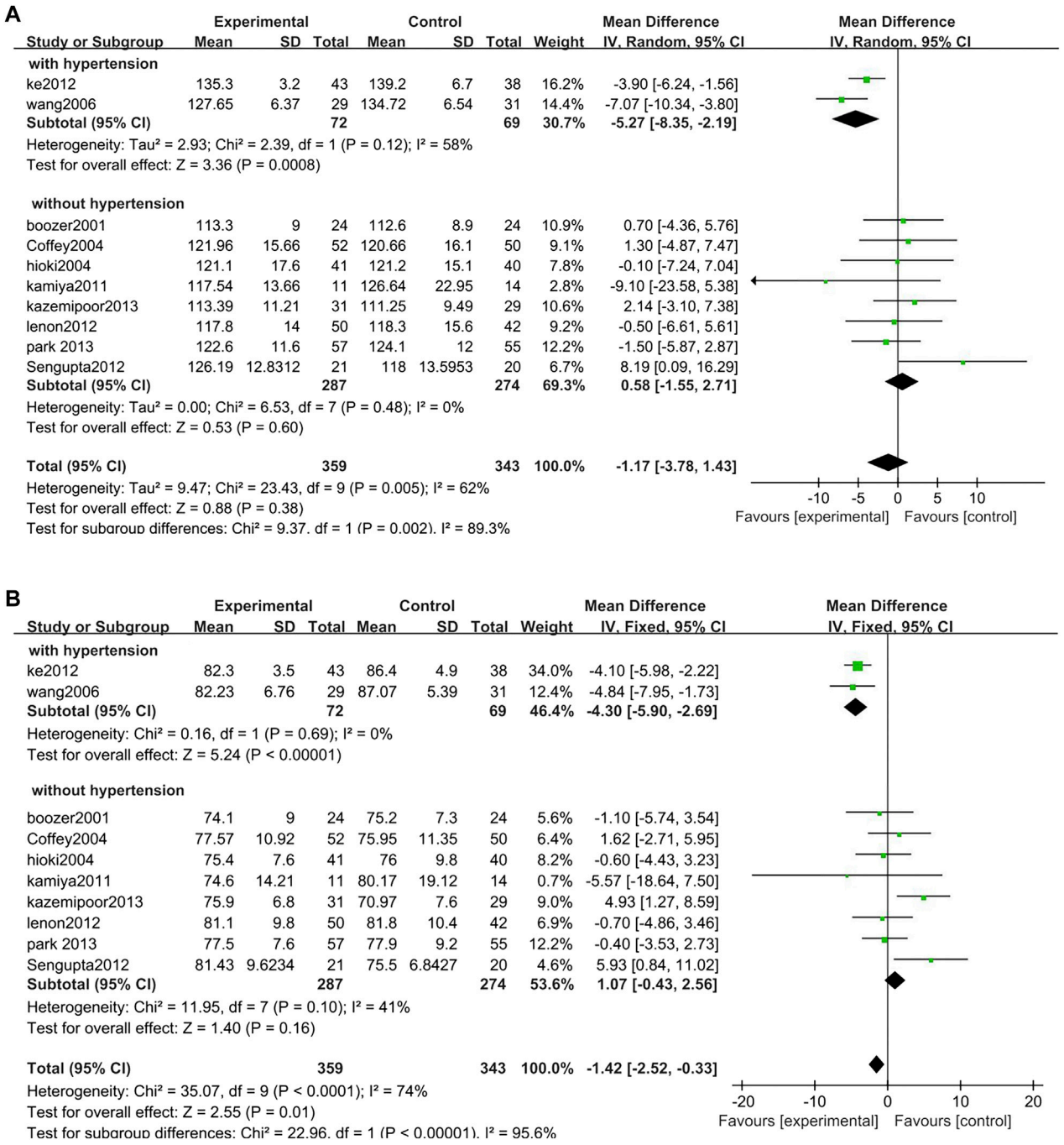

FIGURE 14
Forest plot of blood pressure. Comparison: TCM treatment vs. placeo or blank control. **(A)** Forest plot of SBP. **(B)** Forest plot of DBP.

Compared with control, TCM preparations can reduce FBG and blood pressure, and regulate lipid metabolism disorder in overweight and obese patients with diabetes mellitus. There is no significant difference in liver function before and after the intervention of TCM, which has high safety and fewer adverse reactions.

4.2 Analysis of the curative effect of TCM

4.2.1 Lose weight

In recent years, the BMI of the global population has been increasing (Jaacks et al., 2019). Compared with western countries, the obesity rate in China is low, but the growth

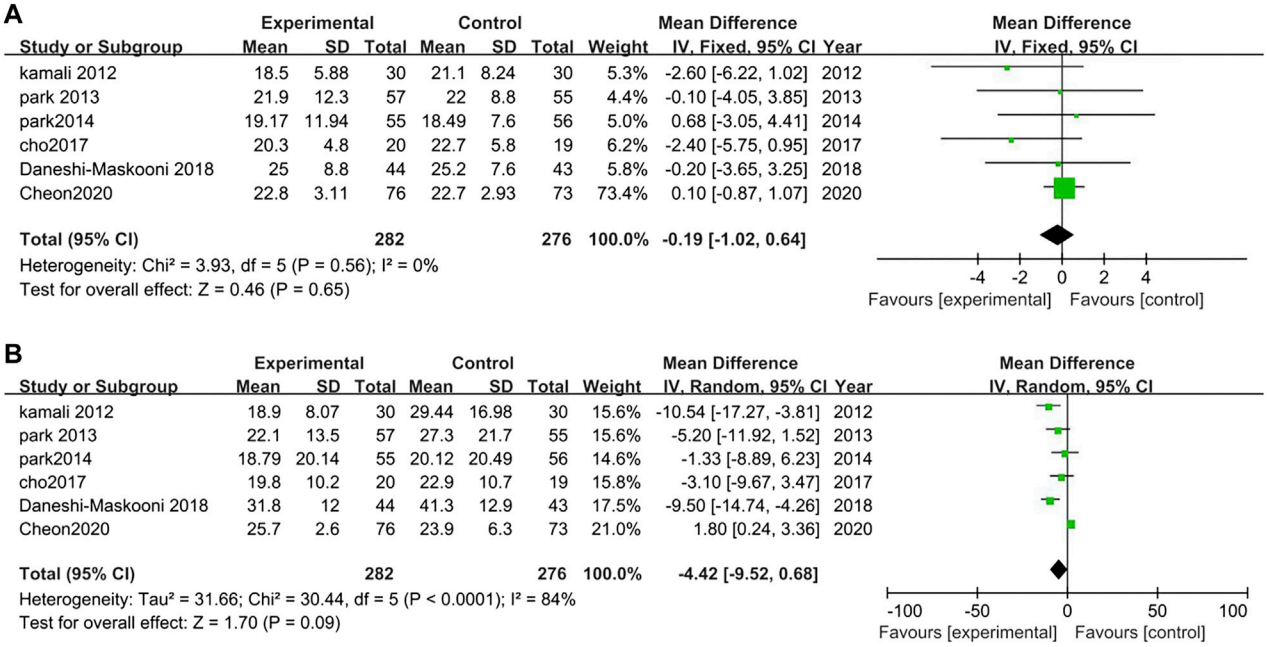

FIGURE 15
Forest plot for safety. Comparison: TCM treatment vs. placeo or blank control.**(A)** Forest plot of AST. **(B)** Forest plot of ALT.

trend is obvious (Wang et al., 2021). From 1989 to 2015, the number of overweight adults increased from 16.8% to 31%, and the number of obese adults increased from 3.8% to 11.3% (Pan et al., 2021). Therefore, it is urgent to explore new therapies that can effectively improve the treatment of obesity.

The systematic evaluation proved that 23 studies analyzed the BMI of 1,036 people in the TCM group and 823 people in the control group. The BMI of the TCM group decreased significantly more than the control group. And the decline was more pronounced in the polyherbal preparations group 20 of the 25 included studies analyzed and compared the weight of 962 people in the TCM treatment group and 755 people in the control group, it was found that the weight loss in the TCM group was significantly more than that in the control group. Likewise, the decline was more pronounced in the polyherbal preparations group. 17 studies analyzed the waist circumference of 869 people in the TCM group and 658 people in the control group. The waist circumference of the TCM group decreased significantly more than the control group. There was also a significant change in hip circumference in the herbal treatment group compared to the control group. However, there is some heterogeneity in the combination of these studies. These studies were from different countries and geographic regions, and the different BMI levels and comorbidity differences in overweight and obese people may be the source of these heterogeneities.

Interestingly, the study found that the overall herbal treatment was superior to the control group, but it appears the herbal compound has a larger variety of drugs and a more complex composition, which may have stronger effects. In addition, herbal compounding will also play a certain synergistic effect on each other compared to single prescriptions, which will form a complex pharmacological network. A single herb may not achieve this effect. In addition, TCM treatment is concerned with the relationship between the combination of ingredients, and an appropriate combination also improves the efficacy to some extent. This complex effect may be the key to impacting the synergistic effect of TCM multi-targets. In theory, choosing and creating a single target additive effect could realize the scientific compatibility of TCM and improve the curative effect and attenuate the toxicity (Weng et al., 2018).

At present, the main methods to treat obesity include lifestyle management, weight-loss drugs, bariatric surgery, reducing food intake and absorption, and improving its utilization (Bray et al., 2018). Weight-loss drugs act through peripheral and central mechanisms. They may achieve different degrees of rapid weight loss by increasing satiety, energy consumption, action pathway, and inhibiting calorie absorption (Heffron et al., 2020). However, they also have the characteristics of large side effects and many contraindications. Weight-loss drugs are suitable for a limited population and can potentially increase the risk of some diseases.

TABLE 2 Adverse reaction of included study.

Study ID	TCM treatment	Control
Coffey et al., (2004)	A total of 1 96 adverse events observed over the course of this study. Of the 1.02 patients in the study, 78 (76%) suffered at least one AE and 30 (29%) suffered at least one PTRAE over the course of the study. There was no difference in the occurrence of any adverse event between the two groups (77% for active vs. 76% for control, PY 0.91). There was no difference in the occurrence of any PTRAE between the two groups (33% for active vs. 26% for control, PY 0.46). Of the 78 subjects who experienced adverse events, 56 had multiple adverse events.	
	One subject had two adverse events classified as serious: 'Low Back Pain' and 'Compression Fracture of LI '	One subject had three adverse events. 'Exacerbated Depression', 'Atrial Fibrillation', and 'Exacerba- tion of Asthma'
Hioki et al., (2004)	loose bowels (n = 3)	No serious adverse effects
Kim et al, (2008)	Palpitation (n = 1), Headache (n = 7), Dull head (n = 4), I nsomnia (n = 4), Dizziness (n = 4), Nervousness (n = 1), Nausea (n = 2), Vomiting (n = 2), Anorexia (n = 1), Constipat ion (n = 12), Dry mouth (n = 6)	Insomnia (n = 2) Nervousness (n = 1) Constipation (n = 3) Eruption (n = 1) Dry mouth (n = 1)
Kamali et al, (2012)	Without statistically significant differences in rates of any adverse events.	
Ke et al, (2012)	There were five patients that felt fatigue, hunger and dizziness, and which was recovered after giving normal diets. Besides, no serious side effect was found.	No serious adverse effects
Sengupta et al, (2012)	There were no major adverse events reported. Some minor adverse events such as gastric irritation, abdominal pain and back pain were reported by few subjects. These minor events were distributed evenly between the placebo and treatment groups.	
Lenon 2012	nausea (n = 4) headache (n = 9)	decreasein appetite (n = 2)
Tong et al, (2013)	There were no medium or serious adverse events reported. Twenty-four mild adverse events (6.69%) were reported in experimental group versus 7 mild adverse events (5.83%) reported (p = 0.743). In the Experimental group, there were two cases with transient slight ALT elevation and two with AST elevation.	
Kazemipoor et al, (2013)	only the placebo participants experienced skin allergy to the placebo product , and no important adverse events were reported during the physical examinations.	
Park et al, (2013)	no adverse effects were reported.	
Park et al, (2014)	epigastric pain (n = 7) headache (n = 2) diarrhea (n = 3) nausea and vomiting (n = 2), palpitations (n = 1)	dyspepsia and epigastric pain (n = 3) and headache (n = 1)
Azushima et al, (2015)	3 patients in the experienced minor adverse events (gastric irritation, constipation, and elevation of serum hepatic enzyme level).	no adverse events reported in the control group
Kudiganti et al, (2016)	7 minor adverse events occurred in 5 people: Acidity (n = 2) Dyspepsia (n = 3) Nausea (n = l) Gastritis (n = 1)	9 adverse events occurred in 6 people : Dyspepsia (n = 2), Nausea (n = 1), Gastritis (n = 1), Pain Headache (n = 1), Itching (n = 1), Rash on forearm (n = 1), Giddiness (n = 1), Feet swelling (n = 1)
Chung et al, (2016)	There were no adverse sign except burning sensation, indigestion and fatigue for severa l volunteer, and no significantly different between two groups.	
Cho et al, (2017)	gastrointestinal symptoms, such as dyspepsia, nausea, epigastricsoreness ,diarrhea ,and constipation (n=4) upper respiratory tract infections (n = 3) headache and dizziness (n = 2) skin rash (n = 2) musculo skeletal pain (n = 1)	gastrointestina l symptoms, such as dyspepsia, nausea, epigastricsoreness, diarrhea, and constipation (n = 8) upper respiratory tract infections (n = 4) headache and dizziness (n = 2) musculo skeletal pain (n =1) fatigue (n = 1)
Zuniga et al, (2017)	two subjects (16.7"/o) reported diarrhea, pyrosis, and polydipsia, and one subject (8.3%) reported abdominal distension and headache	Two subjects (16.7%) from the placebo group reported headache and one patient (8.3%) reported diarrhea and pyrosis.
Daneshi-Maskooni et al, (2018)	No side effects associated with the treatment.	Only one patient reported nausea and constipation in one of his followed up

(Continued on following page)

TABLE 2 (*Continued*) Adverse reaction of included study.

Study ID	TCM treatment	Control
Cheon et al, (2020)	In total, 10 (6.7"/o) participants experienced adverse events during the trial, which included 4 (5.3%) from the	
	Headache (n = I)	Diarrhea (n = 1)
	Diarrhea (n = J)	Aspartate aminotransferase increased (n = 1)
	Herpes zoster (n = 1)	Alanine aminotransferase increased (n =1)
	Cholelithiasis (n = 1)	Concussion (n = 1)
	Dermatitis allergic (n = 1)	Peripheral swelling (n =1)
		Hypertonic bladder (n = 1)
		Uterine leiomyoma (n = 1)
		Uterine polyp (n = 1)

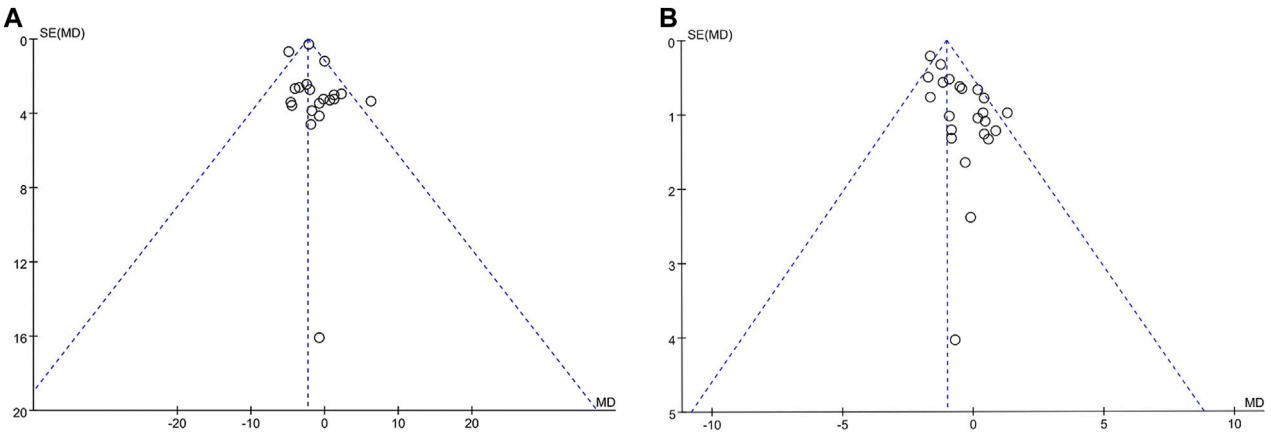

FIGURE 16
Funel plot of publication bias. Comparison: TCM treatment placeo or blank control. **(A)** Funel plot in publication bias in weight. **(B)** Funel plot on publication bias in BMI.

Orlistat may reduce body weight by inhibiting dietary fat absorption (Lavie et al., 2015), increasing adiponectin levels, reducing inflammation, and improving insulin sensitivity (Derosa et al., 2016). It can also improve glucose metabolism and reverse the development of impaired glucose tolerance to diabetes (Torgerson et al., 2004). But orlistat has the risk of causing a significant reduction of fatty vitamins and rare cases of severe liver injury have also been reported (Bray and Ryan, 2012). Lorcaserin promotes satiety by selectively activating 5-HT2C receptors on opioid melanocyte precursor (POMC) neurons in the arcuate nucleus of the hypothalamus (Donnelly et al., 2009), which can effectively reduce weight (Dong et al., 2017) and cardiovascular risk factors (Marso et al., 2016). However, *in vivo* studies have found that Lorcaserin has the risk of cancers, such as colorectal cancer, pancreatic cancer, and lung cancer (Sharretts et al., 2020). Liraglutide is used for weight management in chronic diseases. It achieves weight loss and reduces cardiovascular risk factors by increasing glucose sensitivity and inhibiting glucagon production. It can also reduce liver gluconeogenesis and slow stomach transport, promoting satiety and reducing energy intake (Astrup et al., 2012). But liraglutide is contraindicated in persons with a family history of medullary thyroid cancer or type 2 of multiple endocrine neoplasia. PHEN/TPM ER can stimulate the hypothalamus to release catecholamines and inhibit the reuptake of norepinephrine (Swift et al., 2014), reducing appetite and food consumption to effective weight loss (Garvey et al., 2012). It can also improve blood pressure, blood sugar, high-density lipoprotein, triglyceride, and total cholesterol (Gadde et al., 2011; Allison et al., 2012). However, PHEN/TPM ER has a greater risk of side effects, causing kidney stones and increasing heart rate. Use in the first trimester of pregnancy can increase the risk of cleft lip and cleft palate in infants, and the drug should not be used in

TABLE 3 Grade of the outcomes.

Quality assessment							No of patients		Effect		Quality Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Experimental	Contral	Relative (95% CI)	Absolute	
weight (Better indicated by lower values)											
20	randomised trials	very serious	no serious inconsistency	no serious indirectness	no serious imprecision	none	962	755	–	MD 2.32 lower (2.85 to 1.8 lower)	⊕⊕◯◯ LOW
BMI (Better indicated by lower values)											
23	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	no serious imprecision	none	1036	823	–	MD 1 lower (1.26 to 0.74 lower)	⊕⊕◯◯ LOW
Waist circumference (Better indicated by lower values)											
17	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	no serious imprecision	none	869	658	–	MD 2.62 lower (3.36 to 1.88 lower)	⊕⊕◯◯ LOW
Hip circumference (Better indicated by lower values)											
8	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	very serious[2]	none	317	304	–	MD 3.48 lower (4.13 to 2.83 lower)	⊕⊕◯◯ VERY LOW
waist to hip ratio (Better indicated by lower values)											
7	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	very serious[2]	none	266	245	–	MD 0.01 higher (0 to 0.01 higher)	⊕⊕◯◯ VERY LOW
body fat (Better indicated by lower values)											
10	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	very serious[2]	none	350	339	–	MD 0.38 lower (1.2 lower to 0.44 higher)	⊕⊕◯◯ VERY LOW
Total cholesterol (Better indicated by lower values)											
8	randomised trials	very serious [1]	serious[3]	no serious indirectness	very serious[2]	none	276	263	–	MD 10.45 lower (18.92 to 1.98 lower)	⊕⊕◯◯ VERY LOW
Triglycerides (Better indicated by lower values)											
9	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	very serious[2]	none	297	283	–	MD 4.19 lower (6.35 to 2.03 lower)	⊕⊕◯◯ VERY LOW
high-density lipoprotrein (Better indicated by lower values)											
8	randomised trials	very serious[1]	very serious[4]	no serious indirectness	very serious[2]	none	276	267	–	MD 3.6 higher (0.47 to 6.73 higher)	⊕⊕◯◯ VERY LOW
low-density lipoprotein (Better indicated by lower values)											
7	randomised trials	very serious[1]	serious[3]	no serious indirectness	very serious[2]	none	219	212	–	MD 7.1 lower (16.43 lower to 2.23 higher)	⊕⊕◯◯ VERY LOW
systolic blood pressure (Better indicated by lower values)											
10	randomised trials	very serious[1]	serious[3]	no serious indirectness	very serious[2]	none	359	343	–	MD 3.15 lower (4.56 to 1.74 lower)	⊕⊕◯◯ VERY LOW

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TABLE 3 (*Continued*) Grade of the outcomes.

Quality assessment							No of patients		Effect		Quality Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Experimental	Contral	Relative (95% CI)	Absolute	
diastolic blood pressure (Better indicated by lower values)											
10	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	very serious[2]	none	359	343	–	MD 1.42 lower (2.52 to 0.33 lower)	⊕⊕◯◯ VERY LOW
Alanine aminotransferase (Better indicated by lower values)											
6	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	very serious[2]	none	282	276	–	MD 0.19 lower (1.02 lower to 0.64 higher)	⊕⊕◯◯ VERY LOW
Aspartate aminotransferase (Better indicated by lower values)											
6	randomised trials	very serious[1]	very serious[4]	no serious indirectness	very serious[2]	none	282	276	–	MD 4.42 lower (9.52 lower to 0.68 higher)	⊕⊕◯◯ VERY LOW
dropout rate											
24	randomised trials	very serious[1]	no serious inconsistency	no serious indirectness	no serious imprecision	none	201/1186 (16.9%)	156/979 (15.9%)	RR 1.06 (0.88 to 1.29)	10 more per 1000 (from 19 fewer to 46 more)	⊕⊕◯◯ LOW
								10.1%	6 more per 1000 (from 12 fewer to 29 more)		

[1]most articles are biased
[2]sample size is small
[3]Heterogeneity is large
[4]Heterogeneity is very large

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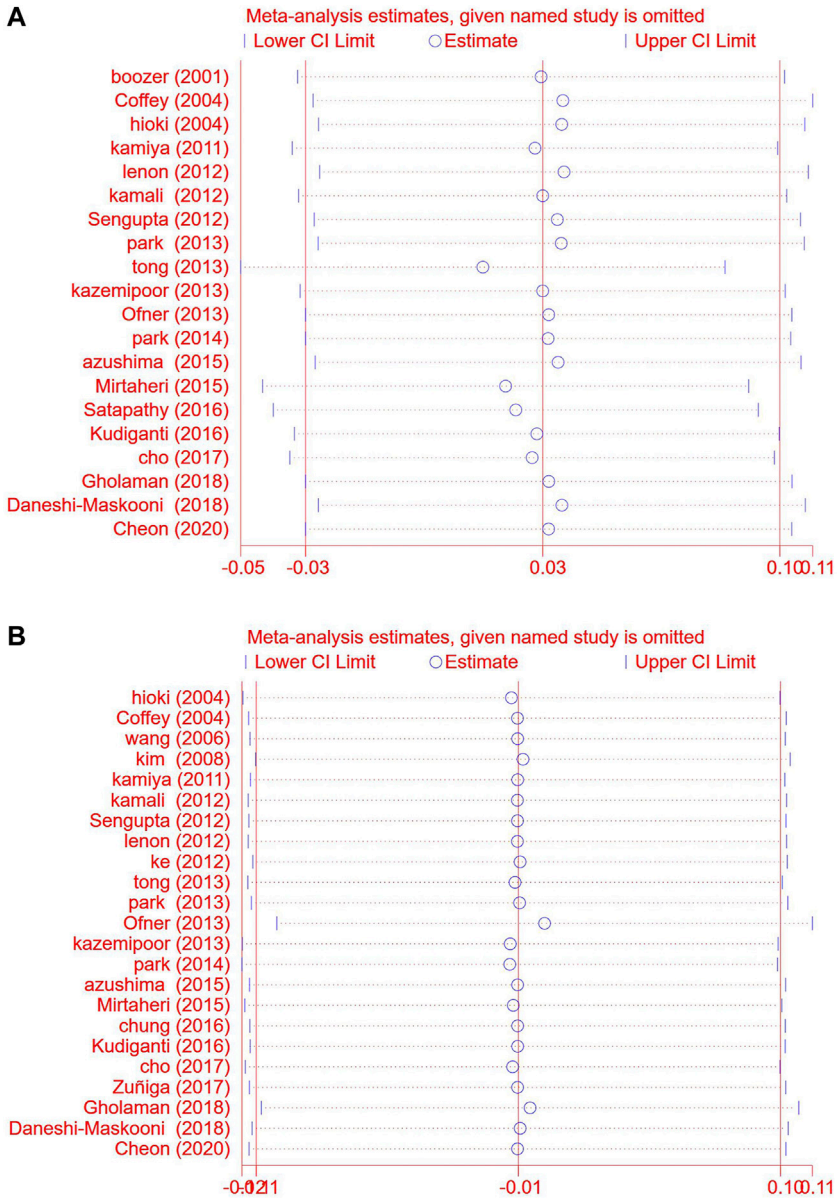

FIGURE 17
.Sensitivity analysis chart. Comparison: TCM treatment placeo or blank control. **(A)** Sensitivity analysis chart of weight. **(B)** Sensitivity analysis chart of BMI.

patients with glaucoma. The studies of naltrexone/bupropion (Greenway et al., 2010) have shown that it can effectively reduce body weight and HbA1c by inhibiting neuronal reuptake of dopamine and norepinephrine (Polak et al., 2006; Hollander et al., 2013). It also reduces waist circumference, fasting blood glucose, insulin level, high-density lipoprotein, and total cholesterol, but it will temporarily increase blood pressure and heart rate.

Surgical treatment of obesity has a definite curative effect. It is the first choice, especially for obese people with a high BMI which seriously affects their health and quality of life. At the same time, bariatric surgery also has some problems such as high perioperative and postoperative risks, certain complications, and difficulty to overcome fear.

Compared with western medicine and surgery, patients using TCM treatment for weight reduction are well tolerated and have no serious adverse effects. It applies to a wide range of people and has no clear contraindications. While reducing body weight, it can also alleviate some discomfort symptoms, such as can't stand the heat, hyperhidrosis, stickiness in the mouth, and so on. TCM

can regulate people's body composition and thus improve their quality of life (Sang et al., 2018).

4.2.2 Regulating glucose and lipid metabolism

The study found that, compared with placebo, TCM preparations treatment can reduce TG and TCHO, and improve HDL in overweight and obese patients. It can also reduce fasting blood glucose in overweight and obese patients with abnormal blood glucose. These results proved that TCM was involved in metabolic pathways *in vivo*, especially glucose and lipid metabolism. Modern pharmacology has also proved that saponins, polysaccharides, alkaloids, polyphenols, and other active ingredients in TCM can lose weight. They fight obesity by suppressing appetite, reducing digestion and absorption of exogenous lipids, and promoting oxidation and consumption of lipids (Zhang et al., 2014).

At the same time, compared with placebo, TCM treatment can also effectively reduce FBG of overweight and obese people with abnormal blood glucose. However, this article is inconclusive as to whether TCM modulates HbA1c, we can explore this further by including more studies in the future.

It can be seen that TCM was also involved in metabolic pathways in the body while exerting weight reduction effects. It takes advantage of the synergistic effect between drugs, participates in the regulation of glucose and lipid metabolism and blood pressure through multiple targets, and improves many potential metabolic risk factors associated with overweight and obesity (He et al., 2016). In addition, TCM can improve people's body composition (Sang et al., 2018) and reduce many discomfort symptoms (Zhang and Shen, 2013). The general population has good tolerance to TCM, and no serious adverse reactions have been found.

4.2.3 Probe into the potential mechanism of TCM

TCM intervention can reduce weight, correct glucose and lipid disorders, and regulate blood pressure compared to control. Its mechanism may be realized by regulating fat metabolism, intestinal flora, and hormone level, but the mechanism for muscle, liver, and pancreas is not clear (Li et al., 2020). Understanding from syndrome differentiation and treatment, Damp Heat Syndrome (DHS) is one of the most common "syndromes" in TCM (Ahirwar and Mondal, 2019), and obesity mostly belongs to "DHS". DHS is mainly characterized by changes in inflammatory factors and abnormal immune function. It is also closely related to oxidative damage, energy metabolism, endotoxin production, blood lipid metabolism, etc., (Guo et al., 2020; Zhang G. D. et al., 2021). In addition, obesity is prone to insulin resistance, which eventually leads to serious metabolic disorders. Anoxia is an important feature of "dampness" (Lu, 2010), "dampness" can lead to circulatory disorder and anoxia of adipose tissue and the small intestine. Also, the consumption of fatty and oily or sweet foods can cause fat accumulation and internal heat (Ahirwar and Mondal, 2019). Excessive production of free fatty acids (FFA) can lead to lipotoxicity or "lung toxicity" in the TCM, they together lead to chronic low-grade systemic inflammation. TCM may act on multiple targets of the pathological pathway of "lipotoxicity (non-toxicity) - inflammation - DHS - insulin resistance - metabolic disease". "Heat clearing" and "dampness clearing" drugs are mainly used to correct the situation of damp heat and relieve the symptoms of "heavy body trapped, fear of heat, thick and greasy tongue coating". These herbal drugs can improve glucose and lipid metabolism, reduce inflammation *in vivo*, inhibit hypoxia inducible factor (HIF), and reverse insulin resistance, to prevent and treat obesity metabolic disorder (Zhang C. H. et al., 2021). A large number of *in vitro* studies and animal experiments show that TCM has the potential for the multi-target treatment of obesity (Li et al., 2020).

4.2.4 Limitations of the study

However, these studies still have many limitations. First of all, the quality of original documents is not high, so the evidence level can be improved by enhancing the quality of TCM clinical trials in the future. Secondly, some high-quality studies of TCM for obesity and its complications are mainly yellow race. Therefore, more extensive studies are needed to clarify the practicality of TCM in different ethnic groups. Third, metabolism-related indicators such as blood pressure, blood glucose, and blood lipids are rarely included in clinical trials, with small sample sizes and large heterogeneity. Consequently, interpretation of the results needs to be cautious, and high-quality and large samples are needed to prove it. Finally, none of the included studies were followed up. It is unclear whether there is a rebound in weight loss and the long-term weight maintenance, so the long-term effects of weight loss with TCM treatment need further study.

5 Conclusion

In this meta-analysis of RCTs, TCM preparations can effectively reduce the weight, BMI, waist circumference, and hip circumference of overweight and obese people compared to control. Meanwhile, TCM preparations can regulate FBG and lipid metabolism and control blood pressure through multi-system treatment. However, long-term effects of TCM on weight loss still need to be further explored, which is also our future research goal.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

Author contributions

LHZ, YH and ZW: study concept and design; ZW, YH, QZ, and LLZ: protocol design. ZW, YH, and QZ: literature retrieval and data extraction. ZW, YH, and QZ: statistical analysis. ZW and YH: interpretation of data and drafting of the manuscript. YH and LHZ: Quality assessment; QZ and MS: adjust the article layout. YH, LLZ, and LHZ: critical revision of the manuscript. ZW: technical support. All authors take responsibility for the integrity of the data and the accuracy of data analysis. ZW, QZ, and LLZ contributed equally to this work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer XL declared a shared affiliation with the author MS to the handling editor at the time of review.

Publisher's note

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EDITED BY
Dongmei Li,
Georgetown University, United States

REVIEWED BY
Yu Zhao,
Shanghai University of Traditional Chinese Medicine, China
Freeborn Rwere,
Stanford University, United States

*CORRESPONDENCE
Hojun Kim,
kimklar@dongguk.ac.kr
Young-Do Nam,
youngdo98@kfri.re.kr

†These authors contributed equally to this work

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Impact of the herbal medicine, *Ephedra sinica* stapf, on gut microbiota and body weight in a diet-induced obesity model

Eun-Ji Song[1†], Na Rae Shin[2†], Songhee Jeon[3], Young-Do Nam[1*] and Hojun Kim[2*]

[1]Research Group of Personalized Diet, Korea Food Research Institute, Wanju-gun, Jeollabuk-do, South Korea, [2]Department of Rehabilitation Medicine of Korean Medicine, Dongguk University, Goyang-si, Gyeonggi-do, South Korea, [3]Department of Biomedical Sciences, Center for Global Future Biomedical Scientists at Chonnam National University, Gwangju, South Korea

Obesity is a chronic metabolic disease caused by excessive body fat and has become a global public health problem. Evidence suggests that obesity and obesity-induced metabolic disorders are closely related to gut microbiota. Bupropion (BP), an antidepressant medicine, and *Ephedra sinica* Stapf [Ephedraceae; Ephedrae Herba], a herbal medicine, are sympathetic stimulants and have weight loss effects. However, to our best knowledge, no studies have simultaneously assessed the effects of drugs and herbal medicines on obesity and gut microbiota. This study aimed to determine the effects of BP and ES on weight loss and re-modulation of host gut microbiota. To test this hypothesis, we fed C57BL/6J mice with a high-fat diet supplemented with bupropion (BP; 30 mg/kg/day) and *Ephedra sinica* Stapf extract (ES; 150 mg/kg/day) *via* oral gavage for eight weeks. Further, we evaluated the effects of BP and ES on body weight and fat accumulation. In addition, we evaluated the effects of BP and ES on gut microbiota using 16S rRNA amplicon sequencing. Our results showed that weight loss was confirmed in both BP and ES; however, it was more pronounced in ES. ES changed the overall composition of the gut microbiota by restoring the relative abundance of *Oscillospiraceae*, *Lachnospiraceae*, and the *Firmicutes*/*Bacteroidetes* ratio, an indicator of gut microbiota dysbiosis. Nine amplicon sequence variants (ASVs) of the gut microbiome were significantly recovered by BP and ES treatment, of which eight ASVs correlated with body weight and fat accumulation. Additionally, three ASVs were significantly recovered by ES treatment alone. In conclusion, the anti-obesity effects of BP and ES, especially fat accumulation, are related to the regulation of gut microbiota. Moreover, ES had a greater influence on the gut microbiota than BP.

KEYWORDS

obesity, anti-obesity drug, gut microbiota, bupropion, Ephedra sinica

Abbreviations: ASVs, amplicon sequence variants; BP, Bupropion; ES, Ephedra sinica Stapf; F/B, Firmicutes/Bacteroidete; FFA, free fatty acid; HFD, high-fat diet; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDL, low-density lipoprotein; PCoA, principal coordinate analysis; SCFAs, short-chain fatty acids.

1 Introduction

Overweight and obesity are global public health problems because obesity is related to chronic, systemic inflammation, leading to insulin resistance and ultimately type 2 diabetes (Rohm et al., 2022). Increased energy intake and reduced expenditure are the primary driving forces of obesity, and changes in exercise or dietary composition are recommended for weight loss (Romieu et al., 2017). Moreover, depending on the severity, prescription drugs or surgery is also recommended for the treatment of obesity (Nudel and Sanchez, 2019; Müller et al., 2022).

