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Real-world evidence of natural products, herbal medicines, and traditional Chinese medicine treatments

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Real-world evidence of natural products, herbal medicines, and traditional Chinese medicine treatments

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Medicinal plant-based drug delivery system for inflammatory bowel disease

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Inflammatory bowel disease (IBD) is a chronic recurrent intestinal disease. The incidence rate of IBD is increasing year by year, which seriously endangers human health worldwide. More and more studies have shown that medicinal plants or their main phytochemicals have great potential in the treatment of intestinal diseases. However, the disadvantages of low oral absorption rate, low biological distribution and low systemic bioavailability limit their clinical application to a certain extent. In recent years, the application of nanotechnology has made it possible to treat IBD. Nanoparticles (NPs) drug delivery system has attracted special attention in the treatment of IBD due to its small size, low immunogenicity, surface modification diversity, targeting and other advantages. Synthetic nanoparticles and extracellular vehicles (EVs) can deliver drug components to colon, and play a role in anti-inflammation, regulation of oxidative stress, improvement of intestinal flora, etc. In addition, some medicinal plants can secrete EVs by themselves, and carry biological molecules with therapeutic effects to act on the intestine. Some clinical trials to evaluate the safety, tolerance, toxicity and effectiveness of EVs-loaded drugs in IBD are also progressing steadily. This review introduces that synthetic nanoparticles and medicinal plants derived EVs can play an important role in the treatment of IBD by carrying the effective active phytochemicals of medicinal plants, and discuss the limitations of current research and future research needs, providing a scientific and reliable basis and perspective for further clinical application and promotion.

KEYWORDS

medicinal plant, traditional Chinese medicine, drug delivery, nanomedicine, extracellular vesicles, synthetic nanoparticles, plant exosome-like nanovesicles, inflammatory bowel disease

1 Introduction

Inflammatory bowel disease (IBD) is a chronic recurrent intestinal disease, which consists of ulcerative colitis (UC) and Crohn's disease (CD). UC affects the colon, while CD can affect any part of the digestive tract from the mouth to the perianal area (Ochsenkuhn and D'Haens, 2011). IBD is a global disease. In recent years, the incidence

rate of IBD has increased year by year, not only in Western countries, but also in developing countries in South America, Asia, Africa and Eastern Europe, which might bring social and economic burden on governments and health systems (Ng et al., 2017). It is believed that its evolution can be divided into four epidemiological stages: disease emergence, disease acceleration, disease deterioration and disease balance (Kaplan and Windsor, 2021). UC and CD share many common pathological and clinical features but their treatment methods are completely different, and their pathogenesis is still unclear (Flynn and Eisenstein, 2019). The current research shows that heredity (host susceptibility) (Mohanan et al., 2018), environment (microorganisms) (Sun et al., 2019), barrier factors (intestinal epithelial and innate immune cells) (Haag and Siegmund, 2015) and other causes may participate in the pathogenesis of IBD. Although traditional drugs used to treat IBD, such as anti-inflammatory drugs, immunosuppressants and glucocorticoids, have been proved to be effective in correcting immune dysregulation and dampening inflammation within the intestinal mucosa they still have some disturbing side effects (toxicities to healthy organs, allergic reactions, and nausea, etc.) (Zhang et al., 2017).

With the development of science and technology, more and more evidence proved that medicinal plants themselves or their main phytochemicals have great potential for the treatment of intestinal diseases. But the shortcomings of low oral absorption rate, low solubility and low bioavailability make it difficult to approve these effective ingredients as drugs in clinical practice. Therefore, it is an important way to find more accurate and effective drug delivery methods. In recent years, the application of nanotechnology has made it possible to treat IBD (Verstockt et al., 2018). Nanoparticle (NP) drug delivery systems have attracted special attention in the treatment of IBD due to their small size, low immunogenicity, stability, surface modification diversity, targeting and other advantages (Zhang et al., 2017). Since the 1990s, synthetic nanoparticles have been widely used in clinical drug delivery (Witwer and Wolfram, 2021). Besides, Extracellular vesicles (EVs)-based therapy is one of the most concerned one at present. EVs are cell-derived membranous structures comprising exosomes (30–200 nm), microvesicles (MVs) (50–1000 nm) and apoptosis bodies (50–2000 nm), which are originated from the endosomal system or released from the plasma membrane or apoptotic cells, respectively. They contain a variety of functional contents mainly proteins, nucleic acids and lipids (van Niel et al., 2018). The EVs used in the research mostly come from mammals, but the possibility of immunogenicity limits their development to a certain extent. EVs derived from natural plants may alleviate the concerns of most mammalian exosomes, while medicinal plants with therapeutic effects themselves may play a greater role in transportation (Karamanidou and Tsouknidas, 2021). The methods of loading drugs into EVs mainly include indirect loading through the source cells and direct loading of EVs after separation. The EVs secreted by varieties cells treated with medicinal plants or effective ingredients can specifically target the disease site, increase the drug content in the colon, prolong the drug retention time in the body, affect the intestinal microenvironment, and reduce the related systemic adverse reactions while improving the efficacy. The highly selective homing ability and specific targeting potential of EVs make them an ideal tool for targeted therapy of IBD. In addition, some medicinal plants with anti-inflammatory effects can secrete EVs, which called plant exosome-like nanovesicles (PELNVs) and carry biological molecules with therapeutic effects to act on intestinal cells (Dad et al., 2021). This review introduces the therapeutic efficacy of EVs and synthetic nanoparticles as a therapeutic drug-delivery loaded with medicinal plant phytochemicals or medicinal plant derived EVs for IBD to clarify its mechanism. At the same time, some ongoing clinical trials for evaluating the safety, tolerance, toxicity and effectiveness of enterovirus loaded drugs in IBD are introduced. These findings provide a scientific and reliable basis for the role of medicinal plant related EVs in IBD and contribute to further clinical application and promotion.

2 Application of synthetic nanoparticles loaded with medicinal plant active ingredients in IBD

Engineering nanomaterials are widely used in the treatment of IBD. Nanoparticle delivery systems (including transferosomes, liposomes, dendrimers, mesoporous silica, solid lipids, microspheres and cellular carriers, etc.) may facilitate targeted delivery of drugs, increase effective concentration and reduce the side effects (Yang and Merlin, 2019). The actions are summarized below and shown in Table 1.

2.1 NPs for the delivery of resveratrol

Resveratrol (RES), a natural (poly) phenol, which exists in the traditional medicinal plant called *Polygonum cuspidatum* Sieb. et Zucc. [Polygonaceae; Polygoni Cuspidati Rhizoma et Radix] (Medium Taxonomic Confidence) and has been proven to prevent and improve intestinal inflammation by interacting with NF-κB, SIRT1, mTOR and HIF-1α, etc., (Nunes et al., 2018). However, the poor water solubility, rapid metabolism and low bioavailability of RES limit its clinical applications (Gowd et al., 2022). Some studies have used chitosan-based composites materials as the carrier of RES and found that RES can be continuously released in the colon, which may have a potential therapeutic effect on IBD. Hydrogel is used as a matrix for controlled release of bioactive molecules to ensure good biocompatibility of biomaterials. Chitosan can tightly adhere to the wall surface of gastrointestinal tract (GIT), and temporarily open the tight connection between epithelial cells, enhancing the drug absorption of intestinal epithelial cells. The cross-linking in the composite can fully inhibit the 3D network of hydrogel and the water flow in the system. The chitosan network is responsible for significantly reducing the drug release rate, making this system a multifunctional tool by extending the retention and delivery time. This study carried out 20 experiments and a Box-Behnken experimental design was used to evaluate the importance of these independent variables related to packaging efficiency (EE). It was found that at RES/polymer ratio of 0.75: 1 w/w, the RES EE value could be enhanced in 24 h and 39°C (Iglesias et al., 2019). In other studies, 3^3 Box Behnken was further used to design colon targeting system to optimize the preparation of RES loaded chitosan-based microcapsules. It was found that 3^3 Box

TABLE 1 Physicochemical characterization and application of NPs as delivery nano-platforms for phytochemicals.

Refs	Drugs	Size (nm)	Nanoparticles	Diseases	Model used	Test sites	Biochemical measurements
Iglesias et al. (2019)	RES	170 (± 90) nm	Chitosan-based composites	IBD	*in vitro*	—	—
Gandhi et al. (2020)	RES	—	Chitosan-based microsponges	Acetic acid-induced colitis	*in vivo*/vitro	Colon tissues	Mucosal ulceration↓, inflammatory cell infiltration↓, submucosal edema↓, goblet hyperplasia↓
Pujara et al. (2021)	RES	165 (± 2) nm	β-Lactoglobulin-Nanosphere-Encapsulated	Spontaneous colitis	*in vivo*/vitro	Colon tissues	IL-10↑, TNF-α↓, IL-1β↓, IL-17↓
Naserifar et al. (2020)	RES	120 ± 7 nm 131 ± 9 nm	PLGA	TNBS-induced colitis	*in vivo*/vitro	Colon tissues	TNF-α↓, SOD↓, MPO↓, IL-6↓
Ohno et al. (2017)	Cur	—	Theracurmin	DSS-induced colitis	*in vivo*	Colonic mucosa, faeces	TNF-α mRNA↓, IL-1β mRNA↓, IL-6 mRNA↓, CXCL1 mRNA↓, CXCL2 mRNA↓, NF-κBp65↓
Plaza-Oliver et al. (2020)	Cur	173 ± 20 nm	NE-ADP	—	*in vitro*	Caco-2 cell	ROS↓
Salah et al. (2022)	Cur	65 nm	NPL	DSS-induced colitis	*in vivo*/vitro	Caco-2 cell, colon tissues	IL-1β↓, IL-6↓, IL-8↓, IL-10↑
Sharma et al. (2019)	Cur	210.56 ± 41.22 nm	SBLNs	DSS-induced colitis	*in vivo*/vitro	Stomach, small intestine, colon	MPO↓, TNF-α↓, protein carbonyl group↓, LPO↓
Wang et al. (2022)	Cur	200 nm	AceKGM	DSS-induced colitis	*in vivo*/vitro	Colon tissues	MPO↓
Mutalik et al. (2016)	Cur	425 nm	PAAm-g-XG copolymer	Acetic acid-induced colitis	*in vivo*/vitro	Colon tissues, plasma	MPO↓, NO↓
Beloqui et al. (2014)	Cur	166 ± 3.0 nm	PLGA/ES100 NPs	DSS-induced colitis	*in vivo*/vitro	Caco-2 cell, colon tissues	TNF-α↓, MPO↓
Diez-Echave et al. (2021)	QT	175.8 ± 0.9 nm	QSFN	DSS-induced colitis	*in vivo*/vitro	Colon tissues	TNF-α↓, IL-1β↓, IL-6↓, MCP-1↓, ICAM-1↓, NLRP3↓, iNOS↓
Khater et al. (2022)	QT	—	CS	DSS-induced colitis	*in vivo*	Colon tissues	ROS↓, NO↓, MDA↓, H_2O_2↓, GSH-Px↑, SOD↑, IL-6↓, TNF-α↓, IFN-γ↓, IL-10↓, CAT↑, MUC-2↑, JAM-2↑, Occludin↑, Nrf2↑, HO-1↑, CD4↓, CD8↓, TLR4↓, iNOS↓, COX2↓
Shen et al. (2021)	QT	220 nm	pH/ROS dual-responsive prodrug micelle	DSS-induced colitis	*in vivo*/vitro	Colon tissues	IL-6↓, TNF-α↓, iNOS↓
Feng et al. (2021)	RMP	205.6 ± 1.86 nm	PLGA	DSS-induced colitis	*in vivo*/vitro	Colon tissues	IFN-γ↓, IL-6↓, IL-10↑, ZO-1↑, occludin↑, acetate↑, propionate↑, butyrate↑
Feng et al. (2021)	RMP	202 nm	PLGA	LPS-induced colitis	*in vivo*/vitro	Jejunum tissues	TNF-α↓, IL-6↓, IL-1β↓, PGE2↓
Feng et al. (2022)	SK	190.3 nm	ES100/HA/CS	TNBS-induced colitis	*in vivo*/vitro	Colon tissues	TNF-α↓, IL-6↓, IL-1β↓, COX-2↓, iNOS↓, IL-10↑, TGF-β↑, ZO-1↑, occludin↑
Zhao et al. (2021)	BBR	230.2 ± 18.1 nm	CS	DSS-induced colitis	*in vivo*/vitro	Colon tissues	Firmicutes↑, proteobacteria↓, TNF-α↓, IL-6↓, TGF-β↓, IL-23↓
Zhang et al. (2018)	Shogaol	249.6 ± 1.3 nm	PLGA/PLA-PEG-FA	DSS-induced colitis	*in vivo*/vitro	Colon tissues	TNF-α↓, IL-6↓, IL-1β↓, iNOS↓, Nrf-2↑, HO-1↑
Nidhi et al. (2017)	Embelin	12.6 ± 2.1 μm	Microspheres	Acetic acid-induced colitis	*in vivo*/vitro	Colon tissues	MPO↓, MDA↓, LPO↓, GSH↑
Tambuwala et al. (2019)	PCT	210–288 nm	Albumin	DSS-induced colitis	*in vivo*/vitro	Colon tissues	p65↓, HIF-1α↓, INF-γ↓, IL-6↓, TNF-α↓, MPO↓
Nguyen et al. (2021)	SM	110 nm	siRNP	DSS-induced colitis	*in vivo*/vitro	Colon tissues	NO↓, IL-1β↓, IL-6↓, TNF-α↓, 2,2-diphenyl-1-picrylhydrazide radical↓

(Continued on following page)

TABLE 1 (*Continued*) Physicochemical characterization and application of NPs as delivery nano-platforms for phytochemicals.

Refs	Drugs	Size (nm)	Nanoparticles	Diseases	Model used	Test sites	Biochemical measurements
Varshosaz et al. (2015)	SM	109 ± 6 nm	Eudragit RL PO NPs	Acetic acid-induced colitis	*in vivo*/vitro	Colon tissues	TNF-α↓, IL-6↓, MPO↓
Miroliaee et al. (2011)	SM	245 ± 82.47 nm	Se	TNBS-induced colitis	*in vivo*	Colon tissues	TNF-α↓, IL-1β↓, NF-κB↓, MPO↓, lipid peroxidation↓, protein carbonyl↓

Notes: ↑, upregulated by drugs; ↓, downregulated by drugs. Abbreviations: PLGA, poly (lactic-co-glycolic acid); PAAm-g-XG, pH-sensitive hydrolyzed polyacrylamide-grafted-xanthan gum; NPs, nanoparticles; NE, nanoemulsions; ADP, ascorbyl-2,6-dipalmitate; OC-B, MPO, myeloperoxidase; NO, nitric oxide; NPL, crosslinked starch nanocarrier; SBLNs, solid binary lipid nanoparticles; PLGA, poly (lactide-co-glycolide) acid; MUC-2, mucin-2; JAM, junction adhesion molecule; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, hemeoxygenase-1; TLR4, toll-like receptor 4; QSFN, Quercetin-loaded silk fibroin nanoparticles; RMP, ramulus mori polysaccharide; CS, chitosan; HA, hyaluronic acid; ES100, Eudragit S100; GSH, glutathione; LPO, lipid peroxides concentration; siRNP, silica-containing redox nanoparticles; Se, Selenium; SCFA, short chain fatty acids.

Behnken could enhance the therapeutic effect of UC. Average weight, friability test (%), hardness are used as evaluation parameters of matrix tables, and hardness of 4.13 ± 0.13 kg/cm^2, friability below 0.69% ± 0.23% and average weight of 499.65 ± 1.35 mg would be satisfactory. This study used appropriate mathematical models to fit drug release data, with the increase of drug concentration, the percentage yield (% Y) of the prepared microsponges and drug loading (DL %) have an increasing trend, which may be related to the increase in viscosity of the dispersed phase and reduced the diffusion rate from viscous solutions into aqueous phase (Gandhi et al., 2020). When RES was encapsulated in β-lactoglobulin (BLG) nanospheres, it was found that the complexation of RES with BLG increased the solubility and stability of RES. RES with BLG increased the aqueous solubility of RES by ≈ 1.7 times with 10% w/w loading. After oral administration, BLG-RES (50 mg/kg) significantly improved the body weight percentage and disease activity index (DAI) of UC mice, upregulated the expression of IL-10, increased the number of goblet cells, restored the destruction of colon epithelium, and produced anti-inflammatory effect (Pujara et al., 2021). In order to protect RES from rapid degradation and increase its intestinal permeability, researchers have developed resveratrol-loaded galactosylated poly (lactic-co-glycolic acid) (PLGA) nanoparticles targeted by folic acid (FA). The release of RES, PLGA-RES and PLGA-FA-RES were 4.5%, 61% and 99% respectively, whether the PLGA encapsulation could retain RES under simulated gastric conditions (HCl 0.1 N, pH 1.2) or release a large amount of RES under simulated intestinal conditions (PBS, pH 7.4). It was found that oral FA-PLGA- RES and PLGA- RES (100 mg RES) could significantly inhibit inflammation (downregulating TNF-α, SOD, MPO and IL-6) and reduce the accumulation of neutrophils and lymphocytes. Compared with non-targeted delivery system, FA-targeted system has the highest efficacy in inhibiting colitis (Naserifar et al., 2020).

2.2 NPs for the delivery of curcumin

Curcumin (Cur) is a bioactive ingredient derived from *Curcuma longa* L. [Zingiberaceae; Curcumae longaae rhizoma] (High Taxonomic Confidence). Traditionally, it has been used to flavor various delicacies in Southeast Asia and Arabian countries (Baliga et al., 2012). Cur has a variety of biological functions, such as anti-inflammatory, anti-cancer, anti-oxidation, and hypoglycemic, etc. A large number of studies have proved that Cur may be interacted with NF-κB, JAKs/STATs, MAPK, PPARγ and TRPV1 to treat IBD and slow down the progress of IBD (Karthikeyan et al., 2021). However, due to of its poor absorption in the GIT, poor stability, low bioavailability and rapid elimination, its traditional application in treatment is limited. Oral a newly developed nanoparticle curcumin (named Theracurmin) can inhibit NF-κB activation and mucosal Tregs induction (increasing expansion of CD4$^+$ Foxp3$^+$ regulatory T cells and CD103$^+$ CD8α-regulatory dendritic cells) in colon epithelial cells, downregulate the expression of mucosal mRNA of inflammatory mediators (TNF-α mRNA, IL-1β mRNA, IL-6 mRNA, CXCL1 mRNA, CXCL2 mRNA), regulate the intestinal flora environment (increasing the abundance of butyrate-producing bacteria and fecal butyrate), further significantly alleviate weight loss, DAI, histological colitis score, and improve mucosal permeability in DSS-induced colitis mice. Although some studies have shown that the absorption efficiency of nanoparticle curcumin in rats and humans is 30-fold higher than that of curcumin powder, this study has not verified the drug loading rate, release rate and safety of nanoparticle curcumin, only compared the efficacy of nanoparticle Cur with that of pure Cur (Ohno et al., 2017). Oxidative stress reaction plays an important role in the development of IBD. In consideration of the antioxidant effects of α-tocopherol nanoemulsions (NE) (a lipophilic antioxidant to scavenge free radicals in hydrophobic environments) and ascorbyl-2,6-dipalmitate (ADP) (maintains the antioxidant properties of ascorbic acid). Some researchers combined these two materials to construct a new type of antioxidant nano lotion nano carrier. They can be combined to construct a new antioxidant nanoemulsion as a novel nanocarrier for delivering Cur in order to play a synergistic role. The study evaluated the stability of NE-ADP in temperature, storage time, normal (non-enzyme-riched) and normal simulated gastric and intestinal fluids (SGF and SIF respectively). It was found that NE-ADP could stably exist in gastrointestinal media and be located in Caco-2 cells, which may play a role in IBD by down regulating intracellular reactive oxygen species (ROS) level (Plaza-Oliver et al., 2020). Crosslinked starch nanocarrier loaded with Cur (NPL/Cur) can also be delivered to TNFα-stimulated Caco-2 epithelial cells, further inhibits the pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 and increases the expression of anti-

inflammatory cytokine IL-10. Oral administration of NPL/Cur can target the colon and deliver Cur deeper into the epithelium to alleviate the symptoms of DSS-induced colitis mice. This study used an *ex vivo* murine colonic explant from an inflammation model and found that NPL/Cur is not only on the surface of epithelial cells, but also can penetrate into the intestinal wall, with stronger targeting (Salah et al., 2022). Curcumin encapsulated by solid binary lipid nanoparticles (C-SBLNs) also has good gastrointestinal stability, and prolonged drug release up to 24 h. Oral administration of C-SBLNs can reduce leukocyte infiltration, oxidative stress and proinflammatory cytokines (TNF-α), restore colon structure in DSS-induced colitis mice (Sharma et al., 2019). Acetylated konjac glucomannan (AceKGM) NPs loaded with Cur were prepared by emulsion solvent evaporation technology, which has the targeting property of colon macrophages. It is selected pH1.2 to simulate SGF and pH7.4 to simulate SIF respectively. It was found that Cur-AceKGM NPs did not release significantly in SGF but released more than 60% in SIF within 24 h, suggesting that Cur-AceKGM NPs may be a suitable delivery carrier for colon targeting. At 48 h, the release of Cur-AceKGM NPs in SIF can reach 81%. Cur-AceKGM NPs can decrease the colon local level of MPO and DAI score, significantly alleviate the symptoms of colitis (Wang et al., 2022). Drug delivery systems based on pH and/or enzyme also have good application prospects in IBD. The pH-sensitive hydrolyzed polyacrylamide-grafted-xanthan gum (PAAm-g-XG) nanoparticles were used to load Cur for colon delivery, and it was found that Cur could be released optimally in the pH 6.8 solution (Compared at pH 1.2, 4.5, 6.8 and 7.2 respectively). Oral Cur/PAAm-g-XG/NPs can reduce the levels of myeloperoxidase (MPO) and nitrite, prevent weight loss, and alleviate the symptoms of acetic acid-induced IBD in rats (Mutalik et al., 2016). Using pH-sensitive polymeric NPs combining both PLGA and a polymethacrylate polymer to delivery Cur can significantly enhanced the penetration of Cur in Caco-2 cell monolayer. In the medium with pH 1.2 and 4.5, CC was not released, but once the pH value reached the neutral value, CC was released rapidly. Oral administration of Cur-NPs can significantly reduce neutrophil infiltration and TNF-α, restore colon structure in DSS induced colitis mice (Beloqui et al., 2014).

2.3 NPs for the delivery of quercetin

Quercetin (QT) is a strong antioxidant related to kaempferol, which is expressed in more than 100 kinds of medicinal plants, such as *Panax notoginseng* (Burkill) F.H.Chen [Araliaceae; Notoginseng radix et rhizoma] (High Taxonomic Confidence), Platycladus orientalis (L.) Franco [Cupressaceae, Platycladi cucumen] (High Taxonomic Confidence), *Ginkgo biloba* L. [Ginkgoaceae, Ginkgo folium] (High Taxonomic Confidence), etc. It can prevent oxidative damage and cell death by scavenging oxygen free radicals, inhibiting xanthine oxidase, lipid peroxidation and chelating metal ions (Boesch-Saadatmandi et al., 2011). Because QT can be absorbed in the stomach and small intestine, metabolized rapidly or degraded strongly, that may lead to less QT entering the colon and limiting its potential use for IBD treatment. The application of nano carriers may well solve this problem. Oral QT-loaded silk fibroin nanoparticles (QSFN) can produce obvious intestinal anti-inflammatory properties by down regulating proinflammatory cytokines (TNF-α, IL-1β, IL-6, MCP-1, ICAM-1, NLRP3, iNOS) and significantly reducing the DAI score of DSS-induced colitis mice. < 40% of loaded QT is released in the GST, while the rest can reach the colon, actively enhancing its anti-inflammatory effect in the damaged area (Diez-Echave et al., 2021). The incidence rate and progression of IBD are closely related to oxidative stress caused by excessive production of ROS. CS loaded with QT (QT-NPs) can downregulate inflammatory cytokines (IL-6, TNF-α, IFN-γ, IL-10) in colitis mice, upregulate tight junction protein (MUC-2, JAM-2, occludin), regulate oxidative stress state (ROS, NO, MDA, H2O2, GSH-Px, SOD, CAT, Nrf2, HO-1), restore the healthy structure of colon tissue in DSS-induced colitis mice. This study also compared the effects of three concentrations of QT-NPs (10 mg/kg, 15 mg/kg, 20 mg/kg), and found that higher concentrations had better effects, showing a concentration dependence (Khater et al., 2022). Some studies have further improved the inflammation targeting function on the basis of CS materials, such as pH/ROS dual-responsive prodrug micelle (GC-B-QT). Anti-inflammatory single drug QT was conjugated to glycol chitosan by aryl boronic ester which can give potential pH/ROS dual responsiveness. In the presence of H_2O_2, the release rate of GC-B-Que under physiological conditions is low (<20 wt%), but it contains 10% at pH 5.8 μM H2O2 was almost completely released in the medium (>95 wt% after 72 h). GC-B-QT micelles tend to accumulate in intestinal inflammatory sites and inhibit inflammatory cytokines (TNF-α, IL-6, iNOS) and showed better therapeutic effect than free drugs (QT and mesalazine) (Shen et al., 2021).

2.4 NPs for the delivery of ramulus mori polysaccharide

Because flora can affect intestinal immune homeostasis, the role of intestinal flora in IBD has been paid more and more attention. Ramulus mori polysaccharide (RMP) is one of the main components of *Morus alba* L. [Moraceae, Mori ramulus] (Medium Taxonomic Confidence). Its surface is porous and spongy and composed of seven monosaccharides: mannose, rhamnose, glucuronic acid, glucose, xylose, galactose and arabinose. It has good anti-inflammatory, antioxidant and hypoglycemic effects. It can also regulate the structure of intestinal flora and prevent intestinal inflammation damage. However, as a non-starch polysaccharide, RMP has low oral bioavailability and short biological half-life, which constructing a nano delivery system may help solve this problem (Yu et al., 2019). RMP was encapsulated into PLGA to form PLGA-RMP. It is found that oral PLGA-RMP can inhibit the expression of IFN-γ and IL-6, upregulate the expression of IL-10, ZO-1 and occludin, adjust the metabolic disorder (up regulating the content of acetate, propionate and butyrate, reducing the diversity and richness of intestinal microbiota), repair intestinal barrier and finally prevent weight loss, reduce the DAI score, and promote the recovery of colon length in DSS-induced colitis mice. This study only observed the effectiveness of PLGA-RMP on IBD and explored its mechanism, but there was no *in vitro* experiment to evaluate the concentration, drug loading rate and release rate of PLGA-RMP (Feng et al., 2021). In mice with IBD induced by LPS, oral administration of PLGA-RMP can also regulate macrophage polarization, inhibit specific

inflammatory cytokines (including TNF-α, IL-6, IL-1β, PGE2), inhibit the activation of $CD3^+CD8^+$ T cells, increase the number of activated Treg in the intestine, and reduce the DAI score and intestinal inflammatory damage. In order to test the potential toxicity of PLGA-RMP, this study treated RAW264.7 macrophages with different concentrations of PLGA-RMP. It is found that PLGA-RMP was non-toxic to macrophages at 125 μg/mL and RMP encapsulated by PLGA show a higher uptake efficiency by macrophages than free RMP (Feng et al., 2021).

2.5 NPs for the delivery of shikonin

Shikonin (SK) was originally extracted from *Arnebia euchroma* (Royle ex Benth.) I.M.Johnst. [Boraginaceae, Arnebiae radix] (Medium Taxonomic Confidence), which has the functions of inhibiting inflammation, regulating immunity and healing wounds. Studies have shown that SK can inhibit NF-κB and STAT-3 pathway to attenuate acute UC in Balb/C mice induced by DSS (Andujar et al., 2012). It can also promote intestinal wound healing *in vitro via* induction of TGF-β release in intestinal epithelial cells (Andujar et al., 2013). SK loaded ES100/HA/CS nanoparticles (SK@SAC) were constructed using CS, hyaluronic acid (HA) and pH responsive polymer Eudragit S100 (ES100), and drug loading efficiency was 6.6%. In TNBS induced IBD mice, oral administration of SK@SAC can reduce the non-specific distribution of other organs by increasing the local drug concentration in the colon. SK@SAC has significant therapeutic effect, that can downregulate proinflammatory mediators (TNF-α, IL-6, IL-1β, COX-2 and iNOS), upregulate anti-inflammatory cytokines (IL-10, TGF-β) and tight junction proteins such as ZO-1 and occludin (Feng et al., 2022).

2.6 NPs for the delivery of berberine

Berberine (BBR) is a quaternary ammonium alkaloid isolated from the medicinal plant *Coptis chinensis* Franch. [Ranunculaceae, Coptidis rhizoma] (Medium Taxonomic Confidence), which is the main effective component against bacteria. It is also expressed in *Phellodendron chinense* C.K.Schneid. [Rutaceae, Phellodendri chinensis cortex] (Medium Taxonomic Confidence), etc. Modern research found that it also has analgesic, anti-inflammatory, anti-cancer, hypoglycemic, anti-hyperlipidemia and other pharmacological effects, which is widely used in IBD. However, the oral absorption of BBR is poor. After injection, BBR quickly enters various organs and tissues. The blood concentration is maintained soon, and the blood concentration is lower than the minimum inhibitory concentration (Habtemariam, 2016). The nanoparticle was constructed stablely from a phenylboronic esters-modified carboxylmethyl chitosan (OC-B) conjugated with BBR (OC-B-BBR) which could respond to the selective degradation of ROS. In the slightly excessive ROS environment, the stimuli-responsive borate ester of OC-B-BBR can be broken to release BBR molecules, and more than 90% of BBR can be released in 24 h incubation. OC-B-BBR can suppress the secretion of some inflammatory cytokines such as TNF-α, IL-6, TGF-β, IL-23 and remodel intestinal microbiota in DSS-induced mice, significantly improved the symptoms and colon injury (Zhao et al., 2021).

2.7 NPs for the delivery of shogaol

Shogaol is the major pharmacologically active compounds of ginger which is broadly used for a wide range illness worldwide. Ginger is the rhizome of *Panax ginseng* C.A.Mey. [Araliaceae, Ginseng radix et rhizoma] (High Taxonomic Confidence), a well-known plant with anti-inflammatory and antioxidant properties. Ginger can inhibit various pro-inflammatory cytokines and the inflammation related pathways, such as TLRs, NF-κB, STATs, NLRPs, MAPKs, and mTOR, which may play an effective anti-inflammatory role in IBD (Lashgari et al., 2022). The active compound 6-shogaol was loaded on PLGA/PLA-PEG-FA nanoparticles (NPs-PEG-FA/6-shogaol). It was found that NPs-PEG-FA/6-shogaol could be absorbed by colon-26 cells and activated Raw 264.7 macrophages. PEG modification can improve the biocompatibility of NPs. NPs, NPs-PEG and NPs-PEG-FA will not change colon-26 cells (24 h–48 h) at any test concentration (up to 1 mg/mL), but NPs can reduce the cell viability of Raw 264.7 macrophage cells at 48 h, while there is no decrease in NPs-PEG or NPs-PEG-FA treatment groups. In addition, NPs-PEG-FA seems to have no obvious toxicity *in vivo*, and has no obvious effect on gastrointestinal tract and main organs such as heart, liver, spleen, lung and kidney. It was found that NPs-PEG-FA/6-shogaol can be taken up by colon-26 cells and activated Raw 264.7 macrophages. Oral NPs-PEG-FA/6-shogaol can downregulate the proinflammatory factors (TNF-α, IL-6, IL-1β and iNOS), upregulate the expression level of anti-inflammatory factors (Nrf-2 and HO-1), alleviate the colitis symptoms and accelerate the wound repair of DSS-induced colitis mice (Zhang et al., 2018).

2.8 NPs for the delivery of embelin

Embelin is a kind of benzoquinone compound extracted from *Embelia ribes* Burm.f.[Primulaceae, Emblica ribes] (Medium Taxonomic Confidence). It is distributed in the east of India to Indonesia and China, which has been used to treat fever, inflammatory diseases and various gastrointestinal diseases for a long time. Modern research has found that embelin also has anti-tumor, anti-inflammatory and analgesic properties. Embelin can significantly reduce the DAI score, inflammatory factors, MPO accumulation and further alleviate weight loss, diarrhea, massive hemorrhage and immune cell infiltration in DSS-induced colitis mice (Kumar et al., 2011). The targeting and effectiveness of embelin can be significantly improved by delivering it *via* nanoparticle loading. Embelin-loaded enteric-coated microspheres can significantly reduce the ulcer activity score, oxidative stress level (downregulate the expression of MPO, MDA, LPO), upregulate the level of glutathione, and reduce the inflammatory reaction in acetic acid-induced colitis rats. Compared with other conventional dosage forms, this method can produce time-dependent and pH-dependent continuous embelin release. Moreover, due to the existence of pH-sensitive and time-controllable polymers, it can effectively prevent

embelin release into the gastric environment (pH 1.2) (Nidhi et al., 2017).

2.9 NPs for the delivery of piceatannol

Piceatannol (PCT) is a resveratrol analogue, mainly derived from the medicinal plants such as Rheum palmatum L. [Polygonaceae, Rhei radix et rhizoma] (Medium Taxonomic Confidence) and *Cinnamomum cassia* Presl [Lauraceae, Cinnamomi ramulus] (Medium Taxonomic Confidence). It is found that PCT has a good therapeutic effect on intestinal inflammation by regulating the activity of transcription factors such as NF-κB, Nrf2, HIF-1α (Ashikawa et al., 2002). PCT also has anti-coliform effect. However, due to the extensive phase II liver metabolism (glucuronization and sulfation) *in vivo*, the bioavailability of PCT is very low. Therefore, improving the bioavailability of PCT is a key concern. Some researchers have used colon-targeted capsule (colon-targeted PCT) to significantly improve the efficacy of PCT (Yum et al., 2015). Compared with capsules, nanoparticles show more prominent advantages, which can provide better healing and convenience, decrease toxicity profiles and reduce cost (Jin et al., 2018). PCT and caffeic acid phenethyl ester (CAPE, from honey bee propolis) with anti-inflammatory effect were loaded onto albumin nanoparticles, and it was found that PCT/CAPE-loaded albumin NPs could enhance the anti-inflammatory potential. PCT content has certain influence on particle size, polydispersity index and zeta potential, but the increase of CAPE content has no significant effect on particle size and polydispersity index. It can downregulate INF-γ, IL-6, TNF-α, MPO level, further alleviate weight loss, improve DAI and colon morphology in DSS-induced colitis (Tambuwala et al., 2019).

2.10 NPs for the delivery of silymarin

Silymarin (SM), a natural flavonoid lignan compound, is an active substance extracted from the dried fruit of the medicinal plant *Silybum marianum* (L.) Gaertn. [Asteraceae, Silybi fructus] (Medium Taxonomic Confidence). Its main components are silybin, isosilybin, silidianin and silychristin. Modern research has found that SM has the functions of antioxidation, liver protection, anti-tumor, and anti-cardiovascular/cerebrovascular diseases. However, due to poor oral bioavailability, the applicability of SM has not been recognized (Al-Drees and Khalil, 2016). SM loaded silica-containing redox nanoparticles (siRNP) can not only improve the bioavailability and colon targeting in colon mucosa of SM, but also enhance the anti-inflammatory and antioxidant capacity. SM@ siRNP can scavenge 2,2-diphenyl-1-picrylhydrazide radical, inhibit NO and proinflammatory cytokine (IL-1β, IL-6, TNF-α), significantly improved the damage of colonic mucosa in DSS-induced colitis mice (Nguyen et al., 2021). The optimised nanoparticles had a loading efficiency of 98.3% ± 12% and release efficiency of 40.8% ± 5.5% at 24 h. Eudragit RL PO nanoparticles loaded with SM (75 mg/kg/day) can significantly reduce TNF-α, IL-6 and MPO activity in colon tissues, improve macroscopic and histopathological scores of acetic acid-induced colitis mice (Varshosaz et al., 2015). Selenium (Se) nanoparticles have more bioavailability with less toxicity. Combined administration of SM and nano Se can improve anti-inflammatory and antioxidant capacity by reducing the expression of TNF-α, IL-1β, NF-κB, MPO, lipid peroxidation and protein carbonyl, finally relieve symptoms of TNBS-induced colitis. Combined therapy may be a good way to treat IBD. However, more basic and clinical studies are needed to confirm the safety and effectiveness of this new combination (Miroliaee et al., 2011).

3 Application of medicinal plant derived EVs in IBD

Although liposomes and other nanoparticles have been used to deliver a variety of therapeutic drugs, but due to their synthetic characteristics, they still have the possibility of time-consuming, low solubility, particle aggregation and toxicity. The optimal design of nanoparticles delivery systems may require a level of complexity similar to the biological environment in order to successfully pass barriers, including clearance, degradation and physical barriers. The type, orientation, density and surface modification of ligands are all factors affecting the successful delivery of nanoparticles. EVs with unique homology and low immunogenicity have better biocompatibility and bioavailability than the traditional synthetic nanoparticles prepared in the chemical environment. At present, most of the EVs used as therapeutic carriers are derived from mammalian cells, but there are relatively few studies on the use of these EVs to load medicinal plants active ingredients for IBD. Modern research has found that not only mammals, but also plants can secrete EVs, which can be obtained with low cost and efficiency without carrying human pathogens. PELNs may have better application prospects in future research, especially the EVs derived from medicinal plants. For example, exosome-like nanovesicles with high yield (17.5 mg/kg) can be isolated from ginger (Witwer and Wolfram, 2021; Li et al., 2022). The actions are summarized below and shown in Table 2.

3.1 Ginger derived EVs

The EVs from ginger, which called ginger exosome-like nanoparticles (GELNs), have the property of targeting intestinal tract. In DSS-induced colitis, oral GELNs can target intestinal macrophages and intestinal stem cells (ISC), increase the survival and proliferation of intestinal epithelial cells, reduce the expression of proinflammatory cytokines (TNF-α, IL-6, IL-1β), and increase the expression of anti-inflammatory cytokines (IL-10, IL-22) and E-cadherin, thus restoring intestinal barrier dysfunction and homeostasis. Furthermore, *in vitro* and *in vivo* wound-healing models, it is demonstrated that GELNs can promote intestinal mucosa healing, upregulate the expression of carbonic anhydrase 1 (CAR1) on the surface of colon intestinal epithelial cells, reestablished normal levels of pro-/anti-inflammatory cytokines and MPO activity, and restored IEC proliferation-apoptosis balance in the intestinal mucosa (Zhang et al., 2016; Zhang et al., 2016). Similarly, for LPS-induced intestinal inflammation, GELNs can be specifically absorbed by intestinal cells through the role of caveolin, further downregulate NF-κB, IL-6, IL-8 and TNF-α in

TABLE 2 Extraction, physicochemical characterization and application of EVs derived from medicinal plants.

Refs	Medicinal plants	Isolation	Size (nm)	Morphology	Diseases	Model used	Test sites	Biochemical measurements
Zhang et al. (2016)	Ginger	DGUC	231.6–292.5 nm	Cup-shaped	DSS-induced colitis	*in vivo*/ vitro	Colon tissues	TNF-α↓, IL-6↓, IL-1β↓, IL-10↑, IL-22↑, E-cadherin↑, CAR1↑
Yin et al. (2022)	Ginger	DGUC	156 ± 36 nm	Spherical	LPS-induced colitis	*in vivo*/ vitro	Caco-2 Cells	TNF-α↓, IL-6↓, IL-8↓, NF-κB↓
Mao et al. (2021)	Ginger	DGUC	284.6 nm	Spherical	DSS-induced colitis	*in vivo*/ vitro	Colon tissues	TNF-α↓, IL-6↓, IL-1b↓, IL-10↑, IL-22↑, NLRP3↓, caspase-1↓, IL-1β↓
Zhang et al. (2017)	Ginger	DGUC	232.7 nm	Cup-shaped	DSS-induced colitis	*in vivo*/ vitro	Colon tissues	CD98↓
Liu et al. (2022)	Turmeric	DGUC	178 nm	Cup-shaped	DSS-induced colitis	*in vivo*/ vitro	Colon tissue	TNF-α↓, IL-6↓, IL-1β↓, NF-κB↓, HO-1↑
Gao et al. (2022)	Turmeric	DGUC	191.7 ± 15.8 nm, 243.9 ± 13.9 nm, 800.5 ± 66.2 nm	Spherical	DSS-induced colitis	*in vivo*/ vitro	Colon tissue	TNF-α↓, IL-6↓, MCP-1↓
Zu et al. (2021)	Tea leaves	DGUC	140.0 nm	Spherical	DSS-induced colitis	*in vivo*/ vitro	Colon tissue	ROS↓, TNF-α↓, IL-6↓, IL-12↓, IL-10↑

Notes: ↑, upregulated by drugs; ↓, downregulated by drugs. Abbreviations: DGUC, Differential density gradient centrifugation; CAR1, carbonic anhydrase 1; HO-1, hemeoxygenase-1; ROS, reactive oxygen species; MCP-1, onocyte Chemoattractant Protein 1

colon tissues, alleviate inflammation (Yin et al., 2022). Some study has compared the EVs derived from four plants such as grape, grapefruit, ginger, carrot, and found that only the macrophages treated with GELNs can significantly increase the expression of HO-1 and IL-10 at the same time, promote Nrf2 nuclear translocation, and also appropriately stimulate the expression of pro-inflammatory factor IL-6 to maintain homeostasis (Mu et al., 2014). Taking advantage of the good compatibility of PELNVs, some drugs were loaded into GELNs to target or synergize the treatment of IBD. Loading TNF-α with GELNs can downregulate NLRP3, caspase-1, IL-1β, TNF-α, IL-6 and IL-1b, upregulate anti-inflammatory substances such as IL-10 and IL-22 in colon tissue of DSS-induced colitis mice. This method avoids adverse reactions caused by immunosuppression in routine administration. In this study, mesoporous silicon, a type of inorganic framework with good biocompatibility, used as a suitable nanocore to embed into GELNs for solving the shortcomings of low drug loading and poor stability (Mao et al., 2021). siRNA antibody CD98 (siRNA-CD98) was loaded into GELNs to construct siRNA-CD98/GELNs. In mice with UC, oral administration of siRNA-CD98/GELNs can effectively target colon tissue, leading to the decrease of effective expression of CD98 in colon, so as to play a role in treating UC (Zhang et al., 2017; Sung et al., 2019; Sung et al., 2020).

3.2 Turmeric derived EVs

Using EVs as delivery carriers can make curcumin more stable, with higher concentration in the blood, higher availability of biological tissues, and stronger anti-inflammatory effect (Sun et al., 2010; Aqil et al., 2017). In acute inflammation induced by LPS, turmeric-derived nanoparticles (TDNPs) showed excellent anti-inflammatory and antioxidant properties. It is proved that oral TDNPs can reduce proinflammatory cytokines (TNF-α, IL-6 and IL-1β), upregulate antioxidant gene HO-1 to relieve colitis in mice and accelerate colitis regression. Further this study was conducted through NF-κB-RE-Luc transgenic mice to demonstrate that inactivation of the NF-κB pathway may the mechanism of TDNPs-treated colitis (Liu et al., 2022). Oral TDNPs can also show excellent anti-inflammatory effects in DSS-induced UC mice by restoring the damaged intestinal barrier, regulating the intestinal microbiota and polarization of and macrophages (promoting the transformation of M1 to M2 phenotype) (Gao et al., 2022).

3.3 Tea leaf derived EVs

Tea originated in China and was first used as a sacrifice. Later, it was used in daily diet, beverages and medical treatment in the world. Tea contains polyphenols, flavones, catechin, caffeine, inositol, folic acid, pantothenic acid and other healthy ingredients. Studies have proved that tea polyphenols can effectively eliminate free radicals in the body, with anti-aging, anti-radiation, anti-cancer, antibacterial and bactericidal effects (Khan and Mukhtar, 2018). Green tea polyphenols (GTPs) can block the transcription of NF-κB activation and upstream of mediated I kappa B kinase complex pathway activities, inhibit the invasion of cytokines and the synthesis of cyclooxygenase-2 (COX-2), further downregulate endotoxin-mediated TNF-α production to treat IBD and its complications (Rahman et al., 2018). Oral administration of the EVs derived from tea leaves can reduce the production of ROS, inhibit the expression of proinflammatory cytokines (TNF-α, IL-6, IL-12), increase the anti-inflammatory activity of macrophages (IL-10), inhibit the inflammatory intestinal response, restore the damaged colon barrier, enhance the diversity and overall

abundance of intestinal microbiota, and thus prevent or alleviate IBD and colitis related colon cancer. At the same time, it was found that the galactose groups on EVs surface could be specifically internalized by macrophages through galactose receptor mediated endocytosis (Zu et al., 2021).

4 Discussion

IBD is a chronic inflammatory disease involving the small intestine and colon. Common symptoms include abdominal pain, diarrhea, fever, vomiting, bloody stool and weight loss, and there is a risk of colorectal cancer (CRC). Because the lesion is mainly located in the colon, colon targeted drug delivery system (DDS) has received extensive attention in the treatment of IBD. Oral administration has become the most preferred method for the treatment of IBD due to its high safety, high compliance and low cost-effectiveness. Conventional preparations, such as capsules, tablets and solutions, are used clinically to deliver anti-inflammatory drugs or immunosuppressants for the treatment of IBD (Yang and Merlin, 2019). In addition, biological agents such as TNF-α antibodies are also used to treat IBD through intravenous (IV) or subcutaneous (SC) injection. However, these methods cannot produce colon targeted drug delivery, and often cause serious systemic side effects (Li et al., 2022). Phytochemicals from medicinal plants can act on various pathogenic and inflammatory targets, but these natural molecules may damage their therapeutic activity before reaching the inflammatory colon (Khare et al., 2020). Recently, drugs-based on NPs have received extensive attention due to their potential to solve such problems. Polymer nanoparticles, such as synthetic polymer-, natural polymer-, polymeric prodrug, hybrid polymer- (including pH-responsive, ROS-responsive, dual-responsive, polymer-lipid) based NPs have been extensively studied in the treatment of IBD. Some targeted ligands, such as monosaccharides, polysaccharides, peptides, folic acid, antibodies and their fragments, can also be used to modify NPs to improve their targeting (Zu et al., 2021). The active ingredients of these medicinal plants can be better absorbed by the whole body in the form of nanoparticles or EVs. Some studies have found that the absorption rate of Cur loaded nanoparticles can be increased by about 3 times compared with pure Cur (Mutalik et al., 2016). Because patients with IBD lack physiological lipids and some lipids have immunomodulatory properties, it is hypothesized that the combination of lipids and hytochemicals in nanocarriers may be a valuable strategy to overcome IBD. Three kinds of lipid-based nanocarriers containing Cur (including self-nanoemulsifying drug delivery systems-SNEDDS, nanostructured lipid carriers-NLC and lipid core-shell protamine nanocapsules-NC) were studied and compared as anti-inflammatory drugs in DSS-induced colitis mice. The efficiency of permeability across Caco-2 cell monolayers was NC > SNEDDS > NLC and Cur suspension. At the same time, it is proved that lipid nanocarriers show increased Cur retention in the intestinal tract, rather than increased Cur permeability, which may provide some new ideas for drug delivery in the future (Beloqui et al., 2016). The synergistic use of some drugs combined with targeting and therapeutic effects has increased the efficacy and has also been applied in IBD. For example, the combination of targeted drug delivery with anti-inflammatory drugs and ROS scavengers has good advantages. Micelles formed by CUR conjugated hydroxyethyl starch as vehicles are used as carriers to further load dexamethasone (DEX) to form HES-CUR nanoparticles (DHC-NPs). DHC-NPs can target the inflammatory colon. When the α-amylase is overexpressed in the inflammatory colon can lead to HES degradation, and the drug is released by amylase reaction from DHC-NPs. Targeting and combination therapy significantly reduce the damage caused by DSS induced UC (Xu et al., 2022).

In addition to synthetic NPs, the presence of EVs also provides a direct drug delivery route to colon mucosa for the treatment of IBD. Compared with the synthetic NPs, which will lead to cell stress, apoptosis, inflammatory body activation and other side effects, EVs are non-toxic, low immunogenicity and can be produced in large scale, may have better application prospects (Hu and Palic, 2020; Radmanesh et al., 2021). In a variety of EVs, plants derived EVs contain bioactive lipids, proteins, ribonucleic acids and other pharmacologically active molecules, which can be used as natural nano carriers. Medicinal plants derived EVs not only have therapeutic effects themselves, but also can package different therapeutic drugs to produce synergistic effects (Di Gioia et al., 2020). However, due to the environmental reasons of the GIT, such as gastric acid, digestive enzymes and intestinal microbiota, the passage of EVs is threatened. Researchers have extracted natural EVs from three kinds of edible tea and found that they have high potential for large-scale production and can accumulate in colitis tissues for up to 48 h, improving the bioavailability of their contents. The particle sizes, polydispersity indices (PDIs), and zeta potentials of all the EVs presented only slight variations in simulated stomach acid and intestinal fluid. How to enhance the stability while ensuring the targeting of EVs is one of the problems to be solved at present (Zu et al., 2021). Since proteins, nucleic acid and lipids are important components of EVs, further quantitative and comparative analysis of these components may improve the understanding of medicinal plants derived EVs biogenesis and intercellular communication mediated by effective components in future research (Kim et al., 2020).

These natural nanoplatforms have great potential for medical transformation. At present, there are also clinical trials for the treatment of intestinal diseases being funded. Some researchers found that the bioavailability of Cur taken orally by IBD patients is limited even at a very high dose of 8–12 g per day. It is further proposed to solve the problem of Cur delivery by using plant exosomes to deliver drugs to colon tumors and normal colon tissues. There are also clinical trials to evaluate the effect of plant derived exosomes or effective active phytochemicals on patients with IBD (https://clinicaltrials.gov/). In a prospective randomized study, the main focus was to compare the improvement of symptoms of IBD patients with ginger exosomes alone, curcumin, and ginger exosomes plus curcumin (NO. NCT04879810). This trial included 90 patients with chronic IBD, using three main stratified factors: race (white and black), sex and IBD type (CD and UC), and using the incidence of bloody stool to justify the sample size. Although limited medicinal plant-based nano preparations have been tested in humans, but the number is few and the evidence is low, there is still an urgent need for new clinical trials to analyze non-toxic natural NPs targeting the colon in order to deliver natural products.

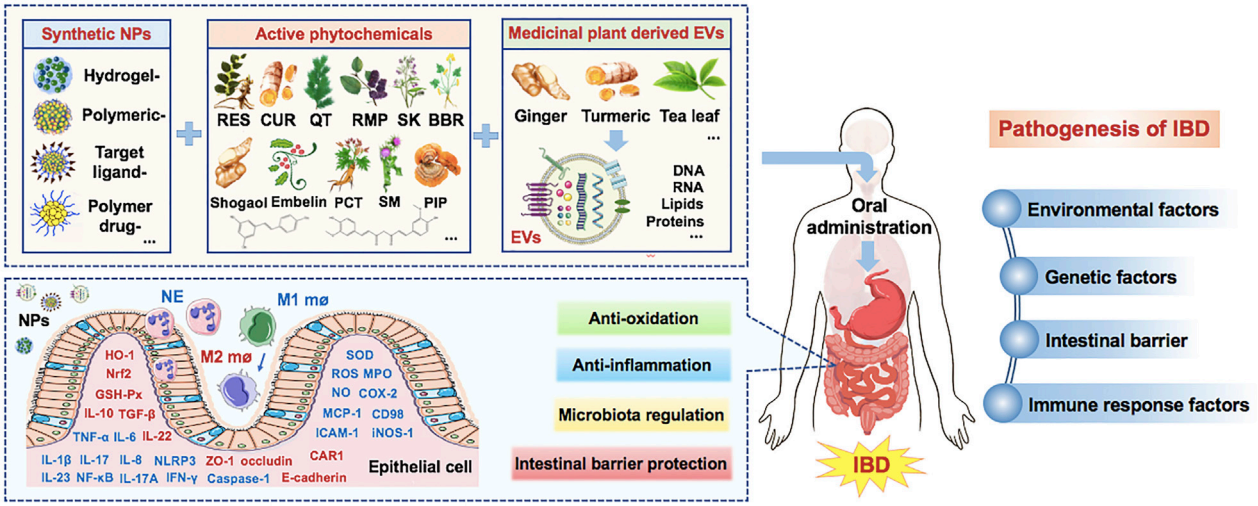

FIGURE 1
Medicinal plant-based drug delivery system for IBD. (Note: Factors in red are upregulated by NPs, while factors in blue are downregulated by NPs. IBD, Inflammatory bowel disease; EVs, extracellular vesicles; RES, resveratrol; Cur, curcumin; QT, quercetin; RMP, ramulus mori polysaccharide; SK, shikonin; BBR, berberine; ROS, reactive oxygen species; NPs, nanoparticles; CAR1, carbonic anhydrase 1; MPO, myeloperoxidase; PCT, piceatannol; SM, silymarin; Se, selenium; SOD, superoxide dismutase; MCP-1, monocyte Chemoattractant Protein 1; ICAM-1, intercellular cell adhesion molecule-1; HO-1, heme oxygenase; Nrf2, Nuclear factor erythroid 2-related factor 2).

During the treatment of IBD, there are still some problems to be considered. First, compared with other drug delivery routes, oral drug delivery route has obvious advantages, such as systemic side effects, simple self-medication, good patient compliance, and has better research and application prospects. In the design of nano-delivery materials, there are still some aspects to be considered. Oral nano-pharmaceuticals should overcome a variety of physical, chemical and biological barriers in GIT, including high acidic environment in the stomach, extreme pH changes and proteolytic enzymes along GIT. For NPs with weak acid or base groups, it should be considered that the change of pH value will affect the changes of ionizable groups and morphology of nanoparticles. For NPs targeting the lamina propria, how to overcome the intestinal epithelium and its mucus secretion layer is a key obstacle to be considered. Due to the short residence time of NPs in the small intestine (3–4 h), this poses additional challenges for the design of this part of NPs. In addition, due to the needs of the lesion site, such as the leakage of epithelial inflammation and the loss of mucous layer, the oral nanodrugs used for the treatment of IBD may first reach the destroyed intestinal barrier and lamina propria passively. It is found that the intestinal flora disorder also plays an important role in the occurrence and development of IBD, while most symbiotic microorganisms inhabit in the mucosal layer of the intestinal cavity. Therefore, when designing some oral NPs which aiming at regulating the intestinal microbiome, targeting mucus layer should be mainly considered (Blanco et al., 2015; Zu et al., 2021). Moreover, the appropriate time for the release of drugs contained in NPs is also critical. NPs should release their drug at the target site of the intestine. Once the drug is released prematurely, it may cause side effects mediated by systemic absorption. In order to prevent premature release, it is necessary to stabilize the drug loading in the nano preparation, but in this way, the drug release will decrease with the increase of the stability of the preparation, which is also to be considered in the future. Next, although most literatures in this review have evaluated the degree of substitution, particle size, surface charge, encapsulation efficiency, loading capacity, release rate and stability of nanomaterials, and screened the best parameters. However, the targeting rate, safety and side effects of oral drugs need further exploration. Some studies only evaluated the possibility of using nanomaterials in the gastrointestinal tract and speculated that the possibility of using nanomaterials in IBD without verification *in vivo* experiments, which is also one of the shortcomings of current research. In addition, recent studies have shown that as a new way of intercellular communication, the components of EVs (including nucleic acid, protein and some other components) participate in the occurrence, development and treatment of IBD. Using EVs-like structure to design new drug formulations may provide new insights for the treatment of IBD. Because of its good biological distribution and inherent biocompatibility, it is of great interest to use EVs containing biological/chemical components as drug delivery carriers. However, at present, there are relatively few studies on EVs carrying medicinal plants and EVs derived from medicinal plants, and more animal and clinical studies are needed to study the effect of EVs on IBD. In addition, in some animal and clinical studies, it is found that combination therapy may be a good way to treat IBD. However, clinical research is needed to confirm the safety and effectiveness of this new combination.

5 Conclusion

This review demonstrates that, as an alternate, Synthetic nanoparticles and medicinal plants derived EVs can play an important role in the treatment of IBD by carrying the effective active phytochemicals. They can treat IBD by inhibiting

inflammation and oxidative stress, regulating the structure of intestinal flora, and repairing the intestinal barrier. However, before they become a mature delivery system for clinical use, there are still many challenges, including how to increase drug concentration without increasing toxic and side effects, improve targeting, improve oral availability, etc. Some clinical trials to evaluate the safety, tolerance, toxicity and effectiveness of EVs-loaded drugs in IBD are also progressing steadily. Nanotechnology can play an important role in improving the therapeutic potential of phytochemicals and medicinal plant derived EVs, so as to develop new therapeutic options for IBD (Figure 1).

Author contributions

NL, MW, and ZL contributed equally and thus share first authorship. All authors contributed to data collection, analysis, drafting and revising the article, gave final approval of the version to be published, agreed to the submitted journal.

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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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Clinical characteristics and survival outcomes in patients aged 75 years or older with advanced colorectal cancer treated using traditional Chinese medicine: an observational retrospective study

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Limited evidence suggests that elderly patients with advanced colorectal cancer (ACRC) may benefit from traditional Chinese medicine (TCM). This study investigated the efficacy and safety of TCM in old ACRC patients treated in the Oncology Department of Xiyuan Hospital between January 2012 and December 2021. The clinical characteristics of these patients were retrospectively reviewed. Their progression-free survival (PFS) and total duration of TCM therapy (TTCM) were analyzed using the Kaplan-Meier curve. Forty-eight patients (F:M 13:35) with a mean age of 78.75 ± 2.99 years (range, 75–87) met the inclusion criteria. There were 18 cases of rectal cancer and 30 of colon cancer. The median PFS was 4 months (range, 1–26; 95% CI 3.26–4.73). The median TTCM was 5.5 months (range, 1–50; 95% CI 1.76–8.24). Subgroup analysis revealed that PFS and TTCM were shorter in patients with bone metastases and an ECOG performance status score of 2–3 ($p < 0.05$). No hematological toxicity or serious adverse reactions occurred during the study period. This real-world study demonstrates that TCM may be a potentially beneficial therapy for old ACRC patients, including when the ECOG performance status score is 2–3.

KEYWORDS

advanced colorectal cancer, elderly, survival outcomes, clinical characteristics, traditional Chinese medicine

1 Introduction

Colorectal cancer (CRC) is one of the most common malignancies and has high morbidity and mortality rates (Song et al., 2020). In China, CRC ranks third among all cancers in terms of overall incidence, third for mortality in women, and fifth for mortality in men (Feng et al., 2019). CRC is the second leading cause of cancer-related death in patients aged 60–79 years and the third leading cause of death in patients aged 80 years and older (Hamed et al., 2022). However, regarding conventional medicine, there are still no guidelines for patients with advanced CRC (ACRC) who are aged ≥ 75 years because patients in this age group are usually excluded from clinical studies (Audisio and Papamichael, 2012). Nevertheless, some elderly patients with an Eastern Co-operative Oncology Group performance status (ECOG-PS) score of 0–1 may be suitable candidates for clinical studies in the Western medicine. Yet, the toxicity of the anti-cancer agents developed in

the West is still greater in elderly patients than in their young counterparts, resulting in reduced quality of life and a shorter life expectancy (Shibutani et al., 2021).

Traditional Chinese medicine (TCM) has been used to treat various diseases for thousands of years in China. The history of treatment of CRC in China can be traced back thousands of years to a description of CRC and its treatment in the classic Chinese book "*Huangdi Neijing*" written 2,000 years ago. TCM is now covered by the medical insurance system in China and is very cost-effective. Therefore, it is readily accessible by the general population. Recent clinical studies have also confirmed that TCM has anti-tumor activity and prolongs survival without serious side effects (Sun et al., 2021). TCM can also improve quality of life, relieve symptoms, and reduce adverse events in patients with ACRC undergoing conventional chemotherapy (Zhang et al., 2018; Yan et al., 2021; Chen et al., 2022); Moreover, TCM can significantly prolong overall survival and progression-free survival (PFS) (Liu et al., 2020; Zhu et al., 2021). There are clinical cases reports of elderly patients with ACRC deriving benefit from TCM, with some taking TCM for up to 50 months. In view of these reports and the lack of data on TCM in elderly patients with CRC, the aim of our study was to explore the efficacy and safety of TCM in elderly patients with ACRC.

2 Materials and methods

2.1 Study design and participants

This observational retrospective study analyzed the clinical data, including for survival, in elderly patients who had been treated with TCM for ACRC in the Oncology Department of Xiyuan Hospital, China Academy of Chinese Medical Sciences between January 2012 and December 2021. The inclusion criteria are as follows: (Song et al., 2020): pathological diagnosis of CRC, (Feng et al., 2019), stage IV disease according to the National Comprehensive Cancer Network guidelines, and (Hamed et al., 2022) age ≥ 75 years. Study participants had received or were continuing to receive TCM whether or not they had a favorable response to conventional chemotherapy, including oxaliplatin, irinotecan, 5-fluorouracil, capecitabine, and anti-vascular endothelial growth factor and anti-epidermal growth factor receptor agents. The primary endpoint was PFS; secondary endpoints included the total duration of TCM (TTCM), disease control rate (DCR), and incidence of treatment-related adverse events (TRAEs).

2.2 Tumor assessment and TCM

Tumor size was assessed in each patient before treatment according to RECIST (Response Evaluation Criteria in Solid Tumors) version 1.1 by spiral computed tomography, which was performed at 3-month intervals from the start of treatment until disease progression or cessation of TCM. TCM is usually decoction that administered to strengthen the spleen and remove phlegm. Each prescription contains 16 natural herbs [*Astragalus* (*Leguminosae*), *Taizishen* (*Pseudostellaria*), *Epimedium* (*The Genus Epimedium*), *Tiannanxing* (*Arisaema*), *Ligustrum lucidum*, *Poria*, *Turmeric* (*Curcuma longa*), *ShiJianchuan* (*Labiatae Juss*)], administered twice a day, half an hour after breakfast and dinner. TCM can be administered as a decoction or as granules depending on the patient's wishes. The TCM dose was adjusted according to each patient's symptoms and tumor size or until the patient was no longer willing to take.

2.3 Data collection

Two investigators collected clinical information for all eligible patients from the outpatient records held at Xiyuan Hospital, Chinese Academy of Chinese Medical Sciences, including sex, age, location of the primary tumor, site(s) of metastasis, whether the primary site was treated surgically, post-visit treatments, ECOG-PS score, PFS, and TTCM.

2.4 Outcomes

The date of the last visit was assumed to be the time of progression if the patient discontinued treatment before the disease had progressed. PFS and TTCM were calculated from the day when treatment was started until disease progression for any reason, until the last dose of TCM, or until death from any cause. DCR is defined in RECIST version 1.1 as the objective response of the disease to an agent that has been administered for 6 months and includes complete response, partial response, stable disease, and progressive disease. TRAEs were assessed according to the Common Terminology Criteria for Adverse Events, version 4.0. The last follow-up was on 31 December 2021.

2.5 Statistical analysis

Patient basic characteristics and the incidence of TRAEs were analyzed descriptively. PFS and TTCM were analyzed by the Kaplan-Meier method. The 95% confidence interval (*CI*) was calculated. Statistical analyses were performed using SPSS version 25.0 software (IBM Corp., Armonk, NY, United States). A p-value <0.05 was considered statistically significant.

3 Results

3.1 Patient characteristics

Forty-eight patients (35 men, 13 women) aged ≥ 75 years with ACRC were eligible for inclusion in our study. The mean patient age was 78.75 ± 2.99 years (range, 75–87). There were 18 cases of rectal cancer and 30 of colon cancer. Thirty-eight patients underwent primary surgery (radical resection, n = 31; palliative surgery, n = 7) and 10 did not. Twenty-two patients received TCM alone and 26 received TCM in combination with conventional medicine. Six of the 48 patients progressed after first-line therapy, 10 were receiving first-line therapy, one was intolerant of first-line therapy and received only one cycle, four were receiving second-line therapy, and one was intolerant of second-line therapy and received only one cycle. Two patients were receiving targeted third-line therapy, one was undergoing radiotherapy for distant lymph node metastasis, one had just completed a course of radiotherapy, one was unable to

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TABLE 1 Basic characteristics of patients at the time of initial treatment.

	N = 48	Percentage (%)
Age (total)	78.75 ± 2.99 (75–87)	
< 80 years old	30	62.5
≥ 80 years old	18	37.5
Gender		
Men	35	72.9
Women	13	27.1
Primary location		
Rectal	18	37.5
Colon	30	62.5
Differentiation		
Moderate	25	52.1
Poorly	12	25
Unknown	11	22.9
Tumor type		
Adenocarcinoma	35	72.9
Other	4	8.3
Unknown	9	18.8
Metastatic site		
Liver	28	58.3
Lung	14	29.2
Peritoneum	6	12.5
Bone	5	10.4
Distant lymph nodes	14	29.2
Single-organ metastasis	27	56.3
Multi-organ metastasis	21	43.8
Primary site surgery		
Radical surgery	31	64.6
Palliative surgery	7	14.6
Not operated	10	20.8
Treatment methods		
TCM	22	45.8
Integrative TCM and Western Medicine	26	54.2
ECOG-PS		
1	20	41.7
2	18	37.5
3	10	20.8

complete radiotherapy because of a low platelet count, three had just undergone radio-frequency ablation for metastases, six had just been diagnosed to have advanced disease and not received conventional chemotherapy, and 12 had sought TCM because they were unwilling or unable to receive conventional therapy. Twenty patients had an ECOG-PS score of 1, 18 had a score of 2, and 10 had a score of 3. The patient characteristics are summarized in Table 1.

3.2 Survival outcomes

Kaplan-Meier curve showed an overall median PFS of 4 months with a mean of 5.3 months (range, 1–26; 95% Cl 3.26–4.73; Figure 1) and a median TTCM of 5.5 months with a mean of 9.45 months (range, 1–50; 95% Cl 1.76–8.24; Figure 2). Subgroup analysis (Table 2) revealed that PFS and TTCM were significantly longer in patients with no bone metastasis, those who received a combination of TCM and traditional medicine, and those who had an ECOG-PS score of 1 than in patients with bone metastases, those who received only TCM, and those with an ECOG-PS score of 2–3 ($p < 0.05$; Figures 3–5).

3.3 Disease control rate

After 6 months, the response to TCM was reassessed by spiral computed tomography. The response could not be assessed in 14 patients because they had been treated for up to 3 months without follow-up. No patient had a complete response, one had a partial response, 15 achieved stable disease, 18 had progressive

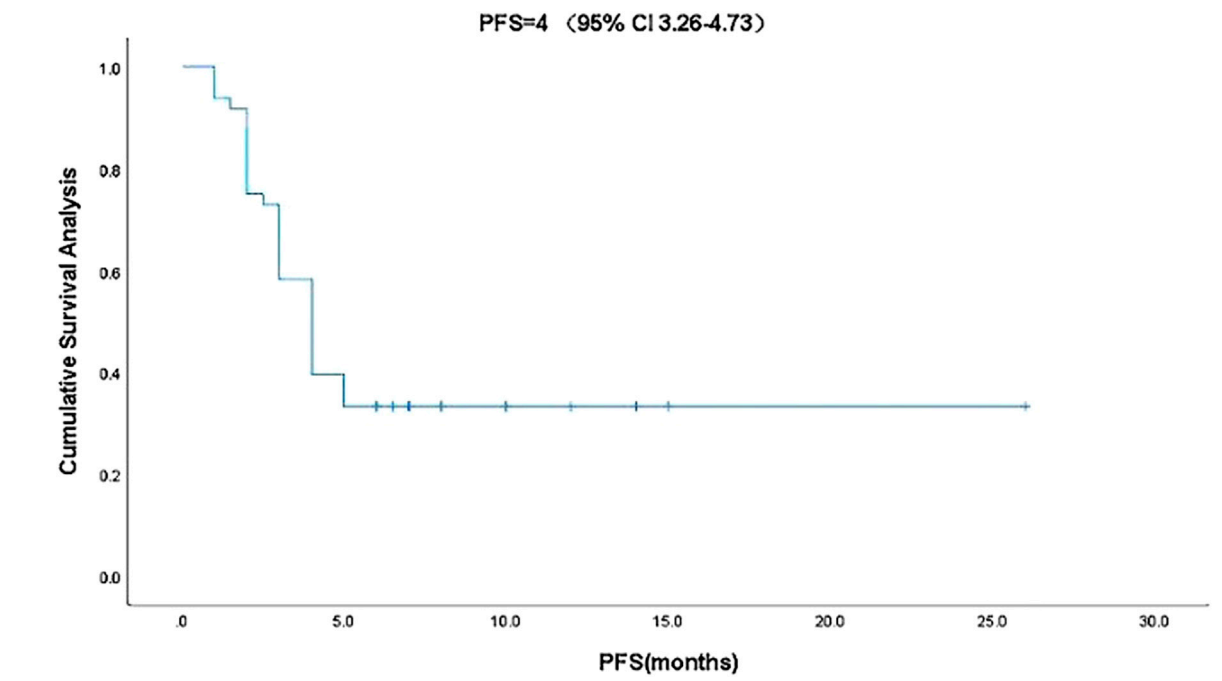

FIGURE 1
Kaplan-Meier plot showing PFS in patients with advanced colorectal cancer who received traditional Chinese medicine. CI, confidence interval; PFS, progression-free survival.

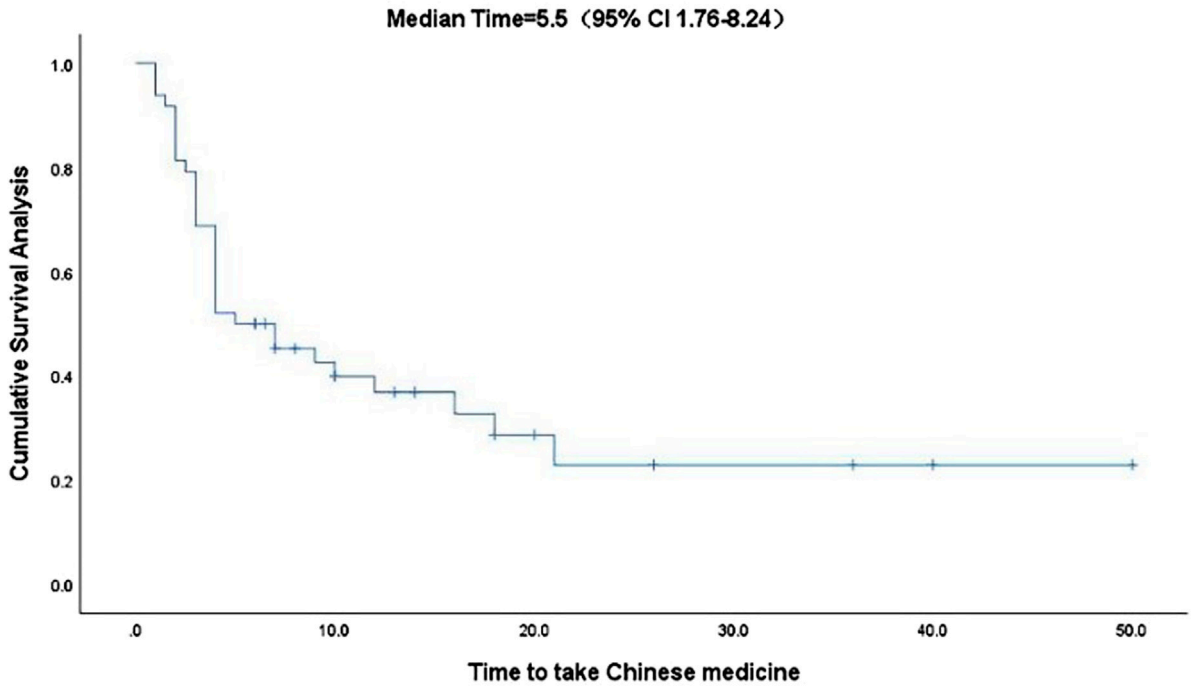

FIGURE 2
Kaplan-Meier plot showing the TTCM in patients with advanced colorectal cancer. CI, confidence interval; TTCM, total duration of traditional Chinese medicine therapy.

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TABLE 2 Subgroup analysis of PFS and TTCM.

	N = 48	Median PFS (months)	Median TTCM (months)	*p*-value (PFS)	*p*-value (TTCM)
Age (total)		4	5.5	0.390	0.945
< 80 years old	30	4	4		
≥ 80 years old	18	3	8.5		
Gender				0.819	0.958
Men	35	4	6.5		
Women	13	4	4		
Primary location				0.370	0.228
Rectal	18	4.5	8.5		
Colon	30	4	4		
Differentiation				0.418	0.151
Moderate	25	4	7		
Poorly	12	4	4		
Unknown	11	3	4		
Tumor type				0.333	0.113
Adenocarcinoma	35	4	6		
Other	4	7	11		
Unknown	9	3	4		
Metastatic site					
Liver	28	3.5	4	0.259	0.249
Lung	14	4.5	7.5	0.656	0.577
Peritoneum	6	4	5.5	0.713	0.700
Bone	5	2	6	**0.021**	**0.015**
Distant lymph nodes	14	4	6.25	0.987	0.64
Single-organ metastasis	27	4	5	0.488	0.343
Multi-organ metastasis	21	4	5	0.488	0.343
Primary site surgery				0.580	0.614
Radical surgery	31	4	7		
Palliative surgery	7	3	3		
Not operated	10	2.5	2.5		
Treatment methods				**0.008**	**0.022**
TCM	22	3	4		
Integrative TCM and Western Medicine	26	4.5	6.5		
ECOG-PS				**0.000**	**0.000**
1	20	5.5	9.5		
2	18	4	4.5		
3	10	2.5	2.5		

Note: Bold represents $p < 0.05$ or $p < 0.1$, which is statistically significant or potentially influential.

disease, and 14 had disease that could not be evaluated (Table 3). The mean follow-up duration was 9.45 months (range, 1–50).

3.4 Safety analysis

Most patients were experienced slightly discomfort, such as fatigue, anorexia, diarrhea, abdominal distension and pain, constipation, and soreness around the abdomen and in the knees. Two patients developed diarrhea while taking TCM, and one developed constipation. Patients with advanced tumors had many symptoms, for which TCM could be ruled out as the cause. No relationship between was identified TCM and liver or kidney function. No obvious hematological toxicity or serious adverse reactions could be attributed to TCM during treatment.

4 Discussion

Currently, there's no treatment guidelines exist for elderly patients with ACRC, mainly because patients aged ≥ 75 years are excluded from participation in clinical trials in the conventional

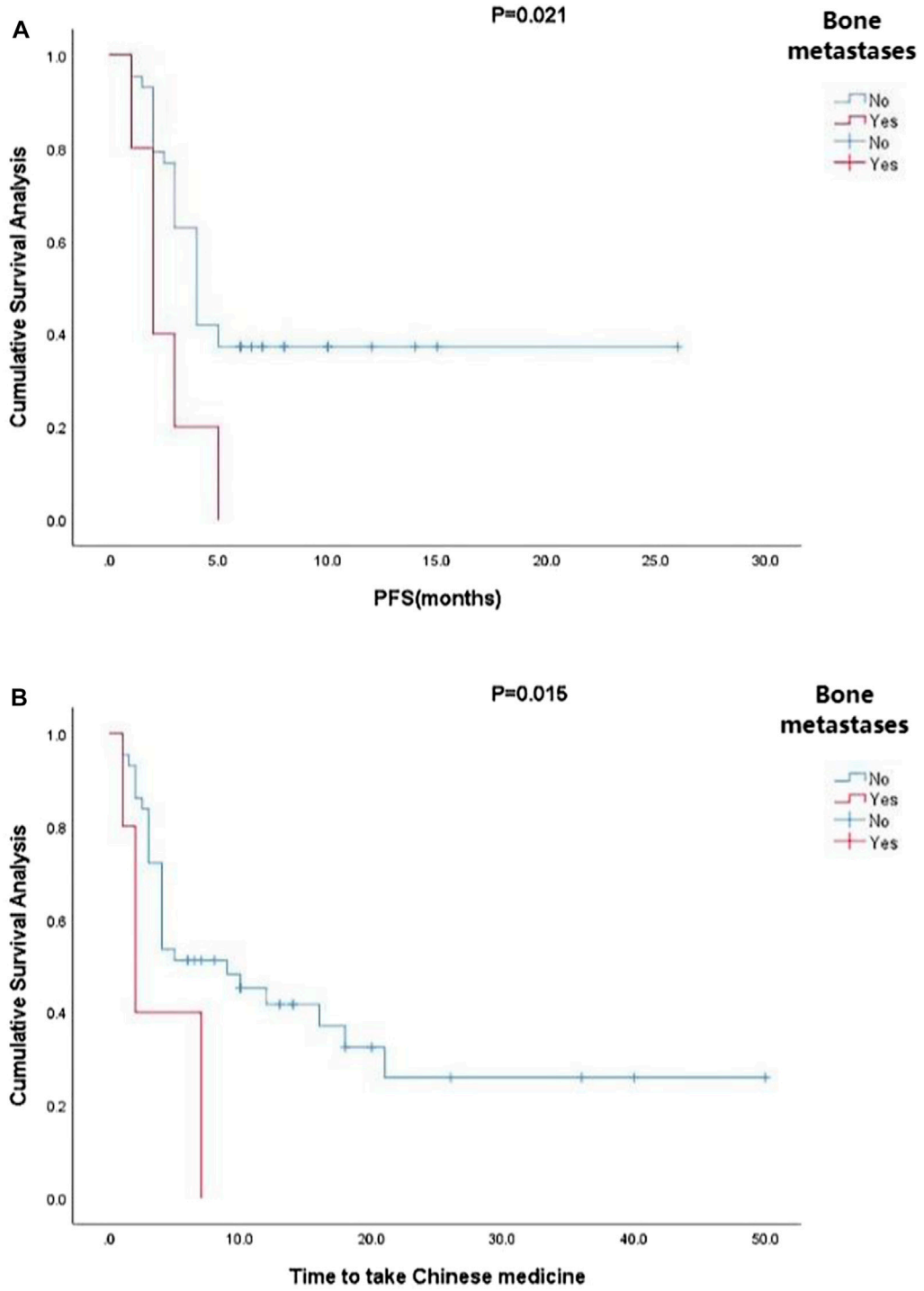

FIGURE 3
Kaplan-Meier plots showing **(A)** PFS and **(B)** TTCM in patients with advanced colorectal cancer and bone metastases. PFS, progression-free survival; TTCM, total duration of traditional Chinese medicine.

medicine as a result of their high likelihood of comorbidities. Therefore, there is little information on treatment for ACRC and its efficacy in this age group. The limited literature available has focused on the short-term effects after surgery or on patients starting treatment at the age of 65–70 years (Cheng et al., 2022; Hashimoto et al., 2022), Or start at age 65 or 70 (Takahashi et al., 2021). Although there are some relevant clinical studies in patients aged ≥ 75 years, either the ECOG-PS score has been around 0–1 (Rosati et al., 2019), adverse events were severe (François et al., 2020), or there was only one metastatic site (Cuccia et al., 2021). Furthermore, most elderly participants in clinical research have undergone strict screening, requiring, for example, an ECOG-PS score of 0–1 or only

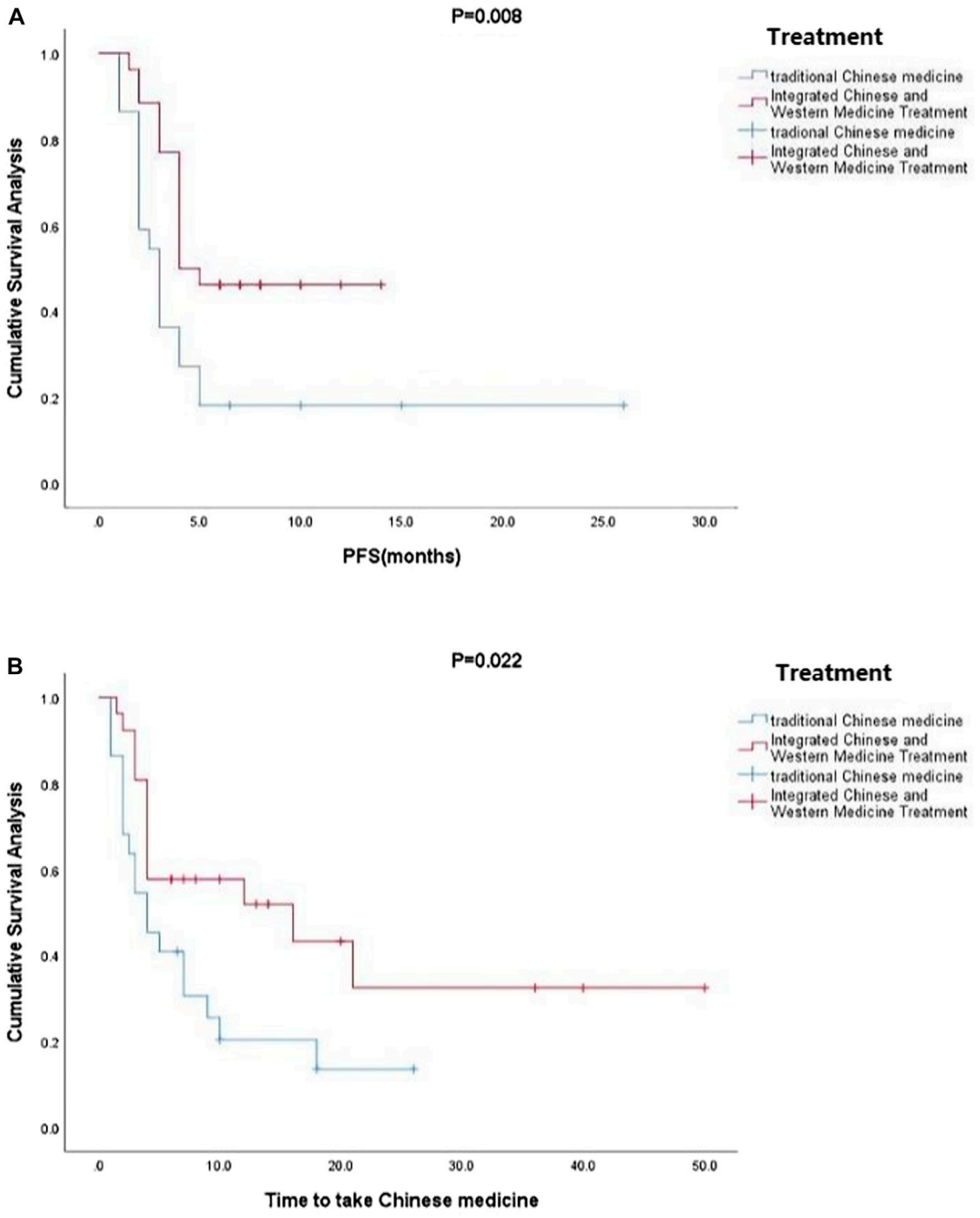

FIGURE 4
Kaplan-Meier plots showing **(A)** PFS and **(B)** TTCM according to whether or not TCM was combined with conventional Western chemotherapy. PFS, progression-free survival; TTCM, total duration of traditional Chinese medicine.

one metastatic site for inclusion. Therefore, most clinical studies in the conventional Western medicine do not include patients aged ≥ 75 years with multiple metastases and an ECOG-PS score ≥ 2. Moreover, older adults may be more concerned about their quality of life and therapies that relieve their symptoms rather than receiving conventional curative Western chemotherapy, targeted therapy, or immunotherapy, which could have a high degree of toxicity and be burdensome for both patients and their families (Kemeny et al., 2003).

Patients treated with TCM do not need to be screened strictly for clinical research purposes. They can receive TCM provided that they can drink and are willing to do so. TCM is an independent factor affecting PFS (11); when combined with chemotherapy and cetuximab or bevacizumab, TCM has been shown to prolong

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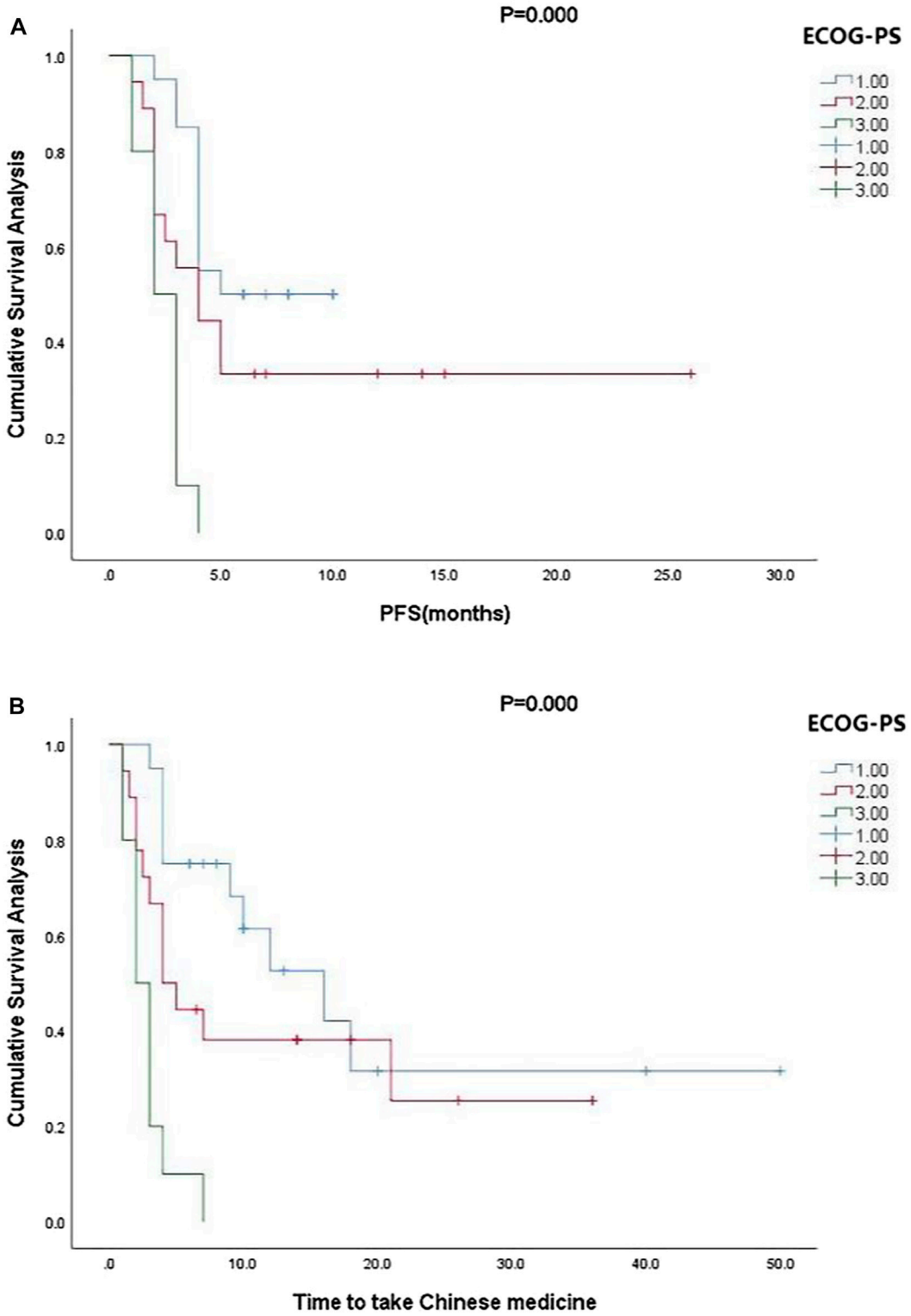

FIGURE 5
Kaplan-Meier plots showing **(A)** PFS and **(B)** TTCM according to ECOG-PS status. ECOG-PS, Eastern Cooperative Oncology Group performance status; PFS, progression-free survival; TTCM, total duration of traditional Chinese medicine.