Herbal medicines are considered a common alternative therapy worldwide based on a thousand-year history and phenotype-based clinical trials (Li and Weng, 2017). Numerous studies have been conducted on the effectiveness and safety of herbal medicines for obesity and metabolic syndromes (Payab et al., 2020). Green tea extract reduces weight, waist circumference, total cholesterol, and low-density lipoprotein (LDL) plasma levels in women with central obesity (Chen et al., 2016). Extract of *Hibiscus sabdariffa* L. [Malvaceae] reduced obesity, abdominal fat, and serum free fatty acid (FFA) as well as improved liver steatosis in obese individuals (Chang et al., 2014). Cinnamon consumption has resulted in substantial improvements in all components of the metabolic syndrome in Asian Indians (Jain et al., 2017). *Ephedra sinica* Stapf [Ephedraceae; Ephedrae Herba] (ES), a traditional Chinese botanical drug, has sympathomimetic effects and has been used to treat colds, arthralgia, edema, and asthma (Rotblatt and Ziment, 2002). Moreover, ES is effective in reducing body weight, body mass, and body fat percentage in women with obesity (Kim et al., 2008; Kim et al., 2014). The *Ephedra* and caffeine mixture improves metabolic parameters such as heart rate, serum cholesterol, triglycerides, glucose, and fasting insulin in overweight and obese premenopausal women (Hackman et al., 2006).

Herbal medicines exert anti-obesity effects *via* various mechanisms, such as appetite suppression, metabolic promotion, reduced fat absorption, increased lipolysis, and decreased lipogenesis (Payab et al., 2020). Among herbal medicines, *Ephedra* is a representative sympathetic stimulant drug. Sympathetic stimulants represent a common type of anti-obesity drugs and can be categorized as appetite suppressants, similar to norepinephrine (Coulter et al., 2018). BP is also a sympathomimetic drug used as an anti-depressant but has an appetite-reducing and weight loss properties and is used alternatively as an anti-obesity drug in combination with naltrexone. (Billes et al., 2014; Barrea et al., 2020). Recently, several studies have investigated the interaction between gut microbiota and the effects of herbal medicines. Gut microbiota digests herbal medicines into active small molecules, which are easily absorbed and have strong physicochemical activities (Wang et al., 2011; Xing et al., 2014; Van Rymenant et al., 2018). Moreover, herbal medicines regulate the composition of gut microbiota and microbe-derived materials, such as short-chain fatty acids (SCFAs), lipopolysaccharides, and hippuric acid, which can induce physiological changes in the host (Chang et al., 2015; Xing et al., 2015; Xu et al., 2015; Chen et al., 2017; Zhang et al., 2018). However, the anti-obesity effect of herbal medicines, such as in sympathetic stimulants, *via* interactions with gut microbiota remains unclear. Therefore, we aimed to identify the influence of ES on gut microbiota by comparing it with bupropion (BP), a different anti-obesity drug.

We hypothesized that ES can modulate the host gut microbiota, which is correlated with body weight and fat accumulation. Therefore, we investigated the anti-obesity effect and gut microbial changes after ES supplementation in a high-fat diet (HFD) model compared with the synthetic drug (BP). This research may provide insights into the application of herbal medicines for the treatment of obesity. To the best of our knowledge, this is the first study that assesses the effects of drugs and herbal medicines on obesity and gut microbiota simultaneously.

2 Materials and methods

2.1 Botanical drug preparation

Ephedra sinica Stapf [Ephedraceae; Ephedrae Herba] (ES) was obtained from Dongguk University Ilsan International Hospital (Goyang, Korea). After washing with distilled water (DW) and oven-dying for 12 h, 500 g of ES was boiled in 4 L of DW for 4 h and filtered using a 300-mesh filter (50 μm). The water extract was concentrated using a vacuum rotary evaporator following lyophilization at 70°C and stored at 20°C for future use. Previous studies confirmed the chemical profile of ES with a major component as a standard (Kim et al., 2014; Wang et al., 2017).

2.2 Animal studies

Six-week-old male C57BL/6 mice obtained from DBL Inc. (Eumseong-gun, Republic of Korea) were acclimatized for 1 week in a controlled environment with a 12 h light/dark cycle at 25°C, 50%–60% relative humidity, and a chow diet (Purina Irradiated Laboratory Rodent Chow, Purina Korea, Seoul, Republic of Korea). The animal study was approved by the Institutional Animal Care and Use Committee (IACUC-2019–06,187) of Dongguk University Ilsan Hospital and was performed according to the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

To assess the efficacy of anti-obesity drug treatment in HFD-induced obese mice, the mice were randomly divided into four

groups: control group (NOR), HFD, HFD + BP, and HFD + ES; $n = 6–8$ per group. The mice in the NOR group were fed a 10% fat control diet (D12450B, Research Diets Inc., New Brunswick, NJ, United States), whereas the other groups were fed a 60% HFD (D12492, Research Diets Inc.) for 4 weeks. Mice in the HFD + BP and HFD + ES groups received BP (30 mg/kg/day) and ES (150 mg/kg/day), respectively, *via* oral gavage. Untreated mice in the NOR and HFD groups were orally administered water. Treatments were administered five times a week for eight weeks.

Body weight was measured weekly. Following termination of the experimental period, all animals were fasted overnight. Thereafter, they were sacrificed with a combination of Zoletil (tiletamine-zolazepam, Virbac, Carros, France) and Rompun (xylazine-hydrochloride, Bayer, Leverkusen, Germany) (1:1, v/v). Blood samples were collected from the central aorta and rapidly transferred to a BD Vacutainer (BD, Franklin Lakes, NJ, United States). Visceral, subcutaneous, and perigonadal adipose tissues were quickly excised, washed in ice-cold PBS (pH 7.4), dried, and weighed.

2.3 16S rRNA gene amplicon sequencing analysis

Fresh fecal samples were collected 1 week before sacrifice and stored at −80 °C until use. Metagenomic DNA was extracted from samples using a QIAamp stool DNA mini kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The V1-V2 region of 16S rRNA genes was amplified with polymerase chain reaction using universal primers (8F and 338R) with barcode sequence for multiplexing reads of each sample. Sequencing reactions were performed using an Ion Torrent PGM system (Thermo Scientific, Wilmington, DE, United States), according to the manufacturer's instructions. Raw sequence reads were quality-filtered, and quality-controlled reads were processed for diversity analysis and taxonomy assignment using the Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline V2022.2 (Bolyen et al., 2019). Taxonomy assignment was conducted with VSEARCH, using the Silva database (Quast et al., 2012; Rognes et al., 2016). Beta diversity using unweighted UniFrac distances was calculated using 25,795 reads per sample, representing the minimum number of features observed in each sample. PICRUSt was employed to predict the functional metagenomic profiles of microbial communities with reference to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2012; Langille et al., 2013).

2.4 Statistical analysis

Data are expressed as the mean ± SEM unless otherwise indicated. Statistical significance of the animal data was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The statistical significance of the microbiota data was evaluated using the Kruskal–Wallis test followed by Dunn's test. Statistical significance was set at $p < 0.05$. The strength of the relationships between the parameters was assessed using Spearman's correlation test with R packages.

3 Results

3.1 Supplementation of BP and ES prevents body weight gain and fat accumulation

BP and ES are sympathetic stimulant drugs with appetite suppressant and anti-obesity effects (Billes et al., 2014; Kim et al., 2014). Therefore, we established an HFD-induced obese mouse model to investigate the effects of BP and ES on obesity. HFD feeding of mice led to significant increase in food intake, body weight, and fat accumulation compared with the normal diet (NOR) group (Figure 1). BP and ES supplementation considerably decreased food intake by 7.37% and 10.68%, respectively, in HFD-induced obese mice (Figure 1A). Notably, BP and ES supplementation affected body weight and fat accumulation upon HFD feeding. BP and ES supplementation significantly reduced body weight gain by 20.81% and 33.61%, respectively, compared with that in the HFD group (Figures 1B,C). In addition, BP and ES supplementation significantly decreased weights of total fat by 22.31% and 26.65%, respectively, visceral fat by 35.67% and 40.78%, respectively, subcutaneous fat by 25.02% and 25.30%, respectively, and perigonadal fat by 15.17% and 22.56%, respectively, compared with the HFD group (Figures 1D–G). BP and ES supplementation have anti-obesity effects by suppressing appetite and preventing body weight gain and fat accumulation, and although not significant, ES supplementation tended to improve weight-related indicators compared with BP supplementation.

3.2 Effect of BP and ES treatment on gut microbiota composition in HFD-induced mice

Accumulating evidence suggests that gut microbiota is involved in drug response and metabolism (Vila et al., 2020; Xie et al., 2020). Therefore, gut microbiota might be a potential target for the treatment of obesity. We examined whether BP and ES alter the composition of gut microbiota using 16S rRNA amplicon sequencing of feces. There was no significant difference in alpha diversity such as Shannon index and observed features among groups (Supplementary Figure S1). The principal coordinate analysis (PCoA) plot showed separation among

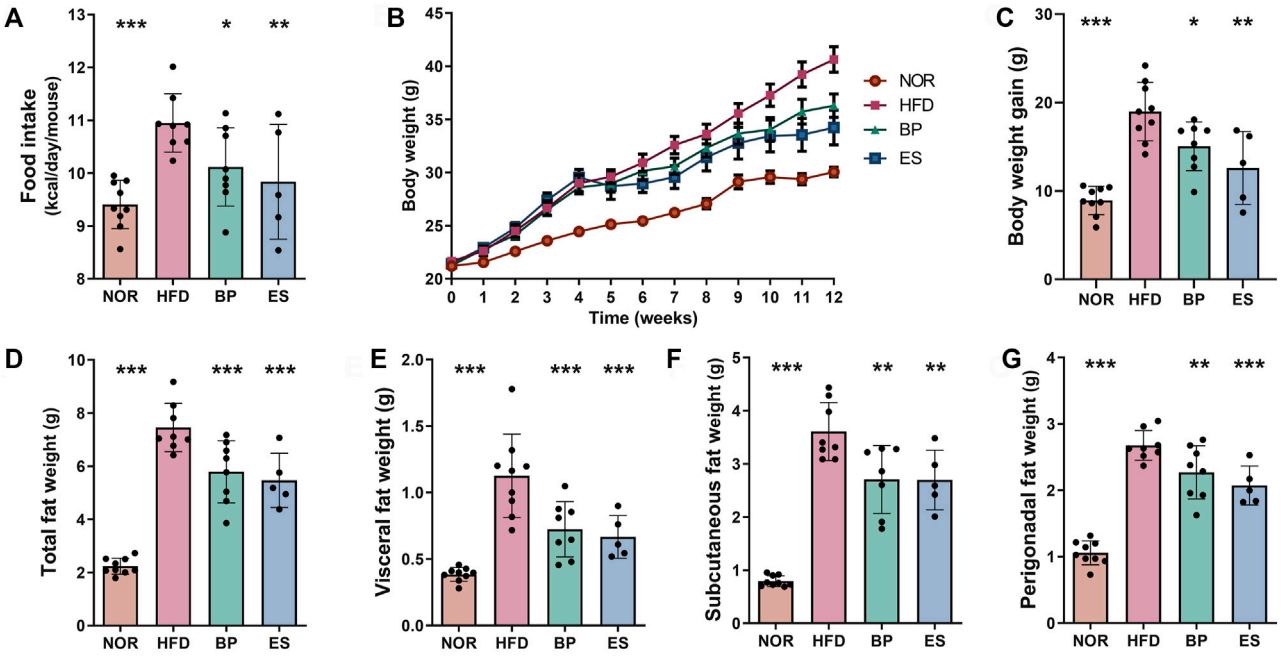

FIGURE 1
Effect of anti-obesity drugs on weight and fat accumulation in a high-fat diet (HFD)-induced obese model after treatment for 8 weeks. **(A)** Food intake. **(B)** Body weight. **(C)** Body weight gain. **(D)** Total fat weight. **(E)** Visceral fat weight. **(F)** Subcutaneous fat weight. **(G)** Perigonadal fat weight. Data are expressed as the mean ± SEM. Statistical significance was assessed using one-way ANOVA. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, vs. HFD. NOR, control group; BP, bupropion; ES, *Ephedra sinica*.

groups, and significance was determined using PC1 and PC2 values on the unweighted UniFrac distance matrix (Figures 2A–C). ES supplementation resulted in a structural shift from the HFD group to the NOR group (Figure 2A). The PC1 value showed no statistically significant difference between the HFD groups (Figure 2B). ES supplementation resulted in a significant difference in PC2 values compared with the HFD group (Figure 2C).

The distribution of bacterial taxa and the relative abundance of bacteria at the phylum level are shown in Figure 2D. The HFD led to a significant increase in the *Firmicutes*/*Bacteroidetes* (F/B) ratio compared with the NOR group (mean ± SD; 0.43 ± 0.19 in NOR vs. 1.22 ± 0.67 in HFD) (Figure 2E). Notably, ES supplementation significantly reduced the F/B ratio compared with that in the HFD group (1.22 ± 0.67 in HFD vs. 0.61 ± 0.38 in ES), however, BP supplementation did not provide a significant result.

The distribution of bacterial taxa and the relative abundance of bacteria at the family level are shown in Figure 2F. HFD feeding led to significant increases in the relative abundance of *Oscillospiraceae* (13.57 ± 5.43 in NOR vs. 23.15 ± 5.95 in HFD) and *Lachnospiraceae* (8.98 ± 5.13 in NOR vs. 17.44 ± 3.42 in HFD) compared with that in the NOR group (Figures 2G,H). Notably, ES supplementation significantly reduced the relative abundance of *Oscillospiraceae* (23.15 ± 5.95 in HFD vs. 13.93 ± 8.98 in ES) and *Lachnospiraceae* (17.44 ± 3.42 in HFD vs. 12.25 ± 8.02 in ES) compared with that in the HFD group; however, BP supplementation did not provide a significant result.

Collectively, ES can restore the gut microbiota dysbiosis such as increased F/B ratio caused by HFD, whereas BP cannot. These results suggest that gut microbiota participates in the response to ES supplementation in terms of recovery from dysbiosis.

3.3 Correlation of gut microbiota with body weight and fat accumulation

The metabolic capabilities and disease associations of specific microbial strains are well established (Yan et al., 2020). Different strains within the same species could be differentially affected by the treatment. To detect specific features associated with the anti-obesity effect of drug supplementation, we found amplicon sequence variants (ASVs) that were significantly restored by drug supplementation and analyzed their correlation with body weight and fat accumulation.

Twelve ASVs were found, and their relative abundances are shown in Figure 3A. HFD feeding led to a significant decrease in the relative abundance of five ASVs and an increase in the relative abundance of seven ASVs compared with the NOR group. ES and BP supplementation significantly restored the relative abundance of ASV0091 and ASV1102 assigned to the species *Bacteroides*

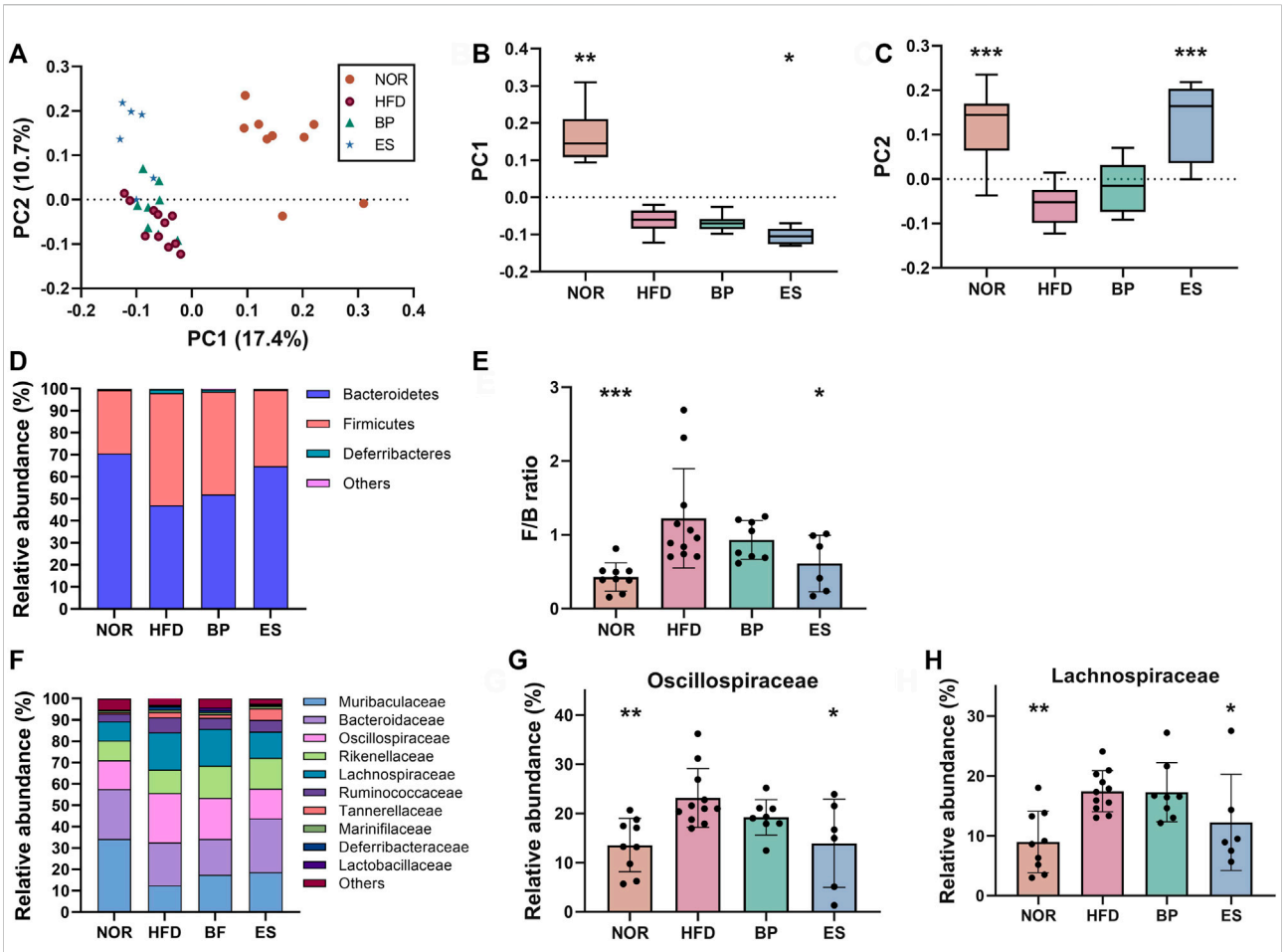

FIGURE 2
Effect of anti-obesity drugs on the structure of gut microbiota in a high-fat diet (HFD)-induced obese model after treatment for 8 weeks. **(A)** Unweighted UniFrac principal coordinate analysis (PCoA) plot based on the amplicon sequence variants (ASV) abundance of each mouse. **(B)** Box plot of PC1 values in each group. **(C)** Box plot of PC2 values in each group. **(D)** The relative abundance at the phylum level significantly changes with HFD. **(E)** The F/B ratio was calculated as a biomarker of gut dysbiosis. **(F)** The relative abundance at the family level significantly changes with HFD. **(G)** The relative abundance of *Oscillospiraceae* in each group. **(H)** The relative abundance of *Lachnospiraceae* in each group. Values are presented as the mean ± SEM. Statistical significance was assessed using the Kruskal–Wallis test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, vs. HFD. NOR, control group; BP, bupropion; ES, *Ephedra sinica*.

intestinalis; ASV6693, ASV1473, and ASV0236 assigned to the family *Muribaculaceae*; ASV1176 and ASV6532 assigned to the family *Oscillospiraceae*; and ASV2122 assigned to the genus *Oscillibacter*. Notably, ASV6795 assigned to the species *Mucispirillum schaedleri*, ASV3708 assigned to the genus *Oscillibacter*, ASV6076 assigned to the family *Peptococcaceae*, and ASV6947 assigned to the family *Oscillospiraceae* were significantly decreased by ES supplementation only.

Furthermore, Spearman's correlation analysis was performed to assess ASVs as biomarkers of body weight and fat accumulation (Figure 3B). We found that 12 ASVs were significantly restored by drug supplementation. Among these, 4 ASVs (ASV0091, ASV0236, ASV1102, and ASV6693) showed a negative correlation and 7 ASVs (ASV6532, ASV2122, ASV6947, ASV1176, ASV3708, ASV6795, and ASV5076) showed a positive correlation with body weight and fat accumulation. In particular, seven ASVs (AS 0091, ASV0236, ASV1102, ASV6947, ASV1176, ASV3708, and ASV6795) showed strong correlation with all fat accumulation indicators.

Collectively, BP, and ES supplementation resulted in specific changes to bacterial ASVs associated with their anti-obesity effects, but the effect of ES supplementation was greater than that of BP supplementation. These results suggest that BP- and ES-induced changes in gut microbiota are linked to the anti-obesity effect.

3.4 Functional metagenome prediction analysis

To evaluate differences in functional attributes of gut microbiota in response to ES supplementation, the predicted

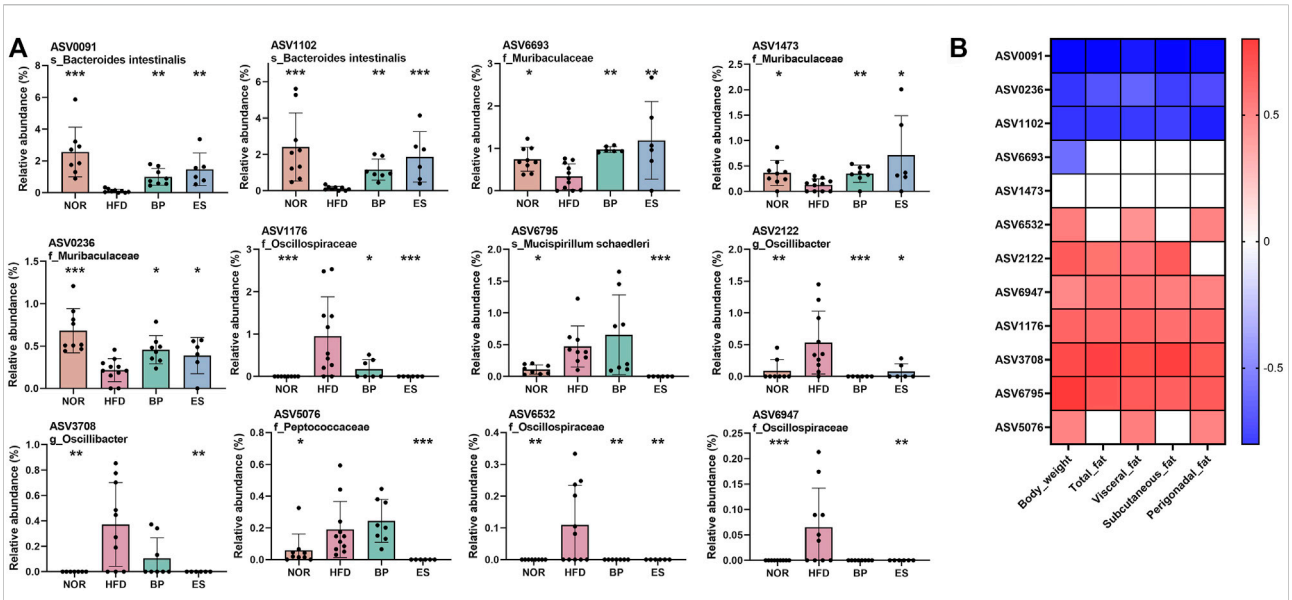

FIGURE 3
Association of microbiota with anti-obesity drugs and obesity-related parameters. **(A)** Significantly different taxa at the amplicon sequence variant (ASV) level among groups. **(B)** A heatmap generated using Spearman correlation showing the association of significantly different taxa among groups with obesity-related markers. Only correlations with a significance of $p < 0.05$ are represented. Color scale indicates the value of the correlation coefficient. Values are presented as the mean ± SEM. Statistical significance was assessed using the Kruskal–Wallis test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, vs. HFD. NOR, control group; BP, bupropion; ES, *Ephedra sinica*.

functional metagenomic profiles based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were generated using PICRUSt. The PCoA plot showed separation among groups based on the Bray–Curtis distance matrix of gene content (Figure 4A). Level 1 results showed that HFD led to a significant increase in "Environmental Information Processing" genes and significant decrease in "Metabolism" and "Organismal Systems" genes (Figure 4B). Notably, ES supplementation significantly restored the "Environmental Information Processing" and "Metabolism" genes; however, BP supplementation did not provide a significant result.

Level 2 results showed that 12 pathways were associated with the restoration of HFD-induced imbalances by BP and ES supplementation (Figure 4C). We detected that the recovery of genes was related to the pathways of membrane transport, signal transduction, and environmental adaptation by ES treatment, which was increased in the HFD group. In contrast, we detected a recovery in amino acid metabolism, energy metabolism, metabolism of terpenoids and polyketides, nucleotide metabolism, digestive system, endocrine system, excretory system, and nervous system, which decreased after HFD feeding with ES treatment. The decrease in enzyme families after HFD feeding was reversed by both BP and ES.

Level 3 results showed that 32 pathways were associated with the restoration of HFD-induced imbalances by BP and ES supplementation (Supplementary Table S1). The phosphatidylinositol signaling system and tryptophan metabolism were decreased by HFD and restored by BP supplementation. Tetracycline biosynthesis and the insulin signaling pathway were increased by HFD and restored by BP supplementation. Twenty-one pathways, including oxidative phosphorylation and vitamin B6 metabolism, were decreased by HFD and restored by ES supplementation. Nine pathways, including ABC transporters, were increased by HFD and restored by ES supplementation.

Collectively, ES supplementation resulted in restoration of functional profiles including ABC transporters and metabolism-related pathways, in particular, energy metabolism and oxidative phosphorylation. These results suggest that ES induces changes in functional profiles of gut microbiota that may contribute to anti-obesity.

4 Discussion

In this study, we hypothesized that some herbal medicines, such as ES, exert anti-obesity effects by inducing changes in gut microbiota. Supplementation with BP and ES prevented body weight gain and fat accumulation. Moreover, ES markedly recovered perturbation of overall composition of gut microbiota, whereas BP and ES considerably recovered members of the gut microbiota, correlated with body weight and fat accumulation, at the ASV level. Only ES significantly recovered predicted pathways, such as environmental information processing and metabolism. These results

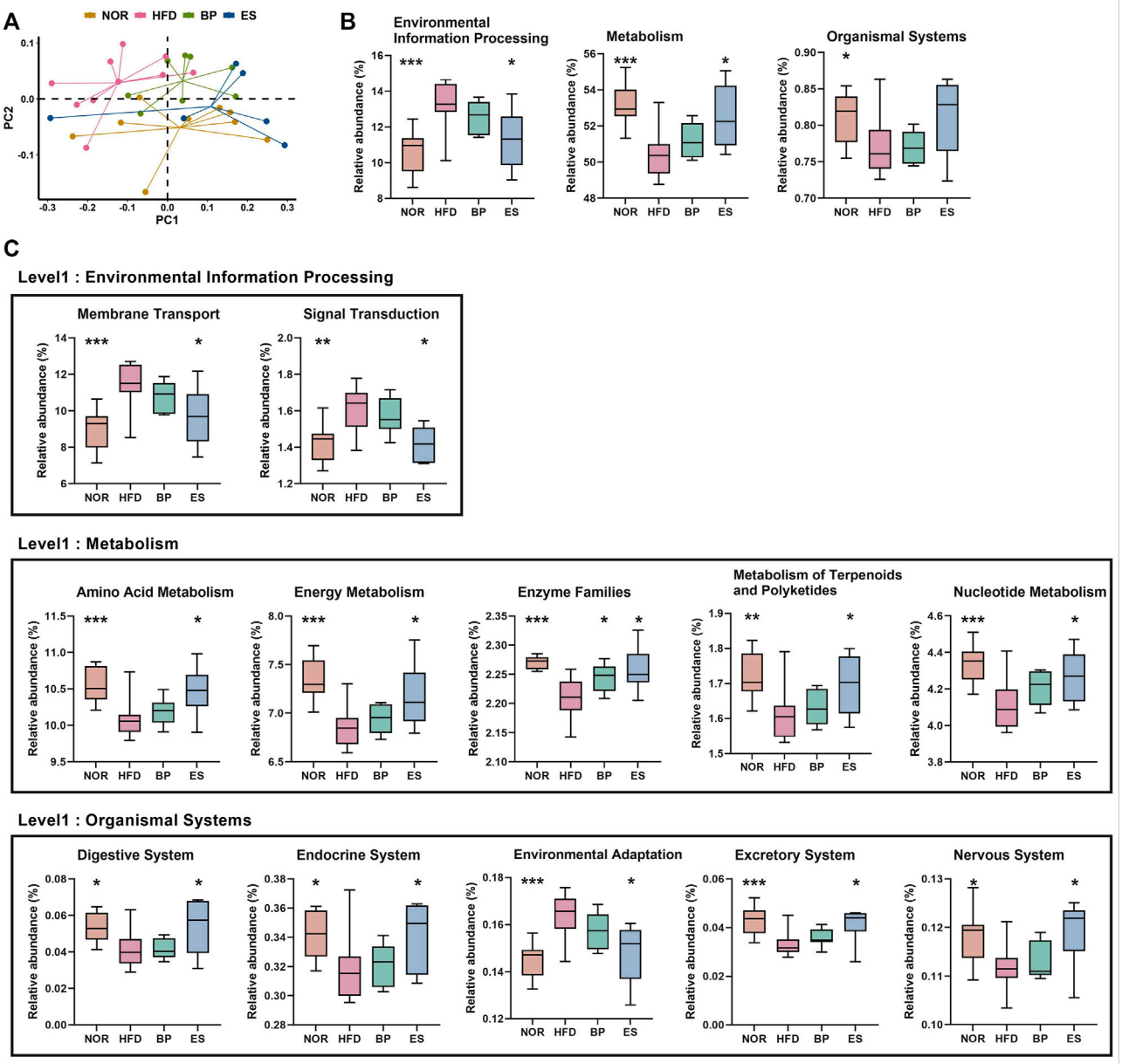

FIGURE 4
Predictive functional profiling of microbial communities by PICRUSt. **(A)** The principal coordinate analysis (PCoA) generated at the Kyoto Encyclopedia of Genes and Genomes (KEGG) level 3. **(B)** The significantly different pathways at KEGG level 1 among groups. **(C)** The significantly different pathways at KEGG level 2 among groups. Values are presented as the mean ± SEM. Statistical significance was assessed using the Kruskal–Wallis test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, vs. HFD. NOR, control group; BP, bupropion; ES, *Ephedra sinica*.

indicated that the anti-obesity effects of BP and ES are related to the regulation of gut microbiota and that ES has more influence on the modulation of gut microbiota than BP.

In our study, we have discovered that gut microbiota responds to ES supplementation in terms of recovery from dysbiosis but not to BP supplementation. Interest in the association between gut microbiota and obesity treatment is increasing because gut microbiota is involved in drug response and metabolism (Vila et al., 2020; Xie et al., 2020). In addition, recent studies have reported changes in gut microbiota composition following treatment with anti-obesity drugs such as metformin, nuciferine, and orlistat in animal models and clinical trials (Pascale et al., 2019; Ke et al., 2020; Wang et al., 2020). Raineri et al. investigated the effects of four anti-obesity drugs (tacrolimus/FK506, BP, naltrexone, and sibutramine), alone and in combination, on the gut

microbiota of obese female rats (Raineri et al., 2022). BP alone had no significant weight change effect and did not affect the changes in gut microbial diversity. The BP and naltrexone combination resulted in increased *Bacteroidetes* and oxidative phosphorylation pathways and decreased the ABC transporter pathway. In our study, although BP exerted anti-obesity effects, such as suppressing appetite and reducing body weight gains and fat accumulation, it did not affect the overall composition of the gut microbiota. Considering our results and those of previous studies, BP alone is insufficient to affect gut microbiota.

A previous study investigated the influence of ES on the composition of gut microbiota in women with obesity (Kim et al., 2014). The impact of ES differed in each subject owing to individual differences in the gut microbiota. However, some bacteria, including *Akkermansia* and *Lactobacillus*, correlated with body weight, body mass index, and body fat percentage after ES consumption. In the current study, we investigated the influence of ES on the composition of gut microbiota in HFD-fed mice. We found that ES changed the overall composition of gut microbiota, and in particular, the F/B ratio-increase due to HFD was also restored. The F/B ratio has been used as a marker of gut microbial dysbiosis and an indicator of obesity (Magne et al., 2020; Stojanov et al., 2020). *Firmicutes* includes numerous known SCFA-producing bacteria, and an increase in the F/B ratio is expected to increase the efficiency of polysaccharide fermentation to SCFA (Louis and Flint, 2009). SCFAs play an important role in various physiological processes, such as host metabolism, gut integrity, glucose homeostasis, lipid metabolism, appetite regulation, and immune function (Morrison and Preston, 2016). However, SCFAs also act as an energy source and increased SCFA production is linked to an increased capacity to harvest energy from the diet (Murphy et al., 2010). Several human studies have shown that the F/B ratio and fecal SCFAs are positively associated (Fernandes et al., 2014; Rahat-Rozenbloom et al., 2014; Goffredo et al., 2016).

In our study, we have discovered that ES induced changes in gut microbiota, and functional profiles of gut microbiota are linked to the anti-obesity effect of ES. *Firmicutes* possess more ABC transporter and phosphotransferase systems compared with *Bacteroidetes* (Mahowald et al., 2009). ABC transporters in *Firmicutes* are often located adjacent to genes encoding glycoside hydrolases, and these two groups may be co-regulated and function together (Rey et al., 2010; Koropatkin et al., 2012). The obese microbiome showed a high enzyme profile of glycoside hydrolases and other enzymes responsible for transport (ABC transporters) and metabolism (α- and β-galactosidases) of products of glycoside hydrolases, resulting in SCFA production (Turnbaugh et al., 2006). We observed a significant increase in the ABC transporter pathway in the HFD group. Consistent with our results, an increased F/B ratio and ABC transporters pathway have been observed in obese and overweight Italian adults (Palmas et al., 2021). Hou et al. observed an association between increased ABC transporter pathway activity and obesity (Hou et al., 2017). Notably, ES supplementation restored the ABC pathway. Our results agreed with previous research that suggested that ES supplementation may have contributed to the modulation of energy harvesting by regulating the ABC transporter pathway and SCFA production, accompanied by restoration of the F/B ratio.

Additionally, we found metabolism-related pathways restored by ES treatment, in particular, energy metabolism and oxidative phosphorylation. Oxidative phosphorylation, also known as the electron transport chain, contributes to ATP synthesis and energy transduction (Nath and Villadsen, 2015). The KEGG pathway for oxidative phosphorylation includes the electron transport chain complexes of NADH dehydrogenase. NADH is a fundamental mediator of energy metabolism (Berger et al., 2004). The pharmacological activation of NADH oxidation has been suggested as a new therapy for the treatment of metabolic syndrome (Hwang et al., 2009). Consistent with our results, exercise enriched the oxidative phosphorylation pathway and reduced fat mass in HFD mice (Lai et al., 2018). *Bupleuri radix* extract also enhanced oxidative phosphorylation and improved lipid disorders in HFD mice (Wu et al., 2021). Although the mechanism of involvement of gut microbiota in the anti-obesity effect of ES remains elusive, our results, in combination with the previous literature, suggest that ES supplementation establishes a gut microbiota community that is adept at oxidative phosphorylation, which may contribute to energy harvesting and reducing fat accumulation. Further confirmation of the role of this pathway in the gut microbiota and the anti-obesity effects of ES remains unexplored.

The ASV with a strong negative correlation between body weight and fat accumulation was assigned to *Bacteroides intestinalis,* which was decreased in the HFD group and restored in both the BP and ES supplementation groups. *Bi73*, a novel gene encoding endoxylanase and esterase, has been identified in transcriptional analysis and is highly upregulated by *B. intestinalis* DSM 17393 (Zhang et al., 2014). Recently, molecular and biochemical analyses have reported a novel trifuctionalendoxylanase/endoglucanase/feruloyl esterase and feruloyl acid (FA)-producing *B. intestinalis* DSM 17393 (Zhang et al., 2022). FA is a plant phenolic acid that is abundant in grains and vegetables (Zhao and Moghadasian, 2008; Molinari et al., 2009). FA suppresses obesity and obesity-related metabolic syndromes in HFD-induced obese mice (Wang et al., 2018). FA also has beneficial effects on diabetes by suppressing oxidative stress, increasing plasma insulin levels, and lowering blood glucose (Roy et al., 2013). Further studies to determine whether *B. intestinalis* is increased by BP and ES supplementation and exerts anti-obesity effects *via* the generation of FA will help better elucidate the mechanism of obesity treatment.

Additionally, an ASV with a strong positive correlation between body weight and fat accumulation was assigned to *Mucispirillum schaedleri*. Consistent with our results, *M. schaedleri* increased in the HFD feeding model and positively correlated with body weight and body fat content (Ussar et al.,

2015; Lee et al., 2018; Du et al., 2021). *M. schaedleri* has also been identified as a pathogen associated with intestinal inflammation and oxidative stress (Berry et al., 2015; Loy et al., 2017). Moreover, the development of CD-like diseases is triggered by the presence of *M. schaedleri* (Caruso et al., 2019). We suggest that the increased *M. schaedleri* in HFD serves as a marker of obesity-related dysbiosis, and reduced *M. schaedleri* by ES supplementation is an indicator of the anti-obesity effect of ES. However, further research is needed to elucidate the causality of the relationship between *M. schaedleri* and obesity.

In summary, we investigated the effects of ES on gut microbiota, body weight, and fat accumulation. Our results demonstrated that ES supplementation had an anti-obesity effect, and the potential mechanisms could be due to restoration of gut microbiota dysbiosis related to body weight and fat accumulation. These findings provide evidence for the application of ES as a therapeutic herbal agent for ameliorating obesity, and gut microbiota could be a target for the anti-obesity effect. Nevertheless, preparations derived from Ephedra has safety concerns. *Ephedra* can cause a quickened heartbeat and elevated blood pressure (Soni et al., 2004). The limitations of this study include that we investigate the effect of ES with only single dose study. The clinical application of *Ephedra* as an anti-obesity drug requires careful attention and approach, and sufficient discussion is needed on dose and side effects test. The limitations of this study also include the lack of well-known positive controls; further studies involving positive controls will help confirm the effect of ES on gut microbiota.

Data availability statement

The raw sequencing data presented in this study are deposited in the DNA Data Bank of Japan under accession number DRA013284.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Dongguk University Ilsan Hospital.

Author contributions

E-JS: Formal analysis, visualization, writing–original draft. NS: Formal analysis, writing–original draft. SJ: resource supervision. Y-DN: Conceptualization, supervision, writing, reviewing, and editing. HK: Conceptualization, supervision, writing, reviewing, and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.1042833/full#supplementary-material

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EDITED BY
Dan Tang,
Guangdong Pharmaceutical University, China

REVIEWED BY
Asim Kumar Mandal,
Brigham and Women's Hospital, United States
Ping Fu,
Sichuan University, China

*CORRESPONDENCE
Wenlong Sun,
512649113@qq.com
Jinyue Sun,
moon_s731@hotmail.com
Xinhua Song,
892442572@qq.com

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New insight into the management of renal excretion and hyperuricemia: Potential therapeutic strategies with natural bioactive compounds

Bendong Yang[1], Meiling Xin[1], Shufei Liang[1], Xiaoxue Xu[1], Tianqi Cai[1], Ling Dong[1], Chao Wang[1], Meng Wang[1], Yuting Cui[1], Xinhua Song[1,2]*, Jinyue Sun[3]* and Wenlong Sun[1,2]*

[1]School of Life Sciences and Medicine, Shandong University of Technology, Zibo, China, [2]Shandong Qingyujiangxing Biotechnology Co., Ltd., Zibo, China, [3]Key Laboratory of Novel Food Resources Processing, Ministry of Agriculture and Rural Affairs/Key Laboratory of Agro-Products Processing Technology of Shandong Province/Institute of Agro-Food Science and Technology, Shandong Academy of Agricultural Sciences, Jinan, China

Hyperuricemia is the result of increased production and/or underexcretion of uric acid. Hyperuricemia has been epidemiologically associated with multiple comorbidities, including metabolic syndrome, gout with long-term systemic inflammation, chronic kidney disease, urolithiasis, cardiovascular disease, hypertension, rheumatoid arthritis, dyslipidemia, diabetes/insulin resistance and increased oxidative stress. Dysregulation of xanthine oxidoreductase (XOD), the enzyme that catalyzes uric acid biosynthesis primarily in the liver, and urate transporters that reabsorb urate in the renal proximal tubules (URAT1, GLUT9, OAT4 and OAT10) and secrete urate (ABCG2, OAT1, OAT3, NPT1, and NPT4) in the renal tubules and intestine, is a major cause of hyperuricemia, along with variations in the genes encoding these proteins. The first-line therapeutic drugs used to lower serum uric acid levels include XOD inhibitors that limit uric acid biosynthesis and uricosurics that decrease urate reabsorption in the renal proximal tubules and increase urate excretion into the urine and intestine *via* urate transporters. However, long-term use of high doses of these drugs induces acute kidney disease, chronic kidney disease and liver toxicity. Therefore, there is an urgent need for new nephroprotective drugs with improved safety profiles and tolerance. The current systematic review summarizes the characteristics of major urate transporters, the mechanisms underlying the pathogenesis of hyperuricemia, and the regulation of uric acid

Abbreviations: ABCG2, ATP-binding cassette transporter, subfamily G, member 2; CKD, chronic kidney disease; GLUT9, glucose transporter member 9; IL-1β, interleukin-1β; IL-6, interleukin-6; JAK/STAT3, Janus kinase/signal transducer and activator of transcription 3, MSU, monosodium urate; NF-κB, nuclear factor-kappa B; NLRP3, NOD-like receptor family pyrin domain-containing 3; NPT, sodium-dependent phosphate cotransporter type; OAT, organic anion transporter; PI3K/AKT, phosphoinositide 3-kinase/protein kinase; PO, potassium oxonate; ROS, reactive oxygen species; LPS, lipopolysaccharide; SNPs, single nucleotide polymorphisms; TCMs, traditional Chinese medicines, TGF-β, transforming growth factor-beta; TNF-α, tumor necrosis factor-alpha; TLR4, Toll-like receptor 4; URAT1, human urate transporter 1; XOD, xanthine oxidoreductase.

biosynthesis and transport. Most importantly, this review highlights the potential mechanisms of action of some naturally occurring bioactive compounds with antihyperuricemic and nephroprotective potential isolated from various medicinal plants.

KEYWORDS

hyperuricemia, urate transporters, natural products, chronic kidney disease, renal urate extraction

Introduction

Uric acid (2,6,8-trioxypurine, $C_5H_4N_4O_3$) is a heterocyclic organic compound with a molecular weight of 168.11 Da. It is the end-product of purine (endogenous and exogenous) metabolism in humans and great apes because of loss-of-function mutations during primate evolution in the gene encoding the uricase enzyme, which oxidizes uric acid to produce more soluble allantoin (Wu et al., 1992; So and Thorens, 2010). Due to uricase inactivation, the serum urate level is 7- to 8-fold higher in humans (≈240–360 μM) than in other mammals (≈30–50 μM in mice) (So and Thorens, 2010). Considering the pKa value of uric acid (5.75) that exists as soluble urate at physiological pH (7.4), more uric acid than urate is present in the urine (pH 5–6), and a pH less than 7.4 in the tissue microenvironment favors urate crystal formation. In humans, the serum urate concentration is approximately 10 times higher than that of ascorbic acid, showing that urate is a potent radical scavenger in plasma. Perhaps 7- to 8-fold higher serum urate levels might have selectively protected the neurons of hominids during evolution from the detrimental effects of reactive oxygen species (ROS) and allowed them to become the most intelligent species on Earth. In addition, elevated serum urate levels have been shown to reduce the risk of neurodegenerative diseases, specifically Alzheimer's disease (Ye et al., 2016), Parkinson's disease (Schwarzschild et al., 2008; Ascherio et al., 2009), and multiple sclerosis (Liu et al., 2012). Recently, integral membrane protein 2B (ITM2B) has been shown to be a potential regulatory link between urate homeostasis and neurodegenerative disorders (Mandal and Mount, 2019). Elevated serum urate levels may have given hominids a selective advantage during evolution. Taken together, these results suggest a potential role of soluble urate in maintaining memory and intelligence.

Approximately two-thirds of serum uric acid in humans is produced endogenously, while the remaining third comes from dietary purines (Schlesinger 2005). Under physiological concentrations, uric acid in its soluble form as urate acts as a protective powerful antioxidant, demonstrating the ability to scavenge ROS such as superoxide, hydroxyl radicals, and singlet oxygen (Ames et al., 1981; Davies et al., 1986), which is closely comparable to the scavenging ability of vitamin C (Ames et al., 1981). However, under conditions of poor solubility and a high uric acid concentration in crude urine, urate crystal deposition occurs in the renal tubular lumens and ureters, which contributes to obstructive nephropathy (Moe et al., 2002). The urate crystals adhere to the surfaces of renal epithelial cells and induce an acute inflammatory response (Koka et al., 2000). In addition to inducing kidney stone formation, such effects can reduce the glomerular filtration rate (Spencer et al., 1976). Urate can also act as a pro-oxidant inside cells, as it can induce the activity of NADPH oxidases, resulting in mitochondrial alterations and endothelial dysfunction (Sánchez-Lozada et al., 2012).

Hyperuricemia is a common finding in patients with metabolic syndrome. Clinically, the prevalence of hyperuricemia is much higher than that of hypouricemia. Hyperuricemia in humans is defined by the serum urate level >7.0 mg/dl among men and >5.7 mg/dl among women. Statistically, the morbidity due to hyperuricemia and gout is higher in men than in women, probably because of the influence of sex hormones. With increasing age after menopause, the discrepancy in prevalence is reduced between men and women (Chen-Xu et al., 2019). The prevalence of hyperuricemia increased from 10.5% to 16.6% in Caucasian or Australian representative populations from 2011 to 2020 (Pathmanathan et al., 2021). In Ireland, the prevalence increased from 19.7% to 25.0% among men and from 20.5% to 24.1% among women between 2006 and 2014 (Kumar A U et al., 2018). The pooled prevalence of hyperuricemia was 19.4% in men and 7.9% in women from 2000 to 2014 and was high in mainland China (Liu et al., 2015). Long-term hyperuricemia can induce renal mitochondrial dysfunction associated with oxidative stress in the renal cortex as well as tubular damage (Cristóbal-García et al., 2015). Moreover, it can lead to urate crystal formation that causes gout (a type of inflammatory arthritis), leading to joint damage, loss of motion and an acute inflammatory response (Koka et al., 2000). Hyperuricemia is the causative risk factor for gout (Choi et al., 2005) and increases the risks for chronic kidney disease (CKD) (Johnson et al., 2018), cardiovascular disease (Feig et al., 2008; Gaffo et al., 2009), hypertension (Feig and Johnson, 2003), insulin resistance (Facchini et al., 1991; Yoo et al., 2005), and diabetic kidney disease (Kim et al., 2012; Qu et al., 2022). Currently, it is recognized that hyperuricemia alone is not sufficient to cause gout and that other factors also play roles in urate crystal formation. It is also acknowledged that lowering serum urate levels is an effective strategy to prevent gout attacks (Shoji et al., 2004).

The normal serum urate level in humans is the result of balance among biosynthesis of uric acid primarily in the liver, reabsorption of urate in renal proximal tubules and secretion in the renal tubules and intestine (Mandal and Mount, 2015). Dysregulation of xanthine oxidoreductase (XOD), the enzyme that catalyzes the endogenous production of uric acid primarily in the liver, and urate transporters that reabsorb urate in renal proximal tubules and secrete urate in renal tubules and the intestine, is the major cause of hyperuricemia, along with variability in the genes encoding these proteins. Notably, most urate is filtered freely in the kidney, with approximately 90% of the urate from glomerular filtrate being reabsorbed *via* urate transporters in the proximal tubules (Alexandru and Moe, 2012). Approximately 70% of the total serum urate in the human body is excreted *via* the kidney, and the rest is excreted *via* intestinal and biliary secretion (Schlesinger 2005). Ultimately, after urate reabsorption, only 3–10% of the filtered urate is eliminated in the urine (Taniguchi and Kamatani, 2008). Abnormalities in urate metabolism and decreased urate metabolite in the kidney are major inducers of hyperuricemia and gout development (Terkeltaub 2003; Liu et al., 2015). Genome-wide association studies have also found associations between polymorphisms in urate transporters and the risk for hyperuricemia/gout (Köttgen et al., 2013; Phipps-Green et al., 2016; Tin et al., 2019). In this review, advances in urate excretion and possible therapeutic herbal extracts are discussed to provide novel insights regarding the development and treatment of hyperuricemia.

Relationship between hyperuricemia and CKD

Long-term hyperuricemia is considered an independent risk factor for the occurrence and progression of CKD (Jalal et al., 2013; Su et al., 2020). The prevalence of hyperuricemia and CKD has been steadily increasing (Levey et al., 2007; Jalal et al., 2013). Patients with long-term hyperuricemia have poor quality of life and high mortality rates. Because of advances in research, CKD can now be detected using simple laboratory tests, and there are treatments to prevent or delay abnormal kidney function, slow the progression of kidney disease, and reduce the risk of CKD. However, the debate regarding whether hyperuricemia plays a causal role in the progression of CKD or is simply a marker of renal dysfunction continues. The causal relationship between hyperuricemia and CKD remains controversial, and the pathophysiological mechanisms of hyperuricemia-induced renal injury are not entirely clear. Serum urate is often elevated in subjects with CKD but is not always associated with the development and progression of CKD. The causal role of hyperuricemia in the progression of CKD has not been fully established. Currently, there is no clear cutoff serum urate level associated with the risk for kidney damage, and there is not sufficient evidence to recommend the widespread use of uric acid-lowering therapy to prevent or slow the progression of CKD. Elevated urate levels in PO-induced (PO, potassium oxonate; a uricase inhibitor) hyperuricemic rats have been shown to cause intrarenal oxidative stress, increased NOX-4 and angiotensin II expression, increased juxtaglomerular renin and decreased nitric oxide bioavailability, renal vasoconstriction, glomerular hypertrophy, glomerulosclerosis and afferent arteriolopathy (Mazzali et al., 2001; Kang et al., 2002; Nakagawa et al., 2003; Sánchez-Lozada et al., 2005; Sánchez-Lozada et al., 2008). Furthermore, treatment with allopurinol partially prevents cortical vasoconstriction and fully prevents arteriolopathy and glomerular hypertension (Nakagawa et al., 2003; Sánchez-Lozada et al., 2005).

Excessive serum urate causes hyperuricemic nephropathy, which is characterized by inflammatory infiltration of macrophages, neutrophils and lymphocytes and tubulointerstitial fibrosis (Lai and Zhou, 2013; Pan et al., 2021a). Recent findings suggest that asymptomatic hyperuricemia has no effect on CKD progression unless urate crystallizes in the kidney (Sellmayr et al., 2020). However, when experimental animals with CKD are made hyperuricemic, renal disease progresses rapidly despite an absence of crystals in the kidney. This experimental results link hyperuricemia with the progression of CKD as in most mammals, uric acid levels are relatively low (compared to those in humans) because of the presence of liver uricase, which degrades uric acid to 5-hydroxyisourate and eventually to soluble allantoin. All humans are essentially "uricase knockouts," exhibiting elevations in serum urate levels that can be treated. Reducing urate levels with losartan may slow renal disease (Miao et al., 2011) and reduce cardiovascular events (Smink et al., 2012). The renoprotective drug febuxostat (a xanthine oxidase inhibitor) does not alleviate the decline in kidney function in patients with stage 3 CKD and asymptomatic hyperuricemia (Kimura et al., 2018). Evidence obtained from basic research suggests that hyperuricemia plays a pathogenic role in the development of CKD and cardiovascular disease by inducing inflammation, endothelial dysfunction, proliferation of vascular smooth muscle cells, and activation of the renin–angiotensin system (Saito et al., 1978; Mazzali et al., 2001; Perlstein et al., 2004). In human first trimester uterine trophoblast cell lines, monosodium urate (MSU) crystals have been shown to induce inflammatory cytokine production in response to activation of the NOD-like receptor superfamily pyrin domain containing 3 (NLRP3) inflammasome (Mulla et al., 2011; Mulla et al., 2013). CKD is most often associated with obesity and metabolic syndrome (Copur et al., 2022), and there is insufficient evidence to suggest that uric acid-lowering therapy can prevent CKD progression.

Relationship between hyperuricemia and insulin resistance

Serum urate is elevated in metabolic syndrome and diabetes (Choi et al., 2007; Choi and Ford, 2007; Copur et al., 2022) as a consequence of insulin resistance and insulin-mediated reductions in urinary urate excretion (Quiñones Galvan et al.,

1995). There is a positive relationship between serum insulin and elevated serum urate levels in healthy individuals and people with diabetes (Mandal et al., 2021). Insulin resistance also leads to impaired urate excretion at a low urinary pH, contributing to the formation of urate stones (Spatola et al., 2017). Genetic variation in insulin signaling pathways is also associated with variations in serum urate levels (Köttgen et al., 2013; Phipps-Green et al., 2016; Tin et al., 2019). These genetic data are consistent with a role of insulin in controlling serum urate levels (Mandal et al., 2021). In an oocyte expression system and transfected cells overexpressing individual urate transporters, insulin was recently suggested to activate both "reabsorptive" urate transporters (GLUT9 and OAT10) and "secretory" urate transporters (OAT1, OAT3, NPT1, and ABCG2) *via* phosphoinositide 3-kinase/protein kinase (PI3K/AKT) and mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathways (Mandal et al., 2021).

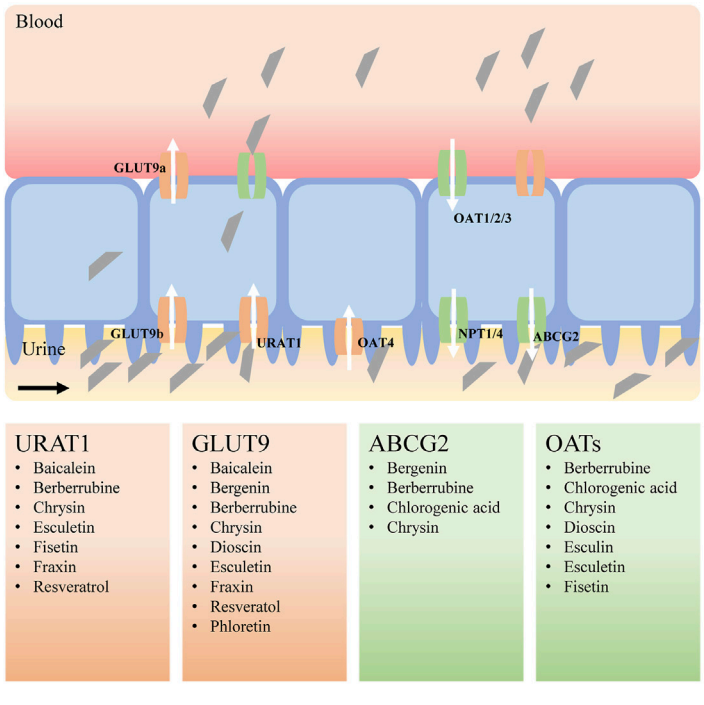
Various urate transporters involved in renal excretion

Well-characterized urate transporters that are involved in reabsorption of urate from the glomerular filtrate in the human renal proximal tubules include human urate transporter 1 (URAT1, encoded by the *SLC22A12* gene), organic anion transporters (OAT10/ORCTL3, encoded by the *SLC22A13* gene, and OAT4, encoded by the *SLC22A11* gene), and glucose transporter member 9 (GLUT9, encoded by the *SLC2A9* gene). The urate transporters that are involved in secretion of urate into the urine from serum include OAT1 (encoded by the *SLC22A6* gene), OAT2 (encoded by the *SLC22A7* gene), OAT3 (encoded by the *SLC22A8* gene), ATP-binding cassette, subfamily G, member 2 (ABCG2, encoded by the *ABCG2* gene), ABCC4 (encoded by the *ABCC4* gene), sodium-dependent phosphate transporters types (NPT1, encoded by the *SLC17A1* gene) and NPT4 (encoded by the *SLC17A3* gene) (Mandal and Mount, 2015; Mandal et al., 2017). Urate reabsorption in the proximal tubule involves the coordinated activity of several transporters. Sodium-dependent reabsorption of organic monocarboxylates by the apical Na^+-dependent monocarboxylate transporters SMCT1 (encoded by the *SLC5A8* gene) and SMCT2 (encoded by the *SLC5A12* gene) (Coady et al., 2004; Srinivas et al., 2005; Mandal et al., 2017) increases the intracellular concentrations of monocarboxylate anions that can then be exchanged with luminal urate *via* the urate–anion exchangers URAT1 and OAT10.

URAT1 (SLC22A12)

URAT1 is expressed in the apical membrane of the proximal tubules in the kidney and has been regarded as the dominant apical urate/anion exchanger in humans mediating urate reabsorption (Figure 1). URAT1 transports urate in exchange for intracellular nicotinate and pyrazinoate, but not lactate (Mandal et al., 2017). In an oocyte expression system, the urate/anion exchange activity of URAT1 has been found to be independent of Na^+ ions, but complete removal of Cl^- ions in the extracellular medium increases the urate uptake activity of URAT1 3- to 4-fold. URAT1 is also transported in exchange for pyrazinoate. Uricosuric drugs such as benzbromarone, probenecid, tranilast (Mandal et al., 2017), lesinurad (Miner et al., 2016b; Zhao et al., 2020), fenofibrate (Uetake et al., 2010), and losartan, an angiotensin II receptor antagonist (Nakashima et al., 1992), are potent inhibitors of URAT1.

Loss-of-function mutations in the *SLC22A12* gene are associated with hypouricemia (Dinour et al., 2011). Hypouricemia has been previously shown to be associated with common variants associated with several single nucleotide polymorphisms (SNPs), including rs12800450 (G65W), rs121907896 (R90H), rs121907892 (W258X), rs139316841 (W277R), rs749900943 (R349W), rs147647315 (R434H), rs201423508 (T450I) and rs747742344 (E458K) (Ichida et al., 2004; Tin et al., 2011; Sakiyama et al., 2016). The identification of missense mutations (T217M, E298D, G269A, R406C, G412A, G444R, G490A, A1145T, and T1289C) in patients with hypouricemia validates URAT1 as the primary reabsorption urate transporter (Enomoto et al., 2002; Ichida et al., 2004; Dinour et al., 2011). In an *SLC22A12*-knockout mouse model, fractional urate excretion was significantly greater than that in wild-type mice, confirming the role of URAT1 in urate reabsorption (Hosoyamada et al., 2010). Based on this information, regulation of the URAT1 expression level and its activity is an effective strategy for maintaining a suitable serum urate concentration. Benzbromarone is a potent uricosuric agent that has been used in the treatment of gout for over 30 years. It functions by increasing urate excretion in human kidney proximal tubules through effective inhibition of the dominant apical (luminal) urate exchanger URAT1 at low doses (Enomoto et al., 2002; Mandal et al., 2017). This blockade reduces urate reabsorption, increasing urate elimination *via* the urine (Enomoto et al., 2002). Benzbromarone was withdrawn from the market by Sanofi-Synthelabo in 2003 after reports of severe hepatotoxicity (Jansen et al., 2004). Aggravation of hepatic steatosis in obese individuals and gastrointestinal problems associated with benzbromarone treatment limit the clinical use of this drug but warrant further affirmation *in vivo* (Heel et al., 1977; Sun et al., 2018). Recently, many studies have indicated that SNPs in the *SLC12A22* gene are strongly associated with hyperuricemia and gout. In an analysis of the Korean Cancer Prevention Study-II (KCPS-II) cohort, sequencing of URAT1 in 68 male Korean subjects revealed that the pattern most strongly associated with hyperuricemia included a common variant consisting of rs7929627 (IVS7-103A/G, noncoding variants),



First-line drugs	Dose	Target
• Allopurinol	-	Xanthine oxidoreductase
• Febuxostat	-	Xanthine oxidoreductase
• Topiroxostat	-	Xanthine oxidoreductase
• Benzbromarone	50-100 μM	URAT1, GLUT9, OAT10, OAT4, OAT1, OAT3, ABCG2, NPT1) (Mandal et al., 2017b)
• Probenecid	1 mM	URAT1, GLUT9 (~50% inhibition), OAT10 (~50% inhibition), OAT4, OAT1, OAT3, ABCG2 (~50% inhibition), NPT1 (~50% inhibition) (Mandal et al., 2017b)
• Tranilast	100 μM	URAT1, GLUT9, OAT10, OAT4, OAT1, OAT3, NPT1) (Mandal et al., 2017b)
• Losartan	100-500 μM	URAT1, OAT10, OAT1, OAT3, ABCG2 (Hamada et al., 2008; Miner et al., 2016b)
• Lesinurad	-	URAT1, OAT4 (Miner et al., 2016b)
• Fenofibrate	-	URAT1 (Uetake et al., 2010)
• Furosemide	-	OAT2 (Sato et al., 2010)

FIGURE 1
Urate transporters and potential natural products for hyperuricemia treatment. URAT1, urate transporter 1; GLUT9, glucose transporter member 9; ABCG2, ATP-binding cassette transporter, subfamily G, member 2; OATs, organic anion transporters; NPTs, sodium-dependent phosphate cotransporter types. The activities of urate transporters were inhibited by benzbromarone (Mandal et al., 2017), probenecid (Mandal et al., 2017), tranilast (Mandal et al., 2017), losartan (Hamada et al., 2008; Miner et al., 2016b), lesinurad (Miner et al., 2016b), fenofibrate (Uetake et al., 2010), furosemide (Sato et al., 2010), respectively.

rs75786299 (IVS3+11A/G, noncoding variants) and rs3825017 (N82N, coding variant). Moreover, rs11602903 (788A/T, promoter variants) and rs121907892 (W258X, coding variant) were negatively correlated with hyperuricemia (Cho et al., 2015). In addition, other SNPs, including rs3825017 (N82N), rs3825016 (C/T), and rs11231825(H142H), are linked with the risk of hyperuricemia and gout (Li et al., 2014; Pavelcova et al., 2020).

GLUT9 (*SLC2A9*)

GLUT9 is a voltage-driven high-capacity urate transporter that is mainly expressed in the proximal tubules of the human kidney, the liver, and the intestine (Figure 1) (Anzai et al., 2008; Döring et al., 2008; Vitart et al., 2008; Wallace et al., 2008). It is the sole transporter that transports reabsorbed urate from the proximal tubular epithelium to the blood. Loss-of-function mutations in GLUT9 have been identified in familial hypouricemia, and SNPs are associated with reduced serum urate, indicating that GLUT9 is a major determinant of serum urate levels (Mandal and Mount, 2015). GLUT9 exists in two isoforms, GLUT9a and GLUT9b, which differ in their amino-terminal cytoplasmic domains; GLUT9a is located in the basolateral membrane, and GLUT9b is located in the apical membrane of the proximal tubules in human kidney (Augustin et al., 2004; Kimura et al., 2014). However, in mice, Glut9a is expressed in the proximal convoluted and straight tubules, and Glut9b is expressed in distal convoluted tubules and connecting tubules (Bibert et al., 2009). Thus, mouse Glut9 in the kidney differs from human GLUT9. Human GLUT9a is 540 amino acids in length and is encoded by 12 exons, whereas GLUT9b is 511 amino acids in length and is encoded by 13 exons of the splice variants of RNA of the *SLC2A9* gene. In both humans and mice, GLUT9b is expressed only in the liver and kidney, whereas GLUT9a is present in many more tissues, such as the liver, kidney, intestine, leukocytes, and chondrocytes (Augustin et al., 2004; Keembiyehetty et al., 2006; Kimura et al., 2014).

Although GLUT9 was initially reported to mediate glucose/fructose transport (Augustin et al., 2004; Doblado and Moley, 2009), it was later confirmed to be a high-capacity robust urate transporter without any detectable glucose/fructose transport activity (Caulfield et al., 2008; Vitart et al., 2008; Mandal et al., 2017; Mandal et al., 2021). Furthermore, *SLC2A9*-knockout mice exhibit increased serum urate levels with impaired enterocyte urate transport kinetics. *SLC2A9*

deficiency in mice can induce the occurrence of early-onset metabolic syndrome, suggesting a role of *SLC2A9* in regulating enterocyte urate clearance (DeBosch et al., 2014). Importantly, uricosuric drugs such as benzbromarone, probenecid, and tranilast (Mandal et al., 2017), but not losartan or lesinurad, are potent inhibitors of GLUT9 (Miner et al., 2016a). In a oocyte expression system, urate uptake activity was found to be almost completely inhibited by 100 μM benzbromarone (Mandal et al., 2017). The uptake of urate mediated by GLUT9 is inhibited by benzbromarone (100 μM) and losartan (1 mM) by approximately 90 and 50%, respectively (Bibert et al., 2009). Moreover, electrophysiological measurements suggest that urate transport by mouse GLUT9 is electrogenic and voltage-dependent but independent of Na^+ and Cl^- transmembrane gradients (Bibert et al., 2009). However, in the oocyte expression system, complete removal of Cl^- ions in the extracellular medium increased the urate uptake activity of GLUT9 3- to 4-fold. Similarly, complete replacement of Na^+ ions by K^+ ions in the extracellular medium increased the urate uptake activity of human GLUT9 4- to 5-fold (Mandal et al., 2017).

ABCG2 (ABCG2)

ABCG2 protein, first identified as a multidrug resistance protein (Doyle et al., 1998) that transports a wide range of structurally and functionally diverse chemotherapeutics, has been characterized as a high-capacity urate secretion transporter (Matsuo et al., 2009; Woodward et al., 2009; Nakayama et al., 2011). ABCG2 is located on chromosome 4q, as identified by genome-wide association studies related to hyperuricemia and gout (Nakayama et al., 2011). It is expressed in the apical membrane of the human kidney proximal tubules (Figure 1) (Huls et al., 2008; Woodward et al., 2009), the intestine and the liver (Nakayama et al., 2011).

In white, African and Asian populations, the SNP rs2231142 in exon 5 of the ABCG2 gene, which generally causes the Gln141Lys amino acid substitution, is the strongest link between ABCG2 and gout/hyperuricemia (Dehghan et al., 2008). Functional studies have shown that the Q141K substitution causes an approximately 53% reduction in ABCG2-mediated urate export activity compared with that of the wild-type protein (Woodward et al., 2009). Approximately one-third of uric acid is excreted from the intestines in humans (Matsuo et al., 2014). Thus, ABCG2 plays a crucial role as an essential renal and intestinal urate exporter, as its dysfunction is associated with abnormal serum uric acid levels and gout/hyperuricemia risk. In an oocyte expression system, benzbromarone and probenecid were shown to inhibit ABCG2 urate export activity (Mandal et al., 2017; Fujita et al., 2019), but lesinurad was not (Miner et al., 2016a).

OATs (SLC22A)

OATs, encoded by *SLC22* family of genes, are expressed in the barrier epithelia of the major excretory organs of the body, the kidney and the liver (Figure 1). These transporters play important roles in renal drug elimination, as they interact with endogenous metabolic end products such as urate and a multitude of widely used drugs, including antibiotics, antihypertensives, antivirals, anti-inflammatory drugs, diuretics and uricosurics (Rizwan and Burckhardt, 2007). OATs also play important roles in both the renal secretion and reabsorption of urate. Five of the characterized OATs are expressed in the renal proximal tubules: OAT1 (encoded by the *SLC22A6* gene), OAT2 (encoded by the *SLC22A7* gene) and OAT3 (encoded by the *SLC22A8* gene) are located in the basolateral membrane, whereas OAT4 (encoded by the *SLC22A11* gene) and OAT10/ORCTL3 (encoded by the *SLC22A13* gene) are located in the apical membrane of the renal proximal tubules (Eraly et al., 2008). The basolateral urate transporters OAT1, OAT2 and OAT3 function in urate secretion (Eraly et al., 2008), transporting urate from blood into proximal tubular cells for secretion at the apical membrane (Mandal et al., 2017). OAT1 and OAT3 are Na^+-independent and exchange urate with divalent anions such as α-ketoglutarate (Aslamkhan et al., 2003; Bakhiya et al., 2003; Sweet et al., 2003), indicating that basolateral entry of urate from the blood into the proximal tubular epithelium is driven by intracellular α-KG during urate secretion. In an oocyte expression system, the urate transport activity of murine OAT3 was found to be cis-inhibited by extracellular salicylate, nicotinate or pyrazinoate anion (Mandal et al., 2017). OAT2 is chloride-dependent and has more restricted substrate specificity than OAT1 and OAT3 (Sato et al., 2010). The urate transport activity of OAT2 is cis-inhibited by the antiuricosuric agents pyrazinecarboxylic acid and nicotinate (Sato et al., 2010), and OAT3 is cis-inhibited by pyrazinecarboxylic acid, nicotinate and salicylate (Mandal et al., 2017). Human OAT4 transports urate in exchange for divalent organic dicarboxylate ions (Hagos et al., 2007; Mandal et al., 2017). OAT10 is a urate transporter and high-affinity urate/nicotinate exchanger dependent on Cl^- ions (Bahn et al., 2008; Mandal et al., 2017). OAT10 and URAT1 share functional similarities, as both of them transport urate and nicotinate and as the urate transport activity of both is trans-stimulated by intracellular nicotinate or pyrazine carboxylate (Mandal et al., 2017). Oat1-and Oat3-null mice exhibit decreased secretion of urate rather than reabsorption (Eraly et al., 2008). Benzbromarone, probenecid and tranilast were shown to inhibit the urate transport activity of OAT10, OAT4, OAT1, and OAT3 in an oocyte expression system (Mandal et al., 2017). Benzbromarone, probenecid, losartan, telmisartan, hydrochlorothiazide, and furosemide have been

shown to inhibit the urate transport activity of OAT2 (Sato et al., 2010). In addition, lesinurad inhibits OAT4 but does not inhibit OAT1 or OAT3 in the clinical setting (Sato et al., 2010).