PFS, improve quality of life, and reduce adverse reactions in patients with ACRC (11). Studies have shown that long-term use of TCM not only has a positive effect on survival in patients with CRC but also helps to reduce the risk of recurrence and metastasis (Wang et al., 2020). Pharmacological studies in animals or cell lines and network research have shown that TCM can prevent metastasis of CRC to the

TABLE 3 Disease response rate.

	n (%)
Complete responses	0
Partial response	1 (2.1)
Stable disease	15 (31.3)
Progressive disease	18 (37.5)
Unevaluable	14 (29.2)

liver by down-regulating the activation of cancer-associated fibroblasts mediated by CRC-derived ITGBL1-loaded extracellular vesicles (Li et al., 2022); thus, TCM has the unique characteristics of being multi-targeted and multi-linked, and may have a comprehensive therapeutic effect. TCM can also inhibit epithelial–mesenchymal transition by downregulating transforming growth factor-beta, thereby inhibiting invasion and metastasis of CRC cells (Ge et al., 2022), inhibit progression of CRC by suppressing CCL2 and preserving progenitor Tex in an obese microenvironment (Xu et al., 2022) and suppress growth and metastasis of 5-fluorouracil-sensitive/resistant CRC by inhibiting the Wnt singling pathway (Zhang et al., 2022).

For the above reasons, this retrospective observational real-world study analyzed the clinical characteristics and survival data for patients aged ≥ 75 years with ACRC whose treatment included TCM. These patients had an overall median PFS of 4 months and a median TTCM of 5.5 months, which is in line with the PFS of 4.3 months reported for patients with wild-type KRAS metastatic CRC and frail older patients treated with panitumumab as a single agent in a study by the Spanish Digestive Oncology Collaborative Group (Sastre et al., 2015). However, the patients in that study were also strictly screened; the highest ECOG-PS score was 2% and 15.2% had adverse reactions that were grade ≥ 3. In contrast, our study included patients who did not undergo strict screening and was open to any patient who chose TCM and attended for follow-up. Our subgroup analysis found that patients without bone metastases had longer PFS and TTCM than those with bone metastases, which is also consistent with another recent report (Lavacchi et al., 2021). Due to the small sample size in our study, there were few patients with bone metastases; nevertheless, of the five patients with multiple bone metastases, four also had liver and lung metastases, pelvic metastases, and distant lymph node metastases. Therefore, no conclusions can be drawn regarding TCM and PFS in patients with CRC and bone metastases. Quality of life and survival in patients with bone metastases is markedly decreased due to pain, walking difficulties, pathological fractures, and neurological impairment (Rocha et al., 2022). Although TCM cannot reverse bone metastasis, it can have a positive effect on expression levels of markers of inflammation, apoptosis, and remission, thereby relieving pain and improving quality of life (Shen et al., 2022).

Studies have shown that patients with liver (Chuang et al., 2020) and peritoneal (Franko, 2018) metastases have a shorter survival time. However, our subgroup analysis found no significant difference in PFS or TTCM according to liver or peritoneal metastasis status. There are several possible explanations for these inconsistent findings. First, our sample size of 48 patients may have been too small to detect a statistically significant effect of TCM. Second, all our patients with liver metastases had metastases at multiple sites, including the lung, peritoneum, and distant lymph nodes. Similarly, patients with peritoneal metastasis also had metastases in the liver and abdominal and pelvic lymph nodes. Therefore, we cannot draw any conclusions regarding survival by simply analyzing liver and peritoneal metastases.

In our study, most of the patients who received TCM alone had an ECOG-PS score of 2–3 ($n = 18$) and most of those who received a combination of TCM and conventional anti-cancer treatment had an ECOG-PS score of 1 ($n = 22$). ECOG-PS is an important determinant of patient survival (LI, 2020), and also one of the important reasons for whether or not Western chemotherapy is recommended. With the exception of four patients who chose not to continue with conventional chemotherapy, those treated with TCM were not candidates for chemotherapy. Our subgroup analysis according to ECOG-PS score confirmed that the better the patient's physical status, the longer the PFS and TTCM (Figure 5). This may be the main reason why PFS was shorter in our patients who were treated with TCM alone than in those treated with TCM in combination with conventional chemotherapy. Median PFS was 2.1 months in rigorously screened patients who received TAS-102 (Cicero et al., 2020). This value is similar to the median PFS of 3 months in our patients who received TCM alone, which had the additional advantages of low cost and fewer side effects. Overall, our research shows that TCM has advantages in treatment.

Surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy remain the mainstay of treatment for CRC. However, for patients who have failed on or are ineligible for these treatments, TCM is their last chance for potentially helpful treatment. Most patients who choose TCM are in a poor physical state and seek TCM to alleviate the toxicity of conventional chemotherapy, have discontinued conventional anti-cancer treatments because of adverse reactions, or are not candidates for these therapies. All the patients in our study had multiple metastases, even if only one organ was involved. Furthermore, their ECOG-PS scores were worse than those in the clinical studies of Western anti-cancer treatments. More than half of our patients had an ECOG-PS score > 1. Many such patients attend for a medical consultation in a wheelchair, or are already bedridden, in which case their family members attend instead. However, even in these circumstances, the median PFS was still 4 months with a median TTCM of 5.5 months, which suggests that TCM is effective in prolonging survival and may improve quality of life.

This study has some advantages and limitations. The study found the potential benefits of TCM for elderly patients with ACRC who are physically weak and unable to receive conventional Western medicine treatment. In China, many elderly patients choose TCM treatment because they cannot tolerate conventional Western medicine treatment. In the course of clinical treatment and follow-up, we found that elderly patients with ACRC seem to benefit from TCM, but there is a lack of relevant data to prove a benefit of TCM for elderly patients with ACRC. Therefore, we carried out such a retrospective data summary analysis. Fortunately, we found a potential benefit of TCM in the elderly. TCM has a potential therapeutic value for the elderly patients with an ECOG score of 2-3 and who cannot tolerate conventional

Western medicine treatment. Therefore, we would like to share this result in the hope that more studies can pay attention to this problem and better serve elderly patients.

However, there are still some shortcomings in this study. First, the study had a retrospective single-center design, which would have introduced a degree of bias. Second, although most elderly patients have comorbidities, these were not included in our analysis. Therefore, while some studies have shown that comorbidities have a marked impact on survival in the elderly (Canoui-Poitrine et al., 2022), we could not draw any conclusions in this regard. Third, the safety of TCM is also one of the topics of concern at present. Although no serious side effects of TCM were found in this study, the interaction between drugs should not be ignored. For this kind of study, we preliminarily found the potential benefits of TCM on elderly patients. However, due to the lack of rigor in design, more rigorous design, scientific statistical processing, strict inclusion of patients, and well follow-up information design is needed in future studies. Multicenter clinical studies in larger samples are required to determine the efficacy and safety of TCM in elderly patients with ACRC.

5 Conclusion

This retrospective observational real-world study suggests that TCM is potential effective in patients aged ≥ 75 years with advanced colorectal cancer. And TCM can be used safely in these patients, even if they have an ECOG-PS score of 2–3.

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 study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Xiyuan Hospital, Chinese Academy of Chinese Medical Sciences. The protocol code 2021XLA095-3 and date of approval is 15 November 2021. According to national legislation, informed consent was not needed due to the retrospective and non-interventional nature of the study and the anonymity of the study data. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization, YW and NC; data acquisition, JW, ZL, TH, and LL; data analysis and interpretation, YW, NC, and ZL; writing and critical revision of the manuscript, YW, NC, and ZL; original draft, JW.

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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 ZW declared a shared parent affiliation with the author at the time of review.

Publisher's note

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Traditional Chinese medicine use is associated with lower risk of pneumonia in patients with systemic lupus erythematosus: a population-based retrospective cohort study

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Objectives: To investigate the association between traditional Chinese medicine (TCM) therapy and the risk of pneumonia in patients with systemic lupus erythematosus (SLE).

Methods: This population-based control study analyzed the data retrieved from the National Health Insurance Research database in Taiwan. From a cohort of 2 million records of the 2000–2018 period, 9,714 newly diagnosed patients with SLE were initially included. 532 patients with pneumonia and 532 patients without pneumonia were matched 1:1 based on age, sex, and year of SLE diagnosis using propensity score matching. The use of TCM therapy was considered from the SLE diagnosis date to the index date and the cumulative days of TCM therapy were used to calculate the dose effect. Conditional logistic regression was used to investigate the risk of pneumonia infection. Furthermore, to explore the severity of pneumonia in SLE, sensitivity analyses were performed after stratification using the parameters of emergency room visit, admission time, and antibiotic use.

Results: TCM therapy for >60 days could significantly reduce the risk of pneumonia in patients with SLE (95% CI = 0.46–0.91; p = 0.012). Stratified analysis showed that TCM use also reduced the risk of pneumonia in younger and female patients with SLE by 34% and 35%, respectively. TCM for >60 days significantly reduced the risk of pneumonia in the follow-up periods of >2, >3, >7, and >8 years. In addition, the exposure of TCM for >60 days reduced the risk of pneumonia in patients with SLE who were treated with antibiotics for moderate or severe pneumonia. Finally, the study found that using formulae to tonify the kidney for more than 90 days and formulae to activate blood circulation for less than 30 days could significantly reduce the risk of pneumonia infection in patients with SLE.

Conclusion: TCM use is associated with a lower risk of pneumonia among patients with SLE.

KEYWORDS

systemic lupus erythematosus (SLE), traditional Chinese medicine (TCM), pneumonia, cohort, infection

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that presents with complex clinical manifestations. SLE can affect all systems of the body, including the kidneys and nervous system (Kiriakidou and Ching, 2020). The estimated worldwide prevalence of SLE is approximately 30–50/100,000 people, and the prevalence is higher in developing countries (Ingvarsson et al., 2016; Tsioni et al., 2015; Li et al., 2012). The 10-year survival rate is approximately 90%, 15-year survival rate is 85%, and 20-year survival rate is 78% (Durcan et al., 2019), indicating that the disease seriously affects the physical and mental health of the patients as well as their quality of life. The current management of SLE is still dominated by glucocorticoids and immunosuppressive agents. However, these agents often cause several side effects, such as a secondary infection (27%), hypertension (11.3%), and osteoporosis (7.5%) (Tektonidou et al., 2015). Among the side effects, infection and lupus nephritis are the main causes of death in SLE (Sciascia et al., 2017). Clinical studies have found that belimumab, the first biologic approved for the treatment of SLE by the Food and Drug Administration, combined with standard therapy can alleviate the disease activity in some patients with SLE and has been shown to have a safety profile similar to a placebo (Stohl et al., 2017). Although pre-emptive antimicrobial therapy and vaccination has become the consensus for preventing infections, the incidence of certain infections, such as pneumonia, remains high and inevitable (Oku et al., 2021). Therefore, there is an urgent need for the development of clinical treatments or drugs with high safety and good efficacy for alleviating SLE and preventing infections.

Traditional Chinese medicine (TCM) has been used for treating several autoimmune diseases (called Bi syndrome in TCM), including SLE, and has shown significant clinical efficacy for thousands of years (Wang et al., 2019). We previously conducted a meta-analysis of 13 randomized, placebo-controlled trials that included 856 participants and revealed that TCM could control disease activity and reduce glucocorticoid dose used among patients with SLE (Wang et al., 2021). A population-based cohort study based on the National Health Insurance Research Database (NHIRD) in Taiwan provided evidence that regular treatment combined with Chinese herbal medicine improves the survival of patients with SLE. The study included several useful TCM formulae, including Zhi Bo Di Huang Wan, Jia Wei Xiao Yao San, Liu Wei Di Huang Wan, Gan Lu Yin, and Yin Qiao San (Ma et al., 2016). However, few studies have evaluated whether TCM can reduce the risk of infections in patients with SLE.

To the best of our knowledge, no large-scale study has evaluated the association between TCM therapy and the risk of pneumonia in patients with SLE. Thus, the present study aimed to provide some evidence regarding the aforementioned association.

Methods

Data sources

The research data of 2 million people from 1 January 2000, to 31 December 2018, were retrieved from NHIRD, Taiwan, out of the 23 million people included in the database. The random sampling method was employed, with serial numbers assigned to each of the 23 million insurance beneficiaries. NHIRD contains information on patient demographics, age, sex, disease diagnoses, number of clinical visits and hospitalizations, prescribed medications (with dosages), and TCM treatment agents administered by registered TCM physicians. Diseases were defined as per the International Classification of Diseases (ICD) Ninth Revision (ICD-9) and 10th Edition (ICD-10) codes (Goulielmos and Zervou, 2020).

Patients

As shown in Figure 1 total of 2 million patients were randomly selected from NHIRD. The included patients were

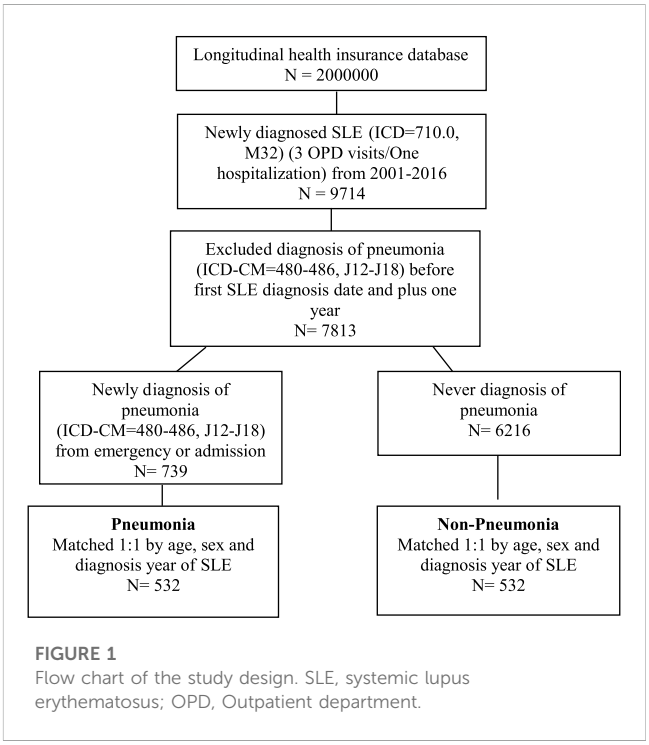

FIGURE 1
Flow chart of the study design. SLE, systemic lupus erythematosus; OPD, Outpatient department.

those who were newly diagnosed with SLE (ICD-9 code = 710.0; ICD-10 code = M32) from January 2001 to December 2016 (n = 9,714). A stringent criterion of requiring at least three outpatient visits or one hospital admission was used for recognizing SLE. In addition, pneumonia (ICD-9 code = 480–486; ICD-10 code = J12–J18) was chosen as the representative infection with SLE. After excluding the diagnosis of pneumonia before the first SLE diagnosis date and 1 year after SLE diagnosis, a total of 7,813 patients with SLE were finally selected as the study group. A total of 739 pneumonia patients with SLE were selected from the emergency or admission group. The index date was defined as the pneumonia onset date (Supplementary Figure S1). Moreover, 6,216 patients with SLE were never diagnosed with pneumonia. After 1:1 propensity score matching using the parameters of age, sex, and SLE diagnosis year, 532 pneumonia and 532 non-pneumonia patients with SLE were included in the analysis.

Traditional Chinese medicine and covariates

TCM use was calculated from the SLE diagnosis date to the index date, and the cumulative days of TCM therapy were used to calculate the dose effect. TCM users were defined as patients who used TCM more than three times. In addition, the cumulative days of TCM therapy were classified as 30, 60, and 90 days. TCM considers SLE as a systemic disease associated with the state of the entire body and implicates toxic heat, blood stasis, and kidney yin deficiency in the pathogenesis of SLE (Sun et al., 2018). Therefore, the TCM formulae were initially classified into three types: those tonifying the kidney (KF), those activating blood circulation (BF), and the "other TCM therapy" group. The classification standards were followed as per the classification criteria described in a previous study (Lin et al., 2021). As a result, the top 20 frequently used Chinese medicine formulae in NHIRD were selected and classified into two groups: the KF and BF groups. The KF group included Guilu Erxian Jiao, Jisheng Shenqi Pill, Qiju Dihuang Pill, Zhibo Dihuang Pill, Liuwei Dihuang Wan, and Zuo Guiwan, whereas the BF group comprised Duhuo Jisheng Decoction, Shujing Huoxue Decoction, Danggui Niantong Decoction, Shaoyao Gancao Decoction, Shentong Zhuyu Decoction, Xuefu Zhuyu Decoction, Xiao Huoluo Dan, and Guizhi Shaoyao Zhimu Decoction (Supplementary Table S1). The baseline comorbidities included hypertension (ICD-9 code = 401–405; ICD-10 code = I10–I15), hyperlipidemia (ICD-9 code = 272; ICD-10 code = E78), chronic liver disease (ICD-9 code = 571; ICD-10 code = K70, K73, K74, K75.4, K75.81, K76.0, K76.89, and K76.9), chronic kidney disease (ICD-9 code = 585; ICD-10 code = N184, N185, N186, and N189), diabetes (ICD-9 code = 250; ICD-10 code = E10, E11, E12, E13, and E14), chronic obstructive pulmonary disease (ICD-9 code = 491, 492, and 496; ICD-10 code = J41–J44), rheumatoid arthritis (ICD-9 code = 714.0; ICD-10 code = M05 and M06), ankylosing spondylitis (ICD-9 code = 720.0; ICD-10 code = M45 and M46), hepatitis B (ICD-9 code = 070.2, 070.3, and V02.61; ICD-10 code = B16.0, B16.1, B16.2, B16.9, B18.0, B18.1, B19.10, B19.11, and Z22.51), hepatitis C (ICD-9 code = 070.41, 070.44, 070.51, 070.54, 070.7, and V02.62; ICD-10 code = B17.10, B17.11, B18.2, B19.20, B19.21, and Z22.52), endocarditis (ICD-9 code = 424.91; ICD-10 code = I39), nephritis (ICD-9 code = 583.81; ICD-10 code = E10.21, E11.21, and N16), glomerulonephritis (ICD-9 code = 580.81, 581.81, and 582.81; ICD-10 code = N08). These comorbidities were identified during at least three outpatient visits or one hospital admission between the SLE diagnosis date and index date. The Anatomical Therapeutic Chemical (ATC) Classification System codes were used for defining drugs. The use of corticosteroids, hydroxychloroquine (ATC code = P01BA02), and methotrexate (ATC code = L04AX03) for at least three times prescription in each kind of medication between the SLE diagnosis date and index date was evaluated.

Statistical analysis

The Chi-squared test and Student's t-test were used to compare continuous and dichotomous data, respectively, between the pneumonia and non-pneumonia groups. Multivariate conditional logistic regression analysis was performed to evaluate the risk of pneumonia infection in patients with SLE after prescribing TCM therapy. The risk was estimated using adjusted odds ratios (aORs) and 95% confidence intervals (CIs). Statistical significance was defined as p-value <0.05, and all data analyses were performed using the statistical package SAS for Windows (Version 9.4, SAS Institute Inc., Carey, NC, United States).

Subgroup analyses were performed to determine how the risk of pneumonia infection in patients with SLE patients differed as per sex, age, and days of TCM use. In addition, the association between the use of different TCM formulae and risk of pneumonia infection was evaluated. Antibiotic use and hospitalization period were analyzed to estimate the severity of pneumonia.

Results

Demographic characteristics and comorbidities

The demographic characteristics of included patients with SLE in the pneumonia and non-pneumonia groups are shown in Table 1. After 1:1 propensity score matching using the parameters of age, sex, and SLE diagnosis year, 532 pneumonia and 532 non-pneumonia patients with SLE were finally included in the study. SLE patients with pneumonia had several comorbidities, including hypertension (p = 0.0014), chronic liver disease (p = 0.0312), chronic kidney disease (p < 0.001), diabetes (p = 0.0002), chronic obstructive pulmonary disease (COPD) (p = 0.0102), and rheumatoid arthritis (p = 0.0308), except ankylosing spondylitis. Moreover, corticosteroids, nonsteroidal anti-inflammatory drugs, and hydroxychloroquine were used more frequently by patients with SLE in the pneumonia group. Finally, TCM was prescribed more frequently to SLE patients with pneumonia in the 60 days stratification groups (p = 0.0268).

TCM use reduced the risk of pneumonia in SLE patients

Conditional logistic regression analysis revealed that TCM use reduced the risk of pneumonia in patients with SLE (aOR (95% CI) = 0.73 (0.55–0.96); p = 0.026). Among the comorbidities, chronic kidney disease (aOR (95% CI) = 2.23 (1.25–3.97); p = 0.007),

TABLE 1 Demographic characteristics of pneumonia and non-pneumonia.

	Non-pneumonia (*N* = 532)	Pneumonia (*N* = 532)	*p*-value
Age			1
<65	413 (77.6)	413 (77.6)	
≥65	119 (22.4)	119 (22.4)	
Mean ± SD	50.6 ± 17	50.6 ± 17	1
Age			1
<50	245 (46.1)	245 (46.1)	
≥50	287 (53.9)	287 (53.9)	
Sex			1
Female	452 (85.0)	452 (85.0)	
Male	80 (15.0)	80 (15.0)	
TCM	295 (55.5)	262 (49.2)	0.0428
TCM (day)			0.0640
None	237 (44.5)	270 (50.8)	
≤30	107 (20.1)	108 (20.3)	
31–60	42 (7.9)	44 (8.3)	
>60	146 (27.4)	110 (20.7)	
TCM (days)			0.0117
None	237 (44.5)	270 (50.8)	
≤30	107 (20.1)	108 (20.3)	
31–60	42 (7.9)	44 (8.3)	
61–90	31 (5.8)	11 (2.1)	
>90	115 (21.6)	99 (18.6)	
TCM (days)			0.1233
None	237 (44.5)	270 (50.8)	
≤90	180 (33.8)	163 (30.6)	
>90	115 (21.6)	99 (18.6)	
TCM (days)			0.0629
None	237 (44.5)	270 (50.8)	
≤30	107 (20.1)	108 (20.3)	
>30	188 (35.3)	154 (28.9)	
TCM (days)			0.0268
None	237 (44.5)	270 (50.8)	
≤60	149 (28.0)	152 (28.6)	
>60	146 (27.4)	110 (20.7)	
Hypertension	149 (28.0)	198 (37.2)	0.0014
Hyperlipidemia	117 (22.0)	140 (26.3)	0.0995
Chronic liver disease	74 (13.9)	100 (18.8)	0.0312
Chronic kidney disease	25 (4.7)	61 (11.5)	<0.001
Diabetes	52 (9.8)	94 (17.7)	0.0002
Chronic obstructive pulmonary disease	40 (7.5)	65 (12.2)	0.0102
Rheumatoid arthritis	53 (10.0)	76 (14.3)	0.0308
Ankylosing spondylitis	15 (2.8)	4 (0.8)	0.0109
Corticosteroids	503 (94.5)	522 (98.1)	0.0019
NSAIDs	512 (96.2)	523 (98.3)	0.0384
Hydroxychloroquine	141 (26.5)	234 (44.0)	<0.001
Methotrexate	33 (6.2)	50 (9.4)	0.0520
Follow-up duration (years)	7.1 ± 4.4	7.1 ± 4.4	0.9358

(Continued on following page)

TABLE 1 (*Continued*) **Demographic characteristics of pneumonia and non-pneumonia.**

	Non-pneumonia (*N* = 532)	Pneumonia (*N* = 532)	*p*-value
SLE year			1.0
2001	96 (18.0)	96 (18.0)	
2002	66 (12.4)	66 (12.4)	
2003	61 (11.5)	61 (11.5)	
2004	50 (9.4)	50 (9.4)	
2005	47 (8.8)	47 (8.8)	
2006	38 (7.1)	38 (7.1)	
2007	30 (5.6)	30 (5.6)	
2008	24 (4.5)	24 (4.5)	
2009	22 (4.1)	22 (4.1)	
2010	25 (4.7)	25 (4.7)	
2011	16 (3.0)	16 (3.0)	
2012	21 (3.9)	21 (3.9)	
2013	13 (2.4)	13 (2.4)	
2014	7 (1.3)	7 (1.3)	
2015	10 (1.9)	10 (1.9)	
2016	6 (1.1)	6 (1.1)	

NSAIDs, Non-steroidal anti-inflammatory drugs; SLE, systemic lupus erythematosus.

diabetes (aOR (95% CI) = 1.84 (1.18–2.87); $p = 0.007$), and COPD (aOR (95% CI) = 1.72 (1.06–2.79); $p = 0.028$) were associated with a higher risk of pneumonia in patients with SLE. Moreover, the use of corticosteroids (aOR (95% CI) = 2.99 (1.31–6.83); $p = 0.009$) and hydroxychloroquine (aOR (95% CI) = 2.02 (1.48–2.74); $p < 0.001$) increased the risk of pneumonia in patients with SLE (Table 2).

Subgroup analyses

Association between TCM use and risk of pneumonia at different sub-doses

The number of days of TCM use was considered as per our previous research (Liang et al., 2021; Lin et al., 2021). Thus, 30 days was selected as an interval, and 30, 60, and 90 days were selected to estimate the risk. When 60 days of TCM use was used for stratification, the statistical difference between the two groups was evident (Table 1). Thus, patients with SLE who cumulatively received TCM for >60 days had a significantly lower risk of pneumonia (aOR (95% CI) = 0.64 (0.45–0.90); $p = 0.011$) (Table 3).

Association between TCM use and risk of pneumonia in different sub populations and follow-up periods

Conditional logistic regression analysis was performed to evaluate the association between TCM use and the risk of pneumonia in patients with SLE according to age, sex, and follow-up duration, which was defined from the SLE diagnosis date to index date. As shown in Table 4, using TCM for >60 days reduced the risk of pneumonia in younger patients with SLE (age <65 years) (aOR (95% CI) = 0.66 (0.44–0.99); $p = 0.042$). In addition, female patients with SLE had a lower risk of pneumonia after using TCM for >60 days (aOR (95% CI) = 0.65 (0.45–0.93); $p = 0.019$). Moreover, patients who used TCM for >60 days and followed-up for >2 (aOR (95% CI) = 0.66 (0.46–0.93); $p = 0.034$), >3 (aOR (95% CI) = 0.64 (0.44–0.93); $p = 0.019$), >7 (aOR (95% CI) = 0.57 (0.35–0.94); $p = 0.028$), and >8 years (aOR (95% CI) = 0.55 (0.32–0.95); $p = 0.032$) had a lower risk of pneumonia (Figure 2; Supplementary Table S2).

Sensitivity analyses

Association between TCM use and risk of pneumonia as per antibiotic use, severity of pneumonia, and days of hospitalization

Antibiotic use and days of hospitalization may vary as per the severity of pneumonia in patients with SLE. Compared with patients with SLE in the non-pneumonia group, TCM use for >60 days reduced the risk of pneumonia with antibiotic use (aOR (95% CI) = 0.60 (0.43–0.84); $p = 0.005$) in pneumonia group. Moreover, TCM use for >60 days reduced the risk of pneumonia in patients with SLE having moderate (pneumonia admission <7 days) (aOR (95% CI) = 0.43 (0.25–0.74); $p = 0.002$) or severe pneumonia [(pneumonia admission ≥7 days) (aOR (95% CI) = 0.42 (0.23–0.74); $p = 0.003$) and (pneumonia admission ≥8 days) (aOR (95% CI) = 0.46 (0.25–0.83); $p = 0.010$)]. However, there were no significant differences between pneumonia patients from the emergency room and non-pneumonia patients ($p = 0.859$ or $p = 0.241$) (Figure 3; Supplementary Table S3).

Association between the use of different TCM formulae and risk of pneumonia

Conditional logistic regression analysis was performed to evaluate the association between the use of different TCM formulae and risk of pneumonia infection. The results showed

TABLE 2 Conditional logistic regression of risk of pneumonia.

	cOR (95% C.I.)	*p*-value	aOR[a] (95% C.I.)	*p*-value
TCM	0.73 (0.55–0.96)	0.026	0.73 (0.55–0.96)	**0.026**
Hypertension	1.78 (1.31–2.421)	<0.001	1.18 (0.81–1.71)	0.397
Hyperlipidemia	1.31 (0.97–1.77)	0.080	1.20 (0.83–1.74)	0.331
Chronic liver disease	1.47 (1.05–2.07)	0.027	1.17 (0.80–1.71)	0.423
Chronic kidney disease	2.80 (1.68–4.67)	<0.001	2.23 (1.25–3.97)	**0.007**
Diabetes	2.17 (1.46–3.22)	<0.001	1.84 (1.18–2.87)	**0.007**
Chronic obstructive pulmonary disease	1.81 (1.17–2.80)	0.008	1.72 (1.06–2.79)	**0.028**
Rheumatoid arthritis	1.52 (1.04–2.23)	0.030	1.33 (0.85–2.10)	0.211
Ankylosing spondylitis	0.27 (0.09–0.80)	0.019	0.34 (0.10–1.08)	0.067
Corticosteroids	3.11 (1.47–6.59)	0.003	2.99 (1.31–6.83)	**0.009**
NSAIDs	2.38 (1.04–5.43)	0.040	1.88 (0.72–4.92)	0.201
Hydroxychloroquine	2.26 (1.72–2.97)	<0.001	2.02 (1.48–2.74)	**<0.001**
Methotrexate	1.59 (1.00–2.53)	0.052	1.04 (0.60–1.78)	0.897

Bold font indicates statistical significance ($p < 0.05$). cOR, crude odds ratio; aOR, adjusted odds ratio; COPD, chronic obstructive pulmonary disease; NSAIDs, Non-steroidal anti-inflammatory drugs.
[a]Adjusted for all variables.

TABLE 3 Conditional logistic regression of risk of pneumonia in different sub-doses (60 days).

	cOR (95% C.I.)	*p*-value	aOR[a] (95% C.I.)	*p*-value
TCM (day)				
None	Reference		Reference	
≤60	0.89 (0.67–1.18)	0.405	0.81 (0.59–1.12)	0.205
>60	0.66 (0.49–0.90)	0.008	0.64 (0.45–0.90)	0.011
Hypertension	1.78 (1.31–2.421)	<0.001	1.19 (0.82–1.73)	0.361
Hyperlipidemia	1.31 (0.97–1.77)	0.080	1.22 (0.84–1.76)	0.302
Chronic liver disease	1.47 (1.05–2.07)	0.027	1.18 (0.81–1.73)	0.399
Chronic kidney disease	2.80 (1.68–4.67)	<0.001	2.22 (1.24–3.95)	0.007
Diabetes	2.17 (1.46–3.22)	<0.001	1.81 (1.16–2.82)	0.009
Chronic obstructive pulmonary disease	1.81 (1.17–2.80)	0.008	1.72 (1.06–2.79)	0.028
Rheumatoid arthritis	1.52 (1.04–2.23)	0.030	1.31 (0.83–2.06)	0.250
Ankylosing spondylitis	0.27 (0.09–0.80)	0.019	0.35 (0.11–1.13)	0.080
Corticosteroids	3.11 (1.47–6.59)	0.003	2.97 (1.30–6.77)	0.010
NSAIDs	2.38 (1.04–5.43)	0.040	1.85 (0.70–4.88)	0.212
Hydroxychloroquine	2.26 (1.72–2.97)	<0.001	2.00 (1.47–2.72)	<0.001
Methotrexate	1.59 (1.00–2.53)	0.052	1.08 (0.62–1.85)	0.794

cOR, crude odds ratio; aOR, adjusted odds ratio; NSAIDs, Non-steroidal anti-inflammatory drugs.
[a]Adjusted for all variables.

that patients with SLE who used the formulae of the KF group for >30 days (OR (95% CI) = 0.49 (0.29–0.82); p = 0.007), >60 days (OR (95% CI) = 0.45 (0.25–0.82); p = 0.008), and >90 days (OR (95% CI) = 0.50 (0.26–0.96); p = 0.038) had a reduced risk of pneumonia. Moreover, patients with SLE who used the formulae of the BF group for ≤30 days had a reduced risk of pneumonia (OR (95% CI) = 0.48 (0.31–0.74); $p < 0.001$) (Figure 4; Supplementary Table S4).

TABLE 4 Conditional logistic regression of risk of pneumonia by age, sex stratification (60 days).

	N	No. of pneumonia	aOR (95% C.I.)	*p*-value
Age <65				
TCM (day)				
None	392	209	References	
≤60	228	112	0.77 (0.53–1.12)	0.167
>60	206	92	0.66 (0.44–0.99)	0.042
Age ≥ 65				
TCM (day)				
None	115	61	References	
≤60	73	40	1.04 (0.52–2.08)	0.920
>60	50	18	0.52 (0.25–1.05)	0.069
Female				
TCM (day)				
None	408	220	References	
≤60	264	134	0.85 (0.60–1.20)	0.344
>60	232	98	0.65 (0.45–0.93)	0.019
Male				
TCM (day)				
None	99	50	References	
≤60	37	18	0.58 (0.21–1.56)	0.277
>60	24	12	0.59 (0.19–1.77)	0.343

[a]Adjusted for hypertension, hyperlipidemia, chronic liver disease, chronic kidney disease, diabetes, chronic obstructive pulmonary disease, rheumatoid arthritis, ankylosing spondylitis, corticosteroids, NSAIDs, hydroxychloroquine, and methotrexate.

Discussion

To the best of our knowledge, this is the first large-scale cohort study to demonstrate that TCM decreases the risk of pneumonia in patients with SLE. The study results revealed that patients with SLE who cummulatively used TCM for >60 days had a significantly lower risk of pneumonia. A 34% and 35% reduced risk of pneumonia, respectively, was observed in younger and female patients with SLE. TCM use for >60 days significantly reduced the risk of pneumonia in the follow-up periods of >2, >3, >7, and >8 years. In addition, TCM use for >60 days reduced the risk of pneumonia in patients with SLE who also used antibiotics for moderate or severe pneumonia. Furthermore, the use of formulae of the KF group for >90 days and use of formulae of the BF group for <30 days significantly reduced the risk of pneumonia infection in patients with SLE.

SLE is a chronic autoimmune disease with multiorgan manifestations. On the one hand, infection is a common triggering factor that activates SLE and also a common cause of mortality in SLE. The Epstein–Barr virus (Truszewska et al., 2021) and some bacterial lipopolysaccharides (Jung and Suh, 2017) have been reported as pivotal factors for inducing SLE. Bonometti et al. illustrated the case of a patient in whom SLE was triggered by coronavirus disease 2019 infection (Bonometti et al., 2020). On the other hand, a secondary infection has become a common cause of mortality (25%–50%) in SLE (Wang et al., 2015; Kedves et al., 2020). A previous study revealed that lung, cutaneous, and urinary tract infections account for more than two-thirds of all infections in SLE (Jeong et al., 2009). The risk factors of infection in patients with SLE include the overall disease activity, higher C-reactive protein levels, higher anti-dsDNA levels, low complement levels, nephritis, daily dose of prednisone>10 mg, and others (Duffy et al., 1991; Suh et al., 2001; Bosch et al., 2006; Jeong et al., 2009). In the present study, the comorbidities of chronic kidney disease, diabetes, and COPD increased the risk of pneumonia infection in patients with SLE. Diabetes itself has been proven to be a major risk factor in pnemonia (for example, coronavirus disease) infection (Abdi et al., 2020). In terms of COPD, the incidence of lower respiratory tract infections was higher in patients with exacerbated COPD (Sethi, 2010). The use of corticosteroids and hydroxychloroquine also significantly increased the risk of pnemonia infection in patients with SLE, which correlated with a previous study (Kang and Park, 2003). This is mainly because immunosuppressive drugs strongly suppress immune responses against microorganisms, thereby facilitating the onset of pneumonia.

SLE affects females more frequently than males, with a ratio of nearly 9:1 (Tsang and Bultink, 2021). SLE is most commonly diagnosed during the reproductive age, which may be owing to endogenous estrogen production, failure in X chromosome inactivation, increased expression of Toll-like receptors, and alterations in microRNA function (Nusbaum et al., 2020; Leosuthamas et al., 2023). Men with SLE have a more aggressive clinical course, which include cardiovascular disease and nephritis. However, musculoskeletal involvement appears to be more common in women patients (Andrade et al., 2007; Crosslin and Wiginton, 2011). In the present study, female patients with SLE who used TCM for >60 days had a 35% reduced risk of pneumonia infection. SLE usually occurs in the women of reproductive age (Harden and

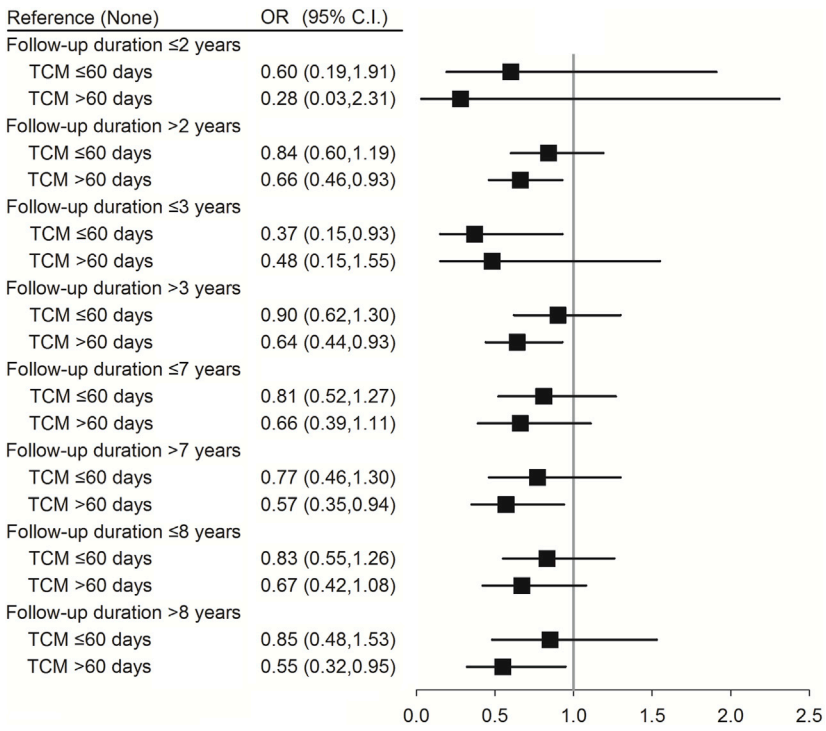

FIGURE 2
Conditional logistic regression of risk of pneumonia by follow-up duration stratification (60 days). †Adjusted for hypertension, hyperlipidemia, chronic liver disease, chronic kidney disease, rheumatoid arthritis, corticosteroids, NSAIDs, and hydroxychloroquine.

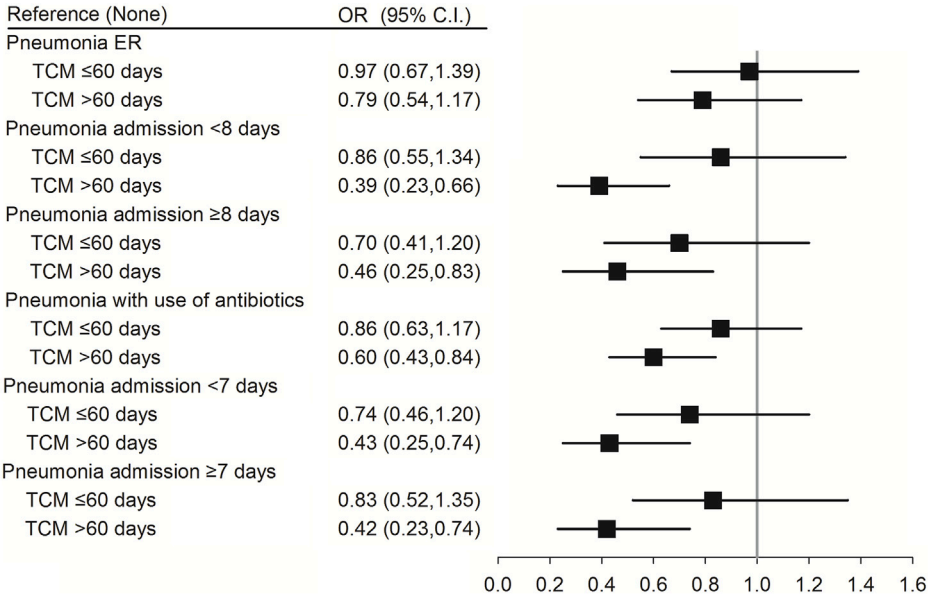

FIGURE 3
Conditional logistic regression of risk of pneumonia by antibiotics use, severity of pneumonia and days of hospitalization. ER, emergency room. †Adjusted for all variables.

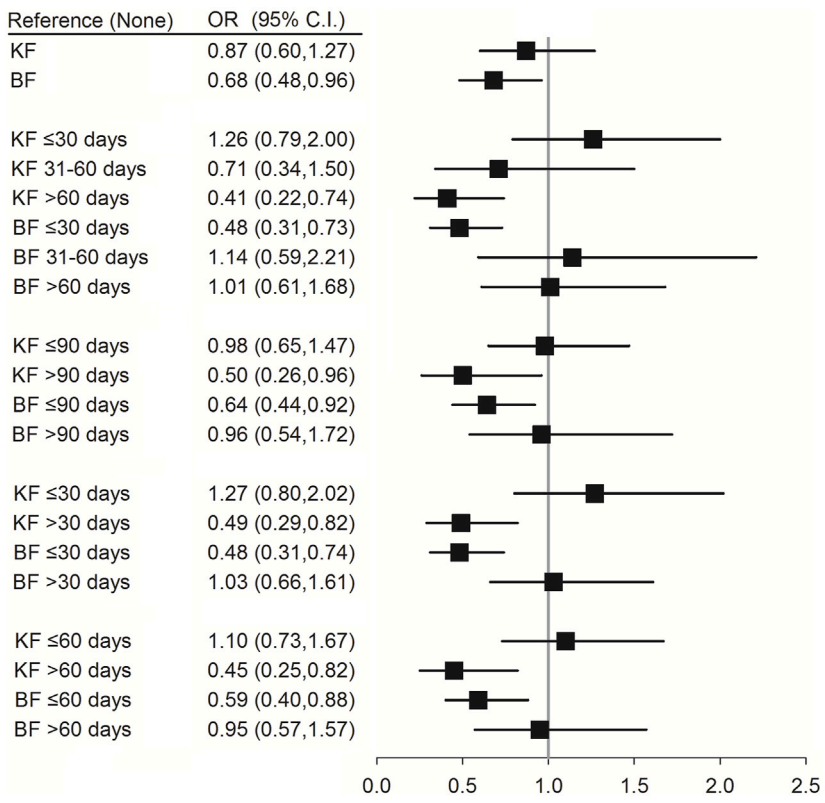

FIGURE 4
Conditional logistic regression of risk of pneumonia by different formulae of TCM. † Adjusted for hypertension, hyperlipidemia, chronic liver disease, chronic kidney disease, diabetes, chronic obstructive pulmonary disease, rheumatoid arthritis, ankylosing spondylitis, corticosteroids, NSAIDs, hydroxychloroquine, and methotrexate. KF: Chinese formulae tonifying the kidney; BF: Chinese formulae activating blood circulation.

Hammad, 2020); therefore, TCM use for >60 days could significantly reduce the risk of pneumonia infection in younger patients with SLE (age <65 years).

The formulae of the KF group have been used for treating chronic diseases, COPD, bone marrow suppression, and osteoprosis (Shi et al., 2014; He et al., 2017; You et al., 2019; Gao et al., 2020). In the present study, the use of formulae of the KF group for >60 days significantly reduced the risk of pneumonia infection in patients with SLE. A similar result was observed with the use of the same formulae for >90 days. Lupus nephritis affects nearly half of the patients with SLE and is one of the most commom contributors of patient mortality in SLE. In addition, SLE usually occurs in female patients during adolescence and pregnancy who are predisposed toward kidney yin deficiency (for patients with deficiency syndrome) and stagnation in the blood (for patients with excess syndrome). Furthermore, female patients easily develop kidney and liver yin deficiency during and after menopause. As per TCM, these deficiencies should be treated with a long course of TCM therapy. The formulae of the BF group have been used for treating rheumatoid arthritis, psoriasis, and coronary heart disease (Qiu et al., 2005; Gong et al., 2021; Liu et al., 2021). In the present study, a 46% reduction in the risk of pneumonia infection was observed in patients with SLE who received BF formulae for <30 days (OR = 0.48; $p \leq 0.001$). BF formulae are usually prescribed for patients with SLE who also have arthritis, facial erythema, and erythema nodosa. Furthermore, these diseases, which belong to excess syndrome, can be ameliorated in a short period of time. However, using these formulae for a long period would consume healthy Qi. Jieduquyuziyin prescription, which contains both herbs from KF and BF formulae, has been shown to ameliorate SLE in MRL/lpr mice by inhibiting the expression of the IRAK1-NF-κB and PI3K/Akt/PGC-1α signaling pathways (Ji et al., 2020; Ji et al., 2022). Therefore, the underlying mechanisms by which TCM therapies may reduce the risk of pneumonia in patients with SLE need further investigations in the future. Moreover, more randomized clinical trials using TCM and therapies and conventional treatment would be the better way to provide the robust evidence.

However, the study had several limitations, which are inherent to observational studies and registries. For instance, detailed information regarding the SLE disease activity and the laboratory results of parameters such as anti-dsDNA, C3, and C4 were lacking. Moreover, the medication doses for each traditional Chinese herbal formula could not be fully accessed in the database. This study used a set of ICD codes to capture all types of pneumonia that occurred 1 year after the diagnosis of SLE, which may have inevitably included pneumonia caused by opportunistic pathogens as well as common pathogens, thereby confounding the results. In addition, the included study population was Taiwanese, and there may be regional differences in other regions of China and other eastern countries, including Korea and Japan, where TCM is popular for treating certain chronic diseases. Finally, the lack of some individual factors, such as the smoking status, blood pressure, body mass index,

patient lifestyle, and environmental factors, may have potentially led to unmeasured confounding.

Conclusion

The present population-based cohort study revealed that the use of TCM could reduce the risk of pneumonia in patients with SLE. In particular, the use of KF formulae for >90 days and BF formulae for <30 days are recommended in the TCM treatment of SLE. Further randomized clinical trials are needed to confirm these results.

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 studies involving human participants were reviewed and approved by Institutional Review Board of the Chung Shan Medical University Hospital, No. CS1-20201. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Study conception: WW and JC-CW. Statistical expertise: Y-HW. Data interpretation: WW and JC-CW. Drafting of article: WW. Collecting references: KY and XY. Critical revision of article: XW and JC-CW. All authors contributed to the article and approved the submitted version.

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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.2023.1185809/full#supplementary-material

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Identifying subgroups of patients with type 2 diabetes based on real-world traditional chinese medicine electronic medical records

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Introduction: Type 2 diabetes (T2D) is a multifactorial complex chronic disease with a high prevalence worldwide, and Type 2 diabetes patients with different comorbidities often present multiple phenotypes in the clinic. Thus, there is a pressing need to improve understanding of the complexity of the clinical Type 2 diabetes population to help identify more accurate disease subtypes for personalized treatment.

Methods: Here, utilizing the traditional Chinese medicine (TCM) clinical electronic medical records (EMRs) of 2137 Type 2 diabetes inpatients, we followed a heterogeneous medical record network (HEMnet) framework to construct heterogeneous medical record networks by integrating the clinical features from the electronic medical records, molecular interaction networks and domain knowledge.

Results: Of the 2137 Type 2 diabetes patients, 1347 were male (63.03%), and 790 were female (36.97%). Using the HEMnet method, we obtained eight non-overlapping patient subgroups. For example, in H3, Poria, Astragali Radix, Glycyrrhizae Radix et Rhizoma, Cinnamomi Ramulus, and Liriopes Radix were identified as significant botanical drugs. Cardiovascular diseases (CVDs) were found to be significant comorbidities. Furthermore, enrichment analysis showed that there were six overlapping pathways and eight overlapping Gene Ontology terms among the herbs, comorbidities, and Type 2 diabetes in H3.

Discussion: Our results demonstrate that identification of the Type 2 diabetes subgroup based on the HEMnet method can provide important guidance for the clinical use of herbal prescriptions and that this method can be used for other complex diseases.

KEYWORDS

type 2 dabetes, real-world clinical data, heterogeneous medical record network method, traditional chinese medcine, enrichment analysis

1 Introduction

Type 2 diabetes (T2D) is the most common type of diabetes and accounts for approximately 90% of all diabetes cases worldwide; T2D is a complex, serious and multifactorial chronic disease that has become an increasingly prevalent health issue and imposes a tremendous economic burden worldwide (Li et al., 2015; International Diabetes Federation IDF, 2019). People with T2D have an approximately 15% higher overall excess mortality risk than people who do not have T2D (Tancredi et al., 2015). Although T2D is defined by a single metabolite, glucose, it is increasingly recognized as a highly heterogeneous disease with varying clinical manifestations (Gregg et al., 2014; World Health Organization, 2019a; Ahlqvist et al., 2021). Therefore, identifying the precise subtypes of T2D patients would be important for preventing serious complications, predicting individualized drug responses and improving health outcomes for patients with diabetes in the early stage and help predict the drug responses of patients with diabetes (Pigeyre et al., 2022; Williams et al., 2022).

Precision medicine has been recognized as a new medical approach for refining the disease taxonomy and improving the healthcare capability (National Research Council US, 2011; Zhou et al., 2018). Recently, several studies have identified new subtypes of T2D through data-driven analysis of a clinical population, which has improved the understanding of T2D with the goal of improving patient care in clinical settings (Li et al., 2015; Ahlqvist et al., 2018). These studies suggested that there are opportunities to further refine the current definition of T2D in real-world clinical settings into additional subtypes (American Diabetes Association, 2010). Traditional Chinese medicine (TCM) is a typical kind of personalized medicine (Jiang et al., 2012; Zhou et al., 2014) that classifies disease conditions into different subtypes (i.e., syndromes) through the comprehensive analysis of symptom phenotypes identified by the four main diagnostic TCM procedures (observation, listening, questioning, and pulse analyses). Furthermore, individualized treatment (in most cases, with herbal prescriptions) would be ordered for patients according to the diagnosis of syndromes. This clinical framework presents a novel view of disease conditions from symptom profiles and herbal prescriptions for patients.

In this study, we collected large-scale real-world TCM clinical data on T2D and used an established heterogeneous medical record network (HEMnet) (Edward et al., 2017) method to identify the clinical subgroups of T2D. Four types of clinical features, namely, symptom phenotypes, syndrome diagnoses, herbal prescriptions and comorbid disease conditions, together with phenotype–genotype associations and botanical drug -efficacy relationships, were incorporated into the HEMnet approach to help identify clinical groups with both clinical meaningfulness and biological insights. Enrichment analysis was used to identify the significant features of the clinical characteristics and molecular pathways of the T2D patient groups. Our findings are expected to help refine the understanding of T2D by both improving personalized treatment and identifying the underlying mechanisms.

2 Materials and methods

2.1 Clinical data and preprocessing

The data of 2137 inpatients diagnosed with T2D were collected from the EMR database of the Second Affiliated Hospital of Shandong University of TCM from 2016 to 2021, which included all inpatient information obtained during hospitalization, such as demographic information, symptoms, laboratory or physical tests, diagnoses and treatment. Because most data were in free text that cannot be used directly for analysis, we used a clinical information extraction tool (Shu et al., 2019) to efficiently extract the biomedical entities (e.g., symptoms, diseases) from these records. Then, to normalize the various clinical term descriptions, we manually checked and standardized the terms "disease", "botanical drug" and "drug" by referring to the 10th Revision of International Classification of Diseases (ICD-10) (World Health Organization, 2019b), the Pharmacopoeia of the People's Republic of China 2020 Revision (ChP 2020) (Chinese Pharmacopoeia Commission, 2020), and DrugBank Online (Wishart et al., 2018), respectively. In addition, diseases with detailed ICD-10 codes were further aggregated into higher level codes. For example, the ICD-10 codes I50.903 and I50.905 were aggregated into ICD-10 code I50.9.

2.2 External data sources

In this study, several external data sources were used to support this research. The efficacy of botanical drugs was extracted from ChP 2020, and human protein–protein interactions (PPIs) were obtained from the STRING database (Szklarczyk et al., 2019). The phenotype–genotype and botanical drug–target associations were extracted from the SymMap database (Wu et al., 2019). The disease–gene associations were extracted from the MalaCards database (Rappaport et al., 2017).

2.3 The HEMnet method

Missing data and semantic mismatch were the two main challenges of EMR analysis. Therefore, we used HEMnet to address the challenges of EMR analysis by leveraging information from several external sources to supplement clinical data (Edward et al., 2017). In our study, we utilized three distinct categories of edges to create the HEMnet (Figure 1). The first two categories PPI and phenotype–genotype were drawn from the external database, while the last category was drawn directly from the EMRs.

1) PPI. This network was based on HumanNet, an external network of protein-encoding genes (Lee et al., 2011). The nodes are proteins, and the undirected edges are the interactions between proteins.
2) Phenotype–genotype associations. This network was obtained from SymMap. The nodes were phenotype or genotype, and the undirected edges were the association of the phenotype and genotype.

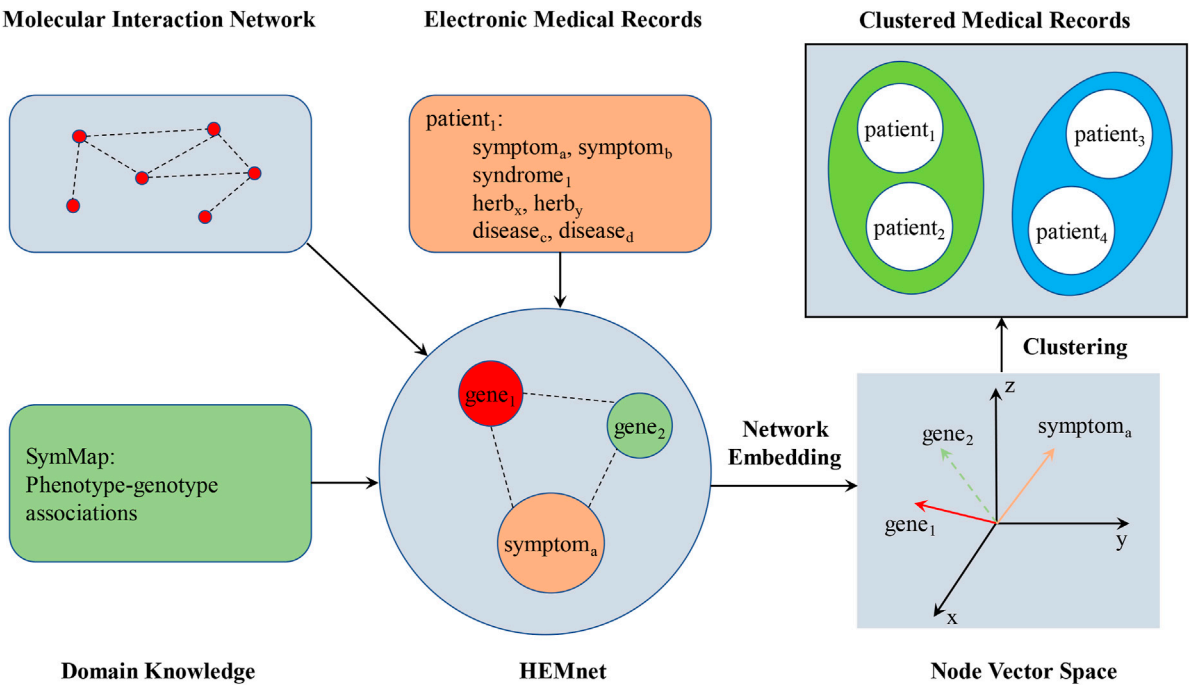

FIGURE 1
The pipeline for the HEMnet.

3) Co-occurrence of clinical entities from the EMR. We directly added the clinical cooccurrence edges of botanical drugs from each medical record. The missing data was one of the main challenges of electronic medical records (EMR) analysis, especially the lack of symptom information. Botanical drugs can represent symptom precision to address missing symptom information in EMR. We repeated this for all clinical features in each patient's medical record.

Then, HEMnet uses an embedding method, ProSNet (Wang et al., 2017), to infer relationships among its constituent nodes. ProSNet takes a heterogeneous network as input, on which it performs a novel dimensionality reduction algorithm to optimize a low-dimensional vector representation for each node. The vectors of two nodes are colocalized in the low-dimensional space if the nodes are close to each other in the heterogeneous network. After generating low-dimensional vector representations of nodes in the HEMnet, a similarity matrix was constructed according to the similarity between every two embedding vector features, which was calculated by cosine similarity. Finally, the similarity matrix was used to fill in missing features of the original patient characteristics and form the phenotypes of patients (Edward et al., 2017).

The K-means clustering (MacQueen, 1967) was used for the patient phenotype. According to the outcomes, the patients were divided into eight non-overlapping subgroups. The t-distributed stochastic neighbour embedding (t-SNE) algorithm (Cieslak et al., 2020) was used to visualize the outcomes.

The chi-square test and relative risk (RR) (Pirhaji et al., 2008; Ouimet et al., 2010) were used to assess the significance of clinical features, including symptom phenotypes, syndrome diagnoses, botanical drugs and comorbidities in eight subgroups. In this study, patients with a certain clinical feature, such as a symptom phenotype, in a particular subgroup as an exposed group, and the remaining patients with this certain clinical feature as the non-exposed group. So RR is defined as $RR = (C_{ij}/C_i)/((C_j-C_{ij})/(N-C_i))$, where C_i is the number of patients in subgroup i, C_j is the number of patients with a clinical feature j, C_{ij} represents the number of patients in subgroup i and with a clinical feature j and N is the total number of patients in the study. A p-value <0.05, which was obtained from the chi-square test, and an RR > 1 indicated that a clinical feature was truly significant.

2.4 Gene ontology (GO) and KEGG pathway enrichment analysis

The GO and KEGG pathway enrichment analysis are useful to trackle the DNA-related and protein-related problems. And they offers considerable power for discovering the biological functions of genes and proteins (Chen et al., 2017). The Gene Ontology (GO) project serves as a comprehensive source for functional genomics. The project creates evidence-supported annotations to describe the biological roles of individual genome products (e.g., genes, proteins, ncRNAs, complexes) (Gene Ontology Consortium, 2015). The KEGG pathway database is the main database in Kyoto Encyclopedia of Genes and Genomes (KEGG), and it consists of manually drawn reference pathway maps together with organism-specific pathway maps (Kanehisa et al., 2017). We obtained enriched GO and KEGG pathways using the Database for Annotation, Visualization, and Integrated Discovery (DAVID), which is a web-based online

TABLE 1 The characteristics of the 2137 T2D inpatients.

Characteristics		n (%)/(mean ± SD)
Sex	Male	1347 (63.03)
	Female	790 (36.97)
Age		66.31 ± 11.44
Age group	<20	1 (0.05)
	20–39	30 (1.40)
	40–59	527 (24.66)
	60–79	1295 (60.60)
	≥80	284 (13.29)
LOS		14.08 ± 9.20
LOS group	1–7	495 (23.16)
	8–14	894 (41.83)
	15–21	391 (18.30)
	22–28	186 (8.70)
	≥29	171 (8.00)
Number of comorbidities	1–5	764 (35.75)
	6–10	1206 (56.43)
	≥11	167 (7.81)

bioinformatics resource that aims to provide tools for the functional interpretation of large lists of genes/proteins (Sherman et al., 2022).

3 Results

3.1 Basic characteristics

As shown in the table below (Table 1), of the 2137 T2D patients, 1347 (63.03%) were male, and 790 (36.97%) were female. The ages of most T2D patients (60.60%) were between 60 and 79 years old. The average length of stay (LOS) was 14.08 ± 9.20, and for most patients (41.83%), LOS was between 8 and 14 days. We counted the distinct number of comorbidities of each patient and found that most patients had 6–10 diagnoses (56.43%).

Then, we analysed the distribution of the top five clinical features including symptom phenotypes, syndrome diagnoses, botanical drugs, and comorbidities (Table 2).

3.2 The result of the HEMnet

With the method introduced in the Materials and Methods, we utilized three distinct categories of edges to create the HEMnet, which contained 5,846 nodes and 125,426 connected edges. There were 3,000 symptom nodes and 2,846 gene nodes. Furthermore, there were 16,641 PPI edges, 8,749 phenotype–genotype edges, and 100,036 symptom edges.

Then, the embedding method ProSNet was used to generate low-dimensional vector representations of nodes in the HEMnet. A similarity matrix was constructed according to the similarity between every two embedding vector features, which was calculated by cosine similarity, and used to fill in missing features of the original patient characteristics to form the patient phenotypes. Finally, using the K-means clustering algorithm, eight non-overlapping patient subgroups were obtained. The t-SNE algorithm was used to visualize the clustering results (Figure 2). The numbers of patients in the eight subgroups were as follows (Table 3): H1 (n = 547, 25.60%), H2 (n = 501, 23.44%), H3 (n = 432, 20.22%), H4 (n = 298, 13.94%), H5 (n = 197, 9.22%), H6 (n = 132, 6.18%), H7 (n = 18, 0.84%), and H8 (n = 12, 0.56%).

3.3 The significant clinical features of the subgroups

We then selected the top 10 clinical features in these modules according to their frequency in each subgroup. Then, the RR and chi-square test (RR > 1 and $p < 0.05$, see Materials and methods) were used to screen the significant clinical features.

Because of fewer patients in H7 and H8 subgroups, it was less meaningful to analyse them. And since this study focused on the precision treatment of comorbidities, the H1, H2, and H4 subgroups with no significant botanical drugs and the H5 subgroup with a lower frequency of botanical drug use were excluded according to the screening results. Finally, H3 and H6 were included for further analysis.

We present the statistically significant botanical drugs, comorbidities, syndromes, and symptoms in H3 and H6 (Table 4, Table 5, Table 6, and Table 7), Poria, Astragali Radix, Glycyrrhizae Radix et Rhizoma, Cinnamomi Ramulus, and Ophiopogonis radix were the significant botanical drugs. Essential (primary) hypertension, atherosclerotic heart disease, heart failure, unstable angina, etc., were the significant comorbidities. Qi-Yin deficiency was the main significant syndrome. And chest tightness, fever, coarse lung breathing, vomiting, expectoration, etc., were the significant symptoms. In H6, Chuanxiong Rhizoma, Gastrodiae Rhizoma, and Baked Ziziphi Spinosae Semen were the significant botanical drugs. Cerebral infarction, sequelae of cerebral infarction and sequelae of intracerebral haemorrhage were the significant comorbidities. Deficient qi and blood stasis was the main significant syndrome. And poor physical activity, fever, slurring of speech, vomiting, etc., were the significant symptoms.

3.4 Significant GO terms and pathways for H3 and H6

In this part, we explored the shared molecular associations between the significant botanical drugs and comorbidities of T2D in H3 and H6. First, we identified the distinct genes associated with each significant botanical drug and comorbidity in H3 and H6 from an external database (see Materials and methods). Then, we obtained the pathways and GO terms for the botanical drugs, comorbidities and T2D in H3 and H6 by the DAVID program (2021, see Materials and methods). Finally, we screened out pathways and GO terms with $p < 0.05$ from botanical drugs, comorbidities and T2D. We identified the overlapping pathways

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TABLE 2 The top five clinical features.

Clinical features		n (%)
Symptom phenotypes	Insomnia	763 (35.70)
	Poor absorbing	487 (22.79)
	Lack of energy	416 (19.47)
	Chest tightness	239 (11.18)
	Constipation	215 (10.06)
Syndrome diagnoses	Deficient qi and blood stasis	618 (28.92)
	Qi-Yin deficiency	247 (11.56)
	Qi stagnation and blood stasis	97 (4.54)
	Blood stasis	77 (3.60)
	Wind and phlegm blocked channel	43 (2.01)
Botanical drug	Poria	1294 (60.55)
	Astragali radix	1133 (53.02)
	Angelicae sinensis radix	1073 (50.21)
	Glycyrrhizae radix et rhizoma	969 (45.34)
	Glycyrrhizae radix et rhizoma praeparata cum melle	848 (39.68)
Comorbidities	Essential (primary) hypertension	1569 (73.42)
	Atherosclerotic heart disease	1127 (52.74)
	Cerebral infarction	743 (34.75)
	Heart failure	664 (31.07)
	Unstable angina	429 (20.07)

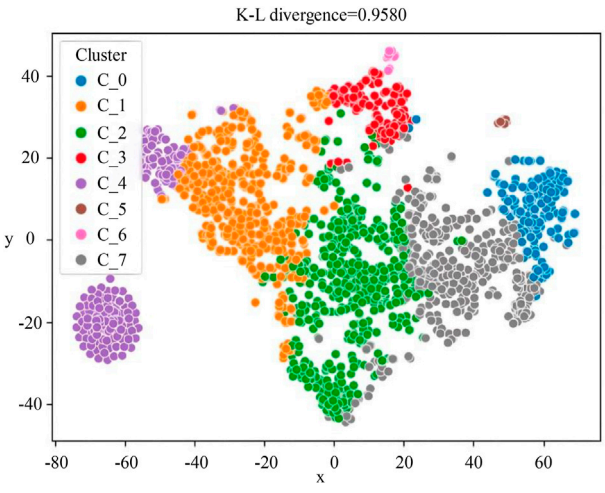

FIGURE 2
The visualized clustering result of HEMnet. The correspondence between the C_0-C_7 clusters in the figure and the H1-H8 subgroups in this paper is as follows: C_0 = H5, C_1 = H2, C_2 = H1, C_3 = H6, C_4 = H4, C_5 = H8, C_6 = H7, C_7 = H3. This picture was to reduce the dimensionality of the patient's characterization vector to a two-dimensional vector for display. So the *x*-axis and *y*-axis represent the patient's characterization vector, and the closer the two points are, the closer the patient's characteristics are.

TABLE 3 The numbers of patients in the eight subgroups.

Subgroups	n (%)
H1	547 (25.60)
H2	501 (23.44)
H3	432 (20.22)
H4	298 (13.94)
H5	197 (9.22)
H6	132 (6.18)
H7	18 (0.84)
H8	12 (0.56)

and GO terms among the botanical drugs, comorbidities, and T2D in H3 and H6 (Table 8 and Table 9). In H3, there were six overlapping pathways and eight overlapping GO terms among the botanical drugs, comorbidities, and T2D. In H6, there were no overlapping pathways among the botanical drugs, comorbidities, and T2D. Therefore, we reported on the pathways that overlapped between the two of them. There was only one overlapping GO term among the botanical drugs, comorbidities, and T2D. For example, most of the pathways and GO functions in H3 were associated with

TABLE 4 The significant botanical drugs in H3 and H6.

Subgroup	Botanical drug	n (%)	*p*	RR
H3	Poria	150 (34.72)	4.35E-03	1.25
	Astragali Radix	131 (30.32)	1.36E-02	1.24
	Glycyrrhizae Radix et Rhizoma	118 (27.31)	5.74E-03	1.29
	Cinnamomi Ramulus	100 (23.15)	1.50E-02	1.29
	Ophiopogonis radix	89 (20.60)	2.72E-02	1.28
H6	Chuanxiong Rhizoma	35 (26.52)	2.84E-02	1.41
	Gastrodiae Rhizoma	28 (21.21)	1.01E-09	3.17
	Baked Ziziphi Spinosae Semen	24 (18.18)	1.62E-03	1.89

TABLE 5 The significant comorbidities in H3 and H6.

Subgroup	Comorbidity	n (%)	*p*	RR
H3	Essential (primary) Hypertension	344 (79.63)	1.07E-03	1.11
	Atherosclerotic Heart Disease	265 (61.34)	6.05E-05	1.21
	Heart Failure	194 (44.91)	3.48E-12	1.63
	Unstable Angina	127 (29.40)	6.09E-08	1.66
	Cardiac Arrhythmia	65 (15.05)	3.24E-04	1.64
	Atrial Fibrillation and Flutter	52 (12.04)	6.80E-03	1.52
H6	Cerebral Infarction	93 (70.45)	6.21E-19	2.17
	Sequelae of Cerebral Infarction	23 (17.42)	3.00E-08	3.21
	Sequelae of Intracerebral Haemorrhage	17 (12.88)	4.60E-25	15.19

T2D, such as type II diabetes mellitus, insulin resistance, glucose metabolic process, and response to glucose. The significant botanical drugs in H3 had some overlapping pathways and GO terms with comorbidities and T2D.

4 Discussion

In recent years, the continual growth of EMR databases has facilitated clinical research, paved the way for data mining applications, and supported population health. However, missing data is the biggest barrier to using EMRs (Kruse et al., 2018). In our study, the problem of missing data and semantic mismatch in EMRs posed a considerable challenge. For example, if T2D was not the primary diagnosis, the patient's T2D-related symptoms would not be recorded in the medical record, which results in incomplete information in the patient's medical record. Furthermore, the overabundant expression of symptoms, diagnoses, botanical drugs, and syndromes in clinical TCM data leads to mismatched records containing semantically similar but lexically distinct terms. Therefore, the problem of missing data and semantic mismatch were solved by standardizing the data and creating the HEMnet to ensure the reliability of the research results (Edward et al., 2017).

Analysing disease comorbidities with EMR data has become popular in real-world clinical settings for chronic disease conditions such as T2D and chronic liver diseases (Li et al., 2015; Ahlqvist et al., 2018; Shu et al., 2019; Mansour Aly et al., 2021). In this manuscript, the HEMnet method was used to identify the eight non-overlapping patient subgroups. Then, H3 and H6 were screened according to a specific screening strategy for subgroups to further analyse the clinical features. For example, cardiovascular disease (CVD), such as atherosclerotic heart disease, heart failure, unstable angina, cardiac arrhythmia, atrial fibrillation and flutter, was a significant comorbidity of T2D in H3. In large prospective trials, T2D has been identified as a significant risk factor for CVD, including stroke, angina, heart failure, myocardial infarction, and atherosclerosis (Emerging Risk Factors Collaboration Sarwar et al., 2010; Peters et al., 2014; Shah et al., 2015; Einarson et al., 2018). Regarding treatment, Poria, Astragali radix, Glycyrrhizae radix et rhizoma, Cinnamomi ramulus, and Ophiopogonis radix were the significant botanical drugs in H3. And studies have shown that these botanical drugs used alone or in combination with other botanical drugs are often used to treat diabetes as well as other disorders (Jia et al., 2003; Li et al., 2004; Lindequist et al., 2005).

Furthermore, to explore the shared molecular associations among the significant botanical drugs, comorbidities and T2D in H3 and H6, we explored the overlapping pathways and GO terms between the significant botanical drugs and comorbidities of T2D in H3 and H6. The significant botanical drugs in H3 had six pathways and eight GO terms that overlapped between comorbidities and T2D. This result

TABLE 6 The significance syndromes in H3 and H6.

Subgroup	Syndrome	n (%)	*p*	RR
H3	Qi-Yin deficiency	63 (14.58)	2.77E-02	1.35
	Qi-blood deficiency	9 (2.08)	2.02E-02	2.96
	Defideficiency of spleen and kidney	9 (2.08)	4.75E-05	8.88
	Wind-cold attacking lung	7 (1.62)	7.31E-03	4.60
	Phlegm-damp obstructing lung	6 (1.39)	1.37E-02	4.74
	Phlegm-heat obstructing lung	6 (1.39)	6.04E-03	5.92
H6	Deficient qi and blood stasis	70 (53.03)	3.44E-10	1.93
	Wind and phlegm bloke channel	13 (9.85)	2.99E-10	6.58
	Phlegm and blood stasis blocking collaterals	8 (6.06)	6.89E-05	4.86
	Blood stasis blocking collaterals	7 (5.30)	1.58E-03	3.94
	Deficiency of liver and kidney	5 (3.79)	6.18E-06	10.85
	Stirring wind due to yin deficiency	4 (3.03)	1.41E-06	20.25
	Kidney deficiency	2 (1.52)	1.60E-03	30.38

TABLE 7 The significant symptoms in H3 and H6.

Subgroup	Symptom	n (%)	*p*	RR
H3	Chest tightness	315 (72.92)	4.56E-33	1.79
	Fever	268 (62.04)	7.32E-10	1.36
	Coarse lung breathing	253 (58.56)	6.36E-25	1.85
	Vomiting	250 (57.87)	2.01E-16	1.60
	Expectoration	242 (56.02)	5.11E-25	1.90
	Dizziness	231 (53.47)	1.50E-09	1.43
	Fatigue	225 (52.08)	5.34E-11	1.49
	Insomnia	224 (51.85)	1.42E-23	1.94
	Cough	218 (50.46)	9.21E-22	1.90
	Headache	156 (36.11)	6.33E-08	1.55
H6	Poor physical activity	103 (78.03)	1.86E-164	14.90
	Fever	78 (59.09)	1.47E-02	1.23
	Slurring of speech	76 (57.57)	3.18E-80	8.55
	Vomitting	69 (52.27)	4.53E-03	1.32
	Poor activity	66 (50.00)	8.53E-66	8.08
	Fatigue	64 (48.48)	1.36E-02	1.29
	Coarse lung breathing	63 (47.73)	9.46E-03	1.31
	Disability of left limbs	55 (41.67)	2.35E-104	21.98
	Choking cough	50 (37.88)	9.95E-36	5.75

indicated that these botanical drugs may have therapeutic effects on comorbidities and T2D via the pathways and GO terms identified in the analysis. For example, the overlapping pathways in H3 inculded insulin resistance which is one shared defect in T2D and Essential (primary) Hypertension. Although the mechanisms by which defective insulin action *per se* contributes to high blood pressure are still somewhat uncertain (Ferrannini and Cushman, 2012). But previous studies have demonstrated that within the physiological concentration range of insulin, it causes slight increases in limb blood flow by enhancing the release of nitric oxide (via stimulation of nitric oxide synthase activity in endothelial cells) and by potentiating acetylcholine-induced vasodilation. In people with insulin resistance, vasodilation in response to supraphysiological insulin concentrations is reduced (Taddei et al., 1995; Yki-Järvinen and Utriainen, 1998; Steinberg and Baron, 2002; Giacco and Brownlee, 2010). Astragaloside Ⅳ (AST Ⅳ, chemical formula: C41H68O14, molecular weight:785), as the primary active ingredient of Astragali radix, has the pharmacological effects of regulating lipid and carbohydrate metabolism and improving insulin resistance. Previous studies have shown that AST Ⅳ improvement of insulin resistance may be related to activation of the IRS1/protein kinase B (AKT) insulin signaling pathway to increase the glucose transporter type 4 (GLUT4) activity, thus increasing glucose uptake and insulin sensitivity (Zhou et al., 2021). So the main findings of the GO and KEGG pathway enrichment analysis require further experimental verification.

Our study has several potential limitations. Our sample included only 2137 hospitalized patients, resulting in an insufficient number of patients with some subtypes of T2D for identification of additional significant TCM phenotypes. In future studies, more patients should be included to ensure the abundance of the results. Another limitation is that Western medicine and laboratory tests were not included in our study. Therefore, the resulting disease subtypes would incorporate little information on these features. In addition, some patients were not given herbal prescriptions. This might affect the results of data mining. Finally, we used EMRs from only one hospital, and the resulting patient subgroups that were identified may not be representative. And further experiments should be performed to verify the results of this paper (Sheng et al., 2021).

TABLE 8 The overlapping pathways among the botanical drugs, comorbidities, and T2D in H3 and H6.

Subgroup	Pathway	Botanical drug	Comorbidity	T2D
H3	cGMP-PKG signalling pathway	1.26E-02	4.25E-13	2.71E-02
	Diabetic cardiomyopathy	3.24E-05	2.27E-03	7.76E-03
	Insulin resistance	1.82E-07	2.30E-03	1.94E-10
	MicroRNAs in cancer	6.13E-03	2.21E-03	1.59E-04
	Regulation of lipolysis in adipocytes	3.43E-04	3.96E-05	1.32E-03
	Type II diabetes mellitus	1.02E-04	1.13E-04	6.42E-14
H6	Adipocytokine signalling pathway	4.96E-02	ns	2.41E-03
	Diabetic cardiomyopathy	ns	6.53E-03	7.76E-03
	FoxO signalling pathway	2.23E-02	ns	1.41E-04

Ns: not significant.

TABLE 9 The overlapping GO terms among the botanical drugs, comorbidities, and T2D in H3 and H6.

Subgroup	GO	Botanical drug	Comorbidity	T2D	Category
H3	glucose metabolic process	1.03E-08	3.24E-03	1.49E-05	BP
	liver development	1.31E-03	1.93E-04	1.13E-03	BP
	negative regulation of gene expression	3.93E-07	3.31E-09	5.93E-04	BP
	positive regulation of cell proliferation	1.51E-15	4.49E-03	1.30E-03	BP
	positive regulation of gene expression	5.73E-16	1.68E-09	9.17E-04	BP
	response to drug	2.72E-33	7.92E-05	4.48E-05	BP
	response to glucose	5.33E-07	5.09E-03	1.68E-08	BP
	response to xenobiotic stimulus	2.51E-30	7.37E-05	2.01E-03	BP
H6	response to xenobiotic stimulus	9.85E-04	3.15E-02	2.01E-03	BP

5 Conclusion

Our results demonstrate that Cardiovascular disease (CVD) and Qi-Yin deficiency syndrome were significant comorbidity and TCM syndrome of T2D in subgroup H3, respectively. Regarding treatment, Poria, Astragali radix, Glycyrrhizae radix et rhizoma, Cinnamomi ramulus, and Ophiopogonis radix were the significant botanical drugs in subgroup H3. In subgroup H6, cerebral infarction and its sequelae, Qi deficiency and blood stasis syndrome were significant comorbidities and TCM syndrome, respectively. Regarding treatment, Chuanxiong rhizoma, Gastrodiae rhizoma, and Baked ziziphi spinosae semen were the significant botanical drugs. So identification of the T2D subgroup based on the HEMnet method can provide important guidance for the clinical use of herbal prescriptions and that this method can be used for other complex diseases.

Data availability statement

The raw dataset obtained from the electronic medical record of the hospital presented in this article is not available because of local legislation and institutional requirements. Requests to access the datasets should be directed to the corresponding author. The external datasets, such as human protein-protein interactions, phenotype-genotype, and botanical drug-target associations supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

SZ was in charge of writing and revising the paper. HL was responsible for the data analysis and data mining of the paper. XJ

was in charge of polishing the paper. XZ extracted the external data sources, such as the efficacy of herbs, human protein-protein interactions, and phenotype-genotype. RL, YL, CL, JC, and GL structured the text and extracted biomedical entities from electronic medical records of traditional Chinese medicine. WZ, QL, LW, XW, and YS standardized the data of symptoms, herbs, syndromes, and diseases. SW and YX were responsible for the design of the paper. All authors contributed to the article and approved the submitted version.

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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 TW declared a shared parent affiliation with the authors XZ, SW to the handling editor at the time of review.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2023.1210667/full#supplementary-material

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A retrospective study of Reyanning mixture in elderly patients infected with SARS-CoV-2 Omicron variant

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Objective: Reyanning mixture has been demonstrated to be effective in treating infected patients during the outbreak pandemic of SARS-CoV-2 Omicron variant of Coronavirus disease 2019 (COVID-19) in Shanghai 2022. The aim of this study is to further investigate the role of Reyanning mixture specifically in the treatment of elderly patients.

Methods: This study enrolled 1,102 elderly patients who were infected with SARS-CoV-2 Omicron variant. Of these, 291 patients received Reyanning mixture in conjunction with conventional Western medicine treatment were assigned to the treatment group, while 811 patients only received conventional Western medicine treatment were assigned to the control group. Clinical parameters including hospitalization duration, viral shedding time, and Cycle Threshold (Ct) values of novel coronavirus nucleic acid tests, as well as adverse events were recorded and analyzed in both groups.

Results: There was no significant difference in baseline characteristics between two groups. In comparison to the control group, the treatment group demonstrated a substantial difference in hospitalization duration (median: 8 days vs. 10 days, HR: 0.638, 95% CI: 0.558–0.731, $p < 0.001$). The treatment group also showed a significantly shorter viral shedding time compared to the control group (median: 7 days vs. 8 days, HR: 0.754, 95% CI: 0.659–0.863, $p < 0.001$). Multivariate Cox proportional-hazards model analysis indicated that the use of Reyanning mixture was closely associated with a reduction in hospitalization duration (HR: 1.562, 95% CI: 1.364–1.789, $p < 0.001$) and viral shedding time (HR: 1.335, 95% CI: 1.166–1.528, $p < 0.001$). In addition, during the treatment process, no serious adverse event occurred in either group.

Abbreviations: TCM, Traditional Chinese medicine; COVID-19, Coronavirus disease 2019; HR, Hazard ratio; CI, Confidence interval; Ct, Cycle Threshold; HPLC, High performance liquid chromatography; ORF, Open reading frame; N, Nucleocapsid.

Conclusion: The improvement of clinical parameters in the treatment group indicate a promising therapeutic benefit of Reyanning mixture for elderly patients infected with SARS-CoV-2 Omicron variant in the present study. Further investigations are required to validate this finding by examining the underlying mechanism and function of Reyanning mixture.

KEYWORDS

Reyanning mixture, elderly patients, hospitalization duration, COVID-19, Omicron variant, viral shedding time

Introduction

The outbreak pandemic of COVID-19 has raised significant impact to public health, resulted in widespread suffering across the globe (Teng et al., 2021; Zhang X. et al., 2022; Huang et al., 2022). During this period, vaccines and medications have been developed and implemented to combat the viral infection (Wang Y. et al., 2020; Xu et al., 2022b; Ciotti et al., 2022; Wong et al., 2022), and the SARS-CoV-2 Omicron variant infection appeared to become less virulent. However, the health of elderly people continues to be challenged, as they are more susceptible to severe outcome subsequent to infection (Zhang et al., 2022a), and available data indicated that advanced age and underlying health conditions confer the greatest risk for developing severe COVID-19 infection, which is linked to increased morbidity and mortality rates (Huemer et al., 2021; Ramasamy et al., 2021; Chen et al., 2022; Tong et al., 2022).

Traditional Chinese medicine (TCM) has been widely recognized as medical science with thousands of years, and incorporated as one of the therapeutic options for addressing COVID-19. Despite studies demonstrating the promising therapeutic effects of TCM and integrated traditional Chinese-Western medicine to treat COVID-19 (Yang Y. et al., 2020; Luo et al., 2020; Zhong et al., 2022), there is limited research specifically focus on the elderly patients' treatment, which is closely associated with reducing the incidence of severe disease and mortality. Reyanning mixture is a Chinese patent medicine, which composed of four botanical drugs: *Taraxacum mongolicum* Hand.-Mazz. [Asteraceae, Herba taraxaci], *Reynoutria japonica* Houtt. [Polygonaceae, Polygoni cuspidati rhizoma et radix], *Sonchus brachyotus* DC. [Asteraceae, Sonchus arenicola Vorosch.], and *Scutellaria barbata* D.Don [Lamiaceae, Herba scutellariae barbatae], has been recommended in "The Diagnosis and Treatment of New Coronavirus Infected Pneumonia of Shaanxi Province (Trial edition 2)" (Li et al., 2021b). And the efficacy in treating SARS-CoV-2 infection has been illustrated in our previous research (Xu et al., 2023). The objective of the current investigation is to examine the correlation between administration of Reyanning mixture and clinical outcomes among elderly patients.

Methods and materials

Recruitment of participants

All data were obtained from individuals who were hospitalized during the pandemic outbreak in Shanghai 2022. The dataset comprised demographic data, medical history, illness status, hospitalization duration, and results from coronavirus nucleic acid testing. This study was applied in compliance with the tenets of Good Clinical Practice and the Declaration of Helsinki, granted ethical approval by the Medical Ethics Committee (Approval number: JJSZYYY20220403) and was based on the registered clinical trial (ChiCTR2200060292). Informed consent has been collected from all patients.

The diagnostic criteria were applied according to the ninth trial edition guidelines for the diagnosis and treatment of COVID-19 (General Office of National Health Commission of the People's Republic of China, Office of National Administration of Traditional Chinese Medicine, 2022), as outlined below:

Diagnostic criteria.

1. Presence of relevant epidemiological history.
2. Presence of at least two of the following mentioned clinical symptoms:
   (i) fever and/or respiratory symptoms, or other clinical manifestations of COVID-19;
   (ii) imaging features in accordance with COVID-19;
   (iii) A normal or decreased total white blood cell count as well as lymphocyte count in the early stage of illness.
3. Presented with one of the following microbiological or serological evidence:
   (i) novel coronavirus nucleic acid detected positively;
   (ii) positive detection of both IgM and IgG antibodies to novel coronavirus in patients who have not been vaccinated.

Clinical presentations were also assessed according to the guideline criteria (General Office of National Health Commission of the People's Republic of China, Office of National Administration of Traditional Chinese Medicine, 2022), and an classification of the severity of the illness is presented below.

1. Mild: A mild clinical symptom without image evidence of pneumonia.
2. Common: Clinical manifestations mentioned above, in addition to image evidence of pneumonia.
3. Severe: Individuals who met one or more criteria as follow:
   (i) Shortness of breath, respiratory rate ≥30 breaths/minute;
   (ii) Oxygen saturation equal or below to 93% in a resting state;
   (iii) Arterial partial pressure of oxygen (PaO2)/fraction of inspired oxygen (FiO2) ≤300 mmHg;
   (iv) Progressive worsening of clinical symptoms and pulmonary imaging showing significant lesion progression >50% within 24–48 h.
4. Critical: Met any condition as follow:

(i) Individuals who suffered respiratory failure with requirement of mechanical ventilation;
(ii) Shock;
(iii) Individuals who suffered multiple organ dysfunction or failure and required intensive care.

We screened the data of all COVID-19 patients who received treatment during hospitalization and finally obtained the cases for this study, based on the criteria as follow:

Inclusion criteria.

1. An individual with an age greater than or equal to 60 years;
2. Patients diagnosed with mild or asymptomatic type of COVID-19 according to the diagnostic criteria;
3. Only received conventional Western medical therapy or combine with Reyanning mixture.

Exclusion criteria.

1. Diagnosed with severe type of COVID-19;
2. Deterioration of clinical symptoms or death within 48 h of admission;
3. Those who were suffering from severe underlying diseases;
4. A severe psychiatric disorder and medication was required;
5. Patient who received other TCM botanical drugs or participated in any other clinical trial beside Reyanning mixture;
6. Received any kind of antiviral, corticosteroid, or monoclonal antibody;
7. Discontinuation, intolerance or refusal to take Reyanning mixture.

The screened and enrolled patients were split into two groups. The treatment group was given Reyanning mixture combine with conventional Western medicine therapy, while the control group received conventional Western medicine based on the Guideline (General Office of National Health Commission of the People's Republic of China, Office of National Administration of Traditional Chinese Medicine, 2022). Furthermore, all participants were categorized into three subgroups according to their age.

Stratum Ⅰ: Age equal to/greater than 60 years and less than 65 years (≥60 years and <65 years).

Stratum Ⅱ: Age equal to/greater than 65 years and less than 70 years (≥65 years and <70 years).

Stratum Ⅲ: Age equal to/greater than 70 years (≥70 years).

Investigational medications

As a Type A extract (Heinrich et al., 2022), Reyanning mixture was produced by Xingfu Pharmaceutical Group Co., Ltd. (Xi'an, Shaanxi, China), received approval from the National Medical Product Administration of China in 2005 (Approval number: Z20050493), and has been included in the Chinese Pharmacopoeia. According to the Chinese Pharmacopoeia (2020 edition), pharmaceutical manufacturer obtained 372 g *Taraxacum mongolicum* Hand.-Mazz. (Asteraceae, Herba taraxaci), 372 g *Reynoutria japonica* Houtt. [Polygonaceae, Polygoni cuspidati rhizoma et radix], 372 g *Sonchus brachyotus* DC. [Asteraceae, Sonchus arenicola Vorosch.], and 186 g *Scutellaria barbata* D.Don [Lamiaceae, Herba scutellariae barbatae]. The four botanical drugs mentioned above were decocted twice with water. The first decoction lasted for 2 hrs, and the second for 1 h. The decoction was filtered, concentrated under reduced pressure to an appropriate volume and combined, centrifuged, filtered, and heated to boiling. Finally, 1,000 mL of the decoction was obtained. The quality control analysis of Reyanning mixture used high performance liquid chromatography (HPLC) (Heinrich et al., 2022) has been reported by Su et al. (2021). The primary metabolites of the Reyanning mixture including polydatin, emodin, luteolin, caffeic acid, and chlorogenic acid, have been qualitatively controlled and quantitatively assessed (Su et al., 2021), which were consistent with the Medicine Standards stipulated by the National Medical Products Administration of China.

The active metabolites such as chlorogenic acid, emodin, and caffeic acid, have been studied for their potential antiviral activity against COVID-19 (Adem et al., 2021; Shao et al., 2022; Wang et al., 2022). Polydatin has been found to bind Spike, ACE2, and ACE2, thereby hindering SARS-CoV-2 (Perrella et al., 2021). Moreover, luteolin has been experimentally evaluated against SARS-CoV-2's RNA-dependent RNA polymerase (Munafò et al., 2022), demonstrating its potential therapeutic effect for COVID-19.

The administration of Reyanning mixture involved oral dosages of 20 mL four times daily, started from the day of enrollment and continued for seven consecutive days. The remaining hospitalization period was followed by standard treatment. A detailed record was kept of the concurrent medications. The hospitalized patients were monitored until discharge, while patients who were discharged within 7 days were subjected to telephone follow-ups to monitor and report any unfavorable incidents. Patients who met the discharge criteria or experienced deterioration of the condition and required hospital transfer were considered to have fulfilled the criteria for study completion.

Evaluation of clinical outcomes

In both groups, pharyngeal swab NATs were performed daily for all the patients. The criteria for discharge was defined according to the Guideline (General Office of National Health Commission of the People's Republic of China, Office of National Administration of Traditional Chinese Medicine, 2022) as follows: 1) Body temperature maintained normal for at least three consecutive days; 2) Respiratory symptoms improved significantly; 3) Significant improvement in acute infiltrative lesions manifested with pulmonary imaging; 4) Two consecutive COVID-19 nucleic acid tests of N gene and ORF gene with a Ct value of ≥35, or two consecutive negative nucleic acid tests (a minimum sampling interval of 24 h was required).

The primary outcome for this study was hospitalization duration and viral shedding time. The hospitalization duration was measured from admission to the date of discharge. Viral shedding time was measured from the first positive result to the date on which the second consecutive negative nucleic acid test result was obtained. Additionally, the Ct values of the ORF and N genes between two groups were analyzed. The secondary outcome was the evaluation of the overall adverse events, adverse events related to the use of Reyanning mixture, as well as the deterioration

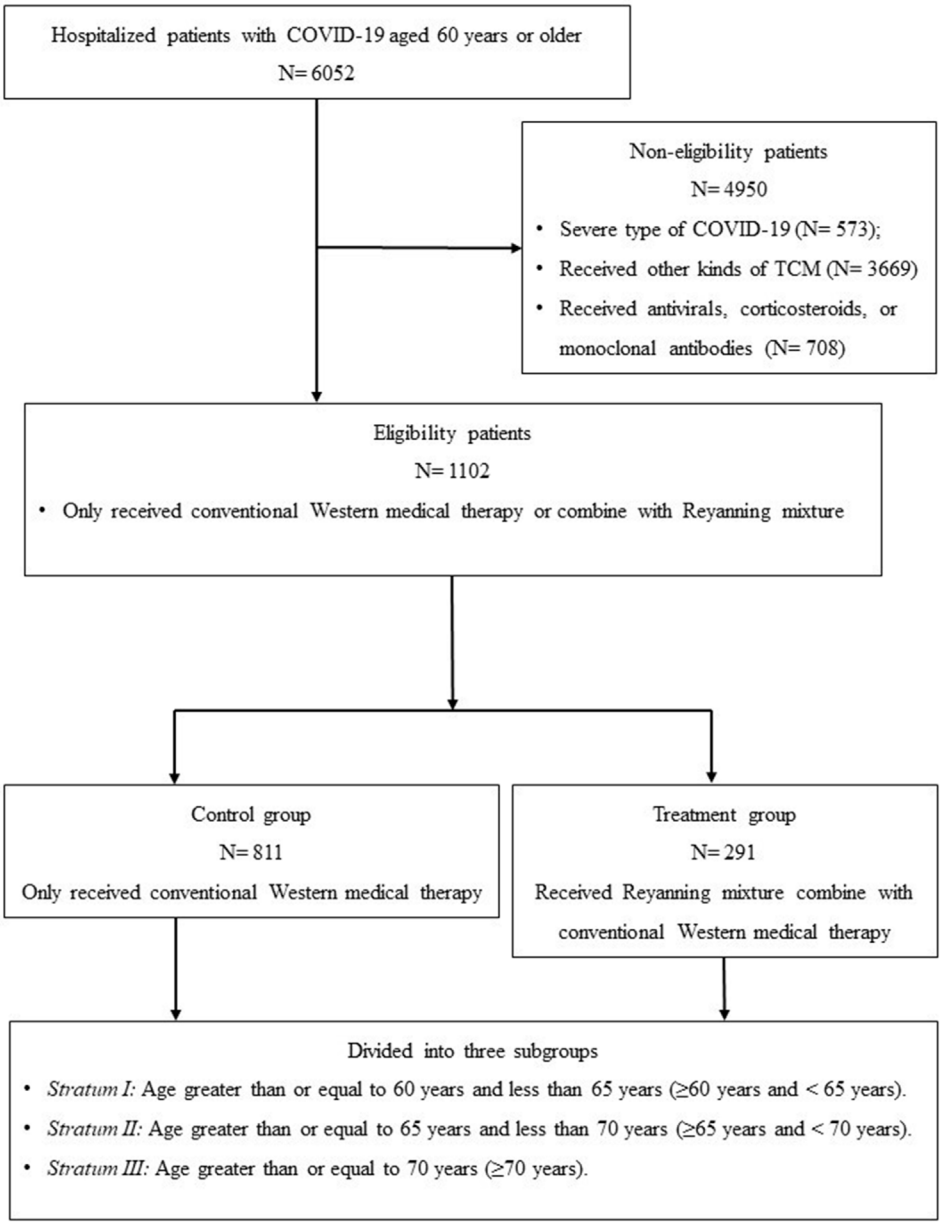

FIGURE 1
The patient screening flow chart.

of illness during the treatment process. All adverse events that occurred during the treatment process were closely monitored by physicians, while the severity, duration, and onset time were carefully recorded.

Statistical analysis

The analyses of the continuous variables (presented as medians, interquartile ranges) were conducted with the Mann-Whitney U test, while the analyses of categorical variables were conducted with the Chi-square test for counts and percentages (%). Cox proportional-hazards models and 95% confidence intervals (CI) were applied to estimate the variables that may impact the outcome. The Kaplan-Meier method was applied to present the time to events with a 95% CI and generate survival curves.

We used Cox proportional-hazards models to perform sensitivity analysis. Firstly, model 1 was established by adjusting only for age to explore the impact of Reyanning mixture on outcome. Then, model 2 was established by adjusting for patient characteristics listed in our study. Next, we selected cases with restricted age range based on age stratification and re-analyzed the association between Reyanning mixture and outcome. Statistical significance was determined by a p-value less than 0.05 in all tests. Statistical analyses were applied with SPSS (version 26.0; IBM Corp.) and R software (version 4.2.1; R Foundation for Statistical Computing).

TABLE 1 Baseline information of Treatment group and Control group.

	Treatment group (*n* = 291)	Control group (*n* = 811)	*p-value*
Gender n (%)			
Female	141 (48.5%)	333 (41.1%)	0.985
Age, median (interquartile ranges)	65 (62, 68)	64 (63, 67)	0.453
Underlying diseases n (%)	109 (37.5%)	255 (31.4%)	0.061
Vaccination status n (%)			0.614
Unvaccinated	92 (31.6%)	235 (28.9%)	
Partially vaccinated	7 (2.4%)	14 (1.7%)	
Full vaccination	75 (25.8%)	235 (28.9%)	
Booster	117 (40.2%)	327 (40.3%)	
Type			0.108
Mild	228 (78.4%)	670 (82.6%)	
Asymptomatic	63 (21.6%)	141 (17.4%)	
Clinical symptoms n (%)			
Cough	182 (62.5%)	533 (65.7%)	0.330
Sputum	51 (17.6%)	161 (19.9%)	0.388
Sore throat	52 (17.9%)	108 (13.3%)	0.059
Fever	97 (33.3%)	226 (27.9%)	0.079
Muscle soreness	63 (21.6%)	161 (19.9%)	0.513
Fatigue	42 (14.4%)	103 (12.7%)	0.453
Shortness of breath	7 (2.4%)	18 (2.2%)	0.855
Apocleisis	8 (2.7%)	20 (2.5%)	0.792

Results

Baseline and clinical characteristics

Between April 1 and 31 May 2022, a total of 6,052 patients aged 60 years or older were diagnosed with SARS-CoV-2 Omicron infection and admitted to the N3 Mobile Cabin Hospital at the Shanghai New International Expo Center. Among these patients, 1,102 met the inclusion criteria and were collected in this study. Of these patients, 291 received Reyanning mixture combined with conventional Western medical therapy were assigned to the treatment group, while the remaining 811 patients received only conventional Western medical therapy and served as the control group. Flow chart for screening was present in Figure 1.

In the treatment group, the cohort was comprised of 150 males and 141 females, with a median age of 65 (62, 68) years. The control group consisted of 478 males and 333 females, with a median age of 64 (63, 67) years. No statistically different was observed between two groups. A total of 109 patients (37.5%) in the treatment group and 255 (31.4%) in the control group had underlying medical conditions, including hypertension, diabetes, cerebrovascular diseases, and cardiovascular diseases, et al., and there was no statistically significant difference between the two groups (p = 0.061). Patients in both groups presented with various clinical syndromes. The most common symptoms of hospitalization included cough, sputum, sore throat, fever, muscle soreness, and fatigue. Regarding vaccination status, 92 patients were unvaccinated, 7 patients were partially vaccinated, 75 received fully dose and 117 received booster within treatment group. In the control group, 235 patients were unvaccinated, 14 had received one dose, 235 had received two doses, and 327 had received a booster. The baseline information of two groups were presented in Table 1.

Primary outcome

The hospitalization duration in the treatment group and the control group were 8 (4, 11) days and 10 (8, 12) days, respectively. Furthermore, the viral shedding time was 7 (3, 10) days in the treatment group and 8 (6, 10) days in the control group, as illustrated in Figure 2. The Kaplan-Meier graph indicated a significant reduction in both hospitalization time and virus clearance time for the treatment group compared to the control group (HR: 0.638, 95% CI: 0.558-0.731, $p < 0.001$; HR: 0.754, 95% CI: 0.659-0.863, $p < 0.001$, respectively) (Figures 3, 4). Furthermore, we collected and analyzed the nucleic acid test results of patients during their

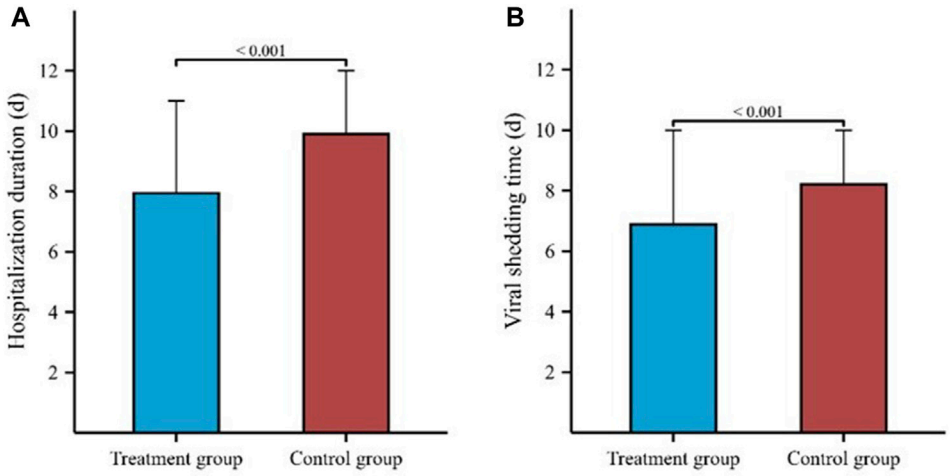

FIGURE 2
Comparison of hospitalization duration and viral shedding time between Treatment group and Control group. **(A)** represented the comparison of hospitalization duration between two groups. **(B)** represented the comparison of viral shedding time between two groups.

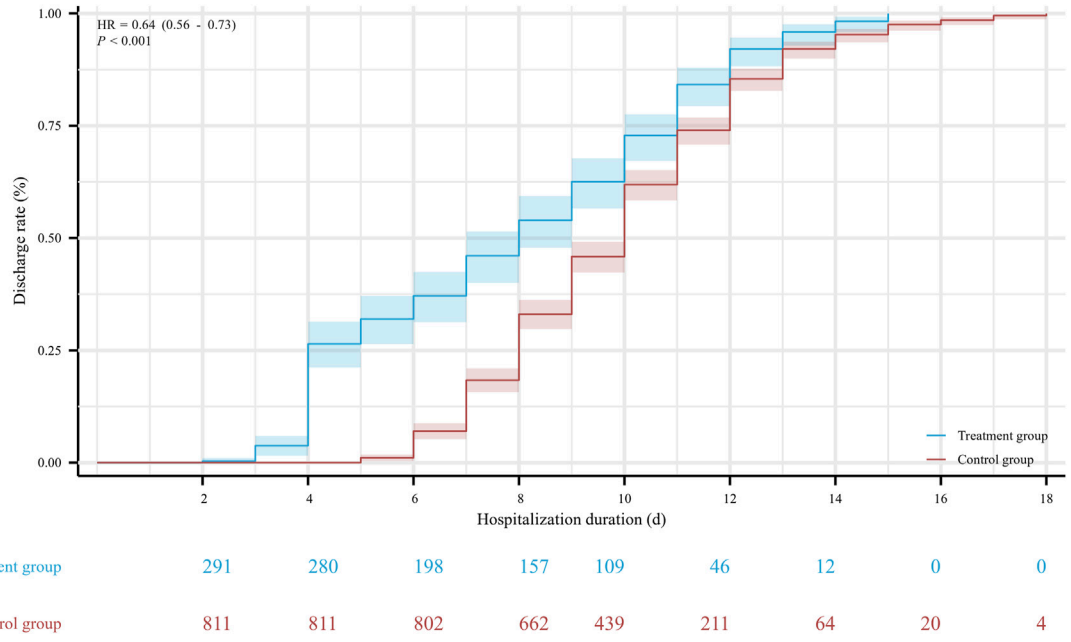

FIGURE 3
The Kaplan-Meier gram for the hospitalization duration between Treatment group and Control group. HR, hazard ratio.

hospitalization for a period of 10 days. Both groups of patients exhibited an upward trend in Ct values of the open reading frame (ORF) and nucleocapsid (N) genes after receiving treatment. Comparison with the control group, the results revealed that patients who received treatment with Reyanning mixture exhibited a more pronounced upward trend in Ct values (Figure 5; Table 2).

The variables of all enrolled patients that may impact hospitalization duration and viral shedding time, including age, gender, disease type, underlying condition, vaccination status, and use of Reyanning mixture, were incorporated into a Cox proportional-hazards model (presented in Table 3), which revealed a significant association between age, vaccination status, the use of Reyanning mixture with hospitalization duration, as well as viral shedding time ($p < 0.05$). After adjustment for age and vaccination status in the multivariate regression analysis, the result suggested that use of Reyanning mixture significantly benefitted the reduction of both

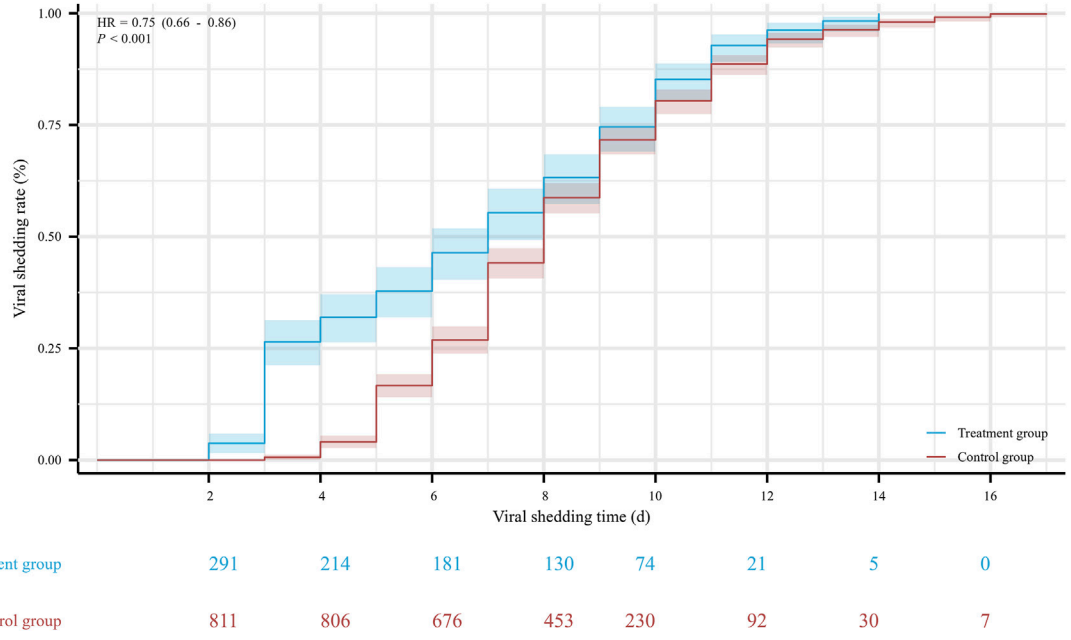

FIGURE 4
The Kaplan-Meier gram for the viral shedding time between Treatment group and Control group. HR, hazard ratio.

hospitalization duration and viral shedding time (HR: 1.562, 95% CI: 1.364-1.789, $p < 0.001$; HR: 1.335, 95% CI: 1.166-1.528, $p < 0.001$, respectively).

In the sensitivity analysis, firstly, we used hospitalization duration as the dependent variable, while group and age as independent variables, and fitted in the Cox proportional-hazards model 1. The result showed a statistical significance, with a β value of 0.433. Then, we included group, age, gender, vaccination status, underlying diseases, and disease type as independent variables and fitted in the Cox proportional-hazards model 2. The result showed a β value of 0.446, with $p < 0.001$, which was consistent with model 1. Similarly, we applied the above method to analyze the association between group and viral shedding time, and the results showed a β value of 0.276 and 0.289, with $p < 0.001$ respectively. The sensitivity analysis manifested the association between the use of Reyanning mixture and outcome was consistent with our main finding (Supplementary Table S1).

Furthermore, we included cases within the age range specified for each subgroup based on the age stratification, conducted independent analysis with multivariate Cox proportional-hazards model and presented in Table 4 to estimate the impact of using Reyanning mixture on the hospitalization duration and viral shedding time. The result still exhibited a significant association between the use of Reyanning mixture and hospitalization duration (HR: 1.844, 95% CI: 1.455-2.338, $p < 0.001$; HR: 1.590, 95% CI: 1.268-1.993, $p < 0.001$; HR: 1.788, 95% CI: 1.201-2.663, $p < 0.05$, respectively) as well as viral shedding time (HR: 1.547, 95% CI: 1.220-1.961, $p < 0.001$; HR: 1.349, 95% CI: 1.075-1.691, $p < 0.05$; HR: 1.534, 95% CI: 1.035-2.274, $p < 0.05$, respectively) in different age stratums.

Secondary outcome

Throughout the treatment process, the most frequently observed adverse events included diarrhea, muscle soreness, and insomnia in both groups (Table 5). No severity adverse event was observed in our series. In addition, no progressive deterioration or death was occurred in the treatment period of our series. Besides, six patients in the treatment group were experienced with slight diarrhea, which was considered as a possible association with Reyanning mixture use. After symptomatic treatment, all adverse events were alleviated.

Discussion

In the present study, we analyzed the data of 1,102 elderly patients from our ward, infected with SARS-CoV-2 Omicron variant. Our findings revealed a strong correlation between the length of hospitalization and viral shedding time in elderly patients, and various factors including age, vaccination status, and the use of Reyanning mixture. Compared to the control group, the use of Reyanning mixture significantly reduced hospitalization duration and viral shedding time in elderly individuals. Additionally, our study analyzed the patients in different age stratums still manifested a reduction in hospitalization duration and viral clearance time with Reyanning mixture treatment. Furthermore, there was no significant adverse event observed during the use of Reyanning mixture, nor any deterioration of the health condition. These results suggested that Reyanning mixture was an effective and safe treatment for elderly patients with COVID-19.

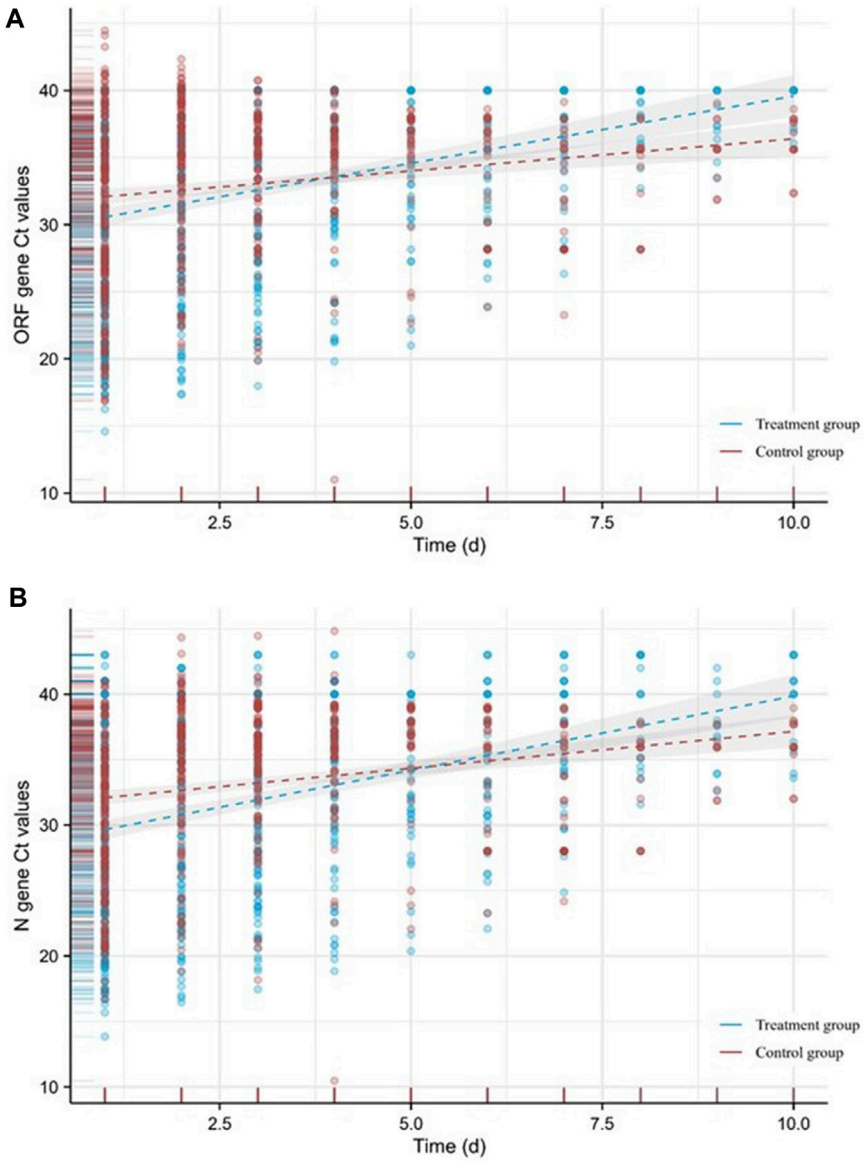

FIGURE 5
Variation of Ct values of the ORF **(A)** and N **(B)** genes.

Since the initial emergence of COVID-19 pandemic in late 2019, multiple variants of SARS-CoV-2 have been identified. In addition to respiratory symptoms, infection can also trigger a cytokine storm, lead to systemic inflammation and damage to multiple organs. The Omicron variant, designated B.1.1.529, was a heavily mutated strain which was deemed a matter of concern on 26 November 2021 by the World Health Organization, posed a high infection risk with serious repercussions (Araf et al., 2022). Despite significant progress in clinical diagnosis and treatment, there were still many uncertainties in the therapy for this variation, and the long-term protection efficacy of current vaccines against viral variants still remained controversies, especially for elderly patients (Meo et al., 2021). In addition, the variant Omicron has been recognized cause more infectious, medical system overload and exhaust, with continuous outbreaks (Shen et al., 2022).

In addition to conventional clinical treatment, a range of initiatives, such as clinical trials and observation, have been undertaken to discover the utility of TCM for COVID-19, especially for those with antiviral and anti-inflammatory properties (Zhang et al., 2022b; Guo et al., 2022), to find alternative approaches to managing the disease course. Furthermore, various botanical drugs, such as Xuebijing injection, Qingfei Paidu decoction, and Lianhua Qingwen capsule, have been investigated in this regard and provided evidences in treating with COVID-19 infection (Li et al., 2021a; Hu et al., 2021; Tianyu and Liying, 2021). Shi J. et al. (2020) performed a retrospective analysis of data from 234 patients diagnosed with COVID-19. The results revealed that patients who received TCM treatment within 3 days of hospital admission exhibited a significant reduction in hospitalization duration, disease course, and nucleic acid negative conversion time, compared to those who received TCM

TABLE 2 Comparison of the ORF/N genes Ct values between the Treatment group and Control group.

Day	ORF gene		*p-value*	N gene		*p-value*
	Treatment group	Control group		Treatment group	Control group	
1	30.56 ± 7.48	30.28 ± 5.99	0.579	30.07 ± 7.87	29.73 ± 5.69	0.515
2	31.91 ± 6.99	32.71 ± 5.35	0.094	31.40 ± 7.46	32.36 ± 5.57	0.057
3	32.51 ± 6.62	33.35 ± 4.64	0.066	32.04 ± 7.11	33.29 ± 4.86	0.001
4	32.36 ± 5.97	33.85 ± 4.39	0.001	31.79 ± 6.29	33.56 ± 4.57	0.001
5	33.24 ± 5.62	34.41 ± 4.18	0.001	32.63 ± 5.89	34.48 ± 4.49	0.001
6	34.87 ± 5.17	34.89 ± 3.82	0.955	34.48 ± 5.59	34.75 ± 3.94	0.593
7	35.89 ± 4.45	34.84 ± 3.58	0.020	35.42 ± 4.88	34.61 ± 3.74	0.112
8	37.35 ± 3.28	35.10 ± 3.58	0.001	37.06 ± 3.91	34.77 ± 3.63	0.001
9	38.50 ± 2.36	36.02 ± 2.95	0.001	38.30 ± 2.97	35.80 ± 3.19	0.001
10	38.92 ± 1.73	36.14 ± 2.49	0.001	38.79 ± 2.64	36.03 ± 2.63	0.001

TABLE 3 Cox proportional-hazards model for hospitalization duration and viral shedding time.

	Hospitalization duration				Viral shedding time			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Age	0.962 (0.945, 0.980)	<0.001	0.963 (0.946, 0.981)	<0.001	0.962 (0.945, 0.980)	<0.001	0.964 (0.947, 0.982)	<0.001
Gender	1.026 (0.911, 1.154)	0.676	0.997 (0.884, 1.126)	0.967	1.045 (0.928, 1.177)	0.465	1.010 (0.895, 1.140)	0.867
Type	1.116 (0.958, 1.299)	0.159	1.146 (0.980, 1.339)	0.088	1.104 (0.948, 1.285)	0.204	1.121 (0.959, 1.310)	0.152
Underlying diseases	1.008 (0.889, 1.143)	0.902	0.948 (0.833, 1.079)	0.417	1.019 (0.899, 1.155)	0.770	0.965 (0.848, 1.098)	0.589
Vaccination status	1.205 (1.059, 1.373)	0.005	1.205 (1.057, 1.373)	0.005	1.189 (1.045, 1.354)	0.009	1.180 (1.035, 1.344)	0.013
Group	1.520 (1.329, 1.740)	<0.001	1.562 (1.364, 1.789)	<0.001	1.307 (1.142, 1.495)	<0.001	1.335 (1.166, 1.528)	<0.001

CI, confidence interval; HR, hazard ratio.

TABLE 4 Cox proportional-hazards model for estimating the impact of using Reyanning mixture on the hospitalization duration and viral shedding time in different age stratums.

	Hospitalization duration				Viral shedding time			
	Univariate		Multivariate		Univariate		Multivariate	
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
60–64 years	1.647 (1.352, 2.005)	<0.001	1.844 (1.455, 2.338)	<0.001	1.395 (1.146, 1.698)	0.001	1.547 (1.220, 1.961)	<0.001
65–69 years	1.587 (1.272, 1.981)	<0.001	1.590 (1.268, 1.993)	<0.001	1.359 (1.090, 1.695)	0.006	1.349 (1.075, 1.691)	0.010
≥70 years	1.771 (1.198, 2.617)	0.004	1.788 (1.201, 2.663)	0.004	1.583 (1.074, 2.332)	0.020	1.534 (1.035, 2.274)	0.033

CI, confidence interval; HR, hazard ratio.

decoction after 7 days or more. Hu et al. (2021) reported that the application of Lianhua Qingwen capsule manifested a significantly increasing in the recovery rate, with shorting the median time of symptom recovery in their study. Zhang et al. (2021) conducted an evaluation of 25 patients diagnosed with mild or common COVID-19 who received treatment with Tanreqing capsule. The result indicated a significant reduction in negative conversion time compared to the group that received conventional Western medicine. Shi N. et al. (2020) reported a series of 782 patients with confirmed COVID-19 and treated with Qingfei Paidu decoction, investigated the association

TABLE 5 Adverse events in the Treatment group and Control group.

Adverse events	Treatment group	Control group	*p*-value
Diarrhea	12 (4.1%)	36 (4.4%)	0.821
Muscle soreness	10 (3.4%)	32 (3.9%)	0.697
Headache	3 (1.0%)	10 (1.2%)	0.784
Insomnia	20 (3.8%)	33 (4.0%)	0.055
Dizziness	4 (1.3%)	9 (1.1%)	0.720
Palpitation	2 (0.7%)	5 (0.6%)	0.896
Stomachache	5 (1.7%)	12 (1.5%)	0.777

between recovery and the treatment initiation time among infected individuals. However, there is a scarcity of research specifically targeting the application of TCM in treating elderly patients with SARS-CoV-2 infection, which remains an important area of study that requires further research.

The Reyanning mixture has been authorized by the National Medical Products Administration of China, in which consists four kind of herbs with a 2:2:2:1 proportion by weight. Previous studies has shown the usage of Reyanning mixture in treating infectious diseases such as fever, pneumonia, tonsillitis suppurative, sore throat, acute pharyngitis, and acute bronchitis (Lyu et al., 2020; Lyu et al., 2022). In a clinical investigation conducted by Yang M. et al. (2020), a series of 49 patients diagnosed with common COVID-19 were enrolled. The administration of Reyanning mixture was found to significantly ameliorate the clinical symptoms and chest images from computed tomography of the COVID-19 patients. However, the study did not specifically examine the correlation between the use of Reyanning mixture and the hospitalization duration or nucleic acid conversion time in elderly patients. Additionally, it should be noted that the sample size was relatively small, with only 26 cases receiving Reyanning mixture. In terms of the botanical drug mechanisms, Bao et al. (2020) assessed the efficacy of Reyanning mixture in a mouse model that was designed to mimic the syndrome of human coronavirus pneumonia. The results demonstrated the effectiveness of Reyanning mixture in the improvement of lung lesions, autoimmune function, enhancing gastrointestinal function, as well as reducing the expression of inflammatory factors. Han et al. (2021) used the network pharmacology approach, exhibited its anti-inflammation effects via regulating the cell proliferation, and the surviving pathways. Wang M. et al. (2020) employed network pharmacology in addition to molecular docking methodologies and forecast the potential application of Reyanning mixture in treating COVID-19. Their findings suggested that the active constituents of Reyanning mixture may exert their therapeutic effects by modulating various targets, including CD40LG, IL2, IL6, IL10, and CXCL10, CXCL8. Nonetheless, it should be noted that the studies still lacked sufficient experimental validation through *in vivo* and *in vitro* assessments.

Recently, we conducted a randomized controlled study comprising of 2,830 patients, and the result indicated that Reyanning mixture represented a safe and effective treatment option for promoting recovery from asymptomatic and mild SARS-CoV-2 Omicron infection, as well as accelerating virus clearance (Xu et al., 2023). As the result of current study, we provided further evidence for the conversion to negative nucleic acid status, as well as a potential efficacious option of TCM for treating COVID-19 in elderly patients. Based on another study we conducted previously (Xu et al., 2022a), the finding indicated that elderly patients had prolonged hospitalization duration and a higher risk of deterioration. Additionally, we observed a correlation between the appliance of TCM and a reduction in hospitalization duration. An earlier study has identified age as an independent risk factor that may prolong the time of viral clearance (Wang K. et al., 2020). Moreover, some scholars have suggested that prolonged viral shedding time may be primarily associated with chronic diseases and low immunity (Hao et al., 2020), which were more prevalent among elderly patients. The result of our present study demonstrated that, in comparison to the control group, a significant reduction in hospitalization time and viral clearance time was observed in the population receiving Reyanning mixture, which could provide potential advantage for COVID-19 treatment in elderly patients.

Our finding provided another evidence of Reyanning mixture and treatment option for elderly patients infected with SARS-CoV-2 Omicron variant. Nonetheless, the study had certain limitations that should not be neglected. Firstly, as a retrospective and observational investigation, it may be subject to inherent biases. Secondly, the research exclusively focused on patients with the Omicron variant in Shanghai, which may restrict the generalizability of the findings. Moreover, the study was restricted in specific population, and the use of conventional Western medicine in the treatment regimen may have confounded the result and introduced potential bias.

In conclusion, given the circumstance of elderly patient, how to respond to SARS-CoV-2 infection and protect the health condition of this population still requires attention and concern. The present finding suggested that Reyanning mixture was a safe and effective treatment option for our series, and provided evidence for the application of Reyanning mixture in the elderly population. Prospective clinical trials with larger sample, targeting specific population, and incorporating more indicators for monitoring and analyzing are needed, and further exploration should be conducted to reveal the underlying mechanism and function of Reyanning mixture.

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 studies involving human participants were reviewed and approved by the Medical Ethics Committee of Jiujiang Hospital of Traditional Chinese Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conception and design: CL, BF, and SZ. Analysis and interpretation of the data: CL and XW. Drafted the article: CL and

XW. Critically revised the article: YF and YS. Reviewed submitted version of manuscript: CL, BF, and SZ. Final approval of the version to be published: BF and SZ. Statistical analysis: XW, WZ, and LW. Administrative, data collection: HW, CC, HY, and HS. All authors contributed to the article and approved the submitted version.

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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.2023.1185122/full#supplementary-material

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Integrated transcriptomic and metabolomic profiles reveal the protective mechanism of modified Danggui Buxue decoction on radiation-induced leukopenia in mice

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Leukopenia caused by radiation hinders the continuous treatment of cancers. Danggui Buxue Decoction (DBD) has been widely used in clinical owing to low toxicity and definite therapeutic effects to increase leukocytes. Meanwhile, icaritin (ICT) has also been proved to have the effect of boosting peripheral blood cells proliferation. However, there is no study to prove the efficacy of MDBD (Modified Danggui Buxue Decoction), a derivative herbal formula composed of DBD and ICT, in the treatment of radiation-induced leukopenia. In this study, we performed a model of 3.5 Gy whole-body radiation to induce leukopenia in mice. The results of pharmacodynamic studies demonstrated that MDBD could significantly increase the white blood cells in peripheral blood by improving the activity of bone marrow nuclear cells, reducing bone marrow damage, modulating spleen index, and regulating hematopoietic factors to alleviate leukopenia. We also analyzed the integrated results of metabolomics and transcriptomics and found that MDBD could relieve leukopenia and alleviate bone marrow damage by targeting steroid biosynthesis and IL-17 signaling pathway, in which the key genes are Jun, Cxcl2 and Egr1. Therefore, our study provides a basis for the effectiveness and compatibility in the combination of traditional Chinese medicine formula and small molecule drugs.

KEYWORDS

leukopenia, radiation, modified Danggui Buxue decoction (MDBD), transcriptomics, metabolomics

Abbreviations: MDBD, modified Danggui Buxue decoction; DBD, Danggui Buxue decoction; ICT, icaritin; WBC, white blood cell; RBC, red blood cell; PLT, platelet; HGB, hemoglobin; GM-CSF, granulocyte-macrophage colony-stimulating factor; G-CSF, granulocyte colony-stimulating factor; IL-6, interleukin-6; TPO, thrombopoietin; BMNCs, bone marrow nuclear cells; HSCs, hematopoietic stem cells; VIP, variable importance in projection; PCA, principal component analysis; PLS-DA, partial least-squares-discriminant analysis; DEGs, different expression genes; KEGG, kyoto encyclopedia of genes and genomes; CFU, colony formation unit.

1 Introduction

Leukopenia is a commonly adverse effect associated with abnormal bone marrow hematopoiesis, especially during cancer-related radiotherapy (Shi C. et al., 2020). Leukocytes depletion is an unintended consequence of radiation toxicity, which results in the continuous decline of leukocytes in peripheral blood. Leukopenia, as the most common hematological toxicity caused by radiotherapy, has always been the main cause of treatment interruption (Franco et al., 2017), which affects the efficacy of radiotherapy and increases treatment time and economic burden. Leukopenia patients are taking a higher risk of infection, which has emerged as a serious threat to prevent patients from recovering (Kuo et al., 2014). Therefore, it is an essential step to reverse leukopenia in cancer treatment. Currently, the first-line drugs for leukopenia treatment include granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), leucogen, vitamin B4, *etc.* (Bayne et al., 2012; Tian et al., 2019). However, numerous side effects appeared in their applications, such as bone tumors, fever, bone pain, and myalgia (Dai et al., 2010). Therefore, traditional Chinese medicine has become an alternative due to its low-toxicity and high-efficacy.

Danggui Buxue Decoction (DBD), a traditional Chinese medicine, has been used for nourishing and enriching the "Blood" for almost 800 years, and its clinical efficacy has been well-documented (Lin et al., 2017). DBD is a simple formula which consists of two botanical drugs: *Radix Angelicae Sinensis* (Danggui, DG) and *Radix Astragali* (Huangqi, HQ) with a weight ratio of 1: 5. A large number of studies have shown that DBD remarkably increases the number of white blood cells, reticulocytes, and bone marrow nucleated cells (Liu et al., 2010; Wang et al., 2020). DBD also significantly affects hematopoietic function by promoting bone tissue regeneration, regulating immune-mediated aplastic anemia, and modulating gut microbiota balance (Yang et al., 2014; Wang et al., 2015; Du et al., 2020). In order to improve the ability to nourish blood, DG and HQ are often used in combination with other botanical drugs in clinical to form modified Danggui Buxue Decoction (MDBD). Traditional Chinese medicine formulas for treating leukopenia, such as Qijiao Shengbai Capsule (Ma et al., 2022) and Qijing Shengbai Granule (Huang, 2019), both contain DG and HQ. Meanwhile, DG and HQ that make up DBD are also the two most frequently used botanical drugs in clinical treatment of aplastic anemia (Dong et al., 2022). Icaritin (ICT) is one of the main effective ingredients of *Herba Epimedii in vivo* which is an important botanical drug that has been used for boosting peripheral blood cells proliferation (Qin et al., 2019). ICT also can improve hematopoietic function of chemotherapy-induced myelosuppression mice by reducing bone marrow depression and improving bone marrow hematopoietic microenvironment (Sun et al., 2018). Our research group has found that ICT may be one of the key pharmacological components of Qijing Shengbai Granule in treating leukopenia in mice (Huang, 2019). In our further research, we found that ICT can exert the best therapeutic effect of increasing white blood cells at 3 mg/kg. ICT is a chemical drug derived from botanical drug, and there are currently no reports of its compatibility with DBD. Meanwhile, it is unclear whether modified Danggui Buxue Decoction (MDBD), consisting of three drugs of DG, HQ and ICT with a weight ratio of 1: 5: 0.003 (DBD: 6 g/kg, ICT: 3 mg/kg), exerts a better efficacy in promoting hematopoiesis.

In this study, we first studied the effect of MDBD on leukopenia in mice induced by radiation. Next, transcriptomic data from bone marrow and metabonomic data from serum were applied to further study the therapeutic mechanism of MDBD. Finally, we applied correlation analysis based on transcriptomics and metabolomics to explore the mechanism of MDBD in treating leukopenia (Figure 1).

2 Materials and methods

2.1 Reagents and materials

DG (origin in Gansu, China, batch number 200807), HQ (origin in Gansu, China, batch number 201215) were provided by Shanghai Hongqiao Traditional Chinese Medicine Co., Ltd. (Shanghai, China). And the above two drugs have all reached the characteristic identification standard described in the 2015 edition of the Chinese Pharmacopoeia. ICT (molecular weight = 368.37, Lot: 21398S1, CAS: 118525-40-9, purity ≥98.0%) was offered by Shanghai Sunny Biotech Co., Ltd. (Shanghai, China). Leucogen tablets were purchased from Jiangsu Jibel Pharmaceutical Co., Ltd. (Zhenjiang, China). The ELISA kits for GM-CSF, interleukin-6 (IL-6), thrombopoietin (TPO) were purchased from Abcam (Cambridge, UK). A cobalt radiation source was provided by the Radiation Center of Naval Medical University (Shanghai, China).

2.2 Preparation of MDBD

First of all, we prepared DBD with DG and HQ. As described in the literature (Kwan et al., 2019; Shi X. Q. et al., 2020), DBD needs to be extracted with an aqueous solution for optimal efficacy. DG (100 g) and HQ (500 g) were immersed into water (1: 8, w/v) for 2 h, and then decocted in boiling water (1: 8, w/v) for three times, 2 h each time. After filtration, the filtrates were combined and concentrated under reduced pressure (60 °C) to a concentration of 1.2 g mL^{-1}. Then, DBD was stored in aliquots at −80 °C for future use. DBD was taken out of the refrigerator and dissolved at 4 °C when using. Then it was diluted with 0.5%CMC-Na solution of equal volume to obtain DBD with a concentration of 0.6 g/mL. Next, ICT powder was dissolved in 0.5% CMC-Na solution to obtain ICT solution with a concentration of 0.3 mg/mL. Finally, we mixed the above DBD solution and ICT solution with equal volume to obtain MDBD, which consisted of three drugs of DG, HQ and ICT with a weight ratio of 1: 5: 0.003.

2.3 Establishment of leukopenia model and drug administration

Six-to eight-week-old male C57BL/6 mice (18–22 g) were offered by Shanghai Lingchang Biotechnology Co., Ltd. (Shanghai, China) (SCXK, 2018-0003), and housed in the environmentally controlled breeding room (humidity: 60% ± 5%, temperature: 22°C ± 2 °C). All experiments were approved by the Medical Ethics Committee of Navy Medical University. After a

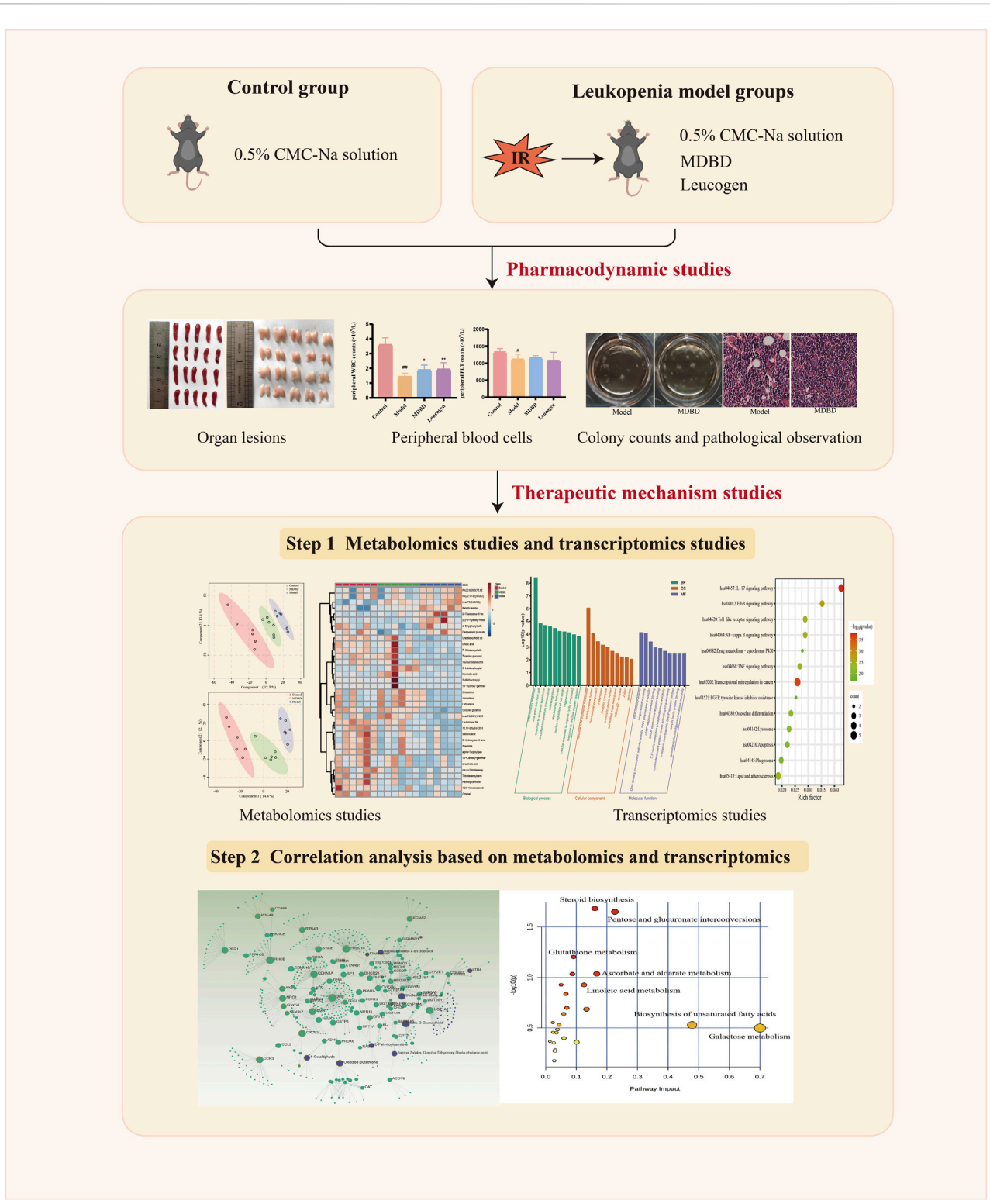

FIGURE 1
The workflow of protective mechanism of MDBD on radiation-induced leukopenia mice.

7-day acclimation period, 40 mice were randomly divided into 4 groups: control group and 3 leukopenia model groups. The three model groups were treated with CMC-Na solution (concentration: 0.5%), MDBD (combine 6 g/kg/day DBD and 3 mg/kg/day ICT) and leucogen (20 mg/kg/day) for 2 days before and for 7 days after radiation, respectively, while the control group was given the same volume of CMC-Na solution by the same gavage method.

Due to the special sensitivity of white blood cells, especially lymphocytes, to radiation, despite individual differences, a dose of 2 Gy can kill 50% of the irradiated population (Pouliliou et al., 2015; Koukourakis and Giatromanolaki, 2021). Meanwhile, some studies have found that in order to observe the response of all blood cells to radiation, especially red blood cells that are not sufficiently sensitive to radiation, the choice of irradiation dose should not be less than 3.5 Gy, and the gender of mice is better for male (Liu et al., 2008). Therefore, in order to obtain a leukopenia model, leukopenia model mice received a 350 cGy total body radiation at the rate of 32.41 cGy/min. Mice in the control group underwent a sham radiation procedure.

2.4 Pharmacodynamics of MDBD

2.4.1 Measurement of peripheral blood cells and organ indexes

On the 14th day after administration, 100 μL of whole blood was collected from the eyeballs with anticoagulant EP tubes after the mice were anesthetized with 4% chloral hydrate (0.05 mL/10 g), and blood routine examination was performed with the hematology analyzer (Mindray, BC-5000Vet, China). The remaining eyeballs blood was collected continuously with EP tubes without anticoagulant for subsequent experiments. Thymus and spleen are important immune organs in mammals, and both of them have lesions in anemic mice (Shi X. Q. et al., 2020). The mice were sacrificed after blood collection, and the spleen and thymus were collected and weighed to calculate the organ index.

2.4.2 Determination of bone marrow cells

Bone marrow cells were collected from femurs for bone marrow nuclear cells (BMNCs) count and cell viability assays. After the mice were sacrificed, they were immersed in 75% alcohol for 3 min immediately. Then, the unilateral femur was removed under sterile conditions. The muscle and connective tissue on the femur were removed as many as possible. Next, carefully cut open both ends of the femur with sterile scissors. Bone marrow cells were flushed from the bone marrow cavity into a sterile EP tube with 1 mL DMEM medium and rinsed 4 to 6 times repeatedly. Afterwards, bone marrow cells were pipetted into a single-cell suspension, resuspend and counted in automated cell counter.

Cell viability assays were performed with Cell Counting Kit-8 reagent (Adamas, Switzerland). BMNCs (100 μL/well) were added to 96-well culture plates at a density of 1×10^7/mL and then incubated with 10 μL CCK-8 reagent at 37 °C for 4 h. Lastly, the absorbance was measured at 450 nm.

2.4.3 Colony-forming units assay

After the cell viability was determined, the remaining cell suspension was filtered through a 70 μm cell strainer. We slowly added the filtered cell suspension to the upper layer of Ficoll lymph separation solution of equal volume, and centrifugated at 2000 r/min for 20 min. Additionally, we carefully suck out the middle milky white cloud layer (bone marrow mononuclear cells), and added 5 times of the volume of DMEM medium to wash twice. Bone marrow cells were diluted with M3434 methylcellulose medium (StemCell, Canada) to a density of 2×10^4/mL. Then the cells were evenly spread in the middle four wells of the 12-well culture plate (with 2 mL PBS added to the surrounding eight wells) with 1 mL per well, and cultured at 37 °C, 5% CO_2 and saturated humidity for 10 days. Finally, we counted the number of colonies under the microscope with different magnification, and the cell cluster containing more than 50 cells was counted as one colony.

2.4.4 ELISA analysis

The blood samples in the EP tube without anticoagulant were placed at room temperature and clotted for 2 h, then centrifuged at 2000 r/min for 15 min. The serum was collected and divided equally, and stored in the −80 °C refrigerator for future use. GM-CSF, TPO and IL-6 in serum were quantified by ELISA kit according to the manufacturer's instructions, and optical density of each sample was measured at 450 nm. Finally, the cytokine level was quantified by standard curve and expressed in pg/mL.

2.4.5 Effects of MDBD on bone marrow histology

Four unilateral femurs of each group of mice were stained with hematoxylin-eosin (H&E) to make pathological sections of bone marrow. Morphological and pathological observation and analysis were carried out under the microscope. Five random visual fields were selected for each femur slice, and the percentage of hematopoietic area in bone marrow sections was measured with image analysis software (Image J) to judge the structural impact of MDBD on bone marrow tissue.

2.5 Untargeted metabolomics analysis

2.5.1 Serum pretreatment and UPLC-Q/TOF-MS analysis

Six serum samples from mice in control, model and MDBD group were randomly selected and thawed at 4 °C before metabolomics analysis. Each serum sample (100 μL) was mixed with four times the amount of methanol and swirled for 5 min, then centrifuged at 12000 rpm for 10 min for protein precipitation. Next, the supernatant was transferred to auto-sampler vials for ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF-MS) analysis. At the same time, we took an equal amount of 10 μL supernatant from each sample and mixed it into QC sample, which were injected once every six samples to monitor the repeatability and stability of instrument.

Chromatographic separation was executed by using an ACQUITY UPLC system (Waters Corp., Milford, United States) with a ACQUITY UPLC HSS T3 (2.1 × 150 mm, 1.8 μm particles, Waters Corp., Milford, United States) chromatographic column with a temperature maintenance of 40 °C. The serum sample injection volume was set to 3 μL and the temperature of autosampler was fixed at 8 °C. The gradient mobile phase was a mixture of 0.1% formic acid in water (phase A) and acetonitrile (phase B), which was pumped at a flowing rate of 0.4 mL/min. The optimal elution procedure of phase B was set as follows: 0–0.5 min, 5%; 0.5–5 min, 5%–70%; 5–11 min, 70%–75%; 11–14 min, 75%–95%; 14–14.5 min, 95%–5%; 14.5–17 min, 5%. Mass spectrometry data detection and acquisition was performed by SYNAPT G2-Si time-of-flight mass spectrometry (Waters Corp., Milford,

United States) coupled with an electrospray ionization source (ESI). Mass spectra obtained in positive and negative ion mode respectively. The temperature of ESI source was set at 120 °C, and the capillary voltage was 2.0 kV. The desolvation gas (nitrogen) temperature was 400 °C with a flow rate of 800 L/h. The flow rate of cone gas was 50 L/h. Mass data were gathered between 50 and 1,000 m/*z* with the 0.2 s scanning time. Leucine enkephalin (LE) was used as the external reference (LockSpray™) to ensure the precision and accuracy of mass information. The flow rate of LE was 5 μL/min with a concentration of 1 μg/mL. The MS collision energy was set from 10 to 45 V.

2.5.2 Data analysis

Raw serum data were performed using Progenesis QI (V2.0, Nonlinear Dynamics Ltd., Newcastle, UK). Next, the pre-processed data were imported into SIMCA 14.1 (Umetric, Umeå, Sweden) and MetaboAnalyst 5.0 for multivariate statistical analysis. At the same time, differential metabolite ions with VIP >1.5 in the OPLS-DA model, *p*-value <0.05 in *t*-test and FC > 1.5 were selected as candidates. After that, the candidate metabolites were screened and identified based on by Human Metabolome database (HMDB) and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Finally, MetaboAnalyst 5.0 was used to analyze the pathway of the screened differential metabolites.

2.6 Transcriptomic analysis

2.6.1 RNA extraction, library construction, and sequencing

Femoral bone marrow cells from 18 mice in control group, model group and MDBD group (n = 6) were selected for transcriptomic analysis. The total RNAs were isolated from bone marrow cells and purified using Trizol (Beyotime, Shanghai, China) reagent following the manufacturer's instruction. Nanodrop 2000 (NanoDrop, Wilmington, DE, United States) was applied to detect the concentration and purity of the extracted RNA. Meanwhile, RNA integrity was detected by agarose gel electrophoresis, and RIN value was determined by Agilent 2,100 system (Agilent Technologies, CA, United States). The total amount of RNA was required to be ≥1 μg and the concentration ≥35 ng for a single database establishment. Sequencing libraries were constructed using Illumina Truseq™ RNA library Prep kit (Illumina, Nebraska, United States) according to manufacturer's protocol.

2.6.2 Data analysis and enrichment analysis for gene expression

The data were uploaded to the online platform of Majorbio Cloud Platform (www.majorbio.com). Differential expression analysis was conducted by DESeq2 software. The significantly different expression genes (DEGs) between groups were determined according to the following criteria: *p*-value ≤0.05 and |log2FoldChange| ≥ 0.585.

In addition, functional-enrichment analysis including GO (Gene Ontology) and KEGG were performed to identify which DEGs were significantly enriched in GO terms and metabolic pathways at *p*-value ≤0.05 compared with the whole-transcriptomic background. GO functional enrichment and KEGG pathway analysis were carried out by Metascape (https://metascape.org). Finally, OmicsNet 2.0 (https://www.omicsnet.ca) was used for correlation analysis of metabolomics and transcriptomics.

2.7 RNA isolation and quantitative real-time PCR

Total RNA was extracted from bone marrow cells of control group, model group and MDBD group with RNAiso Plus (Takara, Japan). Then PrimeScript Master Mix kit (Takara, Japan) was used to generate cDNA. Quantitative real-time PCR (qRT-PCR) was performed using PowerUp™ SYBR™ Green Master Mix (Thermo Fisher, United States) and QuantStudio3 (Applied Biosystems) according to protocol. The primer sequences (Supplementary Table S1) were designed by BioTNT Co., Ltd. (Shanghai, China). The mRNA expression levels of target genes were normalized with the reference gene *β*-actin, and then the results were analyzed using $2^{-\Delta\Delta CT}$ method.

2.8 Statistical analysis

The data was depicted as the means ± SD (standard deviation). Statistical analyses of multiple groups were conducted via one-way ANOVA (one-way analysis of variance) by using the GraphPad Prism 8 software. When *p*-value was less than 0.05, it was considered statistically significant. In PPI analysis, the species were limited to *Homo sapiens* with a minimum required interaction score of 0.400 (medium confidence), and disconnected nodes in the network were hidden.

3 Results

3.1 MDBD increased peripheral WBC counts and spleen index

It is shown in Figures 2A–D that compared with the control group, peripheral WBC ($p < 0.01$) and PLT ($p < 0.05$) were significantly decreased in the model group, which demonstrated that a model of radiation-induced leukopenia was successfully established. After MDBD treatment, the WBC content increased obviously ($p < 0.05$), and PLT also tended to increase (no statistical significance). However, no significant changes were observed in peripheral RBC and HGB under given radiation dose. The differential cell count of WBC showed that radiation could significantly reduce the counts of neutrophils, lymphocytes, monocytes, and eosinophils, but had no significant effect on the counts of basophils and the proportion of each type of cells (Supplementary Figure S1). Furthermore, the administration of MDBD did not significantly change the proportion and quantity of leukocyte cells of each type caused by radiation (Supplementary Figure S1). Compared with the control group, the spleen index and thymus index were significantly decreased ($p < 0.01$) in the model group, which means spleen damage and thymus atrophy. Meanwhile, MDBD could reverse the spleen index to the control

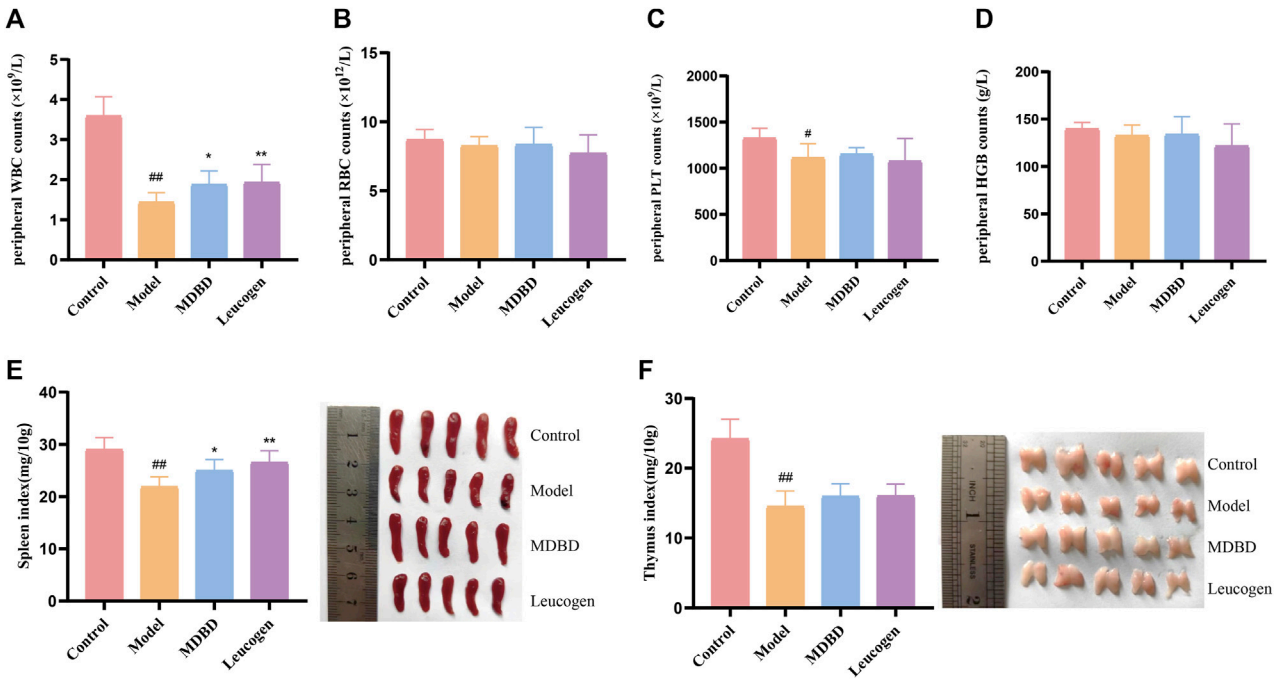

FIGURE 2
Effect of MDBD on peripheral blood cells and organ indexes in leukopenia mice induced by radiation. **(A)** WBC. **(B)** RBC. **(C)** PLT. **(D)** HGB. **(E)** Spleen index. **(F)** Thymus index. After treatment with MDBD, WBC **(A)** and spleen index **(E)** increased compared with model group. The data are expressed as means ± SD. #$p < 0.05$ and ##$p < 0.01$ vs control group; *$p < 0.05$ and **$p < 0.01$ vs. model group.

level ($p < 0.05$) but had no visible effect on the thymus index (Figures 2E,F).

3.2 MDBD increased bone marrow nucleated cells and enhanced cell viability

Peripheral blood cells are derived from hematopoietic stem cells in bone marrow, and the number of BMNCs indirectly reflects hematopoietic function. Compared with the control group, the count and the cell viability of BMNCs were significantly reduced in the model group ($p < 0.01$), reflecting the myelosuppressive effect of radiation. After administration of MDBD, the cell viability enhanced significantly ($p < 0.01$), while the increase of BMSCs count was not significant ($p > 0.05$) (Figures 3A,B).

3.3 MDBD promoted colony formation of bone marrow mononuclear cells

To detect the effect of MDBD on hematopoietic colony formation unit (CFU) *in vivo*, the number of erythroid (CFU-E), myeloid (CFU-GM), megakaryocytic (CFU-MK), mixed (CFU-GEMM) lineages and bone marrow stromal cells (CFU-F) clusters were counted together under the microscope on the 10th day of culture. CFU of bone marrow mononuclear cells was obviously decreased by radiation ($p < 0.01$). After treatment with MDBD, the CFU count increased significantly ($p < 0.05$) (Figure 3C).

3.4 MDBD regulated hematopoiesis-related cytokines levels

Hematopoiesis-related cytokines such as GM-CSF, TPO and IL-6 are important factors regulating hematopoietic function (Liu et al., 2021). The levels of GM-CSF, TPO and IL-6 in serum were determined by ELISA kits. Compared with control group, the levels of GM-CSF and IL-6 in serum were significantly increased respectively after radiation treatment. MDBD reversed the dramatically change of GM-CSF and IL-6. In addition, no significant changes in serum TPO levels were observed (Figures 3D–F).

3.5 MDBD increases the area of bone marrow hematopoietic tissue

In the bone marrow of control group, the distribution of erythroid and granulocyte cells was relatively tight and uniform with intact structure of hematopoietic scaffold, and the proportion of hematopoietic area was more than 70%. Compared with the control group, the hematopoiesis area of the model group decreased obviously concomitant with an increase of vacuoles and adipocytes cells ($p < 0.01$), which indicated that radiation significantly inhibited hematopoiesis. Furthermore, the adipocytes and vacuoles in the MDBD group were reduced compared with the model group accompanied by the structural integrity of the hematopoietic scaffold was improved, and the hematopoietic area was significantly increased ($p < 0.01$) (Figure 3G).

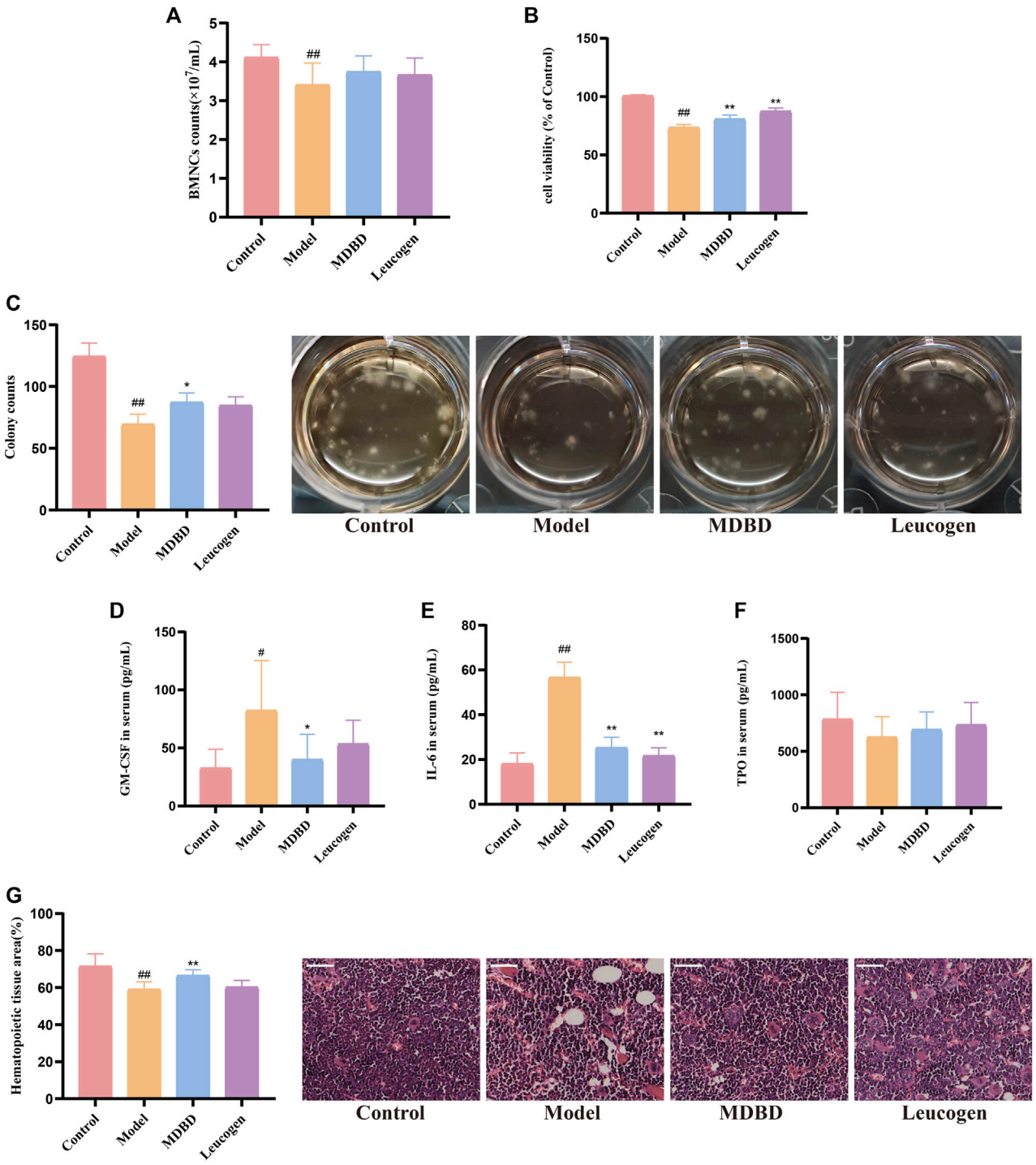

FIGURE 3
The effect of MDBD on the cell number and viability of BMNCs, colony-forming assay of bone marrow mononuclear cells, cytokines concentration and hematopoietic area. **(A)** BMNCs counts. **(B)** Cell viability. **(C)** Colony forming of bone marrow mononuclear cells. **(D)** GM-CSF. **(E)** IL-6 **(F)** TPO. **(G)** Hematopoietic area. The scale in the figures represents 50 μm. The data are expressed as means ± SD. #$p < 0.05$ and ##$p < 0.01$ vs control group; *$p < 0.05$ and **$p < 0.01$ vs model group.

3.6 Metabolomics studies

In order to distinguish the metabolic profiles among control, model and MDBD group, PCA and PLS-DA in ESI^+ mode (positive ion mode) and ESI^- mode (negative ion mode) were performed for cluster analysis. The serum samples between the control, model, and MDBD groups tended to be separated in PCA (Supplementary Figure S2). Meanwhile, the significant separation of different groups in PLS-DA confirmed the reliability and reproducibility of the test method (Figures 4A,B). Furthermore,

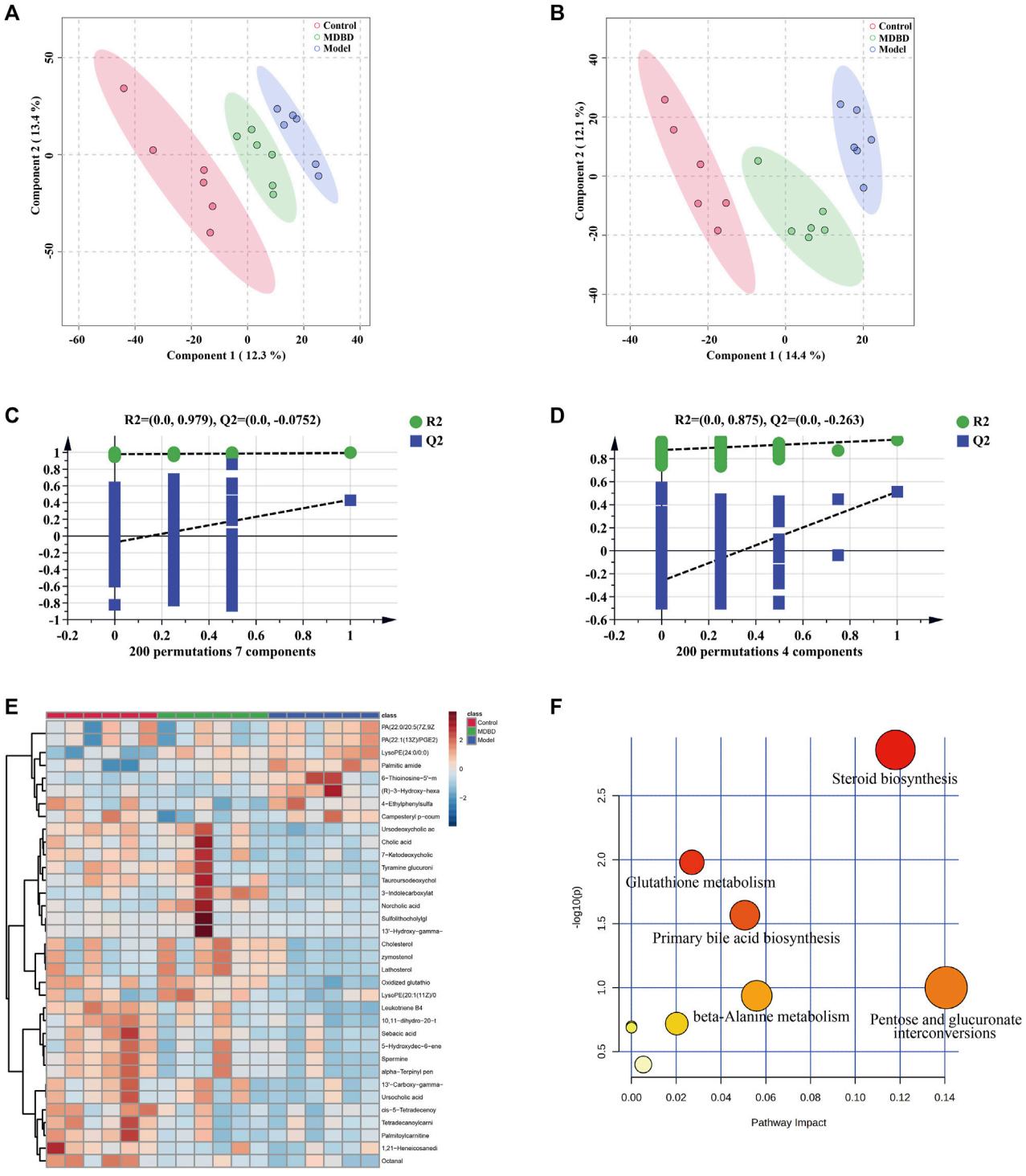

FIGURE 4
MDBD modulated the metabolites in serum of radiation-induced leukopenia mice. **(A)** PLS-DA score plots in ESI + mode. **(B)** PLS-DA score plots in ESI- mode. **(C)** Permutation test of PLS-DA in ESI + mode. **(D)** Permutation test of PLS-DA in ESI- mode. **(E)** Clustering heatmap of potential differential metabolites in serum. **(F)** The key metabolic pathways of differential metabolites.

the permutations plot of PLS-DA showed that the model was non-overfitting and reliable (Figures 4C,D). Metabolites with VIP >1.5, $p < 0.05$ and FC > 1.5 were selected and considered as potential metabolites by matching with substance molecules in HMDB. 35 differential metabolites (Supplementary Table S2) were identified among the control, model and MDBD groups. After administration of MDBD, compared with the model group, all 35 metabolites were inversely regulated by MDBD, and

TABLE 1 Pathways enrichment analysis of differential metabolites in serum of MDBD-treated mice.

NO.	Term	Match status	*p*-value	-Log(*p*)	FDR
1	Steroid biosynthesis	3/42	0.0019464	2.7108	0.1635
2	Glutathione metabolism	2/28	0.012953	1.8876	0.54401
3	Primary bile acid biosynthesis	2/46	0.033327	1.4772	0.93315
4	Pentose and glucuronate interconversions	1/18	0.11055	0.95644	1
5	beta-Alanine metabolism	1/21	0.12786	0.89325	1
6	Arachidonic acid metabolism	1/36	0.20998	0.67783	1
7	Arginine and proline metabolism	1/38	0.22038	0.65683	1
8	Fatty acid degradation	1/39	0.22554	0.64678	1
9	Drug metabolism - other enzymes	1/39	0.22554	0.64678	1
10	Steroid hormone biosynthesis	1/85	0.43203	0.36449	1

14 metabolites of them were significantly regulated by MDBD ($p < 0.05$). To further capture the differences of metabolites among three groups, a heatmap was drawn (Figure 4E). In addition, in order to identify the key metabolic pathways, MetaboAnalyst 5.0 was applied for metabolic pathway enrichment analysis. The main metabolic pathways were enriched as follows: steroid biosynthesis, glutathione metabolism, primary bile acid biosynthesis, pentose and glucuronate interconversions, beta-alanine metabolism, arachidonic acid metabolism, arginine and proline metabolism, fatty acid degradation, drug metabolism-other enzymes and steroid hormone biosynthesis (Figure 4F; Table 1).

3.7 Transcriptomics studies

A total of 2,217 DEGs between model and control group were identified, including 1,029 upregulated and 1,188 downregulated genes (Figure 5A). Meanwhile, 443 DEGs were identified between MDBD treatment and model group, which included 202 upregulated and 241 downregulated genes (Figure 5A). In order to find out DEGs regulated by MDBD, we crossed all the above differential genes and got 158 specific DEGs (Supplementary Table S3, Figure 5B), which were inversely expressed by 148 after MDBD treatment. To further visualize the differences among the DEGs among three groups, a heatmap was drawn (Figure 5E). In order to identify the potential pathway of MDBD, the above 158 specific DEGs were performed for KEGG pathway enrichment analysis. These pathways were mainly enriched in IL-17 signaling pathway, ErbB signaling pathway and Toll-like receptor signaling pathway (Figure 5C). To further understand the cellular processes and function of the DEGs, GO terms enrichment analysis was performed. The GO terms mainly mapped to inflammatory response of BP (biological processes), external side of serum membrane of CC (cell components), and virus receptor activity of MF (molecular functions) (Figure 5D). The results of enrichment analysis indicated that MDBD may influence these biological processes and pathways to increase peripheral WBC in radiation-induced leukopenia mice.

3.8 Integrated analysis of MDBD-Treated radiation-induced leukopenia mice from metabolomics and transcriptomics data

In order to further explore the relationship between DEGs and candidate metabolites, 158 DEGs and 35 differential metabolites were uploaded to OmicsNet 2.0 and MetaboAnalyst 5.0 to obtain the potential relationship between DEGs and candidate metabolites regulated by MDBD. The results of metabolites-genes association network analysis finally focused on the steroid biosynthesis, pentose and glucuronate interconversions, glutathione metabolism (Figures 6A,B; Table 2), among which the key genes were Jun, Cxcl2 and Egr1, as these genes had relatively highest scores. At the same time, in transcriptome analysis, through PPI analysis of 158 DEGs, we can also focus on genes Cxcl2, Egr1 and Jun as key genes (Supplementary Figure S4). The integrated analysis suggested that these pathways and genes may be the key biological process and genes for MDBD to increase WBC in radiation-induced leukopenia mice.

3.9 MDBD regulate the gene expression of Cxcl2, Egr1 and Jun

In order to further study the effects of MDBD on the regulation of key metabolic pathways and the accuracy of the multi-omics integrated analysis, we detected the expression of Cxcl2, Egr1 and Jun, which were key genes in the integrated analysis. The bone marrow cells of radiation-induced leukopenia mice were analyzed by qRT-PCR. Compared with the control group, the expression of Cxcl2, Egr1 and Jun in the model group increased significantly. QJSB significantly regulated the mRNA level of these genes (Figure 7). In addition, the results of qRT-PCR analysis were consistent with those of transcriptomics results, which show the reliability of our conclusions.

4 Discussion

Since the first clinical application of radiotherapy in 1896, radiotherapy has undergone tremendous development

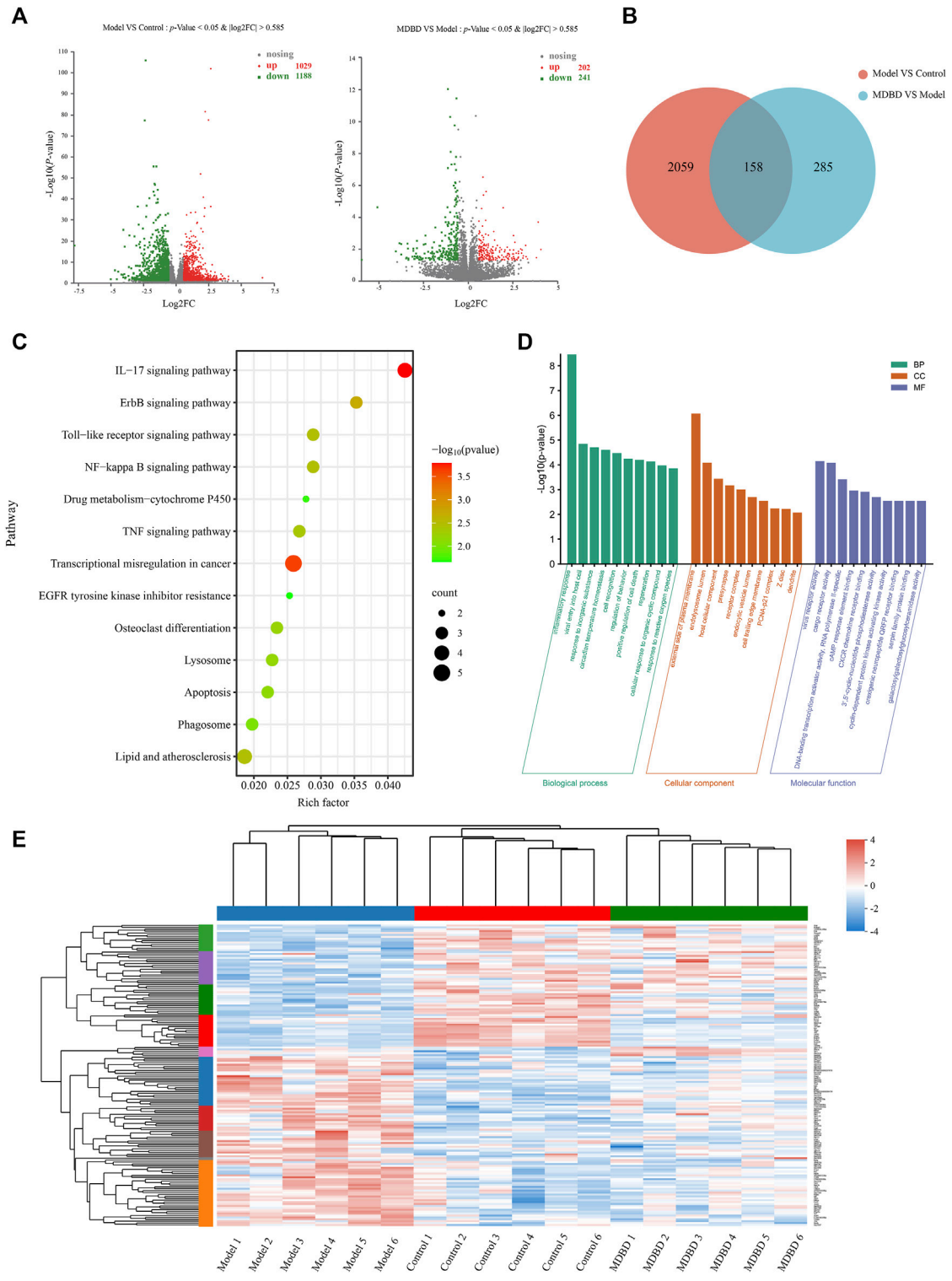

FIGURE 5
Transcriptomics analysis of MDBD on radiation-induced leukopenia mice. **(A)** Relative volcano plot in different groups. **(B)** Veen diagram of differently expressed genes among control, model and MDBD groups. **(C)** KEGG enrichment analysis of differently expressed genes. **(D)** GO enrichment analysis of differently expressed genes. **(E)** Clustering heatmap of DEGs.

(Allen et al., 2017). Currently, radiotherapy is one of the main treatment schedules for approximately 50% of cancer patients, whether used alone or in combination with chemotherapy and surgery to treat a wide range of malignant tumors (Delaney et al., 2005; Citrin, 2017). At the same time, radiation treatments may have chronic or acute side effects, which limit the sustainability

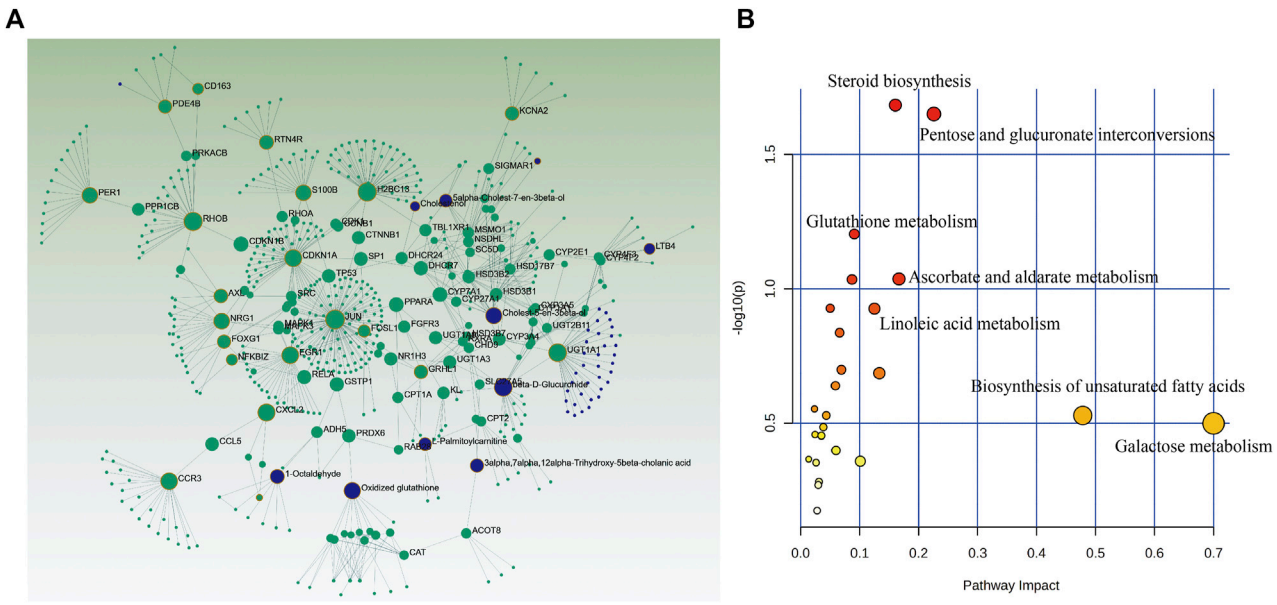

FIGURE 6
Integrated analysis of the differential metabolites and differently expressed genes using OmicsNet 2.0 and MetaboAnalyst 5.0. Metabolites (blue) and genes (green) are presented as nodes and relationships are presented as edges. The input genes and metabolites have a yellow outer circle when having a critical role. **(A)** Integrated analysis network. **(B)** Joint-pathway analysis.