NPTs (SLC17A)

NPT1 and NPT4, encoded by *SLC17A1* and *SLC17A3* genes, respectively, are located at the apical membrane of the proximal tubule and mediate net tubular urate secretion (Figure 1). Genome-wide association studies have identified a region in chromosome 6p23-p21.3, where *SLC17A1* and *SLC17A3* are located, that is associated with serum urate concentrations (Kolz et al., 2009). NPT1 is the first identified member of the *SLC17A* phosphate transporter family. Human NPT1 transports organic anions such as urate, p-aminohippurate and acetylsalicylate (aspirin), and salicylate in a voltage-driven and Cl^--dependent manner (Reimer and Edwards, 2004; Iharada et al., 2010). The identification of a common gain-of-function variant, rs1165196 (T806C), in Japanese patients with significantly decreased risk of renal underexcretion gout enhanced understanding of the physiological role of NPT1 as a renal urate exporter (Chiba et al., 2015). Benzbromarone, probenecid and tranilast have been shown to inhibit the urate transport activity of NPT1 in an oocyte expression system (Mandal et al., 2017). NPT4 is also a voltage-dependent organic anion transporter, similar to porcine OAT1/3, with lower affinity urate transport (Jutabha et al., 2011). Two thiazide drugs, chlorothiazide-2 and trichlormethiazide-3, and two loop diuretics, bumetanide-4 and furosemide-5, have been shown to inhibit the urate transport activity of NPT4 in an oocyte expression system (Jutabha et al., 2011), which suggests the involvement of NPT4 in diuretic-induced hyperuricemia. Two SNPs, rs1165205 within intron 1 of *SLC17A3* and rs116205 identified in the *SLC17A3* gene, correlate well with the changes in serum urate concentration (Riches et al., 2009). The *in vivo* role of NPT4 is supported by the presence of missense mutations (N68H and F304S) in *SLC17A3* in underexcretion-type hyperuricemia patients (Jutabha et al., 2010).

Management of hyperuricemia by herbal extracts and natural bioactive compounds

The field of traditional Chinese medicine (TCM) is valued for its holistic view of the human body. TCM provides a massive amount of information on natural products and disease phenotypes observable as clinical symptoms that are crucial for clinical diagnosis and treatment (Table 1). Various aqueous or ethanolic herbal extracts used in TCM have been demonstrated to be beneficial in various disease conditions. The 70% ethanol extract (comprising polyphenols and flavonoids) of *Eurycoma longifolia*, a tropical medicinal plant, has been reported to significantly reduce serum urate levels by downregulating the protein expression levels of Urat1 and Glut9 in rats with PO-induced hyperuricemia and an adenine-/PO-induced hyperuricemia mouse model (Bao et al., 2019). The macroporous resin extract of *Dendrobium officinale* leaves has been reported to reduce serum urate levels in rats with fructose- and PO-induced hyperuricemia by inhibiting XOD activity and regulating the expression of Abcg2, Urat1, and Glut9 (Wang et al., 2022). Sunflower calathide aqueous extract has been shown to reduce serum urate levels to a degree comparable to that mediated by allopurinol and benzbromarone in rats with yeast extract-induced hyperuricemia with renal injury (Dai et al., 2021). Sunflower calathide aqueous extract has also been shown to downregulate the cytokines COX-2, PGE2, NO, and IFN-γ in lipopolysaccharide (LPS)-treated RAW264.7 cells (Dai et al., 2021). Ethanol extract of the bark of *Liriodendron chinense* (Hemsl.) Sarg has been shown to reduce serum urate in adenine and PO-induced hyperuricemia mice with nephropathy by suppressing the activation of nuclear factor-kappa B (NF-κB) and the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) signaling pathway, reducing inflammatory factor infiltration and urate accumulation in the kidney (Pan et al., 2021a). A polysaccharide (molecular weight, 46.56 kDa) obtained from the green algae *Enteromorpha prolifera*, comprising rhamnose, glucuronic acid, galactose, arabinose, and xylose at a molar ratio of 20.45:12.74:10.99: 5.84:1.95, has been shown to significantly reduce serum urate, serum XOD and hepatic XOD; upregulate the mRNA and protein expression of urate secretion transporters Abcg2, Oat1, and Npt1; and downregulate the urate reabsorption transporter, maintaining the stability of the intestinal flora in mice with hyperuricemia (Li X. et al., 2021). Fufang Zhenzhu Tiaozhi capsule prevents renal injury, inflammation and fibrosis in mice with hypoxanthine- and PO-induced hyperuricemia by promoting urate excretion and inhibiting the PI3K/AKT/NF-κB signaling pathway (Li et al., 2022). The Third National Health and Nutrition Examination Survey (1988–1994), using data from 14,758 men and women aged ≥20 years, showed that coffee consumption is associated with reduced serum urate levels (Choi and Curhan, 2007). Dioscin, a spirostane glycoside with anti-inflammatory and antiallergic properties found in the rhizome of *Dioscorea spongiosa*, has been shown to significantly reduce serum urate by downregulating Glut9 and upregulating Oat1 in rats with PO-induced

TABLE 1 Summary of the effects and potential mechanisms of candidates for hyperuricemia treatment. PO, potassium oxonate; LPS, lipopolysaccharide; URAT1, human urate transporter 1; GLUT9, glucose transporter member 9; ABCG2, ATP-binding cassette transporter, subfamily G, member 2; OAT, organic anion transporter; NPT, sodium-dependent phosphate cotransporter type; NLRP3, NOD-like receptor family pyrin domain-containing 3; IL-6, interleukin-6; IL-1β, interleukin-1β; NF-κB, nuclear factor-kappa B; TGF-β, transforming growth factor-beta; PI3K/AKT, phosphoinositide 3-kinase/protein kinase; JAK/STAT3, Janus kinase/signal transducer and activator of transcription 3, TLR4, Toll-like receptor 4; XOD, xanthine oxidoreductase.

Bioactive compound	Natural resources	Effects	Potential mechanisms	Models	References
Anthocyanin s (flavonoids)	Korean black beans (also found in blue, purple, and red colored fruits, flowers and leaves)	Significantly reduces serum urate levels, antioxidant, neuroprotective	Inhibits XOD, inhibits activation of PI3K/Akt/GSK3β	Mice with yeast extract-induced hyperuricemia	(Ali et al., 2018; Qian et al., 2019)
Chrysin (a flavonoid)	Honey, propolis, mushrooms	Effectively reduces serum urate levels, anti-inflammatory, antioxidant	Inhibits XOD, downregulates URAT1 and GLUT9 expression, upregulates OAT1 and ABCG2 expression, inhibits activation of the PI3K/Akt/mTOR signaling pathway and the NLRP3 inflammasome	High-fructose corn syrup-fed hyperuricemia rats, RAW264.7 cells	(Chen et al., 2021a; Cai et al., 2021; Chang et al., 2021)
Bergenin (a type of polyphenolic compound)	Medicinal plants like *Bergenia crassifolia, Corylopsis spicata, Rodgersia sambucifolia*	Significantly reduces serum urate levels, antiulcerogenic, anti-inflammatory, wound-healing	Induces Abcg2 expression, suppresses Glut9 expression, inhibits nuclear translocation of p53, inhibits activation of PI3K/Akt signaling pathway, decreases the serum levels of IL-6, IL-1β, and TNF-α	Model fed daily with diet mixed with 25% yeast polysaccharide, mice with PO-induced hyperuricemia, HK-2 cells, Caco-2 cells, RAW264.7 cells	Chen et al. (2020)
Baicalein (a bioactive flavonoid)	Roots of the Chinese herbs *Scutellaria baicalensis* and *Scutellaria lateriflora*	Significantly reduces serum urate levels, antioxidant, anti-inflammatory, antihypertensive, anticancer	Inhibits the activity of Glut9 and Urat1, inhibits XOD activity, inhibits activation of the PI3K/AKT/NF-κB pathway	Mice with PO-induced hyperuricemia, human A549 lung adenocarcinoma cells	(Yu et al., 2017; Chen et al., 2021b)
Berberrubine (an isoquinoline alkaloid)	Phellodendri Chinensis Cortex, *Coptis chinensis* Franch and *Phellodendron chinense* Schneid	Significantly reduces serum urate levels, anti-gout	Inhibits hepatic XOD activity, downregulates the GLUT9 and URAT1 expression, upregulates OAT1/3 and ABCG2 expression, reduces inflammatory mediator (IL-1β, IL-6, and TNF-α) levels, suppresses the JAK2/STAT3 signaling pathway	Mice with PO- and hypoxanthine-induced hyperuricemia	(Lin et al., 2021; Cheng et al., 2022a)
Pectolinarigenin (a flavonoid)	*Crisium setidens, Cirsium chanroenicum* and citrus fruits	Significantly reduces serum urate levels, alleviates inflammation and fibrosis	Inhibits the TGF-β expression and phosphorylation of the transcription factors Smad3 and Stat3, LPS-induced NF-κB activation, and synthesis of iNOS, COX-2, IL-6, IL-1β, and TNF-α	Mice with adenine- and PO -induced hyperuricemic nephropathy, uric acid-treated mouse kidney epithelial cells, RAW264.7 and THP1 cell lines	(Ren et al., 2021a; Feng et al., 2022)
Fisetin (a flavonol)	Widely dispersed in fruits (apples, grapes, strawberries), vegetables and nuts	Reduces serum urate, anti-inflammatory, attenuates hyperuricemia-induced kidney injury, improves renal function, and inhibits tumor growth, osteoarthritis, and rheumatoid arthritis	Modulates/restores the expression of Urat1, Oat1, Oat3 and Abcg2; reduces the levels of inflammatory mediators (IL-6, TNF-α); regulates activation of the JAK/STAT3 and TGF-β signaling pathways; inhibits activation of the FGFR1/TLR4/NLRP3 inflammasome pathway	Mice with adenine- and PO-induced hyperuricemic nephropathy, uric acid-stimulated mouse kidney tubular epithelial cells	(Ren et al., 2021b; Huang et al., 2021)
Phloretin (a type of natural phenol)	Leaves of apple and apricot trees	Reduces serum urate levels, antioxidant, anti-inflammatory	Inhibits urate reabsorption mediated by Glut9, suppresses the NLRP3 signaling pathway, inhibits uric acid-induced activation of the ERK/NF-κB pathway	Mice with PO-/adenine-induced hyperuricemia, human umbilical vein endothelial cells	(Liu et al., 2017; Cui et al., 2020)

(Continued on following page)

TABLE 1 (*Continued*) Summary of the effects and potential mechanisms of candidates for hyperuricemia treatment. PO, potassium oxonate; LPS, lipopolysaccharide; URAT1, human urate transporter 1; GLUT9, glucose transporter member 9; ABCG2, ATP-binding cassette transporter, subfamily G, member 2; OAT, organic anion transporter; NPT, sodium-dependent phosphate cotransporter type; NLRP3, NOD-like receptor family pyrin domain-containing 3; IL-6, interleukin-6; IL-1β, interleukin-1β; NF-κB, nuclear factor-kappa B; TGF-β, transforming growth factor-beta; PI3K/AKT, phosphoinositide 3-kinase/protein kinase; JAK/STAT3, Janus kinase/signal transducer and activator of transcription 3, TLR4, Toll-like receptor 4; XOD, xanthine oxidoreductase.

Bioactive compound	Natural resources	Effects	Potential mechanisms	Models	References
Resveratrol (a polyphenol)	Grapes, veratrum and other plants	Reduces serum urate levels, anti-inflammatory, antioxidant, antidiabetic, antiarthritic, anticancer, neuroprotective	Downregulates the expression levels of Glut9 and Urat1; reduces the renal concentrations of IL-6, IL-18, IL-1β and TNF-α; inhibits the Nlrp3 inflammasome and TLR4/MyD88/NF-kB, Nrf2 and NF-κB signaling pathways	Mice with high-fat diet-induced insulin resistance, Sprague–Dawley rat model of sepsis peritonitis, duck ileitis caused by LPS	(Shang et al., 2019; Yang et al., 2021; Zhang et al., 2021)
Chlorogenic acid (a polyphenol)	Medicinal herbs, apples, artichokes, carrots, eggplants, grapes, cherries, honeysuckle, sunflower	Reduces serum urate levels; antioxidant and anti-inflammatory activity; alleviates kidney fibrosis, liver fibrosis, metabolic syndrome, acute kidney injury, and diabetic nephropathy	Downregulates the mRNA expression of secretory urate transporters, inhibits the PI3K/AKT/mTOR and the NF-κB signaling pathways, reduces the mRNA expression of interleukin IL-1β, tumor necrosis factor TNF-α, Nlrp3, and caspase-1, and the TLR4/MyD88/NF-κB signaling pathway in kidney	Mice and rats with PO/hypoxanthine-induced hyperuricemia	(Ye et al., 2017; Zhou et al., 2021)
Esculetin (a natural dihydroxy coumarin)	*Fraxinus rhynchophylla* bark and chicory skin	Significantly reduces serum urate, antioxidant, anti-inflammatory, antiapoptotic, anticancer, antidiabetic, neuroprotective	Inhibits XOD in liver; modulates urate transporters in the kidney; upregulates OAT1, IL-6, IL-1β, and TNF-α; suppresses the expression of inducible nitric oxide synthase and the cyclooxygenase-2 protein by blocking the NF-κB pathway	Mice with PO-induced hyperuricemic nephropathy, mice with unilateral ureteral occlusion, RAW264.7 macrophages, HepG2 and NRK-52E cells	(Hong et al., 2014; Zhu et al., 2016; Wang et al., 2020; Zhang et al., 2022)
Esculin (a coumarin derivative)	*Fraxinus rhynchophylla* bark and chicory skin	Significantly reduces serum urate, anti-inflammatory, antiarthritic and anticancer	Upregulates Oat1	PO-induced hyperuricemic nephropathy	(Li et al., 2011; Zhou et al., 2018)
Fraxin (a coumarin derivative)	*Fraxinus rhynchophylla* bark and chicory skin	Significantly reduces serum urate, anti-inflammatory, antiarthritic and anticancer	Inhibits Glut9 and Urat1	Mice with PO-induced hyperuricemic nephropathy	(Li et al., 2011; Zhou et al., 2018)
Dioscin (a spirostane glycoside)	Rhizome of *Dioscorea spongiosa*	Significantly reduces serum urate, anti-inflammatory, antiallergic	Downregulates Glut9 and upregulates Oat1	Rats with PO-induced hyperuricemia and mice with adenine-/PO-induced hyperuricemia	(Tao et al., 2018; Zhang et al., 2018)
RA-3 (a lanosteryl triterpene)	*Protorhus longifolia* stem bark	Significantly reduces serum urate, nephroprotective and antihyperglycemic	Inhibits XOD, downregulates pGSK-3β and pAKT	Sprague–Dawley rat model of sepsis peritonitis, high-fat diet-fed and streptozotocin-induced T2DM rat model	Hlophe et al., (2020)
Theobromine	Cocoa	Markedly inhibits uric acid nucleation	—	—	Grases et al., (2014)
Caffeine	Coffee	Reduces serum urate	—	Systemic meta-analysis	(Bae et al., 2015; Park et al., 2016)
Curcumin	Rhizome of *Curcuma longa*	Significantly reduces serum urate, modulates the gut microbiota, fortifies the intestinal barrier, attenuates metabolic endotoxemia, protects renal function	—	Rats with PO-induced hyperuricemia	(Zhang et al., 2012; Xu et al., 2021)

hyperuricemia alone and in mice with adenine-/PO-induced hyperuricemia (Tao et al., 2018; Zhang et al., 2018; Li J. et al., 2021). Theobromine, a natural dimethylxanthine present in high amounts in cocoa, may be clinically useful in the treatment of nephrolithiasis, as it inhibits nucleation and urate crystal growth (Grases et al., 2014). Curcumin, a hydrophobic polyphenol extracted from the rhizome of *Curcuma longa*, has been shown to significantly reduce serum urate in PO-induced hyperuricemia rats modulating the gut microbiota, fortifying the intestinal barrier, attenuating metabolic endotoxemia, and consequently protecting renal function (Xu et al., 2021).

These results suggest that TCM herbal extracts have potential benefits in the management of hyperuricemia and related kidney impairment. Due to the complex ingredient structures and compositions of TCM herbal extracts, it is difficult to comprehensively elucidate the mechanisms of the pharmacological effects of the extracts. Identification and exploration of natural bioactive compounds from TCMs might be promising directions for hyperuricemia management.

The PI3K/Akt signaling pathway, a potential target for the treatment of hyperuricemia

Generally, MSU deposition causes acute gout flares and recurrent gout attacks that destroy joints. Macrophages are thought to initiate and drive MSU-induced inflammation. MSU-induced acute gouty arthritis in mice has been shown to cause activation of the PI3K/Akt pathway (Cao et al., 2021). In addition, insulin stimulation of urate uptake in human proximal tubular cells (PTC-05) and HEK293T cells is effectively abrogated by inhibitors of protein tyrosine kinase and PI3K (Mandal et al., 2021). In an oocyte expression system, insulin was shown to stimulate the urate transport activity of GLUT9a, GLUT9b, OAT10, OAT3, OAT1, NPT1, and ABCG2, which are directly activated by insulin signaling through the PI3K/Akt signaling pathway (Mandal et al., 2021). Anthocyanins, a group of natural flavonoids found in Korean black beans (also found in blue-, purple-, and red-colored fruits, flowers, and leaves) that regulate the PI3K/Akt/GSK3β pathways (Ali et al., 2018), have been reported to significantly reduce serum urate levels and XOD activity in the serum and livers of mice with yeast extract-induced hyperuricemia (Qian et al., 2019). Future studies are warranted to investigate whether anthocyanins regulate the expression of urate transporters *via* the PI3K/Akt signaling pathway. Chrysin, a flavonoid compound naturally found in honey, propolis and mushrooms that exerts anti-inflammatory and antioxidant effects, has been shown to effectively reduce serum urate levels by inhibiting the activity of XOD in the liver of high-fructose corn syrup-fed hyperuricemia rats by downregulating the protein expression of Urat1 and Glut9 and upregulating the protein expression of Oat1 and Abcg2 (Chang et al., 2021). The anti-inflammatory effect of chrysin is mediated by the PI3K/Akt/mTOR signaling pathway in RAW264.7 cells (Cai et al., 2021). Bergenin, a type of polyphenol compound with antiulcerogenic, anti-inflammatory, and wound-healing properties that induces ABCG2 expression and suppresses *SLC2A9* expression by inhibiting the nuclear translocation of p53 in HK-2 cells, reduces serum urate levels in mice with yeast polysaccharide-induced hyperuricemia by promoting renal and gut urate excretion (Chen et al., 2020).

The JAK/STAT3 signaling pathway, a potential target for the treatment of hyperuricemia

Kidney fibrosis is a histologic hallmark of CKD that is possibly caused by hyperuricemia. Pharmacological inhibition of the activation of the JAK/STAT3 pathway has been shown to reduce serum urate levels and delay the progression of kidney fibrosis and CKD in adenine- and PO-induced hyperuricemia mouse models (Pan et al., 2021b). Berberrubine is an isoquinoline alkaloid that is the primary metabolite of berberine, the main component of Phellodendri Chinensis Cortex, which is found in *Coptis chinensis* Franch and *Phellodendron chinense* Schneid (Cheng H. et al., 2022). Berberrubine possesses antihyperuricemic and antigout effects and significantly decreases the serum urate levels in mice with PO- and hypoxanthine-induced hyperuricemia (Lin et al., 2021). Berberrubine has also been shown to reduce hepatic XOD activity, downregulate the expression of Glut9 and Urat1 and upregulate the expression of Oat1/3 and Abcg2 at both the protein and mRNA levels in mice with hyperuricemia as well as to suppress activation of the JAK/STAT3 signaling pathway (Lin et al., 2021). The natural flavonoid pectolinarigenin has been reported to significantly reduce serum urate levels in mice with adenine- and PO-induced hyperuricemic nephropathy (Ren et al., 2021a). Pectolinarigenin also inhibits the expression of transforming growth factor-beta (TGF-β)1 as well as the phosphorylation of the transcription factors Smad3 and Stat3, suggesting that suppression of inflammation and fibrosis by pectolinarigenin occurs through inhibition of Smad3 and Stat3 signaling pathway activation in mice with hyperuricemic nephropathy (Ren et al., 2021a). Fisetin (3,3′,4′,7-tetrahydroxyflavone), a naturally occurring flavonol, reduces serum urate by modulating the expression of kidney urate transporters, including Urat1, Oat1/3, and Abcg2, *via* the STAT3 and TGF-β signaling pathways in mice with PO- and adenine-induced hyperuricemia (Ren et al., 2021b). Fisetin treatment also reduces the levels of proinflammatory mediators, including tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6) and monocyte chemoattractant protein-1; attenuates kidney fibrosis; and restores the expression of alpha-smooth muscle actin, collagen I and fibronectin *via* modulation of the STAT3 and TGF-β signaling pathways (Ren et al., 2021b). In a cell-based urate transport assay, fisetin was shown to be a strong URAT1 inhibitor with a half-maximal inhibitory concentration of

7.5 μM (Toyoda et al., 2022). Curcumin has been shown to reduce fructose-induced hyperuricemia and renal endothelial dysfunction by inhibiting activation of NO-mediated JAK/STAT signaling in rats (Zhang et al., 2012).

NLRP3 and the NF-κB/TLR4 signaling pathway, potential targets for the treatment of hyperuricemia

The inflammasome is a by multiprotein cytoplasmic complex that plays important roles in host defense and inflammatory responses through activating caspase-1 and promoting secretion of the proinflammatory cytokines interleukin 1beta (IL-1β) and IL-18 (Franchi et al., 2009). Nod-like receptor protein members, including NLRP1, NLRP3 and NLRC4, and the adaptor ASC (apoptosis-related specific protein) constitute the inflammasome (Franchi et al., 2009). The NLRP3 inflammasome, an intracellular sensor, plays an important role in innate immunity and is therefore the most investigated inflammasome (Franchi et al., 2009). For activation of the NLRP3 inflammasome, activation of NF-κB is required to upregulate the expression of NLRP3, pro-IL-1β, and caspase 1, which is accomplished *via* stimulation of Toll-like receptors (TLRs) (Toma et al., 2010; Qiao et al., 2012). MSU, identified as a danger signal formed after the release of uric acid from dying cells (Shi et al., 2003), has been shown to trigger the cellular inflammatory response through the NLRP3 inflammasome, resulting in the production of the active inflammatory cytokines IL-1β and IL-18 (Martinon et al., 2006). MSU-induced inflammation and oxidative stress proceed through the NF-κB/NLRP3 and Nrf2 pathways, leading to increased production of the inflammatory cytokines IL-1β, IL-6, IL-18, and TNF-α (Alberts et al., 2019; Cheng J.-J. et al., 2022). The bacterial cell wall component crude LPS can also activate the NRLP3 inflammasome (Martinon et al., 2006).

In this review, we have shown the mechanisms by which some natural bioactive compounds suppress activation of the NLRP3 inflammasome in mice with hyperuricemia (Table 1). In a PO-induced hyperuricemia mouse model, reduction of serum urate by treatment with the XOD inhibitor N-(9,10-anthraquinone-2-yl-carbonyl), downregulates Glut9 protein expression and upregulates Oat1 and Oat3 protein expression, resulting in decrease in the levels of TNF-α, IL-6, and other inflammatory factors in the serum and kidney of mice and inhibition of NLRP3 pathway-mediated inflammation (Gao et al., 2022). Chlorogenic acid, a polyphenolic compound found in medicinal herbs with antioxidant and anti-inflammatory activity, has been shown to inhibit XOD and downregulate the mRNA expression of secretory uric acid transporters in a hypoxanthine- and PO-induced hyperuricemia mouse model (Zhou et al., 2021). In addition, chlorogenic acid reduces the mRNA expression of IL-1β, TNF-α, Nlrp3, and caspase-1. It also inhibits activation of the TLR 4/MyD88/NF-κB signaling pathway in the kidney and reduces the mRNA expression of ileal IL-1β and IL-6, resulting in inflammation relief in the above hyperuricemic mice and in LPS-induced acute kidney injury (Ye et al., 2017; Zhou et al., 2021). Berberrubine has been shown to significantly reduce serum urate levels and the levels of inflammatory mediators (IL-1β, IL-6, and TNF-α) in mice with PO- and hypoxanthine-induced hyperuricemia (Lin et al., 2021). Pectolinarigenin, in addition to reducing serum urate in mice with hyperuricemic nephropathy (Ren et al., 2021a), inhibits LPS-induced NF-κB activation by interfering with the degradation of IκB-α and the synthesis of inducible nitric oxide synthase, cyclooxygenase-2, IL-6, IL-1β, and TNF-α in RAW264.7 and THP1 cell lines (Feng et al., 2022). Chrysin improves vascular permeability and alleviates the inflammatory response in lung tissue by suppressing the IRE1α/TXNIP/NLRP3 signaling pathway, thereby alleviating LPS-induced acute lung injury in mice (Chen M. et al., 2021). Bergenin reduced the levels of serum urate, IL-6, IL-1β, and TNF-α in hyperuricemic mice and promotes a polarization shift from the M1 to the M2 phenotype in RAW264.7 cells (Chen et al., 2020). Esculetin is a natural dihydroxy coumarin found in *Fraxinus rhynchophylla* bark and chicory skin that has been shown to reduce serum urate by increasing renal urate excretion, inhibiting XOD expression and activity in the liver, and modulating urate transporters in the kidney (Hong et al., 2014; Wang et al., 2020). Esculetin has been shown to attenuate elevations in the levels of proinflammatory cytokines, including IL-6, IL-1β, and TNF-α, and serum urate and suppress inducible nitric oxide synthase and cyclooxygenase-2 protein expression by blocking the NF-κB pathway and suppressing the generation of proinflammatory mediators, including nitric oxide and prostaglandin E2, in LPS-induced RAW264.7 macrophages and mice (Hong et al., 2014; Zhu et al., 2016). Huang et al. reported that fisetin, a naturally occurring flavonol, inhibits inflammatory responses in experimental periodontitis in rats and LPS-induced human gingival fibroblasts through the FGFR1/TLR4/NLRP3 inflammasome pathway, which mitigates kidney injury to restore the normal expression of Urat1 (Huang et al., 2021). Phloretin, a dihydrochalcone, is a type of natural phenol found in the leaves of apple and apricot trees with antioxidant and anti-inflammatory properties. Phloretin has been shown to effectively attenuate urate-induced renal injury by inhibiting Nlrp3 and urate reabsorption mediated by Glut9 and promoting urinary urate excretion in mice with PO-/adenine-induced hyperuricemia (Cui et al., 2020). In human umbilical vein endothelial cells, phloretin also significantly attenuates proinflammatory factor expression and reduces GLUT9-mediated urate uptake by inhibiting activation of the ERK/NF-κB pathway (Liu et al., 2017). Resveratrol, a polyphenolic,

non-flavonoid plant-derived antitoxin, has been shown to reduce serum urate levels by downregulating the expression levels of Glut9 and Urat1 and to improve kidney inflammation, possibly *via* the TLR4 AND NLRP3 signaling pathways, in mice with high-fat diet-induced insulin resistance (Zhang et al., 2021). In a classic Sprague–Dawley rat model of sepsis peritonitis, resveratrol significantly decreases LPS-induced expression of NF-κB, TNF-α, IL6, IL-1β, and TLR4 and increases the expression of p-PI3K, p-AKT, and p-mTOR in the myocardium (Shang et al., 2019). Thus, resveratrol has the potential to protect the myocardium in sepsis by activating the PI3K/AKT/mTOR signaling pathway and inhibiting the NF-κB signaling pathway and related inflammatory factors (Shang et al., 2019). Resveratrol also reduces LPS-induced inflammation by reducing the levels of inflammatory cytokines (IL-6, IL-18 and TNF-α) in duck ileitis (Yang et al., 2021). Bergenin, a C-glucoside of 4-O-methyl gallic acid isolated from several medicinal plants, has been shown to reduce serum urate levels in a hyperuricemia-induced mouse model by elevating Abcg2 expression in both the kidney and intestine and by suppressing *Slc2a9* expression in the kidney (Chen et al., 2020). Bergenin also reduces the serum levels of IL-6, IL-1β, and TNF-α in hyperuricemic mice (Chen et al., 2020). Baicalein, a bioactive flavonoid exhibiting antioxidant, anti-inflammatory, antihypertensive and anticancer properties, has been reported to significantly reduce serum urate and enhance renal urate excretion in mice with PO-induced hyperuricemia by specifically inhibiting the [^{14}C]-urate uptake activities of Glut9 and Urat1 in a noncompetitive manner, with a half-maximal inhibitory concentration values of 30.17 ± 8.68 and 31.56 ± 1.37 μM, respectively (Chen Y. et al., 2021). Baicalein also downregulates Glut9 and Urat1 expression in the kidney and suppressed XOD activity in the serum and liver (Chen Y. et al., 2021). Cisplatin resistance is one of the major obstacles in the treatment of non-small cell lung cancer. Combining baicalein with cisplatin has been shown to significantly attenuate cisplatin resistance in human A549 lung adenocarcinoma cells through inhibition of the PI3K/AKT/NF-κB pathway (Yu et al., 2017).

Present-day first-line medications for the management of hyperuricemia/gout

It is not clear whether elevated uric acid is the primary cause of kidney disease or the oxidant (H_2O_2) produced as byproduct by the activation of XOD in addition to uric acid. Are uric acid-lowering agents more beneficial than angiotensin-converting enzyme inhibitors in subjects with CKD? At present, the first-line medications for hyperuricemia/gout are XOD inhibitors, such as allopurinol (a purine analog and competitive xanthine oxidase inhibitor), or the more renoprotective febuxostat/topiroxostat (a nonpurine analog), which inhibits uric acid production (Figure 1). Although allopurinol can successfully control serum urate levels in hyperuricemia/gout patients, long-term use of a high dose of allopurinol to promote the dissolution of urate crystals has been linked to an increased risk of liver, kidney and cardiovascular diseases (Arellano and Sacristán, J. A. 1993; Yang et al., 2015). Febuxostat is used only in the subset of CKD patients with impaired renal function due to its hepatic elimination. There is a risk that all xanthine oxidase inhibitors can increase urinary xanthine levels, which can be nephrotoxic. Uricosurics lower uric acid levels by promoting urate excretion through interactions with urate transporters. Uricosuric diuretics such as probenecid and benzbromarone increase urine output, but high-dose or long-term use of diuretics increases the risk of hepatotoxicity and renal failure (Stamp and Chapman, 2014). Probenecid is ineffective in patients with renal impairment (Stamp et al., 2005). Benzbromarone is effective in allopurinol-intolerant patients with renal failure, solid organ transplant or tophaceous/polyarticular gout. In an oocyte expression system, benzbromarone has been found to inhibit ABCG2, the major transporter for urate excretion in the kidney and intestine, along with OAT1, OAT3 and NPT1 (Mandal et al., 2017). Notably, benzbromarone was withdrawn from the market by Sanofi-Synthelabo in 2003, after reports of serious hepatotoxicity. Fenofibrate may reduce serum urate but it is associated with the decline in renal function (Stamp et al., 2005). In transplant recipients, there is a risk of adverse effects and potentially severe interactions between hypouricemic and immunosuppressive drugs (Stamp et al., 2005).

Conclusion

Hyperuricemia is associated with multiple comorbidities, including metabolic syndrome, CKD, urolithiasis, cardiovascular disease, hypertension, dyslipidemia and diabetes, creating a demand for new antihyperuricemic and nephroprotective drugs. Long-term use of high doses of first-line medications for hyperuricemia likely induces acute kidney disease, CKD, liver toxicity, and adverse effects in transplant recipients. Therefore, there is an urgent need for new nephroprotective drugs with improved safety profiles and tolerance. To avoid oxidative stress and excessive inflammatory responses, new therapeutic drugs with strong antioxidant and anti-inflammatory activities would be preferred. There is substantial evidence of naturally occurring bioactive compounds from various medicinal plants with antihyperuricemic and nephroprotective potential that can protect the kidney from various insults and maintain their integrity and functions. The current review has covered some of the compounds that influence the expression and activity of urate transporters, including URAT1, GLUT9, ABCG2, NPTs, and OATs, and their regulatory pathways, such as the PI3K/AKT, JAK/STAT3, NLRP3, and NF-κB pathways.

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SX and SW directed and guided this review. YB wrote and edited the whole article. XM, LS, XX, CT, and DL reviewed and made some comments on the article. All authors read and approved the final article.

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Conflict of interest

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EDITED BY
Dan Tang,
Guangdong Pharmaceutical University, China

REVIEWED BY
Suleiman Muhammad,
Usmanu Danfodiyo University, Nigeria
Mingsan Miao,
Henan University of Traditional Chinese Medicine, China

*CORRESPONDENCE
Yanbing Gong,
gyb_1226@163.com

†These authors have contributed equally to this work and share first authorship

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Efficacy and safety of Keluoxin capsule in combination with Western medicine for diabetic kidney disease: A systematic review and meta-analysis

Wenhua Zhang[1†], Jingxin Zhou[1†], Churan Wang[1], Xu Wang[1], Shuwen Zhang[1], Weiyu Jia[1], Yijia Jiang[1], Lan Lin[2] and Yanbing Gong[1*]

[1]Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China, [2]Guang'anmen Hospital China Academy of Chinese Medicine Sciences, Beijing, China

Objective: Keluoxin capsule (KLXC) has been widely used in diabetic kidney disease (DKD), but its efficacy and safety have not yet been clarified. A systematic review and meta-analysis were performed to assess the efficacy and safety of KLXC for DKD.

Methods: The randomized control trials (RCTs) included KLXC searched from seven major English and Chinese databases up until 3 June 2022. The methodological quality and risk of bias were assessed by version 2 of the Cochrane risk-of-bias tool (RoB 2) for the RCTs from the Cochrane Handbook. The analyses were conducted by RevMan 5.4 and Stata 17.0.

Results: A total of 20 trials with 1,500 participants were identified. The meta-analysis showed that KLXC combined with Western medicine was superior to the use of Western medicine alone for DKD which included improvements in the estimated glomerular filtration rate (eGFR) [MD = 3.04, 95% CI (0.30, 5.78), p = 0.03], reduction in microalbuminuria (mALB) [MD = −25.83, 95% CI (−41.20, −10.47), p = 0.001], urinary albumin excretion rate (UAER) [SMD = −0.97, 95% CI (−1.50, −0.44), p = 0.0003], 24-h urine protein (24hUpro) [SMD = −1.31, 95% CI (−1.82, −0.80), p < 0.00001], serum creatinine (Scr) [MD = −11.39, 95% CI (−18.76, −4.02), p = 0.002], blood urea nitrogen (BUN) [MD = −1.28, 95% CI (−1.67, −0.88), p < 0.00001], fasting blood glucose (FBG) [MD = −0.51, 95% CI (−0.90, −0.11), p = 0.01], total cholesterol (TC) [MD = −1.04, 95% CI (−1.40, −0.68), p < 0.00001], triglycerides (TG) [MD = −0.36, 95% CI (−0.50, −0.23), p < 0.00001], and low-density lipoprotein cholesterol (LDL) [MD = −0.39, 95% CI (−0.71, −0.07), p = 0.02]. Results showed no statistically significant difference in glycated hemoglobin (HbA1c) (p = 0.14) or adverse events (p = 0.81) between the two groups.

Conclusion: The combination of KLXC and Western medicine had a positive effect on DKD. However, due to the high clinical heterogeneity and low quality of included studies, further standardized, large-scale, rigorously designed RCTs

for DKD in the definitive stage are still necessary to achieve more accurate results.

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KEYWORDS

Keluoxin capsule, diabetic kidney disease, systematic review, meta-analysis, Chinese patent medicine

1 Introduction

Diabetic kidney disease (DKD), as one of the most serious microvascular complications of diabetes mellitus (DM), is characterized by persistent albuminuria or reduced estimated glomerular filtration rate (eGFR) due to chronic exposure to hyperglycemia, resulting in progressive alterations in the kidney structure and function (Alicic et al., 2017; Selby and Taal, 2020). Epidemiological studies have shown that approximately 20%–50% of DM developed into DKD, of which 50% progressed to end-stage renal disease (ESRD) requiring dialysis or kidney transplantation with an increased risk of cardiovascular disease and premature mortality (Afkarian et al., 2013; Selby and Taal, 2020). The improvement of health outcomes should refocus on the strategies to control the progression of DKD to ESRD.