TABLE 2 Integrated analysis of 158 DEGs and 35 differential metabolites.

NO.	Term	Total	Expected	Hits	-Log(*p*)
1	Steroid biosynthesis	82	0.60492	3	1.6833
2	Pentose and glucuronate interconversions	32	0.23607	2	1.6503
3	Glutathione metabolism	56	0.41311	2	1.2035
4	Ascorbate and aldarate metabolism	13	0.095902	1	1.0363
5	Drug metabolism-other enzymes	70	0.51639	2	1.0343
6	Arachidonic acid metabolism	81	0.59754	2	0.92718
7	Linoleic acid metabolism	17	0.12541	1	0.92576
8	Primary bile acid biosynthesis	92	0.67869	2	0.83637
9	Mannose type O-glycan biosynthesis	30	0.22131	1	0.69841
10	Glycosphingolipid biosynthesis-globo and isoglobo series	31	0.22869	1	0.68564
11	Glycerolipid metabolism	35	0.2582	1	0.63883
12	beta-Alanine metabolism	44	0.32459	1	0.55261
13	Retinol metabolism	47	0.34672	1	0.52833
14	Biosynthesis of unsaturated fatty acids	47	0.34672	1	0.52833
15	Galactose metabolism	51	0.37623	1	0.49866

of treatment and affect the quality of life, and leukopenia is one of the most common and costly complications (Jairam et al., 2019). As a hematopoietic drug, the substantial efficacy of DBD has been confirmed by a large number of experiments and clinical data (Yang et al., 2009; Zheng et al., 2010). In this study, we successfully demonstrated that the combination of DBD and ICT also could alleviate radiation-induced leukopenia. In our further study (Supplementary Figure S3), it was found that the effect of

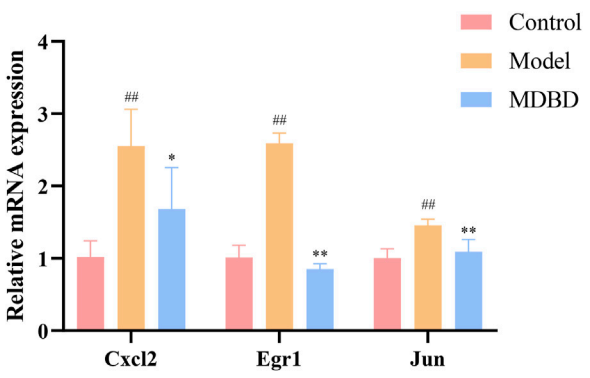

FIGURE 7
The effects of MDBD on the expression of the key genes (Cxcl2, Egr1 and Jun) in bone marrow cells from radiation-reduced leukopenia mice. The data are expressed as means ± SD. #p < 0.05 and ##p < 0.01 vs. control group; *p < 0.05 and **p < 0.01 vs. model group.

increasing WBC with MDBD was better than using DBD or ICT alone, and the effect of medium dose MDBD (6 g/kg DBD +3 mg/kg ICT) was better than that of low dose MDBD (3 g/kg DBD +1.5 mg/kg ICT) and high dose MDBD (12 g/kg DBD +6 mg/kg ICT), which suggests that there may be a synergistic effect between DBD and ICT when used together, thereby improving the efficacy.

In addition, hematopoietic-related cytokines, such as GM-CSF, IL-6 and TPO, had also been detected in the pharmacodynamic study of MDBD due to their important regulatory effects on the hematopoietic system. GM-CSF was originally discovered to be a protein capable of generating granulocyte and macrophage colonies from myeloid precursor cells *in vitro* (Becher et al., 2016). Meanwhile, it was reported that GM-CSF, IL-6 and TPO were regulated by DBD in myelosuppression mice (Liu et al., 2021). However, the over-expression of GM-CSF will over-mobilize HSCs in the temporary non-proliferation phase, which will make them enter the cell proliferation cycle prematurely, resulting in the excessive production and activation of granulocytes and macrophages, ultimately reducing the body's hematopoietic function, and even inducing a variety of hematological diseases (Dhagat et al., 2018). At the same time, studies have shown that excessive secretion of IL-6 will also destroy the homeostasis of HSCs (hematopoietic stem cells), leading to damage to the body's hematopoietic function (O'Hagan-Wong et al., 2016). In our pharmacodynamic study, MDBD reversed the excessive depletion of certain cytokines caused by radiation, which may contribute to the recovery of leukopenia. The mammalian spleen is considered as a secondary peripheral lymphoid organ and a red blood cells bank, which plays an important role in immunity and hematopoiesis (Brendolan et al., 2007). Our study showed that the spleen index significantly improved after administration of MDBD, indicating that MDBD may participate in the regulation of hematopoietic and immune functions to alleviate radiation-induced leukopenia.

HSCs are maintained in the specialized niches of bone marrow throughout life, and long-term maintenance of HSCs is achieved by balancing self-renewal or differentiation with signals that promoted cell division or quiescence (Cordeiro Gomes et al., 2016). Interestingly, MDBD increased cell viability and promoted colony formation of bone marrow mononuclear cells but did not change the number of BMNCs. This indicated that MDBD indirectly increased the hematopoietic capacity of bone marrow cells without changing the quantity. The pharmacodynamic results fully showed that the MDBD designed by our research group could effectively treat radiation-induced leukopenia.

To further explore the potential mechanism of MDBD in the treatment of leukopenia, we adopted metabolomics and transcriptomics techniques. Metabolomics demonstrated that the regulated metabolites were mainly involved in steroid biosynthesis, glutathione metabolism and primary bile acid biosynthesis. As an important derivative of cholesterol, steroids play an active role in regulating the water-salt balance, stress response, metabolism, and in maintaining sexual differentiation (Schiffer et al., 2019). When Xie et al. (Xie et al., 2016) studied the perimenopausal syndrome caused by estrogen deficiency, he found that the modified Danggui Buxue Decoction composed of DG, HQ and *Herba Epimedii* could significantly alleviate the disorder of steroid hormone metabolism in rat serum. Studies suggested that DBD can play the role of estrogen, and the effect of alleviating menopausal syndrome is mainly caused by the calycosin in HQ (Gong et al., 2016). The redox homeostasis of mitochondria is regulated by the rapidly reactive antioxidant system, and glutathione is one of the key substances to maintain the redox homeostasis of mitochondria and repair mitochondrial damage (Mari et al., 2009). The significant regulation of MDBD on glutathione pathway suggests that MDBD may reduce radiation-induced bone marrow injury by regulating the repair of mitochondrial damage, thereby increasing the number of white blood cells in peripheral blood. Bile acid is synthesized by cholesterol in the liver through multi-step enzymatic reaction, which can stimulate the peristalsis of duodenum and colon, and plays an important role in fat metabolism and glucose metabolism (Jia et al., 2018). Study shown that DBD can regulate the homeostasis of intestinal microflora by regulating the biosynthesis of primary bile acids and improve the metabolic abnormalities in mice. It was shown that DBD can regulate the homeostasis of intestinal microflora by regulating the biosynthesis of primary bile acids and improve the metabolic abnormalities in mice (Du et al., 2020). This results also indicate that MDBD may also be involved in regulating intestinal microflora.

Transcriptomics showed that the regulated differential genes mainly participated in IL-17 signaling pathway and ErbB signaling pathway. IL-17 is mainly an inflammatory factor secreted by CD4 T-cell (Th17), which can induce fibroblasts, keratinocytes, endothelial cells, epithelial cells, *etc.*, to synthesize and secrete inflammatory factors such as G-CSF, IL-6, IL-8, MCP-1, PGE2, and is closely related to asthma, rheumatoid arthritis, lupus and other inflammatory diseases (Park et al., 2005). The main function of IL-17 is to induce G-CSF and IL-8 (Kolls and Linden, 2004), as well as chemokine Cxcl1 and Cxcl2 (Onishi and Gaffen, 2010), and participate in the recruitment of neutrophils during tissue inflammation, and finally attract neutrophils and other myeloid cells to the injured tissue to cause inflammatory reaction. Mice with IL-17 receptor deficiency will weaken the host's defense against microbial infection due to the significant reduction of G-CSF in the lung (Ye et al., 2001). Some studies also found that when IL-17 is deficient, mice will show increased resistance to arthritis (Nakae

et al., 2003). The pro-inflammatory ability of IL-17 is the key to its protection, but when the signal pathway of IL-17 is disordered, it may lead to a series of diseases, showing both pathogenic and protective effects on the body (Amatya et al., 2017). The enrichment of IL-17 pathway in transcriptome results indicates that MDBD may participate in inflammatory reaction.

Additionally, bioinformatics analysis indicated that steroid biosynthesis, pentose and glucuronate interconversions, glutathione metabolism may be the key pathways where MDBD exerted its efficacy against leukopenia. The steroid biosynthesis pathway, as the most critical pathway in bioinformatics analysis, has also been proven to be crucial in metabolomics analysis. After irradiation treatment, the content of the three important intermediate products involved in steroid biosynthesis (Cholest-5-en-3beta-ol, Lathosterol, and Cholesterol) decreased. However, MDBD administration can significantly increase the content of these three intermediate products. This result suggests that the steroid biosynthesis pathway may be one of the key pathways in the treatment of leukopenia with MDBD.

In addition, beta-D-Glucuronide, Cholest-5-en-3beta-ol and Oxidized glutathione were the main metabolites and Jun, Cxcl2 and Egr1 were the main genes. As important participants of energy metabolism *in vivo*, beta-D-Glucuronide and Cholest-5-en-3beta-ol metabolic disorder were regulated by MDBD. Oxidized glutathione is one of the main substances that maintain redox homeostasis in the body. It has been reported that DBD pretreatment could enhance the glutathione status of blood cells, thereby improving their resistance to oxidative stress induced damage (Mak et al., 2006). Jun, as the most widely studied member of the transcription factor AP-1 (activator protein-1) family, was involved in a variety of cell activities, such as tumorigenesis, survival, apoptosis, proliferation and histomorphogenesis (Meng and Xia, 2011). Cxcl2 was a potent neutrophil chemoattractant, and this chemokine was almost completely derived from neutrophils (Girbl et al., 2018). Furthermore, Cxcl2 was also a mast cell and macrophage chemokine, which controlled the early stage of neutrophil recruitment during tissue inflammation (De Filippo et al., 2013). Studies have shown that Cxcl2 impairs the function of BMSCs (bone marrow mesenchymal stem cells) and could be used as a serum marker to indicate the BMSCs dysfunctions (Bi et al., 2021). Egr1 was crucial to the quiescence and self-renewal of HSCs (Yang et al., 2022), which were characterized by its self-renewal potential. In addition, Egr1 deficient mice showed a dramatically increase in the steady-state level of dividing HSCs in bone marrow and a significant spontaneous mobilization of HSC into peripheral blood (Min et al., 2008). The qRT-PCR results showed that MDBD could regulate the expression disorder of these main differential genes to approximate normal values to alleviate leukopenia.

5 Conclusion

Despite our growing understanding of pharmacodynamics and functions of MDBD in relieving radiation-induced leukopenia, details of the mechanism of leukocytosis increase remains unclear due to the complexity of the mechanism of the traditional Chinese medicine formula. We used abundant pharmacodynamic data to prove the efficacy of the new prescription MDBD, which was first introduced in treating radiation-induced leukopenia. In addition, the integrated analysis of metabolomics and transcriptomics was conducted to preliminarily explore the protective effect of MDBD on radiation-induced leukopenia in mice through multi-pathways, mainly including steroid biosynthesis and IL-17 signaling pathway, among which the key genes were Jun, Cxcl2 and Egr1.

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: NCBI BioProject (https://www.ncbi.nlm.nih.gov/bioproject/), PRJNA942083.

Ethics statement

The animal study was reviewed and approved by Animal Care and Use Committee of the Naval Medical University, China. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

WZ and XZ conceived the study and reviewed the manuscript; WC drafted manuscript; WC, JX and XW collected and analyzed the data; QD, YS, XX, YW, JW, XW and ZL participated in data collection; JX and YL were involved in figures drawing. All authors contributed to the article and approved the submitted version.

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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.2023.1178724/full#supplementary-material

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Efficacy and safety of Runzao Zhiyang capsule for chronic urticaria: a systematic review and meta-analysis of randomized controlled trials

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Background: Chronic urticaria (CU) is a commonly seen skin disorder featured by recurring wheals, with or without angioedema, lasting for at least 6 weeks. Runzao Zhiyang capsule (RZC) has been widely applied to treat patients with CU. This study is aimed at systematically evaluating the efficacy and safety of RZC in treating CU.

Materials and Methods: Randomized controlled trials (RCTs) of RZC on treating CU from Chinese and English databases were searched. Data were collected by two independent researchers. The Cochrane Collaboration tool was adopted for evaluating the risk of bias. The meta-analysis was performed with Review Manager 5.3 software. Sensitivity analysis and publication bias assessment were conducted by Stata 14.0 software.

Results: Totally 27 studies were included in the analysis, involving 2,703 patients. The pooled results showed that compared with second-generation H1-antihistamines (sgAHs) therapy alone, RZC combined with sgAHs is more effective in improving the total effective rate (RR = 1.32, 95% CI: 1.25 to 1.39, $p < 0.00001$), the quality of life measured by Dermatology Life Quality Index (DLQI) (MD = −2.63, 95% CI: −3.68 to −1.58, $p < 0.00001$) and the serum IFN-γ level (SMD = 3.10, 95% CI: 1.58 to 4.62, $p < 0.0001$), and reducing the recurrence rate (RR = 0.39, 95% CI: 0.27 to 0.55, $p < 0.00001$), the serum total IgE level (SMD = −2.44, 95% CI: −3.51 to −1.38, $p < 0.00001$), the serum IL-4 level (SMD = −2.96, 95% CI: −4.10 to −1.83, $p < 0.00001$), and the incidence of adverse events including dizziness, fatigue, dry mouth, and constipation (RR = 0.53, 95% CI: 0.33 to 0.85, $p = 0.009$; RR = 0.46, 95% CI: 0.26 to 0.84, $p = 0.01$; RR = 0.57, 95% CI: 0.34 to 0.95, $p = 0.03$; RR = 0.24, 95% CI: 0.07 to 0.85, $p = 0.03$).

Conclusion: The current evidence indicates that RZC may be an efficient therapeutic regimen in patients with CU. Nevertheless, owing to the suboptimal quality of the included studies, more large-scale, well-designed RCTs are required to verify the obtained findings.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/; Identifier: CRD42022313177.

KEYWORDS

Runzao Zhiyang capsule, chronic urticaria, antihistamines, randomized controlled trials, meta-analysis

1 Introduction

Urticaria is one of the commonly seen dermatoses, characterized by the sudden development of transient hives (wheals) and/or angioedema (Antia et al., 2018). Based on its duration, it can be subdivided into acute and chronic urticaria. In general, episodes with or without angioedema lasting for over 6 weeks are defined as chronic urticaria (CU) (Zuberbier et al., 2022). CU is characterized by intensely pruritic wheals generally undergoing spontaneous resolution within 24 h, and resolves without residual hyperpigmentation or ecchymoses, which is clinically distinct from the wheals' persistence and purpura/residual hyperpigmentation of urticarial vasculitis (Marzano et al., 2022). It has been shown that the point prevalence of CU in Asian studies is 1.4% in relative to 0.5% and 0.1% in Europe and Northern America, respectively (Fricke et al., 2020). Due to the itching or physical discomfort during outbreaks of CU, recurrent symptoms and long duration, CU imposes a substantial burden on the patients, their families, public health systems, and the society (Gonçalo et al., 2021).

The pathogenesis of CU has not been completely understood. Some studies have demonstrated that mast cells (MCs) and histamine are crucial mediators in etiopathogenesis (Xue et al., 2023). The standard-dosed, second-generation H1-antihistamines (sgAHs) are the first-line pharmacological treatments in guiding the management of CU (Zuberbier et al., 2022). However, licensed doses of sgAHs provide complete symptom relief in less than half of the patients (Agache et al., 2021; Nochaiwong et al., 2021; Patil et al., 2020). When there is no improvement in the clinical symptoms, the dose is increased up to four-fold as second-line therapy (Zuberbier et al., 2022). Among around 50% of patients with CU, symptoms persist even after receiving increased doses of sgAHs or the combination of different sgAHs (Holm et al., 2018; Nochaiwong et al., 2022; Pereyra-Rodriguez et al., 2020). Omalizumab is the monoclonal anti-IgE antibody and is recommended as an add-on therapy for CU in patients who fail to respond to H1-antihistamine according to the EAACI/GA2LEN/EDF/WAO guidelines issued in 2022 (Rubini et al., 2019). Nevertheless, due to the high cost, it is burdensome for most of CU patients (Xiao et al., 2020; Agache et al., 2021). Other drugs, such as cyclosporine and systemic corticosteroids, can also be applied in cases resistant to H1 antihistamines and omalizumab (He et al., 2021). However, cyclosporine is not recommended as a standard treatment because of its systemic adverse effects, such as gastrointestinal symptoms, headache, nephrotoxicity, and elevated blood pressure (Matsubara et al., 2021; Zuberbier et al., 2022). The adverse effect of corticosteroids, such as infection, also limits their clinical application (Yao et al., 2015).

Traditional Chinese medicine (TCM) can be adopted for treating CU following the guideline for the diagnosis and treatment of urticaria in China (2022 edition) (Centre for Urticaria Research of Chinese Society of Dermatology, 2022). As a well-known traditional Chinese patent medicine, Runzao Zhiyang capsule (RZC) is approved for marketing with an approval number of Z20025030 by National Medical Products Administration, and consists of botanical drugs, including *Reynoutria multiflora* (Thunb.) Moldenke [Polygonaceae; Polygoni multiflori radix], *Rehmannia glutinosa* (Gaertn.) DC. [Orobanchaceae; Rehmanniae radix], *Morus alba* L. [Moraceae; Mori folium], *Sophora flavescens* Aiton [Fabaceae; Sophorae flavescentis radix], *Laportea bulbifera* (Siebold & Zucc.) Wedd. [Urticaceae; Laportea herba]. The details of RZC, including the source, composition, description, extraction procedure, its actions, indications, etc., are showed in Supplementary Material S1, the key active ingredients in RZC are summarized in Supplementary Material S2. Modern pharmacological studies have found that several major compounds of RZC, including catalpol, chlorogenic acid, matrine, and formononetin, play a role in inhibiting mast cells degranulation and reducing histamine release (Inami et al., 2013; Lv and Kang, 2016; Xu and An, 2017; Chiu et al., 2021). RZC has immune regulation and anti-inflammation effects, and is often used to relieve itching symptoms of skin disorders (Zheng et al., 2018; Li et al., 2021). Some clinical trials have demonstrated that RZC in combination with sgAHs can significantly improve the efficacy compared with sgAHs alone without serious adverse events. This indicates that RZC may become a potential treatment option for CU (Dou and Zhang, 2019; Ye et al., 2022).

Based on our knowledge, the relatively small sample size and different outcome assessments exist in the single RCT of RZC in treating CU, which failed to offer a systematic and comprehensive assessment of the clinical application of RZC. Till the present, no high-quality systematic reviews on this topic have been published. This study was conducted to critically estimate the efficacy and safety of RZC for treating CU.

2 Materials and methods

This study was performed and reported in line with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) Guidelines (Page et al., 2021) (Supplementary Material S3). It has been registered on the PROSPERO platform (CRD42022313177).

2.1 Eligibility criteria

2.1.1 Types of studies

Only parallel-group RCTs were involved, whereas quasi-RCTs, in which participants were allocated according to the date of birth, hospital record number, or date of admission were not included.

2.1.2 Types of participants

Patients who had a confirmed diagnosis of CU were included irrespective of age, sex, race, and nationality. The recognized diagnostic criteria of CU need to be reported in the included studies, such as guidelines for the diagnosis and therapy of urticaria in China (Centre for Urticaria Research of Chinese Society of Dermatology, 2019) or the EAACI/GA2LEN/EDF/WAO guideline (Zuberbier et al., 2022).

2.1.3 Types of interventions

2.1.3.1 Experimental interventions

The intervention of experimental group was RZC alone or plus sgAHs. Studies with the other TCM treatment in the experimental group (such as other Chinese patent medicine or decoction, Chinese medicine injections, massage, Tai Chi, Qigong, acupuncture, and moxibustion) were not included. There were no limitations on treatment frequency, dosages, and course of RZC.

2.1.3.2 Comparator interventions

The interventions of control group could be placebo, no treatment, or sgAHs. The specific type of sgAHs (Li, 2021; Phinyo et al., 2021) needs to be reported clearly. We investigated the following comparisons:

RZC alone compared with placebo.
RZC alone compared with no treatment.
RZC alone compared with sgAHs.
RZC plus sgAHs compared with sgAHs alone.
RZC plus sgAHs compared with placebo plus sgAHs.

2.1.4 Types of outcome measures

The primary outcome is the total effective rate calculated based on the reduction of the Clinical Symptom Score (CSS) or Urticaria Activity Score (UAS) (Liu et al., 2018). To be specific, the "effectivity" was defined as a more than 60% reduction of CSS or UAS from the baseline for patients (Xiao et al., 2020). The total effective rate is equal to the number of cases labeled as the "effectivity" divided by the total number of cases in one group.

Secondary outcomes include the Dermatology Life Quality Index (DLQI), recurrence rate, serum total immunoglobulin E (IgE) level, serum Interleukin-4 (IL-4) level, serum Interferon-gamma (IFN-γ) level, and adverse reactions.

2.2 Literature search

PubMed, Embase, Cochrane Library, Web of Science, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (VIP), Wanfang Database and the Chinese Biomedical Literature Database (SinoMed) were searched from inception to 12 May, 2023. Due to different search regulations across the databases, a search strategy containing medical subject headings and free text words was used and modified if necessary. The search terms such as urticarial, hive, chronic urticaria, Runzao Zhiyang capsule, traditional Chinese Medicine, Chinese patent medicine were selected. Supplementary Material S4 presents the detailed search strategies. To obtain other potentially eligible trials, the reference lists of the relevant reviews, and some trial registration platforms such as Clinical Trials.gov and the Chinese Clinical Trial Registry were searched manually. In addition, no restriction on publication status or language was required.

2.3 Literature selecting and data extraction

All the studies identified from the electronic search were managed by NoteExpress Version 3.0. After excluding the duplicates, two reviewers (J. Zhang and Y. Wang) independently inspected the titles and abstracts to eliminate the irrelevant studies. Then, full-texts of the remaining studies were downloaded and read to identify the potentially eligible studies. The data of included articles, such as the first author, year of publication, age, sex, sample size, interventions, treatment course, follow-up period, and outcome indicators were extracted by two authors (J. Zhang and P. Lin) independently. Any disagreement between the two reviewers was resolved by the third author (J. Zhai).

2.4 Quality assessment

Following the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2022), the risk of bias in the involved trials was evaluated independently by two investigators (J. Zhang and J. Li). Seven domains, namely, random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources of bias were evaluated. Each of them was classified to be low, unclear, or high risk of bias. The "risk of bias" summary and graph showed the risk of bias assessment. Any disagreement was resolved by the third author (J. Zhai).

2.5 Statistical analysis

Revman 5.3 version software was applied to carry out meta-analyses. For dichotomous outcomes, the treatment effect was estimated with risk ratio (RR) and 95% confidence intervals (CIs). For continuous outcomes, mean difference (MD) or standardized mean difference (SMD) with 95% CIs was used. Chi-square test was conducted and I^2 was estimated to assess the heterogeneity across the studies. If a significant heterogeneity ($p < 0.1$ or $I^2>50\%$) was found, a random effects model was selected to conduct the meta-analysis. Otherwise, a fixed effects model was built. Subgroup analysis was performed based on the course of RZC or the follow-up period if possible. Sensitivity analysis was conducted by Stata 14.0 software. When over ten studies were contained in a meta-analysis, a funnel plot was adopted for evaluating the potential publication bias. Harbord test was carried out to test the publication bias (Sterne et al., 2011).

3 Results

3.1 Literature screening

Totally 314 potentially relevant articles were initially searched. Fifty-four duplicated studies were excluded by NoteExpress software. Then, 216 articles were removed after screening the titles and abstracts. Among the remaining 44 articles, 17 were further excluded after reading the full-texts. Finally, 27 eligible studies were included (Ai, 2020; Bian et al., 2018; Chen et al., 2016; Chen et al., 2020; Cheng, 2019; Du et al., 2018; Feng et al., 2011; Feng et al., 2016; Huang, 2011; Li Y. et al., 2017; Li, 2019; Liu and Li, 2008; Liu and Yang, 2019; Luo et al., 2016; Lv, 2018; Ma et al., 2014; Sun, 2014; Tan, 2018; Tang and Xu, 2021; Wang and Fang, 2013; Wang et al., 2018; Xiao, 2020; Yang, 2019; Zhang, 2010; Zhang,

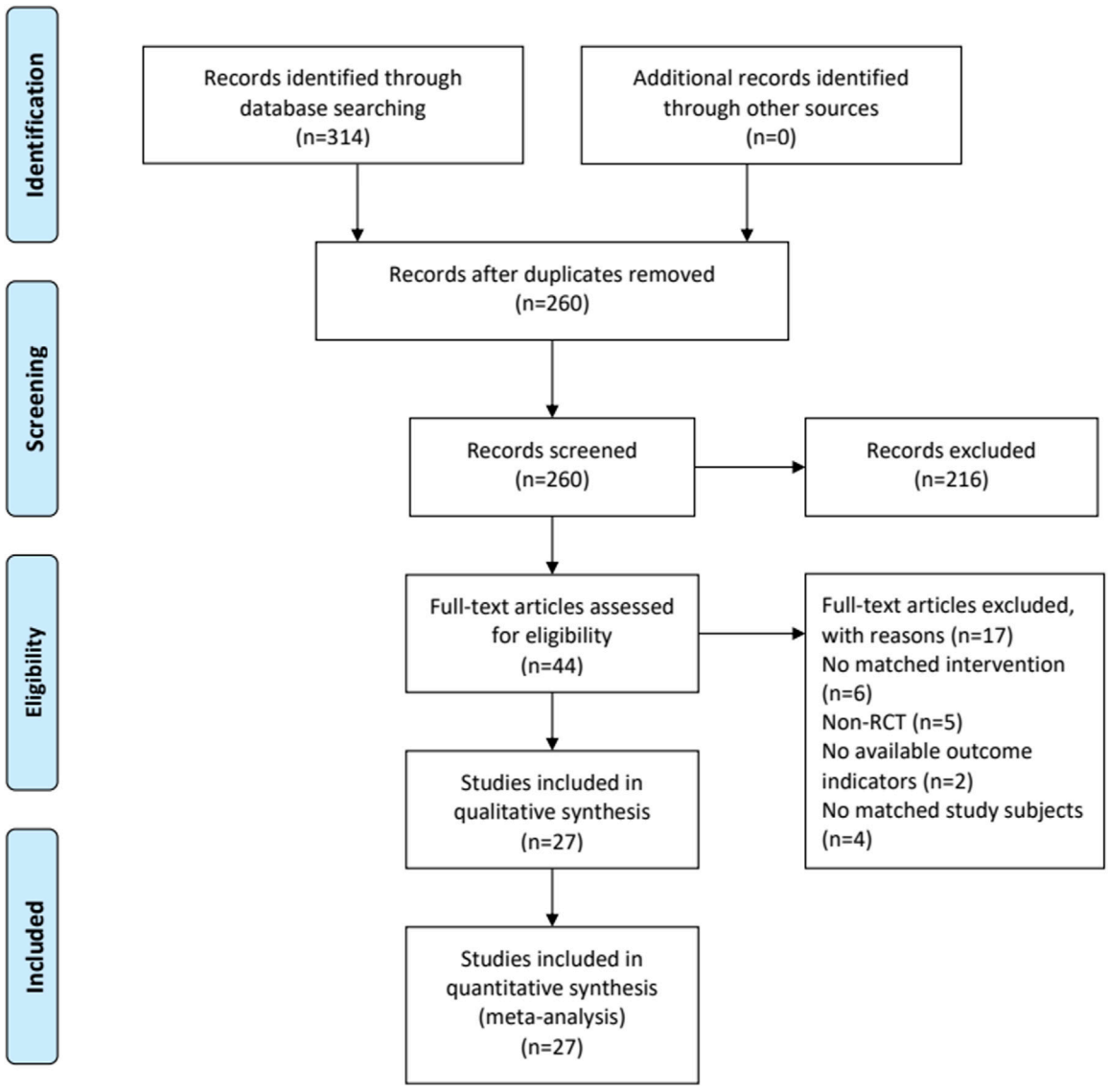

FIGURE 1
The flowchart of study selection.

2014; Zhang, 2020; Zhou, 2020). Figure 1 displays the flowchart of the study selection.

3.2 Study characteristics

All the trials were performed in China. Totally 2,703 patients were enrolled involving 1,368 participants in the RZC plus sgAHs group and 1,335 participants in the sgAHs alone group. They were published from 2008 to 2021. The sample size ranged from 24 to 106. The course of treatment lasted from 2 weeks to 2 months. RZC was taken orally at the dose of 2.0 g three times a day in all the included records. Ten types of sgAHs (desloratadine citrate disodium, cetirizine, desloratadine, levocetirizine, loratadine, ebastine, mizolastine, olopatadine, fexofenadine, and epinastine) were found in the sgAHs alone group. Ten studies reported the duration of follow-up. Only one study (Feng et al., 2011) reported the follow-up period of 7 days, which was the same as the number of studies reporting the follow-up period of 2 months (Bian et al., 2018), 3 months (Feng et al., 2016), and 6–9 months (Zhang, 2014). Six studies (Chen et al., 2016; Liu and Yang, 2019; Luo et al., 2016; Wang, 2018; Wang and Fang, 2013; Li Y. et al., 2017) reported the follow-up periods of 4 weeks to 1 month. Table1 presents the characteristics of the involved studies. The summary of composition characteristics of preparations in all included studies see Supplementary Material S5.

3.3 Assessment of risk of bias

All of the 27 RCTs mentioned the random. Among them, 12 trials showed the use of random number tables (Chen et al., 2016; Chen et al., 2020; Cheng, 2019; Feng et al., 2016; Li Y. et al., 2017; Li, 2019; Tan, 2018; Tang and Xu, 2021; Wang and Fang, 2013; Wang et al., 2018; Xiao, 2020; Zhang, 2020), and one trial used the

TABLE 1 The characteristics of included studies.

First author (publication year)	Sample size (T/C)	Sex (Male/ Female)	Age (years)	Course of disease	Interventions (T)	Interventions (C)	Treatment course	Follow-up period	Outcomes
Yang (2019)	61/61	T:30/31	T: 31.8 ± 10.6	T:3m-7y	RZS 2g tid + IC	Desloratadine tablet 5 mg qd	4w	NR	⑦
		C:29/32	C: 30.5 ± 9.8	C:3m-7y					
Chen et al. (2016)	45/35	NR	T/C: 15-68	T/C:>6w	RZS 2g tid + IC	Desloratadine tablet 5 mg qd	4w	1m	①④⑦
Feng et al. (2011)	50/50	T:24/26	T:18-62	T:6w-12y	RZS 2g tid + IC	Fexofenadine tablet 60 mg bid	4w	7d	①③⑦
		T:22/28	C:18-65	C:6w-14y					
Lv (2018)	59/59	T:29/30	T:18-68	T:4m-2y	RZS 2g tid + IC	Ebastine tablet 20 mg qd	4w	NR	⑦
		C:33/26	C:18-65	C:3m-2y					
Luo et al. (2016)	63/63	NR	16–65	T:2m-4.2y	RZS 2g tid + IC	Mizolastine tablet 10 mg qd	4w	4w	①③⑦
				C:2m-4.2y					
Tan (2018)	50/50	T:27/23	T:21-60	T:4m-4y	RZS 2g tid + IC	Epinastine capsule 10 mg qd	4w	NR	④⑤⑦
		C:28/22	C:22-59	C:3m-4y					
Xiao (2020)	30/30	T:17/13	T: 35.69 ± 2.31	T:2m-4y	RZS 2g tid + IC	Loratadine tablet 5 mg qd	4w	NR	⑦
		C:18/12	C: 36.16 ± 2.14	C:3m-5y					
Li (2019)	50/50	T:26/24	T: 48.56 ± 3.26	T:2m-4y	RZS 2g tid + IC	Olopatadine tablet 5 mg bid	4w	NR	⑦
		C:25/25	C: 49.95 ± 3.86	C:3m-3.5y					
Feng et al. (2016)	60/60	T:32/28	T:21-52	T:7w-2y	RZS 2g tid + IC	Desloratadine citrate disodium capsule 8.8 mg qd	4w	3m	①③④⑦
		C:24/36	C:22-55	C:9w-3y					
Liu and Li (2008)	60/60	T:29/31	T:18-67	T:2m-6y	RZS 2g tid + IC	Loratadine 10 mg qd	4w	NR	①⑦
		C:33/27	C:20-66	C:3m-5y					
Li et al. (2017)	35/35	T:18/17	T: 34.23 ± 9.86	T: (25.72 ± 10.41) m	RZS 2g tid + IC	Fexofenadine tablet 60 mg bid	8w	1m	①③⑦
		C:19/16	C: 33.12 ± 11.13	C: (26.75 ± 14.50) m					
Tang and Xu (2021)	30/30	T:17/13	T: 35.62 ± 3.35	T:3m-6y	RZS 2g tid + IC	Cetirizine 10 mg qd	4w	NR	①②④
		C:18/12	C: 34.42 ± 3.39	C:2m-6y					
Bian et al. (2018)	106/94	T:60/46	T: 34.93 ± 2.53	T:3m-6y	RZS 2g tid + IC	Loratadine tablet 1tablet qd	1m	2m	①③④⑤⑦
		C:56/38	C: 35.13 ± 2.15	C:2m-7y					

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TABLE 1 (*Continued*) The characteristics of included studies.

First author (publication year)	Sample size (T/C)	Sex (Male/Female)	Age (years)	Course of disease	Interventions (T)	Interventions (C)	Treatment course	Follow-up period	Outcomes
Du et al. (2018)	42/42	T:21/21	T: 32.41 ± 1.76	T:1m-8y	RZS 2g tid + IC	Olopatadine tablet 5 mg bid	2w	NR	①②⑤⑥
		C:23/19	C: 32.54 ± 1.89	C:2m-8y					
Liu and Yang (2019)	50/50	T:28/22	T:16-78	T:2m-2y	RZS 2g tid + IC	Levocetirizine 5 mg qd	2m	4w	①③⑦
		T:31/19	C:17-82	C:2m-2y					
Ai (2020)	24/24	T:14/10	T: 35.25 ± 1.39	T:6m-9y	RZS 2g tid + IC	Levocetirizine 10 mg qd	2m	NR	①⑤⑥⑦
		C:13/11	C: 35.12 ± 1.46	C:7m-9y					
Zhou (2020)	55/53	T:31/24	T: 35.26 ± 1.37	T:5m-6y	RZS 2g tid + IC	Ebastine 10 mg qd	4w	NR	②③⑦
		C:30/23	C: 35.19 ± 1.31	C:6m-5.5y					
Ma et al. (2014)	56/54	T:32/24	T:20-65	T:6w-28w	RZS 2g tid + IC	Mizolastine 10 mg qd	4w	NR	①⑦
		C:31/23	C:21-62	C:6w-27w					
Sun (2014)	42/39	T:28/14	T:13-41	T:5m-8y	RZS 2g tid + IC	Desloratadine citrate disodium tablets	2w	NR	①⑦
		C:21/18	C:15-43	C:9m-8y		8.8 mg qn			
Chen et al. (2020)	35/35	NR	T: 38.5 ± 3.1	T:2m-2y	RZS 2g tid + IC	Ebastine 10 mg qd	4w	NR	①②④⑤⑥⑦
			C: 38.5 ± 3.1	C:2m-2y					
Zhang (2020)	45/45	T:23/22	T: 40.25 ± 1.7	T:5m-5y	RZS 2g tid + IC	Levocetirizine 5 mg qd	4w	NR	①
		C:24/21	C: 40.15 ± 1.25	C:6m-5y					
Wang and Fang (2013)	46/46	T:30/16	T: 35.8 ± 1.5	T:2m-5y	RZS 2g tid + IC	Mizolastine tablet 10 mg qd	4w	1m	①③⑦
		C:28/18	C: 35.7 ± 1.6	C:2m-6y					
Cheng (2019)	50/50	T:28/22	T: 26.21 ± 2.11	T:1y-3y	RZS 2g tid + IC	Epinastine tablet 10 mg qd	4w	NR	⑦
		C:27/23	C: 26.26 ± 2.24	C:1y-3y					
Wang et al. (2018)	64/62	NR	T:>16; C:>16	T:>6w; C:>6w	RZS 2g tid + IC	Ebastine 10 mg qd	4w	4w	①②③⑦

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TABLE 1 (*Continued*) **The characteristics of included studies.**

First author (publication year)	Sample size (T/C)	Sex (Male/ Female)	Age (years)	Course of disease	Interventions (T)	Interventions (C)	Treatment course	Follow-up period	Outcomes
Zhang (2014)	42/42	NR	T: 40.62 ± 2.77	T:9m-7y	RZS 2g tid + IC	Ebastine capsule 10 mg qd	1m	6–9 m	②③⑦
			C: 40.62 ± 2.77	C:9m-7y					
Huang (2011)	80/80	T:36/44	T:18-55	T:2m-8y	RZS 2g tid + IC	Mizolastine sustained-release tablets 10 mg qd	4w	NR	①⑦
		C:42/38	C:18-55	C:2m-8y					
Zhang (2010)	38/36	NR	T:16-63	T:2m-3y	RZS 2g tid + IC	Mizolastine sustained-release tablets 10 mg qn	2w	NR	①⑦
			C:16-63	C:2m-3y					

Note: T, treatment; C, control; IC, interventions in control group; qd, quaque die; bid, bis in die; tid, ter in die; qn, quaque nocte; d, day; w, week; m, month; ① Total effective rate; ② Dermatology life quality index (DLQI); ③ Recurrence rate; ④ Serum total IgE level; ⑤ Serum IL-4, level; ⑥ Serum IFN-γ, level; ⑦ The incidence of adverse events.

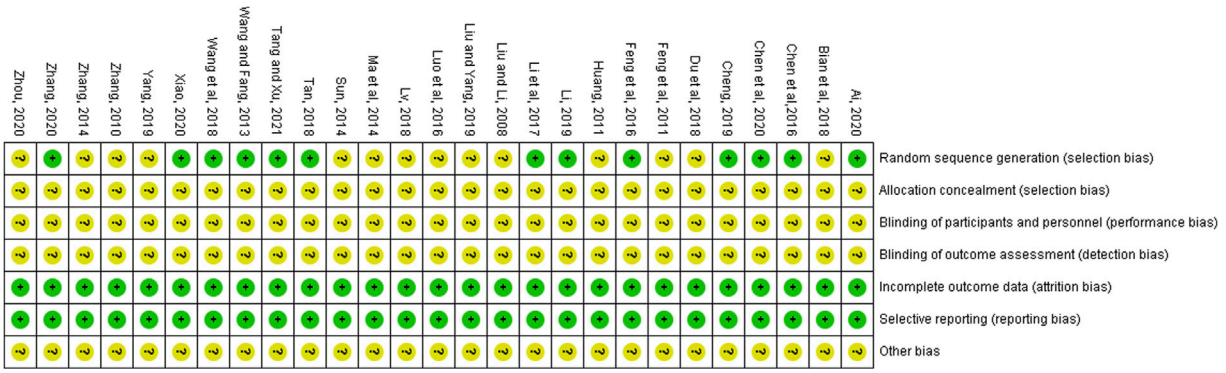

FIGURE 2
Bias risk assessment of included studies.

method of random touch balls (Ai, 2020). However, in the remaining studies, the specific random method was not described (Bian et al., 2018; Du et al., 2018; Feng et al., 2011; Huang, 2011; Liu and Li, 2008; Liu and Yang, 2019; Luo et al., 2016; Lv, 2018; Ma et al., 2014; Sun, 2014; Yang, 2019; Zhang, 2010; Zhang, 2014; Zhou, 2020). None of the studies mentioned allocation concealment, blinding of participants and personnel, as well as blinding of outcome assessment. The 27 included articles had complete data and no selectively reported results. Other risk of biases was classified as unclear due to the insufficient information. Figure 2 presents the results of the risk of bias evaluation.

3.4 Results of meta-analyses

3.4.1 Primary outcomes

3.4.1.1 Total effective rate

Nineteen trials involving 1,911 patients (971 in the RZC plus sgAHs group and 940 in the sgAHs alone group) reported the total effective rate (Ai, 2020; Bian et al., 2018; Chen et al., 2016; Chen et al., 2020; Du et al., 2018; Feng et al., 2011; Feng et al., 2016; Huang, 2011; Li Y. et al., 2017; Liu and Li., 2008; Liu and Yang, 2019; Luo et al., 2016; Ma et al., 2014; Sun, 2014; Tang and Xu, 2021; Wang and Fang, 2013; Wang et al., 2018; Zhang, 2010; Zhang, 2020). According to the result of a meta-analysis involving 19 studies, the combination of RZC and sgAHs could obviously improve the total effective rate compared with sgAHs alone (RR = 1.32, 95% CI: 1.25 to 1.39, $p < 0.00001$, Figure 3). A subgroup analysis was performed in accordance with the course of RZC. The total effective rate in the RZC with a course of 2 weeks plus sgAHs group was notably higher than that in the sgAHs alone group (RR = 1.51, 95% CI: 1.27 to 1.79, $p < 0.00001$). The similar findings were obtained after sgAHs treatment plus RZC with a course of 4 weeks (RR = 1.27, 95% CI: 1.20 to 1.35, $p < 0.00001$), 1 month (RR = 1.34, 95% CI: 1.13 to 1.60, $p = 0.0007$), and 2 months (RR = 1.46, 95% CI: 1.14 to 1.87, $p = 0.003$).

3.4.2 Secondary outcomes

3.4.2.1 DLQI

DLQI is one of the vital indicators which can be adopted for assessing the quality of life in the patients with urticaria. Six studies

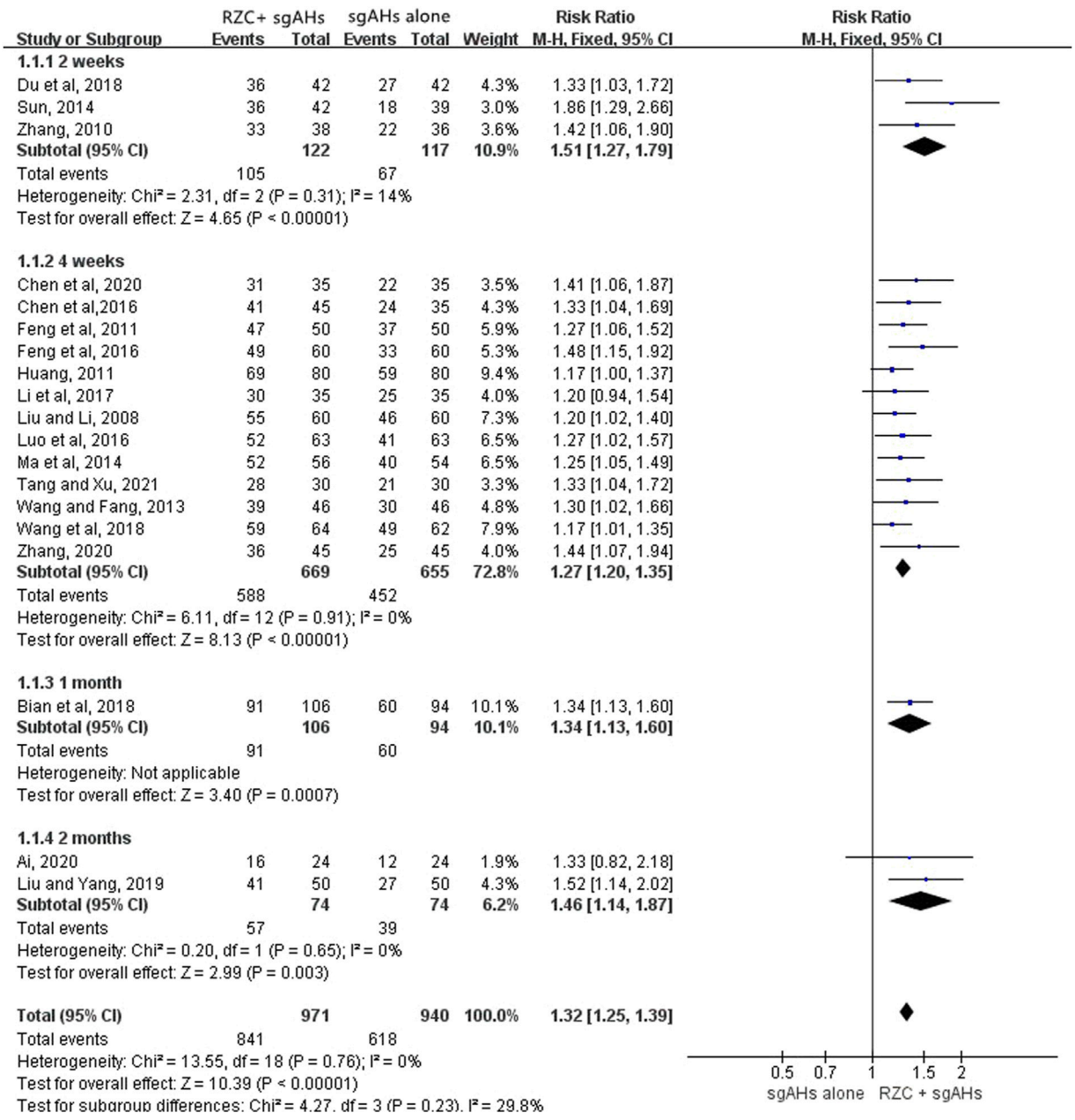

FIGURE 3
Forest plot for total effective rate.

involving 532 participants reported the DLQI (Chen et al., 2020; Du et al., 2018; Tang and Xu, 2021; Wang et al., 2018; Zhang, 2014; Zhou, 2020). The results of a meta-analysis involving six trials suggested that in relative to sgAHs alone, RZC combined with sgAHs significantly improved the quality of life measured by DLQI (MD = −2.63, 95% CI: −3.68 to −1.58, $p < 0.00001$, Figure 4).The results of subgroup analyses showed that RZC combined with sgAHs improved the quality of life measured by DLQI to a great extent compared with sgAHs alone regardless of the treatment course of 2 weeks (MD = −3.41, 95% CI: −3.74 to −3.08, $p < 0.00001$), 4 weeks (MD = −2.30, 95% CI: −3.72 to −0.89, $p = 0.001$), or 1 month (MD = −3.15, 95% CI: −3.62 to −2.68, $p < 0.00001$).

3.4.2.2 Recurrence rate

Ten studies involving 818 participants (447 cases in the RZC combined with sgAHs group and 371 cases in the sgAHs alone group) reported the recurrence rate. One study [Zhou (2020)] reported the number of urticaria recurrence (3 of 55 patients in the RZC plus sgAHs group and 10 of 53 patients in the sgAHs alone group), but did not mention a specific follow-up period, suggesting a lower recurrence rate in the RZC plus sgAHs group relative to the sgAHs alone group (RR = 0.29, 95% CI:0.08 to 0.99, $p = 0.05$).

A meta-analysis involving the remaining nine studies was conducted (Bian et al., 2018; Feng et al., 2011; Feng et al., 2016; Li Y. et al., 2017; Liu and Yang, 2019; Luo et al., 2016; Wang and Fang, 2013; Wang et al., 2018; Zhang, 2014). A subgroup analysis was carried

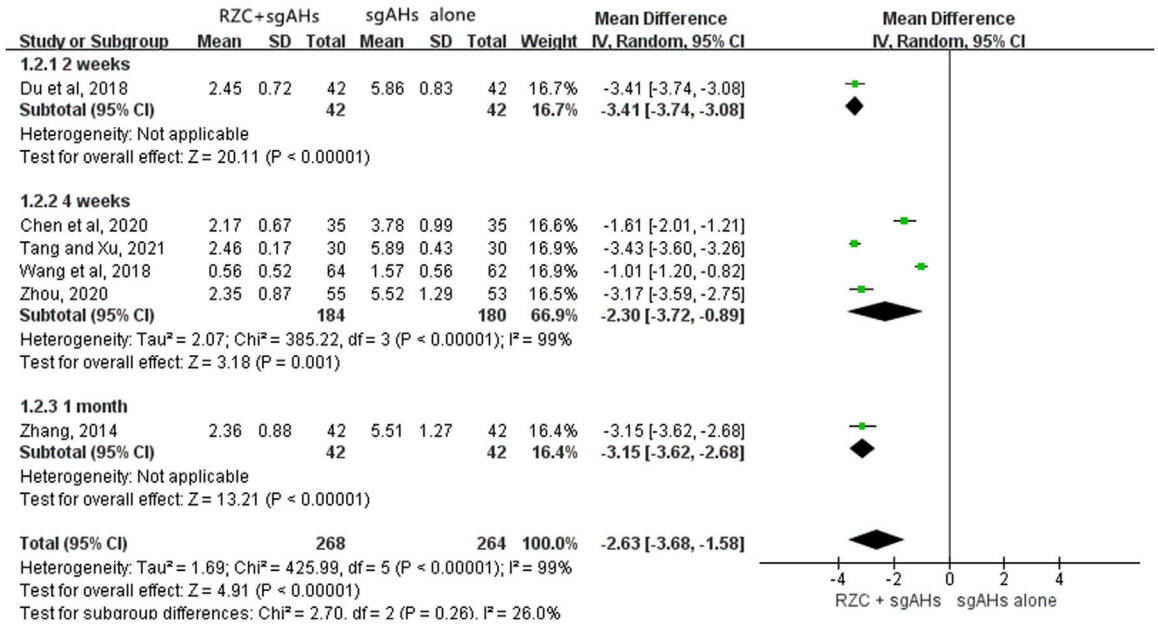

FIGURE 4
Forest plot for DLQI.

out based on the follow-up period. The findings of the meta-analysis involving the nine studies demonstrated that the recurrence rate in the RZC plus sgAHs group was lower than that in the sgAHs alone group (RR = 0.39, 95% CI: 0.27 to 0.55, $p < 0.00001$, Figure 5), with the statistical significance. No statistical difference was found after a follow-up period of 7 days (RR = 0.97, 95% CI: 0.36 to 2.64, $p = 0.95$) or 4 weeks (RR = 0.54, 95% CI: 0.28 to 1.02, $p = 0.06$). However, the recurrence rate in the RZC combined with sgAHs group was significantly lower when compared with that in the sgAHs alone group after the follow-up period of 1 month (RR = 0.29, 95% CI: 0.13 to 0.62, $p = 0.001$), 2 months (RR = 0.34, 95% CI: 0.13 to 0.92, $p = 0.03$), 3 months (RR = 0.18, 95% CI: 0.06 to 0.59, $p = 0.005$), and 6–9 months (RR = 0.31, 95% CI: 0.11 to 0.87, $p = 0.03$).

Table 2 shows subgroup analyses on the recurrence rate based on the course of treatment and follow-up period. A lower recurrence rate was found in the RZC with a course of 4 weeks plus sgAHs group in relative to the sgAHs alone group after a follow-up period of 1 month (RR = 0.28, 95% CI: 0.13 to 0.60, $p = 0.001$) or 3 months (RR = 0.25, 95% CI: 0.08 to 0.81, $p = 0.02$). However, the statistical difference in the recurrence rate was not identified between the two groups after a follow-up period of 7 days (RR = 0.97, 95% CI: 0.36 to 2.64, $p = 0.95$) or 4 weeks (RR = 0.67, 95% CI: 0.32 to 1.39, $p = 0.28$). The recurrence rate in the RZC combined with sgAHs group was lower than that in the sgAHs alone group when patients took RZC for 1 month and underwent a follow-up period of 2 months (RR = 0.34, 95% CI: 0.13 to 0.92, $p = 0.03$) or 6–9 months (RR = 0.31, 95% CI: 0.11 to 0.87, $p = 0.03$). Nevertheless, no obvious difference existed in recurrence rate between the two groups after RZC treatment with a course of 2 months, and a follow-up period of 4 weeks (RR = 0.23, 95% CI: 0.05 to 1.05, $p = 0.06$).

3.4.2.3 Serum total IgE level

Six studies involving 630 participants reported the serum total IgE level with different units, including iu/mL, ng/mL, and pg/mL (Bian et al., 2018; Chen et al., 2016; Chen et al., 2020; Feng et al., 2016; Tan, 2018; Tang and Xu, 2021). Therefore, SMD was used to describe the effects. The pooled result indicated that the RZC combined with sgAHs group obviously decreased the serum total IgE level in relative to the sgAHs alone group (SMD = −2.44, 95% CI: −3.51 to −1.38, $p < 0.00001$, Figure 6). The findings of subgroup analyses demonstrated that the RZC plus sgAHs group could lower the serum total IgE level more than the sgAHs alone group regardless of the treatment course of 4 weeks (SMD = −2.80, 95% CI: −3.81 to −1.78, $p < 0.00001$) or 1 month (SMD = −0.72, 95% CI: −1.00 to −0.43, $p < 0.00001$).

3.4.2.4 Serum IL-4 level

As five studies presented the serum IL-4 level with different units, such as pg/mL, ng/mL and ng/L, SMD was applied to describe the effects (Ai, 2020; Bian et al., 2018; Chen et al., 2020; Du et al., 2018; Tan, 2018). According to the pooled results, the combination of RZC and sgAHs significantly reduced serum IL-4 level compared with the sgAHs alone group (SMD = −2.96, 95% CI: −4.10 to −1.83, $p < 0.00001$, Figure 7). The results of subgroup analyses based on the treatment course were similar regardless of the treatment course of 2 weeks (SMD = −2.32, 95% CI: −2.88 to −1.76, $p < 0.00001$), 4 weeks (SMD = −4.06, 95% CI: −4.61 to −3.51, $p < 0.00001$), 1 month (SMD = −1.44, 95% CI: −1.75 to −1.13, $p < 0.00001$) or 2 months (SMD = −2.96, 95% CI: −3.80 to −2.12, $p < 0.00001$).

3.4.2.5 Serum IFN-γ level

Three studies reported the serum IFN-γ level (Ai, 2020; Chen et al., 2020; Du et al., 2018). The unit "pg/mL" was used in the two studies, with "ng/mL" being used in another one. The result of meta-analysis suggested that the serum IFN-γ level in the RZC combined with sgAHs group was statistically higher than that in the sgAHs alone group (SMD = 3.10, 95% CI: 1.58 to 4.62, $p < 0.0001$, Figure 8).

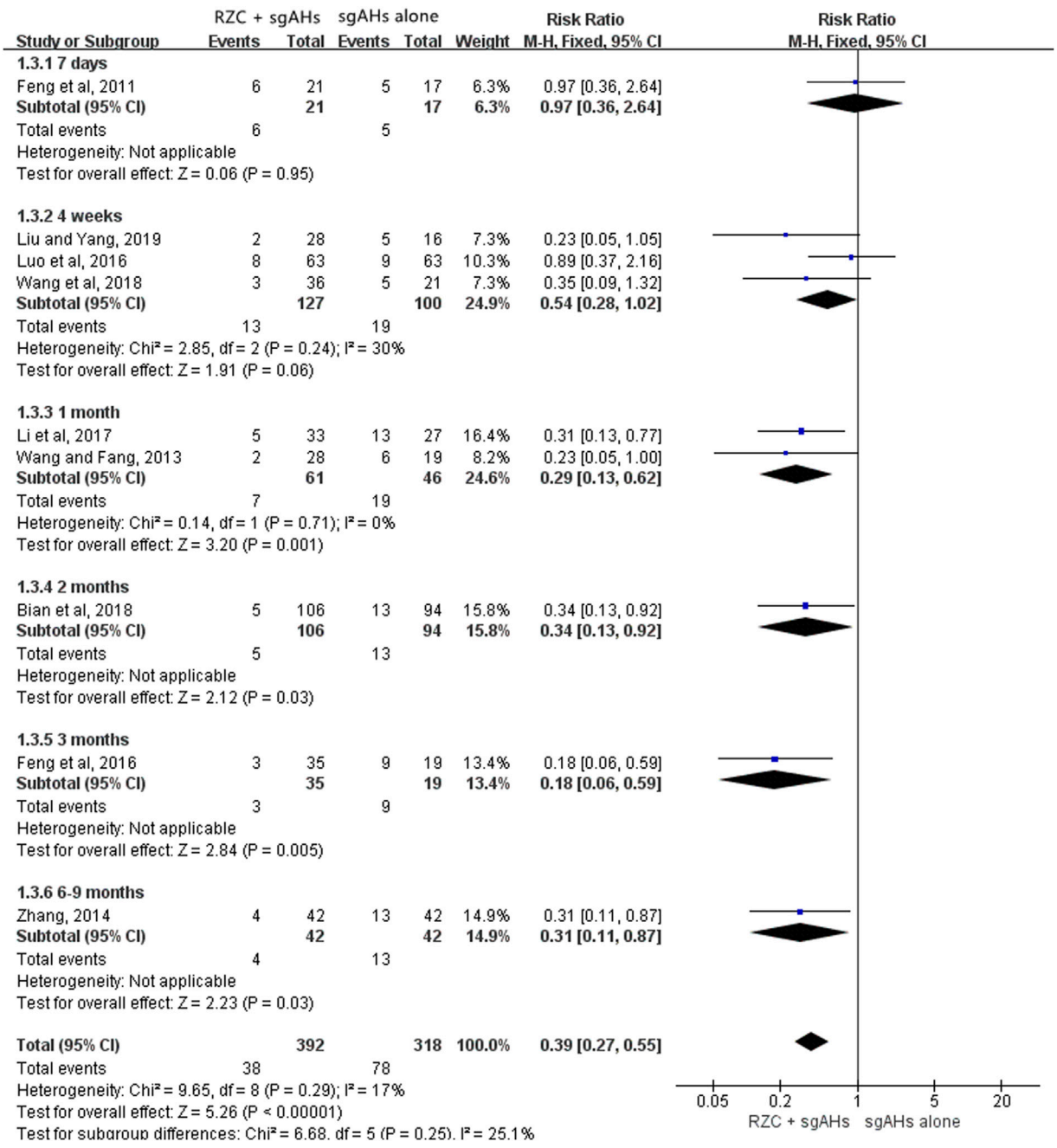

FIGURE 5
Forest plot for recurrence rate.

The findings of subgroup analyses showed that the RZC plus sgAHs group increased the serum IFN-γ level compared with the sgAHs alone group (SMD = 3.29, 95% CI: 2.62 to 3.95, $p < 0.00001$, a treatment course of 2 weeks, SMD = 4.42, 95% CI: 3.53 to 5.30, $p < 0.00001$, a treatment course of 4 weeks, SMD = 1.66, 95% CI: 0.99 to 2.32, $p < 0.00001$, and a treatment course of 2 months).

3.4.2.6 The incidence of adverse events

A total of 24 studies showed adverse events (Ai, 2020; Bian et al., 2018; Chen et al., 2016; Chen et al., 2020; Cheng, 2019; Feng et al., 2011; Feng et al., 2016; Huang, 2011; Li Y. et al., 2017; Li, 2019; Liu and Li, 2008; Liu and Yang, 2019; Luo et al., 2016; Lv, 2018; Ma et al., 2014; Sun, 2014; Tan, 2018; Wang and Fang, 2013; Wang et al., 2018; Xiao, 2020; Yang, 2019; Zhang, 2010; Zhang, 2014; Zhou, 2020). Only one study (Wang et al., 2018) presented that adverse events occurred in 5 of 64 cases in the RZC plus sgAHs group and 8 of 62 cases in the sgAHs alone group, with no obvious difference in the incidence of adverse events between the two groups (RR = 0.61, 95% CI: 0.21 to 1.75, $p = 0.35$). Nevertheless, the specific symptoms were not reported.

Eleven symptoms on adverse events including drowsiness, dizziness, headaches, fatigue, dry mouth, palpitation, diarrhea,

TABLE 2 Subgroup analysis results of the recurrence rate based on course of RZC and follow-up period.

Included study	Follow-up period	Number of recurrence (TG)	Sample size (TG)	Number of recurrence (CG)	Sample size (CG)	RR	95% CI	*p*-value
Treatment course of 4 weeks								
Feng et al. (2011)	7d	6	21	5	17	0.97	0.36–2.64	0.95
Luo et al., 2016; Wang et al., 2018	4w	11	99	14	84	0.67	0.32–1.39	0.28
Li et al., 2017; Wang and Fang, 2013	1m	7	61	19	46	0.28	0.13–0.60	0.001
Feng et al. (2016)	3m	3	25	9	19	0.25	0.08–0.81	0.02
Treatment course of 1 month								
Bian et al. (2018)	2m	5	106	13	94	0.34	0.13–0.92	0.03
Zhang (2014)	6–9m	4	42	13	42	0.31	0.11–0.87	0.03
Treatment course of 2 months								
Liu and Yang (2019)	4w	2	28	5	16	0.23	0.05–1.05	0.06

TG, treatment group; CG, control group; RR, risk ratio; d, day; w, week; m, month.

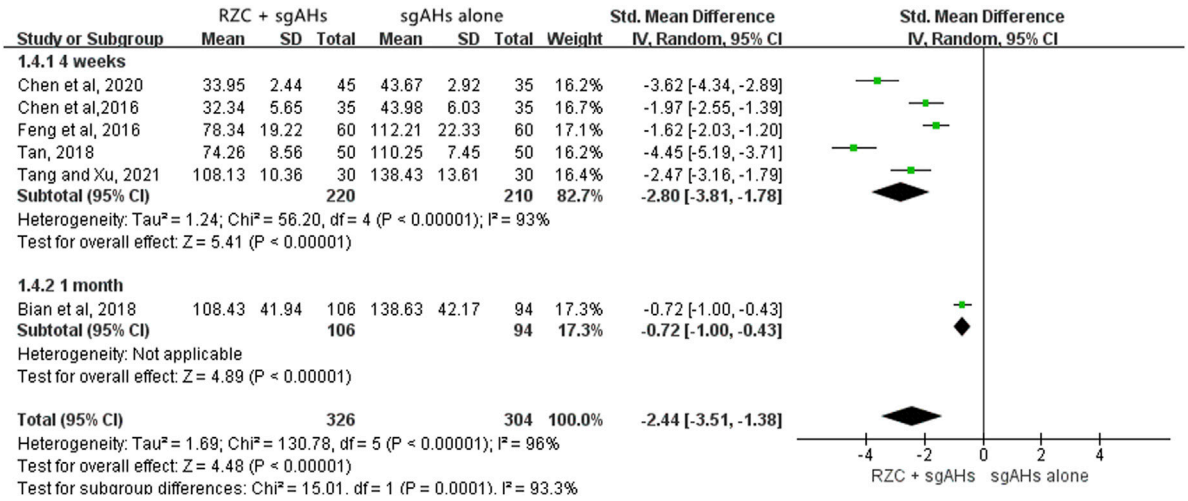

FIGURE 6
Forest plot for serum total IgE level.

stomach discomfort, constipation, nausea and vomiting and sleepiness were involved in the remaining 23 studies. Subgroup analyses were performed according to different symptoms of adverse events. The pooled results indicated that the RZC combined with sgAHs had a lower incidence of dizziness (RR = 0.53, 95% CI: 0.33 to 0.85, $p = 0.009$), fatigue (RR = 0.46, 95% CI: 0.26 to 0.84, $p = 0.01$), dry mouth (RR = 0.57, 95% CI: 0.34 to 0.95, $p = 0.03$), and constipation (RR = 0.24, 95% CI: 0.07 to 0.85, $p = 0.03$) in relative to the sgAHs alone. Nevertheless, no obvious difference was found in the incidence of drowsiness (RR = 0.75, 95% CI: 0.48 to 1.18, $p = 0.22$), headaches (RR = 0.56, 95% CI: 0.12 to 2.57, $p = 0.45$), palpitation (RR = 0.14, 95% CI: 0.01 to 2.70, $p = 0.19$), diarrhea (RR = 2.60, 95% CI: 0.61 to 11.03, $p = 0.19$), stomach discomfort (RR = 1.52, 95% CI: 0.67 to 3.47, $p = 0.32$), nausea and vomiting (RR = 0.50, 95% CI: 0.09 to 2.69, $p = 0.42$), and sleepiness (RR = 0.50, 95% CI: 0.05 to 5.37, $p = 0.57$) between the two groups. The details are presented in Table 3.

3.4.3 Additional analysis

3.4.3.1 Sensitivity analysis

The sensitivity analyses were performed using the method of omitting individual studies one by one to evaluate the influence on pooled results due to the heterogeneity of the meta-analyses on the DLQI, serum total IgE level, serum IL-4 level, and serum IFN-γ level. Then, it was demonstrated that omitting individual trials for the above outcome indicator made no difference to the overall results, suggesting that the pooled results were robust (Figure 9).

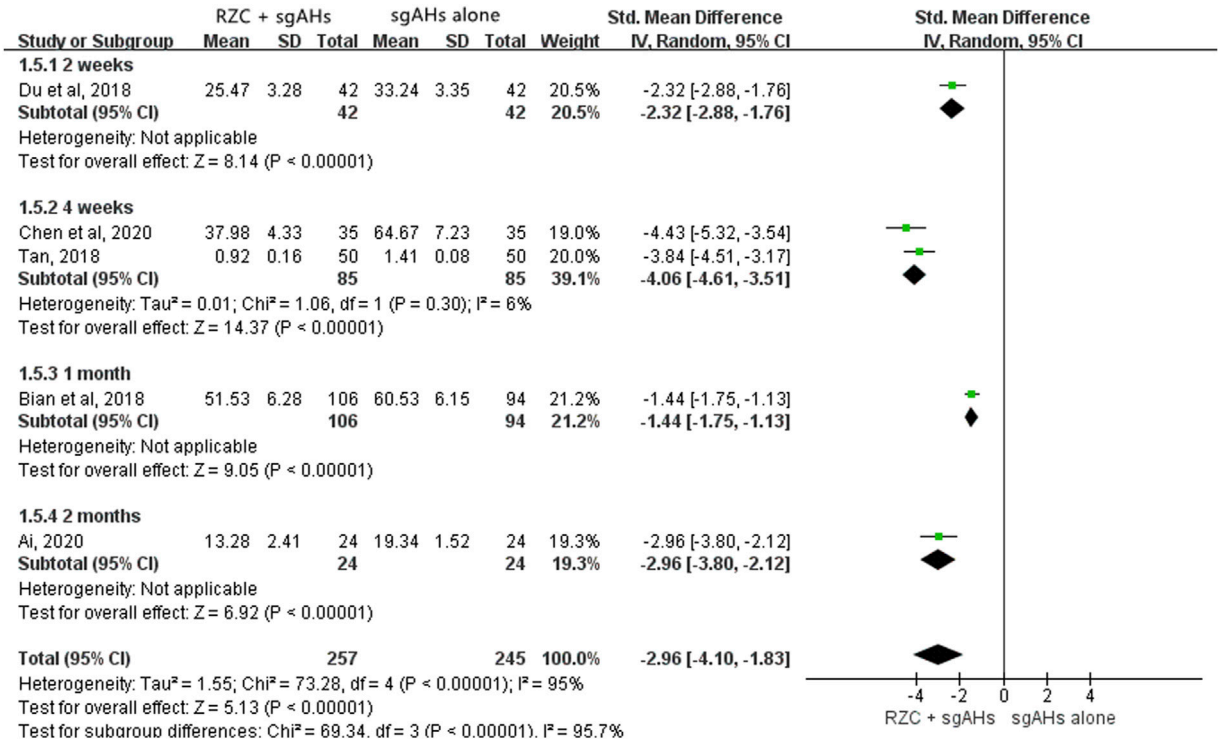

FIGURE 7
Forest plot for serum IL-4 level.

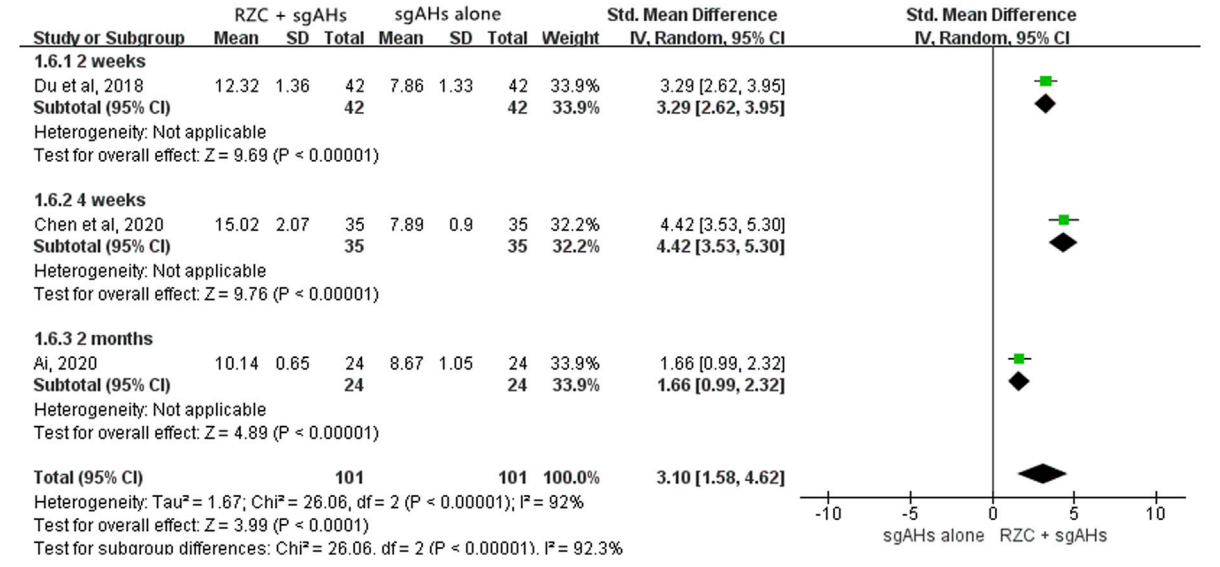

FIGURE 8
Forest plot for serum IFN-γ level.

3.4.3.2 Publication bias

The meta-analyses on total effective rate and the incidence of adverse events included over ten studies. The publication bias of the two meta-analyses was tested. The funnel plot of the total effective rate and the total incidence of adverse events was not visually asymmetric. No significant publication biases were identified for total effective rate (T = 1.83, $p = 0.084$) and the incidence of adverse events (T = 1.14, $p = 0.265$) by Harbord tests. The details are shown in Figure 10.

TABLE 3 The results of the subgroup analysis of the incidence of adverse events.

Adverse event Symptoms	Number of Studies	Heterogeneity test		Effect of the model	Pooled results		*p*-value
		p-value	I^2 (%)		RR	95% CI	
Drowsiness	17	0.93	0	Fixed	0.75	0.48–1.18	0.22
Dizziness	17	0.98	0	Fixed	0.53	0.33–0.85	0.009
Headaches	2	0.16	48	Fixed	0.56	0.12–2.57	0.45
Fatigue	11	0.79	0	Fixed	0.46	0.26–0.84	0.01
Dry mouth	14	0.71	0	Fixed	0.57	0.34–0.95	0.03
Palpitation	1	—	—	Fixed	0.14	0.01–2.70	0.19
Diarrhea	3	0.33	10	Fixed	2.60	0.61–11.03	0.19
Stomach discomfort	8	0.84	0	Fixed	1.52	0.67–3.47	0.32
Constipation	5	0.95	0	Fixed	0.24	0.07–0.85	0.03
Nausea and vomiting	3	0.83	0	Fixed	0.50	0.09–2.69	0.42
Sleepiness	1	—	—	Fixed	0.50	0.05–5.37	0.57

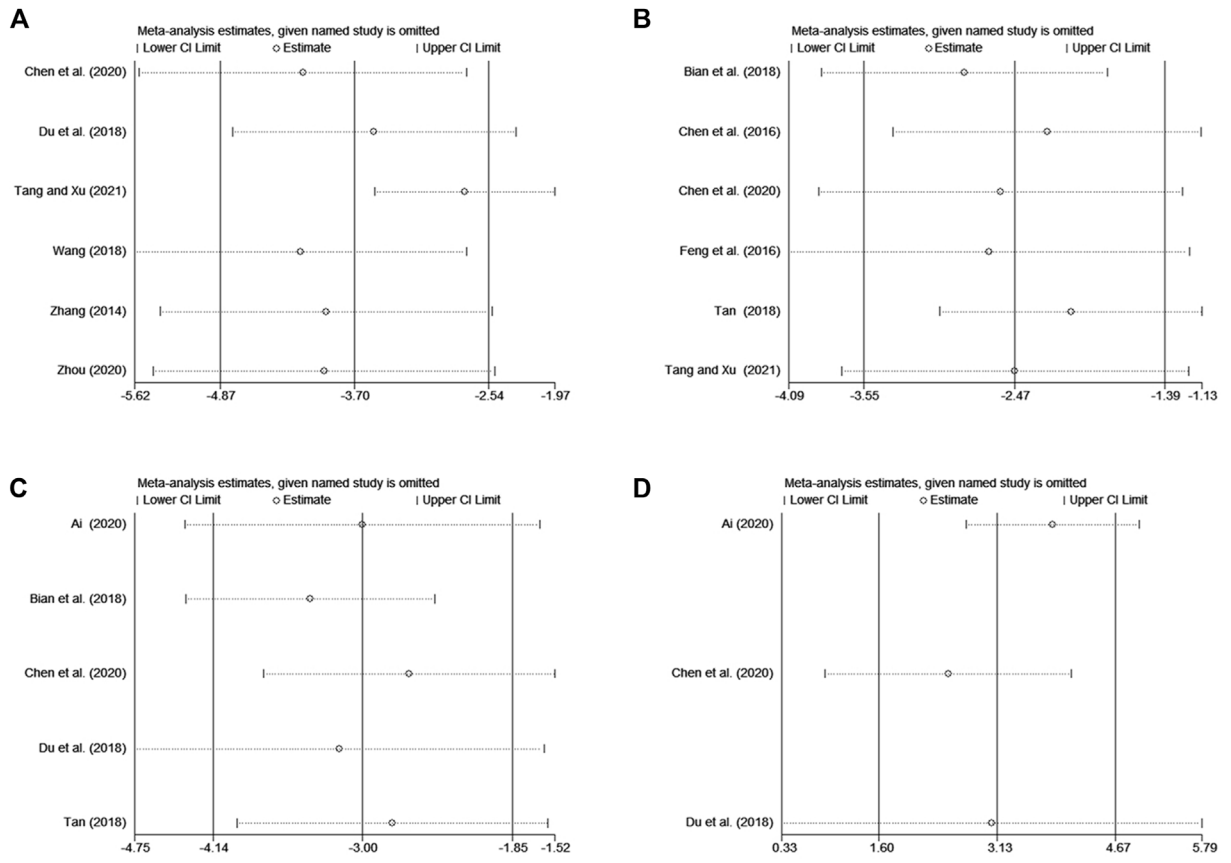

FIGURE 9
The results of sensitivity analysis. **(A)** DLQI, **(B)** serum total IgE level, **(C)** serum IL-4 level, **(D)** serum IFN-γ level.

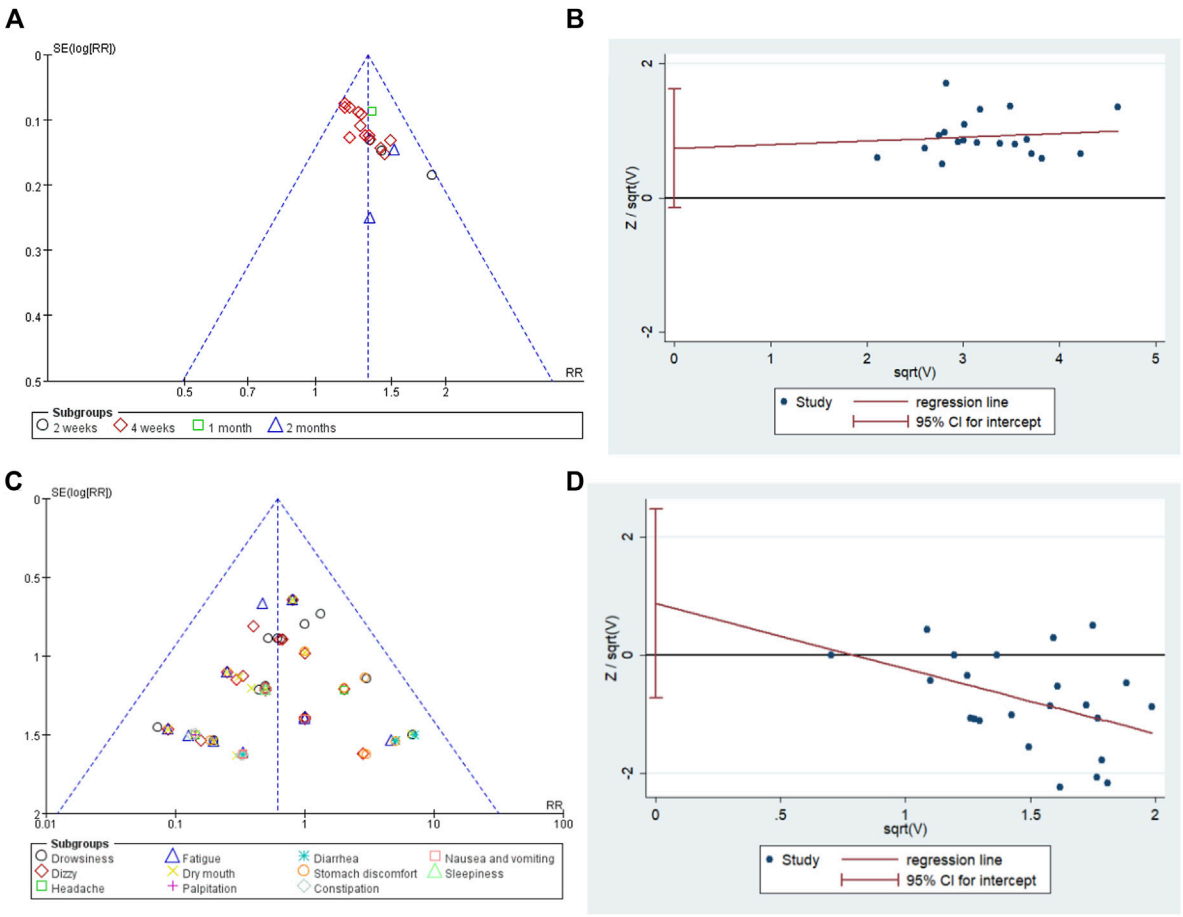

FIGURE 10
The results of publication bias. **(A)** Funnel plot of total effective rate, **(B)** Harbord tests plot of total effective rate, **(C)** funnel plot of the incidence of adverse events, **(D)** Harbord tests plot of the incidence of adverse events.

4 Discussion

4.1 Summary of findings

In this study, 27 RCTs evaluating the efficacy of RZC combined with sgAHs for CU were identified. The results of the meta-analyses suggested that RZC combined with sgAHs therapy significantly improved the total effective rate in relative to sgAHs alone, decreased the recurrence rate, improved the quality of life measured by DLQI and enhanced serum IFN-γ level as well as reduced serum total IgE level and serum IL-4 level. Concerning safety, all the reported adverse events were mild and well tolerated, exerting no influence on the treatment. All the symptoms could spontaneously disappear after drug withdrawal.

Subgroup analyses indicate that compared with the sgAHs alone, RZC plus sgAHs can significantly improve the total effective rate, the quality of life measured by DLQI and serum IFN-γ level, and reduce serum total IgE level and serum IL-4 level when RZC is used for 2 weeks, 4 weeks, 1 month or 2 months. In addition, it suggests that the effect of RZC may be rapid and relatively sustained. Subgroup analyses on the recurrence rate based on the course of treatment and follow-up period demonstrate that combined with sgAHs, RZC exhibits the advantage of reducing long-term recurrence such as that after 1 month follow-up period, while the treatment duration may have no influence on the long-term recurrence rate. Based on the results of meta-analyses, RZC can reduce the incidence of dizziness, fatigue, dry mouth, and constipation. The incidences of other adverse effects including drowsiness, headaches, palpitation, diarrhea, stomach discomfort, nausea and vomiting, and sleepiness, are not of significant difference between the two groups. The safety of RZC may be satisfied.

Sensitivity analysis of DLQI, serum IgE level, serum IL-4 level, and serum IFN-γ level suggest that the pooled results are robust but do not identify the source of heterogeneity. Significant heterogeneity may be associated with factors including fewer included studies, small sample sizes, and inconsistent measurement methods of laboratory test indicators. Further discussion is not performed due to the lack of available data.

4.2 Comparison with previous studies

Several systematic reviews and meta-analyses showed the efficacy and safety of Chinese herbal medicine in combination

with conventional western medicine for CU. However, there were some limitations in the studies that have been published.

Firstly, the course of the disease greater than or equal to 6 weeks is one of the vital items for diagnosing CU. However, participants with the unclear course of illness were all included in most researches (Xiao et al., 2020; Zheng et al., 2022), resulting in a possibility of lacking consistency between the research object and the research topic. The recognized diagnostic criteria, and a well-defined course of the disease are vital parts of our study. Secondly, different from the previous studies (Ke et al., 2021; Lin, 2022), no quasi-randomized trials were considered to ensure the quality of the literature. Thirdly, the prevention of CU recurrence is the advantage of TCM combination therapy. Some factors including the follow-up time and the course of treatment have influence on the recurrence rate to a certain extent, which has not been highlighted in the previously published studies (Liu et al., 2018; Zheng et al., 2022). However, the above factors were still discussed in this review despite being limited by the number of included studies.

4.3 Interpretation

Based on the theory of TCM, yin-blood deficiency and hemopenia generating wind are the primary etiology and pathogenesis of urticaria (Li, 2016). Combined with sgAHs, RZC with the functions of nourishing blood and yin, dispelling wind and arresting itching can increase clinical efficacy to a certain extent. The sgAHs, the preferred treatment for urticaria in modern medicine, are safer than the first-generation agents, while the occurrence of adverse events cannot be ignored. According to subgroup analysis of adverse events, the incidence of dizziness, fatigue, dry mouth and constipation in the RZC plus sgAHs group was notably lower than that in the sgAHs alone group. Based on the TCM theory, *R. multiflora* (Thunb.) Moldenke [Polygonaceae; Polygoni multiflori radix] with the function of supplementing essence blood, *R. glutinosa* (Gaertn.) DC. [Orobanchaceae; Rehmanniae radix] with the function of nourishing yin and generating fluid, and *M. alba* L. [Moraceae; Mori folium] with the function of moistening are the main components of RZC, which are effective in ameliorating dizziness, weakness, dry mouth and constipation (Bian et al., 2018; Cheng, 2019; Tang and Xu, 2021).

At present, the pathogenic mechanisms of CU are unclear. MCs are the essential effector cells in the pathogenesis of CU and can be activated through auto-immunity and non-autoimmunity (Kabashima et al., 2018; Maurer et al., 2019). Auto-immunity, either “autoallergic” (type I, with IgE antibodies to self-antigens/allergens) or “autoimmune” (type IIb, with IgG and IgM autoantibodies to IgE or its high-affinity receptor (FcεRI)) can cause MCs activation and degranulation (Wedi and Traidl, 2021). The autoallergy is a type I, IgE-mediated hypersensitivity reaction against self-antigens (Bracken et al., 2019). Autoallergic CU is related to IgE antibodies directed to self-antigens including thyroid peroxidase, thyroglobulin, and IL-24. It is different from classical type I hypersensitivity and allergy, involving exogenous allergens. Type II autoimmunity is featured by the appearance of IgG autoantibodies activating MCs, especially the IgG-anti-FcεRI and IgG-anti-IgE (Maronese et al., 2023). Positive autologous serum skin test, immunoassays for IgG autoantibodies, and basophil activation tests are the current gold standard for the diagnosis (Kolkhir et al., 2021). CU patients with IgG autoantibodies have been divided into the autoimmune type IIb endotype because type IIb hypersensitivity is featured by an antibody-dependent process where specific IgG antibodies bind to autoantigens to create pathogenic states. It is different from type IIa involving cytolytic destruction of targeted cells (Kolkhir et al., 2022). Not all CU patients can be strictly classified as type I or type IIb autoimmunity. Recently, a milieu of co-existing between two endotypes in the pathophysiology of CU has been confirmed (Maronese et al., 2023). Some non-autoimmunity mechanisms include physical agents, pseudoallergens, infection, the local inflammatory milieu, eosinophils, and different T cell subsets (Kolkhir et al., 2020; Metz et al., 2014). The complex pathogenesis of CU provides an explanation for the existence of cases with refractory to the antihistamine treatment. The combination of RZC and sgAHs therapy plays synergistic effects on the treatment of CU.