Improving albuminuria and reducing eGFR in DKD patients are important treatments to delay the progress to ESRD (Banerjee et al., 2021). Current therapies to postpone the progression of CKD include angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), and sodium–glucose cotransporter 2 (SGLT2) inhibitors (Barrera-Chimal et al., 2021; Wang et al., 2021; Ravindran and Munusamy, 2022). Moreover, the coexistence of multiple risk factors and concurrent comorbidities leads to an increasing number of combined medications, while reduced drug clearance and consequent side effects also limit the choice of treatments (Tuttle et al., 2014). Therefore, the search for additional complementary and alternative combination therapies remains urgently necessary for DKD.

Chinese medicine possessed promising clinical benefits as primary or alternative therapies for DKD with rarely observed adverse effects (Tang et al., 2021). KLXC is a proprietary Chinese medicine widely used in China for DKD. It is composed of *Astragalus membranaceus*, glossy privet fruit, leech, Rheum officinale, Pseudostellaria heterophylla, and Lycii fructus. The overall ingredients of KLXC are listed in Table 1. KLXC has the function of tonifying Qi and Yin and activating the blood circulation to remove blood stasis (Endocrine Professional Committee of the Chinese Society of Integrative Medicine, 2020). Previous clinical studies have demonstrated that KLXC exerts therapeutic effects on DKD by improving glucolipid metabolism, regulating microcirculation, and preventing kidney damage (Lin et al., 2000). KLXC is recommended as a Class 1A proprietary Chinese medicine in the Chinese DKD guidelines (Yu et al., 2022), increasing frequent RCTs on KLXC supplemental therapy for DKD were reported in China. These RCTs have not only demonstrated positive effects of KLXC on glucolipid metabolism but also contributed to improved renal function with mild manageable adverse effects (Zhao et al., 2017; Bai et al., 2019; Yang et al., 2021). However, systematic evaluations of its efficacy and safety for DKD are scarce. Therefore, a systematic review and meta-analysis were conducted to comprehensively evaluate the benefits and drawbacks of KLXC for DKD to provide trustworthy evidence for clinical applications.

TABLE 1 Complete name and species of ingredients of KLXC.

Chinese name	Pharmaceutical name	Species	Family
Huangqi	Astragalus membranaceus	*Astragalus membranaceus* (Fisch.) Bge.	Leguminosae
Nvzhenzi	Glossy privet fruit	*Ligustrum lucidum* Ait.	Myrtaceae
Shuizhi	Leech	*Antiaris toxicaria* (Pers.) Lesch.	Leechidae
Dahuang	Rheum officinale	*Rheum officinale* Baill.	Polygonaceae
Taizishen	Pseudostellaria heterophylla	*Pseudostellaria heterophylla* (Miq.) Pax	Caryophyllaceae
Gouqizi	Lycii fructus	*Lycium barbarum* L.	Solanaceae

2 Methods

2.1 Study registration

The protocol for this study had been registered on INPLASY (ID: INPLASY 2021110067) and conducted on the basis of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocol statement guidelines (Shamseer et al., 2015). As all the research materials required were published studies, no ethical approval was required for conducting this study.

2.2 Inclusion and exclusion criteria

2.2.1 Type of studies

Only RCTs were eligible for inclusion, regardless of the languages. Animal experiments, case reports, nonclinical research, commentaries, and repeated publications were not included. RCTs with incomplete and unavailable important data were excluded.

2.2.2 Type of participants

The study included adult participants aged 18 years or older who were diagnosed with DKD according to the Kidney Disease Outcomes Quality Initiative (KDOQI) criteria (National Kidney Foundation, 2012). There were no restrictions on the type of DM, stage of DKD, gender, nationality, race, education, or job.

2.2.3 Type of interventions

We considered all intervention trials that met the inclusion criteria, which included treatment with KLXC on an unrestricted dosage or course. Comparators consisted of any Western medicine, placebo, or no intervention. Both groups received the same conventional treatments for DKD, which included comprehensive management of glycemia, blood pressure, serum lipid level, lifestyle, and nutrition following the recommendations of the KDOQI clinical practice guidelines. Any herbal or Chinese medicine treatments were excluded from the analysis.

2.2.4 Primary and secondary outcomes

The primary outcomes included eGFR, microalbuminuria (mALB), urinary albumin excretion rate (UAER), and 24-h urine protein (24hUpro). The secondary outcomes included kidney function (Scr, BUN), glucose (FBG, HbA1c), and lipids (TC, TG, and LDL). Additional outcomes were adverse events.

2.3 Search strategy

The China National Knowledge Infrastructure (CNKI), Wanfang data (WF Data), VIP database, SinoMed, PubMed, Web of Science (WOS), and Cochrane Library (Clib) were searched to ensure all possible RCTs on KLXC for DKD, without language restrictions. The time interval for literature searching was from the inception of the libraries to 3 June 2022. The key search terms were "diabetic kidney disease" or "diabetic nephropathy," "keluoxin," and "random." The search strategies are shown in Supplementary Table S1.

2.4 Study selection

Records were extracted from each database and imported into EndNote 20, and duplicates were removed. Two independent reviewers screened the titles and abstracts to exclude irrelevant studies and reviewed full texts according to the inclusion and exclusion criteria. Any disagreements between the two reviewers were resolved by a third reviewer. Additionally, references to the included studies were searched for further potentially relevant articles. If the same clinical data were published more than once, the report that contained the most recent and comprehensive information such as the largest sample size was included.

2.5 Data extraction and analysis

2.5.1 Data extraction

Two reviewers independently extracted the following information from the selected studies with a predefined form: first author, year of publication, country, stage of DKD, study design, sample size, gender, age, dose and course of KLXC, type of control, trial cycle, and outcomes. If information was missing or unavailable directly from the articles, we contacted the corresponding authors to obtain the data and documented all contacts. Studies were excluded if we were unable to obtain the relevant data.

2.5.2 Risk of bias assessment

The methodological quality and risk of bias of the included studies were assessed independently by two reviewers using version 2 of the Cochrane risk-of-bias tool (RoB 2) of the Cochrane Handbook for RCTs (Cumpston et al., 2022). Sources of bias assessment included the randomization process, deviations from the intended intervention, missing outcome data, measurement of the outcome, and selection of the reported result. Any disagreements were resolved by a third reviewer.

2.5.3 Data synthesis and analysis

Statistical analyses were performed using the RevMan software (version 5.4, Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration). Continuous variables that included the primary and secondary outcomes were evaluated by standardized mean differences (SMD) or mean differences (MD) with 95% confidence intervals (95% CIs).

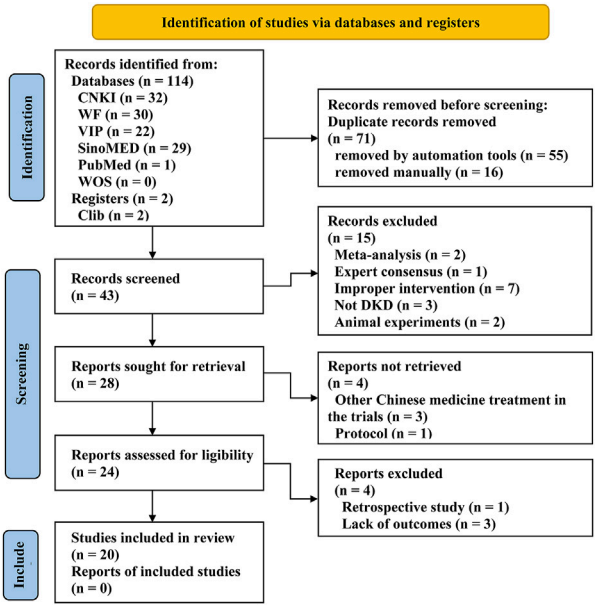

FIGURE 1
Flow diagram of the search for eligible studies.

Dichotomous outcomes were measured by odds ratios (ORs) and their 95% CIs (Bakbergenuly et al., 2019). An OR < 1.00 means that exposure to the risk variable reduces the risk of the event. An OR > 1.00 means that the risk is increased. The statistical significance of an OR is stated along with the OR and its 95% CI. If the 95% CI for the OR includes 1.00, the OR is not statistically significant (Andrade, 2015).

Heterogeneity among studies was assessed by the I^2 test (Higgins and Thompson, 2002), and the data with low heterogeneity (I^2 < 50%, $p \geq 0.1$) were assessed as a fixed effects model. Data with significant heterogeneity (I^2 > 50%, $p < 0.1$) were assessed as a random effects model for meta-analysis. If the data could not be meta-analyzed, descriptive analysis was applied.

2.5.4 Subgroup and sensitivity analysis

If there was high heterogeneity and sufficient data, the subgroup analysis was performed to explore the possible sources of high heterogeneity between studies based on the stage of DKD, age, trial cycle, etc. If heterogeneity was high but data were small, sensitivity analysis was performed by removing each study individually and observing its impact on the overall results.

2.5.5 Publication bias assessment

Outcomes reported more than 10 times (Sterne et al., 2011) were selected to assess publication bias by Egger's test using Stata 16.0, and studies were considered to have publication bias if $p < 0.05$.

3 Results

3.1 Search results

A total of 114 articles were retrieved according to the search queries. After removing 71 duplicates by EndNote and manually, 43 articles remained for further examination. After scanning the titles and abstracts, 19 studies were removed. After reading the full texts, 4 articles were excluded for the ineligible study type ($n = 1$) and missing outcomes ($n = 3$), and ultimately 20 qualified trials (He et al., 2012; Hou, 2012; Liu et al., 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; Chen, 2014; He et al., 2014; Shen, 2015; Wei et al., 2015; Li et al., 2016; Wang, 2016; Zhang, 2016; Guo et al., 2019; Hou, 2019; Chen et al., 2020; Fu et al., 2020; Jin, 2020; Cui, 2021; Yu and Liang, 2021) were identified for further systematic review and meta-analysis. The overall screening process is detailed in the PRISMA (Page et al., 2021) flow diagram (Figure 1).

A total of 20 trials with 1,500 DKD patients (treatment group, 755; control group 745) were included with the intervention duration ranging from 4 weeks to 6 months, and all of the studies were conducted and published in China. There were 18 trials (Liu et al., 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; Chen, 2014; Shen, 2015; Wei et al., 2015; Li et al., 2016; Wang, 2016; Hou, 2019; Chen et al., 2020; Jin, 2020; Cui, 2021; Yu and Liang, 2021) treated with the combination of KLXC and Western medicine in the experiment group, and Western medicine alone in the control group. There was one trial (Hou, 2012) conducted with conventional treatment in the control group and supplemented with KLXC in the experiment group. There was one trial (Zhang, 2016) that had KLXC in the treatment group and KLXC placebo in the control group in addition to Western medicine treatment. The specific characteristics of the included 20 trials are displayed in Table 2.

3.2 Risk of bias

The risk of bias for the included trials is summarized in Figure 2 and Supplementary Figure S1. In the randomization process, there were five trials (Hou, 2012; Li et al., 2016; Zhang, 2016; Cui, 2021; Yu and Liang, 2021) that generated the allocation sequence by random number tables and one trial (Chen, 2014) that was randomized by patients' admission order, while the remaining trials only stated randomization without specific details of stochastic methods. None of the included trials described whether the allocation sequence was concealed. As for deviations from the intended intervention, only one trial performed a double-blind method. In the remaining trials, the participants were aware of their assigned interventions, but there was no information about the care or people delivering the interventions. Thus, there

TABLE 2 The characteristics of the included trials.

Study ID	Stage of DKD	Sample Size (male)		Age (rang/mean, year)	Interventions		Trial Cycle	Indictors			
					T	C		Kidney	glucose	Lipid	Safety
He et al, (2012)	DN III	T: 22 C: 23	T: 42(22) C: 44(21)	T:53.5 ± 8.7 C:55.1 ± 9.1	KLX 2 g tid + Candesartan cilexetil 8 mg qd	Candesartan cilexetil 8 mg qd	2 m	②⑤⑥	⑩	⑬⑭⑮⑯	
	DN IV	T: 21 C: 20						②⑤⑦			
Hou (2012)	DN III DN IV	T:25(12) C:26(17)		T:61.76 ± 9.99 C:62.20 ± 10.96	KLX 2 g tid + Conventional treatments	Conventional treatments	8 w	①②④⑦⑨	⑫		⑰⑱⑲
Liu et al. (2012)	DN III	T: 21 C: 22	T: 39(21) C: 41(20)	T:53.5 ± 8.7 C:55.1 ± 9.1	KLX 2 g tid + Enalapril 10 mg qd	Enalapril 10 mg qd	2 m	②⑤⑥	⑩	⑬⑭⑮⑯	
	DN IV	T: 18 C: 19						②⑤⑦			
Hu et al. (2013)	DN IV	T:32(20) C:28(11)		T:54.71 ± 9.5 C:54.46 ± 9.6	KLX 2 g tid + Valsartan 80 mg qd	Valsartan 80 mg qd	8 w	②⑤⑦			⑰⑱
Ju et al. (2013)	DN III	T:25(14) C:25(13)		T:51.2 ± 6.4 C:52.6 ± 6.2	KLX 2 g tid + Benazepril 10 mg qd	Benazepril 10 mg qd	8 w	②③⑨	⑩⑪⑫	⑬⑭⑮	⑰⑱⑲⑳
Zhou (2013)	DN III	T:54 C:48		T:57.48 ± 6.78 C:58.2 ± 7.16	KLX 2 g tid + Irbesartan 150 mg qd	Irbesartan 150 mg qd	8 w	②⑤⑦			⑰⑱
He et al. (2014)	Early DN	T:22(13) C:23(12)		T:55.3 ± 9.5 C:53.1 ± 8.7	KLX 2 g tid + Candesartan cilexetil 8 mg qd	Candesartan cilexetil 8 mg qd	2 m	②⑥	⑩		⑰
Chen (2014)	DN III DN IV	T:30 C:28		36 ± 22.5	KLX 2 g tid + Alprostadil 10 μg qd	Alprostadil 10 μg qd	15 d	①⑥	⑩	⑬⑭⑮⑯	⑰
Shen (2015)	DN III DN IV	T:40(21) C:40(18)		T:52.7 ± 9.0 C:54.1 ± 8.6	KLX 2 g tid + Candesartan cilexetil 8 mg qd	Candesartan cilexetil 8 mg qd	2 m	②⑤	⑩	⑬⑭⑮⑯	
Wei et al. (2015)	DN III DN IV	T:42(24) C:40		T:37 ± 21.5 C:36.5 ± 20.7	KLX 2 g tid + Alprostadil 10 μg qd	Alprostadil 10 μg qd	4 w	①②③⑧	⑩		
Li et al. (2016)	DN	T:53(30) C:53(32)		T:61.34 ± 10.83 C:63.03 ± 9.16	KLX 2 g tid + Telmisartan 80 mg qd	Telmisartan 80 mg qd	8 w	①⑥⑦		⑬⑭	⑰
Wang (2016)	DN III DN IV	T:50(30) C:50(31)		T:64.5 C:64.2	KLX 2 g tid + Alprostadil 10 μg qd	Alprostadil 10 μg qd	4 w	①②③⑧	⑩		⑱
Zhang (2016)	Early DN	T:75(39) C:74(40)		T:47.4 ± 5.2 C:47.3 ± 5.4	KLX 2 g tid + Irbesartan 150 mg qd	KLX placebo + Irbesartan 150 mg qd	24 w	①②③⑥⑦	⑩⑪	⑬⑭⑮⑯	⑰⑱⑳

(Continued on following page)

TABLE 2 (*Continued*) The characteristics of the included trials.

Study ID	Stage of DKD	Sample Size (male)		Age (rang/ mean, year)	Interventions		Trial Cycle	Indictors			
					T	C		Kidney	glucose	Lipid	Safety
Guo et al. (2019)	DN III DN IV	T:30(16) C:30(17)		T: 55.69±6.32 C: 55.83±6.42	KLX 2 g tid + Compound α-ketoacid tablet 2.52 g tid	Compound α-ketoacid tablet 2.52 g tid	3 m	①②③⑦			⑰
Hou (2019)	DN	T:48(26) C:48(28)		T:53.01 ± 4.87 C:52.18 ± 5.04	KLX 2 g tid + Benazepril 10 mg qd	Benazepril 10 mg qd	1 m	②③	⑩⑪⑫		
Chen et al. (2020)	DN	T:30(16) C:30(17)		T:53.80 ± 6.17 C:53.68 ± 6.09	KLX 2 g tid + Benazepril 10 mg qd	Benazepril 10 mg qd	2 m	①②③⑥⑦			
Fu et al. (2020)	DN	T:43 C:43		38 ± 20.8	KLX 2 g tid + Olmesartan medoxomil 20 mg qd	Olmesartan medoxomil 20 mg qd	6 m	①②③⑧	⑩		
Jin (2020)	DN III	40	T: 40(22) C: 40(23)	T:53.38 ± 5.66 C:53.43 ± 5.51	KLX 2 g tid + Ramipril 5 mg qd	Ramipril 5 mg qd	8 w	②⑤⑥	⑩		⑰
	DN IV	40						②⑤⑦			
Cui (2021)	DN III	T: 31 C: 32	T: 57(32) C: 56(30)	T:59.02 ± 5.28 C:58.75 ± 5.32	KLX 2 g tid + Benazepril 10 mg qd	Benazepril 10 mg qd	4 w	①②③⑥			
	DN IV	T: 26 C: 24									
Yu and Liang (2021)	DN	T:56(29) C: 56(28)		T:60.85 ± 4.62 C:61.73 ± 6.58	KLX 2 g tid + Valsartan 80 mg qd	Valsartan 80 mg qd	8 w	②③④			

Notes: ①clinical symptoms; ②Scr; ③BUN; ④eGFR; ⑤ALB; ⑥UAER; ⑦24hUpro; ⑧mALB; ⑨ACR; ⑩FBG; ⑪postprandial blood glucose; ⑫HbA1c; ⑬TC; ⑭TG; ⑮LDL-C; ⑯HDL-C; ⑰adverse reactions; ⑱liver function; ⑲ECG; ⑳blood, urine, stool routine.

were some marked concerns. In terms of the outcome data, reports with complete results were judged to be low risk, except for one trial (Chen, 2014) without complete data that was judged to be high risk. On the measurement of the outcome, half of the included studies (He et al., 2012; Liu et al., 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; He et al., 2014; Shen, 2015; Hou, 2019; Jin, 2020; Yu and Liang, 2021) took biochemical indicators as outcomes and did not evaluate the effective rates; the remaining studies considered the specific symptoms and biochemical indicators as effective rates, so most were judged to have some concerns. All the outcomes were generated under a prespecified analysis plan and were considered low risk. Overall, only one trial (Zhang, 2016) was classified as low risk, which was conducted with a random numerical table method, double-blinding, placebo-controlled trial and four trials (Hou, 2012; Li et al., 2016; Cui, 2021; Yu and Liang, 2021) that were marked as having some concerns, while the remaining were judged as high risk. In conclusion, most of the involved clinical trials were deemed to be of poor methodological quality.

3.3 Meta-analysis results

3.3.1 The primary outcomes

eGFR was reported in two trials (Hou, 2012; Yu and Liang, 2021). Heterogeneity analysis revealed no significant heterogeneity (p = 0.88, I^2 = 0%), and a fixed effect model

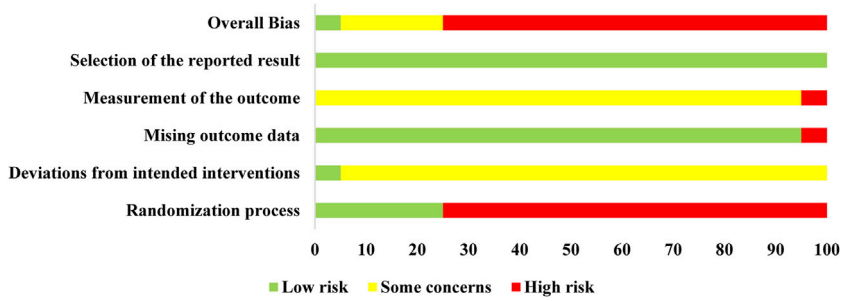

FIGURE 2
Risk of bias assessment in studies.

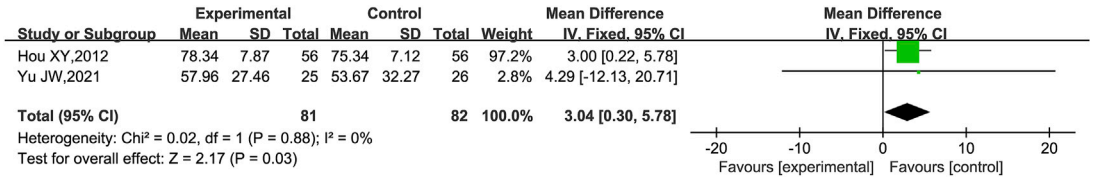

FIGURE 3
Forest plot of eGFR for KLXC + Western medicine vs. Western medicine alone (ml/min).

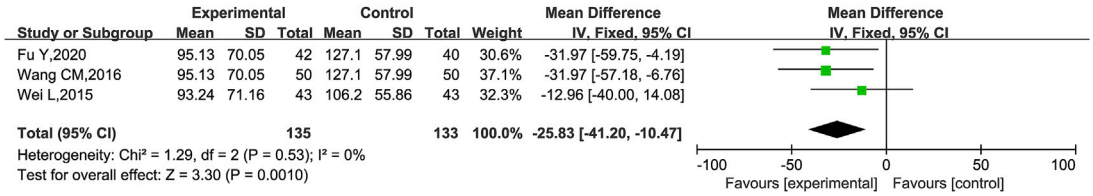

FIGURE 4
Forest plot of t mALB for KLXC + Western medicine vs. Western medicine alone (g/L).

was used for statistical analysis. The results showed that the group receiving KLXC combined with Western medicine was more likely to improve eGFR after 8 weeks of treatment than the group receiving Western medicine alone [MD = 3.04, 95% CI (0.30, 5.78), p = 0.03] (Figure 3).

mALB was reported in three trials (Wei et al., 2015; Wang, 2016; Fu et al., 2020). Heterogeneity analysis revealed no significant heterogeneity (p = 0.53, I^2 = 0%), and a fixed effect model was used for statistical analysis. The results showed that KLXC combined with Western medicine was significantly more effective in reducing mALB than when taking Western medicine alone [MD = −25.83, 95% CI (−41.20, −10.47), p = 0.001] (Figure 4).

UAER was reported in nine trials (He et al., 2012; Liu et al., 2012; Chen, 2014; He et al., 2014; Li et al., 2016; Zhang, 2016; Chen et al., 2020; Jin, 2020; Cui, 2021), of which one trial (Chen, 2014) only stated that UAER was measured but had no data in the results, while the remaining eight trials were included for meta-analysis, of which five (He et al., 2012; Liu et al., 2012; He et al., 2014; Zhang, 2016; Jin, 2020) had UAER in mg/24 h and three trials (Li et al., 2016; Cui, 2021; Chen et al., 2020) had UAER in mg/min. Therefore, SMD was used to evaluate the difference. The result showed that KLXC combined with Western medicine was more effective in reducing UAER than when taking Western medicine alone [SMD = −0.97, 95% CI (−1.50, −0.44), p = 0.0003] (Figure 5). Heterogeneity analysis revealed highly

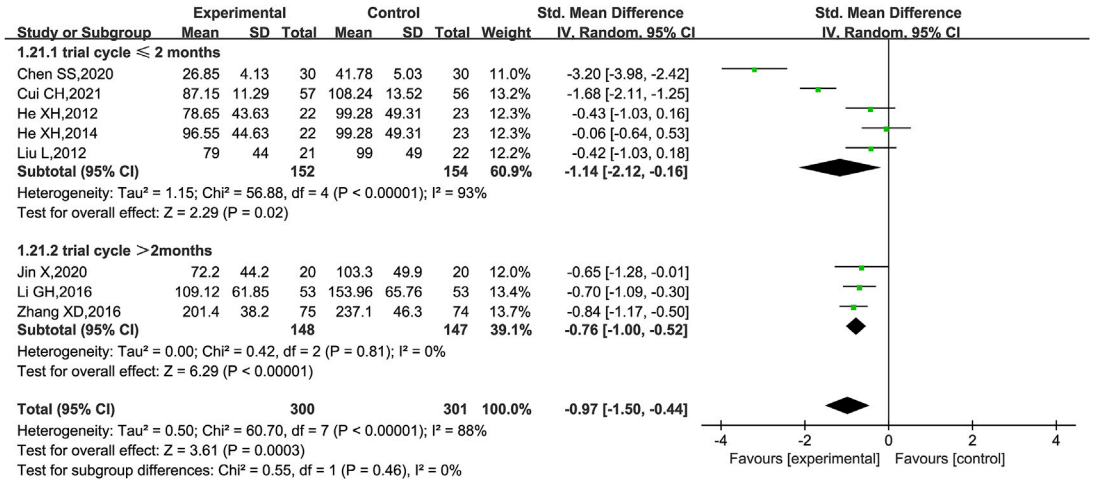

FIGURE 5
Forest plot of UAER for KLXC + Western medicine vs. Western medicine alone.

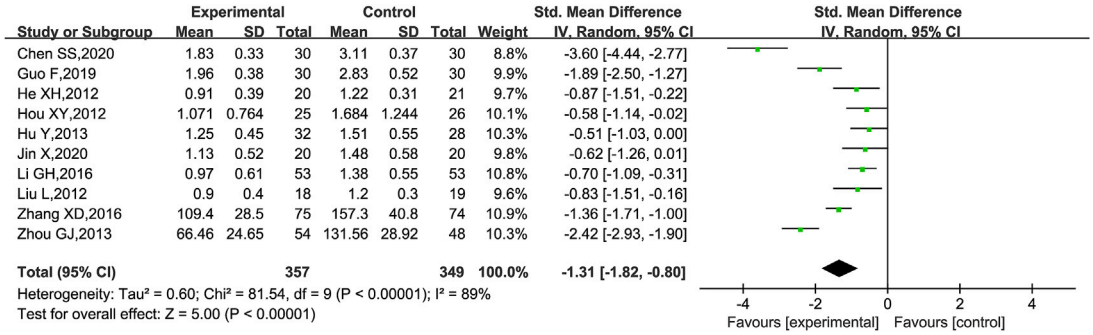

FIGURE 6
Forest plot of 24hUpro for KLXC + Western medicine vs. Western medicine alone.

significant heterogeneity ($p < 0.00001$, $I^2 = 88\%$), thus a random effects model was used for statistical analysis, and a subgroup analysis was performed according to the trial cycle. The result showed that heterogeneity was significantly reduced in the group of trial cycle > 2 months ($p = 081$, $I^2 = 0\%$), therefore the duration of therapy could be a source of high heterogeneity.

24hUpro was reported in 10 trials (Hou, 2012; Liu et al., 2012; Hu et al., 2013; Zhou, 2013; He et al., 2012; Li et al., 2016; Zhang, 2016; Guo et al., 2019; Chen et al., 2020; Jin, 2020), of which 8 trials (Hou, 2012; Liu et al., 2012; Hu et al., 2013; He et al., 2014; Li et al., 2016; Guo et al., 2019; Chen et al., 2020; Jin, 2020) had 24hUpro unit in g and the remaining two (Zhou, 2013; Zhang, 2016) in mg. Thus, the forest plot was assessed by SMD. As shown in Figure 6, those who added KLXC in the experiment group had a significant advantage in reducing 24hUpro [SMD = −1.31, 95% CI (−1.82, −0.80), $p < 0.00001$], but the heterogeneity was high ($p < 0.00001$, $I^2 = 89\%$). Thus, a random effects model was used for statistical analysis. However, in the search for the causes of high heterogeneity, neither subgroup analysis based on age and trial period grouping nor study-by-study deletion for sensitivity analysis can eliminate heterogeneity.

3.3.2 The secondary outcomes

Scr was reported in 18 trials (He et al., 2012; Hou, 2012; Liu et al., 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; He et al., 2014; Shen, 2015; Wei et al., 2015; Wang, 2016; Zhang, 2016; Guo et al., 2019; Hou, 2019; Chen et al., 2020; Fu et al., 2020; Jin, 2020; Cui, 2021; Yu and Liang, 2021), of which 1 trial (Shen, 2015) was excluded as it only had a statistical p-value in the results and no specific data on Scr. The meta-analysis showed that KLXC combined with Western drug was more

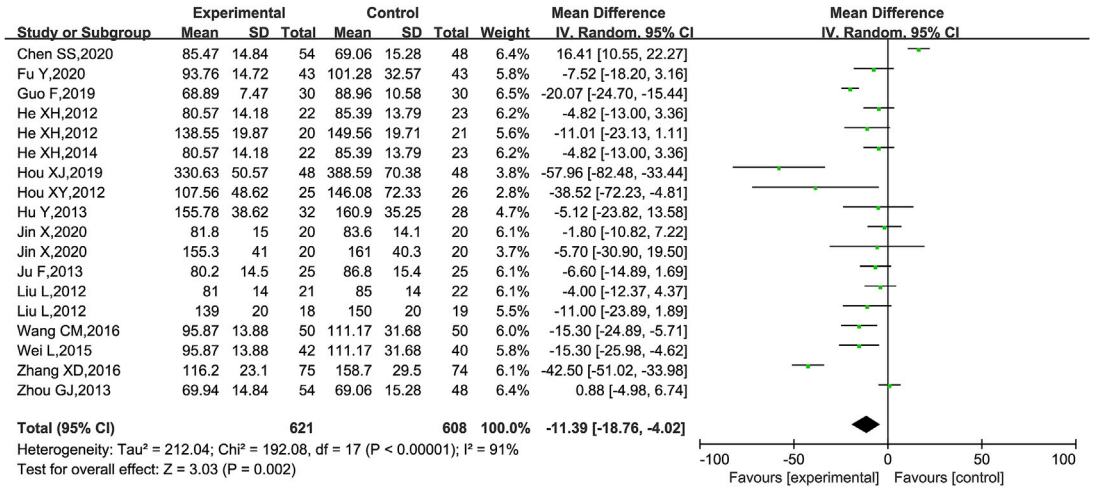

FIGURE 7
Forest plot of Scr for KLXC + Western medicine vs. Western medicine alone (μmol/L).

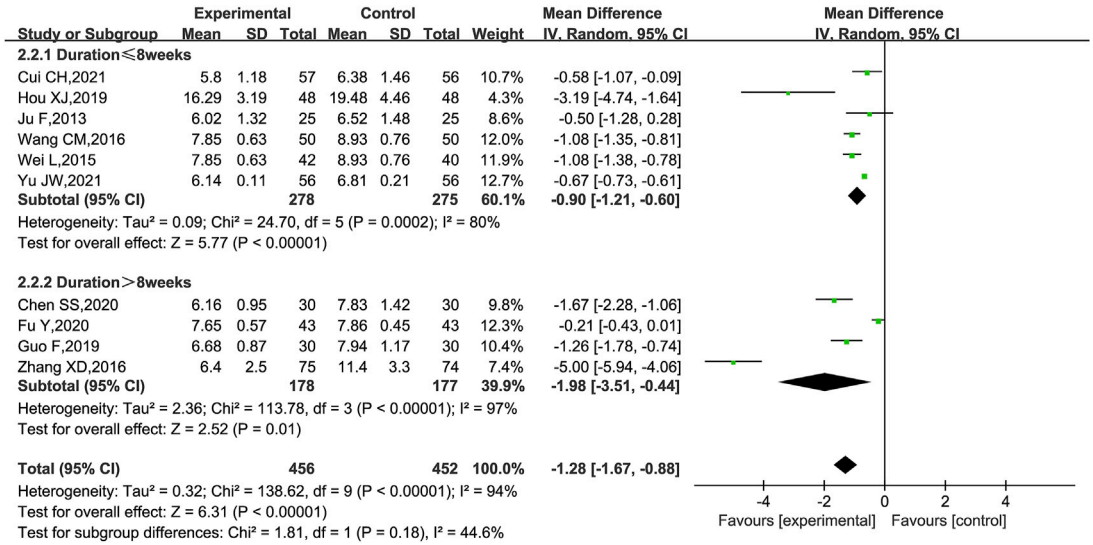

FIGURE 8
Forest plot of BUN for KLXC + Western medicine vs. Western medicine alone (mmol/L).

effective in reducing Scr than when taking Western drug therapy alone [MD = −11.39, 95% CI (−18.76, −4.02), p = 0.002] (Figure 7). Heterogeneity analysis revealed highly significant heterogeneity (p < 0.00001, I^2 = 91%), and a random effects model was used for statistical analysis. In the search for causes of high heterogeneity, heterogeneity remained at a high level, whether subgroup analyses were conducted by age, trial period, DKD stage grouping, or sensitivity analyses were performed by removing trials one by one.

BUN was reported in 10 trials (Hou, 2012; Ju et al., 2013; Wei et al., 2015; Wang, 2016; Zhang, 2016; Guo et al., 2019; Chen et al., 2020; Fu et al., 2020; Cui, 2021; Yu and Liang, 2021). As shown in Figure 8, KLXC combined with Western medicine was more likely to reduce the level of BUN [MD = −1.28, 95% CI (−1.67, −0.88), p < 0.00001] than when taking Western medicine

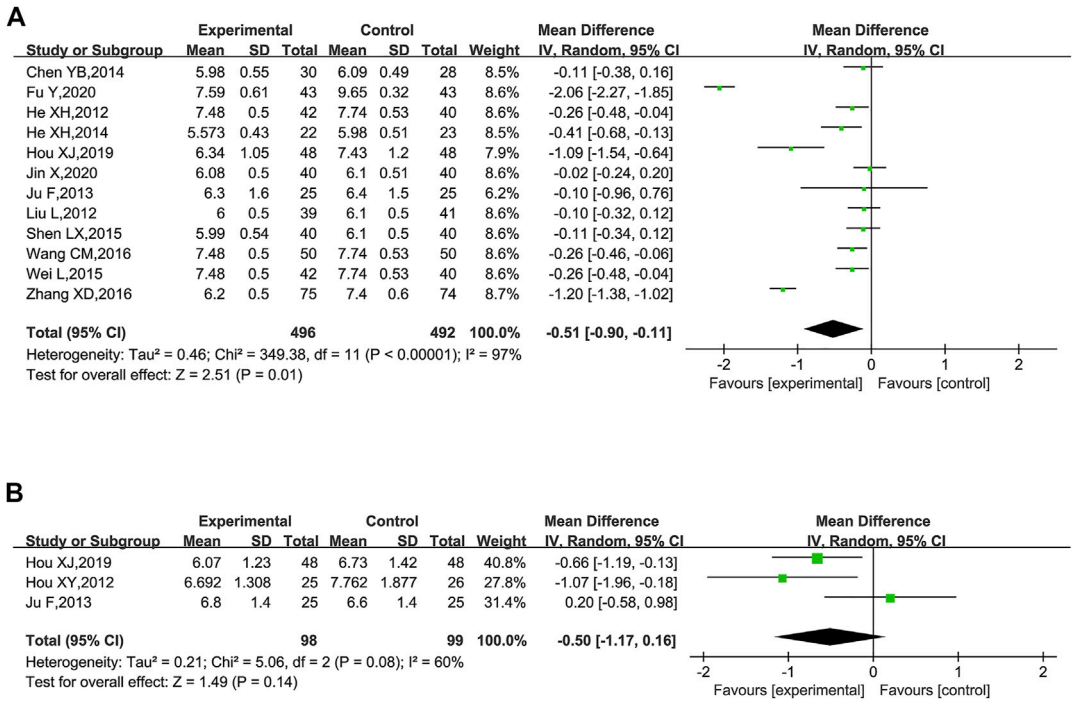

FIGURE 9
Forest plot of glucose for KLXC + Western medicine vs. Western medicine alone. **(A)** Forest plot of FBG for KLXC + Western medicine vs. Western medicine alone. **(B)** Forest plot of HbA1c for KLXC + Western medicine vs. Western medicine alone.

alone, but there was significant heterogeneity between the studies ($p < 0.00001$, $I^2 = 94\%$); a random effects model was used for statistical analysis. When the subgroup analyses were performed according to the trial cycle or stage of DKD, there remained high heterogeneity.