RZC is consisted of *R. multiflora* (Thunb.) Moldenke [Polygonaceae; Polygoni multiflori radix], *R. glutinosa* (Gaertn.) DC. [Orobanchaceae; Rehmanniae radix], *M. alba* L. [Moraceae; Mori folium], *S. flavescens* Aiton [Fabaceae; Sophorae flavescentis radix], *L. bulbifera* (Siebold & Zucc.) Wedd. [Urticaceae; Laportea herba], which exerts its therapeutic effects for CU on multiple targets and pathways (Wang et al., 2023). *Rehmannia glutinosa* (Gaertn.) DC. [Orobanchaceae; Rehmanniae radix] can effectively lower the release of histamine through reducing IgE production and inhabit MCs activation, playing an anti-inflammatory role by improving interleukin-2 (IL-2) function (Sung et al., 2011; Kang et al., 2012). *Reynoutria multiflora* (Thunb.) Moldenke [Polygonaceae; Polygoni multiflori radix] block MCs degranulation and inhibit histamine release by promoting adrenocortical function (Li Y. X. et al., 2017; Lin et al., 2015); meanwhile, it also exerts anti-inflammatory activity by reducing the levels of interleukin-(IL-4), IL-5, IL-13, and other cytokines (Lee et al., 2016). *Sophora flavescens* Aiton [Fabaceae; Sophorae flavescentis radix] suppresses the MC-mediated histamine release. In addition, it also significantly reduces the release of 5-hydroxytryptamine to relieve the pruritus (Yamaguchi-Miyamoto et al., 2003; He et al., 2015). *Morus alba* L. [Moraceae; Mori folium] extract could reduce the plasma levels of IgE and histamine of atopic dermatitis mice induced by the house dust mite (Lim et al., 2014). It also inhibits NF-κB-mediated inflammatory response (Park et al., 2013). The main chemical component of *L. bulbifera* (Siebold & Zucc.) Wedd. [Urticaceae; Laportea herba] is total coumarins, with a content of over 50% (Zhang et al., 2013). The study demonstrated that total coumarins improved the atopic dermatitis symptoms of rats through reducing the production of IL-4, thymic stromal lymphopoietin (TSLP), and IgE and suppressed the pruritus caused by TSLP, and histamine (Yu et al., 2021). *Laportea bulbifera* (Siebold & Zucc.) Wedd. [Urticaceae; Laportea herba] can increase the expression of IL-10 and transforming growth factor-(TGF-) β in dendritic cells and induce the production of $CD4^+CD25^+$Treg cells for anti-inflammatory and immunosuppressive effects (Luo et al., 2011). Therefore, RZC can not only inhibit mast cell activation and degranulation through the auto-immune mechanism but also play a therapeutic role via other non-histamine-dependent pathways, such as anti-inflammatory,

and immunosuppression. This conforms to the findings that RZC plus sgAHs therapy can improve the total effective rate, reduce serum total IgE and IL-4 level, and elevate the serum IFN-γ level.

4.4 Advantages and limitation

This is the first systematic review and meta-analysis on evaluating the efficacy and safety of RZC for CU. In line with the guidelines of the Cochrane Collaboration, it aims to draw more comprehensive and objective conclusions. By adding secondary outcome indicators, such as the expressions of inflammatory cytokines of IL-4 and IFN-γ, the efficacy of RZC combined with sgAHs therapy for CU can be evaluated in a multi-dimensional and multi-level manner. However, several potential limitations need to be pointed out when interpreting the above results.

1) In general, the methodological quality of the contained articles was low. Only less than half of the literature mentioned random sequence generation methods, and none of the included study involved allocation concealment and blinding. 2) All the recruited patients were Chinese individuals, and all the pieces of literature were published in Chinese, which may generate ethnic and geographical biases. 3) Only English or Chinese was used to search the literature, which may cause linguistic deviations. 4) The pooled results of several outcome indicators, such as DLQI, IgE, IL-4 and IFN-γ, suggested relatively high heterogeneity among the included studies. However, possible sources were not successfully analyzed and identified, even though the sensitivity analysis results were reliable.

4.5 Implications for future researches

There are some insights on how to improve the future trials on this topic. Firstly, the purpose should be clearly described, such as evaluate the efficacy of RZC alone or combined with western medicine for CU. Secondly, placebo-controlled RCTs are recommended. It is beneficial for implementing the blind and controlling the placebo effect. Thirdly, sample size should be estimated according to the appropriate parameters. This contributes to avoiding the bias in efficacy estimation. Fourthly, long follow-up period needs to be set up to identify the possible advantage of RZC on reducing the recurrence of CU. Fifthly, a protocol should be registered on an international clinical trial registration platform or published on an international academic journal. Trials should be reported following the Consolidated Standards of Reporting Trials (CONSORT) statement, which is helpful to acquire more detailed information.

5 Conclusion

According to available evidence, we found that RZC plus sgAHs may have more advantages than sgAHs alone in the treatment of CU, especially in improving the total effective rate and the quality of life, decreasing the recurrence rate, enhancing serum IFN-γ level and reducing serum total IgE level and serum IL-4 level. All the reported adverse events were mild and controllable. However, given that some elements of the risk of bias analysis including allocation concealment, blinding are not reported adequately, future trials on this topic need to be conducted more rigorously to obtain high-quality evidence, such as using placebo and long follow-up period, reasonably estimating sample size, registering the protocol, and reporting results according to CONSORT statement.

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

J-FZ and YZ conceived the study. J-FZ, J-BZ, Y-DW, and PL searched databases, screened the studies, extracted the data, assessed the methodological quality, and performed the statistical analysis. All authors contributed to the article and approved the submitted version.

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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.2023.1200252/full#supplementary-material

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Efficacy and safety of combined Chinese and Western medicine in the treatment of knee osteoarthritis: a prospective, multicenter cohort study

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Purpose: To conduct a real-world evaluation of the efficacy and safety of combined Chinese and Western medicine in treating knee osteoarthritis (KOA).

Methods: A multicenter, prospective cohort study design was employed, enrolling 450 KOA patients (Kellgren-Lawrence score of 3 or less). The patients were divided into a Western medicine treatment group (WM group) and a combined Western and traditional Chinese medicine treatment group (WM-CM group). A 6-week treatment plan was administered, and follow-up visits occurred at 2 weeks, 4 weeks, and 6 weeks after initiating treatment. The primary outcome indicator was the total Western Ontario and McMaster Universities Arthritis Index (WOMAC) score after 6 weeks of treatment. Secondary outcome indicators included WOMAC subscales for pain, stiffness, and joint function, visual analogue scale (VAS) score, physical component summary (PCS), mental component summary (MCS), and clinical effectiveness. The incidence of drug-related adverse events was used as a safety evaluation indicator.

Results: A total of 419 patients were included in the final analysis: 98 in the WM group and 321 in the WM-CM group. The baseline characteristics of the two groups were comparable, except for the incidence of stiffness symptoms and stiffness scores. After 6 weeks of treatment, the WM-CM group exhibited superior results to the WM group in improving the total WOMAC score (24.71 ± 1.38 vs.

Abbreviations: KOA, knee osteoarthritis; WM group, Western medicine treatment group; WM-CM group, Combined Western and traditional Chinese medicine treatment group; PCS, Physical Component Summary; MCS, Mental Component Summary; WOMAC, Western Ontario and McMaster Universities Arthritis Index; VAS, the visual analogue scale; SF-36, 36-item short form; physical functioning; RP, physical functioning; BP, physical pain; CH, general health; VT, vitality; SF, social functioning; RE, emotional functioning; MH, mental health; GLMM, generalized linear mixed models; K-L, Kellgren-Lawrence; TCM, traditional Chinese medicine; NSAIDs, Nonsteroidal Antiinflammatory Drugs.

16.36 ± 0.62, $p < 0.001$). The WM-CM group also outperformed the WM group in WOMAC pain and joint function scores, VAS score, PCS score, MCS score, and clinical effectiveness ($p < 0.05$), which was consistent with the findings of the main evaluation index. Subgroup analysis indicated that the combined Chinese and Western medicine treatment showed more pronounced benefits in patients under 65 years of age and in those with a Kellgren-Lawrence (K-L) classification of 0-I. Throughout the study, no adverse effects were observed in either group.

Conclusion: The combination of Chinese and Western medicine demonstrated superiority over Western medicine alone in relieving knee pain symptoms, improving knee function, and enhancing the quality of life for KOA patients with a K-L score of 3 or less. Moreover, the treatment exhibited a good safety profile.

Clinical Trial Registration: (https://www.chictr.org.cn/), identifier (ChiCTR1900027175).

KEYWORDS

knee osteoarthritis, combined Chinese and Western medicine, prospective cohort, efficacy, real-world study

1 Introduction

Knee osteoarthritis (KOA) is one of the most common musculoskeletal disorders and a major cause of disability in elderly individuals (Collaborators, 2018; Jang S., 2021; Lundberg M., 2022). With the accelerated aging process, the growth of the obese population and the increasing life expectancy, the prevention and treatment of KOA are facing a serious challenge (Mahmoudian A., 2021). According to statistics, KOA has affected more than 250 million people worldwide (Vos T., 2012), and in China, the prevalence of symptomatic KOA in people over 65 years of age is 60%, and the detection rate of radiological KOA is as high as 80% (Huang D., 2018). Moreover, the number of patients with KOA will continue to increase in the future, and it is estimated that it will be close to 400 million by 2030 (Pereira D., 2011; Zhang Z., 2020).

KOA is characterized by a long, irreversible and incurable course, causing pain, reduced mobility and even disability, which places a great burden on patients' physical and mental health and seriously interferes with their quality of life (Wang X., 2016; Feng X.Q., 2022). In addition, the high medical costs and corresponding indirect costs of KOA not only increase the economic burden of individuals and families but also have a negative impact on the national healthcare system and increase socioeconomic costs (Tang X., 2016).

Conservative therapy is now becoming increasingly important in the long-term management of KOA as the first line of treatment to slow disease progression and avoid or delay knee replacement surgery (Lim W.B., 2022). Western conservative treatment is mainly based on pain control and cartilage nutrition protocols, with the point of action localized to the joint. In fact, KOA involves multiple lesions and complex pathological changes, which are the result of multiple pathogenic factors intertwined and acting over a long period of time, and the efficacy of localized, single western medicine treatment is limited (Li R., 2013).

Traditional Chinese medicine (TCM) has a history of thousands of years in treating KOA, with a wide variety of therapeutic approaches that can play a role in holistic conditioning, and it is widely used in China and other Asian countries. Although there is preliminary evidence that TCM has the advantage of significant efficacy and low adverse effects and can improve the clinical efficacy of KOA in conjunction with Western medicine, overall, the evidence from high-quality clinical studies is still very limited (Chen B., 2016; Zhang J.H., 2019; Deng Y.L., 2021). Therefore, based on the concept of addressing the characteristics of individualized complex interventions and holistic efficacy evaluation of TCM, we conducted a prospective, multicenter cohort study following the requirements of modern clinical epidemiology and evidence-based medicine to further evaluate the clinical efficacy and safety of combined Chinese and Western medicine in the treatment of KOA from a macroscopic perspective to support its early intervention in KOA.

2 Methods and materials

2.1 Study design

This study was conducted as a prospective, multicenter cohort study from January 2021 to October 2022 in three medical institutions: the First Affiliated Hospital of Jinan University, the Third Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, and Foshan Hospital of Traditional Chinese Medicine. It was approved by the Ethics Committee of the First Hospital of Jinan University (KY-2019-036), and registration was completed at the China Clinical Trials Registry (ChiCTR1900027175). All subjects provided written informed consent.

2.2 Inclusion and exclusion criteria

The inclusion criteria for the study population were as follows: 1) meeting the diagnostic criteria for KOA and having a Kellgren-Lawrence (K-L) radiological diagnosis grade of III or less, based on the Guidelines for the Treatment of Knee Osteoarthritis with Integrative Medicine (Committee, 2018); 2) being aged 30 years

TABLE 1 The frequency of drug usage during treatment.

Medication type	Overall no. (%)	WM group no. (%)	WM-CM group no. (%)
Sodium Citrate	377 (90.0)	93 (94.9)	284 (88.5)
Glucosamine	119 (28.4)	43 (43.9)	76 (23.7)
NSAIDs			
Overall use of NSAIDs	230 (54.9)	75 (76.5)	155 (48.3)
Aceclofenac	102 (24.3)	7 (7.1)	95 (29.6)
Diclofenac Sodium	56 (13.4)	41 (41.8)	15 (4.7)
Etoricoxib	26 (6.2)	7 (7.1)	19 (5.9)
Celecoxib	22 (5.3)	10 (10.2)	12 (3.7)
Flurbiprofen Gel Patch	21 (5.0)	19 (19.4)	2 (0.6)
Loxoprofen Sodium	16 (3.8)	7 (7.1)	9 (2.8)
Meloxicam	14 (3.3)	3 (3.1)	11 (3.4)
Others	5 (1.2)	5 (5.1)	0 (0.0)
Oral Chinese medicine			
Duhuo Jisheng Mixture or Decoction			61 (19.0)
Yuxuebi Tablet			49 (15.3)
Gulong Capsule			29 (9.0)
Wangbi Tablet			23 (7.2)
Yougui Capsule			19 (5.9)
Zhongtongan Capsule			17 (5.3)
Zhuifeng Tougu Capsule			13 (4.0)
Hugu Capsule			13 (4.0)
Tenghuang Jiangu Tablet			12 (3.7)
Dahuoluo Capsule			10 (3.1)
Xianling Gubao Capsule			7 (2.2)
Others			19 (5.9)
External Chinese medicine			
Jianbu Xiaozhong Zhitong Oil			110 (34.3)
Warming Jingjintong Plaster			100 (31.2)
Cooling Blood Swelling Ointment			30 (9.3)
Cooling Jingjintong Plaster			18 (5.6)
Wentong Ointment			14 (4.4)
Li Guanghai Dieda Qufeng Ointment			13 (4.0)
Tianbai Golden Plaster			13 (4.0)
Antai Gel Ointment			12 (3.7)
Huoxue Powder			11 (3.4)
Daiwenjiu Ointment			11 (3.4)
Others			35 (10.9)

TABLE 2 Details of the most common oral and topical traditional Chinese medicines in this study.

Medication type	Ingredients	Percentage (%)	Processing	Approval number	Executive standard	Usage and dosage
Oral Chinese medicine						
Duhuo Jisheng Mixture*	Angelica Biserrata (R.H.Shan and C.Q.Yuan) C.Q.Yuan and R.H.Shan[Apiaceae; Angelicae Pubescentis Radix (Du-Huo)]	9.7	Gentiana Macrophylla Pall.[Gentianaceae; Gentianae Macrophyllae Radix (Qin-Jiao)], Paeonia Lactiflora Pall.[Paeoniaceae; Paeoniae Radix Alba (Bai-Shao)] and Eucommia Ulmoides Oliv.[Eucommiaceae; Eucommiae Cortex (Yan-Du-Zhong)] are extracted with 70% ethanol; volatile oils are extracted from Angelica Biserrata (R.H.Shan and C.Q.Yuan) C.Q.Yuan and R.H.Shan[Apiaceae; Angelicae Pubescentis Radix (Du-Huo)], Asarum Heterotropoides F.Schmidt[Aristolochiaceae; Asari Radix Et Rhizome (Xi-Xin)], Neolitsea Cassia (L.) Kosterm.[Lauraceae; Cinnamomi Ramulus (Gui-Zhi)], Saposhnikovia Divaricata (Turcz. Ex Ledeb.) Schischk.[Apiaceae; Saposhnikoviae Radix (Fang-Feng)], Angelica sinensis (Oliv.) Diels[Apiaceae; Angelicae Sinensis Radix (Dang-Gui)] and Conioselinum Anthriscoides "Chuanxiong"[Apiaceae; Chuanxiong Rhizome (Chuan-Xiong)]; all the medicines are decocted with water and mixed with ethanol extracts and volatile oils.	Z10983003	Chinese Pharmacopoeia 2015 Edition Part One	20 mL each time, 3 times a day
	Taxillus Chinensis (Dc.) Danser[Loranthaceae; Taxilli Herba (Sang-Ji-Sheng)]	6.4				
	Gentiana Macrophylla Pall.[Gentianaceae; Gentianae Macrophyllae Radix (Qin-Jiao)]	6.4				
	Saposhnikovia Divaricata (Turcz. Ex Ledeb.) Schischk.[Apiaceae; Saposhnikoviae Radix (Fang-Feng)]	6.4				
	Asarum Heterotropoides F.Schmidt[Aristolochiaceae; Asari Radix Et Rhizome (Xi-Xin)]	6.4				
	Angelica sinensis (Oliv.) Diels [Apiaceae; Angelicae Sinensis Radix (Dang-Gui)]	6.4				
	Paeonia Lactiflora Pall.[Paeoniaceae; Paeoniae Radix Alba (Bai-Shao)]	6.4				
	Conioselinum Anthriscoides "Chuanxiong"[Apiaceae; Chuanxiong Rhizome (Chuan-Xiong)]	6.4				
	Rehmannia Glutinosa (Gaertn.) DC.[Orobanchaceae; Rehmanniae Radix Praeparata (Shu-Di-Huang)]	6.4				
	Eucommia Ulmoides Oliv.[Eucommiaceae; Eucommiae Cortex (Yan-Du-Zhong)]	6.4				
	Cyathula Officinalis K.C.Kuan [Amaranthaceae; Cyathulae Radix (Chuan-Niu-Xi)]	6.4				
	Codonopsis Pilosula (Franch.) Nannf.[Campanulaceae; Codonopsis Radix (Dang-Shen)]	6.4				
	Carapichea Ipecacuanha (Brot.) L.Andersson [Polyporaceae; Poria (Fu-Ling)]	6.4				
	Glycyrrhiza Glabra L.[Fabaceae; Glycyrrhizae Radix Et Rhizoma (Gan-Cao)]	6.4				
	Neolitsea Cassia (L.) Kosterm.[Lauraceae; Cinnamomi Ramulus (Gui-Zhi)]	6.4				

(Continued on following page)

TABLE 2 (*Continued*) Details of the most common oral and topical traditional Chinese medicines in this study.

Medication type	Ingredients	Percentage (%)	Processing	Approval number	Executive standard	Usage and dosage
Yuxuebi Tablet*	Boswellia Sacra Flück. [Burseraceae; Olibanum (Ru-Xiang)]	4.5	Take Cyathula Officinalis K.C.Kuan [Amaranthaceae; Cyathulae Radix (Chuan-Niu-Xi)] and half the amount of Salvia Miltiorrhiza Bunge [Labiatae; Salviae Miltiorrhizae Radix Et Rhizoma (Dan-Shen)] and Astragalus Mongholicus Bunge[Fabaceae; Astragali Radix(Zhi-Huang-Qi)], and grind them into fine powder. Decoct the remaining drugs and concentrate under reduced pressure to make a clear paste. The paste is mixed with crude drug powder to make granules, and then compressed into tablets.	Z20050762	State Food and Drug Administration Drug Standards YBZ28152005-2009Z	5 tablets at a time, 3 times a day
	Clematis Chinensis Osbeck [Ranunculaceae; Clematidis Radix Et Rhizoma (Wei-Ling-Xian)]	11.2				
	Carthamus Tinctorius L. [Asteraceae; Carthami Flos (Hong-Hua)]	7.5				
	Salvia Miltiorrhiza Bunge [Labiatae; Salviae Miltiorrhizae Radix Et Rhizoma (Dan-Shen)]	14.9				
	Commiphora Myrrha (T.Nees) Engl.[Burseraceae; Myrrha(Zhi-Mo-Yao)]	4.5				
	Cyathula Officinalis K.C.Kuan [Amaranthaceae; Cyathulae Radix (Chuan-Niu-Xi)]	11.2				
	Conioselinum Anthriscoides 'Chuanxiong'[Apiaceae; Chuanxiong Rhizome (Chuan-Xiong)]	11.2				
	Angelica sinensis (Oliv.) Diels [Apiaceae; Angelicae Sinensis Radix (Dang-Gui)]	7.5				
	Curcuma Longa L.[Zingiberaceae; Curcumae Longae Rhizoma(Jiang-Huang)]	7.5				
	Cyperus Rotundus L.[Cyperaceae; Cyperi Rhizoma(Zhi-Xiang-Fu)]	9.0				
	Astragalus Mongholicus Bunge [Fabaceae; Astragali Radix(Zhi-Huang-Qi)]	11.2				
External Chinese medicine						
Jianbu Xiaozhong Zhitong Oil[§]	Styrax benzoin Dryand.[Styracaceae; Benzoinum(An-Xi-Xiang)]		Styrax benzoin Dryand.[Styracaceae; Benzoinum(An-Xi-Xiang)] benzoin with ethanol for 7 days, and keep the filtrate. Crush Capsicum annuum L.[Solanaceae; Capsici Fructus(La-Jiao)] into fine powder, add Turpentine Oil (Song-Jie You) and soak for 7 days, and keep the filtrate. Mix the Above-mentioned filtrate with L-Menthol (Bo-He-Nao), Clove Oil (Ding-Xiang You), Borneolum Syntheticum (Bing-Pian) and Eucalyptus Oil (An You).	ZB20150003 (Guangdong batch number)	Registration Standards for Preparations in Medical Institutions	3 to 4 times a day
	Capsicum annuum L.[Solanaceae; Capsici Fructus(La-Jiao)]					
	L-Menthol (Bo-He-Nao)					
	Clove Oil (Ding-Xiang You)					
	Borneolum Syntheticum (Bing-Pian)					
	Eucalyptus Oil (An You)					
	Turpentine Oil (Song-Jie You)					

(Continued on following page)

TABLE 2 (*Continued*) Details of the most common oral and topical traditional Chinese medicines in this study.

Medication type	Ingredients	Percentage (%)	Processing	Approval number	Executive standard	Usage and dosage
Warming Jingjintong Plaster[$]	Cullen corylifolium (L.) Medik.[Fabaceae; Psoraleae Fructus(Bu-Gu-Zhi)]	7.5	Except for Camphor(Racemic) (Zhang-Nao(He-Cheng)), Borneolum Syntheticum (Bing-Pian), L-Menthol (Bo-He-Nao), and Turpentine Oil (Song-Jie You), the rest drugs are crushed into coarse powder, and extracted with ethanol to make a clear paste. Add carbomer to the clear paste and soak for 24 h, then add Camphor(Racemic) (Zhang-Nao(He-Cheng)), Borneolum Syntheticum (Bing-Pian), L-Menthol (Bo-He-Nao), Turpentine Oil (Song-Jie You), and mix well.	ZB20150006 (Guangdong batch number)	Registration Standards for Preparations in Medical Institutions	3 to 4 times a day
	Astragalus Mongholicus Bunge [Leguminosae; Astragali Radix(Huang-Qi)]	7.5				
	Phellodendron amurense Rupr.[Rutaceae; Phellodendri Amurensis Cortex(Guan-Huang-Bo)	6.0				
	Achyranthes bidentata Blume [Amaranthaceae; Achyranthis Bidentatae Radix(Niu-Xi)]	6.0				
	Dipsacus asper Wall. ex DC.[Dipsacaceae; Dipsaci Radix(Xu-Duan)]	6.0				
	Eucommia Ulmoides Oliv.[Eucommiaceae; Eucommiae Cortex (Du-Zhong)]	4.5				
	Drynaria roosii Nakaike [Polypodiaceae; Drynariae Rhizoma(Gu-Sui-Bu)]	4.5				
	Chaenomeles speciosa (Sweet) Nakai[Rosaceae; Chaenomelis Fructus(Mu-Gua)]	6.0				
	Spatholobus suberectus Dunn [Fabaceae; Spatholobi Caulis(Ji-Xue-Teng)]	4.5				
	Zanthoxylum nitidum (Roxb.) DC.[Rutaceae; Zanthoxyli Radix(Liang-Mian-Zhen)]	4.5				
	Vincetoxicum mukdenense Kitag.[Apocynaceae; Cynanchi Paniculati Radix Et Rhizoma(Xu-Chang-Qing)]	3.0				
	Rheum officinale Baill.[Polygonaceae; Rhei Radix Et Rhizoma(Da-Huang)]	3.0				
	Carthamus Tinctorius L.[Asteraceae; Carthami Flos (Hong-Hua)]	1.5				
	Zingiber officinale Roscoe [Zingiberaceae; Zingiberis Rhizoma(Gan-Jiang)]	1.5				
	Capsicum annuum L.[Solanaceae; Capsici Fructus(La-Jiao)]	1.5				
	Camphor(Racemic) (Zhang-Nao(He-Cheng))	11.2				
	Borneolum Syntheticum (Bing-Pian)	7.5				
	L-Menthol (Bo-He-Nao)	4.1				
	Turpentine Oil (Song-Jie You)	9.7				

Note: *Drugs' information comes from "China Pharmacopoeia 2020 edition"; [$]Drugs' information comes from "Guangdong Province Medical Institution Preparation Standard". Referring to the publication method of "Chinese Pharmacopoeia", we briefly describe the prescription and preparation method if it involves confidential technology.

or older; 3) not having plans for surgery in the near future and requiring conservative treatment; and 4) demonstrating good compliance and the ability to cooperate with the completion of clinical visits.

Exclusion criteria were as follows: 1) having a history of knee trauma or surgery in the last 6 months, along with knee fracture, dislocation, or septic knee arthritis; 2) receiving arthroscopic treatment or intra-articular injection in the last 3 months; 3) receiving hormone therapy in the last 1 month; 4) having undergone knee replacement; 5) having comorbidities such as tumor, tuberculosis, hemophilic arthritis, rheumatoid arthritis, systemic lupus erythematosus, or ankylosing spondylitis; 6) having serious gastrointestinal diseases, severe psychiatric disorders, significant infectious diseases, or severe pathologies affecting vital organs such as the heart, liver, kidneys, or others; 7) having a history of severe allergy to TCM; 8) having local skin ulcers or eczema; and 9) being deemed unsuitable for inclusion in the study by the investigator.

2.3 Therapeutic strategy

The patients were divided into a Western medicine treatment group (WM group) and a combined Western and traditional Chinese medicine treatment group (WM-CM group) according to the actual treatment protocol used in the clinic. All subjects received basic treatment, including health education and exercise instruction. In the WM group, NSAIDs, sodium glutamate and glucosamine were used as the main treatment drugs according to the relevant guidelines (Bannuru R.R., 2019; Association, 2021; Zhang Z., 2021), while the WM-CM group used a combination of Western and traditional Chinese medicine according to the comprehensive treatment plan we developed in the early stage. All patients included in the study received a 6-week treatment, and the medication was administered following the recommended conventional dosage as per the instructions. The types of medications used during treatment were shown in Table 1. TCM therapy mainly includes commercially available Chinese patent medicines and hospital preparations that have been used for many years. We provided detailed information on the most frequently used oral and topical traditional Chinese medicines in Table 2, including their composition, proportions, dosage, usage, manufacturing processes, and quality control aspects. The detailed information for the other traditional Chinese medicines can be found in Supplementary Tables S1, S2 of the supplementary materials.

2.4 Observation indicators

Patients were evaluated before treatment and at 2, 4, and 6 weeks of treatment.

The primary outcome measure was the total Western Ontario and McMaster Universities Arthritis Index (WOMAC) score (ranging from 0 to 96, with higher scores indicating more severe symptoms). The primary end point is the WOMAC total score after 6 weeks of treatment.

Secondary efficacy indicators included the following:

1) WOMAC subscale scores, including WOMAC pain score (ranging from 0 to 20, with higher scores indicating more severe pain), WOMAC stiffness score (ranging from 0 to 8, with higher scores indicating more severe knee stiffness), and WOMAC joint function score (ranging from 0 to 68, with higher scores indicating poorer knee function);
2) The visual analogue scale (VAS) score (ranging from 0 to 10, with higher scores indicating more severe pain);
3) 36-item short form (SF-36) scores, divided into physical component summary (PCS) and mental component summary (MCS); the former includes four dimensions of physical functioning (PF), role-physical (RP), bodily pain (BP), and general health (GH), and the latter includes four dimensions of vitality (VT), social functioning (SF), role-emotional (RE), and mental health (MH), ranging from 0 to 100, with higher scores indicating better status on that dimension; and
4) Clinical effectiveness. As described in the literature (Angst F., 2001; Nishida Y., 2021), an improvement in the WOMAC total score by more than 12% from the baseline value was defined as effective, and an improvement in the WOMAC total score by more than 50% from the baseline value was defined as significant.

An adverse event was defined as any undesirable medical occurrence associated with the treatment protocol, leading to a persistence or worsening of the patient's symptoms that required additional interventions (Deyle G.D., 2016). For this study, patients were asked to report at each follow-up visit any adverse outcomes, including complications, signs or symptoms, that they perceived to be related to their treatment. The incidence of adverse events was considered an indicator for safety evaluation.

2.5 Sample size

G*Power 3.1.2 software was used to calculate the sample size for this study. Based on the relevant literature (Cao Y., 2005; Deyle G.D., 2020) and the previous study of our group, assuming that the mean WOMAC values of the WM group and the WM-CM group were equal at baseline and that a 12% difference between the two groups was maintained after each treatment, setting a standard deviation of 7 and a mean correlation of 0.681 between repeated measures, and considering a 10% missed visit rate, then with a sample size of 92 cases per group, there is a 90% test efficacy to find the difference between groups. Therefore, we set the minimum sample size at 92 cases and adjusted the sample size appropriately according to the actual situation.

2.6 Statistical analysis

SPSS 27.0 software was used for statistical analysis. Data analysis is based on all populations completing the study unless otherwise noted. Hypothesis testing was uniformly performed using a two-sided test, with $p < 0.05$ indicating a statistically significant result. Baseline information was described as the mean ± standard deviation or frequency and composition ratio. Two groups were compared using independent samples t tests or Mann–Whitney U rank sum tests for measurement data and chi-square tests or Fisher's

exact tests for count data. Repeated measures data were compared between groups using generalized linear mixed models (GLMM). The model included treatment, time, and the interaction of treatment with time as fixed effects and patient-specific random intercepts.

3 Results

3.1 Population characteristics

A total of 450 patients were enrolled in this study, and 419 subjects completed the entire study process, eventually forming the analysis cohort, consisting of 98 patients in the WM group and 321 patients in the WM-CM group. Among them, there were 100 patients from Foshan Hospital of Traditional Chinese Medicine, including 14 in the WM group and 86 in the WM-CM group; 106 patients from the First Affiliated Hospital of Jinan University, including 70 in the WM group and 36 in the WM-CM group; and 213 patients from the Third Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, including 14 in the WM group and 199 in the WM-CM group.

The patient follow-up process and lost visits are presented in Figure 1. Detailed baseline characteristics of the patients are summarized in Table 3. The prevalence of knee stiffness symptoms was higher in the WM-CM group (p = 0.003), while the WOMAC stiffness score was higher in the WM group (p = 0.019); otherwise, the rest of the baseline characteristics were comparable.

3.2 The primary outcome measure

The mean total WOMAC values at baseline were 37.62 ± 1.702 in the WM group and 36.23 ± 0.923 in the WM-CM group, and the differences were not statistically significant. After 6 weeks of treatment, the mean WOMAC total score decreased to 24.71 ± 1.38 in the WM group and 16.36 ± 0.62 in the WM-CM group. The mean total WOMAC value was significantly lower in the WM-CM group than in the WM group ($p < 0.001$) (Table 4). Indeed, from the second week of treatment, there was a significant difference in the total WOMAC values of the two groups, and this trend persisted until the sixth week (Figure 2; Supplementary Table S3).

3.3 The secondary outcome measure

3.3.1 WOMAC pain score

Figure 3 demonstrates the overall change in the WOMAC pain scores of both groups during the treatment period. With consistent baseline scores, the mean WOMAC pain score was significantly lower in the WM-CM group than in the WM group after 6 weeks of treatment (4.1 ± 0.165 vs. 5.39 ± 0.342, p = 0.001) (Table 4).

3.3.2 WOMAC stiffness score

Figure 4 demonstrates the overall change in WOMAC stiffness scores over the treatment period for both groups. The large difference in baseline WOMAC stiffness scores between the two groups did not allow direct comparison of scores at 2, 4, and 6 weeks of treatment. Therefore, a comparison of the difference from baseline scores at 2, 4, and 6 weeks of treatment between the two groups was performed instead, and test scores were adjusted using the Bonferroni method. The results showed that the difference between the stiffness values at baseline and after treatment was slightly larger in the WM-CM group than in the WM group, but the difference was not statistically significant ($p > 0.017$) (Table 5).

3.3.3 WOMAC physical function score

Figure 5 demonstrates the overall changes in the WOMAC joint function scores of the two groups during the treatment period. With consistent baseline scores, the mean WOMAC joint function score was significantly lower in the WM-CM group than in the WM group after 6 weeks of treatment (11.45 ± 0.438 vs. 17.7 ± 0.986, $p < 0.001$) (Table 4).

3.3.4 VAS score

The VAS scores of both groups showed a significant decrease with the prolongation of treatment time, but the rate of decrease was faster in the WM-CM group (Figure 6). The intragroup and intergroup multiple comparisons of VAS scores between the two groups at each timepoint are detailed in Supplementary Table S3. After 6 weeks of treatment, the mean VAS score of the WM-CM group decreased to 2.24 ± 0.072, which was much lower than that of the WM group, which was 2.97 ± 0.149 ($p < 0.001$) (Table 4).

3.3.5 PCS score

As shown in Figure 7, with consistent scores at baseline, the PCS scores of patients in the WM-CM group began to be gradually higher than those in the WM group after 2 weeks of treatment, but the difference was not statistically significant (p = 0.135). After 4 weeks of treatment, the PCS scores of patients in the WM-CM group were significantly higher than those in the WM group (p = 0.008), and after 6 weeks of treatment, the difference between the two groups was even more significant ($p < 0.001$) (Table 4; Supplementary Table S3). Details of the results of the 4 dimensions PF, RP, BP, and GH at each follow-up timepoint, as well as multiple comparisons within and between groups, are provided in the supplemental file.

3.3.6 MCS score

As shown in Figure 8, the MCS scores in the WM-CM group showed a steady upward trend with the prolongation of treatment time, and after 6 weeks of treatment, the mean MCS scores of the two groups began to show significant differences (p = 0.009) (Table 4). Details of the results of the 4 dimensions VT, SF, RE, and MH at each follow-up timepoint, as well as multiple comparisons within and between groups, are provided in the supplemental file.

3.3.7 Clinical efficiency

As shown in Table 6, the effective rate of the WM-CM group was as high as 95%, which exceeded that of the WM group (80.6%), and the difference was statistically significant ($p < 0.001$); the significant response rate of the WM-CM group was 63.6%, which exceeded that of the WM group (32%), and the difference was statistically significant ($p < 0.001$).

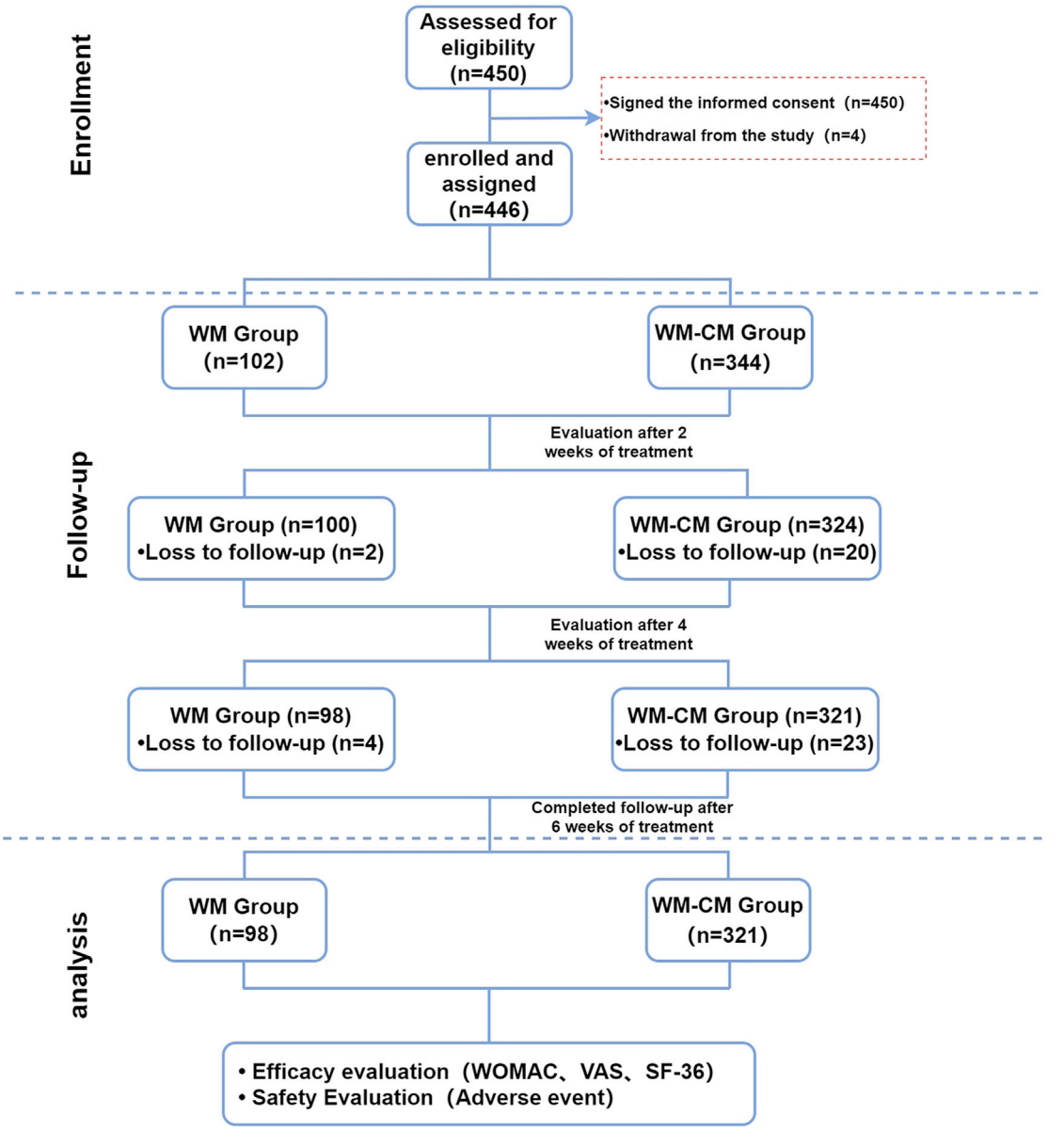

FIGURE 1
Flow chart of the study.

3.4 Subgroup analysis

To examine the robustness of the results, we stratified patients by age, sex, and K-L grade and then compared the differences in changes in WOMAC total score between the two groups compared with baseline after 6 weeks of treatment in different subgroups using GLMM. As shown in Figure 9, in both the under-65 and over-65 age groups, the difference in WOMAC total score at baseline and after 6 weeks of treatment was larger in the WM-CM group than in the WM group, and the difference was significant ($p < 0.001$). The difference was more pronounced in the under-65 patient group. For both male and female patients, the improvement in WOMAC total score after 6 weeks of treatment was significantly better in the WM-CM group than in the WM group ($p < 0.05$). For patients with K-L classification 0-I, the WM-CM group performed better ($p < 0.001$), and for patients with grades II-III, the difference in WOMAC total score at baseline and after 6 weeks of treatment was slightly greater in the WM-CM group than in the WM group, but the difference was not significant ($p = 0.091$).

3.5 Safety evaluation

We focused on monitoring potential drug-related adverse events such as local pain and swelling from injections, gastrointestinal discomfort, cardiovascular events, and skin sensitization. No drug-related adverse events occurred in either group throughout the

TABLE 3 Baseline characteristics of all the subjects.

Characteristics	WM group (n = 98)	WM-CM group (n = 321)	*p*
Age, years	61.37 ± 8.09	60.98 ± 7.70	0.831
BMI, $kg \cdot m^{-2}$	24.13 ± 3.28	23.61 ± 2.99*	0.138
Sex, no. (%), male/female	23 (23.5)/75 (76.5)	70 (21.8)/251 (78.2)	0.729
Disease duration, no. (%)			
<1 month	5 (5.1)	8 (2.5)	0.064
1–3 months	6 (6.1)	7 (2.2)	
4–6 months	5 (5.1)	10 (3.1)	
>6 months	82 (83.7)	296 (92.2)	
K-L grade, no. (%)			
0	4 (4.1)	38 (6.9)	0.162
I	74 (75.5)	186 (63.2)	
II	16 (16.3)	80 (24.6)	
III	4 (4.1)	17 (5.3)	
Main symptoms of knee, no. (%)			
Pain	97 (99.0)	318 (99.1)	1.000
Restricted movement	66 (67.3)	247 (76.9)	0.056
Stiffness	32 (32.7)	159 (49.5)	**0.003**
Swelling	38 (38.8)	138 (43.0)	0.459
Crepitus	25 (25.5)	112 (34.9)	0.083
Soreness and weakness	29 (29.6)	124 (38.6)	0.104
Baseline measure			
WOMAC total score	37.62 ± 14.74	36.23 ± 12.71	0.461
WOMAC pain score	8.62 ± 4.12	8.35 ± 3.26	0.705
WOMAC stiffness score	2.50 ± 1.61	2.05 ± 1.69	**0.019**
WOMAC physical function score	26.50 ± 10.59	25.83 ± 9.00	0.536
VAS score	5.07 ± 1.72	5.23 ± 1.46	0.352
PCS score	44.51 ± 18.48	41.67 ± 16.53	0.197
MCS score	68.28 ± 19.98	66.10 ± 20.54	0.427

Note: Continuous variables are expressed as the mean ± standard deviation. Bolded values represent $p < 0.05$ for comparison between the two groups. * Values were obtained based on data from 319 patients.

treatment cycle, and no patient discontinued the study due to adverse events.

4 Discussion

KOA has become a medical problem plaguing the world due to its high morbidity, high disability, high health hazard and high economic burden. Currently, Western drug treatment of KOA is based on Nonsteroidal Anti-inflammatory Drugs (NSAIDs), glucosamine, and sodium citrate injections. Among them, NSAIDs are recognized as first-line agents, but gastrointestinal and cardiovascular safety risks exist (Blumenthal K.G., 2017; Lim W.B., 2022), while the use of glucosamine and sodium citrate injections is controversial in clinical practice due to varying efficacy reported in different studies (Clegg D.O., 2006; Fransen M., 2015; Jevsevar D., 2015; Reginster J.Y., 2017; Honvo G., 2019; Peng B., 2019; Cai Z., 2022). Recently, there has been increasing evidence that TCM therapy is effective in preventing and treating KOA, improving clinical efficiency and reducing adverse effects (Zhu G.Q., 2014; Wu H.Y., 2015; Yu T. M, 2018), especially with Chinese patent medicines such as Xianling Gubao capsules, Zhuangguguanjie capsules, Jintiange capsules, Xiaotong patch and compound Nanxing analgesic ointment, which are recommended in many clinical guidelines in China (project group, 2021; Chen W.H., 2020; Association, 2021).

TABLE 4 Primary and Secondary outcomes at 6 Weeks.

Outcome	WM group	WM-CM group	*p*
WOMAC total score	24.71 ± 1.38	16.36 ± 0.62	<0.001
WOMAC pain score	5.39 ± 0.342	4.1 ± 0.165	0.001
WOMAC physical function score	17.7 ± 0.986	11.45 ± 0.438	<0.001
VAS score	2.97 ± 0.149	2.24 ± 0.072	<0.001
PCS score	49.09 ± 1.939	59.75 ± 1.304	<0.001
MCS score	71.83 ± 2.131	78.36 ± 1.284	0.009

Note: All 419 patients were included in the analysis.
The least-squares mean ± SD, was calculated based on generalized linear mixed model, and the *p* values were adjusted with the use of Bonferroni correction for multiple comparisons.

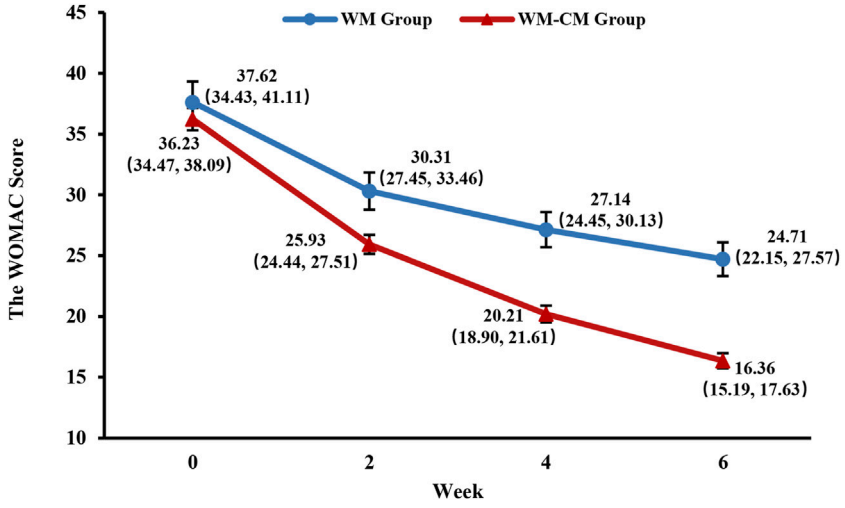

FIGURE 2
Overall trend of the WOMAC total scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

Most of the TCM preparations observed in this study have been proven to be effective in treating KOA in previous clinical researches (project group, 2021; Zhao J., 2022; Zhang W., 2016; Wu K.Y., 2015; Li X.L., 2019; Tan W.X., 2014; Guo X.X., 2017; Kang X.Z., 2011). However, it is important to note that these findings were obtained in the context of clinical trials and require verification in real-life clinical practice. The treatment process of TCM is highly individualized and complex, and the real medical environment is significantly more intricate than the controlled setting of a clinical trial. Therefore, real-world studies are essential to truly and comprehensively evaluate the effectiveness of complex TCM intervention programs. As far as we know, there have been very few large-scale real-world studies evaluating the overall efficacy of TCM combined with chemotherapy in the KOA field. Our early initiation of this research has the potential to garner attention from medical professionals, patients, and administrative personnel alike towards the benefits of TCM. We hope that the results of this research will receive the attention they deserve, enabling more KOA patients to benefit from the integration of TCM into their treatment plans.

In this study, we investigated the efficacy and safety of combined Chinese and Western medicine protocols for KOA patients with K-L score of 3 or less based on real clinical treatment scenarios. It was found that the combination of Chinese and Western medicine had significant advantages over Western medicine alone in relieving pain, improving knee function, and improving quality of life, and there was no significant difference between the two groups in relieving stiffness symptoms, but the interference caused by the insufficient reliability of the stiffness dimension of the WOMAC scale could not be excluded (Van de Graaf V.A., 2014), which needs to be further determined by combining other scales in future studies. In addition, subgroup analysis found that the benefit of the combination therapy group was more pronounced in younger patients under 65 years of age and in patients with milder disease with a K-L grade of 0-I. To some extent, this suggests an early intervention of TCM therapy.

The functions of the drugs used in this study are mainly to dispel wind and dampness, activate blood to relieve pain, and tonify the liver and kidney. “Jin-Gu theory” in TCM can make a reasonable explanation for why these drugs are selected in clinical practice. This

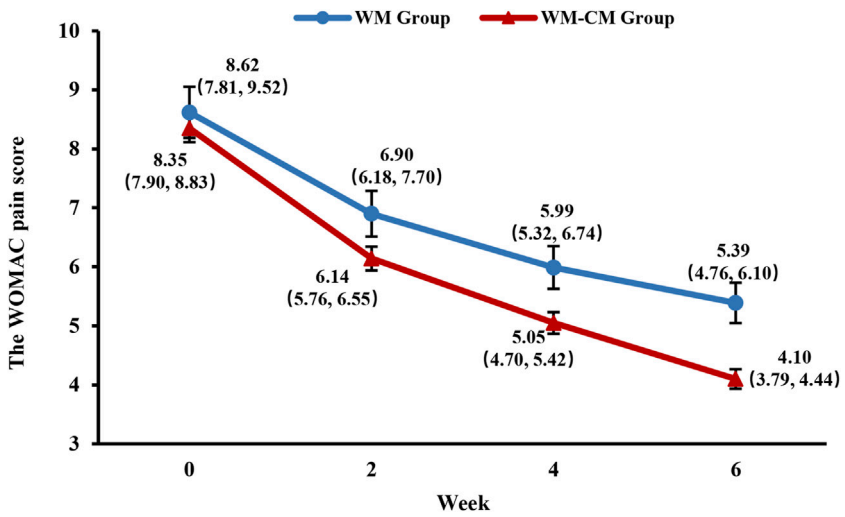

FIGURE 3
Overall trend of the WOMAC pain scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

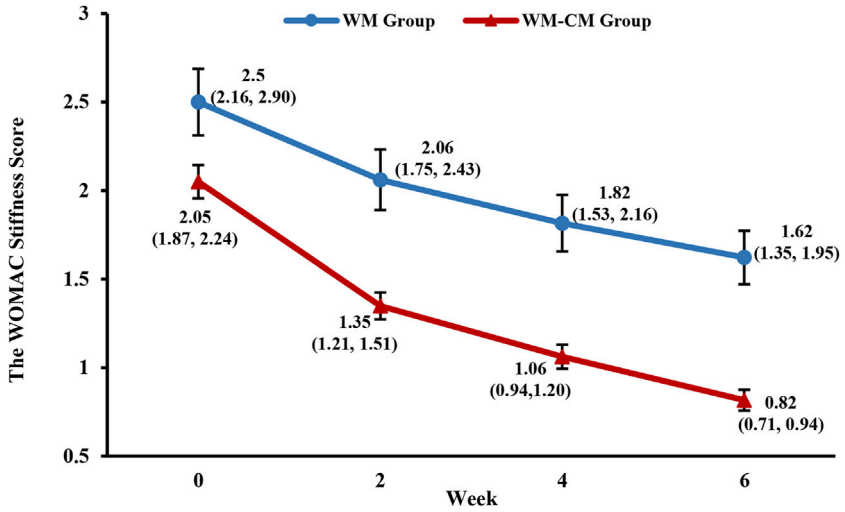

FIGURE 4
Overall trend of the WOMAC stiffness scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

TABLE 5 Comparison of the changes in WOMAC stiffness score from baseline.

	The changes in WOMAC stiffness score from baseline		*p*
	WM group	WM-CM group	
2 weeks	−0.44 ± 1.075	−0.70 ± 1.237	0.08
4 weeks	−0.68 ± 1.476	−0.99 ± 1.337	0.1
6 weeks	−0.88 ± 1.542	−1.23 ± 1.455	0.048

Note: The Mann–Whitney U rank sum test was used for comparisons between groups, adjusted by the Bonferroni method, with a test level of 0.017.

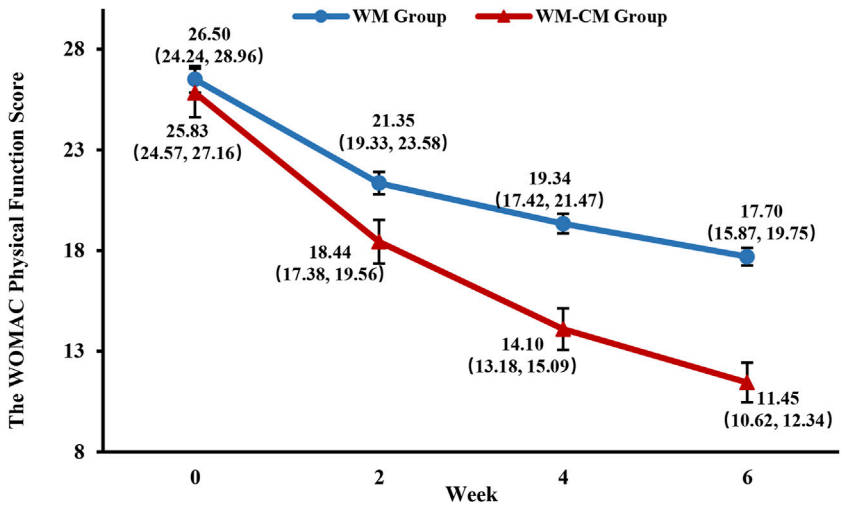

FIGURE 5
Overall trend of the WOMAC physical function scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

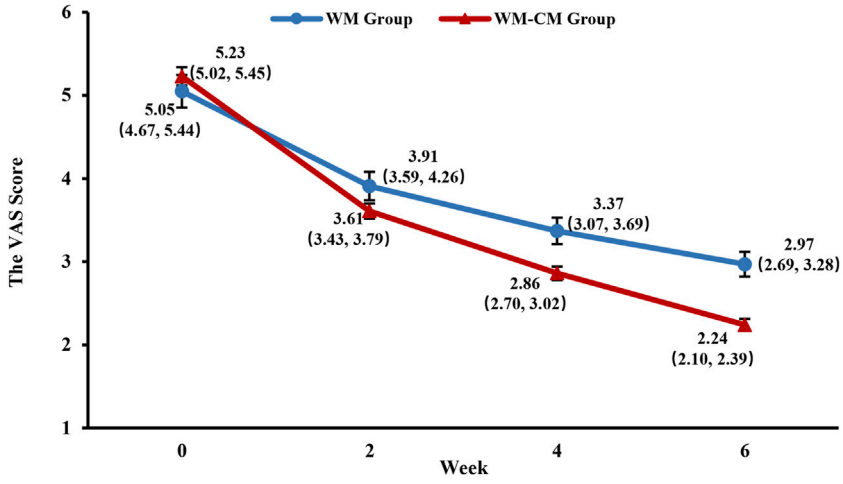

FIGURE 6
Overall trend of the VAS scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

theory regards KOA as a kind of "Jin-Gu" disease, and "Jin-Gu" refers to both the location of the disease and the different stages of the disease. When the disease is located in the "Jin", it mainly affects the soft tissues around the knee. At this time, the disease is still in the early stage, which is an excess syndrome. It is necessary to use medicines for dispelling wind and dampness, and promoting blood circulation to soothe sinews and harmonize collaterals. When the disease is located in the "Gu", the disease progresses and involves articular cartilage and bone tissue, and some symptoms of deficiency syndrome appear. Therefore, medicines for tonifying the liver and kidney are often chosen to protect cartilage and strengthen the bone.

Although most TCM doctors prescribe prescriptions based on TCM theory, the understanding of the active ingredients and targets of TCM is of great significance for elucidating the drug mechanism and even for the development of TCM. At present, studies have pointed out that Duhuo Jisheng Decoction and Yougui Pill can regulate the Wnt/β-catenin signaling pathway, thereby inhibiting the synovial inflammation and protecting articular cartilage (Lyu S., 2017; Yan C.L., 2018; Shi Q., 2022). Wangbi Tablet inhibits cartilage damage and inflammatory response by down-regulating NF-κB and p38-MAPK signaling pathways (Li H., 2021). The potential mechanism of Zhuifeng Tougu Pill against KOA may be the inhibition of TLR4/MyD88/NF-kB signaling pathway and inflammatory cytokines (Xu X., 2022). External preparations such as Wentong ointment and Daiwenjiu Ointment may promote the apoptosis of inflammatory cells, reduce the inflammatory response and inhibit neovascularization (Peng Y.L., 2014; Zhang L.W., 2022). Some botanical drugs frequently appear in these TCM prescriptions,

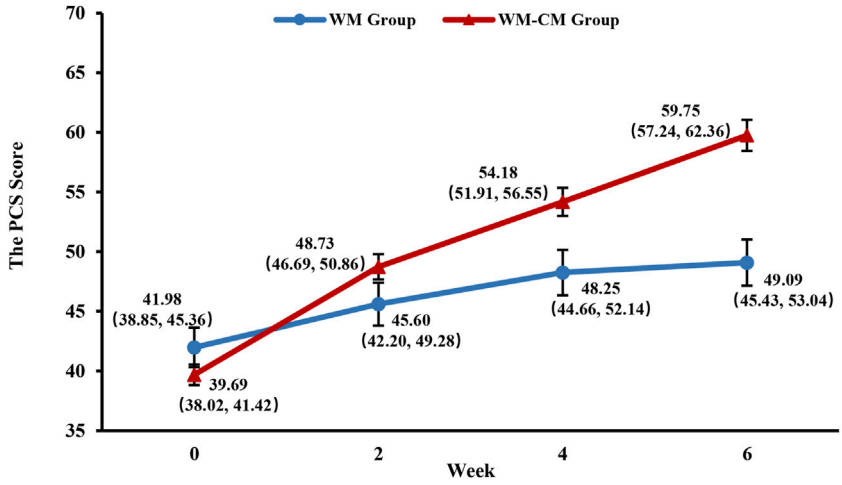

FIGURE 7
Overall trend of the PCS scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

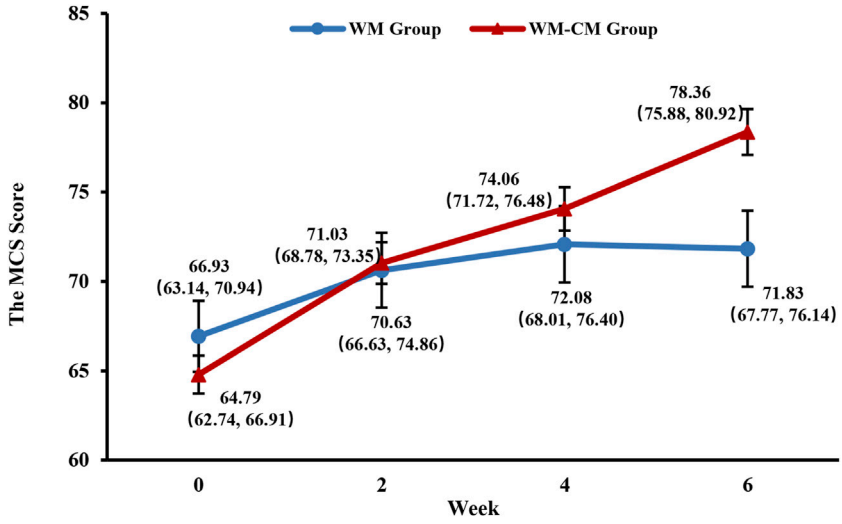

FIGURE 8
Overall trend of the MCS scores. Note: The values shown are least-squares mean calculated based on generalized linear mixed model, with 95% confidence intervals (indicated by error bars) in parentheses.

TABLE 6 Comparison of clinical efficiency between groups.

	WM group (n = 98)	WM-CM group (n = 321)	χ^2	*p*
effective	79 (80.6%)	305 (95%)	20.346	<0.001
significant	31 (32%)	204 (63.6%)	31.056	<0.001

showing immense potential in the treatment of KOA. Rehmanniae Radix Praeparata (Shu-Di-Huang) and Notopterygii Rhizoma Et Radix (Qiang-Huo) show significant anti-inflammatory effects (Pan T., 2017; Jhun J.Y., 2018). Drynariae Rhizoma (Gu-Sui-Bu) and its active components exhibit properties such as inhibiting inflammatory reactions, improving oxidative stress, suppressing cell apoptosis, regulating autophagy, influencing hormone levels, and enhancing microcirculation (Yang Y.J., 2021). Sodium ferulate, abundant in Angelicae Sinensis Radix (Dang-Gui), demonstrates remarkable anti-inflammatory and anti-apoptotic characteristics, while its polysaccharide component promotes the biosynthesis of proteoglycans in cartilage matrix (Magdalou J., 2015). Safflower

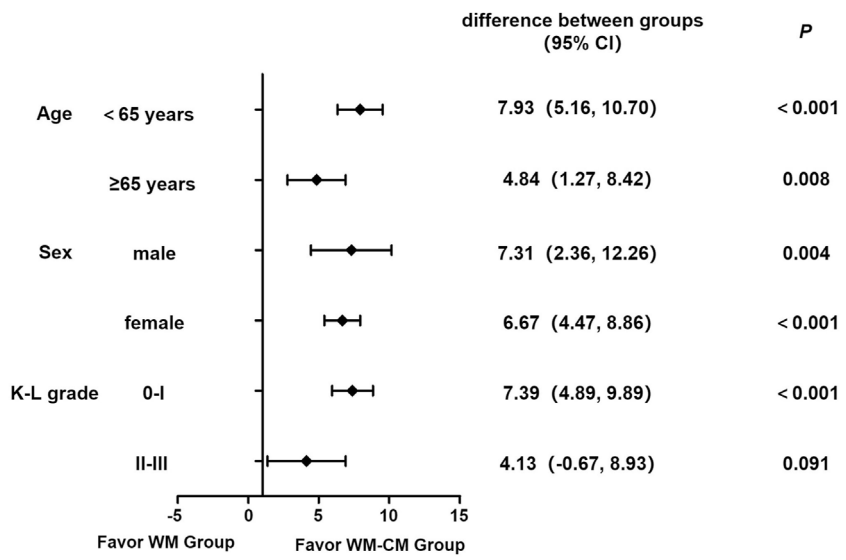

FIGURE 9
Forest plot for subgroup analysis. Note: The forest plot shows the differences in the changes in WOMAC total score between the two groups compared with baseline after 6 weeks of treatment stratifying by different subgroups. The difference between groups is calculated based on the least squares mean using GLMM, with a 95% confidence interval in parentheses. *p* values were Bonferroni corrected at a significance level of 0.05.

yellow from Carthami Flos (Hong-Hua) protects chondrocytes and inhibits inflammation by regulating the NF-κB/SIRT1/AMPK pathway and ER stress (Wang C., 2020).

When selecting evaluation indicators, we refer to previous internationally influential literature and Chinese guidelines (Lu Z., 2018; Chen W.H., 2020; Belk J.W., 2021). In this study, the total score of WOMAC scale was used as the main efficacy index, which can comprehensively reflect the overall state of the knee joint of patients, and has high sensitivity and reliability for elderly patients and patients with mild symptoms (Walker L.C., 2018). The VAS score is mainly to quantify and visualize the pain symptoms of patients (Xu X.M., 2021), which is simpler and easier to understand than the WOMAC scale, and can be mutually corroborated with the WOMAC pain score. In addition, considering that chronic pain is significantly related to psychological depression and seriously affects the quality of life of patients, we also adopted the SF-36 scale, which is often used in combination with the WOMAC scale in the evaluation of the efficacy of KOA (Angst F., 2001).

We chose mature products that have been marketed for many years or in-hospital preparations developed from classical prescriptions or experimental prescriptions, and administered them following the recommended conventional methods stated in the instruction manual. This approach has three major advantages. Firstly, it provides clear side effect profiles, enabling us to better monitor adverse events and determine the correlation between adverse events and the medication. This study focused on monitoring all possible adverse events, and no drug-related adverse events occurred in either group throughout the treatment cycle. This strongly demonstrates the safety of short-term drug application as observed in our study. Secondly, the protocol is easy to replicate as the selected drugs are readily available, making it possible to repeat the study and apply the research plan more easily to a broader context. Thirdly, it reflects the real-world application of the medication, providing direct reference evidence for clinical treatment plan development.

Although this study controlled confounding factors through prospective design and statistical processing, it still has the common limitations of real-world studies. It is difficult to achieve randomization and blinding in real-world studies. Compared with randomized controlled trials, there are more confounding factors and bias. For example, our research center comprises two traditional Chinese medicine hospitals and one comprehensive hospital. Patients seen at the traditional Chinese medicine hospitals tend to lean towards receiving combined Chinese and Western medicine treatment, while patients visiting the comprehensive hospital are more inclined to undergo Western medicine treatment. Patient treatment preferences could introduce some bias into the study. In addition, the subjects of this study mainly come from three medical institutions, limiting the generalizability of the research conclusions to other populations. Therefore, cautious consideration is necessary when extrapolating the research conclusions to other populations. Moreover, due to the complexity of the intervention measures, the sample size currently included is still insufficient for conducting subgroup analysis of treatment regimens to further explore the differences in therapeutic effects among various approaches and their associations with disease phenotypes. Nonetheless, the results of our study do provide preliminary confirmation of the overall advantages of integrated Chinese and Western medicine in treating KOA. In the future, we will include more research centers and continue to increase the sample size to enhance the objectivity and rigor of our conclusions. The accumulation of research data will also facilitate further prescription analysis, helping us identify drugs with greater potential research value, leading to targeted randomized controlled trials and mechanism studies.

5 Conclusion

In summary, our findings demonstrate that TCM can be an important complementary therapy to conventional Western conservative treatment and should be used early and promptly. This conclusion still needs to be validated by further large cohort studies or randomized controlled trials.

Data availability statement

The original contributions presented in the study can be found in the article and in the Supplementary Material. The data underlying this article will be shared on reasonable request to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Hospital of Jinan University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

R-HZ and X-FZ provided oversight and leadership responsibilities for planning and executing the research activities. LH offered guidance in revising the manuscript and enhancing its quality. Q-YY designed the entire clinical study, analyzed the data, and drafted the paper. X-LH, Y-MY, B-LZ, TL, W-QZ, H-YW, Z-FZ, B-JL, Y-WX, A-LW, YL, QL, and Z-LZ conducted research across various centers and participated in data collation. QL, L-YL, X-YL, P-PW, and LY were involved in manuscript revision. All authors contributed to the article and approved the submitted version.

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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.2023.1176980/full#supplementary-material

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The influence of adjunctive traditional Chinese medicine therapy on survival in primary liver cancer: a real-world study based on electronic medical records

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Background: Traditional Chinese medicine (TCM) effectively improves the survival rate and quality of life of primary liver cancer patients, but high-level evidence is lacking.

Patients and methods: Patients were selected from 5 tertiary hospitals in Henan Province, China. Two thousand sixty-seven patients with primary liver cancer were included in the study. The electronic medical records (EMRs) of the patients were collected. Patients who received adjunctive TCM treatment and underwent treatment cumulative time for more than 1 month were classified as the TCM intervention cohort. Patients who did not receive adjunctive TCM treatment or underwent treatment cumulative time for less than 1 month were classified as the non-TCM intervention cohort. The main outcome indicators were the survival rate and overall survival time. The propensity score inverse probability weighting method was used to balance the differences between the groups.

Results: The primary cohort comprised 2,067 patients, including 462 patients who received adjunctive TCM treatment and 1,605 patients who did not receive adjunctive TCM treatment. The results of the Kaplan–Meier survival curve indicated that the survival rate and median survival time of the exposure group before and after propensity score weighting were greater than those of the control group ($p < 0.0001$). Univariate Cox regression analysis after propensity score weighting showed that adjunctive TCM treatment was an independent protective factor for survival [regression coefficient = −0.215, hazard ratio (HR) = 0.8066, 95% confidence interval (CI) (0.6609–0.9844)].

Abbreviations: AFP, alpha-fetoprotein; ASAM, average standardized absolute mean difference; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; CT, computed tomography; DBIL, direct bilirubin; EMRs, electronic medical records; GBM, generalized boosted model; HR, hazard ratio; HCC, hepatocellular carcinoma; KS, Kolmogorov–Smirnov; OR, odds ratio; P, probability; RCT, randomized controlled trial; SD, standard deviation; SE, standard error; TACE, transarterial chemoembolization; TCM, traditional Chinese medicine.

Conclusion: Adjuvant treatment with TCM has a protective effect on the prognosis of patients with primary liver cancer; it can reduce the mortality and prolong the survival time.

KEYWORDS

traditional Chinese medicine, primary liver cancer, real-world study, survival analysis, electronic medical record

1 Introduction

Liver cancer is predicted to be the fifth most important cancer worldwide, and it was the third leading cause of cancer death worldwide in 2020, with approximately 564,000 new cases and 549,000 deaths that year. Approximately 81% of cases occur in less developed countries, with 54% occurring in China (Zhou et al., 2019; Sung et al., 2021). Traditional Chinese medicine (TCM) is widely used in Chinese and East Asian societies, and the combination of TCM and conventional cancer treatment has a good effect in clinical practice. TCM treatments have targeted stimulation of the host immune response for cytotoxic activity against liver cancer by inhibiting proliferation and promoting the apoptosis of tumour cells (Cao et al., 2013; Ling et al., 2014a; Wu et al., 2014), thereby alleviating chemoradio therapy-related or gene therapy-related side effects (Konkimalla and Efferth, 2008; Ling et al., 2014b). However, these studies on TCM treatment for primary liver cancer have only been conducted in laboratories, with only one multicentre randomized control trial having evaluated the effects of TCM use in preventing recurrence after resection of small hepatocellular carcinoma (Zhai et al., 2013). To date, high-level evidence-based medicine evidence is still lacking.

For a long time, the clinical evaluation of TCM has remained at the empirical level, with related research results and clinical experience mostly presented in the form of case reports, which are considered the lowest level of evidence in evidence-based medicine, so the credibility of TCM is not optimal (Li et al., 2017). The higher the level of evidence in evidence-based medicine, the more conducive the findings are to guiding clinical practice. High-quality evidence is mostly found in randomized controlled trials, but this type of trial requires strict inclusion and exclusion criteria to eliminate nonspecific factors to the greatest extent possible. Although high-level clinical evidence has laid the foundation for the formulation of clinical practice guidelines for TCM, its extrapolation to actual medical practice has always been questionable (Liu et al., 2017).

With the rapid development of information technology and the advent of big data, high-tech approaches such as big data, artificial intelligence, internet, and cloud computing continue to emerge. The external environment of real-world research has undergone significant changes, and high-quality clinical big data are now easier to obtain. In addition, continuous improvements in research methods have made research results more reliable. Real-world studies have become an important supplement and continuation of randomized controlled trials (RCTs), and they are also a suitable method for evaluating curative effects within the context of the characteristics of TCM diagnosis and treatment (Fu et al., 2019; Fu et al., 2020). In recent years, hospital information systems at all levels have also been widely used and gradually improved. A patient's complete medical records can be preserved, transmitted, managed and shared through an electronic medical record (EMR) system. Massive amounts of EMR data have laid a solid data foundation for knowledge discovery in the medical field (Zhao et al., 2019). Compared with other types of data, EMR data have the characteristics of a large data volume, objectivity, and convenient storage and transmission. Therefore, real-world research based on EMR data has gradually become a popular research topic.

This study was based on real-world clinical EMRs and used the generalized boosted model (GBM) propensity score weighting method to address the problems of nonrandom and confounding factors, which were found in real-world TCM clinical data. This study was performed with the aims of observing the efficacy of adjunctive TCM treatment in primary liver cancer patients and providing a practical basis for future real-world studies.

2 Materials and methods

2.1 Study design

A retrospective cohort study design was used in this study. Five tertiary hospitals in Henan Province were selected, including 3 hospitals that integrated Chinese and Western medicine and 2 hospitals that used Western medicine. Primary liver cancer patients who were hospitalized from 2015 to 2017 were selected. The patients were divided into an exposed group and a nonexposed group according to the application of TCM, and the survival condition of the patients was observed. The data were real-world clinical EMR data based mainly on the structured EMR system of the hospital, and they were extracted from the medical record homepage in the medical record system and from the hospitalization information. This study was approved by the Ethics Committee of The First Affiliated Hospital of Henan University of Chinese Medicine (No. 2017HL-077), written informed consent was not required for this study.

2.2 Patients

This study mainly included inpatients with primary liver cancer in 5 tertiary hospitals from 2015 to 2017. A total of 2,067 patients were enrolled. The inclusion criteria were as follows: ①patients diagnosed with primary liver cancer consistent with the "Guidelines for the Standardized Pathological Diagnosis of Primary Liver Cancer (2011 Edition)" (Bureau of Medical Administration, National Health and Family Planning Comission of the People's Republic of China, 2017); ②patients aged ≥18 years old; ③patients with complete relevant examination results and hospitalization

information; ④patients and their families who were willing to cooperate with follow-up visits and follow-up calls, the data from which were included in the collected information. The exclusion criteria were as follows: ①patients with malignant tumours of other sites or with serious organic diseases of the heart, kidney, and other organs; ②patients whose original data were severely insufficient; and ③patients who were only recorded for the first time and subsequently lost to follow-up.

2.3 Data source

The data of this study were based mainly on real-world clinical EMRs. Through data collection and data preprocessing, the information in the clinical EMRs was extracted, and the data were cleaned and standardized. Finally, a relatively standardized database was established. The patient information collected in the study included the medical records of patients with primary liver cancer admitted to 5 tertiary hospitals from 2015 to 2017, as well as the telephone follow-up information for patients from August 2018 to March 2019. The EMR information mainly included basic demographic information, disease-related information (family history, drinking history, history of illness), indicators related to disease progression (Child–Pugh classification, Barcelona Clinic Liver Cancer (BCLC) staging, Chinese staging, complications, etc.), admission/discharge information, and disease treatment-related information (surgery treatment and Chinese medicine intervention). The patient follow-up information obtained by telephone follow-up mainly included the patient's final outcome, time of diagnosis, time of death and information related to the patient's out-of-hospital medication use.

2.4 Exposure

In this study, adjunctive TCM treatment was regarded as the exposure factor, and the cohort was divided according to the degree of adjunctive TCM treatment received by primary liver cancer patients. Based on former methods, TCM users were identified as those who had received TCM and treatment cumulative time for more than 1 month, whereas those treatment cumulative time for less than 1 month were considered to be non-TCM users. (Shi et al., 2013; Lin et al., 2015; Tsai et al., 2016; Li et al., 2019). TCM treatment included TCM decoction treatment, Chinese patent medicine treatment, and TCM characteristic therapies (acupuncture, massage, external treatment, etc.).

The collection of exposed drugs and time is divided into two parts: 1) during hospitalization: according to the prescribed dose and time of medication recorded in the hospital electronic medical record information; 2) After discharge: supplement according to the type of medication and medication time of the patient obtained by telephone follow-up.

2.5 Outcome variables

The main observation indicators in this study were the survival rate and overall survival time of primary liver cancer patients. The overall survival time of patients during the study period were recorded. The starting point of survival time was the time at which primary liver cancer was diagnosed, and the end point was the time when the patient died of liver cancer or the end of the study period.

2.6 Quality control

The data were exported from the "case registration system" by data engineers. The design of data extraction documents covers all the key information to be collected, and the extraction of data is carried out by designated professionals using the "manual double entry mode" entry to ensure the accuracy of data. A standardized dictionary was used to standardize data processing to ensure that the data governance process records were complete and traceable. Detailed follow-up contents and follow-up plans were determined. Follow-up data were obtained using the hospital follow-up system. Patients who failed to return to the clinic on time were followed up by telephone by professionals. There are inspection documents in each step of data verification, cleaning and transformation to avoid missing steps. The quality controller regularly monitors the data collection process and randomly selects the collection form to ensure the authenticity and integrity of the data.

2.7 Statistical analysis

According to the inclusion and exclusion criteria and the TCM exposure criteria, the data of patients exposed and not exposed to TCM were analyzed in this study. First, we statistically described the baseline conditions of all patients. Quantitative data were described by the mean ± standard deviation or median (upper and lower quartiles), and comparisons between groups were performed using a *t*-test or Wilcoxon rank sum test; qualitative data were analyzed using frequencies or percentages. Comparisons between groups were performed using the Chi-square test or Fisher's exact test.

Screening for confounding factors was mainly based on the results of the comparison between the baseline groups, removing variables with $p < 0.05$, and combining the relevant literature and the recommendations of clinicians to choose individual variables. To balance confounding factors, the GBM propensity score weighting method was adopted. The weights were calculated by stable weighting method. The mean stabilized weight and the standard deviation of the stabilized weights were estimated to assess the validity of the positivity assumption. If the mean of the stabilized weights is far from one or if there are very extreme values, then this can be indicative of non-positivity or that the propensity score model has been misspecified. Otherwise, the positivity assumption is valid. In this study, the difference in propensity scores before and after weighting was represented by the Kolmogorov-Smirnov test statistic, Kolmogorov-Smirnov test is a useful non-parametric hypothesis test, which is mainly used to test whether a set of samples come from a certain probability distribution (one-sample K-S test). Or to see if two samples have the same distribution (two-sample K-S test). And a KS statistic <0.05 was regarded as equality between groups (Yang et al., 2017; Dai et al., 2020; Xu et al., 2020).

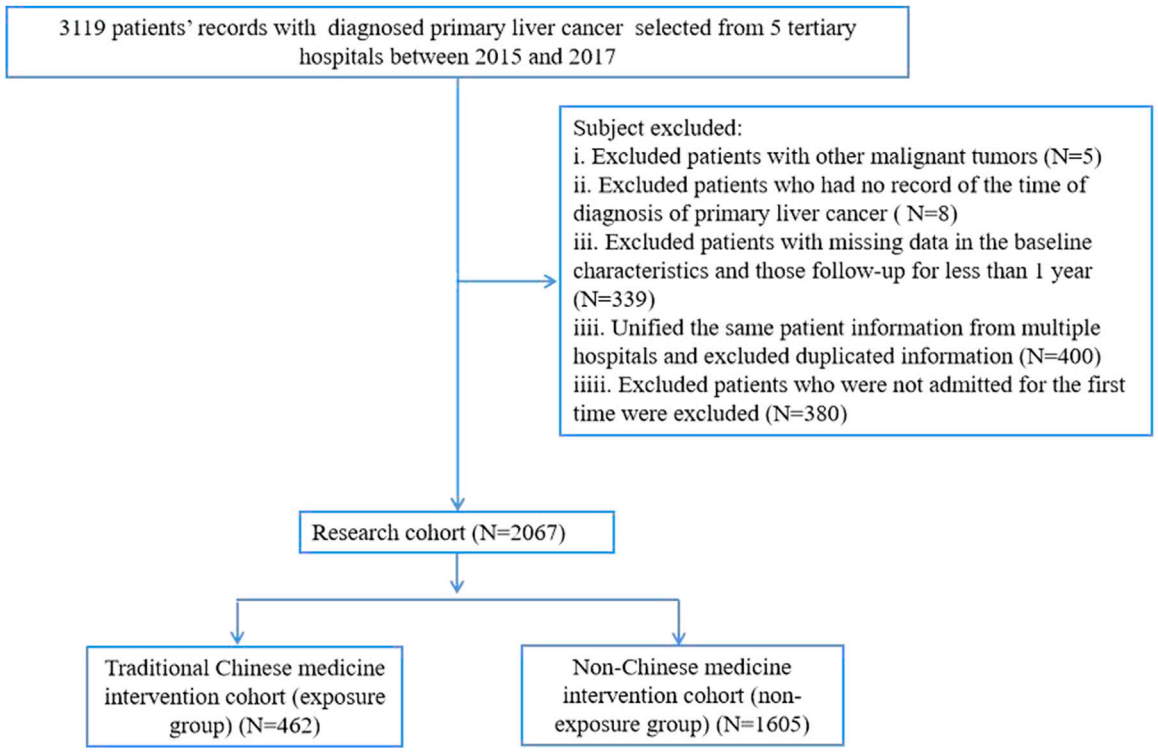

FIGURE 1
Chart of research cohort creation.

The Kaplan–Meier method was used to calculate the survival rate and draw a survival curve, and the survival rate was compared between groups by the log-rank test. Cox proportional hazard regression analysis was performed to observe the influence of adjunctive TCM intervention on the survival outcome and survival time of primary liver cancer patients and related risk factors. The BCLC stage was used as a subgroup to analyze the effect of traditional Chinese medicine treatment on the survival rate and overall survival time of primary liver cancer in different subgroups.

Sensitivity analysis of potential confounding recognition: Propensity score weighting methods can adjust for observable variables, but not for unobserved factors, namely, potential bias. The presence of potential bias can lead to the phenomenon that individuals with the same observed values of the covariate have different treatment assignment probabilities, that is, treatment assignment depends on the unobserved covariate. Therefore, we needed to identify possible potential confounding factors; sensitivity analysis tests whether a model is sensitive to potential confounding bias by sequentially removing confounding variables from the model.

In addition, taking into account that unbalanced confounding factors remained after propensity score weighting, as well as clinical experience and the single-factor analysis results, the factors that had an important impact on the outcome were screened out: cancer thrombus and liver cirrhosis. We carried out a cox multivariate analysis after propensity score weighting, to further explore the impact of traditional Chinese medicine treatment on the survival of primary liver cancer, and used this as a sensitivity analysis. See the table in the annex.

3 Results

3.1 Study population

From 2015 to 2017, approximately 3,114 hospitalized patients were included from the 5 tertiary hospitals included in the study. Among them, 5 patients were excluded because they had other malignancies; 8 patients were excluded for lack of a time of diagnosis; 339 patients were excluded with missing data in the baseline characteristics and those follow-up for less than 1 year; During unified the same patient information from multiple hospitals, 400 patients were excluded for duplicated information; 380 patients were excluded because they were not admitted for the first time. Thus, 2,067 patients were ultimately included in the cohort, see as Figure 1.

3.2 Patient characteristics

Table 1 shows the baseline demographic characteristics and clinical characteristics of the study subjects before and after propensity score weighting. Among the 2,067 study subjects included, according to the exposure standards, 462 patients were treated with adjunctive TCM therapy; they had an average age of 57.51 years (standard deviation, 10.91), with 79% being male and 21% being female. A total of 1,605 patients did not use adjunctive TCM treatments; they had an average age of 56.23 (standard deviation 11.19) years, with 82% being male and 18% being female. Patients who used adjunctive TCM therapy were more likely to have the following characteristics: family history of liver cancer or viral hepatitis; history of hepatitis C, cirrhosis, or alcoholic

TABLE 1 Baseline demographic and clinical characteristics of patients before and after propensity score weighting.

Variable	Unweighted				Propensity score weighted			
	Adjunctive TCM users	TCM nonusers	ks	ks.pval	Adjunctive TCM users	TCM nonusers	ks	ks.pval
	(*N* = 462)	(*N* = 1,605)			(*N* = 462)	(*N* = 1,605)		
Age, mean (SD)	57.51 ± 10.95	56.23 ± 11.19	0.05	0.15	56.62 ± 11.07	56.35 ± 11.1	0.03	0.81
Sex, 100%								
Male	365 (79)	1316 (82)	0.02	0.28	370 (80)	1300 (81)	0.01	0.56
Female	97 (21)	289 (18)	0.02	0.28	92 (20)	305 (19)	0.01	0.56
Career, %								
Worker	11 (2)	32 (2)	0	0.74	11 (2)	32 (2)	0	0.86
Farmer	213 (46)	867 (54)	0.07	0	236 (51)	851 (53)	0.02	0.5
Leader	5 (1)	16 (1)	0	0.43	5 (1)	16 (1)	0	0.86
Teacher	5 (1)	16 (1)	0	0.47	5 (1)	16 (1)	0	0.69
Company employee	32 (7)	96 (6)	0.01	0.55	23 (5)	96 (6)	0.01	0.5
Other	134 (29)	417 (26)	0.03	0.23	134 (29)	433 (27)	0.02	0.38
Retiree	55 (12)	144 (9)	0.03	0.08	42 (9)	144 (9)	0	0.91
Medical insurance, %								
Provincial medical insurance	55 (12)	127 (8)	0.04	0.01	42 (9)	127 (8)	0.01	0.56
City medical insurance	46 (10)	127 (8)	0.02	0.19	37 (8)	127 (8)	0	0.89
NCMS[a]	259 (56)	963 (60)	0.04	0.17	273 (59)	963 (60)	0	1
None	92 (20)	337 (21)	0.02	0.38	92 (20)	337 (21)	0.01	0.58
Drinking history, %	148 (32)	562 (35)	0.03	0.27	166 (36)	562 (35)	0.01	0.8
Family history, %								
Liver cancer	55 (12)	144 (9)	0.03	0.05	51 (11)	144 (9)	0.02	0.41
Viral hepatitis	92 (20)	225 (14)	0.06	0.01	74 (16)	241 (15)	0.02	0.46
Medical history, %								
Hepatitis B	347 (75)	1220 (76)	0.01	0.58	347 (75)	1236 (77)	0.02	0.44
Hepatitis C	42 (9)	80 (5)	0.03	0	28 (6)	80 (5)	0.01	0.58
Liver cirrhosis	273 (59)	562 (35)	0.25	0	208 (45)	610 (38)	0.07	0.02
Alcoholic hepatitis	9 (2)	16 (1)	0.01	0.01	5 (1)	16 (1)	0.01	0.2
Complications, %								
Hepatic encephalopathy	28 (6)	48 (3)	0.04	0	18 (4)	48 (3)	0.01	0.26
Pulmonary infection	42 (9)	64 (4)	0.04	0	32 (7)	80 (5)	0.02	0.15
Upper gastrointestinal bleeding	18 (4)	64 (4)	0.01	0.58	14 (3)	64 (4)	0	0.61
Disease classification, %								
Child-Pugh stage								
A	259 (56)	819 (51)	0.05	0.09	259 (56)	835 (52)	0.05	0.1

(Continued on following page)

TABLE 1 (*Continued*) Baseline demographic and clinical characteristics of patients before and after propensity score weighting.

Variable	Unweighted			ks.pval	Propensity score weighted			ks.pval
	Adjunctive TCM users	TCM nonusers	ks		Adjunctive TCM users	TCM nonusers	ks	
	(*N* = 462)	(*N* = 1,605)			(*N* = 462)	(*N* = 1,605)		
B	106 (23)	385 (24)	0.01	0.64	97 (21)	401 (25)	0.03	0.19
C	65 (14)	273 (17)	0.04	0.08	60 (13)	273 (17)	0.03	0.15
BCLC stage								
0	9 (2)	32 (2)	0.01	0.32	14 (3)	32 (2)	0.01	0.23
A	102 (22)	385 (24)	0.01	0.53	106 (23)	385 (24)	0	0.83
B	134 (29)	353 (22)	0.07	0	120 (26)	353 (22)	0.03	0.16
C	116 (25)	514 (32)	0.06	0	129 (28)	498 (31)	0.03	0.26
D	65 (14)	241 (15)	0.01	0.65	65 (14)	225 (14)	0.01	0.66
Liver cancer stage								
I	116 (25)	433 (27)	0.02	0.46	125 (27)	433 (27)	0	0.85
II	125 (27)	305 (19)	0.08	0	102 (22)	321 (20)	0.02	0.41
III	116 (25)	465 (29)	0.04	0.11	134 (29)	449 (28)	0	0.86
IV	65 (14)	289 (18)	0.04	0.02	60 (13)	289 (18)	0.04	0.06

liver disease; and complications of hepatic encephalopathy or abdominal infection. Patients treated with TCM assistance may have had higher BCLC staging or Chinese staging.

The baseline treatment characteristics of patients before and after propensity score weighting are shown in Table 2. Some treatments, such as surgical excision, transcatheter arterial chemoembolization, hepatic artery embolism, radiofrequency ablation and microwave ablation, etc., were balanced after propensity score weighting.

Figure 2 shows the *p* value and uniform distribution value of the confounding variables before and after weighting of the two groups. After propensity score weighting, the difference between the baseline confounding variables between the two groups was close to that expected with random assignment.

The mean stabilized weight and the standard deviation of the stabilized weights were estimated to assess the validity of the positivity assumption. If the mean of the stabilized weights is far from one or if there are very extreme values, then this can be indicative of non-positivity or that the propensity score model has been misspecified. Otherwise, the positivity assumption is valid. The distribution of the stable weights is shown in Figure 3, which has a mean of 0.91 and a standard deviation of 0.26, supporting the positivity hypothesis.