FBG was reported in 12 trials (He et al., 2012; Liu et al., 2012; Ju et al., 2013; Chen, 2014; He et al., 2014; Shen, 2015; Wei et al., 2015; Wang, 2016; Zhang, 2016; Hou, 2019; Fu et al., 2020; Jin, 2020). These studies plus KLXC with Western medicine showed a slight decrease in FBG when compared to taking Western medicine alone [MD = −0.51, 95% CI (−0.90, −0.11), $p = 0.01$], but with significant heterogeneity between the studies ($p < 0.00001$, $I^2 = 97\%$) (Figure 9A). Also, heterogeneity remained high when further subgroup analyses were performed for age and glucose grouping.

HbA1c was reported in three trials (Hou, 2012; Ju et al., 2013; Hou, 2019). The forest plot illustrated no statistically significant difference in HbA1c between the two groups ($p = 0.14$), and the heterogeneity test suggested a high degree of heterogeneity ($p = 0.08$, $I^2 = 60\%$) (Figure 9B). When each study was removed in turn for sensitivity analysis, similar heterogeneity remained.

TC were reported in seven trials (He et al., 2012; Liu et al., 2012; Ju et al., 2013; Chen, 2014; Shen, 2015; Li et al., 2016; Zhang, 2016). The combination of KLXC with Western medicine significantly reduced the TC levels when compared to taking Western medicine alone [MD = −1.04, 95% CI (−1.40, −0.68), $p < 0.00001$], but there was slightly higher heterogeneity between the two studies ($p = 0.03$, $I^2 = 57\%$) (Figure 10A).

TG was reported in seven trials (He et al., 2012; Liu et al., 2012; Ju et al., 2013; Chen, 2014; Shen, 2015; Li et al., 2016; Zhang, 2016). The heterogeneity test suggested a high degree of heterogeneity ($p < 0.00001$, $I^2 = 91\%$), and sensitivity analysis was carried out by excluding trials one by one. Heterogeneity was significantly reduced after removing the study reported by Li et al. (2016) ($p = 0.97$, $I^2 = 0\%$). As shown in Table 2, different from the other 6 trials, Li's research did not explicitly report the stage of DKD, which might contribute to high heterogeneity. A fixed effects model was used for meta-analysis after removing Li's report. The result showed that the combination of KLXC with Western medicine was more effective in reducing the TG levels than when taking Western medicine alone [MD = −0.36, 95% CI (−0.50, −0.23), $p < 0.00001$] (Figure 10B).

LDL was reported in six trials (He et al., 2012; Liu et al., 2012; Ju et al., 2013; Chen, 2014; Shen, 2015; Zhang, 2016). Significant heterogeneity was detected among these studies ($p < 0.00001$, $I^2 = 89\%$). When sensitivity analysis was

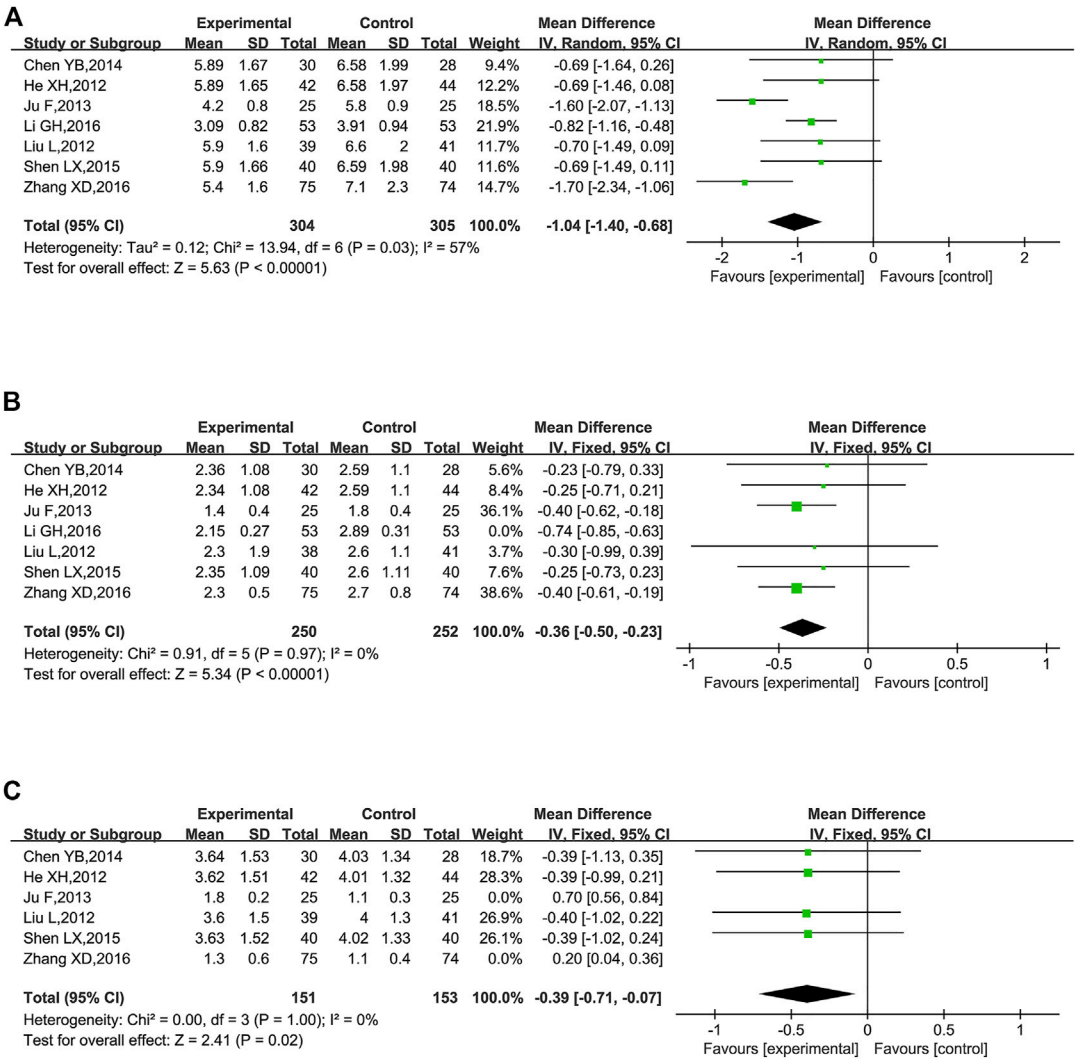

FIGURE 10
Forest plot of lipids for KLXC + Western medicine vs. Western medicine alone (mmol/L). **(A)** Forest plot of TC for KLXC + Western medicine vs. Western medicine alone. **(B)** Forest plot of TG for KLXC + Western medicine vs. Western medicine alone. **(C)** Forest plot of LDL for KLXC + Western medicine vs. Western medicine alone.

performed by deleting the studies one by one, heterogeneity remained high. When the two reported studies of Zhang (2016) and Ju et al. (2013) (p = 1.00, I^2 = 0%) were removed simultaneously, the heterogeneity was significantly reduced and we found that both studies met the lowest threshold of LDL for atherosclerotic cardiovascular disease (Grundy et al., 2019), and the remaining four trials had hypercholesterolemia of LDL. Thus, the level of LDL might be the main source of high heterogeneity. A fixed effect model was used for meta-analysis after moving the two studies (Ju et al., 2013; Zhang, 2016). The result showed that KLXC combined with Western medicine was more effective in reducing LDL than taking Western medicine alone [MD = −0.39, 95% CI (−0.71, −0.07), p = 0.02] (Figure 10C).

3.3.3 Other outcomes

Adverse events were reported in 10 trials (Hou, 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; Chen, 2014; He et al., 2014; Li et al., 2016; Zhang, 2016; Guo et al., 2019; Jin, 2020), of which one trial (Chen, 2014) was excluded because the adverse reactions were planned to be assessed in the protocol but not reported in the results, and the remaining nine trials (Hou, 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; He et al., 2014; Li et al., 2016; Zhang, 2016; Guo et al., 2019; Jin, 2020) were analyzed for adverse reactions. There were two cases of diarrhea (Hou, 2012; Jin, 2020), two cases of nausea and vomiting (Guo et al., 2019; Jin, 2020), one case of skin rash (Lin et al., 2000), and one case of hypercalcemia (Guo et al., 2019) in the group with the combination of KLXC and Western medicine. There

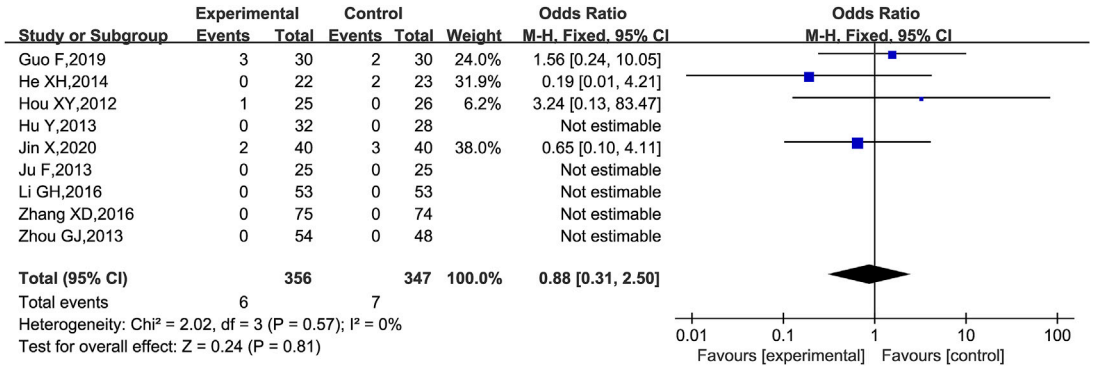

FIGURE 11
Forest plot of adverse events for KLXC + Western medicine vs. Western medicine alone.

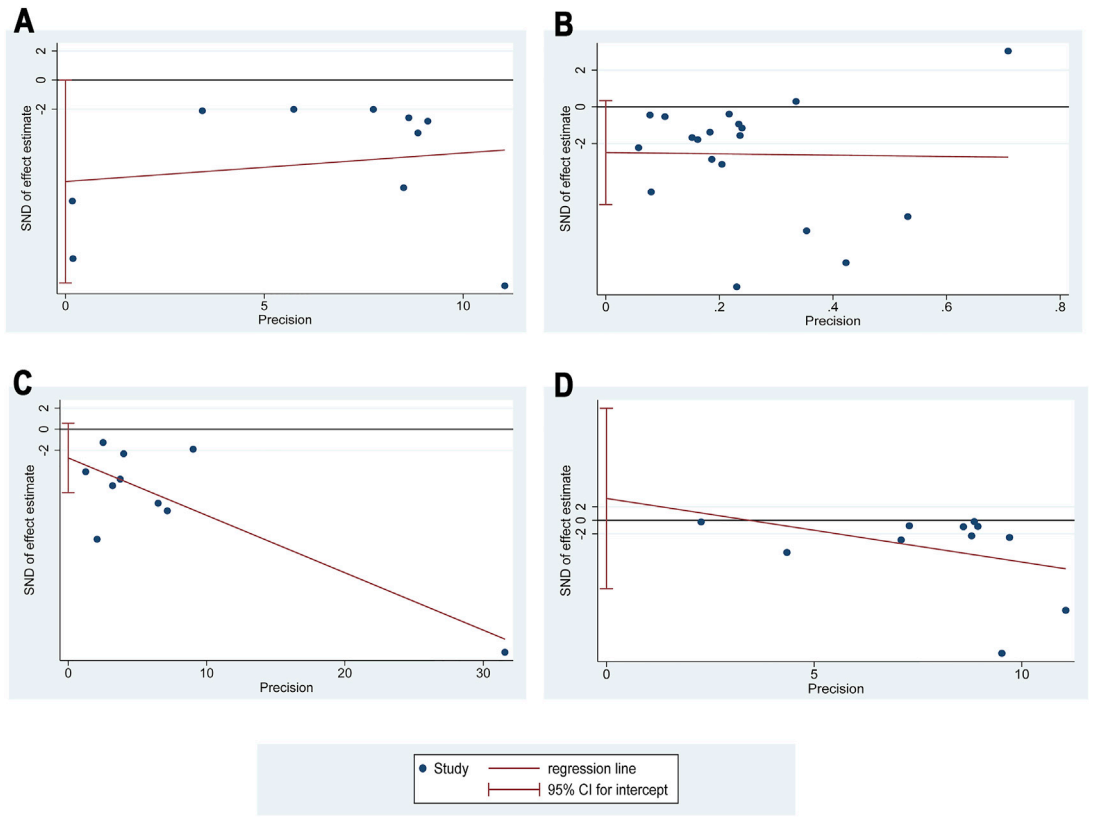

FIGURE 12
Egger's publication funnel plot. **(A)** Egger's funnel plot of 24hUpro. **(B)** Egger's funnel plot of Scr. **(C)** Egger's funnel plot of BUN. **(D)** Egger's funnel plot of FBG.

were two cases of abdominal irritation (He et al., 2014), two cases of nausea and vomiting (He et al., 2012; Hou, 2012; Hu et al., 2013; He et al., 2014; Guo et al., 2019; Hou, 2019; Jin, 2020), two cases of hypercalcemia (Guo et al., 2019), and one case of cough (Jin, 2020) in the control group treated with Western medicine alone. Five trials (Hou, 2012; Hu et al., 2013; Ju et al., 2013; Zhou, 2013; Zhang, 2016) monitored liver function before and after the intervention, and none reported abnormal liver functions. All adverse effects disappeared after appropriate treatment and continued medication, except for one case in the experiment

group that was discontinued due to diarrhea (Hou, 2012). According to the forest plot results, the OR for the adverse events was 0.88 and the 95% CI for the OR was [0.31, 2.50] which included 1.00, implying that the OR was not statistically significant. Thus, there was no statistical difference in the adverse events between the experiment group (KLXC + Western medicine) and control group (Western medicine alone) [OR 0.88 (0.31, 2.50), p = 0.81]. The heterogeneity analysis revealed no significant heterogeneity (p = 0.57, I^2 = 0%) (Figure 11).

3.3.4 Publication bias assessment

At least 10 studies reported 24hUpro (10 RCTs), Scr (18 RCTs), BUN (10 RCTs), and FBG (10 RCTs). So these four outcomes were selected for Egger's test assessment to assess publication bias by Stata 17.0 (Figure 12). The results showed that the p-values were 0.05 (24hUpro), 0.081 (Scr), 0.092 (BUN), 0.6 (FBG), and these p-values were not less than 0.05, indicating that there was no significant publication bias among the studies.

4 Discussion

4.1 Summary of evidence

The coexistence of multiple risk factors and emergence of multiple complications in DKD increased the complexity of management and the diversity of medications (Jiang et al., 2020). It is increasingly emphasized that simultaneous control of blood pressure, glucose, and lipids can help maximize the benefits for DKD patients (Ueki et al., 2021). Therefore, there is an urgent need to comprehensively evaluate the efficacy and safety of medications with multiple therapeutic effects through high-quality evidence-based medicine. In this review, we systematically analyzed the 20 included RCTs that compared the effects of the addition of KLXC on renal functions, glucose, and lipid levels in 1,500 DKD patients. This analysis revealed a significant advantage of the combination of KLXC and Western medicine on renal functions (eGFR, mALB, UAER, 24hUpro, Scr, and BUN), FBG, and lipids (TC, TG, and LDL). In addition, the combination therapy also demonstrated better efficacy in the clinical efficiency rate involving both clinical symptoms and biochemical indicators. Thus, KLXC exhibited significant comprehensive adjuvant treatment advantages for DKD.

The natural progression of DKD was described as progressive albuminuria followed by a decline in eGFR (Tuttle et al., 1990). With the intensive management of DKD, which included intensive glucose and lipid control, improvement in blood pressure, and the use of drugs such as ARBs, ACEI, and SGLT2, the prevalence of albuminuria decreased. However, the increasing incidence of reduced eGFR (Afkarian et al., 1988-2014) and the mechanism still remains unclear and may be related to retinopathy and macrovascular disease (de Boer et al., 2011). In this systematic review, we found that the addition of KLXC reduced both proteinuria (mALB, UAER, and 24hUpro) and the improved eGFR when compared to taking Western drugs alone, which might be the therapeutic advantage of KLXC. However, there was high heterogeneity in UAER and 24hUpro. The trial cycle may be the main heterogeneity of UAER, however, we failed to find the source of heterogeneity in 24hUpro. Recent mechanistic studies have shown that KLXC not only protected the glomerulus but also inhibited the tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) overexpression, increased matrix metalloprotein-9 (MMP-9) expression, and protected tubular basement membrane in rats. It was also associated with protein kinase R–like ER kinase–activating transcription factor 4–CCAAT/enhancer-binding protein homologous protein (PEPK-ATF4-CHOP) pathway–mediated apoptosis of renal tubular epithelial cells and endoplasmic reticulum stress (Wei, 2022). Meanwhile, the constituent Chinese medicines in KLXC also proved to be beneficial for DKD. The animal experiments showed that the active ingredients of *A. membranaceus* were effective in reducing FBG and albuminuria levels, reversing the glomerular hyperfiltration state, and ameliorating the pathological changes of early DKD in rat models (Zhang et al., 2009) and network pharmacology revealed that the targets of *A. membranaceus* for DKD were related to some biological process such as inflammatory response, angiogenesis, and oxidative stress reaction (Guo et al., 2020). Moreover, a meta-analysis revealed that *A. membranaceus* preparations were effective and tolerable for the short-term reduction of albuminuria and Scr in DKD patients (Zhang et al., 2019). Animal experiments showed that hirudin, a natural compound of the leech, inhibited inflammation and podocyte apoptosis in DKD rats *via* the p38 mitogen–activated protein kinase/nuclear factor κB (MAPK/NF-κB) pathway (Han et al., 2020). Rheum officinale reduced glucose and lipids and inhibited oxidative stress in diabetic rats, thereby alleviating nephrotic damage (Hosseini et al., 2017).

DKD frequently coexisted with multiple metabolic disorders. In addition to hyperglycemia, other factors such as hypertension, dyslipidemia, and an unhealthy lifestyle were related to the pathogenesis of DKD (Jiang et al., 2020). It is widely known that hyperglycemia is a primary persistent driver of DKD and intensive glycemia undoubtedly helps control the progression of DKD. We found that KLXC decreased the level of FBG but had no significant reduction of HbA1c which reflect the 120-day average of glucose rather than the point value. The HbA1c may be influenced by the erythrocyte renewal cycle, hemoglobin concentration, acid–base balance, and medicines, which are the main reasons for the inaccuracy of HbA1c (Ansari et al., 2003). Meanwhile, the source for the high heterogeneity of FBG and HbA1c has not been found in this study because of inadequate sample and documentation.

A meta-analysis revealed that high TG levels were an independent risk factor for DKD over 4 years (Russo et al., 2016). Lipotoxicity caused by lipid abnormalities and renal lipid accumulation proved to be associated with damage to the tubule and glomerulus, which promoted kidney dysfunction (Opazo-Ríos et al., 2020). Meanwhile, insulin resistance caused by dyslipidemia also contributed to kidney dysfunction by inflammatory or podocyte damage, which promoted glomerular hyperfiltration and vascular permeability (De Cosmo et al., 2013; Artunc et al., 2016). In this meta-analysis, the combination of KLXC and Western medicine significantly decreased the levels of TC, TG, and LDL more than when taking Western medicine alone. We judged that the higher heterogeneity may be caused by the duration of intervention and the baseline of the lipids.

Thus, KLXC combined with Western medicine showed a significant advantage of the comprehensive therapy for DKD. However, there were some heterogeneities in the glucolipid metabolism outcomes which might be related to various factors such as the stage of DKD, basal values of lipids and glucose, and trial period. Therefore, the factors that affected the results and inclusion of a strict baseline for specific indicators should be refined in future RCTs.

Apart from efficacy, the safety of KLXC should be an equally important consideration, which not only helps find ways to alleviate adverse effects but also guarantees the safety of the drug. However, only half of the included RCTs recorded specific adverse reactions and the corresponding numbers of which five RCTs monitored liver function (Table 2). Based on the adverse reactions reported in the 10 trials, a total of 4 patients in the experiment group experienced gastrointestinal discomfort, which may be related to the greater stimulation of the animal drug or laxative effect of Rheum officinale (Wang et al., 2012). One patient in the experiment group suffered from hypercalcemia, and further research is still required to reveal the possible mechanism. Also, one patient presented with skin rash, which may also be related to drug-induced immune reaction. Although these symptoms were alleviated or disappeared after drug reduction, discontinuation, or symptomatic treatment, there is still a great demand for comprehensive monitoring of side effects and detailed documentation in reports, which includes but is not limited to the symptoms, liver and kidney functions, ECG, and other physical signs. This will help improve the awareness of adverse drug reactions and stimulate research on the mechanisms of adverse reactions.

4.2 Strengths and limitations

This is the first systematic review and meta-analysis of KLXC for DKD in English. Compared with a similar meta-analysis published in China in 2019[10], our study updated four RCTs from 2019 onwards and added the systematic evaluation of eGFR, mALB, UAER, and HbA1c. Bai's research reported no significant difference between group comparisons in 24hUpro, but in our research, the combination of KLXC and Western medicine showed better efficacy than taking Western medicine alone as the sample increased. In addition, we performed subgroup and sensitivity analyses to analyze the high heterogeneity of the outcomes and prospected some implications for future studies on KLXC.

However, our results might be limited by the following aspects. Firstly, most of the included trials were not standard RCTs and were more biased toward real-world studies, and only one trial was randomized and double blinded with high evidence support. Although randomizations were shown in the trials, none of them described the specific randomization method and the concealment of the randomization sequence was a serious problem in many studies. Secondly, none of the studies included in this review had follow-up at the end of the intervention, which was not conducive in observing long-term time effects and dose effects. Thirdly, all the included trials were conducted and published in China, and conclusions may not exclude bias due to geographical and ethnic influences.

4.3 Implication

In addition, the greater benefit of this article might be awareness of current problems and areas for further improvement. 1) Clinical trials that include randomization, allocation concealment, and blinding should be strictly designed and described in detail in the report. 2) The stage of DKD included in the study should be clarified. 3) The assessment of clinical effective rates should be comprehensive and should include a composite of symptoms, blood pressure, renal function, glucose, lipids, and other indicators. 4) Long-term follow-up after the trials are also necessary, especially for end-point events.

5 Conclusion

In conclusion, the systematic review and meta-analysis suggest that KLXC combined with Western medicine is superior to taking Western medicine alone during conventional therapy. However, due to the high clinical heterogeneity and unstandardized nature of the included trials, large-scale, randomized, double-blind, multicenter RCTs at different stages of DKD are required to evaluate or confirm the current results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Author contributions

Conception and design: WZ, JZ, LL, and YG; collection and assembly of data: WZ, CW, XW, and SZ; data analysis and interpretation: WZ, JZ, YG, CW, WJ, and YJ; manuscript writing: all authors; final approval of manuscript: all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fphar.2022.1052852/full#supplementary-material

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EDITED BY
Dan Tang,
Guangdong Pharmaceutical University, China

REVIEWED BY
Mingsan Miao,
Henan University of Traditional Chinese Medicine, China
Yu Wu,
Affiliated Hospital of Nanjing University of Chinese Medicine, China

*CORRESPONDENCE
Rensong Yue,
songrenyue@cdutcm.edu.cn

†These authors have contributed equally to this work

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Efficacy of traditional Chinese medicine injection for diabetic kidney disease: A network meta analysis and systematic review

Caiyi Long[1,2†], Haoyue Feng[1,2†], Zheng Liu[1,2], Zihan Li[1,2], Jun Liu[3], Yayi Jiang[1,2] and Rensong Yue[1*]

[1]Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China, [2]Chengdu University of Traditional Chinese Medicine, Chengdu, China, [3]People's Hospital of Nanjiang County, Bazhong, China

Background: Diabetic kidney disease (DKD) is an important public health problem worldwide that increases the mortality of patients and incurs high medical costs. Traditional Chinese Medicine injections (TCMIs) are widely used in clinical practice. However, their efficacy is unknown owing to a lack of definitive evidence. This study conducted a network meta-analysis (NMA) to evaluate the efficacy and safety of traditional Chinese medicine injections in the treatment of DKD to provide a reference for clinical treatment.

Methods: Total 7 databases had been searched, which included PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), Chinese scientific journal database (VIP), WanFang, and SinoMed. Only randomised controlled trials (RCT) had been included for analysis. The retrieval time limit was from the establishment of the database until 20 July 2022. Cochrane Risk of Bias 2.0 tool was used to evaluate the quality of the studies. Network meta-analyses, and Trial Sequential Analyses (TSA) were used to analysis the effectiveness of the included RCTs for DKD. The Stata 15.1 and R 4.0.4 were used to perform the network meta-analysis. Sensitivity analysis was used to assess the robustness of the findings. The effect of the intervention evidence are summarized on the basis of the minimum background framework.

Results: NMA showed that the total effective rate of SMI, DCI, DHI, HQI, and SKI combined with alprostadil injection (PGE1) was better than PGE1 single used. Based on the surface under the cumulative ranking curve values, PGE1+DHI was the most effective for urinary albumin excretion rate and 24 h urinary albumin, PGE1+HQI was the most effective for the total response rate and β2-MG, and PGE1+SKI was the most effective for serum creatinine and blood urea nitrogen. Cluster analysis found that PGE1+HQI and PGE1+SKI could be the best treatments

Abbreviations: 24 h Alb, 24 h urinary albumin; 95% CI, 95% confidence intervals; β2-MG, urinary β2-microglobulin; BUN, blood urea nitrogen; CNKI, China National Knowledge Infrastructure; DCI, Danshen-Chuanxiongqin injection; DHI, Danhong injection; DKD, Diabetic kidney disease; HQI, Huangqi injection; MD, mean differences; NMA, network meta-analysis; PGE1, Prostaglandin E1; PROSPERO, International Prospective Register of Systematic Reviews; RAAS, reninangiotensin-aldosterone system; RCT, randomised controlled trial; RR, risk ratio; Scr, serum creatinine; SGLT-2, sodium-dependent glucose transporter 2; SKI, Shenkang injection; SMD, standardised mean differences; SMI, Salvia miltiorrhiza injection; SUCRA, Surface under the cumulative ranking curve; SXTI, Shuxuetong injection; TCM, Traditional Chinese medicine; TCMI, Traditional Chinese medicine injection; TSA, Trial Sequential Analyses; UAER, urinary albumin excretion rate; VIP, Chinese scientific journal database; XBJI, Xuebijing injection.

in terms of primary outcome measures. PGE1+SKI was found to be most effective on glomerular filtration function. PGE1+DHI was most effective for urinary protein-related indices.

Conclusion: The efficacy of TCMI combined with PGE1 was higher than PGE1 single used. PGE1+HQI and PGE1+SKI were the most effective treatments. The safety of TCMI treatment should be investigated further. This study needs to be validated using large-sample, double-blind, multicentre RCTs.

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KEYWORDS

traditional medicine, diabetic kidney disease, randomized controlled trial, network meta-analysis, injections

1 Introduction

Diabetic kidney disease (DKD) is one of the most serious microvascular complications of diabetes and has become a global public health challenge. 10.5% of adults have diabetes (Sun et al., 2022), and 40% of them developed into DKD (Alicic et al., 2017). This situation causing a heavy socioeconomic burden (de Boer et al., 2011; Afkarian et al., 2016; Kramer et al., 2018; Kume et al., 2019). DKD caused worse prognosis and increased risk of death in diabetic patients (Groop et al., 2009; Fox et al., 2012; Penno et al., 2018; Skupien et al., 2019). Preventing and delaying DKD progression is important in disease management for diabetes patients.

Currently, the main treatment methods for DKD are renin-angiotensin-aldosterone system (RAAS) blockers to regulate blood pressure, sodium-dependent glucose transporter 2 (SGLT-2) inhibitors, intensive insulin therapy to control blood glucose and intensive life management to improve obesity (Navaneethan et al., 2021). Urinary albumin is an important indicator for the evaluation and early diagnosis of DKD; a reduction in its levels can also alleviate DKD (Foundation, 2012). Recent studies have shown that Prostaglandin E1 (PGE1) can improve insulin resistance (Wei et al., 2018), reduce proximal tubular apoptosis (Mou et al., 2018; Zhang et al., 2020), and prevent vascular, glomerular, tubular, and interstitial changes(Bersani-Amado et al., 2020). A meta-analysis showed that PGE1 may positively affect DKD by reducing the urinary albumin excretion rate (UAER) and proteinuria (Wang et al., 2010).

Modern drugs mainly focus on delaying the disease process; hence, reversing DKD is a challenge and many new drugs are not approved for patients with an eGFR <30 mL/min. Traditional Chinese medicine (TCM) is widely used in the clinical prevention and treatment of DKD in China and has synergistic effects and safety advantages. The specific chemical mechanism of DKD protection by Chinese herbal medicine has been reviewed (Tang et al., 2021), which includes anti-inflammatory and antioxidant effects, inhibition of mesangial cell expansion, and reduction of podocyte injury (Xue et al., 2019; Zhong et al., 2019; Yang et al., 2022). Traditional Chinese medicine injection (TCMI) is a patented traditional Chinese drug registered by the National Medical Products Administration. In clinical practice, it is often combined with modern drug therapy to treat DKD. In recent years, several studies have demonstrated the efficacy of various TCMIs for the treatment of DKD (Yin et al., 2014; Liao et al., 2017; Wang et al., 2021; Xie et al., 2021).

The specific efficacies and therapeutic advantages of TCMIs are unclear, which causes clinical application problems. This study is the first article to systematically evaluate and compare the clinical efficacies, laboratory indicators, and safety of several commonly used TCMIs in combination with PGE1. The purpose of this study was to provide sufficient clinical evidence for TCM medicine and to provide a reference for the clinical use of TCMIs in the treatment of DKD.

2 Materials and methods

2.1 Standard evaluation of traditional Chinese medicine

In order to make the study more accurate and reproducible, this study reported traditional Chinese medicine injections by referring to The ConPhyMP consensus (Heinrich et al., 2022). At the same time, we standardized the scientific names of botanical drug components with reference to Rivera et al. (2014). And validated in the databases of "Plant of the World Online" (http://www.plantsoftheworldonline.org) and "The World Flora Online" (WFO, http://www.worldfloraonline.org/). Summary tables describing the composition of agents and how they were reported in the original study were prepared in accordance with the principles described in the four pillars of ethnopharmacology. The composition and standard name of each injection are shown in Table 1. Other details are shown in Supplementary Tables S12, S13 (page 142–147).

2.2 Systematic review protocol and registration

The network meta-analysis was registered with the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42022348333. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), its protocols, and the PRISMA-extension statement

TABLE 1 Composition of the traditional Chinese medicine injections.

Drug name	Botanical plant names	Species	Plant parts used
Danshen injection	*Salvia miltiorrhiza Bunge*	*Lamiaceae*	*Salviae miltiorrhizae radix et rhizoma*
Danshen-Chuanxiongqin injection	*Salvia miltiorrhiza Bunge*	*Lamiaceae*	*Salviae miltiorrhizae radix et rhizoma*
	Ligustrazine	—	—
Danhong injection	*Salvia miltiorrhiza Bunge*	*Lamiaceae*	*Salviae miltiorrhizae radix et rhizoma*
	Carthamus tinctorius L.	*Asteraceae*	*Carthamus tinctorius L. flower buds*
Huangqi injection	*Astragalus mongholicus Bunge*	*Fabaceae*	*Astragalus mongholicus Bunge radix et rhizoma*
Shenkang injection	*Salvia miltiorrhiza Bunge*	*Lamiaceae*	*Salviae miltiorrhizae radix et rhizoma*
	Astragalus mongholicus Bunge	*Fabaceae*	*Astragalus mongholicus Bunge radix et rhizoma*
	Carthamus tinctorius L.	*Asteraceae*	*Carthamus tinctorius L.radix et rhizoma*
	Rheum palmatum L	*Polygonaceae*	*Rheum palmatum L radix et rhizoma*
Shuxuetong injection	*Hirudo*	*Hirudinidae*	—
	Pheretima	*Megascolecidae*	—
Xuebijing injection	*Carthamus tinctorius L.*	*Asteraceae*	*Carthamus tinctorius L. flower buds*
	Salvia miltiorrhiza Bunge	*Lamiaceae*	*Salviae miltiorrhizae radix et rhizoma*
	Angelica sinensis (Oliv.) Diels	*Apiaceae*	*Angelica sinensis (Oliv.) Diels radix et rhizoma*
	Paeonia lactiflora Pall.	*Paeoniaceae*	*Paeonia lactiflora Pall. radix et rhizoma*
	Ligusticum chuanxiong Hort.	*Apiaceae*	*Ligusticum chuanxiong Hort. et rhizoma*

for network meta-analysis to report the current results (Shamseer et al., 2015; Page et al., 2021).

2.3 Literature search

This study searched PubMed, Embase, Cochrane Library, CNKI, VIP, Wanfang, and SinoMed databases, total 7 databases. The main search terms included "injection*," "Diabetic Nephropathies," "Nephropathies, Diabetic," "Nephropathy, Diabetic," "Diabetic Kidney Disease," "Kidney Disease, Diabetic," "Alprostadil," "PGE1alpha," "Prostaglandin E1alpha," "PGE1," "Lipo-PGE1" and others. References from previous systematic reviews and meta-analyses with similar topics were scanned for supplementation in the preliminary screening stage, references from eligible articles were scanned for supplementation in the full-text screening stage, and unpublished studies were not retrieved. The detailed search strategy is presented in Supplementary Tables S3–S10 (page 131–136). The retrieval time for each database was from database construction until 20 July 2022.

2.4 Inclusion and exclusion criteria

Inclusion criteria were determined based on PICO: (a) type of included studies: randomised controlled trials (RCTs); (b) patients: the subjects of the study were those who met the requirements of the DKD diagnostic criteria—no limitations existed in age, sex, or nationality; (c) interventions: in the treatment group, the intervention was TCMI + PGE1, which could be combined with conventional treatment (including the control of blood glucose, blood pressure, and blood lipids). The control group was treated with PGE1 in combination with conventional treatment; (d) outcome measures: the primary outcomes in this study were total effective rate (the calculation formula was as follows: total effective rate = marked effective rate + effective rate, markedly effective was defined as the main symptoms disappeared, and at least 50% reduction in the urine protein, or blood urea nitrogen (BUN) returned to normal, a decrease in at least 88.4 mmol L^{-1} of serum creatinine (Scr). Effective treatment showed that the main clinical symptoms were improved, the degree of urinary protein reduction was more than 33.3%, and BUN and Scr were decreased. The secondary outcomes included UAER, BUN, 24 h urinary albumin (24 h Alb), and urinary β2-microglobulin (β2-MG) levels. Studies that included only one outcome measure were eligible for inclusion. (e) The number of papers on the same TCMI should be greater than or equal to two.

The following exclusion criteria were used: (a) repeated articles; (b) incomplete or incorrect data; (c) non-conforming studies (including reviews, systematic reviews, meta-analyses, animal experiments, conference abstracts, reports, letters, case reports, etc.).