3.3 Primary outcomes

3.3.1 Survival analysis

3.3.1.1 Kaplan–Meier analysis

Kaplan–Meier analysis was used to calculate the survival rate and draw a survival curve, and the survival rate was compared between groups by the log-rank test, as shown in Figure 4. Before and after propensity score weighting, the median survival time of the TCM intervention group was longer than that of the control group, and the difference was statistically significant ($p < 0.001$).

3.3.1.2 Cox regression analysis after the GBM trend score increased

The results of Cox univariate analysis before and after propensity score weighting are shown in the Table 3. The covariate-adjusted propensity score weighted Cox regression analysis showed that the regression coefficient for adjunctive TCM treatment was negative, and the difference was statistically significant ($p = 0.0224$). This suggests that adjuvant treatment with TCM has a protective effect on the prognosis of primary liver cancer patients; it can prolong the survival time and reduce mortality.

3.3.2 Supplementary analysis

3.3.2.1 Hierarchical analysis--BCLC stage

We used BCLC stage as a subgroup to explore the effect of TCM treatment on the prognosis of different stages of liver cancer. As shown in Figure 5, we plotted the survival curves of different stages of BCLC treated by propensity score weighting method and the results of Log-rank test, indicating that TCM treatment has a good effect on patients with different stages of BCLC of primary liver cancer.

3.3.2.2 TCM treatment time

The treatment time of traditional Chinese medicine is also a key factor affecting the efficacy of traditional Chinese medicine. Previously, we divided the traditional Chinese medicine adjuvant treatment group and the non-traditional Chinese medicine adjuvant

TABLE 2 Baseline treatment characteristics of patients before and after propensity score weighting.

Variable	Unweighted				Propensity score weighted			
	Adjunctive TCM users	TCM nonusers	ks	ks.pval	Adjunctive TCM users	TCM nonusers	ks	ks.pval
	(*N* = 462)	(*N* = 1,605)			(*N* = 462)	(*N* = 1,605)		
Surgical treatment, %								
Surgical excision	129 (28)	610 (38)	0.09	0	162 (35)	594 (37)	0.02	0.53
Interventional therapy, %								
Transcatheter arterial chemoembolization	199 (43)	562 (35)	0.08	0	166 (36)	562 (35)	0	0.87
Hepatic artery infusion chemotherapy	92 (20)	337 (21)	0.01	0.62	88 (19)	337 (21)	0.02	0.39
Hepatic artery embolism	23 (5)	16 (1)	0.04	0	14 (3)	32 (2)	0.01	0.1
Local ablation, %								
Radiofrequency ablation	74 (16)	161 (10)	0.06	0	60 (13)	177 (11)	0.02	0.32
Microwave ablation	28 (6)	48 (3)	0.03	0	18 (4)	64 (4)	0.01	0.58
Antiviral therapy,%								
Entecavir	249 (54)	770 (46)	0.07	0.01	240 (52)	754 (47)	0.05	0.14
Lamivudine	46 (10)	80 (5)	0.05	0	32 (7)	80 (5)	0.02	0.16
Adefovir dipivoxil	65 (14)	128 (8)	0.07	0	55 (12)	128 (8)	0.03	0.08
Radiation therapy, %								
IODINE-125 implantation	42 (9)	112 (7)	0.02	0.24	32 (7)	112 (7)	0	0.85
Molecular targeting drug, %								
Sorafenib	23 (5)	96 (6)	0.01	0.4	28 (6)	96 (6)	0	0.94
Immunotherapy, %								
Interferon alph	9 (2)	0 (0)	0.02	0	5 (1)	16 (1)	0.01	0.16
Thymosin α1	69 (15)	161 (10)	0.05	0	65 (14)	161 (10)	0.03	0.06

treatment group according to the treatment time. Here, we focused on the analysis of the relationship between the treatment time and the outcome of patients with primary liver cancer. We divided the duration of TCM treatment into four levels: level 1 for less than 3 months, level 2 for 3 months to 6 months, level 3 for 6 months to 1 year, and level 4 for more than 1 year. As shown in Table 4, a treatment duration of up to 6 months, 1 year, and even more than 1 year had a better effect compared with a treatment duration of less than 3 months.

3.3.2.3 Commonly used TCMs in patients with primary liver cancer

Commonly used TCMs in patients with primary liver cancer are shown in Table 5. The most common prescriptions of the TCM users were Hua Chan Su Jiao Nang, Fu Fang Ban Mao Jiao Nang, Yang Zheng Xiao Ji Jiao Nang, Ruan Gan Wan, Huaier Granule and Biejiajian Pills, which were used by 297 TCM users (29.6%), 172 TCM users (17.1%), 67 TCM users (6.7%), 63 TCM users (6.3%), 62 TCM users (6.2%) and 60 TCM users (6.0%) respectively (Table 5). Of the most common TCMs, 4 were herbal formulae, and 2 were single herbs.

4 Discussion

Adjunctive TCM treatment of primary liver cancer is widely used in China. Inpatients with liver cancer generally use TCM, such as TCM injections, for adjuvant treatment. The "Norms for the diagnosis and treatment of primary liver cancer (2019 edition)" pointed out that the treatment of liver cancer with traditional Chinese medicine can improve clinical symptoms, improve body resistance and quality of life, and reduce the adverse reactions caused by radiotherapy and chemotherapy. However, clinical studies on the therapeutic effects of TCM in patients with primary liver cancer remain scant, and to the best of our knowledge the majority are either case discussions or descriptive outcomes of a few patients. Although RCT studies are generally accepted as the gold standard, there are few RCT

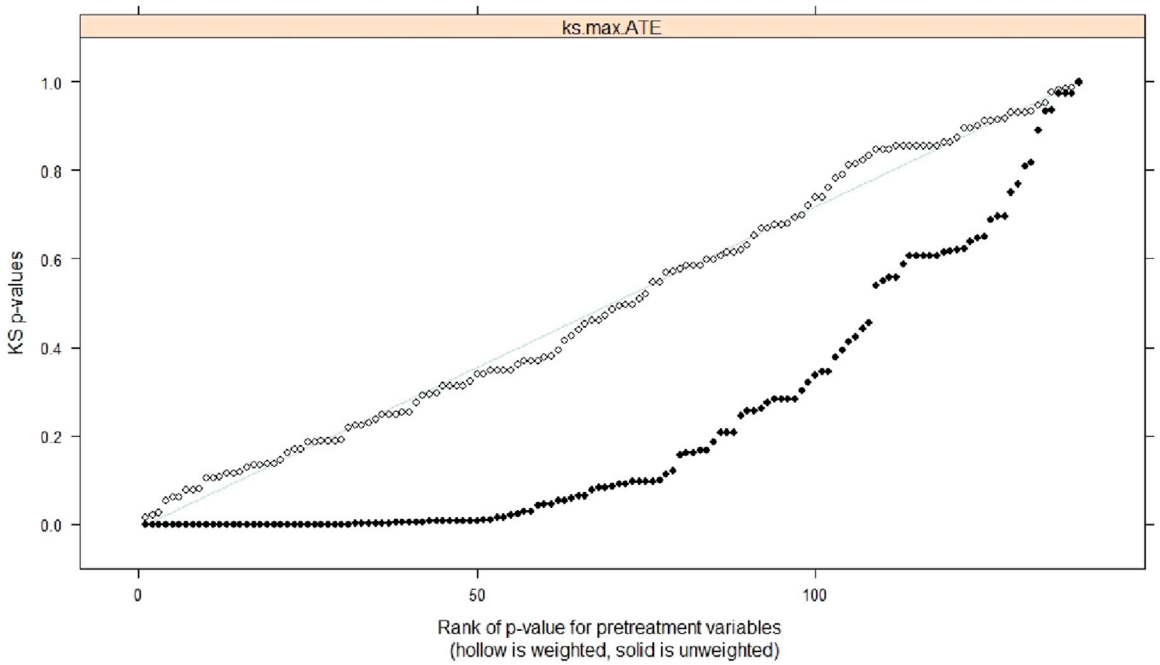

FIGURE 2
Comparison of the *p*-value and the uniform distribution value of the difference test between the two groups of confounding variables before and after weighting.

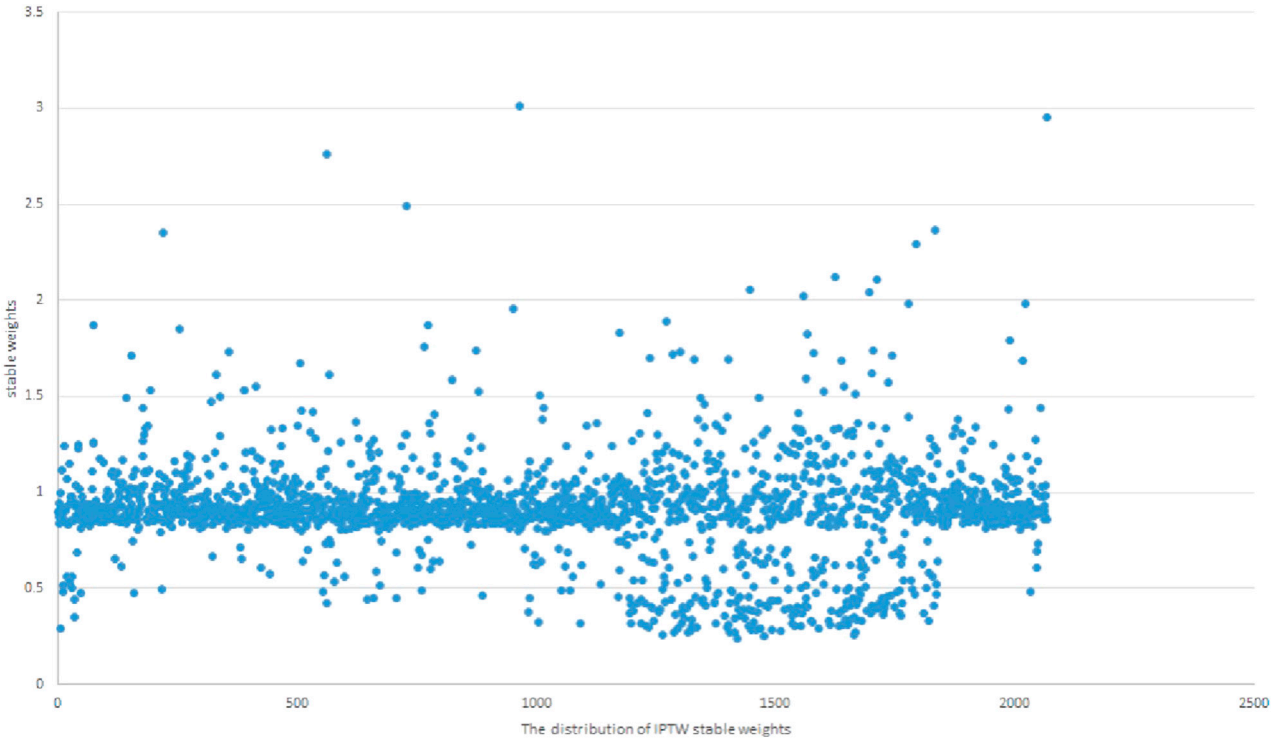

FIGURE 3
The distribution plot of the IPTW stable weights.

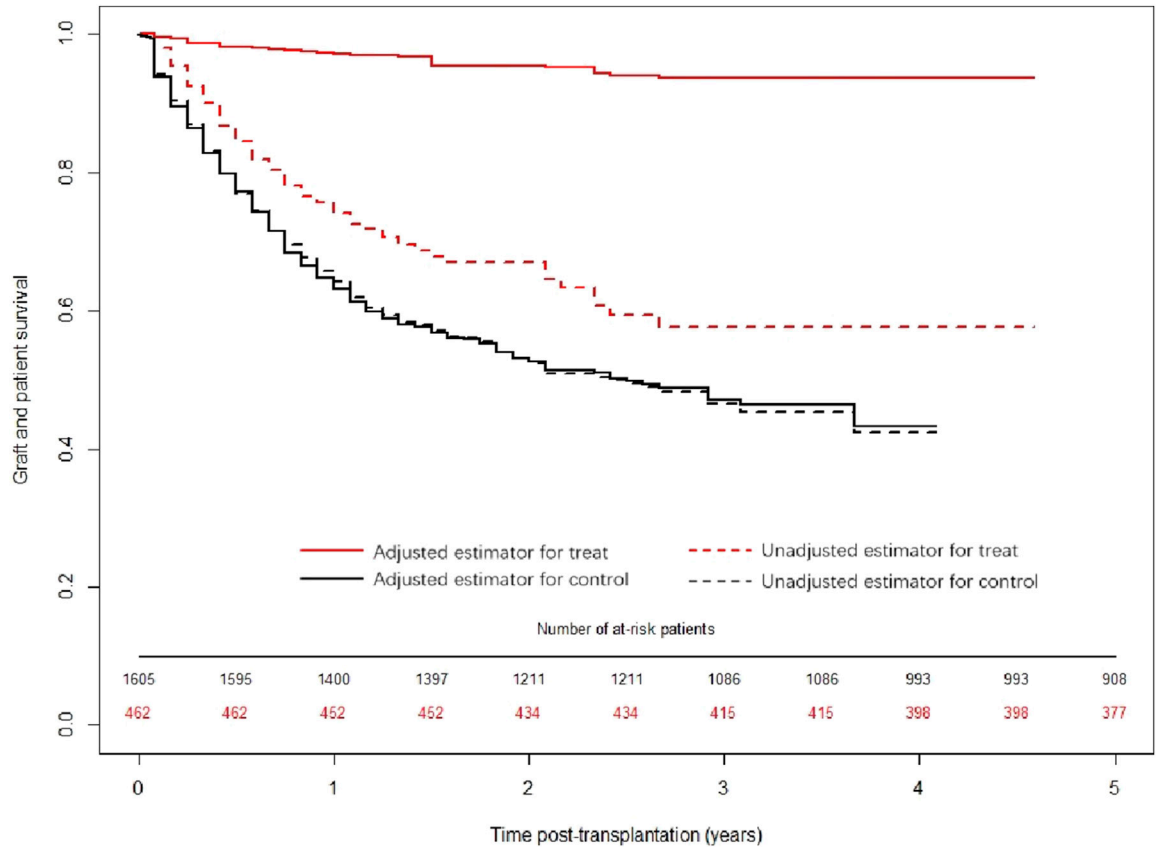

FIGURE 4
Distribution of the survival curves of the exposure group and control group before and after propensity score weighting.

TABLE 3 Univariate Cox regression analysis before and after GBM propensity score weighting.

	Unweighted			Propensity score weighted		
Variable	Beta (SE)	HR (95% CI)	*P*	Beta (SE)	HR (95% CI)	*P*
Adjunctive TCM therapy	−0.4003	0.6701 (0.5561–0.8075)	<0.001	−0.215	0.8066 (0.6609–0.9844)	0.0344

studies on TCM treatment of primary liver cancer, and some of the studies are not standardized and do not fully follow the principle of randomized control. In addition, this method has some limitations in the application of traditional Chinese medicine research, and its extrapolation is poor.

This study is a large-scale, real-world study based on clinical EMRs investigating the association between adjunctive TCM therapy and the survival of patients with primary liver cancer. More importantly, the GBM propensity score weighting method was used to balance the influence of confounding factors to achieve the "effect of postmortem randomization" and enhance the credibility of the evidence. The results of the study show that Chinese medicine interventions are independent protective factors in the survival of patients with primary liver cancer. The median survival time of the Chinese medicine-assisted intervention group was longer than that of the nonintervention group, and the survival rate was also improved. Our findings on the effects of TCM on the mortality of patients with primary liver cancer were similar to those of population-based studies. These studies used a retrospective cohort study to observe the effect of adjunctive TCM treatment on the survival of patients with advanced primary liver cancer, and the results all suggested that adjunctive TCM treatment can improve the survival rate of patients with primary liver cancer (Qiu et al., 2014; Liao et al., 2015), but these studies did not use appropriate methods to control confounding factors. In contrast with their studies, the GBM propensity score weighting method was adopted in this study, which controlled the confounding factors between the groups well, resulting in stronger evidence. In addition, some studies have shown that the use of Chinese medicine compounds in the treatment of primary liver cancer has a good effect (Shi et al., 2013; Li, 2016; Shi et al., 2016). A randomized controlled trial of TCM combined with transarterial chemoembolization (TACE) in the treatment of patients with unrespectable HCC showed that TCM can stimulate the host immune response by causing cytotoxic activity in liver cancer (Cho and Chen, 2009). In addition, some studies have shown that unit traditional Chinese medicine combined with targeted drugs has a remarkable effect on the treatment of HCC. As a zingiberaceae plant,

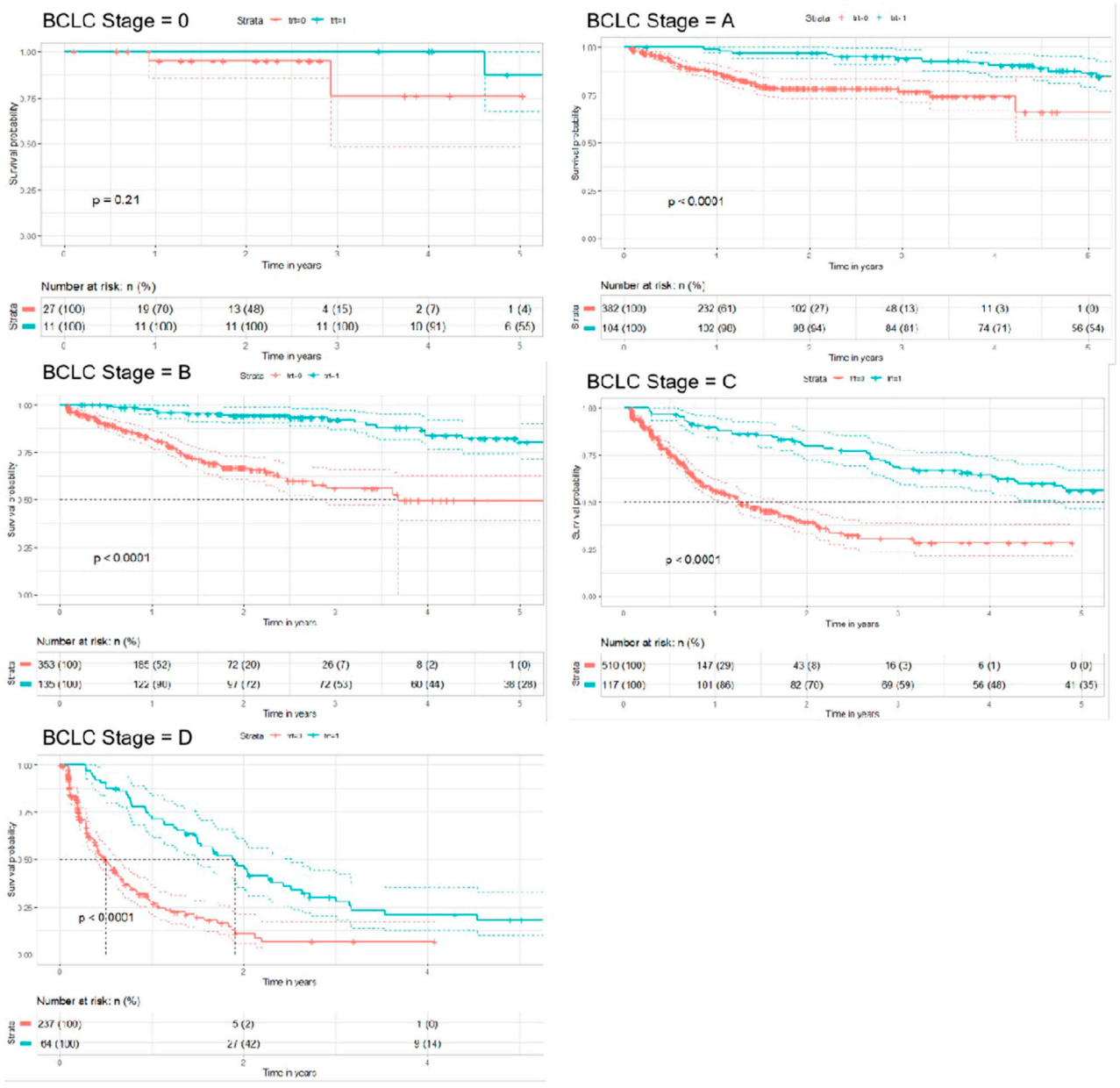

FIGURE 5
Survival curves after propensity score weighting for different stages of BCLC.

TABLE 4 Association of TCM treatment duration with the prognosis of patients with primary liver cancer after GBM weighted.

Variable	Beta	HR (95% CI)	*P*
Time-to-treatment			
3 months ~ 6months	−0.356	0.701 (0.446–1.101)	0.1226
6 months ~ 12 months	−0.799	0.450 (0.269–0.751)	0.0023
>12 months	−1.357	0.257 (0.146–0.452)	<0.001

the extract of Zedoary turmeric has the effect of inhibiting tumor growth, enhancing human immune system and reversing multiple drug resistance after the use of chemotherapy drugs. Chinese scholars have found that elemene injection combined with molecular targeted drugs can effectively improve the disease control rate and prolong the survival of patients with liver cancer (Sun and Zhang, 2012). In addition, as for the anti-tumor mechanism of traditional Chinese medicine, some studies have found that the immunomodulatory function of an approved Chinese medicine formula, compound kushen injection (CKI) acts on macrophages and $CD8^+$ T cells to reshape the immune microenvironment of HCC, which improves the therapeutic outcomes of low-dose sorafenib and avoids adverse chemotherapy effects. It shows that traditional Chinese medicines with immunomodulatory properties can potentiate chemotherapeutic drugs and provide a promising approach for HCC treatment (Yang et al., 2020).

TABLE 5 Commonly used TCMs in patients with primary liver cancer.

TCM name	Ingredients or generic name	Functional classification	No. of users	%
Cinobufagin Capsules	Toad Skin [Bufonidae; Dried toad skin]	Detoxicate disperse swelling and relieve pain	297	29.6
Compound Cantharidin Capsules	Mylabris [Meloidae; Mylabris phalerata Pallas]; Ginseng [Araliaceae; Ginseng Radix et Rhizoma]; Astragalus membranaceus [Leguminosae; Astragalus membranaceus Radix et Rhizoma]; Eleutherococcus senticosus [Araliaceae; Eleutherococcus senticosus Radix et Rhizoma]; Trigone [Sparganiaceae; Sparganium stoloni erum, Dry tubers of Sparganium stoloni erum]; Scutellaria barbata [Lamiaceae; Whole grass of Scutellaria barbata]; Curcuma phaeocaulis Valeton [Zingiberaceae; Curcuma phaeocaulis Valeton Radix et Rhizoma]; Cornus officinalis [Cornaceae; Mature pulp of Cornus officinalis]; Ligustrum lucidum [Oleaceae; Mature fruit of Ligustrum lucidum]; Bear bile Powder [Ursidae; Dry Bile of Brown Bear and Black Bear]; Licorice [Leguminosae; Licorice Radix et Rhizoma];	Breaking blood stasis attack poison and corrode sore	172	17.1
Yang zheng Xiao ji Capsules	Astragalus membranaceus [Leguminosae; Astragalus membranaceus Radix et Rhizoma]; Ligustrum lucidum [Oleaceae; Mature fruit of Ligustrum lucidum]; Ginseng [Araliaceae; Ginseng Radix et Rhizoma]; Ganoderma lucidum [Ganodermataceae; The fruiting body of Ganoderma lucidum]; Curcuma phaeocaulis Valeton [Zingiberaceae; Curcuma phaeocaulis Valeton Radix et Rhizoma]; Atractylodes macrocephala [the composite family; Atractylodes macrocephala Radix et Rhizoma]; Hedyotis diffusa Willd [Rubiaceae; The Whole Grass of Hedyotis diffusa Willd]; Scutellaria barbata [Lamiaceae; The Whole Grass of Scutellaria barbata]; Gynostemma pentaphyllum [Cucurbitaceae; The Whole Grass of Gynostemma pentaphyllum]; Poria cocos [Polyporaceae; Sclerotium of Poria cocos]; Gallusgallusdomesticus Brisson [Phasianidae; Dry sac intima of chicken]; Duchesnea indica [Rosaceae; The Whole Grass of Duchesnea indica]; Solanum lyratum [Solanaceae; The Whole Grass of Solanum lyratum]; Artemisia capillaris Thunb [the composite family; The Whole Grass of Artemisia capillaris]; Cynanchum paniculatum [Apocynum; Cynanchum paniculatum Radix et Rhizoma]; Eupolyphaga steleophaga [Periplanetidae; Female dried body of Eupolyphaga steleophaga]	Enforcing spleen and nourishing kidney, transform stasis and resolve toxin	67	6.7
Ruan Gan Wan	Carapax trionycis [Trionychidae; The back shell of turtle]; Carapax Testudinis [Emydidae; Turtle belly armor]; Anis pentadactyla Linnaeus [Manidae; The scales of Anis pentadactyla Linnaeus]; Angelica sinensis [Umbelliferae; Angelica sinensis Radix]; Oyster [Ostreidae; The shell of an oyster]; Peach kernel [Rosaceae; Dry mature seeds of Prunus persica]; Gallusgallusdomesticus Brisson [Phasianidae; Dry sac intima of chicken]; Licorice [Leguminosae; Licorice Radix et Rhizoma], Etc.	Activating blood circulation todissipate blood stasis, soften hardness and dissipate mass	63	6.3
Huaier granule	Sophora auricula mycoplasm [Auriculariaceae; Fruiting body of Trametes robiniophila]	strengthen the body resistance to consolidate the constitution,·blood quickening and dissipate mass	62	6.2

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Several differences exist between the current study and previous population-based studies. First, the current study data were extracted from real-world clinical data based on electronic medical records with 2067 primary liver cancer patients who were more representative. Second, the electronic medical records included basic demographic information, disease information, laboratory examination information and treatment-related information, and it was possible to obtain all possible confounding factors, with further comprehensive control of these factors to reduce the occurrence of confounding bias. Third, the GBM propensity score weighting method was used to balance the influence of confounding factors, which can achieve the "effect of postmortem randomization" and enhance the credibility of the evidence.

This study also has some shortcomings. First, detailed patient discharge information, such as the type of medication, dosage, frequency, and duration of use, could not be accurately obtained. Electronic medical record data can only be used to collect information about patient medication use during hospitalization, not after discharge. The researcher's prescribing frequency, the number of patients and the memories of patients or family members were used to infer the patient's medication status at discharge. This causes a certain bias. Second, because the data were collected retrospectively, the patient's disease was classified and staged. The information was stratified by the researchers based on the patient's medical record information, making the results easily affected by the medical records. Besides, the inverse probability weighting method eliminates the influence of known covariates on the target effect, and the average efficacy of the various treatments obtained is comparable. In this case, the conclusion that TCM adjuvant therapy has a better effect is based on the average effect of the overall population, rather than on the individual. In addition, the inverse probability weighting method of propensity score was used in this study to control the confounding factors. This method can only control the known confounding factors, but cannot control the unknown confounding factors. However, in observational studies, there may be unknown confounding factors. Finally, in this study, the compositions of the Chinese medicine prescriptions that were taken were not recorded in detail, and the medication information could not be analysed in depth.

5 Conclusion

This study was based on real-world clinical EMRs and used the GBM propensity score weighting method to effectively control for nonrandom and confounding factors in the data; the effect of adjunctive TCM treatment on the survival of primary liver cancer patients was also explored. In this preliminary study, it was found that adjuvant traditional Chinese medicine therapy may improve the survival rate and prolong the survival time of patients with primary liver cancer. However, the specific effect of TCM interventions still needs to be further studied. This study provides a practical basis for future real-world research.

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 Ethics Committee of The First Affiliated Hospital of Henan University of Chinese Medicine (No. 2017HL-077). Written informed consent from the patients/participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

RZ conducted data analysis and article writing; LW and YL polished and perfected the article; MS carried out the overall research design; WY and YF contributed to data quality control; QG, JF, YX, and XX contributed to the case collection; All authors contributed to the article and approved the submitted version.

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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.2023.1231933/full#supplementary-material

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Current state of research on the clinical benefits of herbal medicines for non-life-threatening ailments

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Herbal medicines are becoming increasingly popular among patients because they are well tolerated and do not exert severe side effects. Nevertheless, they receive little consideration in therapeutic settings. The present article reviews the current state of research on the clinical benefits of herbal medicines on five indication groups, psychosomatic disorders, gynecological complaints, gastrointestinal disorders, urinary and upper respiratory tract infections. The study search was based on the database PubMed and concentrated on herbal medicines legally approved in Europe. After applying defined inclusion and exclusion criteria, 141 articles were selected: 59 for psychosomatic disorders (100% randomized controlled trials; RCTs), 20 for gynecological complaints (56% RCTs), 19 for gastrointestinal disorders (68% RCTs), 16 for urinary tract infections (UTI, 63% RCTs) and 24 for upper respiratory tract infections (URTI) (79% RCTs). For the majority of the studies, therapeutic benefits were evaluated by patient reported outcome measures (PROs). For psychosomatic disorders, gynecological complaints and URTI more than 80% of the study outcomes were positive, whereas the clinical benefit of herbal medicines for the treatment of UTI and gastrointestinal disorders was lower with 55%. The critical appraisal of the articles shows that there is a lack of high-quality studies and, with regard to gastrointestinal disorders, the clinical benefits of herbal medicines as a stand-alone form of therapy are unclear. According to the current state of knowledge, scientific evidence has still to be improved to allow integration of herbal medicines into guidelines and standard treatment regimens for the indications reviewed here. In addition to clinical data, real world data and outcome measures can add significant value to pave the way for herbal medicines into future therapeutic applications.

KEYWORDS

herbal medicine, clinical benefits, psychosomatic disorders, gynecological complaints, gastrointestinal disorders, urinary tract infections, upper respiratory tract infections

1 Introduction

Plant derived drugs have been used since humans have started treating physical and mental illnesses. They are part of Traditional Medicine in different cultures all over the world (Yuan et al., 2016). Since then, medicine and treatment procedures have evolved and while in Traditional Medicine a holistic approach of life focusing on health and its maintenance was common philosophy, present Modern Medicine has a clear emphasis on unravelling the changes leading to disease and eradiating it (Fries, 2019). Traditional medicine has a rigorous algorithm of identifying the root of the disease, which is based on traditional concepts, which, unfortunately, are considered obsolete nowadays, despite their practical longevity (e.g., acupuncture, ayurveda). The problem is that this traditional medical epistemology is not fully understood and science has limited tools to "translate" it into modern terms.

With the success of synthetic drugs along with the design of targeted therapies interfering specifically with the respective disease-related signaling pathways, herbal medicines have been eliminated from modern rational treatment strategies. The most important obstacles for the use in novel therapy strategies is that markers to measure clinical efficacy of herbal medicine have not been developed so far. Markers of efficacy of herbal drugs could also be useful to distinguish between patients who could benefit from a therapy with herbal medicines from those who will not. First preclinical studies already indicate that those markers or "signatures" (e.g., mRNA, miRNA) could be found in the future (Bachmeier et al., 2007; Bachmeier et al., 2008; Bachmeier et al., 2009; Bachmeier et al., 2010; Killian et al., 2012; Kronski et al., 2014).

In the last years, more and more patients report on the perceived efficacy of herbal drugs and praise the absence of undesired side effects and the good tolerability.

The following section provides insights into the standard therapies of selected ailments for which herbal medicines may be a rational alternative.

1.1 Indications suitable for treatment with herbal medicines

Herbal medicines are in particular suitable for the treatment of non-life-threatening conditions for which knowledge from traditional use is available pointing to their clinical benefits in treating the respective ailment (Wachtel-Galor and Benzie, 2011). This applies especially to psychosomatic disorders, gynecological complaints, and upper respiratory tract infections. However also for other diseases like gastrointestinal diseases, urinary tract infections herbal medicines have been clinically applied and—as we will show in this review—with some success.

Standard Care of psychosomatic disorders comprises the application of synthetic psychotropic drugs and psychotherapy (Laux, 2021). Psychotropic drugs are used not only for the treatment of depressive disorders and anxiety, but also for sleep disorders, excitation and chronic pain (Gründer and Benkert, 2012). However undesired adverse events having negative impact on quality of life can occur like, e.g., weight gain, sexual dysfunction, sedation, headache and tremor (Grunze et al., 2017). In addition their use, in particular benzodiazepines, can lead to addiction and drug abuse (Soyka and Mann, 2018) and interactions with other medication has to be taken into consideration especially in older multimorbid patients (Burkhardt and Wehling, 2010). About 23% of all over 70-year-old people have psychosomatic disorders with about 40% requiring therapy (Haupt and Vollmar, 2008). In this context herbal medicines represent an interesting alternative to avoid the above-mentioned problems with standard synthetic drugs. However, they do not belong to standard therapy-options and therefore are underrepresented in therapy-guidelines (Bittel et al., 2022). Nevertheless they play an important role in self-medication of patients (Stange, 2014) probably due to their favorable ratio between benefit and side-effects.

Gynecological complaints include, e.g., menopausal and premenstrual symptoms. According to the German medical guideline for post- and perimenopause, vasomotor symptoms of the peri- and post-menopause such as hot flushes and sweating should be treated with hormone therapy for menopause (hormone replacement therapy; HRT), if not contraindicated (AWMF, 2020). The side effects of HRT include edema, joint pain, psychological symptoms or even thrombosis and breast cancer (Maclennan et al., 2004). Herbal medicines, on the other hand, are characterized by a low risk of adverse events which increases patients' adherence and in consequence prevents therapy discontinuations (AWMF, 2020). Premenstrual syndrome (PMS) is characterized by recurring physical and psychological symptoms in the days before menstruation. There are currently no medical guidelines in German-speaking countries for the treatment of PMS. Systematic reviews on hormonal treatments (oral contraceptives, progesterone and estrogen) (Ford et al., 2006; Lopez et al., 2007; Naheed et al., 2013; Kwan and Onwude, 2015) and acupuncture/acupressure (Armour et al., 2018) point to ambiguous evidence. Treatment with serotonin reuptake inhibitors was shown to be effective but was associated with frequent side effects, e.g., nausea and asthenia (Marjoribanks et al., 2013).

Gastrointestinal diseases include several conditions like irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), liver disease (hepatitis), and functional dyspepsia (FD).

Beside dietary changes, stress management and psychotherapy, severe cases of IBS and IBD require additional medication to reduce inflammation or to slow down the intestinal irritations. However patients often complain about the side effects of medical treatment like, e.g., dizziness or weight gain (particularly caused by steroids), or undesired fatigue, headache, and/or tiredness associated with the intake of methotrexate (Feagan et al., 1995). Common types of hepatitis are viral hepatitis B and C. Antiviral therapy represents the treatment of choice to fight the virus caused disease. However, poor tolerability and significant adverse effects that include, for example, headaches, dizziness, depression, and irritability often lead to treatment discontinuation, further decreasing response rates (Cornberg et al., 2002). FD is a common gastrointestinal disorder treated by proton pump inhibitors (PPI) or H2 receptor antagonist, and/or treatment with tricyclic antidepressants or prokinetic agents. As in all cases, adverse side effects may occur ranging from dizziness to the development of diabetes mellitus type 2 (Yuan et al., 2021).

Urinary tract infections (UTI) with estimated 150 million cases worldwide each year reflect the most common outpatient infections (Zavala-Cerna et al., 2020). Women are more susceptible than men with a lifetime incidence of 50%–60%. Application of antibiotics

represents the standard treatment regimen to overcome the infection. However, serious side effects, predominantly exerted on the digestive system, may outweigh the benefits of this drug class. Most importantly, routine use of antibiotics bears the risk to trigger the selection of resistant strains. Hence, avoiding antibiotic treatment of UTI has gained high priority among the urologic community (Jung et al., 2023). Lower urinary tract symptoms (LUTS) caused by benign prostatic hyperplasia (BPH) requires a medical therapy which aims to reduce the BPH-related complications. A range of synthetic drugs is available to treat this condition. However, these have a range of side effects, including postural hypotension, dizziness, asthenia, abnormal ejaculation, intraoperative floppy iris syndrome (α1-blocker), or decreased libido, gynecomastia, and erectile dysfunction (5α-reductase inhibitors) (Cheng et al., 2020). Due to this, patients often discontinue treatment.

The most common acute upper respiratory infections include bronchitis, rhinosinusitis and common cold. Common cold or acute viral rhinosinusitis is triggered by a viral infection/inflammation of the nose and by definition has a duration up to 10 days. According to Jaume and co-workers (Jaume et al., 2020) the recommended therapy (mainly symptomatic) contains of paracetamol, NSAIDs, second-generation antihistamines to reduce symptoms the first 2 days; nasal decongestants with small effect in nasal congestion in adults; combination of analgesics and nasal decongestants; ipratropium bromide for reducing rhinorrhea; probiotics; zinc when administered the first 24 h after the onset of symptoms; nasal saline irrigations; and some herbal medicines. About 5% of adults have an episode of acute bronchitis each year. An estimated 90% of these seek medical advice for the same (Saust et al., 2018). Acute bronchitis is caused by infection of the large airways commonly due to viruses and is usually self-limiting. Bacterial infection is uncommon. Still, often antibiotics are prescribed, despite lacking effectiveness (Tanner and Karen Roddis, 2018). Most medical guidelines advice a "wait-and-see" policy, the use of antihistamines and cough medicines is discouraged.

1.2 Objectives

In the last decade we experienced a renaissance of herbal medicines with a rising demand especially for the treatment of the before-mentioned indications. This implicates that there is an urgent need for a scientific progress towards a rational phytotherapy, which will combine the benefits of "Modern Medicine" with the "Traditional Knowledge" on the therapeutic benefits of herbal medicines.

In order to create a basis of knowledge to build upon novel interdisciplinary research ideas towards the establishment of herbal medicines into rational therapeutic strategies, we extracted information from clinical studies. Thereby we aimed to get an overview on.

- which herbal medicines have been studied so far for which ailment
- which outcomes have been studied
- what quality level (level of evidence) the published studies have

Answering these questions, we create a comprehensive critical picture of the current knowledge on clinical efficacy and benefits as well as on failures and possible adverse events. Based on the results of these studies we give recommendations for practitioners and patients.

2 Methods

2.1 Search strategy and selection of scientific reports

Information on the therapeutic use of herbal medicines in different ailments was collected from scientifically published articles by conducting a search in the database PubMed for each of the five indication groups according to the following inclusion and exclusion criteria.

2.1.1 Inclusion criteria

1. Herbal Medicine

   AND

2. Disorders/complaints (see section "Indications Suitable for Treatment with Herbal Medicines"). Depending on the ailment, the term "herbal medicine" was combined with a, b, c, d, or e respectively:
   a. Psychosomatic symptoms (depressive disorder, sleeping disorders/insomnia, anxiety, cognitive impairment)
   b. Gynecological complaints (climactic symptoms, menstrual symptoms, premenstrual syndrome)
   c. Gastrointestinal disorders/dyspepsia
   d. Urinary tract infections
   e. Upper respiratory tract infections

   AND

3. Clinical Trial

   Exclusion criteria
   a. Reports in languages other than German or English language
   b. No full-text available
   c. Study protocols
   d. Traditional medicine (e.g., Traditional Chinese Medicine, Ayurveda, *etc.*),
   e. Aroma therapy
   f. Dietary supplements
   g. Self-made extracts and preparations
   h. Adjuvant treatment with herbal medicine
   i. Herbal medicines without market access in the EU
   j. *In vivo*/*in vitro* studies (pre-clinical studies)
   k. Homeopathy
   l. Acupuncture/acupressure
   m. Children and youth (under the age of 18 years)
   n. Healthy volunteers
   o. Primary preventive interventions (incl. Pre-post-operative complaints)
   p. Predominant comorbidities
   q. Case studies/case reports
   r. Televised, internet-based or web-based trials

Reasons for exclusion criteria:

a, b: Authors should be able to read and understand the full text; c: clinical results should have been obtained from a study; d, e, f, g, h, i: selected in order to filter all available information on

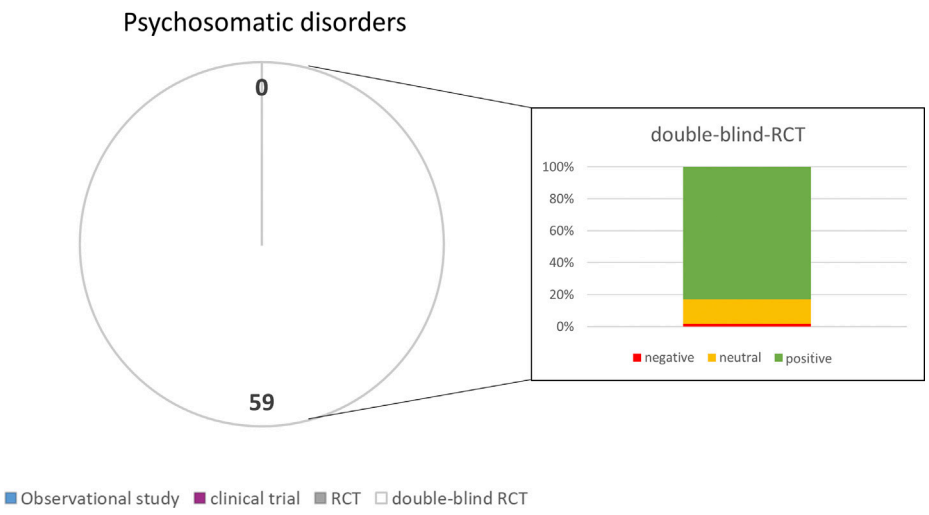

FIGURE 1
Numbers of studies and outcomes.

legally approved (in Europe in particular in Germany) herbal medicines or the respective standardized extract (HMPC Monographs of the European Medical Agency - EMA) only; j: preclinical evidence should be excluded; k, l: alternative naturopathic therapy forms should be excluded; m: children should be excluded due to different drug metabolism; n, o: healthy volunteers should be excluded in order to obtain information on clinical therapeutic benefits; p: predominant comorbidities should be excluded because they can affect the efficacy of the herbal drug in particular when co-administered with other drugs; q; clinical benefits from single cases are difficult to generalize; r: excluded for methodological reasons, e.g., data interpretation.

2.2 Data extraction and quality assessment of scientific reports

To get an overview on the characteristics of all included articles, a table was created for each indication group containing information on the publication, the study design, the population and treatment duration, the indication and the primary outcome, the herbal medicine and comparison treatment (comparator) as well as the results. Furthermore, we performed a quality assessment of the collected reports according to the following scoring method.

- 1 point for an observational study or a pre-post observational comparison
- 2 points for a clinical trial
- 3 points for a randomized controlled trial plus 1 additional point for blinding

Thereby, a score between 1 and 4 was obtained indicating the quality for all scientific reports; respectively publications with the highest level of evidence (RCT + blinded) had a scoring value of four points (see Figures 1–5).

3 Results

3.1 Psychosomatic disorders

A search for publications with the terms "psychosomatic disorder" and "herbal medicine" yielded only 64 results. Therefore, the search was extended with more specific terms (see inclusion criteria) yielding in 4.440 hits for depressive disorder, 1.907 hits for sleeping disorders, 2.380 hits for anxiety and 1.374 hits for cognitive impairment including Alzheimer's disease. After eliminating all publications according to the exclusion criteria 59 publications remained. Among those, 39 studies were related to depressive disorders, 4 to sleeping disorders, 6 to anxiety and 10 to cognitive impairment and Alzheimer's disease (neurological disorders). Most of them were double blind randomized controlled trials (quality group 4). For the treatment of depressive disorders predominantly *Hypericum perforatum* L (St. John's Wort; SJW) was used and only few studies examined the clinical benefits of *Rhodiola rosea* L (Rosewood). *Valeriana officinalis* L (Valerian Root) and *Humulus lupulus* L (Hops) extracts were preferred for the treatment of sleeping disorders, while for anxietyextracts of Lavandula angustifolia (Lavender) were studied. Extracts of *Ginkgo biloba* L (Maidenhair Tree) were used in clinical studies with patients having neurological disorders (cognitive impairment and Alzheimer's disease). Supplementary Table S1 provides an overview of the studies, their characteristics and results (see also Figure 1).

3.1.1 Depressive disorders

The use of herbal medicines in depressive disorders is well examined and in particular the clinical benefits of SJW are well

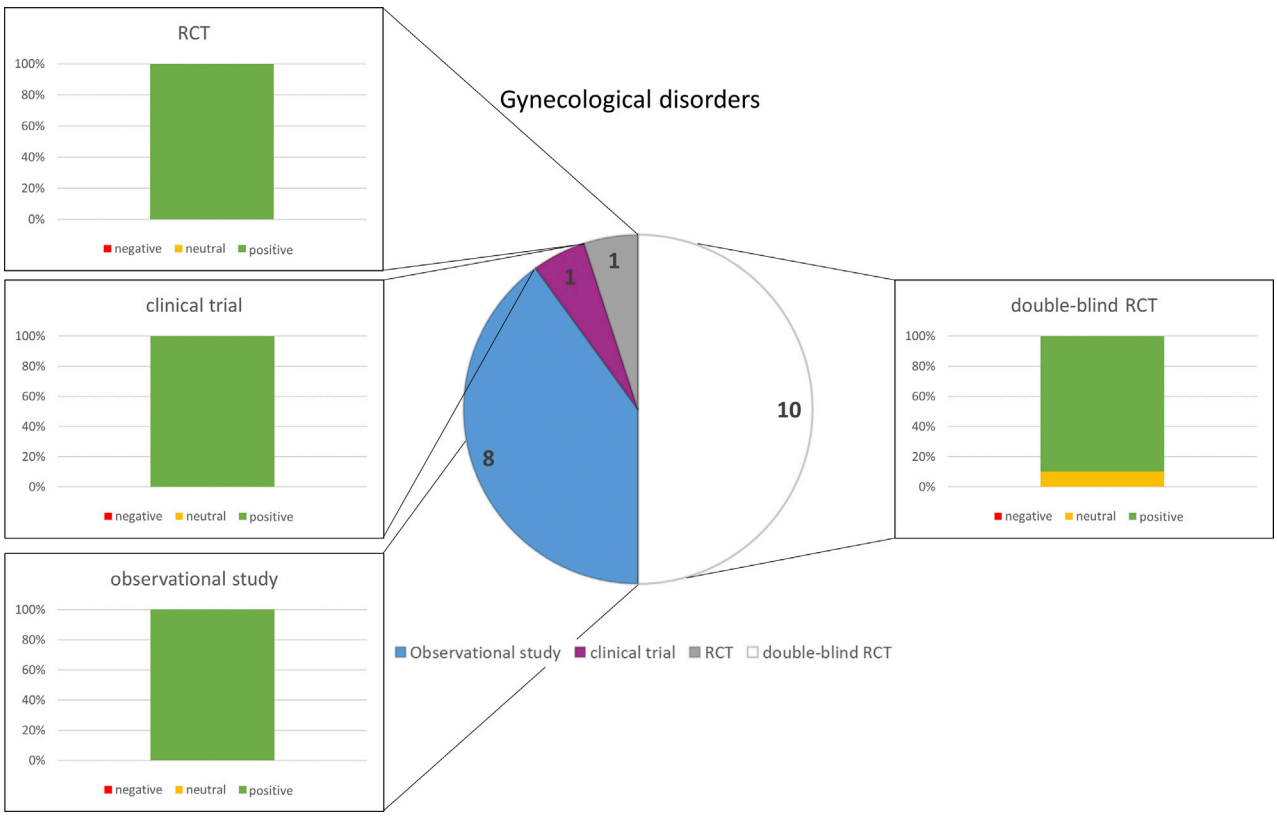

FIGURE 2
Numbers of studies and outcomes.

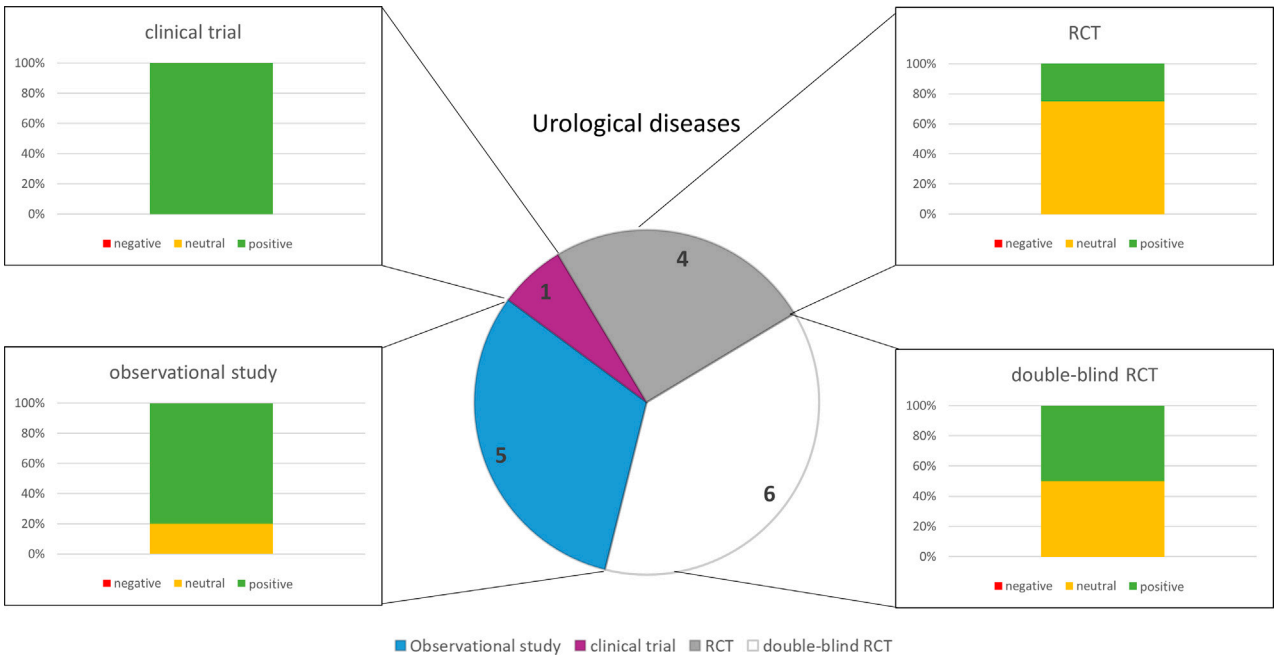

FIGURE 3
Numbers of studies and outcomes.

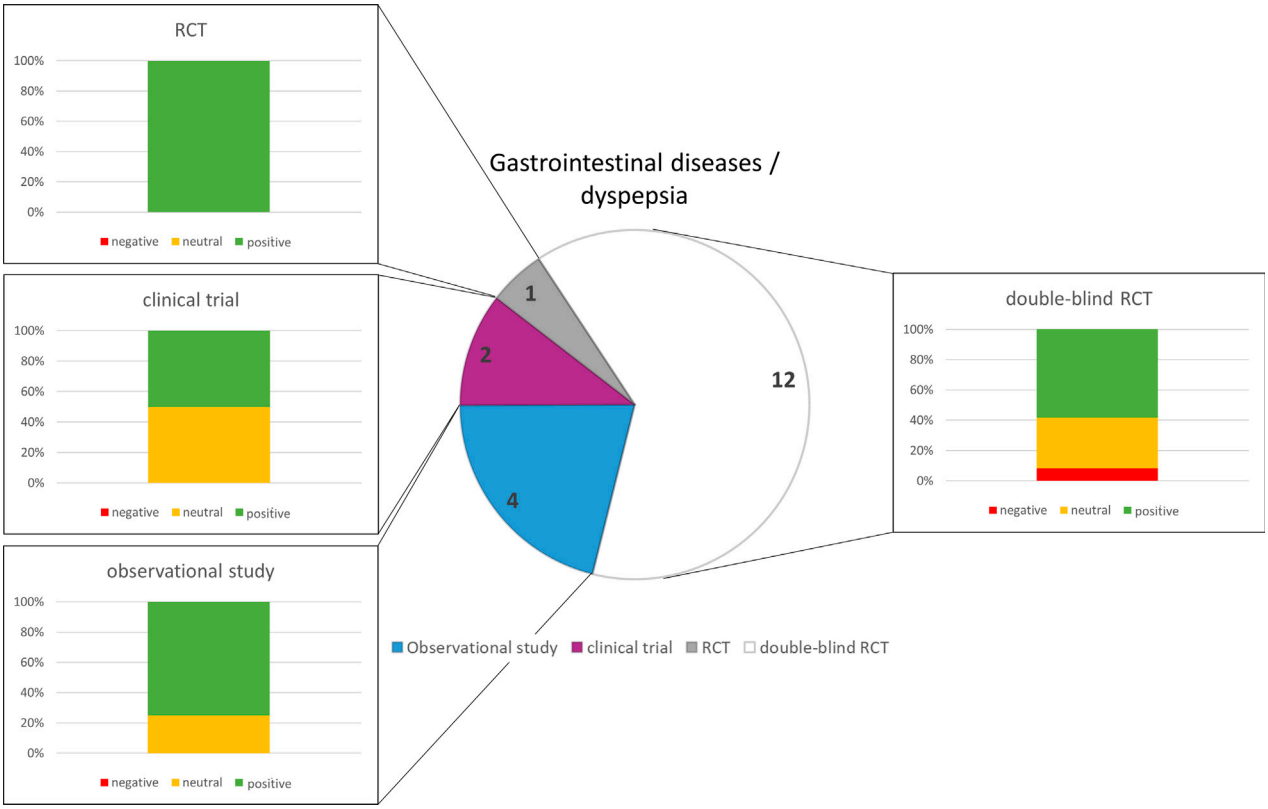

FIGURE 4
Numbers of studies and outcomes.

supported by clinical studies of high quality. All 37 selected studies on the use of SJW in depressive disorders ranging from mild to severe forms have been double-blind randomized controlled trials (quality group 4). Study duration was predominantly between 4 and 8 weeks and only few studies examined the effects for longer time periods of up to 6 months. The majority of the studies reported positive therapeutic effects concerning Hamilton depression rating scale (HAMD) as primary outcome parameter and only 5 of them (Shelton et al., 2001; Davidson et al., 2002; Bjerkenstedt et al., 2005; Moreno et al., 2006; Rapaport et al., 2011) did not demonstrate superiority as compared to placebo or pre-post.

In six studies (published predominantly before the year 2000) comparing SJW with tricyclic anti-depressive drugs the clinical benefits of the herbal drug in respect to placebo or in pre-post comparison was at least equal to the synthetic drug no matter if it was imipramine (Vorbach et al., 1994; Vorbach et al., 1997; Philipp et al., 1999; Woelk, 2000), maprotiline (Harrer et al., 1994) or amitriptyline (Wheatley, 1997). However, with regards to tolerability, SJW was clearly superior to any of the tricyclic antidepressants.

The more recent studies compared the efficacy of SJW with the selective serotonin reuptake inhibitors (SSRI) paroxetine, sertraline, citalopram and fluoxetine. In most of the 18 studies the therapeutic benefits of SJW were at least equal to those of the SSRIs (Harrer et al., 1999; Berger et al., 2000; Brenner et al., 2000; Friede et al., 2001; van Gurp et al., 2002; Bjerkenstedt et al., 2005; Gastpar et al., 2005; Szegedi et al., 2005; Anghelescu et al., 2006; Gastpar et al., 2006; Sarris et al., 2012). In two studies SJW was even superior to fluoxetine (Fava et al., 2005) or paroxetine (Seifritz et al., 2016) in reducing depressive symptoms. In one study the responders of a previous study were included in a further RCT testing the efficacy of SJW against citalopram. Here the numbers of patients with relapse was lower in the SJW group as compared to citalopram (Singer et al., 2011). The results of one study indicated that SJW was less efficacious than both fluoxetine and placebo, however in this study the group on SJW had the lowest remission rates (Moreno et al., 2006). In two studies no statistical differences in HAMD scores between SJW, placebo and citalopram (Rapaport et al., 2011) or sertraline (Davidson et al., 2002) could be found with adverse effects in the SJW and the SSRI groups.

In most of the above-mentioned studies, comparing the efficacy of SJW to standard therapy, a placebo group was included. However, in 13 studies SJW was tested exclusively against placebo whereby two of these studies examined the efficacy of different dosages of SJW extract (Laakmann et al., 1998; Kasper et al., 2006). In these studies, the higher concentrations had the better clinical benefits. In a continuation study of the effect of SJW in long term treatment a higher dosage (1,200 mg/d) was not superior to the lower one (600 mg/d) (Kasper et al., 2007). Interestingly the higher dosages were still well tolerated although mild adverse events related to gastrointestinal disorders were observed in a small portion of the patients (Kasper et al., 2006). In only one of our selected studies SJW was not effective in comparison to placebo for the treatment of major depression but safe and well tolerated (Shelton et al., 2001). In

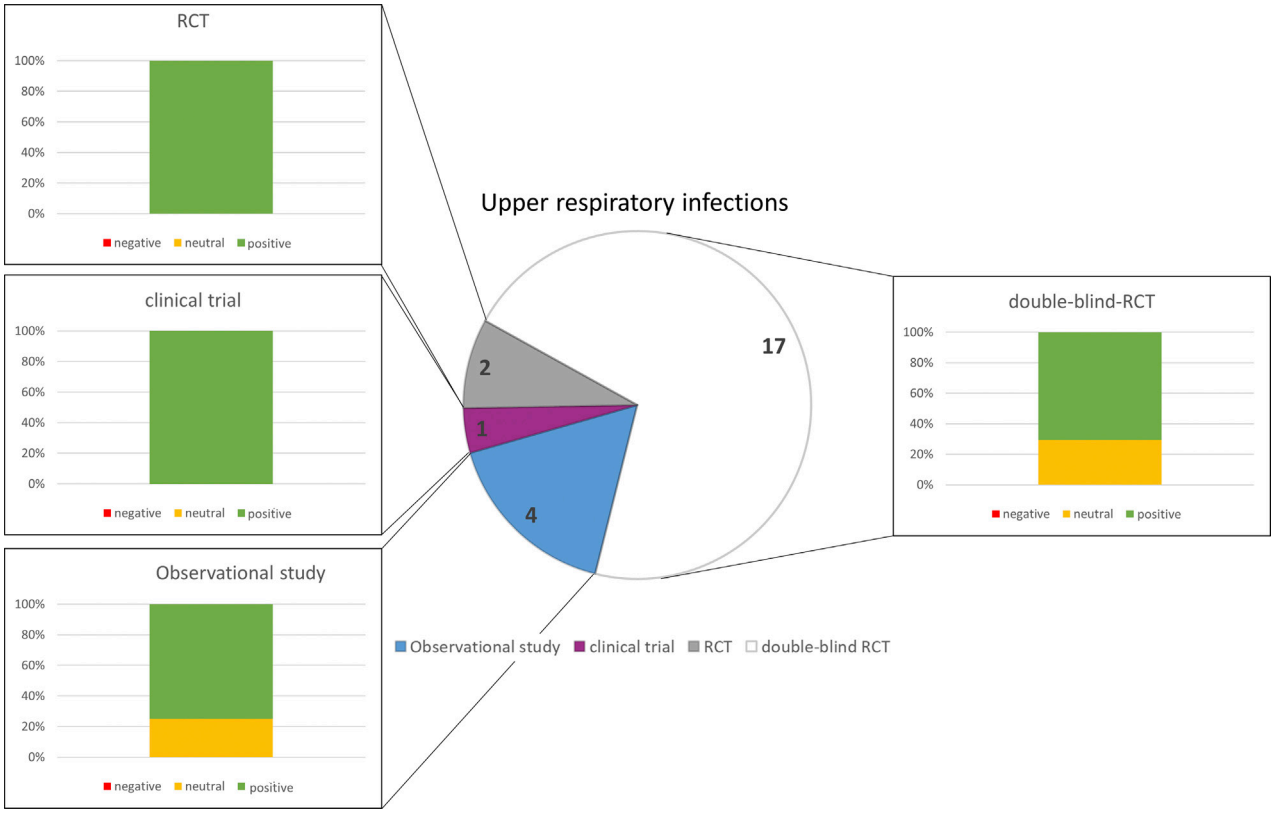

FIGURE 5
Numbers of studies and outcomes.

all other studies SJW was superior to placebo no matter if given in low (Laakmann et al., 1998; Lecrubier et al., 2002; Randlov et al., 2006), medium (Kasper et al., 2006; Kasper et al., 2007; Mannel et al., 2010) or in high (Hansgen et al., 1994; Harrer et al., 1994; Sommer and Harrer, 1994; Kalb et al., 2001; Uebelhack et al., 2004; Kasper et al., 2006; Kasper et al., 2007; Kasper et al., 2008) dosages.

For the efficacy of Rhodiola rosea in treatment of depressive disorders only few studies were performed so far. Therefore, a clear conclusion cannot be drawn, especially as the outcomes are not homogenous. While one study investigating the efficacy of R. rosea against placebo and the SSRI sertraline reported on a statistically not-significant inferiority of the herbal medicine (Mao et al., 2015) another study demonstrated clinical benefits concerning the symptoms of depression, insomnia, emotional instability and somatization against placebo. In this study two dosages of R. rosea were tested and the higher dose (680 mg/d) showed even positive effects on self-esteem (Darbinyan et al., 2007).

3.1.2 Sleeping disorder

Interestingly the search for qualitatively high clinical studies (according to our inclusion and exclusion criteria) revealed only few studies. The majority of them investigated the efficacy of valerian alone (Donath et al., 2000) or in combination with hops (Koetter et al., 2007) compared to placebo (Donath et al., 2000; Koetter et al., 2007) or to oxazepam (Dorn, 2000; Ziegler et al., 2002). All studies reported clinical benefits, however while the one research group reported that valerian alone was efficacious against insomnia (Donath et al., 2000) the other group reported on clinical benefits only in combination with hops (Koetter et al., 2007). Both study designs were placebo-controlled. In comparison to oxazepam valerian was not inferior and both therapy options improved sleep quality (SF-B) in a similar fashion (Dorn, 2000; Ziegler et al., 2002).

3.1.3 Anxiety

Herbal Medicines with lavender extracts were clinically studied for the treatment of anxiety. Between 2010 and 2019 six qualitatively high studies performed in Germany, Austria and Switzerland reported on the beneficial effects of lavender against symptoms of anxiety with improvements on the Hamilton anxiety rating (HAMA) scale as primary outcome (Kasper et al., 2010; Woelk and Schlafke, 2010; Kasper et al., 2014; Kasper et al., 2015; Kasper et al., 2016; Seifritz et al., 2019) and all studies used the same extract (WS1265). Four of the 6 studies were performed by the same group, however the study design differed. In these studies the efficacy of lavender was either compared to placebo (Anghelescu et al., 2006; Kasper et al., 2010; Kasper et al., 2016; Seifritz et al., 2016) and/or to paroxetine (Kasper et al., 2014) and lorazepam (Woelk and Schlafke, 2010). Overall, the lavender preparation was regarded as efficacious and safe.

3.1.4 Neurological disorders (cognitive impairment and Alzheimer)

We selected 10 studies investigating the efficacy of ginkgo biloba extract in the treatment of cognitive impairment and Alzheimer's

Disease (AD) with 8 of them testing against placebo (Le Bars et al., 1997; Le Bars et al., 2002; Le Bars, 2003; van Dongen et al., 2003; Schneider et al., 2005; Napryeyenko et al., 2007; Gavrilova et al., 2014; Gschwind et al., 2017), one against rivastigmine (Nasab et al., 2012) and one against donepezil (Mazza et al., 2006). In three of the studies two different ginkgo extracts did not show superiority over placebo regarding the primary outcome. In detail 5 of the studies showed that extracts of ginkgo biloba lead to a decrease in NPI composite score (Gavrilova et al., 2014) improved significantly ADAS-Gog and GERRI (Le Bars et al., 1997; Le Bars et al., 2002; Le Bars, 2003), or the SKT test battery (Napryeyenko et al., 2007) as outcome parameters. In three studies ginkgo extracts did not show superiority over placebo regarding the primary outcome parameters ADAS-cog (Schneider et al., 2005), gait analyses (Gschwind et al., 2017) or SKT test-battery (van Dongen et al., 2003), whereby in one of these studies the primary outcome parameter ADAS-cog also declined in the placebo group rendering the results of the study inconclusive (Schneider et al., 2005). With respect to the AD conventional medication rivastigmine, ginkgo biloba extract was inferior regarding the primary outcome parameters MMSE and SKT test-battery (Nasab et al., 2012). Finally one study in which gingko biloba was more efficacious than placebo and equal to the second generation cholinesterase inhibitor donepezil (Mazza et al., 2006) was heavily criticized by two other groups (Corrao et al., 2007; Korczyn, 2007), making it difficult to estimate if the use of ginkgo containing herbal medicines are justified for the treatment of mild to moderate AD.

3.2 Gynecological complaints

Of 383 search hits, 20 articles met the inclusion criteria. Eleven studies were related to menopausal symptoms and nine to PMS. Most were double-blind randomized controlled trials or observational studies (Figure 2). The studies on menopausal symptoms reported mainly positive results and the results concerning PMS were exclusively positive (Figure 2). The tested phytopharmaceuticals contained *Cimicifuga racemosa* (L.) (Black cohosh) (10 studies) and *Salvia officinalis* (Sage) (1 study) for the treatment of menopausal symptoms and *Vitex agnus-castus* L (VAC, Chaste tree) (8 studies) and SJW (1 study) for PMS. Supplementary Table S2 provides an overview of the study characteristics and results.

3.2.1 Menopausal symptoms

In studies examining the clinical benefits of black cohosh for the treatment of menopausal symptoms, sample sizes ranged from n = 62 to n = 6,141. Treatment duration was between 12 weeks and 9 months. The herbal drug dosages ranged from 20 to 127.3 mg.

In comparison to HRT, the benefit-risk-balance points to significant non-inferiority and superiority of black cohosh (Bai et al., 2007). In three other studies menopausal complaints improved overall, but differences between black cohosh and HRT were not significant (Wuttke et al., 2003; Nappi et al., 2005; Friederichsen et al., 2020). The combination of black cohosh with SJW significantly reduced menopausal complaints and was superior to transdermal estradiol (Briese et al., 2007). Independent of a high or low dose, menopausal complaints decreased significantly (Liske et al., 2002; Drewe et al., 2013). Adverse events rates were lower in the low dose group (Drewe et al., 2013) or similar to the high dose group (Liske et al., 2002). Menopausal symptoms decreased significantly more for black cohosh compared to placebo (Osmers et al., 2005). In another study with 62 participants, the difference between the symptom scores just approached significance (Wuttke et al., 2003). Interestingly, this also applies to the comparison of conjugated estrogens and placebo. Adverse events rates did not differ significantly between black cohosh and placebo (Wuttke et al., 2003; Osmers et al., 2005). Significant and clinically relevant reductions in menopausal symptoms (Vermes et al., 2005) or higher quality of life (Julia Molla et al., 2009) were observed after treatment with black cohosh compared to therapy start. Sage taken for 8 weeks significantly decreased the number of menopausal hot flushes from week to week (Bommer et al., 2011). Observed treatment-related adverse events were mild and occurred in only one person. However, no comparison was made to another treatment or placebo.

3.2.2 Premenstrual syndrome

Eight studies dealt with the treatment of PMS with VAC. The sample sizes ranged from n = 43 to n = 1,634. Treatment duration was three cycles; Berger et al. (2000) added three subsequent cycles without treatment. The administered dosages ranged from 1.6 to 20 mg extract.

Results of studies comparing VAC with pyridoxine or placebo were similar. PMS symptom reduction was significantly more pronounced for VAC compared to pyridoxine (Lauritzen et al., 1997) or placebo (Schellenberg, 2001; Bachert et al., 2009; Barrett et al., 2010; Schellenberg et al., 2012). Rates of adverse events were similar between groups in each study (Loch et al., 2000; Schellenberg, 2001; Barrett et al., 2010; Schellenberg et al., 2012). Schellenberg et al. (2012) compared a VAC reference dose to a lower and higher dose; the results were in favor for the reference dose compared to the low dose. No significant differences between the high and reference dose emerged. The number of participants with adverse events was slightly elevated for the high dose. In single-arm studies, symptoms of PMS significantly decreased after three cycles of VAC treatment (Berger et al., 2000; Loch et al., 2000; Momoeda et al., 2014). Only mild PMS-like adverse events were observed. Berger et al. demonstrated a gradual symptom return after therapy completion (Berger et al., 2000). PMS symptoms were significantly higher compared to the end of the treatment, but still 20% lower than at baseline.

A clinical study testing the efficacy of SJW in treating mild PMS (Canning et al., 2010) demonstrated significant improvements in physical (e.g., food craving) and behavioral (e.g., confusion) symptoms compared to placebo. The effect on mood (e.g., irritability) and pain (e.g., cramps) was not significant.

3.3 Gastrointestinal disorders

A search for publications with the search terms “gastrointestinal disorder” and “herbal medicine” yielded a total of 19 results after applying the exclusion criteria. Of these, eight studies were related to hepatic disorders, three publications dealt with IBD, two studies focused on IBS, and

six studies had been done on FD. Most of them were done in a double-blinded randomized controlled manner (n = 12) (Figure 3). *Silybum marianum* (L.) Gaertn (Silymarin, milk thistle) was used in patients suffering from a hepatic disease. Patients with IBD were treated with Artemisia absinthium L (wormwood) or *Potentilla erecta* (tormentil). The standardized extract STW 5 containing *Iberis amara* (bitter candytuft), *Glycyrrhiza glabra* L (Liquorice), *Carum carvi* L (caraway), *Mentha ×piperita* (peppermint), *Melissa officinalis* L (lemon balm), *Matricaria chamomilla* (chamomile), *Angelica archangelica* (wild celery), *Chelidonium majus* (greater celandine) and milk thistle has been applied in IBS and FD. The same has been done with the standardized extract STW 5-II which in contrast to STW 5 is free of wild celery, greater celandine, and milk thistle. SJW has been used to treat patients suffering from IBS. A combination of the standardized extracts WS 1340 (peppermint oil) and WS 1520 (caraway oil) was used for patients with FD. Supplementary Table S3 and Figure 3 provide an overview of the study characteristics and results.

3.3.1 Hepatic disease

Trials on steatohepatitis, cirrhosis and different kinds of hepatitis (n = 18) included patient cohorts ranging from 14 to 200 participants, all of them aged >18 years. Patients were treated with silymarin orally or intravenously (Pares et al., 1998; Tanamly et al., 2004; Ferenci et al., 2008; Hawke et al., 2010; Fried et al., 2012; Adeyemo et al., 2013; Fathalah et al., 2017; Tanwar et al., 2017) with dosages ranging from 280 to 2,100 mg/day or 5–20 mg/kg/day, respectively. Six studies compared the HM group to a placebo group (Pares et al., 1998; Tanamly et al., 2004; Hawke et al., 2010; Fried et al., 2012; Adeyemo et al., 2013; Tanwar et al., 2017). Silymarin did not reduce virus titers and/or serum alanine transaminase (ALT) in patients with Hepatitis C and non-alcoholic Steatohepatitis C, compared to placebo (Adeyemo et al., 2013). The same observation has been made by others (Hawke et al., 2010). Furthermore, the integration of silymarin into a PEGylated (Peg)-interferon based regimen did not improve the outcome of HCV patients in terms of HCV RNA suppression and Enhanced Liver Fibrosis score performance (Tanamly et al., 2004). There was also no effect of silymarin on HCV patients who were previously unsuccessfully treated with interferon (multicenter, double-blind, placebo-controlled trial) (Fried et al., 2012). Although HCV-patients reported to "feel better" after 12 months of silymarin therapy in a further study, symptoms and quality of life (QOL) scores did not differ between the silymarin and the placebo group (Tanamly et al., 2004). Treatment with silymarin was also well tolerated over a period of 2 years. However, the course of liver cirrhosis in this patient cohort has not been improved (Pares et al., 1998). Contrasting these results, dose escalating studies on HCV cirrhotic patients revealed positive effects of silymarin or silibinin (also milk thistle), in a way that high-dosed silymarin (1,050 mg/day) improved QOL and biochemical parameters of chronic HCV-decompensated cirrhotic patients with no serious adverse events (Ferenci et al., 2008; Fathalah et al., 2017) compared to low-dosed silymarin (420 mg/day). Notably, silibinin exerted a dose-dependent antiviral effect on Peg-interferon/ribavirin non-responders (Ferenci et al., 2008; Fathalah et al., 2017).

3.3.2 Inflammatory bowel disease (IBD)

Between 2007 and 2009, three clinical trials on CD or IBD have been conducted, two in Germany (quality groups 1 and 2) and one in the United States (quality group 4) (Huber et al., 2007; Omer et al., 2007; Krebs et al., 2010). Patients were treated with wormwood or tormentil for 3–10 weeks. A total of 30 patients were treated with wormwood or placebo (Omer et al., 2007; Krebs et al., 2010). In this context, wormwood decreased tumor necrosis factor alpha levels and the CD activity index score, whilst scores for IBD questionnaire and Hamilton depression scale have been improved, compared to the controls (Omer et al., 2007; Krebs et al., 2010). Daily intake of tormentil reduced clinical activity index scores in all patients, however, during the wash out phase scores increased again. Tormentil has been proven to be safe for ulcerative colitis patients in dosages up to 3,000 mg/day (Huber et al., 2007).

3.3.3 Irritable bowel syndrome (IBS)

Symptoms of IBS were treated with STW 5 and STW 5-II or SJW (both studies were quality group 4) (Madisch et al., 2004b; Saito et al., 2010). The clinical trial carried out by Madisch et al. compared the effects of the treatment group with those of bitter candytuft mono-extract and placebo. STW 5 and STW 5-II (60 drops/day over 4 weeks) significantly reduced the total abdominal pain and the IBS score compared to placebo and bitter candytuft mono-extract (Madisch et al., 2004b). The study carried out by Saito and others investigated the clinical efficacy of SJW pointing to a lower effect as compared to placebo (Saito et al., 2010).

3.3.4 Functional dyspepsia (FD)

Six studies on patients suffering from FD were performed, including treatment with either a WS 1520/WS 1340 combination (n = 3) (Madisch et al., 1999; Rich et al., 2017; Storr and Stracke, 2022) or with STW 5 (von Arnim et al., 2007) and/or STW 5-II (n = 3) (Rösch et al., 2002; Madisch et al., 2004a). WS 1340/WS 1520 was documented to be a "valuable" (Storr and Stracke, 2022) or an "effective" therapeutic regimen (Rich et al., 2017), as it relieved pain and improved disease-specific QOL, compared to placebo. The primary outcome of WS 1340/WS 1520 was also proven to be comparable to the prokinetic agent cisapride (Madisch et al., 1999).

It is to be noted that the use of cisapride has meanwhile be restricted by the EMA due to the risk of potentially life-threatening cardiac arrhythmia [https://www.ema.europa.eu/en/medicines/human/referrals/cisapride].

Similar results have been presented in the STW 5 and STW 5-II trials. The gastrointestinal symptom score was significantly lowered when compared to the placebo group (Madisch et al., 2004a; von Arnim et al., 2007), with a therapeutic response comparable to cisapride (Rösch et al., 2002).

3.4 Urinary tract infection (UTI) and lower urinary tract symptoms (LUTS)

Initial search on herbal drugs in urologic clinical trials pointed to 263 manuscripts published between 1983 and 2022. Narrowing the search to "herbal medicine" (HM) 18 relevant publications were identified. One publication was nearly identical to another one and,

therefore, has not been taken care of in this chapter, one article only reviewed former trials (16 publications remaining). All of them were related to lower urinary tract infection (UTI), or acute uncomplicated cystitis, respectively. Four different HM have been applied, either compared to placebo or guideline-based treatment (n = 12).

3.4.1 Urinary tract infections (UTI)

Several studies investigated the standardized herbal extract BNO 1045 which contains *Centaurium erythraea* Rafin, herba (Centaury); Levisticum officinale Koch, radix (Lovage); and Rosmarinus officinalis L., folium (Rosemary). In two studies, the clinical benefits of BNO 1045 in preventing UTI in high-risk women undergoing urodynamic studies (UDS) (Miotla et al., 2018) or urogynecological surgeries (Wawrysiuk et al., 2022) was evaluated. High-risk women were defined as: age over 70, elevated postvoid residual urine>100 mL, recurrent UTI, pelvic organ prolapse (POP) ≥II in POP-Q scale, and neurogenic bladder. No statistical differences in UTI incidence were found between patients receiving antibiotics or BNO 1045. No superiority of antibiotics over BNO 1045 has been confirmed as well in a subsequent prospective study on postoperative UTI after midurethral sling surgery (MUS) (Rechberger et al., 2020). In another study, an herbal mixture based on D-mannose, Arctostaphylos uva-ursi, Betula pendula, and Berberis aristata was compared to BNO 1045 in reducing symptoms of UTI after MUS (Rechberger et al., 2022). The rationale was based on the EAU 2022 guidelines which recommended D-mannose as prophylaxis of UTI. In this context, BNO 1045 was proven to be similar effective, compared to the herbal mixture. The use of BNO 1045 has been documented here to be a potential and valuable alternative to antibiotics for UTI prevention. All four trials have been carried out in the same institution involving the same main investigators which were (partially) associated with the manufacturer of BNO 1045.

A randomized, double-blind, multicenter Phase III clinical trials compared the efficacy and of BNO 1045 to antibiotics concerning symptoms and recurrence rates in women with uncomplicated UTI. Based on the endpoints "UTI-recurrence" and "additional antibiotics use", BNO 1045 was proven to be non-inferior to antibiotic treatment (Wagenlehner et al., 2018). In a retrospective cohort study, data from outpatients in Germany with at least one diagnosis of acute cystitis or UTI and a prescription of either BNO 1045 or standard antibiotics were analyzed (Holler et al., 2021). Compared to antibiotics, BNO 1045 was associated with significantly fewer recurrence rates of UTI and with reduced additional antibiotic prescription. BNO 1045 was propagated to be an effective and safe symptomatic treatment option for acute cystitis or UTI.

In an open-labeled, randomized, controlled trail the effect of BNO 1045 to prevent recurrences of cystitis in younger women was evaluated (Sabadash and Shulyak, 2017). All patients received an antibacterial therapy, the test group was additionally treated with BNO 1045. The integration of BNO 1045 prevented bacteriuria and recurrent cystitis episodes more frequently (primary outcome), compared to the control group without BNO 1045. This may indicate superiority of the combination therapy. However, interpretation of the results of the study is limited due to the lack of blinding on both sides - patients and physicians. A further study without any involvement of the manufacturer (no conflicts of interest noted) included younger women with acute uncomplicated cystitis. All patients received the same therapy, the nonsteroidal anti-inflammatory drug ketoprofen in combination with BNO 1045 (Kulchavenya, 2018). Quite interestingly, although the majority of the patients responded well to the therapy, the investigators also observed patients who only slightly responded, or did not respond to treatment at all. The authors concluded that uncomplicated cystitis might be cured by BNO 1045 instead of antibiotics which may be required only in minor cases. Still, the data seems to be over-interpreted, since patients were treated with both ketoprofen and BNO 1045 which does not allow to conclude to one drug alone.

Aside from BNO 1045, further herbal medicines have been investigated in clinical studies. Tablets with a standardized herbal extract containing *Armoraciae rusticanae* radix (Horseradish root) (80 mg) and *Tropaeoli majoris* herba (Nasturtium) (200 mg) have been applied to patients suffering from chronically recurrent UTI symptoms, with the result that recurrent UTI symptoms were less, compared to the placebo group (Albrecht et al., 2007). However, a subsequent trial failed to demonstrate non-inferiority of this extract to antibiotics due to a poor recruitment rate (Stange et al., 2017). Actually, no respective clinical trials with sufficient statistical power are underway.

3.4.2 Lower urinary tract symptoms *LUTS*

Clinical studies have also been conducted with an herbal medicine containing the standardized extracts WS 1473 *Sabal serrulata* Schult.f (Sabal fruit) (160 mg) and WS1031 *Urtica dioica* L (Urtica root) (120 mg). All studies were related to the treatment of lower urinary tract symptoms (LUTS) caused by benign prostatic hyperplasia (BPH). The study protocols (placebo-controlled, double-blind, multicentric) were similar in all trials with the International Prostate Symptom Score (I-PSS), quality of life index, uroflow and sonographic parameters as the outcome measures for treatment efficacy. In one study (Lopatkin et al., 2005) patients were randomized to either the herbal medicine (WS 1473 and WS1031) (treatment group) or placebo (control group) while in another study patients received either WS 1473 and 1031 or the α1-adrenoceptor antagonist tamsulosin (Engelmann et al., 2011). A further study was based on the previous mentioned study (Lopatkin et al., 2005), whereby all patients were offered participation in a further 48-week follow-up with WS 1473/1031 (Lopatkin et al., 2007). Independent on the study design, it was concluded that WS 1473/1031 is superior to the placebo, and not inferior to tamsulosin in the treatment of LUTS. In a later re-evaluation of the data sets, WS 1473/1031 was shown to significantly improve nocturnal voiding frequency compared to placebo, with similar effects compared to tamsulosin or the 5α-reductase inhibitor finasteride (Oelke et al., 2014). No further studies have been enrolled since then. However, a database search in 2022 including 3,000 private practices in Germany revealed a significant association between WS 1473/1031 prescription and reduced incidence of urinary incontinence and urinary retention compared to tamsulosin and tamsulosin/dutasteride (5α-reductase blocker), as well as reduced incidence of erectile

dysfunction compared to dutasteride (Madersbacher et al., 2023). In all four studies the manufacturer of the extract was involved.

One observational study was investigating the effectiveness of a standardized herbal extract containing a combination of *Cucurbita pepo* L (Marrow), *Rhus aromatica* bark (Fragrant sumac), and hops, in women with overactive bladder (Gauruder-Burmester et al., 2019). Of the 113 patients included, nearly the half (61 patients) used concomitant medications (e.g., antihypertensive, levothyroxine, lipid/cholesterol lowering agents, low dose ASS, NSAIDS) within the frame of a routine clinical setting. Considering the noninterventional character of this study, the herbal combination was demonstrated to improve overactive bladder symptoms and quality of life. A controlled study has not yet been initiated.

3.5 Upper respiratory tract infections (URTI)

The search on herbal medicines for the indication Upper Respiratory Infections revealed 24 publications.

The most common indications studied for the effectiveness of herbal medications were sinusitis, viral acute Rhisosinusitis (ARS) and common cold (N = 13), bronchitis (N = 8), and less frequently on acute cough (N = 2) and Acute lower and upper tract respiratory infections (N = 1) and chronic rhinosinusitis (N = 1). Most of them (N = 18) were double-blind randomized placebo-controlled trials, there were also randomized controlled trials that compared herbal medication to other herbal medication (N = 2) or to antibiotics (N = 1). Other study designs involved prospective cohorts (N = 3) and one retrospective cohort.

3.5.1 Sinusitis/common cold and chronic rhinosinusitis

Studies on treatment of acute sinusitis and acute rhinosinusitis used a follow-up period between 7 and 14 days, with the (adapted) Sinusitis Severity Score (SSS) (N = 2), the Major Symptom Score (MSS) (N = 4), the Total Symptom Score (N = 1) and facial pain relief (N = 1) as primary endpoints. All studies reported significantly improvement of the intervention group over the placebo or control group.

The treatment of acute sinusitis and acute rhinosinusitis with Eps 7630 (standardized root extract of *Pelargonium sidoides* DC (Pelargonium) was studied in two double blind randomised placebo controlled trials (Bachert et al., 2009; Dejaco et al., 2019) and in one prospective (Perić et al., 2020), randomized, open-label, non-inferiority study comparing study medication to Amoxicillin All three studies reported a significant superiority resp. Non-inferiority for Eps 7630. The use of the standardized herbal extract BNO 1016 (*Primulae flos* (Primrose), *Gentiana lutea* Ruiz and Pav. Ex G.Don (Yellow gentian), *Rumicis herba* (Sorrel), *Sambuci flos* (Elderflower) and verbenae herba (Vervain) was tested in two randomised placebo controlled trials (Jund et al., 2015), one of which was blinded (Jund et al., 2015). Both studies showed stronger impact on the symptom score for BNO 1016 compared to placebo. One more study tested BNO 1016 in a multicenter, prospective, open-label study comparing its effect to intranasal fluticasone furoate, with patients in both groups showing improvement (Passali et al., 2015). ELOM-080 (standardized herbal drug preparation containing specially destilled oils from *Eucalyptus* (Eucalypt) and Citrus ×sinensis (Sweet orange) and *Myrtus* (Myrtle) and *Citrus limon* (L.) Osbeck (Lemon oil)) was evaluated once in a double blind randomised placebo controlled trial (Federspil et al., 1997) and once in a prospective, non-interventional parallel-group trial where the control group received BNO 1016 (Gottschlich et al., 2018). In both studies BNO 1016 showed superior results.

The use of extracts containing Echinacea for the treatment of common cold was positively tested in two studies, reporting on total number of facial tissues used in three to 7 days after intervention start (Naser et al., 2005) and on the Total Daily Symptom Scores (TDSS) after 7 days (Goel et al., 2004). No statistically significant differences were observed between treatment groups for the total symptom score (SS) after 14 days. In two other studies testing capsules/pills containing *Echinacea angustifolia* root and *Echinacea purpurea* root and *E. purpurea* herb there was no statistically significant difference between the intervention and placebo group concerning severity and duration of self-reported symptoms (Barrett et al., 2002) or global severity (Barrett et al., 2010).

In a double blind randomised placebo controlled trial BNO 1016 was tested for the treatment of chronic rhinosinusitis. The results reveal that the herbal drug was not superior over placebo regarding the Major Symptom Score (MSS) in week 8 and week 12 (Palm et al., 2017).

3.5.2 Bronchitis

For bronchitis, nine studies were included, of which six were double-blind randomized placebo-controlled trials, testing EPs 7630 (N = 5) (Matthys et al., 2003; Chuchalin et al., 2005; Matthys and Heger, 2007; Matthys et al., 2010; Kähler et al., 2019) or ELOM-080 (N = 1) (Gillissen et al., 2013). The prospective observational studies included a standardized syrup of *Hedera helix* L (Ivy leaves) (N = 1) (Fazio et al., 2009), pills with ethanolic Ivy-leaves dry extracts (N = 1) (Hecker et al., 2002) and EPs 7630 (N = 1) (Matthys and Heger, 2007).

Using a follow-up period of 7 days to 4 weeks, all but one (double-blinded placebo controlled trial) (Matthys et al., 2003) reported positive effects of the study medication on either Bronchitis Severity Scores, change of symptoms and coughing frequency.