2.5 Study selection and data extraction

Two researchers (CYL and HYF) from related disciplines independently screened and crosschecked for inclusion. In the case of disagreement, a third researcher (RSY) can judge and provide a solution. Preliminary screening was carried out

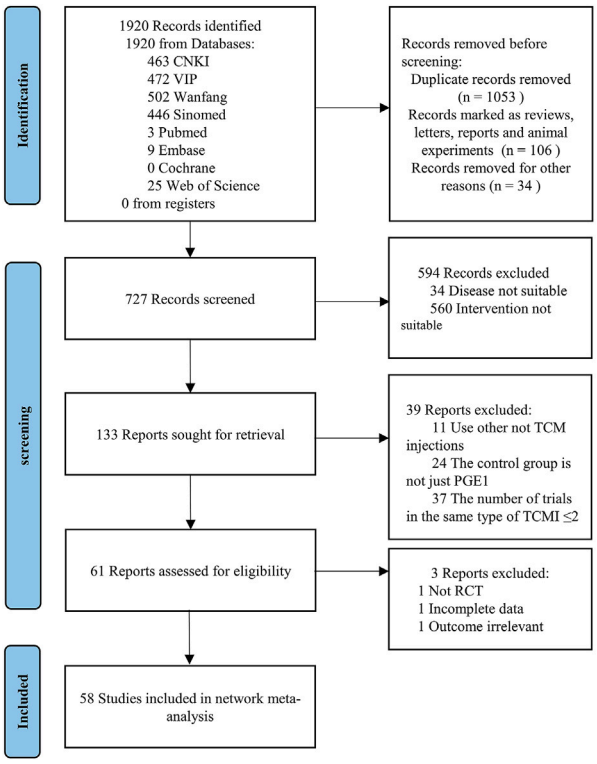

FIGURE 1
Study flow diagram.

according to the title and abstract, and the included studies were then determined by reading the full text. Two researchers used uniform criteria for data extraction: the first author, year of publication, classification of DKD, duration of DM, sample size, male-to-female ratio, age, interventions, course of treatment, and outcomes.

2.6 Risk of bias assessment and quality assessment

The quality of the included studies was assessed by two investigators (CYL & HYF) using the Cochrane Risk of Bias 2.0 tool (Sterne et al., 2019) which included the randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome, selection of the reported result, selection of the reported result, and overall bias. The risk of bias was classified as "low risk," "high risk," and "some concerns." We used the GRADE method for the entire network to provide a framework for the deterministic rating of each paired comparison evidence, divided into high, medium, low or very low (Puhan et al., 2014; Brignardello-Petersen et al., 2019).

2.7 Statistical analysis

This study used R4.0.4 and Stata15.1 software to calculate and draw graphs. For binary results, the combined results were calculated as odds ratio (OR). For continuous outcomes, this study used mean differences (MD), and standardised mean differences (SMD) were used when data units were inconsistent. All results are shown with 95% confidence intervals (95% CI). The league table was calculated using the Markov chain Monte Carlo method of the random-effects model through R4.0.4. The number of iterations was set to 200,000, and the first 100,000 iterations were used in the annealing algorithm to eliminate the influence of the initial values. Network diagrams were constructed using the Stata software to compare different interventions. Surface under the cumulative ranking curve (SUCRA) probability values were used to rank the detected treatments, with SUCRA values of 100% and 0% assigned to the best and worst treatments, respectively. Cluster analysis was used to compare the efficacy of TCMIs for different functions. The minimal contextualization framework was developed based on the results of SUCRA and GRADE assessments (Brignardello-Petersen et al., 2020). The bias information criterion was used to compare the fit of consistent and inconsistent models, and Cochran's I^2 statistic was used to assess statistical heterogeneity, with low, medium, and high I^2 values of 25, 50, and 75%, respectively (Higgins et al., 2003). Funnel plots were used to detect publication bias in the primary outcome measures. Sensitivity analyses were carried out by excluding studies with a high-risk bias and those with courses of treatment that did not fall within 14–30 days. According to the information collected so far, TSA version 0.9 beta was used to calculate and draw the required information size and trial sequential monitoring boundaries.

3 Results

3.1 Study selection and characteristics

A total of 1920 studies were initially identified from the search, and 727 studies were retained after excluding duplicate literature, animal experiments, meetings, reports, and letters by screening titles and abstracts. After reading the titles and abstracts of the 727 studies, 34 studies in which disease was not suitable and 580 studies in which intervention was not suitable (including 184 studies with only PGE1 without comparison, 25 studies not combining PGE1, and 351 studies not combining TCMIs) were excluded, and 133 studies were retained. After reading the full text of the remaining literature, 11 studies without TCMI, 24 not only used PGE1 in the control group, and 37 studies in which the number of studies with the same type of TCMI ≤2 were excluded. Among the remaining 61 studies, one without an after-before control, one with incomplete data, and one with irrelevant outcome indicators from the input data were excluded. Finally, 58 studies from 2002 to 2022 were retained (Xie and Zhang, 2002; Ru et al., 2008b; Gong and Xie, 2009; Wang et al., 2009; Min et al., 2010; Zhao and Dong, 2010; Pang et al., 2011; Wu, 2011; Xing, 2012; Zhou and Lai, 2012a; b; Han and Zhang, 2013; Lin, 2013; Liu, 2013; Pu et al., 2013; Zhang et al., 2013; Ding, 2014; Lan, 2014; Mei et al., 2014; Wang et al., 2014; Yin, 2014; Zhang, 2014; Zhou et al., 2014; Cai et al., 2015; He, 2015; Li and Li, 2015; Liu and Guo, 2015; Yang et al., 2015; Cao et al., 2016; Fang et al., 2016; Li et al., 2016; Liu et al., 2016; Liu and Sun, 2016; Zhang, 2016; Zhang and Peng, 2016; Cui et al., 2017; Jiang and Qu, 2017; Liu, 2017; Mai et al., 2017; Zhang, 2017b; Zhang, 2017a; Zhong, 2017; Chen and Fu,

TABLE 2 Characteristics of the studies included in this network meta-analysis.

Study	Classification of DKD	Duration of DM		Sample size		Sex(M/F)		Age (mean or range)		Interventions		Course of treatment	Outcomes
		T	C	T	C	T	C	T	C	T	C		
Zhoufangmin (2012)	NA	NA	NA	60	60	37/23	35/25	60.2 ± 9.4	58.6 ± 8.7	PGE1 (4 mL/d) + SMI(20 mL/d)	PGE1 (4 mL/d)	28d	②③④⑤
Hanpeng (2013)	III-IV	8.25 ± 0.44		40	40	44/36		55.92 ± 11.40		PGE1 (2 mL/d) + SMI(1 mL/d)	PGE1 (2 mL/d)	15d	①
Wangqun (2014)	III	NA	NA	16	18	32/21a		56.3 ± 16.8		PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	14d	②③⑤
Caiwenting (2015)	NA	10.7 ± 3.4	9.7 ± 3.4	40	40	24/16	22/18	58 ± 9.7	53.6 ± 4.7	PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	28d	②③④⑤
Hemingwu (2015)	NA	6–13	7–14	40	40	21/19	18/22	53.2 ± 7.6	51.7 ± 8.4	PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	28d	①
Liugang (2015)	early stage	NA	NA	52	52	28/24	30/22	59.33 ± 5.16	60.25 ± 4.94	PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	30d	①②③④
Liuying (2017)	early stage	NA	NA	29	29	14/15	15/14	58.65 ± 6.23	59.36 ± 6.54	PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	30d	①
Zhangyarong (2017)	early stage	NA	NA	43	43	51/35		48.21 ± 4.67		PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	28d	①③
Zhongchao (2017)	III	9.2 ± 1.1	9.3 ± 1.2	47	47	28/19	31/16	62.21 ± 2.21	63.34 ± 2.18	PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (2 mL/d)	28d	①
Chenxuan (2018)	III	NA	NA	49	49	29/20	27/22	61.5 ± 2.0	60.2 ± 4.5	PGE1 (2 mL/d) + SMI (20 mL/d)	PGE1 (2 mL/d)	28d	②③④
Liangliang (2018)	NA	NA	NA	48	48	21/27	22/26	55.7 ± 3.3	55.9 ± 2.5	PGE1(4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	28d	①
Wanganwen (2018)	I-III	8.38 ± 4.22	8.38 ± 4.22	38	38	22/16	23/15	63.28 ± 8.45	63.28 ± 8.45	PGE1 (4 mL/d) + SMI (20 mL/d)	PGE1 (4 mL/d)	28d	②③
Yangxu (2015)	early stage	9.8 ± 1.9	9.0 ± 1.7	20	20	12/8	10/10	9.8 ± 1.9	9.0 ± 1.7	PGE1 (2 mL/d) + DCI(10 mL/d)	PGE1 (2 mL/d)	14d	②⑥
Zhangyin (2016)	NA	3.5 ± 1.4	4.2 ± 3.1	42	42	27/15	24/18	56.7 ± 0.8	56.5 ± 0.8	PGE1 (1 mL/d) + DCI(5 mL/d)	PGE1 (1 mL/d)	28d	①②③
Cuiyi (2017)	early stage	NA	NA	51	51	27/24	26/25	61.5 ± 14.2	60.3 ± 14.7	PGE1 (2 mL/d) + DCI(10 mL/d)	PGE1 (2 mL/d)	30d	②③
Maigaoyang (2017)	III	10.2 ± 4.0	10.6 ± 4.2	35	35	24/11	22/13	59.1 ± 9.3	58.2 ± 8.9	PGE1 (4 mL/d) + DCI(20 mL/d)	PGE1 (4 mL/d)	14d	②③④⑤
Shenjinsong (2018)	early stage	10.3 ± 3.9	10.2 ± 3.6	20	20	11/9	9/11	58.1 ± 9.1	57.9 ± 8.7	PGE1 (4 mL/d) + DCI(20 mL/d)	PGE1 (4 mL/d)	28d	①②⑥
Subaoting (2018)	early stage	5.21 ± 1.08	5.30 ± 1.01	44	44	26/18	24/20	60.42 ± 5.75	60.29 ± 5.90	PGE1 (2 mL/d) + DCI(10 mL/d)	PGE1 (2 mL/d)	28d	②③④
Wangjing (2021)	early stage	11.47 ± 1.66	11.65 ± 1.72	65	65	34/31	33/32	64.14 ± 3.12	64.58 ± 3.17	PGE1 (2 mL/d) + DCI(10 mL/d)	PGE1 (2 mL/d)	21d	②③④⑦
Fanmin (2010)	III	NA	NA	92	67	44/48	36/31	56–91	57–85	PGE1 (2 mL/d) + DHI(30–50 mL/d)	PGE1 (2 mL/d)	14d	②③④⑤
Puhongmei (2013)	III	NA	NA	26	26	NA	NA	NA	NA	PGE1 (2 mL/d) + DHI(20 mL/d)	PGE1 (2 mL/d)	90d	②④⑤⑦
Liqiuxia (2015)	III	11.2 ± 2.3	10.4 ± 2.6	24	24	12/12	13/11	55.7 ± 7.5	56.8 ± 7.2	PGE1 (2 mL/d) + DHI(40 mL/d)	PGE1 (2 mL/d)	14d	①④⑥
Liuqingyuan (2016)	III	13.21 ± 8.25	11.35 ± 9.18	24	21	11/13	10/11	52.10 ± 11.86	52.50 ± 12.16	PGE1 (2 mL/d) + DHI(20 mL/d)	PGE1 (2 mL/d)	14d	②③④⑤⑦
Liuzhen (2016)	III-IV	5.65 ± 1.90	5.54 ± 1.85	15	15	9/6	8/7	59.94 ± 5.21	60.24 ± 5.13	PGE1 (2 mL/d) + DHI(20–40 mL/d)	PGE1 (2 mL/d)	21d	①②③④⑤
Jiayinji (2018)	IV	8.8 ± 1.3	7.7 ± 1.5	35	36	18/17	18/18	62.7 ± 2.4	65.2 ± 2.1	PGE1 (4 mL/d) + DHI(30 mL/d)	PGE1 (4 mL/d)	28d	①②⑥
Linchenxin (2019)	NA	14.62 ± 3.05	14.58 ± 3.10	32	32	17/15	19/13	82.09 ± 4.42	82.11 ± 4.23	PGE1 (2 mL/d) + DHI(20 mL/d)	PGE1 (2 mL/d)	14d	①④⑤⑥⑦
Xiaoqianfeng (2019)	NA	7.2 ± 2.1	7.1 ± 1.8	40	40	22/18	24/16	58.3 ± 7.6	60.8 ± 8.2	PGE1(2 mL/d) + DHI(20–40 mL/d)	PGE1 (2 mL/d)	21d	①②③④⑦
Xiebinxuan (2002)	NA	3.52	3.48	20	10	12/8	7/3	64.32 ± 6.94	63.47 ± 7.11	PGE1 (20 mL/d) + HQI (40 mL/d)	PGE1 (20 mL/d)	21d	③⑥⑦
Zhaolijun (2010)	early stage	10 ± 3	8 ± 3	169	157	82/87	84/73	55 ± 12	57 ± 10	PGE1(100 mL/d) + HQI (60 mL/d)	PGE1 (100 mL/d)	14d	⑥⑦
Wuyanbo (2011)	III	NA	NA	40	40	22/18	23/17	55.3 ± 6.9	56.9 ± 7.1	PGE1(2 mL/d) + HQI (30 mL/d)	PGE1 (2 mL/d)	14d	②③④

(Continued on following page)

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TABLE 2 (*Continued*) Characteristics of the studies included in this network meta-analysis.

Study	Classification of DKD	Duration of DM		Sample size		Sex(M/F)		Age (mean or range)		Interventions		Course of treatment	Outcomes
		T	C	T	C	T	C	T	C	T	C		
Linjixiang (2013)	IV	NA	NA	45	45	22/23	24/21	64.1 ± 11.2	63.4 ± 10.6	PGE1(4 mL/d) + HQI (15 mL/d)	PGE1 (4 mL/d)	15d	①②⑥
Liusiyan (2013)	NA	2–13	2–11	16	17	10/6	11/6	44.6 ± 7.1	44.5 ± 7.2	PGE1(2 mL/d) + HQI (30 mL/d)	PGE1 (2 mL/d)	14d	①②③④⑦
Zhoubin (2014)	NA	NA	NA	30	30	15/15	16/14	41.2 ± 11.3	43.1 ± 9.8	PGE1(4 mL/d) + HQI (20 mL/d)	PGE1 (4 mL/d)	28d	①②③⑥
Fangwenjuan (2016)	NA	12.1 ± 2.3		40	40	47/33		61.9 ± 3.6		PGE1(2 mL/d) + HQI (30 mL/d)	PGE1 (2 mL/d)	14d	①④⑥
Zhangliang (2016)	NA	2–8	3–8	45	45	28/20	27/18	46–68	45–70	PGE1(2 mL/d) + HQI (30 mL/d)	PGE1 (2 mL/d)	28d	①②③④⑤⑦
Xufei (2018)	III	9.8 ± 2.7	10.2 ± 2.8	43	43	26/17	27/16	58.4 ± 8.5	59.2 ± 9.1	PGE1(4 mL/d) + HQI (30 mL/d)	PGE1 (4 mL/d)	28d	②③④⑤⑦
Wanghuibin (2009)	III	NA	NA	28	16	14/14	7/9	47 ± 7	45 ± 10	PGE1(2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	14d	②③⑥⑦
Pangjialiang (2011)	III	8.1 ± 4.6	8.3 ± 5.1	30	30	16/14	17/13	53.5 ± 7.3	52.2 ± 6.8	PGE1(2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	14d	⑦
Zhanghong (2013)	NA	3.45 ± 0.76		30	30	14/16	17/13	53.73	52.41	PGE1(2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	28d	②③⑥
Dingxuemei (2014)	III-IV	NA	NA	36	34	NA	NA	NA	NA	PGE1(2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	28d	②③⑦
Lanchunying (2014)	III-IV	10.0 ± 3.0		40	40	19/21	22/18	55.0 ± 6.0		PGE1 (2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	28d	①②③⑥
Meidongdong (2014)	II-IV	10.1 ± 2.5	10.4 ± 2.7	20	20	12/8	12/8	60.9 ± 8.7	61.6 ± 7.6	PGE1 (2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	14d	②③⑥
Yinlili (2014)	NA	7.1 ± 0.5		44	44	NA	NA	50.43 ± 6.90		PGE1 + SKI(100 mL/d)	PGE1	NA	①
Zhangbailing (2014)	IV	13.9 ± 4.3		35	35	38/32		54.5 ± 8.3		PGE1 (2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	28d	②③④⑥
Zhangyunpin (2017)	NA	7.6 ± 2.3	7.5 ± 2.1	58	58	26/22	28/20	64.8 ± 8.3	64.7 ± 9.5	PGE1 (2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	28d	①②③
Quanye (2018)	NA	8.6 ± 2.4	8.1 ± 2.3	30	30	18/12	17/13	64.8 ± 8.7	64.4 ± 8.5	PGE1 (2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	14d	①②③⑥
Zhengwenwu (2018)	early stage	4.35 ± 1.26	4.61 ± 1.18	30	30	16/14	17/13	58.37 ± 4.49	58.19 ± 4.21	PGE1 (2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	28d	②③
Wangxiaojun (2019)	NA	7.5 ± 1.2	7.7 ± 1.3	50	50	31/19	32/18	65.1 ± 3.1	65.3 ± 3.2	PGE1(2 mL/d) + SKI(100 mL/d)	PGE1 (2 mL/d)	30d	①
Zhangmailang (2019)	III-IV	NA	NA	48	48	25/23	26/22	54.26 ± 13.85	53.79 ± 13.42	PGE1 (2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	30d	①②③⑥
Wenxusheng (2021)	IV	9.69 ± 2.26	10.23 ± 2.13	36	36	25/17	23/13	51.46 ± 3.37	52.37 ± 3.11	PGE1 (2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	28d	①②④⑥⑦
Niexing (2022)	NA	NA	NA	50	50	29/21	27/23	63.12 ± 7.71	63.85 ± 7.62	PGE1 (2 mL/d) + SKI(60 mL/d)	PGE1 (2 mL/d)	28d	②③⑥
Rujianyong (2008)	III	12 ± 10	13 ± 9	64	64	34/30	35/29	58 ± 10	56 ± 9	PGE1 (2 mL/d) + SXTI(6 mL/d)	PGE1 (2 mL/d)	56d	②③④⑤
Gongyun (2009)	III	NA	NA	36	30	NA	NA	NA	NA	PGE1 (4 mL/d) + SXTI(6 mL/d)	PGE1 (4 mL/d)	15-20d	③④⑦
Xingyelan (2012)	III-IV	6.2 ± 2.6	5.7 ± 2.8	60	60	38/22	47/13	66.4 ± 9.7	65.2 ± 9.8	PGE1 (20 mL/d) + SXTI(6 mL/d)	PGE1 (20 mL/d)	28d	②③⑤⑦
Tiantian (2018)	III-IV	5.71 ± 0.72	5.68 ± 0.70	57	57	34/23	37/20	56.24 ± 6.12	55.78 ± 7.32	PGE1 (4 mL/d) + SXTI(2 mL/d)	PGE1 (4 mL/d)	14d	①②③⑤
Caoli (2016)	IV	8.3 ± 1.2	9.2 ± 1.3	33	31	18/15	17/14	54.2 ± 9.86	53.9 ± 12.5	PGE1 (2 mL/d) +XBJI (10 mL/d)	PGE1 (2 mL/d)	14d	②③④⑤⑥
Liqing (2016)	NA	5.9 ± 2.6	5.6 ± 2.7	30	30	17/13	13/17	65.6 ± 7.3	63.1 ± 9.5	PGE1 (2 mL/d) + XBJI (40 mL/d)	PGE1 (2 mL/d)	7d	②③⑥⑦
Jiangqiang (2017)	NA	6.02 ± 2.20	6.11 ± 2.44	78	78	48/30	44/34	59.54 ± 10.13	60.52 ± 10.34	PGE1 (4 mL/d) + XBJI (50 mL/d)	PGE1 (4 mL/d)	14d	②③④⑥

1 Total effective rate,② Serum Creatinine,③ Blood Urea Nitrogen, ④ Urinary Albumin excretion rates, ⑤ Urinary beta 2-microglobulin,⑥ 24h Urine Albumin, ⑦ Adverse reactions, SMI, Salvia miltiorrhiza injection; DCI, Danshen-Chuanxiongqin injection; DHI, Danhong injection; HQI, Huangqi injection; SKI, Shenkang injection; SXTI, Shuxuetong injection; XBJI, Xuebijing injection; PGE1, alprostadil injection.

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2018; Jia, 2018; Liang, 2018; Shen, 2018; Su, 2018; Tian et al., 2018; Wang, 2018; Xu and Liu, 2018; Ye, 2018; Zheng et al., 2018; Lin et al., 2019; Wang et al., 2019; Xiao, 2019; Zhang and Nan, 2019; Wang, 2021; Wen et al., 2021; Nie, 2022). The specific screening process is shown in Figure 1.

58 articles included in this study included a total of 4808 subjects and seven types of TCMIs, namely *Salvia miltiorrhiza* injection (SMI; 12 RCTS) (Zhou and Lai, 2012a; Han and Zhang, 2013; Wang et al., 2014; Cai et al., 2015; He, 2015; Liu and Guo, 2015; Zhang, 2017a; Liu, 2017; Zhong, 2017; Chen and Fu, 2018; Liang, 2018; Wang, 2018), Danshen-Chuanxiongqin injection (DCI; 7 RCTs) (Yang et al., 2015; Zhang and Peng, 2016; Cui et al., 2017; Mai et al., 2017; Shen, 2018; Su, 2018; Wang, 2021), Danhong injection (DHI; 8 RCTs) (Min et al., 2010; Pu et al., 2013; Li and Li, 2015; Liu et al., 2016; Liu and Sun, 2016; Jia, 2018; Lin et al., 2019; Xiao, 2019), Huangqi injection (HQI; 9 RCTs) (Xie and Zhang, 2002; Zhao and Dong, 2010; Wu, 2011; Lin, 2013; Liu, 2013; Zhou et al., 2014; Fang et al., 2016; Zhang, 2016; Xu and Liu, 2018), Shenkang injection (SKI;15 RCTs) (Wang et al., 2009; Pang et al., 2011; Zhang et al., 2013; Ding, 2014; Lan, 2014; Mei et al., 2014; Yin, 2014; Zhang, 2014; Zhang, 2017b; Ye, 2018; Zheng et al., 2018; Wang et al., 2019; Zhang and Nan, 2019; Wen et al., 2021; Nie, 2022), Shuxuetong injection (SXTI; 4 RCTs) (Ru et al., 2008a; Gong and Xie, 2009; Xing, 2012; Tian et al., 2018), and Xuebijing injection (XBJI; 3 RCTs) (Cao et al., 2016; Li et al., 2016; Jiang and Qu, 2017). The course of treatment ranged from 7 days to 3 months. The basic characteristics are shown in Table 2 and the comparative associations between each intervention and each outcome measure are shown in Figure 2. In addition, we collected the specific intervention method of each included study (Supplementary Table S14, page148–153).

3.2 Bias risk assessment and the grade of evidence

Among the 58 included studies, 15 studies described the methods used to generate the allocation sequence (Min et al., 2010; Zhou and Lai, 2012a; Pu et al., 2013; Yang et al., 2015; Cao et al., 2016; Liu et al., 2016; Zhang and Peng, 2016; Cui et al., 2017; Jiang and Qu, 2017; Liu, 2017; Mai et al., 2017; Zhong, 2017; Su, 2018; Tian et al., 2018; Wang, 2018), five studies were not random (Han and Zhang, 2013; Cai et al., 2015; Zhang, 2017a; Liang, 2018; Wen et al., 2021), the remaining studies did not explicitly address the random approach. None of the studies stated a pre-established research plan or analysis protocol. Overall, nine studies had a high risk (Han and Zhang, 2013; Lin, 2013; Yin, 2014; Cai et al., 2015; He, 2015; Zhang, 2017a; Zhang, 2017b; Liang, 2018; Wen et al., 2021), 49 studies had some concerns of bias(Xie and Zhang, 2002; Ru et al., 2008b; Gong and Xie, 2009; Wang et al., 2009; Min et al., 2010; Zhao and Dong, 2010; Pang et al., 2011; Wu, 2011; Zhou and Lai, 2012a; Xing, 2012; Liu, 2013; Pu et al., 2013; Zhang et al., 2013; Ding, 2014; Lan, 2014; Mei et al., 2014; Wang et al., 2014; Zhang, 2014; Zhou et al., 2014; Li and Li, 2015; Liu and Guo, 2015; Fang et al., 2016; Li et al., 2016; Liu et al., 2016; Liu and Sun, 2016; Zhang, 2016; Zhang and Peng, 2016; Cui et al., 2017; Jiang and Qu, 2017; Liu, 2017; Chen and Fu, 2018; Jia, 2018; Shen, 2018; Su, 2018; Tian et al., 2018; Xu and Liu, 2018; Ye, 2018; Zheng et al., 2018; Lin et al., 2019; Wang et al., 2019; Xiao, 2019; Zhang and Nan, 2019; Wang, 2021; Wen et al., 2021; Nie, 2022),. The results of the risk of bias assessment of the included studies are shown in Supplementary Figure S1 (page 3), Supplementary Table S1 (page 3–102). There are only indirect comparisons between TCMIs, which results in a very low-quality rating for pairwise comparisons, the details of evidence evaluation utilizing GRADE is available in the Supplementary Material (Supplementary Table S2, page 122–130).

3.3 Results of network meta-analysis

3.3.1 Primary outcome measures

3.3.1.1 Total effective rate

A total of 27 RCTs (Han and Zhang, 2013; Lin, 2013; Liu, 2013; Lan, 2014; Yin, 2014; Zhou et al., 2014; He, 2015; Li and Li, 2015; Liu and Guo, 2015; Fang et al., 2016; Liu and Sun, 2016; Zhang, 2016; Zhang and Peng, 2016; Zhang, 2017a; Zhang, 2017b; Liu, 2017; Zhong, 2017; Jia, 2018; Liang, 2018; Shen, 2018; Tian et al., 2018; Ye, 2018; Lin et al., 2019; Wang et al., 2019; Xiao, 2019; Zhang and Nan, 2019; Wen et al., 2021) reported the total effective rate, including six TCMIs and seven interventions. Five TCMIs combined with PGE1 were better than PGE1 alone, including PGE1+ DCI (RR: 1.17, CI: 1.02, 1.37), PGE1+DHI (RR: 1.28, CI: 1.13, 1.46), PGE1+HQI (RR: 1.43, CI: 1.26, 1.66), PGE1+SKI (RR: 1.2, CI: 1.12, 1.3), PGE1+SMI (RR: 1.24, CI: 1.15, 1.35), and PGE1+HQI were better than PGE1+DCI (RR: 1.23, CI: 1, 1.51), which suggested advantages in improving clinical symptoms (Table 3). According to the results of the SUCRA ranking (Table 4; Figure 3), PGE1+HQI (97.4%) was the best treatment, followed by PGE1+DHI (70.1%) and PGE1+SMI (54.9%).

3.3.1.2 Scr

A total of 45 RCTs (Xie and Zhang, 2002; Ru et al., 2008b; Wang et al., 2009; Min et al., 2010; Wu, 2011; Zhou and Lai, 2012a; Xing, 2012; Lin, 2013; Liu, 2013; Pu et al., 2013; Zhang et al., 2013; Ding, 2014; Lan, 2014; Mei et al., 2014; Wang et al., 2014; Zhang, 2014; Zhou et al., 2014; Cai et al., 2015; Liu and Guo, 2015; Yang et al., 2015; Cao et al., 2016; Li et al., 2016; Liu et al., 2016; Liu and Sun, 2016; Zhang, 2016; Zhang and Peng, 2016; Zhang, 2017a; Zhang, 2017b; Cui et al., 2017; Jiang and Qu, 2017; Mai et al., 2017; Chen and Fu, 2018; Jia, 2018; Shen, 2018; Su, 2018; Tian et al., 2018; Wang, 2018; Xu and Liu, 2018; Ye, 2018; Zheng et al., 2018; Xiao, 2019; Zhang and Nan, 2019; Wang, 2021; Wen et al., 2021; Nie, 2022) reported the Scr, including seven TCMIs and 8 interventions. 4 TCMIs combined with PGE1 were better than single used PGE1, including PGE1+DCI (RR: −1.34, CI: −2.11, −0.56), PGE1+DHI (SMD: −0.87, CI: −1.7, −0.03), PGE1+SKI (SMD: −1.78, CI: −2.39, −1.18), and PGE1+SMI (SMD: −0.83, CI: −1.6, −0.06). PGE1+SKI was superior to PGE1+HQI (SMD: −1.13, CI: −2.12, −0.16), indicating its advantages in improving Scr (Table 3). According to the results of the SUCRA ranking (Table 4; Figure 3), PGE1+SKI (90.7%) was the best treatment, followed by PGE1+DCI (69%), and PGE1+ SXTI (68.7%).

3.3.2 Secondary outcome measures

3.3.2.1 BUN

A total of 40 RCTs (Xie and Zhang, 2002; Ru et al., 2008b; Gong and Xie, 2009; Wang et al., 2009; Min et al., 2010; Wu, 2011; Xing, 2012; Zhou and Lai, 2012a; Liu, 2013; Zhang et al., 2013; Ding, 2014; Lan, 2014; Mei et al., 2014; Wang et al., 2014; Zhang, 2014; Zhou

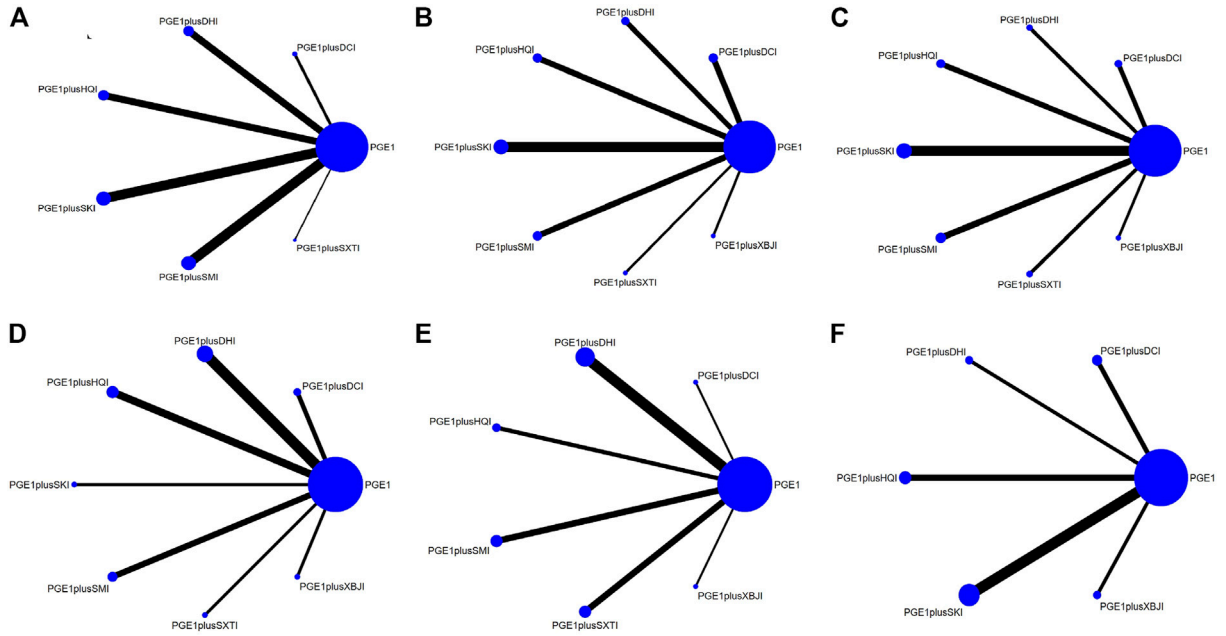


FIGURE 2
Network diagrams for different outcomes.

et al., 2014; Cai et al., 2015; Liu and Guo, 2015; Cao et al., 2016; Li et al., 2016; Liu et al., 2016; Liu and Sun, 2016; Zhang, 2016; Zhang and Peng, 2016; Cui et al., 2017; Jiang and Qu, 2017; Mai et al., 2017; Zhang, 2017a; Zhang, 2017b; Chen and Fu, 2018; Su, 2018; Wang, 2018; Xu and Liu, 2018; Ye, 2018; Zheng et al., 2018; Xiao, 2019; Zhang and Nan, 2019; Wang, 2021; Nie, 2022) reported the BUN, including 7 TCMIs and 8 interventions. The results showed that PGE1+DCI (SMD: −1.11, CI: −1.93, −0.29), PGE1+SKI (SMD: −1.16, CI: −1.73, −0.61), and PGE1+SXTI (SMD: −1, CI: −1.91, −0.09) were better than single used PGE1 (Table 3). According to the results of the SUCRA ranking (Table 4; Figure 3), PGE1+SKI (78.6%) was the best treatment, followed by PGE1+DCI (72.6%) and PGE1+SXTI (64.1%).

3.3.2.2 UAER

A total of 25 RCTs (Ru et al., 2008b; Gong and Xie, 2009; Min et al., 2010; Wu, 2011; Zhou and Lai, 2012a; Liu, 2013; Pu et al., 2013; Zhang, 2014; Cai et al., 2015; Li and Li, 2015; Liu and Guo, 2015; Cao et al., 2016; Fang et al., 2016; Liu et al., 2016; Liu and Sun, 2016; Zhang, 2016; Jiang and Qu, 2017; Mai et al., 2017; Chen and Fu, 2018; Su, 2018; Xu and Liu, 2018; Lin et al., 2019; Xiao, 2019; Wang, 2021; Wen et al., 2021) reported UAER, including 7 TCMIs and 8 interventions. The results showed that using PGE1+DCI (SMD: −1.47, CI: −2.48, −0.47), PGE1+DHI (SMD: −1.57, CI: −2.25, −0.9), PGE1+HQI (SMD: −1.34, CI: −2.14, −0.56), and PGE1+SMI (SMD: −1.08, CI: −1.94, −0.22) were better than single used PGE1(Table 3). According to the results of the SUCRA ranking (Table 4; Figure 3), PGE1+DHI (81.8%) was the best treatment, followed by PGE1+DCI (75%) and PGE1+HQI (68.9%).

3.3.2.3 β2-MG

A total of 15 RCTs (Ru et al., 2008b; Min et al., 2010; Zhou and Lai, 2012a; Xing, 2012; Pu et al., 2013; Wang et al., 2014; Cai et al., 2015; Cao et al., 2016; Liu et al., 2016; Liu and Sun, 2016; Zhang, 2016; Mai et al., 2017; Tian et al., 2018; Xu and Liu, 2018; Lin et al., 2019) reported β2-MG, including 6 TCMIs and 7 interventions. On the one hand, using PGE1+DHI (SMD: −1.37, CI: −2, −0.81), PGE1+HQI (SMD: −1.79, CI: −2.69, −0.89), and PGE1+SXTI (SMD: −1.43, CI: −2.16, −0.73) was better than single used PGE1. On the other hand, PGE1+HQI (SMD: 1.35, CI:0.21, 2.53) and PGE1+DHI (SMD:0.92, CI:0.04, 1.93) were better than PGE1+SMI, indicating excellent performance in improving β2-MG (Table 3). According to the results of the SUCRA ranking (Table 4; Figure 3), PGE1+HQI (92.7%) was the best treatment, followed by PGE1+SXTI (77.1%) and PGE1+DHI (72.8%).