3.5.3 Acute cough

The treatment of acute cough with EA-575 (standardized extract from *H. helix* L.) was tested against placebo in one double blind randomized placebo controlled trial and reported a significantly better improvement of cough severity (CS) assessed by Visual Analogue Scale (VAS) in the intervention group after 1 week as compared to placebo (Schaefer et al., 2016).

3.5.4 Acute lower and upper tract respiratory infections

We included one retrospective cohort study comparing people with acute lower and upper tract respiratory infections who were prescribed a phytopharmaceutical to those who were not prescribed such drugs. They found that extract EPs 7630 (description see 3.5.1) (odds ratio (OR) 0.49 [95% CI: 0.43–0.57]) and thyme extract (OR 0.62 [0.49–0.76]) compared to no phytopharmaceutical prescription

exhibited the strongest decrease in antibiotics prescriptions among patients treated by general practitioners (Martin et al., 2020).

4 Discussion

The aim of this review is to depict the current evidence for the therapeutic efficacy of herbal medicines. Therefore, we conducted a literature search with defined inclusion and exclusion criteria in particular to select information from clinical studies with high levels of evidence and legally approved (in Europe) herbal medicines. Certainly, life-threatening disease are not suitable for the treatment with herbal medicines. This is the reason why we limited our perspective on psychosomatic disorders, gynecological complaints, gastrointestinal disorders and common infectious diseases of the urinary and the upper respiratory tract. Additionally, we concentrated on clinical trials with adult patients. It is to be emphasized that respective studies using herbal drugs have also been done in children with psychosomatic diseases (Verlaet et al., 2017; Schloss et al., 2021), IBS (Menon et al., 2023), gastrointestinal disorders (Michael et al., 2022), UTIs (Ching, 2022), and URIs (Mancak Karakus et al., 2023) to mention only some examples.

The use of herbal medicines in the treatment of psychosomatic disorders is widespread and accordingly a high number of clinical studies was available for our analysis. In our literature search, the term "psychosomatic disorders" has been chosen. This term has not been clearly defined but is related to diseases which involve both physical and psychological illness. In other words, the respective symptoms are caused by mental processes and not directly by a physical disorder. The hits we got are based on this "terminology". In contrast, the term "mental illnesses" which also includes psychological or behavioral manifestations is strictly defined as "health conditions with changes in emotion, thinking or behavior" (Stein et al., 2021). However, even this definition is problematic, since there are concerns about specific conditions, the discrimination between independent biological entities or value-laden social constructs, and the defined indicators of dysfunction (Stein et al., 2021). Independent on these concerns, we did not apply this search term. Therefore, we cannot exclude that (very few) articles have not been discovered with our search strategy.

For the treatment of depressive disorders, St. John's wort is well-established and the studies we selected were predominantly positive regarding improvement of symptoms. Concurrently, SJW is well tolerated and in the majority of the studies at least equal to conventional medication like tricyclic anti-depressants and selective serotonin reuptake inhibitors, which exhibit in part notable adverse events impacting patients' quality of life of (Voican et al., 2014; Jakobsen et al., 2017).

In contrast evidence for insomnia and anxiety was thinner. It would be worthwhile to study the use of herbal drugs as alternative medication for the treatment of sleeping disorders, as for elderly people or long term use conventional hypnotics are not always the best option (Wortelboer et al., 2002; Cheng et al., 2020). All the studies we included were using valerian root extract alone or in combination with Humulus lupulus extract and showed positive effects on sleep without notable side effects. The few studies we selected for anxiety demonstrated efficacy of lavender extract (Lavandula angustifolia) and also here we had a homogenous picture of good efficacy along with good tolerability.

Several years ago, consistent beneficial effects of Ginkgo biloba for patients with cerebral insufficiency were proven in a systematic review (Kleijnen and Knipschild, 1992). However, the methodologic quality of many trials was considered to be poor. Moreover, the studies entailed a heterogeneous collection of target health problems, ranging from overt dementia to noncognitive manifestations of brain dysfunction, such as vertigo and tinnitus. More recently, the results of several new Ginkgo biloba trials have been published, most of them focusing on dementia, and showing positive effects. Probably the most talked about is the trial of the North American EGb Study Group, which was published in the JAMA in 1997 and showed a modest improvement of the cognitive performance and the social functioning of the demented patients involved (Le Bars et al., 1997), which is well in line with the studies we have collected.

In addition, menopausal symptoms and premenstrual syndrome are suitable for treatment with herbal medicines. In the here collected studies, no overall negative effects were observed and adverse events did not occur more frequently than in the comparison groups. A consistent picture emerged when comparing herbal treatment with synthetic drugs or placebo: while herbal drugs and treatment with, e.g., HRT or pyridoxine showed equal efficacy, herbal treatment was in general superior to placebo administration, except for one study.

Effective treatment of menopausal symptoms with black cohosh is supported with multiple study designs. Regardless of the study quality, there are no contradictory results.

The evidence for the treatment of PMS with VAC initially appears similar to that of black cohosh for menopausal symptoms. However, the sample sizes have been insufficient and there was a complete lack of comparisons of VAC with other therapies. Also of interest are the hints on the importance of the dose and continuous administration. A higher dosage did not have a higher efficacy compared to the standard dosage, but slightly more participants experienced adverse events (Momoeda et al., 2014). This suggests a preference for the standard dosage of VAC. Continuous use of VAC is recommended, as it has been shown that symptoms increase significantly, even if they are still lower than before therapy (Bachert et al., 2009).

However, further research is needed for both gynecological indications. Only one study each on sage for menopausal symptoms and SJW for premenstrual symptoms was found (Lauritzen et al., 1997; Lauritzen et al., 1997; Adeyemo et al., 2013). The trend-setting results point to positive effects which have to be confirmed.

For gastrointestinal disorders herbal drugs were, at least partially, shown to be similar efficacious as the standard treatment. Selected, non-toxic plant derived natural compounds may, therefore, replace synthesized drugs which are associated with undesired negative side effects and the therapeutic potential of the compounds may depend on both the plant extract and the type of disease to be treated. Indeed, SJW was not efficacious in treating IBS, whereas WS 1340/WS 1520 and STW 5 and STW 5-II showed efficacy in both IBS and FD. Considering the broad spectrum of gastrointestinal complaints, therapy of severe liver disease may

require more effort than treatment of moderate dyspepsia and, hence, herbal medicine may not replace standard therapy.

As no standard therapy has so far been established for FD (Madisch et al., 2018) and IBS (Lacy et al., 2021) the design of clinical studies is difficult, making it impossible to compare the phytodrug group with a "reference" cohort, and to finally assess the value of the phytodrugs.

Particular attention should be given to STW 5 containing greater celandine which has been related to liver and biliary tract disorders (Zielińska et al., 2018). Therefore, careful preclinical examination of potential toxic properties of a compound of question is necessary before starting clinical trials.

Overall, most of the studies were well designed (multicenter, double-blind, placebo-controlled trials) with large cohorts. Considering the low side effects and often significant improvements, it might be useful to conduct further studies to either gain more detailed information about herbal medicine or to transfer the knowledge to diseases with a similar cluster of symptoms, so that distinct ailments might particularly benefit from herbal medicine (Chey et al., 2015).

With respect to urinary tract infections (UTI), herbal medicines have been proven to be similar effective as antibiotics. Undoubtedly, the data encourages further research on herbal medicines as alternatives to antibiotics in acute lower uncomplicated UTI (Wagenlehner et al., 2018). The use of herbal medicines has also been considered to be a good and safe alternative to perioperative antibiotic prophylaxis (Miotla et al., 2018). However, whether herbal medicines may reduce or even replace antibiotics in future guideline-based regimen requires more prospective studies conducted on large groups of participants (Wawrysiuk et al., 2022).

It is important to note in this context that one study discriminated between HM responders and non-responders (Kulchavenya, 2018). This phenomenon is highly important, since it indicates that the application of HM in general might be restricted to a subset of patients. Unfortunately, no ongoing trials have been enrolled in this matter, and none of the publications cited here discussed the problem of acquired or innate resistance, at least from a theoretical point of view.

LUTS caused by BPH was treated differently than UTI, since the complications of BPH, namely, urinary incontinence, polyuria, urinary retention, and erectile dysfunction, have to be targeted. The clinical trials published so far point to the benefit of herbal medicines in reducing BPH symptoms. However, it is not clear yet whether the integration of herbal medicines may allow to reduce or even to avoid the use of standard medical therapeutics in this case.

Overall, several clinical studies conducted in the last years document a beneficial role of herbal medicines in the treatment of UTI and LUTS.

Upper Respiratory Infections (URIs) are a frequent cause of troublesome symptoms, that might be appropriately treated with herbal medicine. Most studies included in this paper evaluated herbal medicines for the treatment of acute bronchitis or common cold and acute sinusitis or rhinosinusitis.

The majority of the studies we included for the treatment of acute bronchitis tested *P. sidoides* against placebo and reported a statistically significant decrease of bronchitis symptoms and/severity. This is in line with the results of a systematic review and meta-analysis (Agbabiaka et al., 2008), although a more recent systematic review judged that the evidence was of low quality (Timmer et al., 2013). Evidence for other herbal medicines in the treatment of acute bronchitis was scarce.

For the treatment of common cold we found some indications of effectiveness of *P. sidoides*, Eucalyptus, sweet orange, myrtle and lemon oil (ELOM-080) and for Gentianae radix, Primulae flos, Sambuci flos, Rumicis herba and verbenae herba (BNO 1016). A recent systematic review with network meta-analysis, showed very little solid evidence of herbal medicine *versus* placebo for common cold, with only *P. sidoides* and *Andrographis paniculata* showing a reliable decrease of symptoms. Better results were found for herbal medicine *versus* placebo concerning health related quality of life (HRQoL) (in particular *Spicae aetheroleum*) and for symptoms (Cineole and *P. sidoides*) (Hoang et al., 2023). A further systematic review reported on the efficacy of *P. sidoides* (liquid and tablet preparation) for the treatment of acute bronchitis, showing a positive results with, however, low evidence quality (Timmer et al., 2013).

Although herbal medicines are considered to be safe in principle, this might not always be the case. Some herbal compounds are suspected to be carcinogenic and/or hepatotoxic. Herbal products have also been shown to inhibit and/or induce drug-metabolizing enzymes (Moreira et al., 2014). This has to be taken into account, since herbal medicines are often used in combination with conventional drugs. In this context, preparations with SJW may reduce the efficacy of chemotherapy and of anticoagulants but enhance the one of certain consciousness-lowering agents (e.g., sedative medicines, antidepressants) (Nicolussi et al., 2020; Scholz et al., 2021). Due to potential liver toxicity of chelidonium majus, preparations containing more than 2.5 mg daily dose of whole chelidonium alkaloids had to be withdrawn, and for all preparations with lower daily doses, their instruction leaflet must include warnings on liver toxicity (Rosien, 2019). Therefore, the drug's safety must always be carefully investigated and guaranteed by the producers and the regulatory authorities.

The analysis of the outcomes in the selected disorders reflects that herbal medicines are most efficacious for the treatment of URTI (Figure 5), followed by gynecological complaints (Figure 2) and psychosomatic disorders (Figure 1). For the treatment of urological diseases (Figure 4) in particular UTI and LUTS, we could select only 16 studies according to our strict inclusion/exclusion criteria and therefore more studies of high quality have to be performed to gain a better insight into the efficacy of herbal drugs for these ailments. Gastrointestinal diseases hold a special position as only the added value of the phytodrugs to the conventional therapy was tested. In addition, the number of studies we selected was small (Figure 3), making it difficult to judge the efficacy of herbal drugs for this indication.

This report on the current state of research on the clinical benefits of herbal medicines for non-life-threatening ailments has some limitations.

1. The literature search had to be restricted to Pubmed, because other relevant databases like e.g., EMBASE or CINAHL have not been accessible to the authors.
2. Further limitations are the small cohorts in some of the studies
3. Or that the results/outcomes of some studies have been re-analyzed from previous studies.

4. A general obstacle of data interpretation is that for some indications, in particular for gastrointestinal diseases, herbal medicines are predominantly co-administered with standard therapy, which makes it difficult to estimate the clinical benefit of the phytodrug alone.

5 Perspective

Our literature research gives insights into applied herbal medicines for selected indications, the study outcomes and their quality. Based on our results, we (the authors) provide an overview for patients and healthcare practitioners which extracts can be recommended for the treatment if which disorder/complaint (Supplementary Table S1).

In this context we recommend in particular *H. perforatum* L. for depressive disorder, *V. agnus castus* L. for menstrual complaints, *Cimicifica racemose* (L.) for menopausal symptoms, a combination of *I. amara* L., *M. chamomilla* L., *Mentha ×piperita* L., *C. carvi* L., *G. glabra* L. and *M. officinalis* L., for functional dyspepsia, a combination of *C. erythraea*, *Levisticum officinale* W.D.J.Koch and *Rosmarinus officinalis* L. for uncomlicated urinary tract infections, *P. sidoides* DC. for bronchitis and sinusitis and finally *H. helix* for cough (Supplementary Table S1). These recommendations are based on studies with the highest levels of evidence (RCTs).

However, evidence for efficacy of herbal medicines is still not satisfying in order to integrate them in conventional medicine guidelines and standard treatment regimen, which is the reason why statutory health insurances do not reimburse the costs. In fact, herbal medicines are highly popular and accepted among patients, since their application is safe since they do not exert severe side-effects. Especially when conventional medical therapies fail due to undesired side effects having a negative impact on the quality of life, patients are willing to purchase herbal medicines at their own expense. Often doctors do not know about the self-medication activities of their patients and in consequence cannot monitor the treatment with herbal medicines and possible interactions with other drugs.

The discrepancy between available results from clinical research and the use of herbal medicines under everyday conditions shows that we need to perform more interdisciplinary research studies in the future in order to collect scientific sound evidence on their benefits. Clinical research can provide information on the efficacy of phytodrugs and the importance of genetic dispositions and metabolism as well as possible interactions with other medicines. For effectiveness under everyday conditions (from bedside to practice), methods of health services research are necessary. With the help of these, the outcomes of herbal medicines can be recorded from different perspectives, in particular those of the patients (patient-reported outcomes (PROs)). For longitudinal observations, analyses of health insurance and sales volume data are also relevant, using prescriptions and the over-the-counter sales to get a picture on the needs of the patients and the acceptance of phytotherapy by healthcare practitioners. In order to pave the way for the integration of herbal medicines into therapy guidelines and regimens, findings from clinical studies should be carefully evaluated for their transferability to everyday healthcare within the scope of health services research. This way could lead to novel rational efficacious therapy strategies with less side-effects and better compliance of the patients.

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

SS: Investigation, Formal analysis, Writing–Original Draft. JR: Investigation, Formal analysis, Writing–Original Draft, Visualization. MA: Methodology, Investigation, Formal analysis, Writing–Original Draft. RB: Investigation, Formal analysis, Writing–Original Draft. BB: Conceptualization, Methodology, Investigation, Formal analysis, Writing–Original Draft, Supervision. All authors contributed to the article and approved the submitted version.

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.2023.1234701/full#supplementary-material

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Glossary

AAMI	Age-Associated Memory Impairment
AD	Alzheimer's Disease
ADAS-Cog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
ADCS-CGIC	Alzheimer's Disease Cooperative Study-Clinical Global Impression Of Change
ADR	Adverse Drug Reaction
AE	Adverse Effects
ADAS-Gog	Alzheimer's Disease Assessment Scale-Cognitive Subscale
ALT	Alanine Transaminase
ARS	Acute Rhisosinusitis
BDI	Beck-Depressions-Inventar
BEB	Complaint Inventory for symptoms of depression ("Beschwerdeerfassungsbogen")
B-L/BfS	Von Zerssen's Adjective Mood Scale
BPH	Benign Prostatic Hyperplasia
BSS	Bowel Symptome Score
BSS	Bronchitis Symptom Score
CAI	Clinical Activity Index
CD	Crohn's Disease
CDAI	Crohn's Disease Activity Index
CG	Control Group
CGI	Clinical Global Impression
CGI-I	Clinical Global Impression of Improvement
CGI-S	Clinical Global Impression of Severity
CS	Cough Severity
DDS	Dyspeptic Discomfort Score
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised
DSR	Daily Symptom Report
ELF	Enhanced Liver Fibrosis
EPS	Epigastric Pain Syndrome
EMA	European Medicines Agency
FAS	Full Analysis Set
FD	Functional Dyspepsia
GAD	Generalized Anxiety Disorder
GAD	General Alzheimer's Disease
GAF	Global Assessment of Functioning
GERRI	Geriatric Evaluation by Relative's Rating Instrument
GIS	Gastrointestinal Symptom Score
GPA	Global Patient's Self-Assessment
HAMA	Hamilton Rating Scale for Anxiety
HAMD	Hamilton Rating Scale for Depression
HM	Herbal Medicine
HRQoL	Health Related Quality of Life
HRT	Hormone Replacement Therapy
HVC	Viral Hepatitis C
HVB	Viral Hepatitis B
IBDQ	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
ICIQ	International consultation on incontinence modular questionnaire
IDS-C	Inventory of Depressive Symptomatology- Clinician-Rated
IDS-SR	Inventory of Depressive Symptomatology- Self-Report
I-PSS	International Prostate Symptom Score
IBD	Inflammatory Bowel Disease
IBS	Irritable Bowel Syndrome
IG	Intervention Group
KMI	Kupperman Menopause Index
LAD	Left Alzheimer's Disease
LUTI	Lower Urinary Tract Infection
LUTS	Lower Urinary Tract Symptoms
MADRS	Montgomery-Asberg Depression Rating Scale
MCI	Mild Cognitive Impairment
MHT	Menopausal Hormone Therapy
MMDQ	Moos' Menstrual Distress Questionnaire
MMSE	Mini-Mental State Examination
MOS	Mean Opinion Score
MRS	Menopausal Rating Score
MSS	Major Symptom Score
MUS	Mid-Urethral Sling
NAA	Nuremberg Gerontopsychological Rating Scale for Activities of Daily Living
NAI	Nuremberg Gerontopsychological Inventory
NDI	Nepean Dyspepsia Index
NPI	Neuropsychiatric Inventory
NSAID	non-steroidal anti-inflammatory drug
OAB	Overactive Bladder
OR	Odds Ratio
PDS	Postprandial Distress Syndrome
PEG	Polyethylene glycol
PHF	Pressure, Heaviness and Fullness
PHQ-9	Patient Health Questionnaire 9
PMS	Premenstrual Syndrome
PMSD	Premenstrual Syndrome Diary

PMTS	Premenstrual Tension Syndrome
POP	Pelvic Organ Prolapse
PPI	Proton Pump Inhibitors
PRO	Patient-Reported Outcome
PSQI	Pittsburgh Sleep Quality Index
PSWQ-PW	Penn State Worry Questionnaire
PSYCHE	Psychiatry Educator
QOL	Quality of Life
Q-LES-Q	Quality of Life Enjoyment and Satisfaction Questionnaire
RAD	Right Alzheimer's Disease
RCT	Randomized Controlled Trials
SAS	Self-Rating Anxiety Scale
SCL-58	Symptom Check List- 58
SF-36	Short Form 36 Health Survey
SF-B	Sleep Questionnaire B
SKT	german: Syndrom-Kurztest, Cognitive Test Battery
SS	Symptom Score
SSRI	Selective Serotonin Reuptake Inhibitors
SSS	Sinusitis Severity Score
SWS	Slow-Wave Sleep
TDSS	Total Daily Symptom Score
TIB	Time In Bed
TSIRHF	Total Score of the mean number of Intensity-Rated Hot Flushes
TSS	Total Symptom Score
UDS	Urodynamic Studies
URTI	Upper Respiratory Tract Infections
UTI	Urinary Tract Infections
VaD	Vascular Dementia
VAS	Visual Analogue Scale
WBS	Wellbeing Scale

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Effects of potentilla discolor bunge extracts on oxidative stress and glycolipid metabolism in animal models of diabetes: a systematic review and meta-analysis

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Background/aim: Potentilla discolor Bunge (PDB) is an ancient herb of traditional Chinese medicine. Studies have suggested that extracts of PDB may ameliorate diabetes mellitus (DM). This study aimed to systematically assess the efficacy of PDB extracts on glycolipid metabolism and oxidative stress in animal models of diabetes and to provide evidence-based references for the use of PDB extracts.

Methods: This study followed the PRISMA 2020 guidelines. Studies were searched from eight databases until January 2023. Statistical analysis was performed using StataSE 15.0 and RevMan 5.3. The standard mean difference (SMD) and 95% confidence intervals (CI) were computed using the random-effects model. SYRCLE's risk of bias tool was used to assess the risk of bias.

Results: In total, 32 studies with 574 animals were included. The findings demonstrated that PDB extracts considerably lowered fasting blood glucose (SMD: −3.56, 95%CI: −4.40 to −2.72, $p < 0.00001$); insulin resistance (SMD: −3.19, 95% CI: −5.46 to −0.92, $p = 0.006$), total cholesterol (SMD: −2.18, 95% CI: −2.89 to −1.46, $p < 0.00001$), triglyceride (SMD: −1.48, 95% CI: −2.01 to −0.96, $p < 0.00001$), low-density lipoprotein cholesterol (SMD: −1.80, 95% CI: −2.58 to −1.02], $p < 0.00001$), malondialdehyde (SMD: −3.46, 95% CI: −4.64 to −2.29, $p < 0.00001$) and free fatty acid levels (SMD: −3.25, 95%CI: −5.33 to −1.16, $p = 0.002$), meanwhile, increased insulin sensitivity index (SMD: 2.51 95% CI: 1.10 to 3.92, $p = 0.0005$), body weight (SMD:1.20, 95% CI: 0.38 to 2.01, $p = 0.004$), and the levels of high-density lipoprotein cholesterol (SMD: 1.04, 95% CI: 0.40 to 1.69, $p = 0.001$), superoxide dismutase (SMD:2.63, 95% CI: 1.53 to 3.73, $p < 0.00001$), glutathione peroxidase (SMD: 1.13, 95%CI: 0.42 to1.83, $p = 0.002$), and catalase (SMD:0.75, 95% CI: 0.11 to 1.40], $p = 0.02$).

Conclusion: These findings suggest that PDB extracts can ameliorate DM by improving glycolipid metabolism and oxidative stress. PDB may be a promising medication for DM; however, due to significant heterogeneity between studies, these findings should be interpreted with caution. In addition, future well-

designed trials should determine which components of the PDB play a major role in ameliorating DM and whether these benefits persist in humans.

Systematic Review Registration: https://www.crd.york.ac.uk/prospero, CRD42023379391

KEYWORDS

potentilla discolor bunge, diabetes mellitus, flavonoids extracts, aqueous extracts, animal models, meta-analysis

1 Introduction

Diabetes mellitus (DM) is characterized by abnormally increased blood glucose levels due to various etiologies. Approximately 700 million people will have DM by 2040, according to the International Diabetes Federation (Saeedi et al., 2019). DM is one of the most common chronic metabolic diseases. According to the latest data, metabolic diseases were responsible for 18.6 million deaths in 2019, with 43.6% increase since 1990. The burden of metabolic diseases has been on the rise for the past 30 years, especially among men and regions with low-middle socio-demographic index (Hu et al., 2023). Worldwide, nearly 90% of diabetic patients have type 2 diabetes mellitus (T2DM) (Zheng et al., 2018). Data from the global burden of metabolic diseases between 2000 and 2019 revealed that the prevalence rates of T2DM were 5,282 per 100,000 males, and 4,907 per 100,000 females, with an annual increase of 1.56% in global burden each year (1.64% in males and 1.51% in females) (Chew et al., 2023). Considering the high and rapidly increasing prevalence of DM, it imposes a heavy financial burden on healthcare systems. Except for hyperglycemia, DM causes complications, such as retinopathy, nephropathy, neuropathy, and atherosclerotic ischemia (Cundy et al., 2021; Slomski, 2022; Cai et al., 2023; Jonas et al., 2023). Additionally, patients with DM are more likely to suffer from neoplastic diseases, depression, and tuberculosis (Riza et al., 2014; Graham et al., 2020; He S. et al., 2022; Guo et al., 2022; Kramer et al., 2022; Ottaiano et al., 2022; Sharma et al., 2022). Despite the many effective medications developed, in contrast to expectations, the proportion of patients with well-controlled blood glucose has not increased (Nauck et al., 2021). This condition may be related to severe adverse drug reactions or other reasons (Taylor et al., 2015; Arcani et al., 2017; Vos and Rutten, 2017; Razavi-Nematollahi and Ismail-Beigi, 2019). Optimization of DM treatment remains a major public health issue worldwide. The search for new appropriate antidiabetic drugs remains an active area of research and development.

Currently, herbal medicines have been attracting attention worldwide and are increasingly used as complementary and alternative therapies for DM (Zhang and Jiang, 2012; Nie et al., 2019). *Fan Bai Cao* refers to Potentilla discolor Bunge (PDB), a member of the Rosaceae family distributed in the northern temperate zone. It was usually applied to treat hepatitis, diarrhea, or traumatic hemorrhage in the past (Tomczyk and Latté, 2009). However, the potential of PDB in treating metabolic diseases, especially DM, is becoming increasingly apparent (Zhang et al., 2010; Song et al., 2012; Li et al., 2014; Li et al., 2020; Luo et al., 2020). PDB contains more than ten types of constituents (Mou et al., 2020; Qin et al., 2020), among which eight can inhibit α-glucosidase (Gao et al., 2021). Seven triterpenoids have been isolated from PDB, and four can inhibit the protein tyrosine phosphatase-1B (PTP1B) that can prevent insulin receptor-insulin binding to cause insulin resistance and T2DM (Cui, 2016). Animal studies showed that water extract of PDB can improve glucose and lipid metabolism, enhance insulin sensitivity, promote glycogen synthesis, and inhibit gluconeogenesis (Li et al., 2020). The total flavonoids and triterpenoids from PDB had hypoglycemic and hypolipidemic effects and potent anti-oxidative stress properties in streptozotocin-and high-fat diet (HFD)-induced animal models of diabetes. (Zhang et al., 2010). Therefore, it is worthwhile investigating the effects of PDB extracts on DM.

Although existing studies suggest that PDB extracts have great potential in ameliorating DM, controversies existed among studies (Zhang et al., 2010; Li et al., 2014; Li et al., 2020). In addition, a systematic review and meta-analysis based on preclinical studies have not been conducted to synthesize evidence on the effects of PDB extracts on DM. Hence, this study aimed to conduct a comprehensive systematic review and meta-analysis to analyze the effects of PDB extracts on DM by pooling data from relevant animal studies. This study may provide evidence for future application of PDB extracts and determine future research direction.

2 Materials and methods

This study was registered on the PROSPERO platform (registration number: CRD42023379391) and was conducted according to the guidelines for preferred reporting items for systematic reviews and meta-analyses (Page et al., 2021). The checklist is available in Supplementary Material S1.

2.1 Search strategy

Two authors (YY and WD) independently searched PubMed, Wanfang database (Wanfang), Cochrane Library, Embase, Information Chinese Periodical Service Platform (VIP), Web of Science, China National Knowledge Internet (CNKI), and Baidu Academic database to identify relevant animal studies published in English and Chinese from inception till 30 January 2023, without publication time restriction. Additional eligible studies were identified by searching the references list of the included studies and in the unpublished gray literature. We used MeSH and free-text words appropriately adapted for each database. The following keywords were used

("diabetes mellitus") AND (" Potentilla discolor Bunge" OR "herba potentilla" OR "potentilla discolor decoction"). Supplementary Material S2 provides a detailed search strategy for PubMed.

2.2 Inclusion and exclusion criteria

2.2.1 Inclusion criteria

1) participants: animals with diabetes regardless of their species, age gender, or disease induction method; 2) intervention: the experimental group was treated with PDB or its extracts regardless of timings, frequencies, and dosages; 3) control group: diabetic model animals induced by the same methods and treated with vehicle or no treatment; 4) outcomes: fasting blood glucose (FBG), total cholesterol (TC), insulin sensitivity index (ISI), body weight, fasting insulin (FINS), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), insulin resistance (IR), low-density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA), nitric oxide synthase (NOS), superoxide dismutase (SOD), nitric oxide (NO), catalase (CAT), glutathione peroxidase (GSH-px), and free fatty acid (FFA).

2.2.2 Exclusion criteria

1) the PDB extracts were mixed with other compounds of traditional Chinese medicine; 2) case reports; 3) therapeutic drugs were administered to the control group; 4) animals were not diabetic models; 5) conference paper; 6) lack of a control group; 7) review articles; and 8) *in vitro* or clinical studies.

2.3 Data extraction

The retrieved studies were managed using Endnote software (Version X9). Two authors (YY and WD) assessed the remaining studies after eliminating duplicates. Subsequently, titles and abstracts were screened, and the full-text version of potentially eligible studies was further reviewed. The following information was then used to create an Excel form: publication year, author, animal's species, body weight and age, route of drug administration, intervention (form and dosage), sample size (intervention group/model group), methods for inducing DM, the modeling standard, the duration of treatment, and the outcomes. Disagreements were solved by consulting the corresponding author (QC). For studies with insufficient data, we emailed the authors to request specific information. We analyzed the data that were already available if there was no response.

2.4 Risk of bias assessment

Two researchers (YY and YW) independently assessed the bias of the included studies using the risk of bias tool of SYRCLE (Hooijmans et al., 2014). Sequence generation, baseline characteristics, assignment concealment, random housing, blinding of caregivers, investigators, and outcome assessors, random outcome assessment, incomplete outcome data, selective outcome reporting, and other types of bias were all included in the evaluation. Each item received a low, high, or uncertain ratings for its bias risk. If a study was judged to be low risk in one category, then the study received a point. Higher scores indicated higher quality. The corresponding author (QC) was consulted to discuss any disagreements.

2.5 Data synthesis and analysis

All analyses were performed using StataSE 15.0 and Review Manager 5.3. As we included various animal species and experimental models, a random effect model was used to pool the data. The SMD and 95% CI were used to illustrate the effect size. A p-value less than 0.05 was considered as the threshold for statistical significance. If the data were presented as a standard error, the standard deviation was calculated using the formula from the Cochrane Handbook for Systematic Reviews of Interventions (www.training.cochrane.org/handbook). I^2 and Cochran's Q statistics were used to assess the heterogeneity, and $I^2 > 50\%$ and $p < 0.05$ indicated significant heterogeneity. To further explore the sources of heterogeneity, meta-regression was performed for the indicators containing 10 or more studies based on animal species, type of PDB extracts, treatment duration, and subgroup analyses were also conducted based on the types of PDB extracts, animal species, and treatment duration (2–4 weeks or 6–12 weeks). Sensitivity analyses were performed to verify the stability of the overall findings by progressively removing studies. Due to the limited number of studies reporting the IR, NO, NOS, and CAT levels, subgroup and sensitivity analyses were not conducted. Egger's test was used to assess publication bias. The trim-and-fill method was used to assess the effect of publication bias on outcomes if the p-value in Egger's test was less than 0.05.

3 Results

3.1 Search results

In total, 456 studies were identified, including 74 from PubMed, 13 from Embase, eight from Web of Science, 0 from Cochrane Library, 136 from CNKI, 66 from VIP, 123 from Wanfang, and 136 from CNKI, and six from the Baidu Academic Database and references cited in the included studies. Using Endnote X9, 275 duplicates were removed. After reviewing the titles and abstracts, 114 articles were removed. Thirty-two studies with 574 animals were eventually included in this systematic review and meta-analysis after reviewing the full text of 67 papers. Figure 1 depicts the comprehensive selection procedure.

3.2 Characteristics of the included studies

The included studies were published between 2004 and 2021. The animal models included in these studies were rats or mouse models. Wistar rats were used in 16 studies (Guo and Cui, 2004; Li, 2004; Guo et al., 2005; Zhang and Shen, 2005; Bao et al., 2006; Cui et al., 2007; Hong et al., 2007; Piao et al., 2007; Zong et al., 2007; Ma

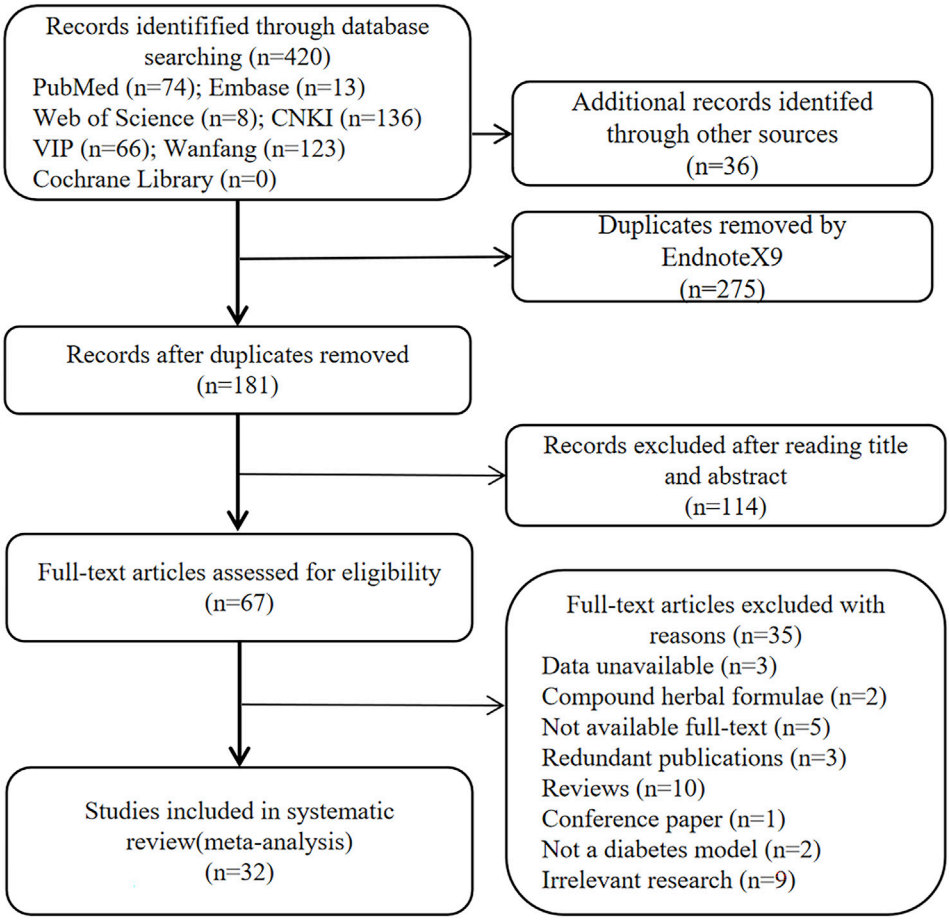

FIGURE 1
Flowchart for selection of studies.

and Cui, 2008; Sun et al., 2010; Yuan et al., 2010; Zhang et al., 2010; Cao and Zhang, 2011; Yan et al., 2012; Su et al., 2016); Sprague-Dawley rats (SD rats) were used in seven studies (Cheng and Li, 2011; Hu et al., 2014; Ding et al., 2016; Liu et al., 2016; Luo et al., 2020; Tan et al., 2020; Shi, 2021); C57BL/6 mice (db/db mice) were used in four studies (Yan et al., 2011; Li et al., 2014; Li et al., 2020; Kong et al., 2021). Two studies (Yan et al., 2011; Kong et al., 2021) used spontaneous T2DM model mice. Institute of Cancer Research (ICR) mice were used in one study (Li et al., 2017); obese-diabetic (Ob-db) mice were used in one study (Song et al., 2012); Kunming mice were used in three studies (Wang et al., 2008; Zeng et al., 2017; Qu et al., 2018). Animals' weight ranged from 18 to 250 g. The gender of animals was not disclosed in two studies (Zhang and Shen, 2005; Zong et al., 2007). Nine studies used both male and female animals (Guo and Cui, 2004; Guo et al., 2005; Bao et al., 2006; Cui et al., 2007; Ma and Cui, 2008; Sun et al., 2010; Yuan et al., 2010; Cao and Zhang, 2011; Zeng et al., 2017), and the remaining studies used male animals. Fourteen studies (Guo and Cui, 2004; Guo et al., 2005; Bao et al., 2006; Cui et al., 2007; Ma and Cui, 2008; Yuan et al., 2010; Cao and Zhang, 2011; Yan et al., 2011; Yan et al., 2012; Li et al., 2014; Ding et al., 2016; Li et al., 2020; Luo et al., 2020; Kong et al., 2021) reported the age of the experimental animals, which ranged from 3 to 8 weeks. The length of treatment ranged from 2 to 8 weeks. Regarding the specific constituents of PDB composition, twenty studies (Guo and Cui, 2004; Li, 2004; Guo et al., 2005; Zhang and Shen, 2005; Bao et al., 2006; Cui et al., 2007; Hong et al., 2007; Piao et al., 2007; Zong et al., 2007; Ma and Cui, 2008; Yuan et al., 2010; Cao and Zhang, 2011; Yan et al., 2011; Song et al., 2012; Yan et al., 2012; Li et al., 2014; Ding et al., 2016; Zeng et al., 2017; Li et al., 2020; Luo et al., 2020) used aqueous extracts of PDB (the concentrated liquid extract of the whole plant after boiling) with doses ranging from 0.3 to 30 g/kg.d; Eleven studies (Wang et al., 2008; Sun et al., 2010; Zhang et al., 2010; Cheng and Li, 2011; Hu et al., 2014; Liu et al., 2016; Su et al., 2016; Li et al., 2017; Tan et al., 2020; Kong et al., 2021; Shi, 2021) used flavonoids from PDB with doses ranging from 0.054 to 36 g/kg.d; Two studies (Zhang et al., 2010; Qu et al., 2018) used triterpenes extracts from PDB with doses ranging from 0.501 to 0.3 g/kg.d. Concerning the diabetes induction model, most studies injected streptozotocin (15–100 mg/kg) in combination with a high-fat diet or a high-fat, high-sugar diet; One study (Li et al., 2014) used a high-fat diet alone to induce diabetes; Two studies (Hong et al., 2007; Piao et al., 2007) used two injections of alloxan to induce diabetes; Four studies (Li, 2004; Wang et al., 2008; Zeng et al., 2017; Qu et al., 2018) used a single injection of alloxan (120–200 mg/kg) to

TABLE 1 Basic characteristics of the included studies.

Study	Animal	Drug route	Age (week)	Weight (g)	Gender	Number (I/M)	Intervention group	Model group	Duration	Induction method	Modeling standard	Outcomes	Intergroup differences
Yuan et al. (2010)	Wistar rat	Gavage	4	60	No restriction	7/7	Aqueous extract of PDB (30 g/kg·d)	Same volume of saline	4 weeks	HFSD + i.p.STZ (30 mg/kg)	11.1 mmol/L ≤ PBG	1.Body weight↑; 2.FBG↓; 3.FINS↑	1.$p < 0.01$; 2.$p < 0.01$ 3.$p < 0.01$
Bao et al. (2006)	Wistar rat	Gavage	8	200 ~ 250	No restriction	10/10	Aqueous extract of PDB (6 g/kg.d)	Same volume of saline	8 weeks	HFSD + i.v.STZ (15 mg/kg)	11.1 mmol/L < FBG <33.3 mmol/L	1.NO↓; 2.MDA↓; 3.NOS↓; 4.SOD↑	1.$p < 0.05$; 2.$p < 0.05$ 3.$p > 0.05$; 4.$p < 0.05$
Piao et al. (2007)	Wistar rat	Gavage	NR	190 ~ 230	Male	10/10	Aqueous extract of PDB (9 g/kg.d)	Same volume of distilled water	4 weeks	i.p.Alloxan (120 mg/kg) +(100 mg/kg). Two injections in total	11.1 mmol/L < FBG	1.FBG↓; 2.TC↓; 3.TG↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$
Zhang et al. (2010)	Wistar rat	Gavage	NR	200–250	Male	10/10	Flavonoids from PDB (369 mg/kg.d) Triterpenes extract from PDB (501 mg/kg.d)	0.5% sodium carboxymethylcellulose	15 days	HFD + i.v.STZ (35 mg/kg)	7.0 mmol/L < FBG < 33.3 mmol/L	1.TC↓; 2.TG↓; 3.LDL-C↓; 4.HDL-C↑; 5.NO↓; 6.MDA↓; 7.SOD↑; 8.GSH-px↑; 9.Body weight↓	1.$p < 0.01$; 2.$p < 0.01$ 3.$p < 0.01$; 4.$p < 0.01$ 5.$p < 0.01$; 6.$p < 0.01$ 7.$p < 0.01$; 8.$p < 0.01$; 9.$p < 0.01$
Cao et al. (2011)	Wistar rat	Gavage	8	NR	No restriction	10/10	Aqueous extract of PDB (18 g/kg.d)	Same volume of saline	8 weeks	HFD + i.v.STZ (18 mg/kg)	11.1 mmol/L < FBG	1.FBG↓; 2.FINS↓; 3.TC↓; 4.TG↓; 5.ISI↑	1.$p < 0.05$; 2.$p < 0.01$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$
Cui et al. (2007)	Wistar rat	Gavage	8	200 ~ 250	No restriction	10/10	Aqueous extract of PDB (12 g/kg.d)	Same volume of saline	8 weeks	HFSD + i.v.STZ (15 mg/kg)	11.1 mmol/L < FBG<33.3 mmol/L	ISI↑	$p < 0.01$
Guo et al. (2004)	Wistar rat	Gavage	8	200 ~ 250	No restriction	10/10	Aqueous extract of PDB (12 g/kg.d)	Same volume of saline	8 weeks	HFSD + i.v.STZ (15 mg/kg)	11.1 mmol/L ≤ FBG<33.3 mmol/L	FINS↓	$p < 0.01$
Guo et al. (2005)	Wistar rat	Gavage	8	200 ~ 250	No restriction	10/10	Aqueous extract of PDB (12 g/kg.d)	Same volume of saline	8 weeks	HFSD + i.v.STZ (30 mg/kg)	11.1 mmol/L ≤ FBG < 33.3 mmol/L	1.TG↓; 2.TC↓; 3.HDL-C↓; 4.LDL-C↓	1.$p < 0.01$; 2.$p < 0.01$ 3.$p < 0.05$; 4.$p < 0.05$
Ma et al. (2008)	Wistar rat	Gavage	8	200 ~ 250	No restriction	10/10	Aqueous extract of PDB (90 g/kg.d)	Same volume of saline	8 weeks	HFSD + i.v.STZ (15 mg/kg)	11.1 mmol/L < FBG < 33.3 mmol/L	1.MDA↓; 2.GSH-px↑; 3.SOD↑; 4.CAT↑	1.$p < 0.01$; 2.$p < 0.01$ 3.$p < 0.01$; 4.$p < 0.05$
Zong et al. (2007)	Wistar rat	Gavage	NR	180 ~ 220	NR	10/10	Aqueous extract of PDB (12 g/kg.d)	Same volume of water	8 weeks	HFD + i.v.STZ (20 mg/kg)	11.1 mmol/L ≤ FBG<30 mmol/L	FINS↓	$p < 0.01$
Wang et al. (2008)	Kunming mice	Gavage	NR	18 ~ 22	Male	10/8	Flavonoids from PDB (216 mg/kg.d)	Each mouse was given 0.4 mL normal saline	2 weeks	i.p.Alloxan (120 mg/kg)	11.1 mmol/L ≤ FBG	1.FBG↓; 2.FINS↑; 3.Body weight↑	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$

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TABLE 1 (*Continued*) Basic characteristics of the included studies.

Study	Animal	Drug route	Age (week)	Weight (g)	Gender	Number (I/M)	Intervention group	Model group	Duration	Induction method	Modeling standard	Outcomes	Intergroup differences
Hong et al. (2007)	Wistar rat	Gavage	NR	190 ~ 230	Male	10/10	Aqueous extract of PDB (9 g/kg.d)	Same volume of distilled water	4 weeks	i.p.Alloxan (120 mg/kg) +(100 mg/kg). Two injections in total	11.1 mmol/L ≤ FBG	1.FBG↓; 2.TC↓; 3.TG↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$
Hu et al. (2014)	SD rat	Gavage	NR	180 ~ 220	Male	8/8	Flavonoids from PDB (216 mg/kg.d)	Same volume of water	4 weeks	HFSD + i.v.STZ (30 mg/kg)	11.1 mmol/L ≤ FBG	1.Body weight↑; 2.FBG↓; 3.TG↓; 4.TC↓; 5.HDL-C↑; 6.LDL-C↓; 7.FINS↓; 8.IR↓; 9.FFA↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p > 0.05$; 4.$p < 0.05$ 5.$p < 0.05$; 6.$p > 0.05$ 7.$p < 0.05$; 8.$p < 0.05$ 9.$p > 0.05$
Ding et al. (2016)	SD rat	Gavage	4	120-140	Male	7/7	Aqueous extract of PDB (4 g/kg.d)	Same volume of water	4 weeks	HFD + i.p.STZ (35 mg/kg)	16.7 mmol/L ≤ RBG	1.Body weight↑; 2.RBG↓; 3.TG↓; 4.TC↓; 5.HDL-C↓; 6.LDL-C↓	1.$p > 0.05$; 2.$p < 0.01$ 3.$p > 0.05$; 4.$p < 0.05$ 5.$p > 0.05$; 6.$p > 0.05$
Cheng et al. (2011)	SD rat	Gavage	NR	160 ~ 200	Male	10/10	Flavonoids from PDB (320 mg/kg.d)	Same volume of distilled water	12 weeks	HFSD + i.p.STZ (40 mg/kg)	16.7 mmol/L ≤ RBG	1.FBG↓; 2.FINS↓; 3.ISI↑; 4.CAT↑; 5.MDA↓; 6.GSH-px↑	1.$p < 0.01$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.01$; 6.$p < 0.01$
Yan et al. (2011)	C57BL/KsJ-db/db Mice	Gavage	6	40.4 ~ 49	Male	6/6	Aqueous extract of PDB (400 mg/kg.d)	Same volume of sterile water	4 weeks	Spontaneous type 2 diabetic mellitus	-	1.FBG↓; 2.FINS↓; 3.ISI↑	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$
Yan et al. (2012)	Wistar rat	Gavage	3	49 ~ 57	Male	7/7	Aqueous extract of PDB (400 mg/kg.d)	Sterile water (10 mL/kg.d)	4 weeks	HFD + i.p.STZ (35 mg/kg)	11.1 mmol/L < FBG<33.3 mmol/L	1.Body weight↑; 2.FBG↓; 3.TG↓; 4.TC↓; 5.HDL-C↑; 6.LDL-C↓; 7. FFA↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$; 6.$p < 0.05$ 7.$p < 0.05$
Liu et al. (2016)	SD rat	Gavage	NR	180 ~ 220	Male	10/10	Flavonoids from PDB (54 mg/kg.d)	Giving 2 mL drinking water	4 weeks	HFSD + i.p.STZ (30 mg/kg)	11.1 mmol/L < FBG	1.FBG↓; 2.FINS↓; 3.MDA↓; 4.SOD↑; 5.GSH-px↑	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$
Zhang et al. (2005)	Wistar rat	Gavage	NR	NR	NR	10/10	Aqueous extract of PDB (12 g/kg.d)	Same volume of saline	8 weeks	HFSD + i.v.STZ (15 mg/kg)	11.1 mmol/L ≤ RBG<33.3 mmol/L	1.NOS↑; 2.NO↑	1.$p < 0.01$; 2.$p < 0.01$
Li et al. (2014)	C57BL/6 mice	Free feeding	4	NR	Male	6/6	HFD + Aqueous extract of PDB (The dose of PDB was not disclosed)	HFD alone	8 weeks	HFD	NR	1.Body weight↓; 2.FBG↓; 3.FINS↓; 4.IR↓; 5.TG↓; 6.TC↓; 7.LDL-C↓; 8.HDL-C↑	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$; 6.$p < 0.05$ 7.$p < 0.05$; 8.$p < 0.05$

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TABLE 1 (*Continued*) Basic characteristics of the included studies.

Study	Animal	Drug route	Age (week)	Weight (g)	Gender	Number (I/M)	Intervention group	Model group	Duration	Induction method	Modeling standard	Outcomes	Intergroup differences
Li et al. (2017)	ICR mice	Gavage	NR	28–32	Male	8/8	Flavonoids from PDB (100 mg/kg.d)	Giving saline (0.1 mL/10 g)	6 weeks	HFD + i.p.STZ (100 mg/kg)	11.1 mmol/L < FBG	FBG↓	$p < 0.001$
Li et al. (2020)	C57BL/6J mice	Gavage	5	NR	male	8/8	Aqueous extract of PDB (400 mg/kg.d)	Same volume of saline	8 weeks	HFD + i.p.STZ (40 mg/kg)	11.1 mmol/L < FBG	1.Body weight↑; 2.FINS↑; 3.TG↓; 4.TC↓; 5.HDL-C↓; 6.LDL-C↓; 7.FFA↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$; 6.$p < 0.05$ 7.$p < 0.05$
Li et al. (2004)	Wistar rat	Gavage	NR	190 ~ 210	male	10/10	Aqueous extract of PDB (5.4 g/kg.d)	Same volume of distilled water	3 weeks	i.p.Alloxan (120 mg/kg)	10.mmol/L < FBG	1.FBG↓; 2.MDA↓; 3.SOD↑	1.$p < 0.01$; 2.$p < 0.05$ 3.$p < 0.01$
Zeng et al. (2017)	Kunming mice	Gavage	NR	25 ~ 30	No restriction	10/10	Aqueous extract of PDB (300 mg/kg.d)	Same volume of saline	4 weeks	i.p.Alloxan (200 mg/kg)	11.1 mmol/L < FBG	FBG↓	$p < 0.01$
Qu et al. (2018)	Kunming mice	Gavage	NR	20 ~ 23	Male	10/10	Triterpenes extract from PDB(200 mg/kg.d)	Same volume of saline	3 weeks	i.p.Alloxan (200 mg/kg)	11.1 mmol/L < FBG	1.Body weight↑; 2.FBG↓	1.$p < 0.01$; 2.$p < 0.01$
Shi et al. (2021)	SD rat	Gavage	NR	160 ~ 200	Male	7/7	Flavonoids from PDB (160 mg/kg.d)	Same volume of saline	4 weeks	HFSD + i.p.STZ (30 mg/kg)	16.7 mmol/L < FBG	1.Body weight↑; 2.FBG↓; 3.FINS↓; 4.TG↓; 5.TC↓; 6.ISI↑	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$; 6.$p < 0.05$
Sun et al. (2010)	Wistar rat	Gavage	NR	180 ~ 220	No restriction	8/8	Flavonoids from PDB (36 g/kg.d)	Same volume of saline	2 weeks	HFSD + i.p.STZ (50 mg/kg)	11.1 mmol/L < FBG	1.FBG↓; 2.FINS↓; 3.ISI↑; 4.SOD↑; 5.MDA↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.05$ 5.$p < 0.05$
Kong et al. (2021)	db/db Mice	Gavage	7	30 ~ 40	Male	6/6	Flavonoids from PDB (400 mg/kg.d)	Same volume of distilled water	4 weeks	Spontaneous type 2 diabetic mellitus	-	1.FBG↓; 2.TG↓; 3.TC↓; 4.HDL-C↑; 5.LDL-C↓; 6.FINS↓; 7.IR↓; 8.SOD↑; 9.MDA↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p < 0.01$ 5.$p > 0.05$; 6.$p < 0.01$ 7.$p < 0.01$; 8.$p < 0.05$ 9.$p < 0.05$
Luo et al. (2020)	SD rat	Gavage	4	120 ~ 140	Male	8/8	Aqueous extract of PDB (4 g/kg.d)	Same volume of saline	4 weeks	HFD + i.p.STZ (35 mg/kg)	16.7 mmol/L < RBG	1.Body weight↑; 2.TC↓; 3.TG↓; 4.HDL-C↓; 5.LDL-C↓	1.$p > 0.05$; 2.$p < 0.05$; 3.$p > 0.05$; 4.$p > 0.05$; 5.$p < 0.05$
Song et al. (2012)	Ob-db mice	Gavage	NR	20 ~ 25	Male	8/8	Aqueous extract of PDB (2 g/kg·d)	Distilled water (0.5 mL/days)	4 weeks	HFD + i.p.Alloxan (60 mg/kg)twice	11.1 mmol/L ≤ FBG	1.Body weight↓; 2.FBG↓; 3.TC↓; 4.TG↓; 5.SOD↑; 6.MDA↓; 7.FFA↓	1.$p > 0.05$; 2.$p < 0.01$ 3.$p < 0.01$; 4.$p < 0.05$ 5.$p < 0.05$; 6.$p < 0.05$ 7.$p < 0.05$

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induce diabetes; One study (Song et al., 2012) used alloxan combined with high-fat diet. The majority of the included studies used FBG≥11.1 mmol/L as the threshold for successful modeling, and four studies (Cheng and Li, 2011; Ding et al., 2016; Luo et al., 2020; Shi, 2021) used FBG or random blood glucose (RBG) ≥ 16.7 mmol/L as the threshold for successful modeling. One study (Li et al., 2014) did not state the criteria for modeling. The details are presented in Table 1.

3.3 Quality of the included studies

All studies were scored between 3 and 5 points; thirteen studies (Guo and Cui, 2004; Guo et al., 2005; Zhang and Shen, 2005; Bao et al., 2006; Cui et al., 2007; Ma and Cui, 2008; Yuan et al., 2010; Cao and Zhang, 2011; Cheng and Li, 2011; Hu et al., 2014; Li et al., 2014; Zeng et al., 2017; Kong et al., 2021) obtained three points; Fifteen studies (Li, 2004; Zong et al., 2007; Wang et al., 2008; Sun et al., 2010; Zhang et al., 2010; Song et al., 2012; Ding et al., 2016; Liu et al., 2016; Su et al., 2016; Li et al., 2017; Qu et al., 2018; Li et al., 2020; Luo et al., 2020; Tan et al., 2020; Shi, 2021) were scored four points; Four studies (Hong et al., 2007; Piao et al., 2007; Yan et al., 2011; Yan et al., 2012) received five points. For sequence generation, five studies (Hong et al., 2007; Piao et al., 2007; Yan et al., 2011; Yan et al., 2012; Su et al., 2016) were determined as low risk for using a random number table method, and four studies (Song et al., 2012; Hu et al., 2014; Li et al., 2020; Kong et al., 2021) that did not use randomization were considered high risk; The remaining studies only reported random assignments and did not specify a specific random sequence method; therefore, they were classified as having an unclear risk. Eighteen studies (Li, 2004; Hong et al., 2007; Piao et al., 2007; Zong et al., 2007; Wang et al., 2008; Sun et al., 2010; Zhang et al., 2010; Yan et al., 2011; Song et al., 2012; Yan et al., 2012; Ding et al., 2016; Liu et al., 2016; Li et al., 2017; Qu et al., 2018; Li et al., 2020; Luo et al., 2020; Tan et al., 2020; Shi, 2021) reported similar baseline characteristics between groups before the experiment and were identified as low risk. None of the studies clarified whether the assignment of different groups was adequately masked; none of the articles mentioned whether random housing and random outcome assessment were implemented; Similarly, none of the studies provided adequate details about the procedures used for blinding researchers, caregivers, and outcome measurement. Thus, all studies considered the risks of these items to be unclear. All studies reported expected outcomes, and none selectively reported data; hence, they were considered low risk. No study was identified as having a significant risk of additional bias. A thorough evaluation of the quality of the included studies is shown in Figure 2.

3.4 Results of meta-analyses

3.4.1 Effect on fasting blood glucose (FBG)

Twenty-one studies reported data on the effectiveness of PDB extracts on FBG levels. Their findings revealed that FBG level in the treatment group was considerably lower than that in the control group (SMD: −3.56 [95%CI: −4.40, −2.72], $p <$

TABLE 1 (*Continued*) Basic characteristics of the included studies.

Study	Animal	Drug route	Age (week)	Weight (g)	Gender	Number (I/M)	Intervention group	Model group	Duration	Induction method	Modeling standard	Outcomes	Intergroup differences
Su et al. (2016)	Wistar rat	Gavage	NR	195 ~ 206	Male	10/10	Flavonoids from PDB (12 g/kg·d)	Same volume of distilled water	4 weeks	HFSD + i.p.STZ (40 mg/kg)	11.1 mmol/ L ≤ FBG	1.FBG↓; 2.FINS↓; 3.ISI↑; 4.SOD↑; 5.MDA↓	1.$p < 0.01$; 2.$p < 0.01$ 3.$p < 0.01$; 4.$p < 0.01$ 5.$p < 0.01$
Tan et al. (2020)	SD rat	Gavage	NR	180 ~ 220	Male	9/9	Flavonoids from PDB (216 mg/kg·d)	Saline (2 mL/kg)	8 weeks	HFSD + i.p.STZ (30 mg/kg)	11.1 mmol/ L < FBG	1.Body weight↑; 2.FBG↓; 3.TC↓; 4.TG↓; 5.HDL-C↑; 6.LDL-C↓; 7.FFA↓	1.$p < 0.05$; 2.$p < 0.05$ 3.$p < 0.05$; 4.$p > 0.05$ 5.$p < 0.05$; 6.$p < 0.05$ 7.$p < 0.05$

Abbreviations: CAT, catalase; FBG, fasting blood glucose; FFA, free fatty acid; FINS, fasting insulin; GSH-px, glutathione peroxidase; HDL-C, high-density lipoprotein cholesterol; HFD, high-fat diet; HFSD, high fat and sugar diet; I, intervention group; ICR, institute of cancer research; IR, insulin resistance; ISI, insulin sensitivity index; i.p., intraperitoneal; i.v., intravenous; LDL-C, low density lipoprotein cholesterol; MDA: malondialdehyde; M, model group; NO, nitric oxide; NOS, nitric oxide synthase; NR, not report; Ob-db, obese-diabetic; PBG, postprandial blood glucose; PDB, potentilla discolor bunge; RBG, random blood glucose; SD, sprague-dawley; STZ, streptozotocin; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride.

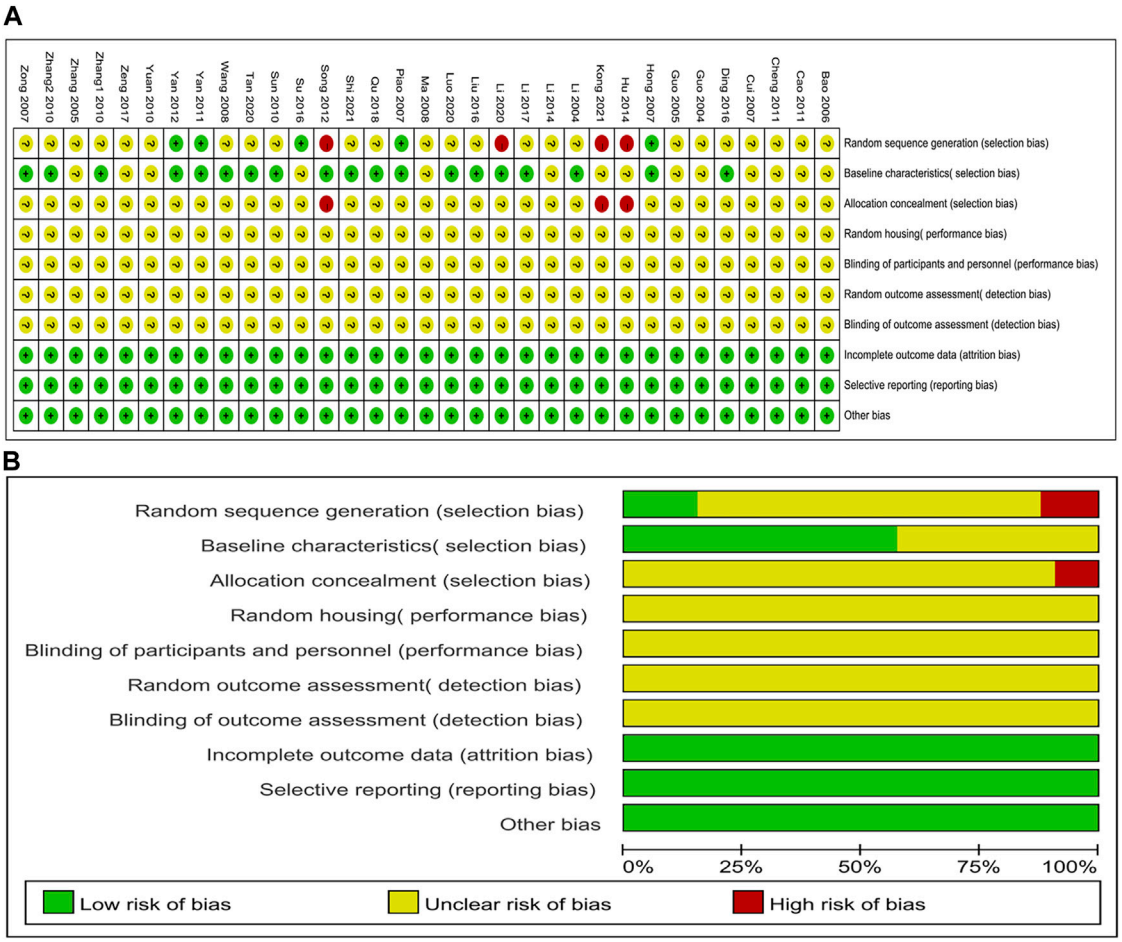

FIGURE 2
Evaluation of risk of bias. **(A)** summary for each risk of bias item for each study; **(B)** graph for each risk of bias item presented as percentages.

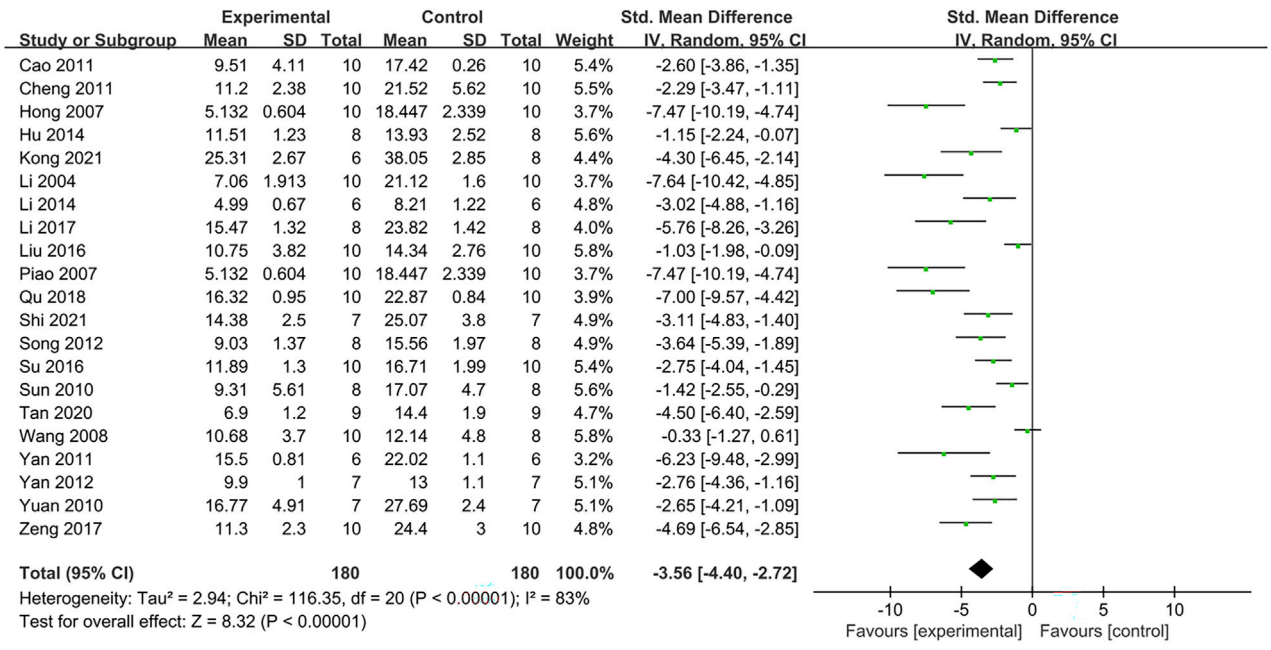

FIGURE 3
The effect of PDB extracts on FBG.

TABLE 2 The results of subgroup analyses.

Parameter		Subgroup	Study (n)	Sample (n)	Effect estimate	*p*-Value	I^2	$P_{heterogeneity}$
FBG	Animal species	Wistar rat	9	164	−4.02 [−5.32, −2.72]	<0.0001	81%	<0.00001
		SD rat	5	88	−2.22 [−3.32, −1.12]	<0.001	72%	0.006
		Kunming mice	2	38	−3.55 [−10.08, 2.98]	0.29	96%	<0.00001
		db/dbmice (Spontaneous T2DM)	2	26	−4.89 [−6.68, −3.09]	<0.00001	0%	0.33
		C57BL/6 mice	1	12	−3.02 [−4.88, −1.16]	0.001	—	—
		ICR mice	1	16	−5.76 [−8.26, −3.26]	<0.00001	—	—
		Ob-db mice	1	16	−3.64 [−5.39, −1.89]	<0.0001	—	—
	Type of PDB	Flavonoids from PDB	10	172	−2.38 [−3.28, −1.47]	<0.00001	78%	<0.00001
		Aqueous extract of PDB	10	168	−4.47 [-5.65, −3.29]	<0.00001	72%	0.0002
		Triterpenes extract from PDB	1	20	−7.00 [−9.57, −4.42]	<0.00001	—	—
	Duration	2–4 weeks	16	274	−3.63 [−4.68, −2.59]	<0.00001	86%	<0.00001
		6–12 weeks	5	86	−3.33 [−4.42, −2.24]	<0.00001	55%	0.07
FINS	Animal species	Wistar rat	6	110	−1.14 [−2.62, 0.33]	0.13	88%	<0.00001
		SD rat	4	68	−1.35 [−1.89, −0.80]	<0.00001	0%	0.82
		db/db mice (Spontaneous T2DM)	2	24	−12.81 [−31.30, 5.69]	0.17	90%	0.001
		C57BL/6 mice	2	28	−0.14 [−5.86, 5.58]	0.96	96%	<0.00001
		Kunming mice	1	16	4.71 [2.59, 6.83]	<0.0001	-	-
	Type of PDB	Flavonoids from PDB	8	132	−1.41 [-2.65, −0.17]	0.03	85%	<0.00001
		Aqueous extract of PDB	7	114	−0.70 [−2.69, 1.28]	0.49	92%	<0.00001
	Duration	2–4 weeks	9	138	−1.21 [−3.05, 0.63]	0.20	91%	<0.00001
		6–12 weeks	6	108	−0.97 [−2.22, 0.29]	0.13	86%	<0.00001
ISI	Animal species	Wistar rat	5	90	2.24 [0.63, 3.86]	0.007	88%	<0.00001
		SD rat	1	20	1.36 [0.37, 2.36]	0.007	—	—
		db/dbmice (Spontaneous T2DM)	1	12	11.03 [5.53, 16.53]	<0.0001	—	—
	Type of PDB	Flavonoids from PDB	4	70	2.84 [0.66, 5.03]	0.01	90%	<0.00001
		Aqueous extract of PDB	3	52	2.37 [0.00, 4.73]	0.05	87%	<0.00001
	Duration	2–4 weeks	4	62	5.10 [1.25, 8.96]	0.009	92%	<0.00001
		6–12 weeks	3	60	1.11 [0.41, 1.81]	0.002	35%	0.21
TG	Animal species	Wistar rat	5	104	−1.31 [−2.07, −0.55]	0.0007	68%	0.008
		SD rat	6	98	−1.07 [−1.77, −0.36]	0.0003	60%	0.03
		db/dbmice (Spontaneous T2DM)	1	12	−1.27 [−2.56, 0.02]	0.05	—	—
		C57BL/6 mice	2	28	−4.96 [−6.67, −3.26]	<0.00001	0%	0.77
		Ob-db mice	1	16	−1.73 [−2.92, −0.53]	0.0005	—	—
	Type of PDB	Flavonoids from PDB	5	80	−0.69 [−1.15, −0.22]	0.004	0%	0.47
		Aqueous extract of PDB	10	168	−2.08 [−2.83, −1.33]	<0.00001	70%	0.0005
		Triterpenes extract from PDB	1	20	−0.37 [−1.25, 0.52]	0.42	—	—
	Duration	2–4 weeks	10	172	−1.29 [−1.85, −0.72]	<0.00001	64%	0.002
		6–12 weeks	5	86	−2.17 [−3.50, −0.85]	0.001	80%	0.0004

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TABLE 2 (*Continued*) The results of subgroup analyses.

Parameter		Subgroup	Study (n)	Sample (n)	Effect estimate	*p*-Value	I^2	$P_{heterogeneity}$
TC	Animal species	Wistar rat	5	104	−1.68 [−2.69, −0.67]	0.001	79%	0.0003
		SD rat	6	98	−2.00 [−3.11, −0.88]	0.0005	76%	0.0008
		db/dbmice (Spontaneous T2DM)	1	12	−3.45 [-5.47, −1.42]	0.0009	—	—
		C57BL/6 mice	2	28	−8.52 [−20.40, 3.36]	0.16	90%	0.002
		Ob/db mice	1	16	−2.52 [−3.92, −1.11]	0.0004	—	—
	Type of PDB	Flavonoids from PDB	5	80	−2.48 [-3.95, −1.02]	0.0009	80%	0.0005
		Aqueous extract of PDB	10	168	−2.32 [−3.24, −1.40]	<0.00001	77%	<0.0001
		Triterpenes extract from PDB	1	20	−0.36 [-1.25, 0.53]	0.43	—	—
	Duration	2–4 weeks	10	172	−2.07 [−2.94, −1.21]	<0.00001	79%	<0.00001
		6–12 weeks	5	86	−2.51 [−3.93, −1.09]	0.0005	78%	0.001
HDL-C	Animal species	Wistar rat	3	64	1.17 [0.16, 2.19]	0.02	72%	0.01
		SD rat	4	64	0.26 [-0.58, 1.10]	0.55	63%	0.05
		db/dbmice (Spontaneous T2DM)	1	12	2.01 [0.51, 3.50]	0.009	—	—
		C57BL/6 mice	2	28	2.63 [−0.19, 5.45]	0.07	79%	0.03
	Type of PDB	Flavonoids from PDB	4	66	0.95 [0.42, 1.48]	0.0004	1%	0.39
		Aqueous extract of PDB	6	92	1.34 [0.08, 2.60]	0.04	83%	<0.0001
		Triterpenes extract from PDB	1	20	0.25 [−0.63, 1.13]	0.001	—	—
	Duration	2–4 weeks	6	102	0.72 [−0.08, 1.52]	0.08	72%	0.001
		6–12 weeks	4	66	1.60 [0.65, 2.55]	0.0009	58%	0.07
LDL-C	Animal species	Wistar rat	3	64	−2.10 [−3.71, −0.50]	0.01	84%	0.0003
		SD rat	4	64	−1.23 [-2.02, −0.44]	0.002	49%	0.12
		db/dbmice (Spontaneous T2DM)	1	12	−1.04 [−2.29, 0.20]	0.10	—	—
		C57BL/6 mice	2	28	−5.25 [-10.67, 0.17]	0.06	83%	0.02
	Type of PDB	Flavonoids from PDB	4	66	−0.91 [−1.52, −0.31]	0.003	25%	0.26
		Aqueous extract of PDB	6	92	−3.17 [−4.63, −1.71]	<0.0001	77%	0.0006
		Triterpenes extract from PDB	1	20	−0.77 [−1.69, 0.14]	0.10	—	—
	Duration	2–4 weeks	6	102	−1.29 [-2.13, −0.44]	0.003	71%	0.002
		6–12 weeks	4	66	−2.74 [−4.14, −1.34]	<0.0001	78%	<0.0001
MDA	Animal species	Wistar rat	6	126	−3.95 [−5.75, −2.15]	<0.0001	90%	<0.00001
		SD rat	2	40	−2.71 [−3.61, −1.80]	<0.00001	0%	0.72
		db/dbmice (Spontaneous T2DM)	1	12	−6.76 [−10.24, −3.27]	0.0001	—	—
		Ob/db mice	1	16	−1.47 [−2.62, −0.33]	0.01	—	—
	Type of PDB	Flavonoids from PDB	6	108	−3.56 [-5.20, −1.92]	<0.0001	84%	<0.00001
		Aqueous extract of PDB	4	76	−4.16 [-6.40, −1.91]	0.0003	85%	0.0002
		Triterpenes extract from PDB	1	20	−0.86 [−1.78, 0.07]	0.07	—	—
	Duration	2–4 weeks	7	134	−3.05 [−4.38, −1.72]	<0.00001	85%	<0.00001
		6–12 weeks	3	60	−4.36 [−6.14, −2.59]	<0.00001	66%	0.05

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TABLE 2 (*Continued*) The results of subgroup analyses.

Parameter		Subgroup	Study (n)	Sample (n)	Effect estimate	*p*-Value	I^2	$P_{heterogeneity}$
SOD	Animal species	Wistar rat	6	126	2.89 [1.40, 4.39]	0.0001	89%	<0.00001
		SD rat	1	20	1.38 [0.38, 2.37]	0.007	—	—
		db/dbmice (Spontaneous T2DM)	1	12	5.62 [2.65, 8.58]	0.0002	—	—
		Ob/db mice	1	16	1.62 [0.45, 2.80]	0.007	—	—
	Type of PDB	Flavonoids from PDB	5	88	3.07 [1.24, 4.90]	0.001	87%	<0.00001
		Aqueous extract of PDB	4	76	3.08 [1.26, 4.89]	0.0009	85%	0.0002
		Triterpenes extract from PDB	1	20	0.34 [−0.54, 1.23]	0.45	—	—
	Duration	2–4 weeks	7	134	2.98 [1.56, 4.41]	<0.0001	88%	<0.00001
		6–12 weeks	2	40	2.05 [1.25, 2.85]	<0.00001	0%	0.43
GSH-px	Animal species	Wistar rat	2	50	0.83 [−0.10, 1.75]	0.08	65%	0.06
		SD rat	2	40	1.60 [0.75, 2.45]	0.0002	24%	0.25
	Type Of PDB	Aqueous extract of PDB	1	20	1.90 [0.81, 3.00]	0.0007	—	—
		Flavonoids from PDB	3	60	1.19 [0.27, 2.11]	0.01	61%	0.08
		Triterpenes extract from PDB	1	20	0.31 [−0.57, 1.19]	0.49	—	—
	Duration	2–4 weeks	2	50	0.88 [−0.14, 1.91]	0.09	71%	0.03
		6–12 weeks	2	40	1.52 [0.79, 2.24]	<0.0001	0%	0.46
FFA	Animal species	Wistar rat	1	14	−5.92 [−8.71, −3.13]	<0.0001	—	—
		SD rat	2	34	−0.97 [-2.62, 0.68]	0.25	79%	0.03
		Ob/db mice	1	16	−1.96 [−3.22, −0.71]	0.002	—	—
		C57BL/6 mice	1	16	−11.20 [−15.78, −6.63]	<0.00001	—	—
	Type of PDB	Flavonoids from PDB	2	34	−0.97 [-2.62, 0.68]	0.25	79%	0.03
		Aqueous extract of PDB	3	46	−5.94 [-10.70, −1.18]	0.01	90%	<0.0001
	Duration	2–4 weeks	3	46	−2.33 [−4.79, 0.13]	0.06	88%	0.0002
		6–12 weeks	2	34	−6.25 [−15.41, 2.92]	0.18	93%	0.0001
Body weight	Animal species	Wistar rat	3	58	1.91 [0.05, 3.76]	0.04	88%	<0.00001
		SD rat	5	78	0.64 [0.10, 1.18]	0.02	25%	0.25
		C57BL/6 mice	2	28	−0.22 [−9.51, 9.07]	0.96	97%	<0.00001
		Kunming mice	2	38	2.79 [1.84, 3.74]	<0.00001	0%	0.53
		Ob/db mice	1	16	−0.01 [−0.99, 0.97]	0.99	—	—
	Type of PDB	Flavonoids from PDB	5	86	1.08 [0.32, 1.85]	0.006	61%	0.04
		Aqueous extract of PDB	7	102	1.19 [−0.50, 2.88]	0.17	89%	<0.00001
		Triterpenes extract from PDB	2	40	1.54 [−1.44, 4.52]	0.31	92%	0.0003
	Duration	2–4 weeks	10	172	1.22 [0.45, 1.98]	0.002	79%	<0.00001
		6–12 weeks	3	46	0.45 [−3.77, 4.66]	0.83	93%	<0.00001

0.00001; I^2 = 83%, P_{he}<0.00001) (Figure 3). Except for the results of two animal studies on Kunming mice, other subgroup analyses showed that PDB extracts significantly reduced FBG levels (Table 2).

3.4.2 Effect on fasting insulin (FINS)

For comparing FINS, data from fifteen animal studies were combined. There was no significant difference between the intervention group and the control group (SMD: −1.04, [95%CI:

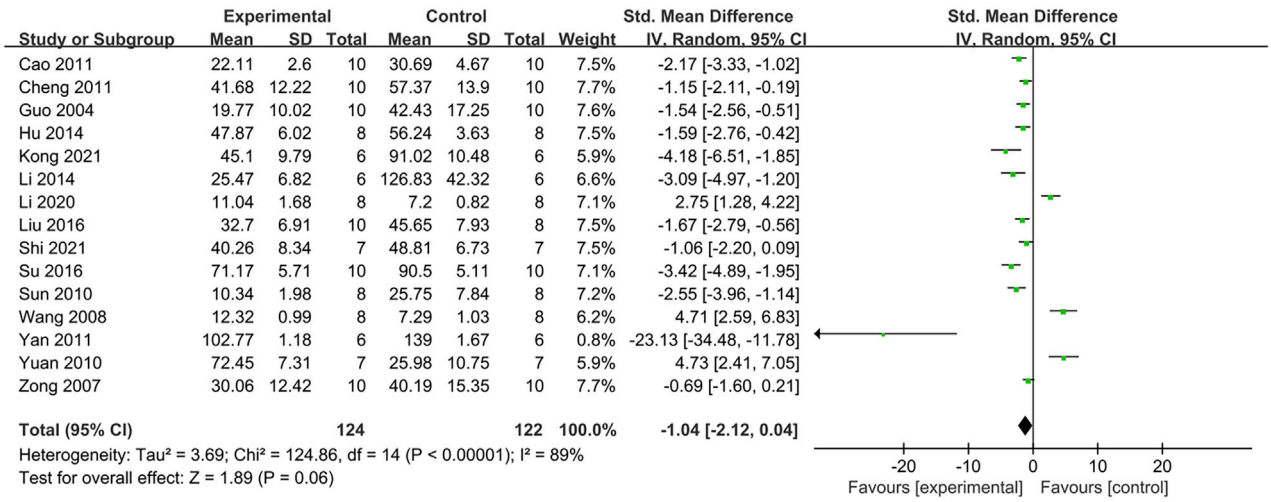

FIGURE 4
The effect of PDB extracts on FINS.

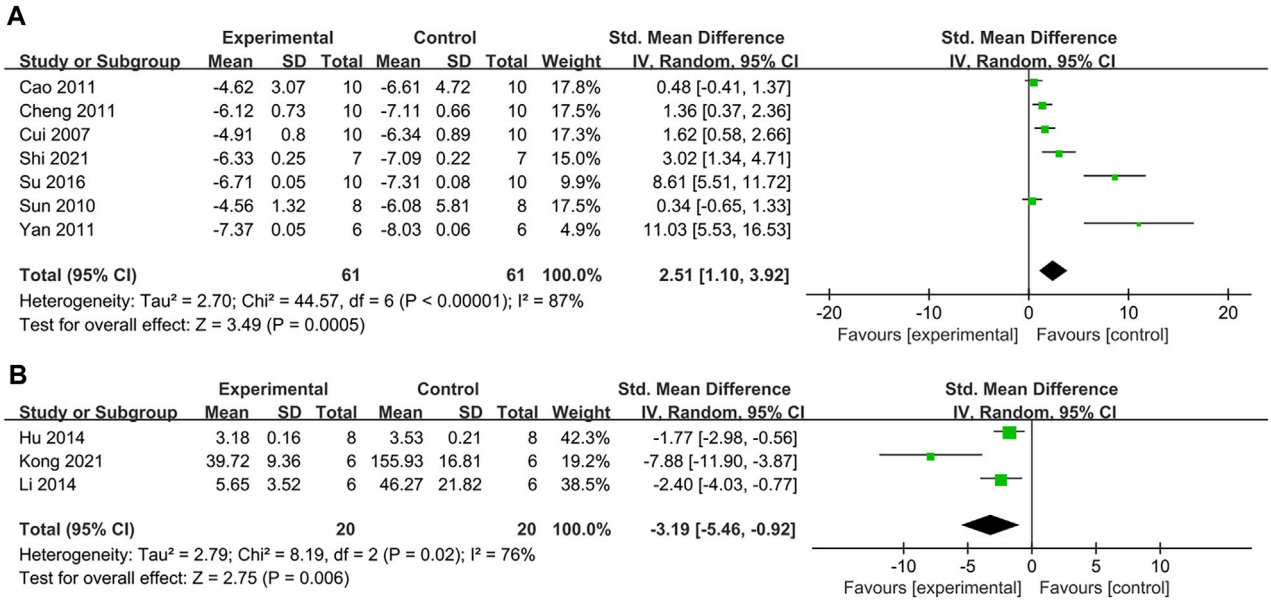

FIGURE 5
(A) The effect of PDB extracts on ISI; **(B)** The effect of PDB extracts on IR.

−2.12, 0.04], $p = 0.06$; $I^2 = 89\%$, $P_{he}<0.00001$) (Figure 4). In subgroup analyses, results from SD rats showed that PDB extracts suppressed insulin secretion (SMD: −1.35, [95%CI: −1.89, −0.80], $p < 0.00001$; $I^2 = 0\%$, $P_{he} = 0.82$). Eight studies of flavonoids from PDB obtained similar results. Interestingly, one study on Kunming mice showed that PDB extracts increased insulin secretion (Table 2).

3.4.3 Effect on insulin sensitivity index (ISI)

The effect of PDB extracts on ISI was reported in seven trials. The overall analysis demonstrated that PDB extracts improved ISI in animal models of diabetes (SMD: 2.51 [95% CI: 1.10, 3.92], $p = 0.0005$; $I^2 = 87\%$, $P_{he} < 0.00001$) (Figure 5A). The subgroup analyses were consistent with the overall results, except the results of three studies that used the aqueous extracts of PDB. The results of three studies showed no significant difference between the control and treatment groups (Table 2).

3.4.4 Effect on insulin resistance (IR)

Three studies reported the effectiveness of PDB extracts on IR. As shown in Figure 5B, the pooled results showed that compared with the control group, PDB extracts significantly decreased the IR (SMD: −3.19 [95% CI: −5.46, −0.92], $p = 0.006$; $I^2 = 76\%$, $P_{he} = 0.02$).

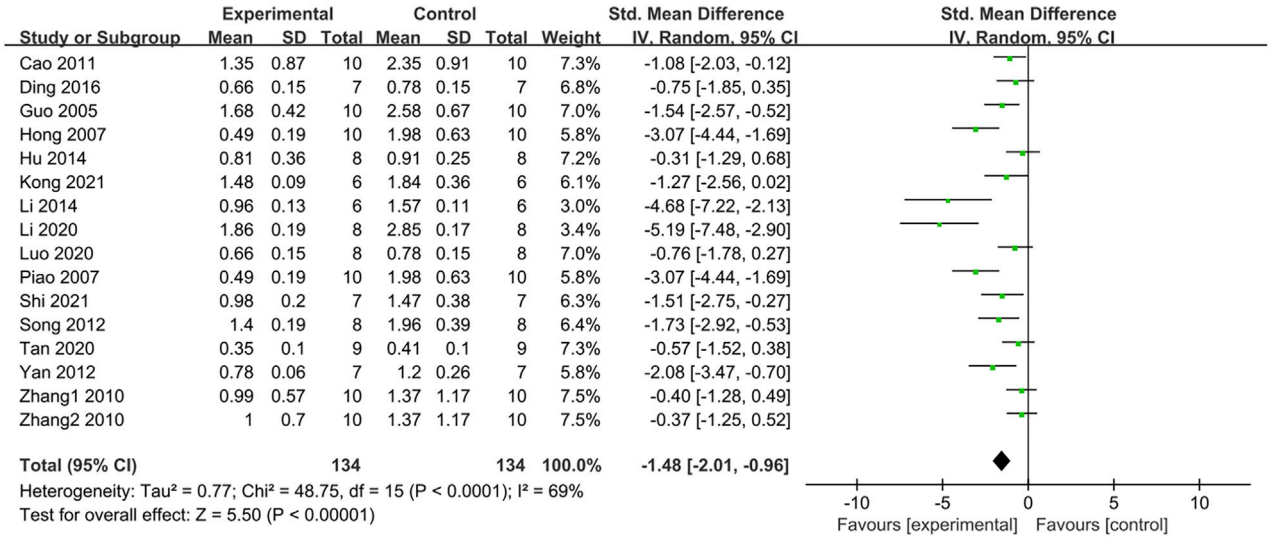

FIGURE 6
The effect of PDB extracts on TG.

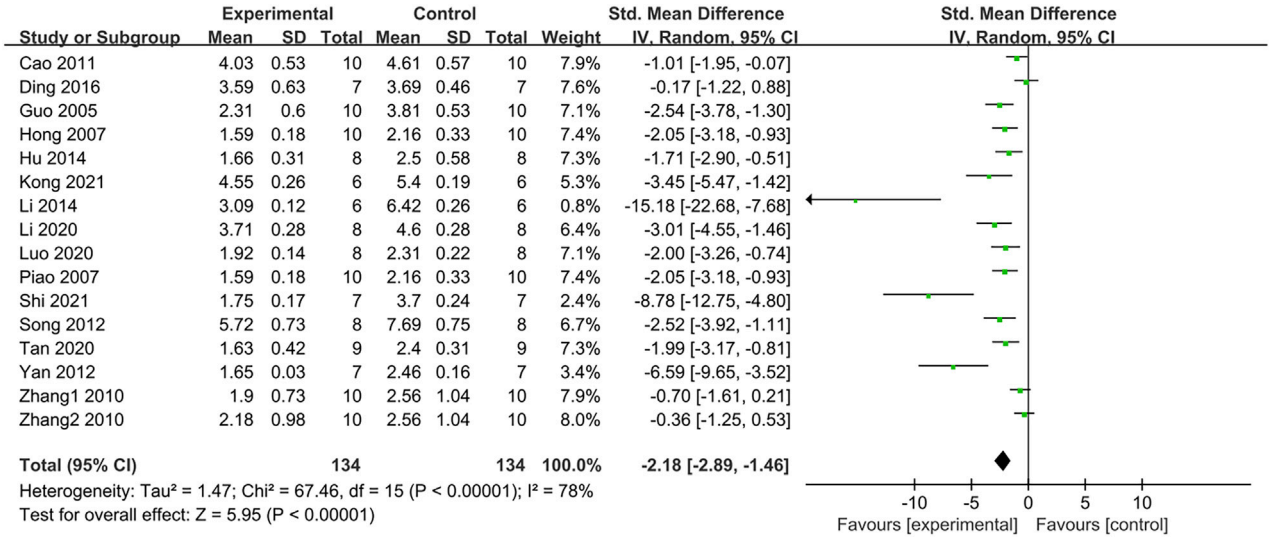

FIGURE 7
The effect of PDB extracts on TC.