3.3.2.4 24 h Alb

A total of 24 RCTs (Xie and Zhang, 2002; Wang et al., 2009; Zhao and Dong, 2010; Lin, 2013; Zhang et al., 2013; Lan, 2014; Mei et al., 2014; Zhang, 2014; Li and Li, 2015; Yang et al., 2015; Cao et al., 2016; Fang et al., 2016; Li et al., 2016; Zhang and Peng, 2016; Cui et al., 2017; Jiang and Qu, 2017; Jia, 2018; Shen, 2018; Ye, 2018; Lin et al., 2019; Zhang and Nan, 2019; Wen et al., 2021; Nie, 2022) reported 24 h Alb, including five TCMIs and six interventions. Using PGE1+DHI (SMD: −2.49, CI: −3.96, −1.03), PGE1+HQI (SMD: −1.15, CI: −2.28, −0.03) was better than using PGE1 alone. PGE1+DHI (SMD: 2.15, CI: 0.48, 3.84) was better than PGE1+SKI, indicating that it may be better to improve 24 h Alb (Table 3). According to the results of the SUCRA ranking (Table 4; Figure 3), PGE1+DHI (96.4%) was the best treatment, followed by PGE1+XBJI (63.7%) and PGE1+HQI (61%).

TABLE 3 League table for all outcome measures.

Total effective rate	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1	PGE1	**1.17 (1.02, 1.37)**	**1.28 (1.13, 1.46)**	**1.43 (1.26, 1.66)**	**1.2 (1.12, 1.3)**	**1.24 (1.15, 1.35)**	1.18 (0.99, 1.42)	—
PGE1plusDCI	—	PGE1plusDCI	1.09 (0.9, 1.34)	**1.23 (1, 1.51)**	1.03 (0.86, 1.21)	1.06 (0.89, 1.24)	1.01 (0.8, 1.28)	—
PGE1plusDHI	—	—	PGE1plusDHI	1.12 (0.93, 1.36)	0.94 (0.8, 1.08)	0.97 (0.83, 1.13)	0.92 (0.74, 1.15)	—
PGE1plusHQI	—	—	—	PGE1plusHQI	0.84 (0.71, 0.97)	0.87 (0.73, 1)	0.82 (0.65, 1.04)	—
PGE1plusSKI	—	—	—	—	PGE1plusSKI	1.03 (0.93, 1.15)	0.98 (0.82, 1.2)	—
PGE1plusSMI	—	—	—	—	—	PGE1plusSMI	0.95 (0.79, 1.16)	—
PGE1plusSXTI	—	—	—	—	—	—	PGE1plusSXTI	—
PGE1plusXBJI	—	—	—	—	—	—	—	PGE1plusXBJI
Serum Creatinine	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1	PGE1	**−1.34 (-2.11, -0.56)**	**−0.87 (-1.7, -0.03)**	−0.65 (−1.42, 0.13)	**−1.78 (-2.39, -1.18)**	**−0.83 (-1.6, -0.06)**	−0.91 (−2.09, 0.28)	−1.1 (−2.27, 0.07)
PGE1plusDCI	—	PGE1plusDCI	0.48 (−0.67, 1.62)	0.69 (−0.41, 1.8)	−0.44 (−1.43, 0.54)	0.51 (−0.59, 1.6)	0.43 (−0.98, 1.86)	0.24 (−1.17, 1.65)
PGE1plusDHI	—	—	PGE1plusDHI	0.22 (−0.93, 1.36)	−0.91 (−1.95, 0.11)	0.03 (−1.1, 1.17)	−0.04 (−1.49, 1.42)	−0.24 (−1.68, 1.21)
PGE1plusHQI	—	—	—	PGE1plusHQI	**−1.13 (-2.12, -0.16)**	−0.19 (−1.28, 0.91)	−0.26 (−1.68, 1.16)	−0.46 (−1.86, 0.95)
PGE1plusSKI	—	—	—	—	PGE1plusSKI	0.94 (−0.03, 1.93)	0.87 (−0.45, 2.21)	0.67 (−0.64, 2)
PGE1plusSMI	—	—	—	—	—	PGE1plusSMI	−0.07 (−1.48, 1.34)	−0.27 (−1.67, 1.14)
PGE1plusSXTI	—	—	—	—	—	—	PGE1plusSXTI	−0.2 (−1.87, 1.47)
PGE1plusXBJI	—	—	—	—	—	—	—	PGE1plusXBJI
Blood Urea Nitrogen	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1	PGE1	**−1.11 (-1.93, -0.29)**	−0.73 (−1.66, 0.19)	−0.59 (−1.34, 0.17)	**−1.16 (-1.73, -0.61)**	−0.66 (−1.35, 0.03)	**−1 (-1.91, -0.09)**	−0.83 (−1.89, 0.22)
PGE1plusDCI	—	PGE1plusDCI	0.38 (−0.86, 1.61)	0.52 (−0.59, 1.64)	−0.06 (−1.06, 0.93)	0.45 (−0.62, 1.53)	0.11 (−1.11, 1.34)	0.28 (−1.05, 1.61)
PGE1plusDHI	—	—	PGE1plusDHI	0.15 (−1.05, 1.34)	−0.43 (−1.52, 0.65)	0.08 (−1.08, 1.23)	−0.26 (−1.57, 1.04)	−0.1 (−1.5, 1.31)
PGE1plusHQI	—	—	—	PGE1plusHQI	−0.58 (−1.53, 0.36)	−0.07 (−1.09, 0.95)	−0.41 (−1.59, 0.77)	−0.25 (−1.54, 1.05)
PGE1plusSKI	—	—	—	—	PGE1plusSKI	0.51 (−0.38, 1.41)	0.17 (−0.9, 1.25)	0.33 (−0.86, 1.53)
PGE1plusSMI	—	—	—	—	—	PGE1plusSMI	−0.34 (−1.48, 0.8)	−0.17 (−1.44, 1.08)
PGE1plusSXTI	—	—	—	—	—	—	PGE1plusSXTI	0.16 (−1.23, 1.57)
PGE1plusXBJI	—	—	—	—	—	—	—	PGE1plusXBJI
Urinary Albumin excretion rates	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1	PGE1	**−1.47 (-2.48, -0.47)**	**−1.57 (-2.25, -0.9)**	**−1.34 (-2.14, -0.56)**	−0.97 (−2.22, 0.26)	**−1.08 (-1.94, -0.22)**	−0.72 (−1.95, 0.5)	−0.65 (−1.86, 0.57)
PGE1plusDCI	—	PGE1plusDCI	−0.1 (−1.31, 1.1)	0.13 (−1.15, 1.4)	0.5 (−1.11, 2.09)	0.39 (−0.93, 1.7)	0.75 (−0.83, 2.34)	0.82 (−0.75, 2.4)

(Continued on following page)

TABLE 3 (*Continued*) League table for all outcome measures.

Total effective rate	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1plusDHI	—	—	PGE1plusDHI	0.23 (−0.81, 1.26)	0.6 (−0.81, 2.01)	0.49 (−0.59, 1.59)	0.85 (−0.54, 2.26)	0.92 (−0.46, 2.32)
PGE1plusHQI	—	—	—	PGE1plusHQI	0.37 (−1.1, 1.84)	0.26 (−0.9, 1.44)	0.63 (−0.83, 2.09)	0.7 (−0.75, 2.16)
PGE1plusSKI	—	—	—	—	PGE1plusSKI	−0.11 (−1.61, 1.41)	0.26 (−1.48, 2.01)	0.33 (−1.41, 2.07)
PGE1plusSMI	—	—	—	—	—	PGE1plusSMI	0.37 (−1.14, 1.85)	0.43 (−1.06, 1.92)
PGE1plusSXTI	—	—	—	—	—	—	PGE1plusSXTI	0.07 (−1.65, 1.8)
PGE1plusXBJI	—	—	—	—	—	—	—	PGE1plusXBJI
Urinary beta 2-microglobulin	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1	PGE1	−0.72 (−1.99, 0.53)	**−1.37 (-2, -0.81)**	**−1.79 (-2.69, -0.89)**	—	−0.44 (−1.16, 0.31)	**−1.43 (-2.16, -0.73)**	−0.57 (−1.84, 0.7)
PGE1plusDCI	—	PGE1plusDCI	−0.65 (−2.08, 0.71)	−1.07 (−2.62, 0.48)	—	0.28 (−1.16, 1.77)	−0.71 (−2.17, 0.74)	0.15 (−1.64, 1.95)
PGE1plusDHI	—	—	PGE1plusDHI	−0.42 (−1.46, 0.69)	—	**0.92 (0.04, 1.93)**	−0.07 (−0.96, 0.89)	0.8 (−0.56, 2.24)
PGE1plusHQI	—	—	—	PGE1plusHQI	—	**1.35 (0.21, 2.53)**	0.36 (−0.79, 1.5)	1.22 (−0.34, 2.79)
PGE1plusSKI	—	—	—	—	PGE1plusSKI	—	—	—
PGE1plusSMI	—	—	—	—	—	PGE1plusSMI	−0.99 (−2.05, 0.01)	−0.13 (−1.61, 1.32)
PGE1plusSXTI	—	—	—	—	—	—	PGE1plusSXTI	0.86 (−0.59, 2.33)
PGE1plusXBJI	—	—	—	—	—	—	—	PGE1plusXBJI
24 h Urine Albumin	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
PGE1	PGE1	−0.83 (−2.08, 0.43)	**−2.49 (-3.96, -1.03)**	**−1.15 (-2.28, -0.03)**	−0.34 (−1.17, 0.49)	—	—	−1.26 (−2.7, 0.18)
PGE1plusDCI	—	PGE1plusDCI	−1.67 (−3.6, 0.25)	−0.32 (−2.03, 1.35)	0.48 (−1.02, 1.99)	—	—	−0.43 (−2.35, 1.47)
PGE1plusDHI	—	—	PGE1plusDHI	1.34 (−0.49, 3.19)	**2.15 (0.48, 3.84)**	—	—	1.23 (−0.81, 3.29)
PGE1plusHQI	—	—	—	PGE1plusHQI	0.81 (−0.57, 2.21)	—	—	−0.11 (−1.93, 1.72)
PGE1plusSKI	—	—	—	—	PGE1plusSKI	—	—	−0.92 (−2.58, 0.74)
PGE1plusSMI	—	—	—	—	—	PGE1plusSMI	—	—
PGE1plusSXTI	—	—	—	—	—	—	PGE1plusSXTI	—
PGE1plusXBJI	—	—	—	—	—	—	—	PGE1plusXBJI

Significant effects are printed in bold.
SMI, Salvia miltiorrhiza injection; DCI, Danshen-Chuanxiongqin injection; DHI, Danhong injection; HQI, Huangqi injection; SKI, Shenkang injection; SXTI, Shuxuetong injection; XBJI, Xuebijing injection; PGE1, alprostadil injection.

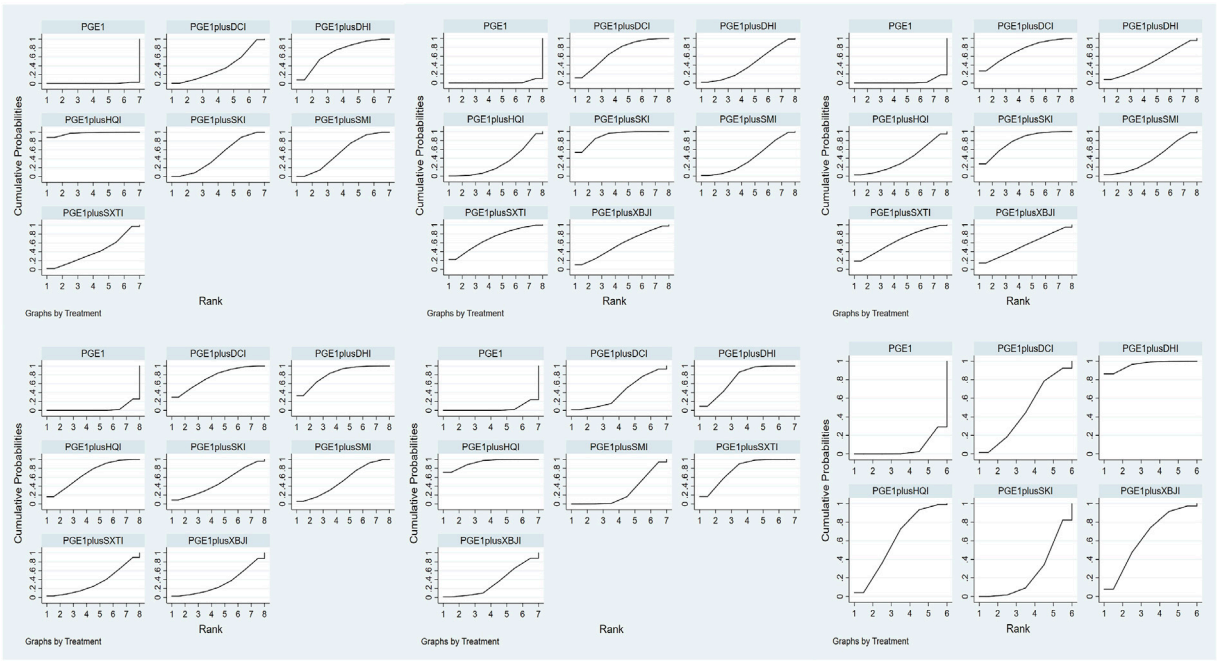
3.4 Cluster analysis

Cluster analysis was used to analyse the interventions for multi-dimensional outcomes and identify the best intervention measures under clustering of primary outcome indicators, glomerular filtration function, and urinary protein-related indicators. In terms of the primary outcome measure (total effective rate and Scr), PGE1+HQI and PGE1+SKI may be the best treatments (Figure 4). In terms of glomerular filtration function (Scr and BUN), PGE1+SKI was the best treatment (Figure 5). In terms of urinary protein-related indicators (24 h Alb & UAER), PGE1+DHI was the best treatment (Figure 6).

TABLE 4 Results of the surface under the cumulative ranking curve (SUCRA) (%).

	PGE1	PGE1plusDCI	PGE1plusDHI	PGE1plusHQI	PGE1plusSKI	PGE1plusSMI	PGE1plusSXTI	PGE1plusXBJI
总有效率	0.6	37.5	70.1	97.4	48.1	54.9	41.4	—
Scr	1.5	69	42.5	30.9	90.7	40.9	68.7	55.8
BUN	2.8	72.6	47.3	37.8	78.6	42.1	64.1	54.7
UAER	4	75	81.8	68.9	48.8	53	35.2	33.3
β2-MG	4.4	40.8	72.8	92.7	—	27.8	77.1	34.3
24h Alb	6.3	47.1	96.4	61	25.4	—	—	63.7

Red is the most likely to be the best intervention, yellow is second and green is third.
SMI, Salvia miltiorrhiza injection; DCI, Danshen-Chuanxiongqin injection; DHI, Danhong injection; HQI, Huangqi injection; SKI, Shenkang injection; SXTI, Shuxuetong injection; XBJI, Xuebijing injection; PGE1, alprostadil injection.



A: Total effective rate, B: Serum creatinine, C: Blood urea nitrogen, D: Urinary albumin excretion rate, E: β2-microglobulin, F: 24h Urine Albumin

FIGURE 3
Plot of the surface under the cumulative ranking curves.

3.5 Minimally contextualized framework

PGE1 was selected as the reference group. Based on the comparison of whether the 95% confidence interval of the effect size of the reference group intersected the decision threshold for each intervention, the intervention measures were divided into "Category 0," which had no difference compared with the intervention group, and "Category 1," which was better than the intervention group. Then, a secondary classification was conducted based on the differences between the interventions. The intervention with the smallest effect size in Category 1 was taken as the reference, and the intervention with better effect was classified into Category 2. Interventions were then classified into high and low reliability categories based on GRADE classification, and the consistency of classification was checked by ranking results. In this study, those interventions with the highest ranking were ensured to be among the most effective (see Table 5).

3.6 Heterogeneity and consistency tests

This study found that most of the heterogeneity in the heterogeneity assessment was mild to moderate, in which the total effective rate was not substantially heterogeneous, and consistent models fit similarly or better than inconsistent models. See details in Supplementary Figures S2–S7 (page 103–108) and Supplementary Table S13 (page 103–108).

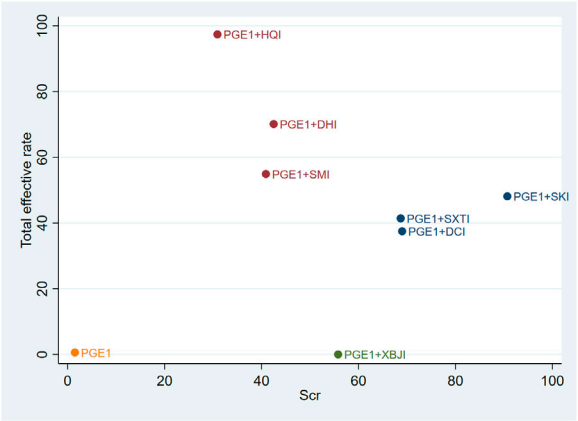


FIGURE 4
Cluster diagram of primary outcome indicators.

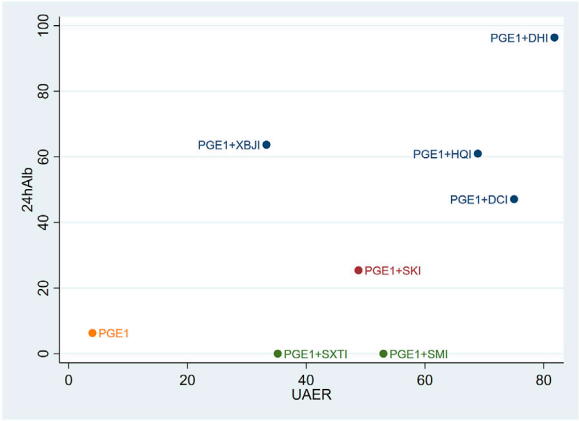


FIGURE 6
Clustering diagram of 24h Alb and UAER.

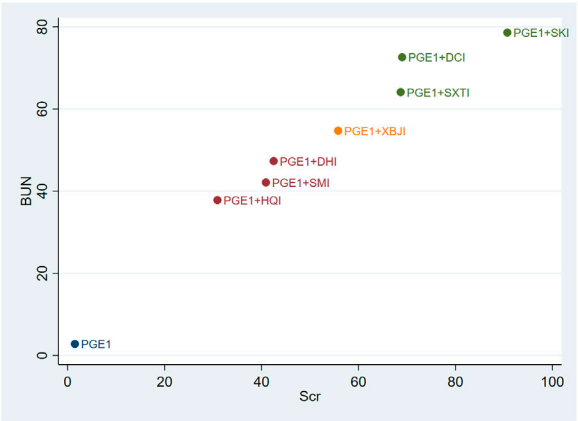


FIGURE 5
Clustering diagram of Scr and BUN.

3.7 Safety

Seventeen RCTs (Xie and Zhang, 2002; Gong and Xie, 2009; Wang et al., 2009; Zhao and Dong, 2010; Pang et al., 2011; Xing, 2012; Liu, 2013; Pu et al., 2013; Ding, 2014; Li et al., 2016; Liu et al., 2016; Zhang, 2016; Xu and Liu, 2018; Lin et al., 2019; Xiao, 2019; Wang, 2021; Wen et al., 2021) reported the specific adverse reactions and safety of TCMIs, including pain, redness, and swelling at the injection site; dizziness; headache; vomiting; and diarrhoea. Only a descriptive analysis was performed because the description criteria of the various studies were not uniform. The specific information is given in Table 6.

3.8 Sensitivity analysis

Four studies had treatment durations that were not in the 14 days–30 days range (Ru et al., 2008b; Pu et al., 2013; Yin, 2014; Li et al., 2016). A study was included in the primary outcome, the total effective rate (Yin, 2014). The findings indicated that excluding this study did not significantly change the overall analysis. 3 studies were included in the primary outcome measure Scr (Ru et al., 2008b; Pu et al., 2013; Li et al., 2016). And the outcomes demonstrated that removing these studies did not significantly change the overall analysis.

Nine studies were identified as having a high risk of bias (Han and Zhang, 2013; Lin, 2013; Yin, 2014; Cai et al., 2015; He, 2015; Zhang, 2017a; Zhang, 2017b; Liang, 2018; Wen et al., 2021), 7 studies were included in the primary outcome measure total response rate (Han and Zhang, 2013; Lin, 2013; Yin, 2014; He, 2015; Zhang, 2017a; Zhang, 2017b; Wen et al., 2021), and 4 studies were included in the primary outcome measure Scr (Lin, 2013; Cai et al., 2015; Zhang, 2017b; Wen et al., 2021). The results revealed that removing these studies separately had no discernible impact on the overall analysis.

3.9 Publication bias

In this study, the funnel plots of the total effective rate (Figure 7) and Scr (Figure 8) were plotted. The results showed that the distribution of the total effective rate and Scr funnel plots were roughly symmetric, without obvious small-sample effect and publication bias.

3.10 Trial sequential analysis

For each of the groups of PGE1+SMI vs. PGE1, PGE1+DHI vs. PGE1, PGE1+SKI vs. PGE1, and PGE1+XBJI vs. PGE1, the cumulative Z-curve of Scr crosses the trial sequential monitoring and the required information size, indicating that SMI, DHI, SKI, and XBJI are effective for reducing Scr. Furthermore, the evidence of DCI, HQI, and SXTI was not sufficient. In terms of the total effective rate, the cumulative Z-curve crossed the trial sequential monitoring and the required information size in the comparisons of PGE1+HQI vs. PGE1 and PGE1+SKI vs. PGE1, which proved to be beneficial to the total effective rate, while the evidence of other injections was insufficient (Supplementary Figures S8–S20, page 109–121).

10.3389/fphar.2023.1028257

TABLE 5 Final classification of 7 interventions for Diabetic kidney disease.

	Certainty of the evidence, and classification of intervention	Intervention	Intervention vs. PGE1 SMD (95% CI)	SUCRA
	High certainty (moderate to high certainty evidence)			
	Category 2: among the most effective	PGE1plusHQI(M)	1.43 (1.26, 1.66)	97.4
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusDHI(M)	1.28 (1.13, 1.46)	70.1
		PGE1plusSKI(M)	1.2 (1.12, 1.3)	48.1
Total effective rate	Low certainty (low to very low certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusSMI(L)	1.24 (1.15, 1.35)	54.9
		PGE1plusDCI(L)	1.17 (1.02, 1.37)	37.5
	Category 0: among the least effective	PGE1plusSXTI(VL)	1.18 (0.99, 1.42)	41.4
	High certainty (moderate to high certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusSMI(M)	−0.83 (−1.6, −0.06)	40.9
	Low certainty (low to very low certainty evidence)			
	Category 2: among the most effective	PGE1plusSKI(L)	−1.78 (−2.39, −1.18)	90.7
Serum Creatinine	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusDCI(VL)	−1.34 (−2.11, −0.56)	69
		PGE1plusDHI(L)	−0.87 (−1.7, −0.03)	42.5
	Category 0: among the least effective	PGE1plusHQI(L)	−0.65 (−1.42, 0.13)	30.9
		PGE1plusSXTI(VL)	−0.91 (−2.09, 0.28)	68.7
		PGE1plusXBJI(L)	−1.1 (−2.27, 0.07)	55.8
	High certainty (moderate to high certainty evidence)			
Blood Urea Nitrogen	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusSKI(M)	−1.16 (−1.73, −0.61)	78.6
	Category 0: among the least effective	PGE1plusDHI(M)	−0.73 (−1.66, 0.19)	47.3
		PGE1plusSMI(M)	−0.66 (−1.35, 0.03)	42.1
	Low certainty (low to very low certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusDCI(L)	−1.11 (−1.93, −0.29)	72.6
		PGE1plusSXTI(L)	−1 (−1.91, −0.09)	64.1
	Category 0: among the least effective	PGE1plusXBJI(L)	−0.83 (−1.89, 0.22)	54.7
		PGE1plusHQI(L)	−0.59 (−1.34, 0.17)	37.8

(Continued on following page)

TABLE 5 (*Continued*) Final classification of 7 interventions for Diabetic kidney disease.

	Certainty of the evidence, and classification of intervention	Intervention	Intervention vs. PGE1 SMD (95% CI)	SUCRA
	Low certainty (low to very low certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusDHI(L)	−2.49 (−3.96, −1.03)	96.4
		PGE1plusHQI(L)	−1.15 (−2.28, −0.03)	61
24h Urine Albumin	Category 0: among the least effective	PGE1plusXBJI(VL)	−1.26 (−2.7, 0.18)	63.7
		PGE1plusDCI(L)	−0.83 (−2.08, 0.43)	47.1
		PGE1plusSKI(L)	−0.34 (−1.17, 0.49)	25.4
	High certainty (moderate to high certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusDHI(M)	−1.57 (−2.25, −0.9)	81.8
		PGE1plusDCI(M)	−1.47 (−2.48, −0.47)	75
Urinary Albumin excretion rates	Category 0: among the least effective	PGE1plusXBJI(M)	−0.65 (−1.86, 0.57)	33.3
	Low certainty (low to very low certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusHQI(L)	−1.34 (−2.14, −0.56)	68.9
	Category 0: among the least effective	PGE1plusSKI(L)	−0.97 (−2.22, 0.26)	48.8
		PGE1plusSXTI(L)	−0.72 (−1.95, 0.5)	35.2
	High certainty (moderate to high certainty evidence)			
Urinary beta 2-microglobulin	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusHQI(M)	−1.79 (−2.69, −0.89)	92.7
	Category 0: among the least effective	PGE1plusSMI(M)	−0.44 (−1.16, 0.31)	27.8
	Low certainty (low to very low certainty evidence)			
	Category 1: inferior to the most effective, or superior to the least effective	PGE1plusSXTI(L)	−1.43 (−2.16, −0.73)	77.1
		PGE1plusDHI(L)	−1.37 (−2, −0.81)	72.8
	Category 0: among the least effective	PGE1plusDCI(VL)	−0.72 (−1.99, 0.53)	40.8
		PGE1plusXBJI(L)	−0.57 (−1.84, 0.7)	34.3

H, high certainty evidence; M, moderate; L, low; VL, very low.

4 Discussion

4.1 Discussion of survey results

Our study found that SKI had obvious overall advantages in improving Scr and BUN levels. SKI is composed of *Salvia miltiorrhiza Bunge, Astragalus mongholicus Bunge, Carthamus tinctorius L., Rheum palmatum L*, and was approved to use on chronic kidney disease by China's State Food and Drug Administration in 1999 (Licence No. YBZ08522004). The main components of SKI can reduce albuminuria, inhibit fibrosis, improve microcirculation, and regulate renal haemodynamic, and showed effects on glomerular and tubular lesions (Huang et al., 2012; Wang et al., 2012; Sun et al., 2016; Xu et al., 2020). A study showed that SKI can prevent renal tubular cell senescence under hyperglycaemia situation by reducing the expression of ageing

TABLE 6 Summary of adverse drug events.

Type of interventions	Number of RCTs	Groups	Total sample size	Incidence	Detailed ADR events (number of cases)
PGE1+SMI vs. PGE1	0	PGE1+SMI	0	—	—
		PGE1	0	—	—
PGE1+DCI vs. PGE1	1	PGE1+DCI	65	3.08%	Anorexia (1), constipation(1)
		PGE1	65	4.62%	Diarrhea (1), Dizziness(1), Fever(1)
PGE1+DHI vs. PGE1	4	PGE1+DHI	147	0.00%	None
		PGE1	144	0.00%	None
PGE1+HQI vs. PGE1	5	PGE1+HQI	433	16.86%	Dizziness (19), headache (17), pain at the injection site (36), allergies (1)
		PGE1	415	14.70%	Dizziness (24), pain at the injection site (27), redness at the injection site (4), vomiting (5), diarrhea (1),
PGE1+SKI vs. PGE1	4	PGE1+SKI	136	0.74%	Pain and flushing at the injection site (1)
		PGE1	116	0.00%	None
PGE1+SXTI vs. PGE1	2	PGE1+SXTI	96	0.00%	None
		PGE1	90	0.00%	None
PGE1+XBJI vs. PGE1	1	PGE1+XBJI	30	0.00%	None
		PGE1	30	0.00%	None

SMI, Salvia miltiorrhiza injection; DCI, Danshen-Chuanxiongqin injection; DHI, Danhong injection; HQI, Huangqi injection; SKI, Shenkang injection; SXTI, Shuxuetong injection; XBJI, Xuebijing injection; PGE1, alprostadil injection.

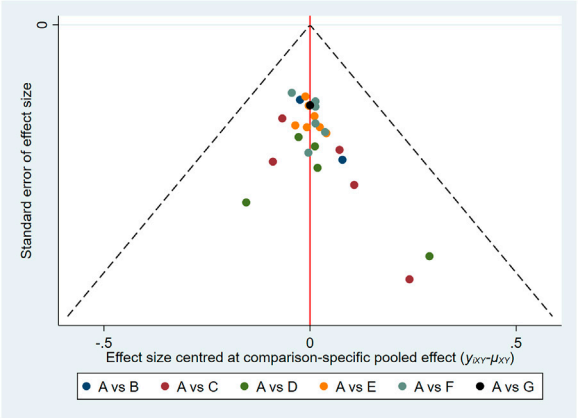


FIGURE 7
Funnel plots for total effective rate.

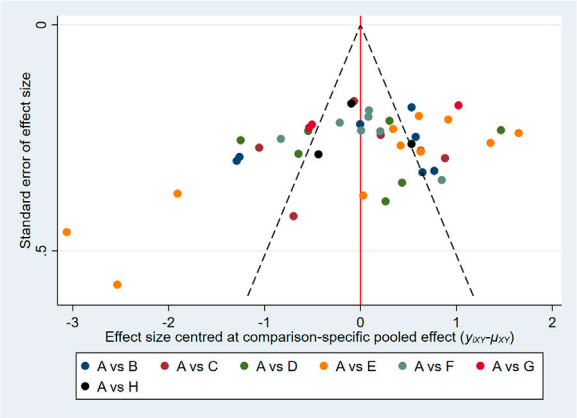


FIGURE 8
Funnel plots for Scr.

markers P16INK4, cyclin D1, DcR2, and SA-β-Gal activity (Fu et al., 2019). It also can inhibit renal fibrosis and oxidative stress by downregulating the TGF-β/Smad3 signalling pathway. It brought significantly effects on improving Scr and BUN and alleviating renal injury (Wu et al., 2015; Wang et al., 2021). It also reduces glomerular hyperfiltration, hypertension, and hyperfusion situation (Zou et al., 2020).

DHI most effective on improving urinary protein levels. The main components in DHI are *Salvia miltiorrhiza Bunge* and *Carthamus tinctorius L.* Studies showed they can improve energy metabolism, oxidative stress, and autophagy and restore mitochondrial energy (Guo et al., 2021; Zeng et al., 2021). Study showed that DHI can inhibit glomerular hypertrophy, and markedly reduce urinary protein excretion in db/db mice (Liu et al., 2015). It also can delay the progression of renal injury by upregulating microRNA-30D-5P and targeting JAK1 (Deng et al., 2022).

HQI was the most effective for the total effective rate and β2-MG. The main component in HQI is *Astragalus mongholicus Bunge.* Main compounds of *Astragalus mongholicus Bunge* are polysaccharides, astragalus saponins, and flavonoids (Li et al.,

2014), which inhibit oxidative stress (Ma et al., 2013), immune adjustment (Cho and Leung, 2007), anti-inflammatory (Zhang et al., 2003), and protect vascular endothelial cells (Wang et al., 2013; Zhu et al., 2013).

Studies have found that DKD occurs earlier than glomerular disease (Magri and Fava, 2009; Hasegawa et al., 2013). Therefore, the proximal renal tubules may be a new therapeutic target for the treatment of DKD. *Astragalus mongholicus Bunge* can ameliorate renal tubular injury and reduce the area, lumen, and wall to nearly normal (Sun et al., 2016), which is consistent with the results of this study.

4.2 Relationships and comparisons with other studies

To the best of our knowledge, this study is the first to compare the differences in the efficacy of TCMIs in the treatment of DKD through a network meta-analysis. Most of the previous studies (Zhang et al., 2022; Zhao et al., 2022), only conducted systematic reviews and network meta-analyses on TCM decoction. Those studies could not have stable quality control due to the diversity of ingredients and dose variability. The composition of TCMIs is more stable than TCM decoctions, which has quantitative significance. We comprehensively studied the RCTs of TCMIs combined with PGE1 in the treatment of DKD and ranked the advantage of each outcome index of different TCMIs to guide the clinical use.

4.3 Implications for clinical practice

This study found that SKI + PGE1 most effective on glomerular filtration function, DHI + PGE1 most effective on urinary protein, and HQI + PGE1 most effective on total effective rate and reduce clinical symptoms. TCMIs can effectively solve different problems of DKD. Non-study showed the effects of combination of multiple TCMIs in the treatment of DKD. This may be related to the complexity of the components, interactions, and other factors, which need to be further explored in subsequent studies.

Xie et al. found that the UAER of the 3 weeks treatment group decreased the fastest (Xie et al., 2021). A meta-analysis of the treatment of DKD with HQI found that the efficacy of a long course (>4 weeks) was better than that of a short course (<4 weeks) (Zhang and Kong, 2018). In this review, the duration of treatment in the 54 included studies focused on 1 month, 2 studies had longer treatment periods (Ru et al., 2008b; Pu et al., 2013), 1 study was in 7 days (Li et al., 2016) and 1 study was not mentioned (Yin, 2014).

Allergic reactions are the most common adverse events of using TCMIs (Wen et al., 2020). According to the studies included in this review, the adverse reactions of TCMIs are mild and can be relieved or eliminated by reducing the dosage, stopping medication, or symptomatic treatment (Xie and Zhang, 2002; Zhao and Dong, 2010; Pang et al., 2011; Zhang, 2016; Xu and Liu, 2018). The safety of TCMIs greatly improved by standardized the use in clinical application (Li et al., 2022). Li et al. improved the quality standard of solvent-enhancing polysorbate 80 in TCMI to reduce anaphylactic reactions (Li, 2018). However, the adverse reactions of patients still need to be concerned to avoid medical accidents.

TCMIs were widely used and effective in clinical practice. However, it was found that the specific extract components, complex pharmacological mechanisms and methodological descriptions of the botanical drugs were not clear in this included studies. In the future, relevant studies should follow the suggestions of consensus (Heinrich et al., 2020) and conduct more critical pharmacological studies on TCMIs.

4.4 Limitations

This study had the following limitations: (a) less reports on adverse reactions, and most of the studies had no clear safety assessment; (b) most of the literatures were “some concerns” in the risk assessment of bias and the quality of literatures was not high; (c) Have clinical heterogeneity due to the differences in botanical drug doses and treatment courses; (d) all included literatures were in China.

5 Conclusion

This study suggests that the combination of TCMIs and PGE1 provide additional benefits to patients with DKD. In terms of different outcome indicators, SKI had more effective on improving glomerular filtration function, DHI more effective on reducing urinary protein, and HQI more effective on improving renal tubular function. Despite the low incidence of adverse events, only a few studies have evaluated the safety of TCMIs. Further research on TCMIs treatment is needed for better understanding about TCMIs and guide the clinical application.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Author contributions

CL, HF, and RY designed the study and drafted the manuscript. CL and HF systems searched literature and extracted data. CL reviewed the included studies and performed statistical analyses. ZL, JL, ZHL and YJ provided useful suggestions and substantial revisions based on the content of the article. All listed authors made substantial, direct and intellectual contributions to the work and were approved for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1028257/full#supplementary-material

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