3.4.5 Effect on triglyceride (TG)

The pooled results of fifteen studies with 16 groups indicated that PDB extracts significantly decreased TG levels (SMD: −1.48, [95% CI: −2.01, −0.96], $p < 0.00001$; $I^2 = 69\%$, $P_{he} < 0.0001$) (Figure 6). Subgroup analyses also showed significant reductions in TG levels with PDB extracts, except one study on spontaneous T2DM and one study on PDB-derived triterpenes (Table 2).

3.4.6 Effect on total cholesterol (TC)

Fifteen studies with 16 groups explored the effects of PDB extracts on TC. The combined data showed that the TC level in the intervention group was much lower than in the control group (SMD: −2.18, [95%CI: −2.89, −1.46], $p < 0.00001$; $I^2 = 78\%$, $P_{he} < 0.00001$) (Figure 7). However, the subgroup analysis of two studies on C57BL/6 mice showed no significant difference in TC level between the treatment group and the control group. A study with PDB-derived triterpenes showed similar results. The remaining subgroup analyses were consistent with the overall results (Table 2).

3.4.7 Effect on high-density lipoprotein cholesterol (HDL-C)

The pooled data from 10 studies with 11 groups suggested that PDB extracts significantly increased HDL-C levels compared to the control group (SMD: 1.04, [95% CI: 0.40, 1.69], $p = 0.001$; $I^2 = 71\%$,

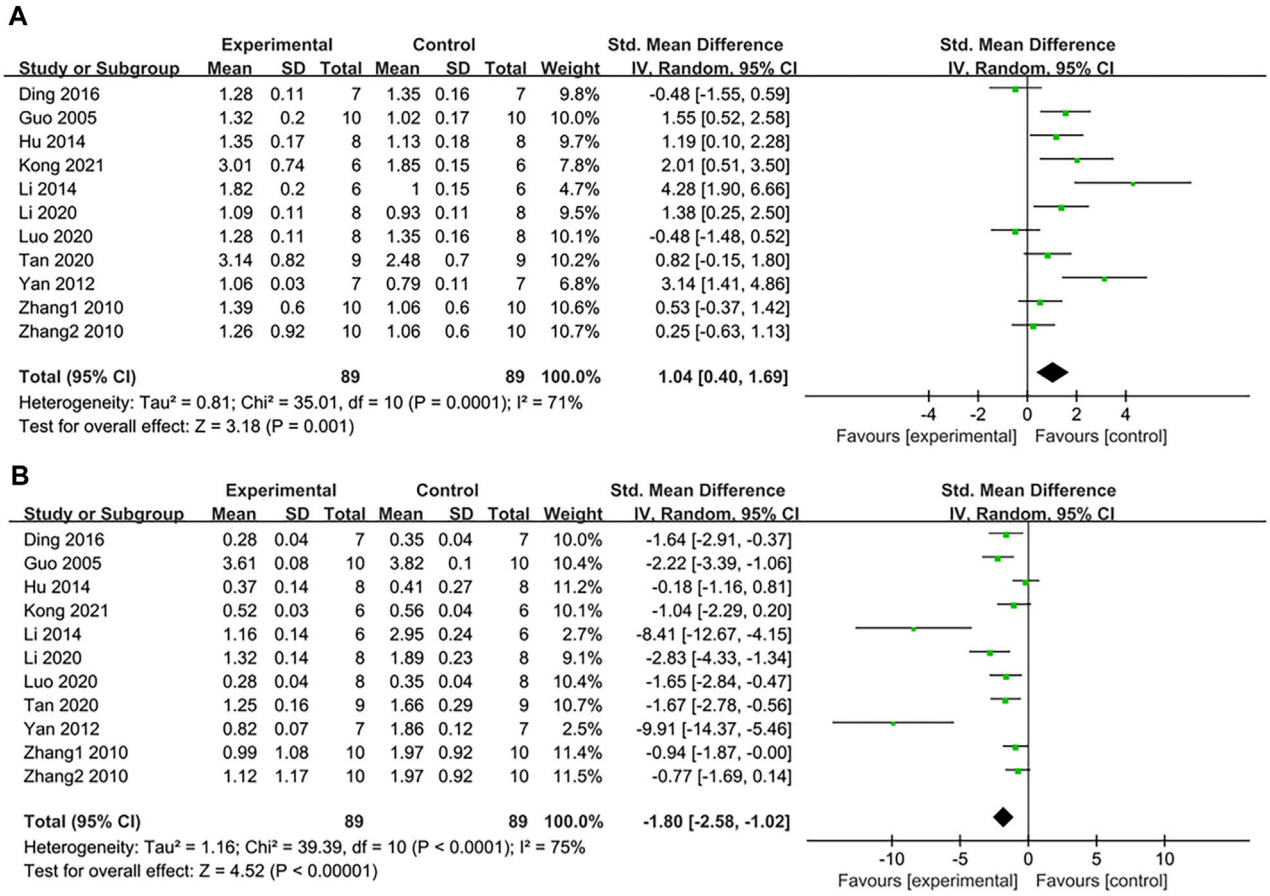

FIGURE 8
(A) The effect of PDB extracts on HDL-C; **(B)** The effect of PDB extracts on LDL-C.

P_{he} = 0.0001) (Figure 8A). Nevertheless, subgroup analyses of studies on SD rats and C57BL/6 mice, studies using aqueous extracts of PDB, and studies with treatment duration of shorter than 6 weeks indicated that there was no significant difference in HDL levels between the treatment group and the control group (Table 2).

3.4.8 Effect on low-density lipoprotein cholesterol (LDL-C)

The effect of PDB extracts on LDL-C levels was examined in ten studies with eleven groups. Compared to the control group, PDB extracts significantly lowered LDL-C levels (SMD: −1.80, [95% CI: −2.58, −1.02], $p < 0.00001$; $I^2 = 75\%$, $P_{he}<0.0001$) (Figure 8B). Except for the results of two studies on C57BL/6 mice, one study on spontaneous T2DM mice, and one study on PDB-derived triterpenes extracts, other subgroup analyses showed that PDB extracts significantly reduced LDL-C levels (Table 2).

3.4.9 Effect on malondialdehyde (MDA)

As shown in Figure 9A, the pooled result of ten studies with 11 groups exhibited that PDB extracts effectively decreased MDA levels compared to the control group (SMD: −3.46, [95% CI: −4.64, −2.29], $p < 0.00001$; $I^2 = 85\%$, $P_{he}<0.00001$). Except one study that used PDB-derived triterpenes, all subgroup analyses supported the overall results (Table 2).

3.4.10 Effect on superoxide dismutase (SOD)

SOD level was assessed in nine studies with ten groups. The results indicated that PDB extracts significantly increased SOD levels (SMD: 2.63, [95% CI: 1.53, 3.73], $p < 0.00001$; $I^2 = 86\%$, $P_{he} < 0.00001$) (Figure 9B). All subgroup analyses showed similar results except one study with PDB-derived triterpenes (Table 2).

3.4.11 Effect on glutathione peroxidase (GSH-px)

In four studies with five groups, GSH-px was measured. Their findings revealed that GSH-px levels were higher in the treatment group than in the control group (SMD: 1.13, [95% CI: 0.42, 1.83], $p = 0.002$; $I^2 = 61\%$, Phe = 0.04) (Figure 10A). All subgroup analyses showed similar results except one study with PDB-derived triterpenes and two studies on Wistar rats (Table 2).

3.4.12 Effect on catalase (CAT)

Two studies assessed the effect of PDB extracts on CAT. Their results showed that CAT levels were higher in the treatment group than in the control group (SMD: 0.75, [95% CI: 0.11, 1.40], $p = 0.02$; $I^2 = 0\%$, Phe = 0.86) (Figure 10B).

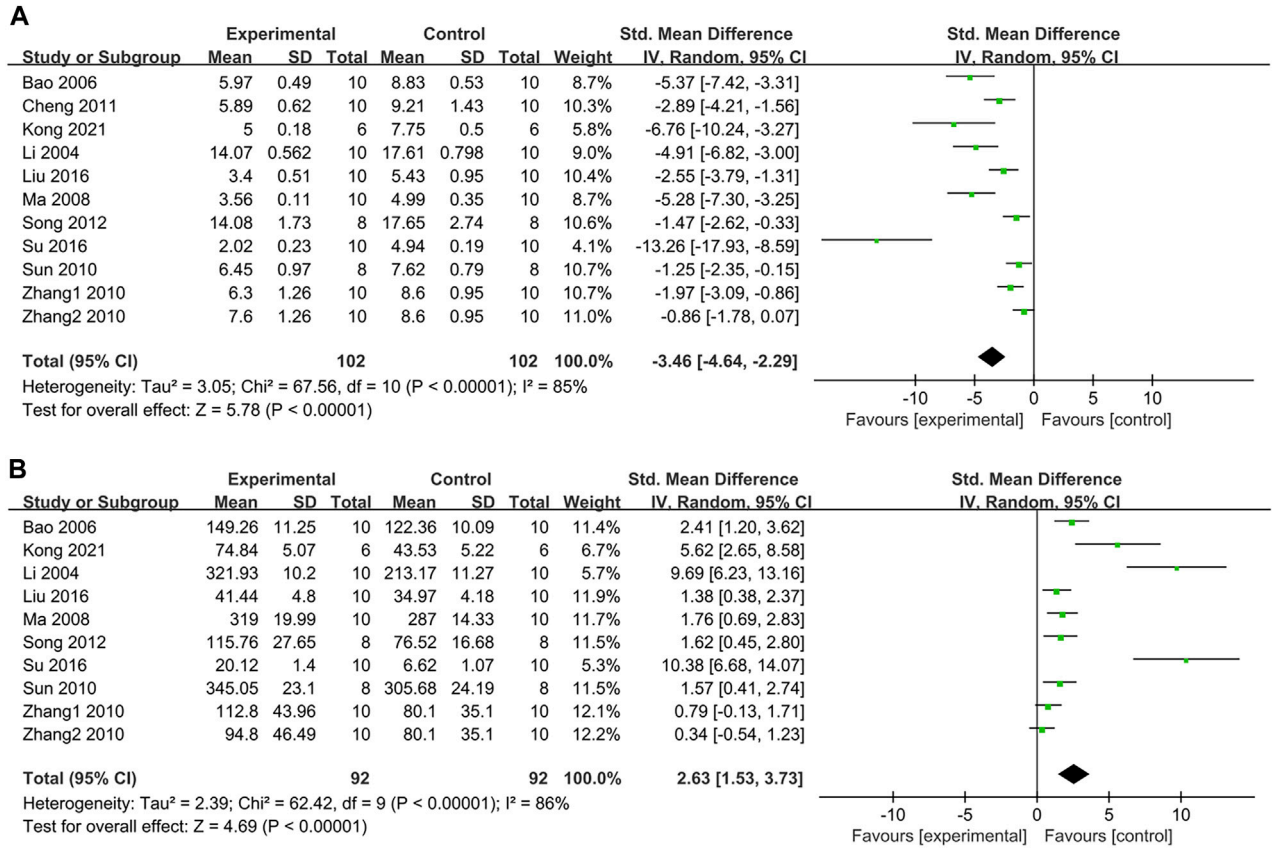

FIGURE 9
(A) The effect of PDB extracts on MDA; **(B)** The effect of PDB extracts on SOD.

3.4.13 Effect on nitric oxide (NO)

Three studies with four groups measured the effect of PDB extracts on NO levels. There was no significant difference between the treatment group and the control group according to the combined results (SMD: −0.91, [95% CI: −2.37, 0.56], p = 0.22; I^2 = 88%, P_{he}<0.0001) (Figure 10C).

3.4.14 Effect on nitric oxide synthase (NOS)

Two studies compared NOS levels between the treatment group and the control group. The pooled results showed that there was no significant difference in NOS levels between the two groups (SMD: 0.67, [95% CI: −0.87, 2.21], p = 0.39; I^2 = 81%, P_{he} = 0.02) (Figure 10D).

3.4.15 Effect on free fatty acid (FFA)

Five studies reported the effect of PDB extracts on FFA. The combined results showed that FFA levels were considerably lower in the treatment group (SMD: −3.25, [95% CI: −5.33, −1.16], p = 0.002; I^2 = 89%, P_{he}<0.00001) (Figure 10E). Subgroup analysis revealed no significant difference in FFA levels between the intervention group and the control group in studies on SD rats treated with PDB-derived flavonoids. Surprisingly, subgroup analyses based on the duration of treatment revealed that PDB extracts had no discernible impact on FFA compared to the control group (Table 2).

3.4.16 Effect on body weight

Body weight was assessed in 13 studies with 14 groups. Compared with the control group, PDB extracts significantly accelerated weight gain (SMD: 1.20, [95% CI: 0.38, 2.01], p = 0.004; I^2 = 84%, P_{he}<0.00001) (Figure 11). Subgroup analyses based on animal species showed no significant difference in body weight between the intervention and control groups in C57BL/6 mice and Ob/db mice. Studies with treatment durations longer than 6 weeks revealed similar results. Subgroup analysis based on the components of PDB revealed that only PDB-derived flavonoids increased body weight compared to the control group (Table 2).

3.5 Results of meta-regression

In order to better find significant influencing factors affecting the heterogeneity of results, meta-regression analysis was performed on indicators containing 10 or more studies based on animal species, type of PDB extracts, and treatment duration. As presented in Table 3, the results of regression analysis showed that the constituent of PDB extracts was a significant factor responsible for the heterogeneity of the effect of PDB extracts on TG (p = 0.04). The remaining indicators showed that heterogeneity was not significantly associated with the constituent of PDB extracts,

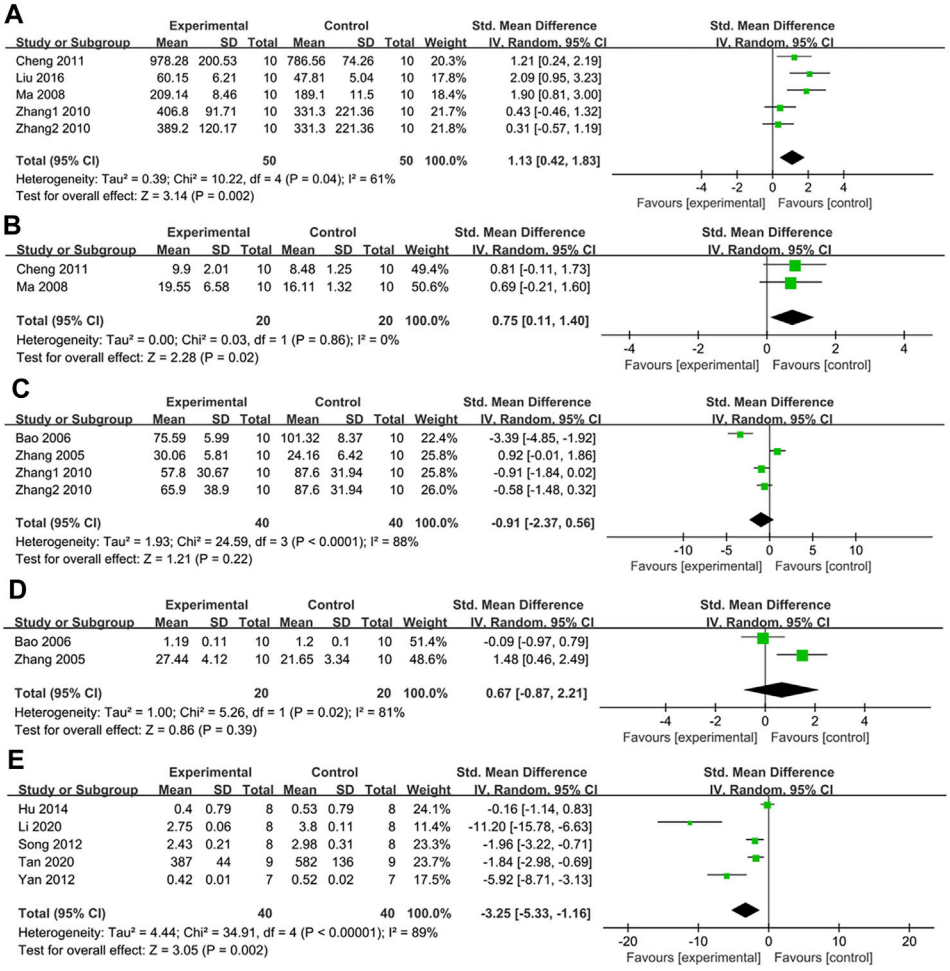

FIGURE 10
(A) The effect of PDB extracts on GSH-px; **(B)** The effect of PDB extracts on CAT; **(C)** The effect of PDB extracts on NO; **(D)** The effect of PDB extracts on NOS; **(E)** The effect of PDB extracts on FFA.

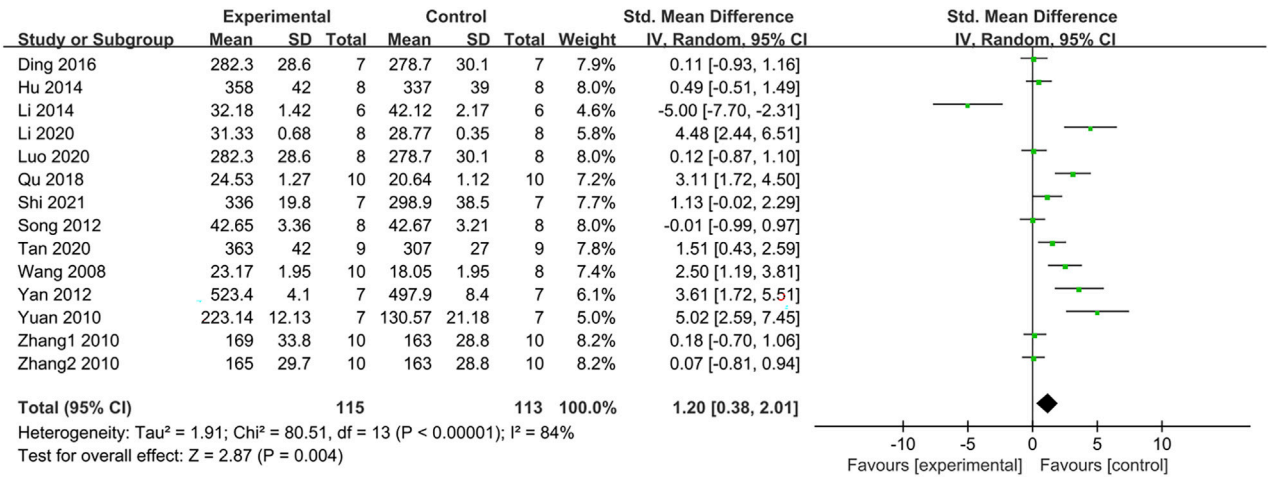

FIGURE 11
The effect of PDB extracts on body weight.

TABLE 3 The results of meta-regression analyses.

Parameter	Variable	Coefficient	Std. Err	*t*	*p*-Value	95% CI
FBG	Type of PDB	0.491	0.701	0.70	0.493	−0.977, 1.958
	Duration	0.044	0.205	0.22	0.832	−0.385, 0.473
	Animal species	−0.005	0.254	−0.02	0.984	−0.537, 0.5278
FINS	Type of PDB	−0.479	2.147	−0.22	0.827	−5.116, 4.159
	Duration	−0.029	0.382	−0.08	0.940	−0.855, 0.796
	Animal species	0.324	0.807	0.40	0.694	−1.419, 2.068
TG	Type of PDB	0.989	0.424	2.33	0.04	0.080, 1.899
	Duration	−0.203	0.151	−1.35	0.20	−0.527, 0.119
	Animal species	−0.228	0.291	−0.78	0.45	−0.853, 0.397
TC	Type of PDB	0.566	0.910	0.62	0.54	−1.386, 2.518
	Duration	−.192	0.270	−0.71	0.49	−0.772, 0.387
	Animal species	−0.479	0.463	−1.04	0.32	−1.471, 0.513
HDL-C	Type of PDB	−0.389	0.606	−0.64	0.54	−1.760, 0.982
	Duration	0.209	0.160	1.31	0.22	−0.153, 0.571
	Animal species	0.705	0.311	2.27	0.05	0.002, 1.409
LDL-C	Type of PDB	1.072	0.624	1.72	0.12	−0.339, 2.484
	Duration	−0.265	0.177	−1.50	0.17	−0.665, 0.135
	Animal species	−.744	0.608	−1.22	0.25	−2.119, 0.631
Body weight	Type of PDB	0.126	0.900	0.14	0.89	−1.835, 2.087
	Duration	−0.116	0.320	−0.36	0.72	−0.813, 0.582
	Animal species	0.262	0.305	0.86	0.41	−0.402, 0.927
SOD	Type of PDB	−1.051	1.767	−0.60	0.57	−5.127, 3.024
	Duration	−0.010	0.542	−0.02	0.99	−1.260, 1.239
	Animal species	−0.634	1.107	−0.57	0.58	−3.187, 1.919
MDA	Type of PDB	1.144	1.525	0.75	0.47	−2.307, 4.594
	Duration	−0.181	0.315	−0.57	0.58	−0.894, 0.532
	Animal species	0.476	1.011	0.47	0.65	−1.812, 2.764

treatment duration, and animal species. The results of meta-regression were almost consistent with those of subgroup analyses.

3.6 Sensitivity analysis

Sensitivity analyses were performed to investigate the stability of the results. After excluding the studies one by one, the results revealed that the effects of PDB extracts on FINS were unstable. Three studies showed that PDB extracts increased insulin secretion and significantly affected the results of FINS. After removing these articles one by one, the combined results were reversed and showed that PDB and its extracts significantly reduced FINS in animal models of diabetes (Supplementary Material S3). After sensitivity analyses, the remaining results remained unchanged, proving that the findings were stable. The details of all sensitivity analyses are shown in Supplementary Material S3.

3.7 Publication bias

Nine outcomes were measured in ten or more studies, including FBG, MDA, HDL-C, FINS, TC, TG, LDL-C, SOD, and body weight. Hence, Egger's test and funnel plot were performed. No publication bias was found regarding the impact of PDB extracts on FINS ($p = 0.899$) and body weight ($p = 0.108$). However, potential publication bias existed regarding FBG ($p < 0.001$), TG ($p < 0.001$), TC ($p < 0.001$), HDL-C ($p < 0$ .001), LDL-C ($p < 0.001$), MDA ($p < 0.001$), and

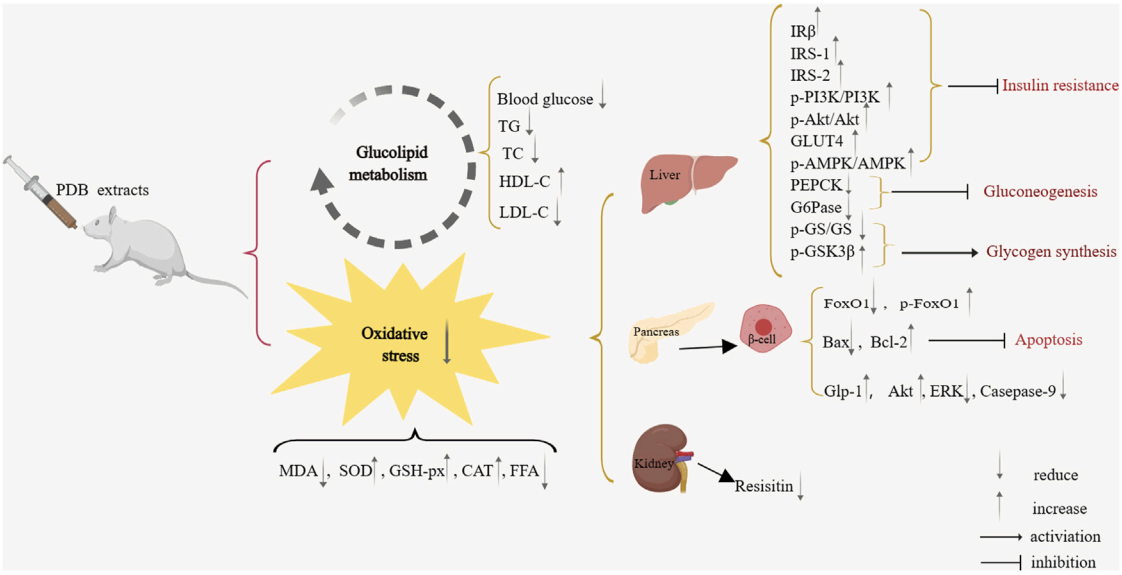

FIGURE 12
The mechanism of PDB extracts in ameliorating diabetes mellitus.

SOD ($p < 0.001$). Details are provided in Supplementary Material S4. Then, the trim-and-fill method was used to determine the impact of publication bias on the outcomes. Trim-and-fill methods for FBG, TG, TC, LDL-C, MDA, and SOD showed no trimming, and the results were unchanged. Regarding HDL-C, the result was also not reversed after filling in one study. Hence, the findings of this meta-analysis were all robust. Detailed results of the trim-and-fill method are provided in Supplementary Material S5.

4 Discussion

To our knowledge, this is the first preclinical systematic review and meta-analysis that evaluated the efficacy of PDB extracts on oxidative stress and glycolipid metabolism in animal models of diabetes. In total, 32 studies with 574 animals were included, and 16 outcomes were analyzed. DM is characterized by chronic hyperglycemia and is closely related to dyslipidemia. Our study demonstrated that the PDB extracts increased HDL-C levels while decreasing TC, TG, LDL-C, and FBG levels. Although there was publication bias, the results did not change after the trim-and-fill method, suggesting their reliability. Additionally, the sensitivity analysis indicated that the outcomes were stable. These demonstrated that the extracts of PDB have great potential in improving glycolipid metabolism. Overall pooled data revealed no discernible difference in FINS between the control and treatment groups. Sensitivity analysis suggested that after removing three studies, the combined results showed that PDB extracts reduced insulin secretion. The findings of these three studies demonstrated that PDB extracts enhanced insulin secretion. After careful consideration, these articles were not removed because, as a compensatory mechanism, FINS increases or decreases in different stages of DM. When insulin resistance is dominant, FINS shows a compensatory increase; insulin secretion decreases when the islet β-cell is damaged. In this study, the methods of animal modeling were different. Some animals were given single or double injections of Alloxan or STZ, resembling type 1 diabetes, in which β cell damage is dominant. (Syed, 2022). Under this situation, PDB extracts promoted the repair of β cells and increased insulin secretion. Some animals were fed a high-fat diet and received low doses of STZ or alloxan, resembling the T2DM model, characterized by insulin resistance and partial dysfunction of β cells. (Stumvoll et al., 2005). In this case, PDB extracts reduced insulin resistance and increased insulin sensitivity, thus reducing the secretion of FINS. Therefore, the increase and decrease in FINS after using PDB extracts are not contradictory. Weight loss is a typical symptom of DM. According to our study, PDB extracts can dramatically enhance body weight. PDB was shown to improve the diversity, composition, and structure of intestinal flora in rats with T2DM, which may affect body weight (He S. Y. et al., 2022). Nevertheless, more studies are needed to uncover the precise mechanism.

Oxidative stress, a significant contributor to DM, is caused by increased production of reactive oxygen species (ROS), decreased levels of endogenous antioxidants, or both (Zhang et al., 2020). In normal conditions, endogenous antioxidants such as SOD, GSH-px, and CAT can protect against oxidative stress (Banerjee and Vats, 2014; Mansuroğlu et al., 2015). Hyperglycemia and FFA can lead to ROS overproduction (Inoguchi et al., 2000; Zhang et al., 2020); Uncontrolled oxidative stress can increase MDA levels (Del Rio et al., 2005), causing insulin resistance and β cell malfunction (Zhang et al., 2020). This study showed that PDB extracts

could considerably lower MDA and FFA levels while raising SOD, GSH-px, and CAT levels. These findings indicate that the PDB extracts possess obvious antioxidant properties. According to the existing literature, some studies have explored the specific mechanisms by which PDB meliorates DM. Most of these studies showed that PDB reduces oxidative stress. PDB extracts also activated protein kinase B (Akt) and AMP-activated protein kinase (AMPK) and inhibited phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) expression in the liver to prevent hepatic gluconeogenesis, decrease glycogen synthase (GS) phosphorylation and enhance glycogen synthase kinase 3β (GSK3β) phosphorylation to promote glycogen synthesis (Li et al., 2020). Furthermore, PDB extracts promoted the expression of insulin receptor β (IRβ), insulin receptor substrate-1 (IRS-1), insulin receptor substrate-2 (IRS-2), and glucose transporter-4 (GLUT4) in the liver to enhance insulin sensitivity and reduce insulin resistance (Hu et al., 2014; Kong et al., 2021). One study showed that PDB extracts upregulated the expression of glucagon-like peptide-1 (GLP-1) and Akt and downregulated the expression of the extracellular signal-regulated kinase (ERK) and caspase-9 to protect β cells (Tan et al., 2020). Besides, PDB extracts reduced the expression of Bax and increased the expression of Bcl-2 to inhibit the apoptosis of β cells (Liu et al., 2016). In addition, decreasing the expression of FOXO1 and increasing the expression of p-FoxO1 are other mechanisms by which PDB protects β cells (Ding et al., 2016; Luo et al., 2020). Only one study investigated the effects of PDB extracts on the kidney, showing that PDB significantly reduced the mRNA expression of resistin in perirenal adipose tissue of rats to reduce insulin resistance (Zong et al., 2007). These mechanisms are shown in Figure 12. Except for ameliorating oxidative stress, whether PDB extracts improve DM by reducing inflammation is still unknown. More studies should be conducted in the future.

This systematic review also has its limitations. Firstly, most of the included studies were published in Chinese, which may lead to language bias. Second, the risk of bias evaluation revealed that the majority of studies did not report the baseline data of the animals, the method of randomization, and whether blinding was used for caregivers and assessors; therefore, a majority of studies scored three to four points out of 10, this may reduce the credibility of the results. Third, there was a lack of data on the adverse effects of PDB extracts. The absence of safety data hampered our evaluation of the long-term tolerability of PDB extracts. Fourth, although subgroup analyses and meta-regression were performed according to animal species, constituents of PDB extracts, and duration of treatment, heterogeneity did not significantly decrease, which may be due to other factors, such as modeling and statistical methods. Thus, it is difficult to find the most prominent factors affecting heterogeneity. Therefore, the random-effects model was used to avoid the influence of heterogeneity on the results as much as possible. Fifth, although 32 studies were included in this systematic review, not all disclosed desired outcomes. Therefore, some indicators, such as NO and NOS, may have been misinterpreted due to the limited number of articles.

The findings of this study may have some implications for future research. The extracts discussed in this systematic review included aqueous extracts, flavonoids, and triterpenes. The aqueous extracts are provided by concentrating the liquid after boiling PDB. Its components are undoubtedly more complex than flavonoids and triterpenes. Flavonoids are found in almost all parts of PDB, constituting approximately 20% of PDB extract, with many pharmacological and physiological activities (Fu, 2017; Qin et al., 2020). Subgroup analyses showed that flavonoids and aqueous extracts were equally effective on most indicators. Therefore, we speculated that PDB ameliorates DM primarily through flavonoids. Although, further studies are needed to verify this hypothesis. Hong *et al.* found seven flavonoid monomers in PDB (Hong et al., 2013). Li et al. found six triterpenoid monomers in PDB (Li et al., 2013). Previous studies have roughly verified the anti-diabetic efficacy of PDB extracts. Further studies are needed to determine which monomer is the most effective for DM. In addition, whether PDB extracts exert the same therapeutic effects in the human body remains to be verified with well-designed RCTs in the future.

5 Conclusion

Current evidence suggests that PDB extracts can improve glycolipid metabolism and oxidative stress, alleviate pancreatic β-cell injury, reduce insulin resistance, and increase insulin sensitivity in diabetic animals. Taken together, the results suggested that PDB might be a promising medication for treating DM. However, the findings should be interpreted with caution due to the significant heterogeneity between studies and the low-to-moderate quality of studies. In addition, it is necessary to investigate which constituent of PDB plays the major role in ameliorating DM and whether these benefits can be reproduced in the human body.

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

Conceptualization, YY and QC; data collection, YY and WD; statistic analysis, YY and YW; draft the manuscript, YY, WD and YW; visualization, CZ; supervision, QC; All authors contributed to the article and approved the submitted version.

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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.2023.1218757/full#supplementary-material

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Efficacy and safety of the Chinese herbal medicine Xiao Yao San for treating anxiety: a systematic review with meta-analysis and trial sequential analysis

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Introduction: The effectiveness and safety of the Chinese herbal medicine (CHM) Xiao Yao San (XYS) used for treating anxiety disorders are still unknown. Thus, we conducted this systematic review with meta-analysis and trial sequential analysis (TSA) to determine its safety and efficacy.

Methods: We searched 12 databases for relevant studies from the inception of each database till 10 August 2023. We selected randomized controlled trials to compare the efficacy and safety of XYS (including XYS only and XYS + anxiolytics) to those of anxiolytics in patients with anxiety.

Results: We found 14 trials with 1,256 patients in total that met the requirements for inclusion. We assessed the majority of studies (8 out of 14) as being at high risk of bias; 6 were assessed as having a moderate risk of bias. Three trials compared oral XYS to anxiolytic medication, and 11 trials compared oral XYS plus anxiolytics to anxiolytic treatment alone. The pooled results showed that the efficacy of treatment in the XYS + anxiolytics groups was significantly higher than that of the anxiolytics alone group (RR = 1.19; 95% CI: [1.13, 1.26]; $p < 0.00001$; $I^2 = 0$) and the adverse event rates in the XYS + anxiolytics groups were significantly lower than those in the anxiolytics alone group (RR = 0.44; 95% CI: [0.28, 0.82]; $p = 0.001 < 0.05$; $I^2 = 13$). The efficacy of treatment in the XYS alone groups was also significantly higher than that of the anxiolytics alone groups (RR = 5.41; 95% CI: [2.23, 13.11]; $p < 0.0001$; $I^2 = 0$). However, there was no statistical difference between the adverse events of the XYS alone group and the anxiolytics alone group, although the incidence of adverse events in the XYS alone group was lower than that in the anxiolytics alone group. The results of the TSA confirmed the above findings.

Conclusion: The use of XYS combined with anxiolytics for treating anxiety was found to be safe and effective. However, although XYS alone is effective in the treatment of anxiety disorder, more large-scale research is needed to investigate adverse events.

Systematic Review Registration: https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=350358, identifier CRD42022350358.

KEYWORDS

anxiety disorder, Chinese herbal medicine, Xiao Yao San, systematic review, meta analysis, trial sequential analysis

1 Introduction

Anxiety disorder is a common psychiatric disorder, which has become a major public global health problem. Anxiety disorder has high morbidity and ranked 24th in terms of disability-adjusted life years (DALYs) in the global prevalence estimates and disability weights in 2019 (Collaborators, 2022). Since the onset of the COVID-19 pandemic, the prevalence of anxiety disorders has increased significantly. In 2020, the prevalence of anxiety disorders worldwide was 26% (Collaborators Covid- Mental Disorders, 2021). It was estimated that due to the COVID-19 pandemic, around 76 million new cases of anxiety disorder would arise globally (Collaborators Covid- Mental Disorders, 2021).

Anxiety disorder is characterized by excessive anxiety and worry. It affects daily physical, psychological, and social functions, and its pathophysiological mechanism is not clear. Studies on the pathophysiological mechanism of anxiety involve genetics, basic neuroscience, transformation research of functional MRI, etc. (Penninx et al., 2021).

Psychotherapy, drug therapy, and combination therapy are recommended for the clinical treatment of anxiety disorder (Slee et al., 2019; Penninx et al., 2021). Psychotherapy is the first-line treatment for anxiety disorder, but popularizing it in clinical practice was difficult due to the limited resources of psychotherapy, the lack of qualification and experience of clinicians, the time required to show effect, and the cost to patients (Moritz et al., 2019). Drug treatment is often regarded as the first choice for treating anxiety disorder, as it is cheap and accessible (Slee et al., 2019). The drugs used for treating anxiety disorders include antidepressants, benzodiazepines (BZs), other anxiolytics (buspirone, agomelatine, pregabalin, and quetiapine), etc. (Slee et al., 2019).

Antidepressants, including selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (SNRIs), and tricyclic antidepressants (TCAs), are often used for treating anxiety disorders. However, at the beginning of treatment, antidepressants may have adverse reactions, such as increased anxiety symptoms, irritability, insomnia, dizziness, and nausea. The onset of anti-anxiety effects has a latency of 2–6 weeks (Slee et al., 2019). Discontinuation of anxiolytics may cause withdrawal symptoms, and adverse events such as sexual dysfunction and gastrointestinal bleeding might occur, which can affect the compliance of patients with medication (Baldwin et al., 2011). Benzodiazepines can produce anti-anxiety effects within 30–60 min, but may affect cognitive function, produce dizziness, prolong reaction time, and other adverse reactions. Short-term administration of benzodiazepines for treating anxiety disorders is usually safe and effective, and maintenance treatment is needed to weigh the risks and benefits (Bandelow et al., 2022). Other anxiolytics (buspirone, agomelatine, pregabalin, and quetiapine) have adverse reactions, such as hepatotoxicity, weight gain, and discontinuation symptoms, which might reduce the patient's medication compliance (Baldwin et al., 2011; Freiesleben and Furczyk, 2015; Maneeton et al., 2016).

Based on the limitations of clinical treatment methods, as prospective therapeutic and preventative strategies for anxiety, complementary and alternative medicine (CAM) and traditional Chinese medicine (TCM) have received attention. XYS is a traditional Chinese medicine prescription, first recorded in the "Taiping Huimin Heji Jufang". The eight Chinese herbs that are found in XYS are Chai Hu (Radix Bupleuri), Bai Shao (Radix Paeoniae alba), Dang Gui (Radix Angelicae sinensis), Bai Zhu (Atractylodes Ovata), Fu Ling (Poria Cocos), mint (Herba Menthae), licorice (Radix Glycyrrhizae), and Stewed ginger (Rhizoma Zingiberis Recens). All significant XYS components, including paeoniflorin, quercetin, luteolin, farnesin, aloe emodin, glyasperin C, and kaempferol, were identified by the UPLC-Q-TOF/MS analysis. The primary chemicals were flavonoids (Yuan et al., 2020). For thousands of years, XYS has been used to treat mental illnesses and functional gastrointestinal disorders (Liu et al., 2021; Zhu et al., 2022). Several studies have also shown that using XYS to treat anxiety results in anxiolytic activity. Additionally, the administration of XYS to treat anxiety was found to cause anxiolytic activity (Cao et al., 2016). Some animal studies have shown that XYS can reduce the increase in synuclein and corticosterone caused by chronic stress. It can also downregulate protein phosphatase 2A (PP2A) in the hippocampus to produce anti-anxiety and neuroprotective effects (Cao et al., 2016).

XYS is frequently used for treating mental illnesses. Although XYS is frequently used to treat depression (Yuan et al., 2020; Liu et al., 2021), its effectiveness in treating anxiety disorders is still unclear (Ding et al., 2013; Wang, 2014). In several randomized controlled trials (RCTs), XYS had positive curative effects on anxiety disorder (Xiong and Song, 2019; Zhou et al., 2021). However, more evidence is needed to confirm the safety and effectiveness of XYS in the treatment of anxiety disorders. Therefore, we performed a systematic review through meta-analysis to compile the evidence for the treatment of anxiety disorder with XYS to objectively assess the literature. This review might serve as a reference for future therapeutic medication and clinical research. We also performed trial sequential analysis (TSA) to estimate the sample size.

2 Materials and methods

The literature review did not require ethical approval. The Preferred Reporting Items for systematic reviews and meta-analyses (PRISMA) were followed in the planning, execution, and reporting of the findings of this study (Page et al.). The PRISMA checklist is available in the Supplementary Material. The protocol is registered in PROSPERO under the registration number CRD42022350358 (Gao et al., 2021).

2.1 Literature search strategy

In total, 12 databases, including Embase, MEDLINE, Cochrane Library, PsycINFO, The Allied and Complementary Medicine Database (AMED), PubMed, the Web of Science, Scopus, the China National Knowledge Infrastructure database (CNKI), Wanfang Data, the China Biomedical Medicine database (SinoMed), and VIP Chinese Medical Journal Database(CMJD), were searched from their inception to 10 August 2023. Keywords or forms of keywords used in the database searches included Anxiety, anxiety disorders, xiaoyao san, xiaoyao formula, kami-shoyo-san, gamisoyo-san, soyo-san, kamo-soyo-san. A copy of the search strategies used for each database is shown in Supplementary Material. To obtain more information, reference materials, and conference proceedings were personally reviewed. When necessary, we contacted the associated authors to obtain any material that was omitted or incomplete. Chinese characters were used to search for terms in the electronic databases in China.

2.2 Inclusion criteria

The studies considered for additional analysis were based on the following criteria: 1) All studies were randomized controlled trials (RCTs). 2) Participants: The International Classification of Diseases, the Diagnostic and Statistical Manual of Mental Disorders, and the Chinese Classification of Mental Disorder require that all enrolled patients meet specific diagnostic criteria for anxiety; 3) Patients in the intervention groups received either XYS (no restrictions on dosage, formula, or dosage form) or XYS combined with anxiolytics, whereas, the control group received only anxiolytics; 4) Results: At least one important outcome with valid and readily accessible data.

2.3 Exclusion criteria

Cases that matched the criteria below were disqualified: 1) Anxiety disorder along with other conditions, including Parkinson's disease, dermatitis, cerebral infarction, etc. 2) Insufficient information on the medication used in the experiment; 3) Experiments in which the baseline data for the observation group and the control group were distinct and incomparable; 4) The treatment group was administered Chinese patent medications other than XYS; 5) Incomplete information or mistakes; 6) No extractable outcome indicators were available; 7) Repetitive publication of studies retaining one article; 8) Non-journal papers.

2.4 Outcome measures

The Clinical Efficacy Rate, the score on the Hamilton Anxiety Rating Scale (HAMA), and the Adverse Effects Rate were the main outcomes. The secondary outcomes included the SAS score, the PSQI score, and the TESS score.

2.5 Data extraction and quality assessment

Using the inclusion and exclusion criteria mentioned above, two researchers, Yifan Wang and Xiaofeng Chen, independently downloaded articles from the databases and extracted the data using the template data extraction form. Basic study parameters (diagnosis criteria, research duration, and total sample size), interventions (drugs, dosages, formulae, and dosage form), and outcomes were included in the data that were taken from each report. The baseline patient characteristics included gender distribution, disease course, and age. Two researchers cross-checked and consulted with each other and with another researcher (Prof. Guo), or, if necessary, the authors of the studies were contacted to settle disagreements in the included papers.

The Cochrane Risk of Bias tool (Higgins et al., 2022) was used to assess the methodological quality of the selected studies. To assess the quality of evidence and interpret the results, we applied six RoB criteria (random sequence generation, allocation concealment, blinding of participants, blinding of outcome assessors, incomplete outcome data, and selective outcome reporting) to categorize each trial. A trial would be categorized as having a low risk of bias if none of the domains above were rated as high risk of bias, and two or fewer were classified as unclear risk. A trial would be categorized as having a moderate risk of bias if one domain was rated as high risk of bias, one or fewer domains were classified as unclear risk, or no domains were rated as high risk of bias but three or fewer were classified as unclear risk. All other cases were considered to fall under the category of high risk of bias (Sbidian et al., 2023). Two researchers, Jialin Wang and Jia Xing, independently assessed the risk of bias. Prof. Guo, another author, was also invited to settle any disagreements.

2.6 Data synthesis and statistical analysis

The risk ratio (RR) and mean differences (MD) were used in the meta-analysis, which was conducted using RevMan 5.4.1 (The Cochrane Collaboration). The overall mean differences and the related 95% confidence intervals were calculated using the random-effects model when heterogeneity ($I^2 > 50\%$) was found between various treatment groups in the included studies. Otherwise, the fixed-effects model was used to perform the calculations. All differences were considered to be statistically significant at $p < 0.05$. Different studies had different definitions of effective rate, and we speculated that significant clinical heterogeneity was present. Hence, we used the random effects model (Higgins et al., 2022). Publication bias was examined by conducting funnel plot analyses if the number of studies was ≥10. Sensitivity analyses were conducted to examine the impact of individual studies on the overall effect estimate.

2.7 Trial sequential analysis

To reduce the possibility of a false-positive or false-negative conclusion, the Copenhagen Trial Unit, Centre for Clinical Intervention used TSA Version 0.9.5.10 Beta to perform the Trial Sequential Analysis (TSA) (Pogue and Yusuf, 1997; Brok et al., 2008). The models for this TSA set the type I error (α) at 0.05 and a power of 80% (two-sided) for all outcomes. The sequential monitory boundary varied each analysis in this TSA, and the usual significance boundary was −1.96 to 1.96.

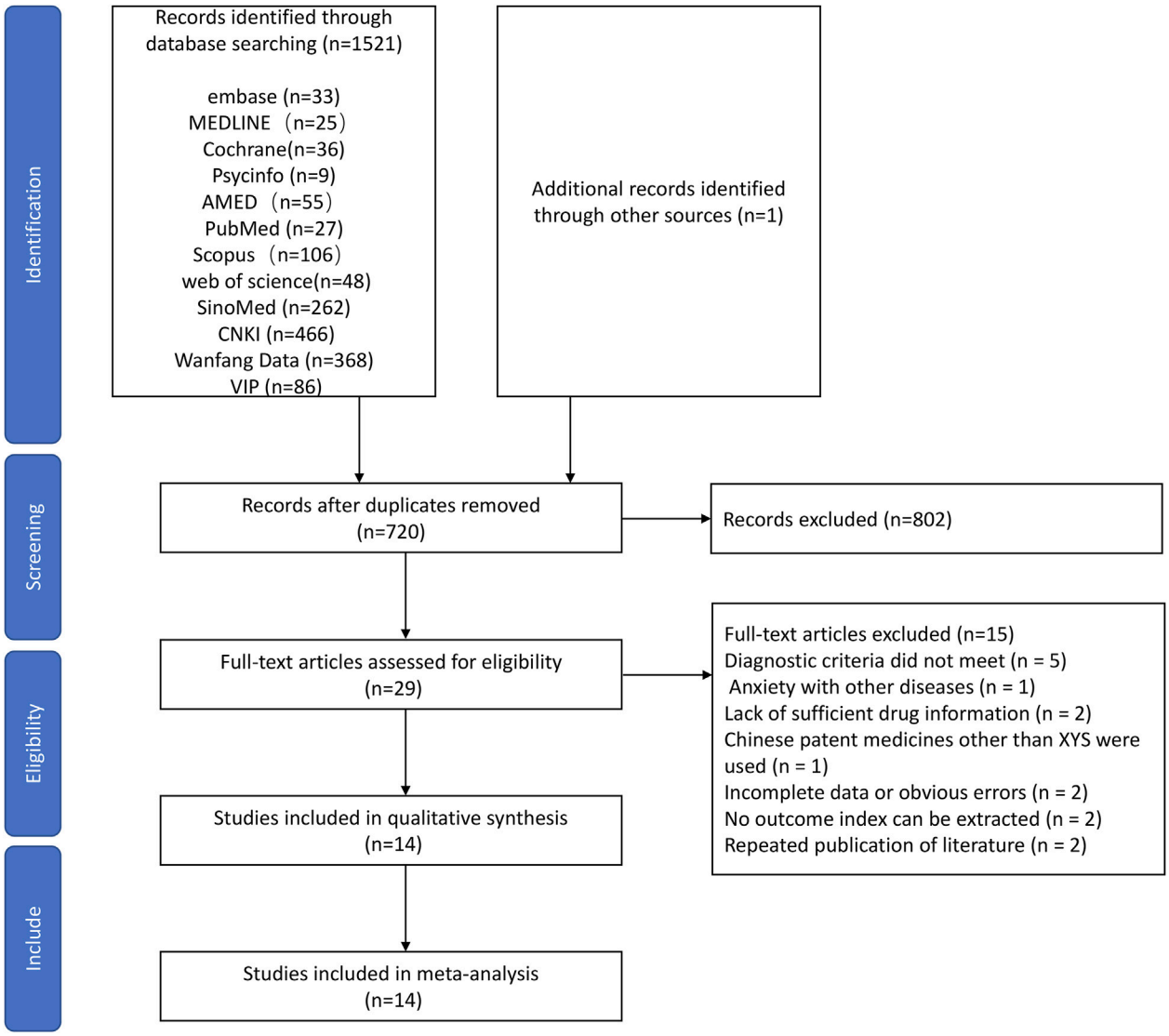

FIGURE 1
A flow diagram of the screening process used to select the articles in this study.

3 Results

3.1 Study selection

Using the predefined search strategy, we obtained 1,522 studies in total. After removing duplicates, 720 studies remained. After excluding 691 articles by screening the title and abstract, 29 articles were left for a full-text analysis of the duplicates. In total, 15 papers were rejected. The diagnostic standards were not met in five studies; various diseases and anxiety were recorded in 1 studies; inadequate medication information was recorded in two studies; other Chinese patent drugs besides XYS were administered to the treatment group in one study; incomplete information or glaring mistakes were found in two studies; no outcome index could be retrieved from two studies; repeated publication of data in two study. Finally, 14 RCTs (Wang et al., 2010; Han et al., 2011; Ding et al., 2013; Lv et al., 2013; Wang, 2014; Zhu, 2015; Chen and Du, 2016; LI and Qin, 2016; Deng et al., 2017; Li et al., 2017; Shi, 2018; Xiong and Song, 2019; Zhou et al., 2021; Li et al., 2022) were eligible for the study. The screening process of the systematic review is shown in Figure 1.

The selected RCTs included 1,256 people, 633 of whom were in the intervention groups, and 623 were in the control groups. The baseline information for the treatment and control groups was similar. Anxiolytics were used in the treatment plans for the individuals in the control groups. Chinese herbal medicine XYS or XYS combined with anxiolytic drugs was administered to the patients in the treatment groups. The data on the characteristics of those studies are summarized in Table 1.

3.2 Risk of bias assessment

Six studies (Deng et al., 2017; Li et al., 2017; Shi, 2018; Xiong and Song, 2019; Zhou et al., 2021; Li et al., 2022) explained the randomization procedure (random number table). The patients were divided into groups by parity order of enrollment (Wang,

TABLE 1 Characteristics of the included studies.

Study ID	Diagnostic criteria	Sample size		Course of disease (month)		Mean age (year)		Male Proportion(Male %)		Intervention		Course of treatment (week)	Outcomes
		Trial	Control	Trial	Control	Trial	Control	Trial	Control	Trial	Control		
Deng et al. (2017)	CCMD-3	59	57	18.18 ±13.56	17.93 ±12.84	43.81 ±10.23	44.53 ±11.65	44.07%	52.63%	XYS + C	Paroxetine	4	CER, HAMA-14, AER
Ding et al. (2013)	CCMD-3	37	38	25.15 ±10.84(day)	27.21 ±6.72(day)	29.46 ±6.82	27.62 ±4.24	NR	NR	XYS + C	Buspirone + Lorazepam	8	CER, HAMA-14, SAS
Han et al. (2011)	CCMD-2R	40	40	18–155	12–168	22–77	23–76	42.50%	37.50%	XYS + C	Deanxit	12	CER, HAMA-14
Li and Qin (2016)	CCMD-3	32	32	57.6 ±28.8	58.8 ±30.0	41.6 ±7.8	42.1 ±7.2	46.88%	43.75%	XYS + C	Buspirone	6	CER, HAMA-14, TESS
Li et al. (2022)	CCMD-3	40	40	≥1	≥1	48.50 ± 1.25	46.00 ± 1.06	42.50%	40.00%	XYS + C	Buspirone	6	CER, HAMA-14, AER
Lv et al. (2013)	CCMD-3	36	35	12.3 ±NR	11.2 ±NR	35.5 ±NR	34.4 ±NR	33.30%	40.00%	XYS + C	Deanxit + psychological counseling	8	CER, HAMA-14
Wang (2014)	CCMD-3	30	30	18–204	12–180	21–75	23–72	33.30%	40.00%	XYS + C	Paroxetine	8	CER, HAMA-14
Wang et al. (2010)	CCMD-3	30	30	NR	NR	48 ±NR	51 ±NR	53.33%	50.00%	XYS + C	Deanxit	6	CER, HAMA-14
Xiong and Song. (2019)	CCMD-3	100	100	NR	NR	57.8 ±6.5	55.4 ±6.2	65.00%	62.00%	XYS + C	Deanxit(+Tandospirone for severe patients)	8	CER, HAMA-14, AER, PSQI
Zhou et al. (2019)	CCMD-3	35	35	4.37 ±2.52	4.37 ±2.52	44.86 ±10.81	46.29 ±12.28	34.29%	28.57%	XYS + C	Mirtazapine	4	CER, HAMA-14, AER, PSQI
Zhu (2015)	CCMD-3	28	28	61.68 ±29.52	61.68 ±29.52	42.86 ±14.12	42.86 ±14.12	NR	NR	XYS + C	Buspirone	6	CER, HAMA-14, TESS
Chen and (2016)	DSM-IV	78	76	23.28 ±5.72	23.12 ±3.37	43.65 ±8.01	43.93 ±10.54	49.38%	51.28%	XYS	Deanxit	6	CER, HAMA-14, SAS
Li et al. (2017)	CCMD-3	48	42	17.86 ±7.34	17.76 ±7.84	55.15 ±11.50	54.71 ±10.77	39.58%	42.86%	XYS	Lorazepam	4	CER, HAMA-14, AER, TESS
Shi (2018)	ICD-10	40	40	NR	NR	33.4 ±12.8	34.3 ±12.4	47.50%	45.00%	XYS	Deanxit	4	CER, HAMA-14

Abbreviations: AER, adverse events rates; CCMD, Chinese classification of mental disorders; CER, clinical efficacy rates; DSM, diagnostic and statistical manual of mental disorders; ER, efficacy rates; HAMA-14, Hamilton Anxiety Scale-14; ICD, international statistical classification of diseases and related health problems; NR, not reported; PSQI, pittsburgh sleep quality index; SAS, Self-Rating Anxiety Scale; TESS, treatment emergent symptom scale.

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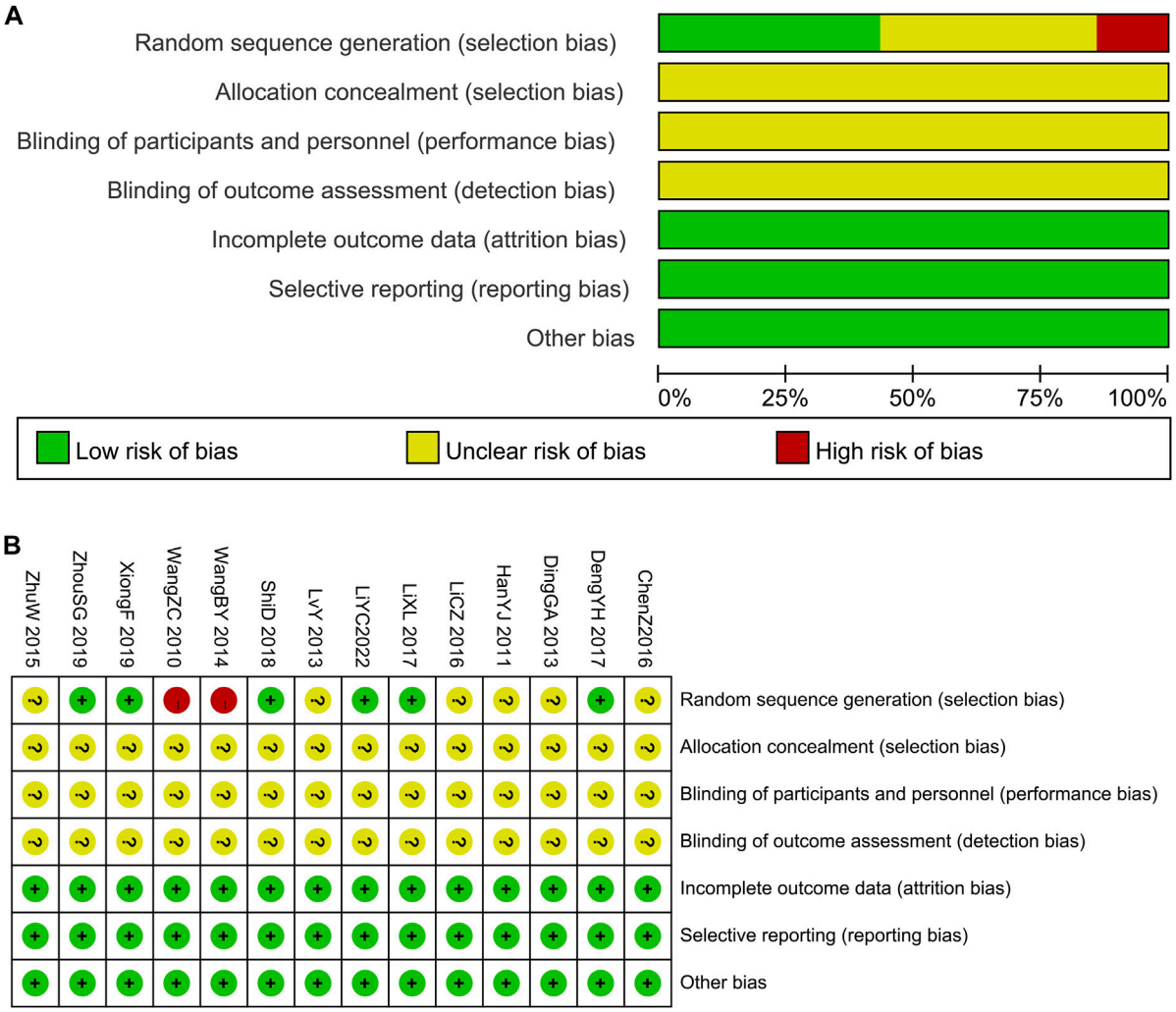

FIGURE 2
The risk of methodological bias in the included studies. **(A)** Risk of bias graph. **(B)** Risk of bias summary.

2014), and it was considered as high risk. Additionally, the other studies did not outline their randomization approach completely. Uncertainties were recorded regarding allocation concealment and outcome assessment blinding. These studies were not double-blinded. Complete data and reliable results were available for all articles included, as stated in the section on techniques. No differences occurred in the baseline data of these studies. The results of the risk of bias assessment are presented in Figure 2.

In terms of overall bias risk, six studies (Deng et al., 2017; Li et al., 2017; Shi, 2018; Zhou et al., 2021; Li et al., 2022) were considered to have a moderate risk of bias, while eight studies (Wang et al., 2010; Han et al., 2011; Ding et al., 2013; Lv et al., 2013; Wang, 2014; Zhu, 2015; Chen and Du, 2016; LI and Qin, 2016) were considered to have a high risk of bias.

3.3 Outcome measures

The outcomes of treatment with XYS combined with anxiolytics were compared to the outcomes of treatment with anxiolytics in 11 of 14 studies, whereas three studies compared the outcomes of treatment with oral XYS alone to those of treatment with anxiolytics.

The HAMA scores and clinical symptoms were the main means to gauge the clinical effectiveness. Eight studies (Wang et al., 2010; Ding et al., 2013; Wang, 2014; Zhu, 2015; LI and Qin, 2016; Zhou et al., 2021; Li et al., 2022) reported the clinical efficacy rate by a HAMA score reduction rate >25%. A HAMA score reduction rate of >30% was reported as the clinical effectiveness rate in one study (Chen and Du, 2016).

In a study (Deng et al., 2017), the clinical efficacy rate was calculated using a HAMA score drop rate of >30% and combined with the clinical symptoms. Using the clinical symptoms, two studies (Han et al., 2011; Lv et al., 2013) reported the clinical effectiveness rate. A decrease in the Chinese medicine syndrome scale score of >30%, along with the clinical symptoms, was used in one study (Li et al., 2017) to determine the clinical effectiveness rate. One study (Shi, 2018) found that a reduction rate of >25% in the Chinese medicine syndrome scale score indicated therapeutic efficacy.

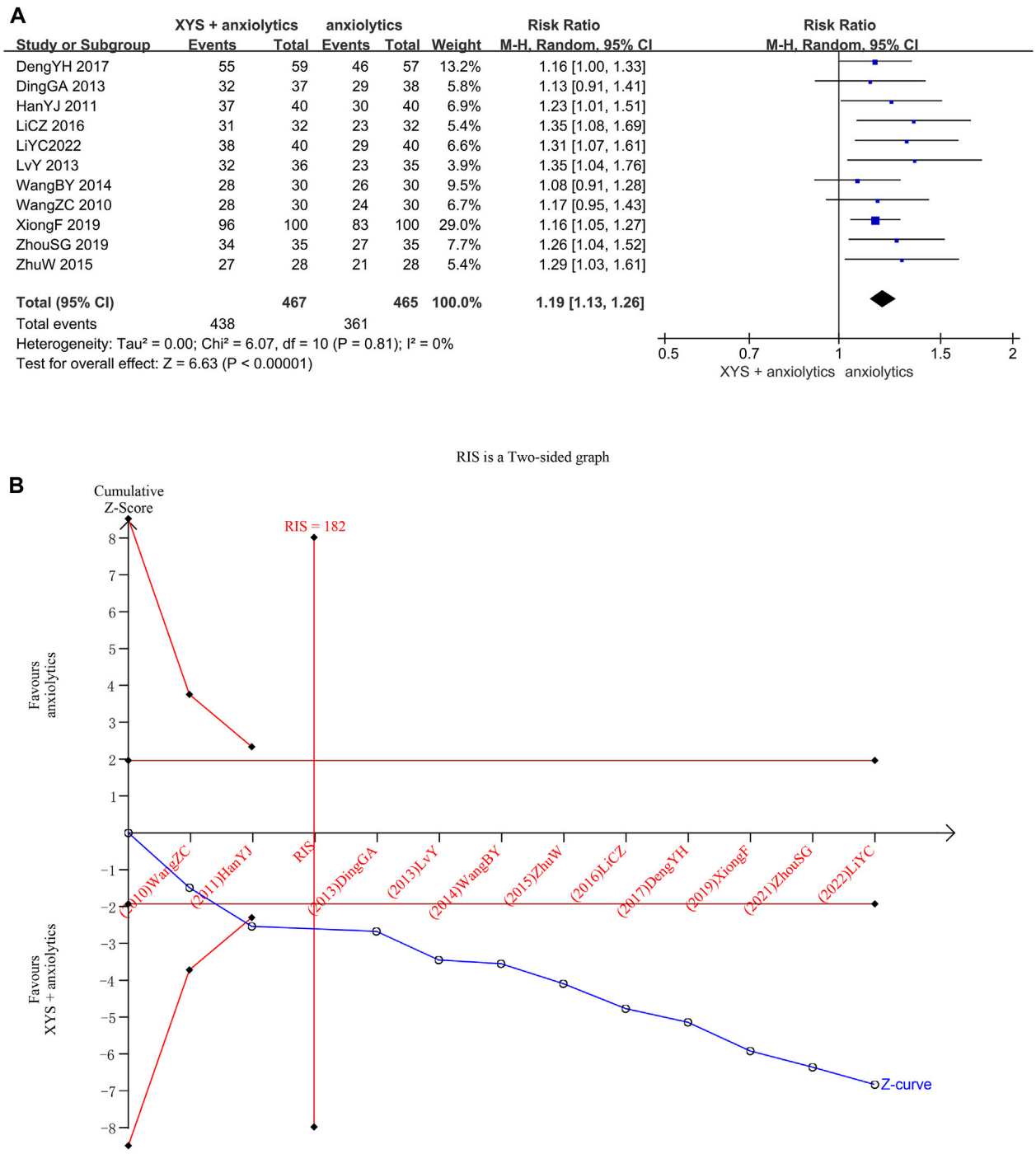

FIGURE 3
Data analysis of clinical efficacy rates (XYS combined with anxiolytics vs. anxiolytics alone). **(A)** Meta-analysis. **(B)** Trial sequential analysis. The blue line represents the cumulative number of cases in the meta-analysis, also called the "Z-curve". The horizontal straight red line shows the conventional test boundary, which is a traditional significant horizontal line (α = 0.05). The twisted red line represents the trial sequential monitoring boundary, which is an interface curve formed by correcting the random error generated by the meta-analysis. The vertical, straight red line represents the required information size (RIS) and refers to the number of cases required for meta-analysis to obtain statistically significant differences. The cumulative Z-curve surpassed the point of no return and increased to the necessary information size.

3.3.1 Comparison of XYS combined with anxiolytics to anxiolytics alone

3.3.1.1 Clinical efficacy rates

In 11 studies (932 patients), the clinical efficacy rates were reported. Between different investigations, heterogeneity was not detected ($Chi^2 = 6.07$, $p = 0.81$, $I^2 = 0\%$). The efficacy achieved in the treatment groups was considerably higher than that in the control groups, determined by the results of the random effects model (RR = 1.19, 95% CI: [1. 13, 1. 26], $p < 0.00001$; Figure 3A). Publishing bias was not found (the Egger's test yielded a p-value of

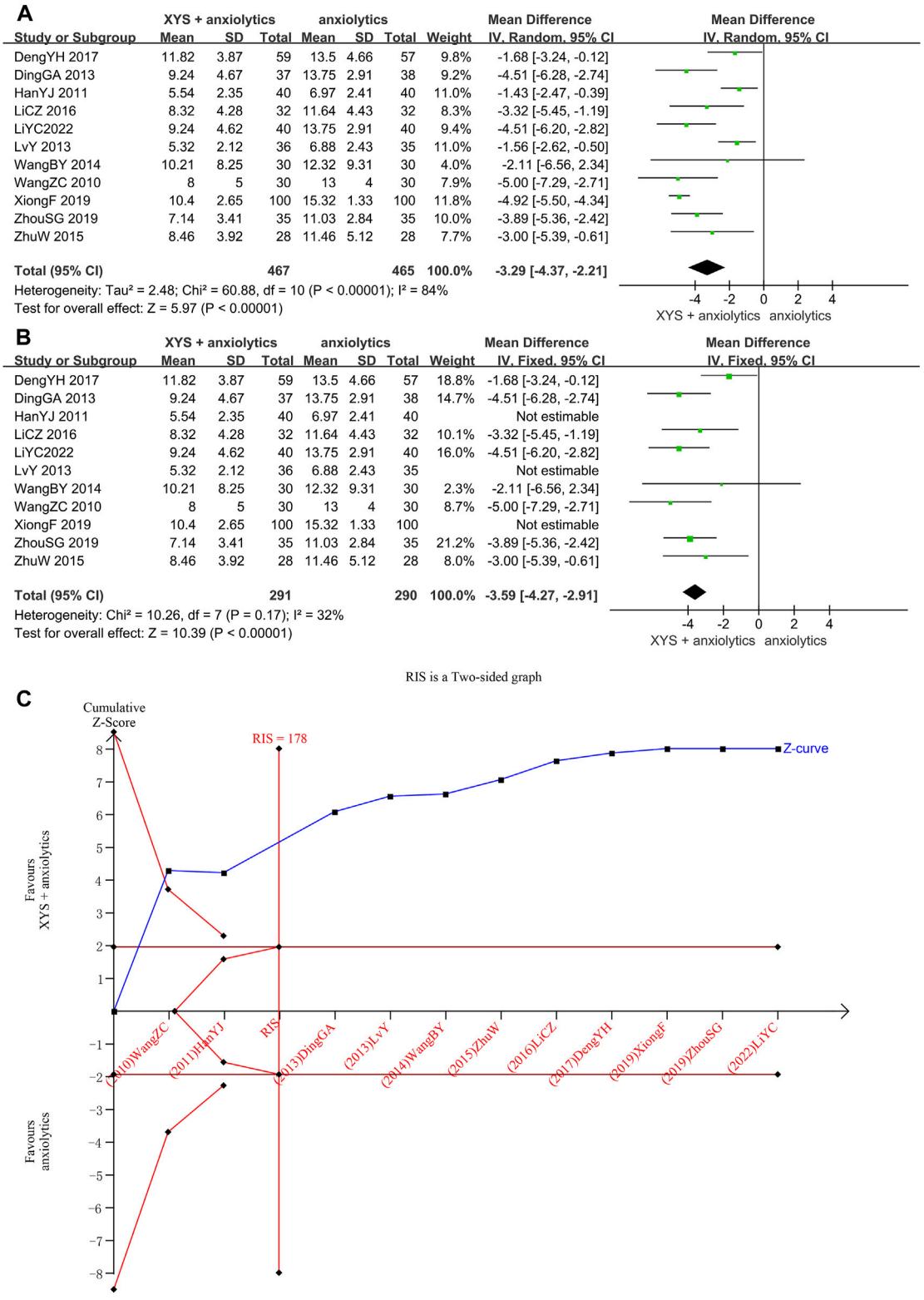

FIGURE 4
Data analysis of the HAMA-14 scores (XYS combined with anxiolytics vs. anxiolytics alone). **(A)** Before eliminating sources of heterogeneity. **(B)** After eliminating sources of heterogeneity. **(C)** Trial sequential analysis. The annotations of the red line and blue line in TSA are the same as those in Figure 3.

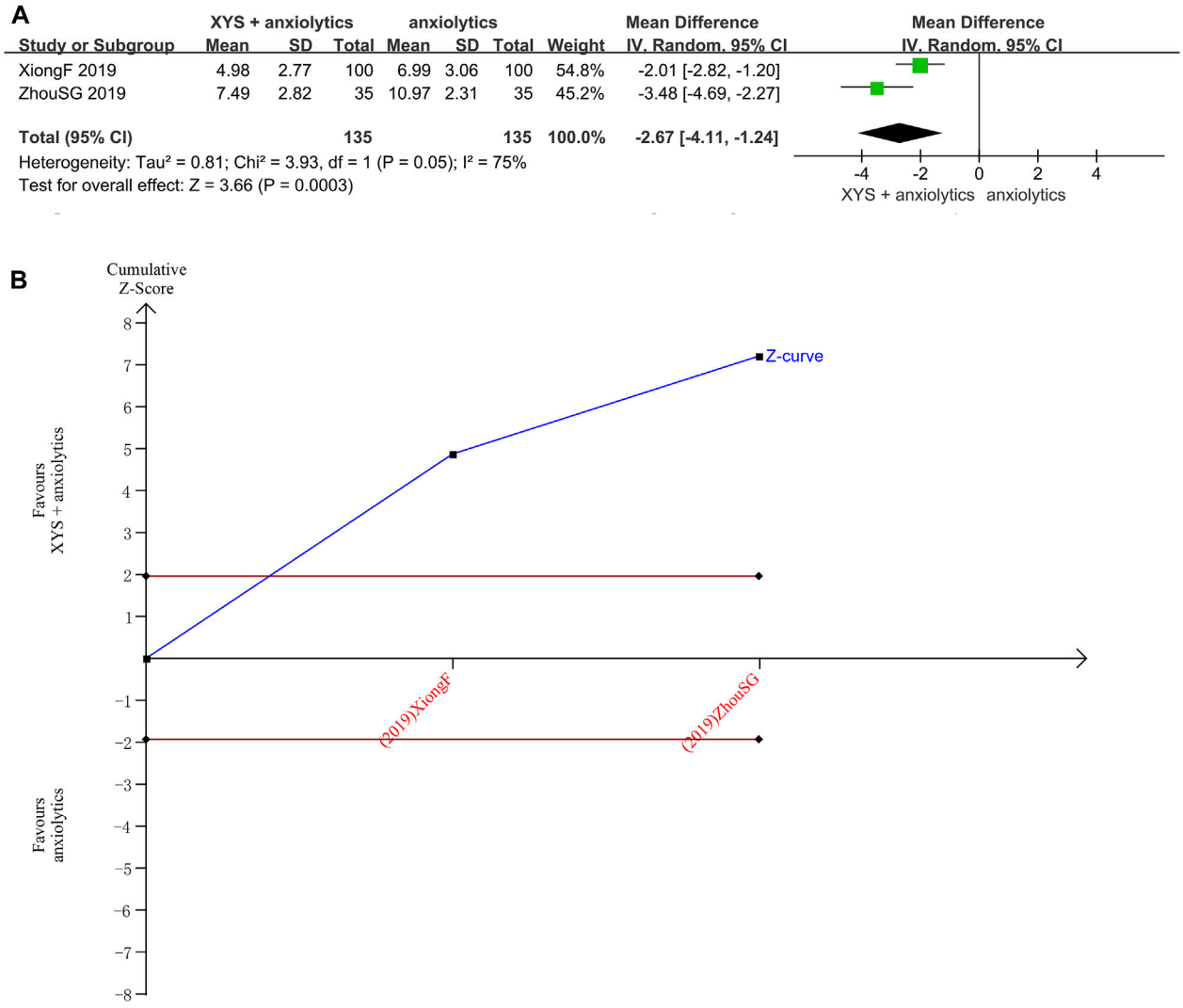

FIGURE 5
Data analysis of the PSQI scores (XYS combined with anxiolytics vs. anxiolytics alone). **(A)** Meta-analysis. **(B)** Trial sequential analysis. The annotations of the red line and blue line in TSA are the same as those in Figure 3.

0.054.), as determined by the symmetry of the funnel plot (Supplementary Figure S1).

We confirmed the reliability of the meta-analysis through TSA. The cumulative Z-curve crossed the trial sequential boundary, and the traditional boundary after the second study was included, indicating a low probability of false positives. When the third study was included, the cumulative sample size crossed the required information size (RIS) (182). The results of TSA matched those of the meta-analysis, suggesting that compared to the effects of anxiolytics alone, the effects of XYS + anxiolytics significantly improved the anxiety symptoms of patients with emotional disorders; the results were stable, and the evidence was reliable (Figure 3B).

3.3.1.2 HAMA scores

The HAMA scores were provided in 11 studies (Wang et al., 2010; Han et al., 2011; Ding et al., 2013; Lv et al., 2013; Wang, 2014; Zhu, 2015; LI and Qin, 2016; Deng et al., 2017; Xiong and Song, 2019; Zhou et al., 2021; Li et al., 2022), with significant heterogeneity among these studies ($p < 0.00001$, $I^2 = 84\%$). The random-effects meta-analysis estimated that the HAMA scores were significantly lower in the XYS combined with the anxiolytics group relative to the anxiolytics group ([MD = −3.29, 95% CI: −4.37, −2.21, $p < 0.00001$]; Figure 4A). A visual analysis of the funnel plot revealed some level of publishing bias (Supplementary Figure S2). Nevertheless, Egger's test failed to reach statistical significance ($p = 0.372$). We tried reducing heterogeneity by classifying by the course of disease and interventions but failed. The sensitivity analysis showed that three studies (Han et al., 2011; Lv et al., 2013) were the sources of heterogeneity. One study (Han et al., 2011) had the longest course of treatment (12 weeks) and the lowest HAMA score before treatment. The HAMA score before treatment was 14.97 ±2.79 in the treatment group and 14.83 ±2.61 in the control group. In contrast, the lowest score in other studies was 19.37 ±4.50 in the treatment group and 20.12 ±5.29 in the control group (Zhou et al., 2021). In one study (Lv et al., 2013), the patients in the treatment group and the control

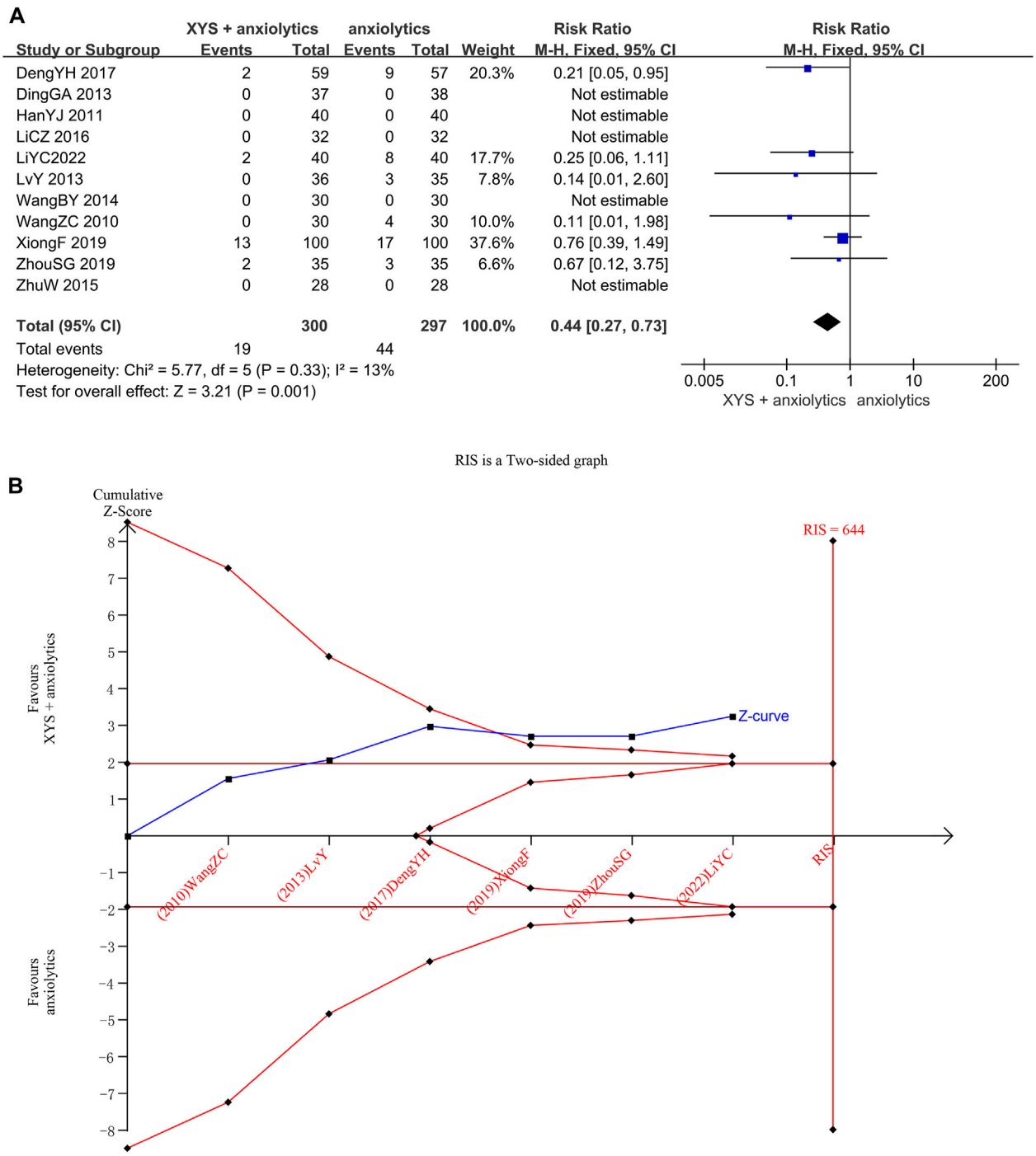

FIGURE 6
Data analysis of Adverse Events Rates (XYS combined with anxiolytics vs. anxiolytics alone). **(A)** Meta-analysis. **(B)** Trial sequential analysis. The annotations of the red line and blue line in TSA are the same as those in Figure 3.

group were treated via psychological counseling. In one study (Xiong and Song, 2019), the administration of oral buspirone citrate in patients with severe anxiety caused heterogeneity. A meta-analysis of the other seven studies suggested that oral XYS combined with anxiolytics treatment has higher efficacy than anxiolytics treatment alone as a means of lowering anxiety disorder patient HAMA with low heterogeneity ([MD = −3.59, 95% CI: −4.27, −2.91, $p < 0.00001$]; $I^2 = 32\%$; Figure 4B).

Through TSA, we confirmed the validity of the meta-analysis. For the first study, the cumulative Z-curve crossed the traditional and trial sequential boundaries, indicating a low likelihood of false positives. The total sample size reached the RIS (178) when the third study was added. The data were reliable, and the TSA results were stable, which showed that XYS combined with anxiolytics could considerably lower the HAMA score of patients with anxiety symptoms compared to anxiolytics (Figure 4C).

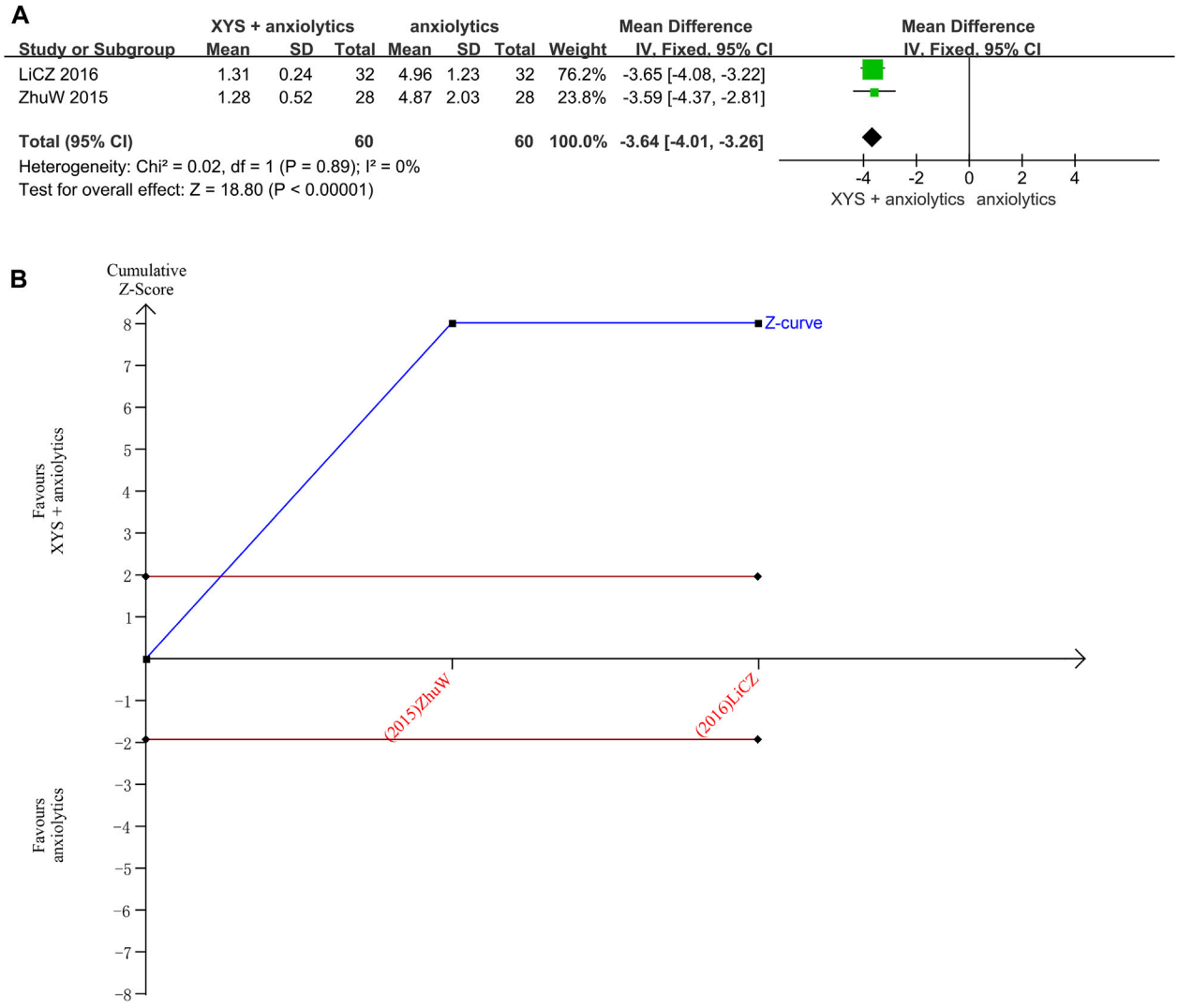

FIGURE 7
Data analysis of TESS scores (XYS combined with anxiolytics vs. anxiolytics alone). **(A)** Meta-analysis. **(B)** Trial sequential analysis. The annotations of the red line and blue line in TSA are the same as those in Figure 3.

3.3.1.3 SAS scores

One RCT study reported the SAS scores (Ding et al., 2013). As we could not conduct a meta-analysis and trial sequential analysis, descriptive analysis was performed, which showed significantly lower SAS scores in the XYS combined with the anxiolytics group relative to the anxiolytics group ([MD = −4.56, 95% CI: −7.19, −1.93]), $p = 0.0007$).

3.3.1.4 PSQI scores

The PSQI scores were reported in two studies (Zhou et al., 2021) with significant heterogeneity between those studies ($p = 0.05$, $I^2 = 75\%$). The random-effects meta-analysis estimated that the PSQI scores were significantly lower in the treatment group relative to that in the control group ([MD = −2.67, 95% CI: −4.11, −1.24, $p < 0.00001$]; Figure 5A). The source of heterogeneity was probably the administration of tandospirone to patients with severe anxiety (Xiong and Song, 2019).

The TSA analysis for PSQI scores showed that the cumulative Z-curve exceeded the traditional significance boundary, but the sequential monitoring boundary was not rendered because the first information fraction exceeded 100%, which indicated that the first study had sufficient statistical power for a meta-analysis. The TSA suggested that compared to the anxiolytics, XYS combined with anxiolytics significantly decreased the PSQI score of patients with anxiety symptoms, the results were stable, and the evidence was reliable (Figure 5B).

3.3.1.5 Adverse events rates

Adverse event rates were reported in Six studies (Wang et al., 2010; Lv et al., 2013; Deng et al., 2017; Zhou et al., 2021; Li et al., 2022). No heterogeneity was detected when evaluating these studies ($p = 0.33$, $I^2 = 13\%$), and the results were thus analyzed using a fixed-effects model. The pooled meta-analysis showed that the adverse event rates in the treatment group were significantly lower than

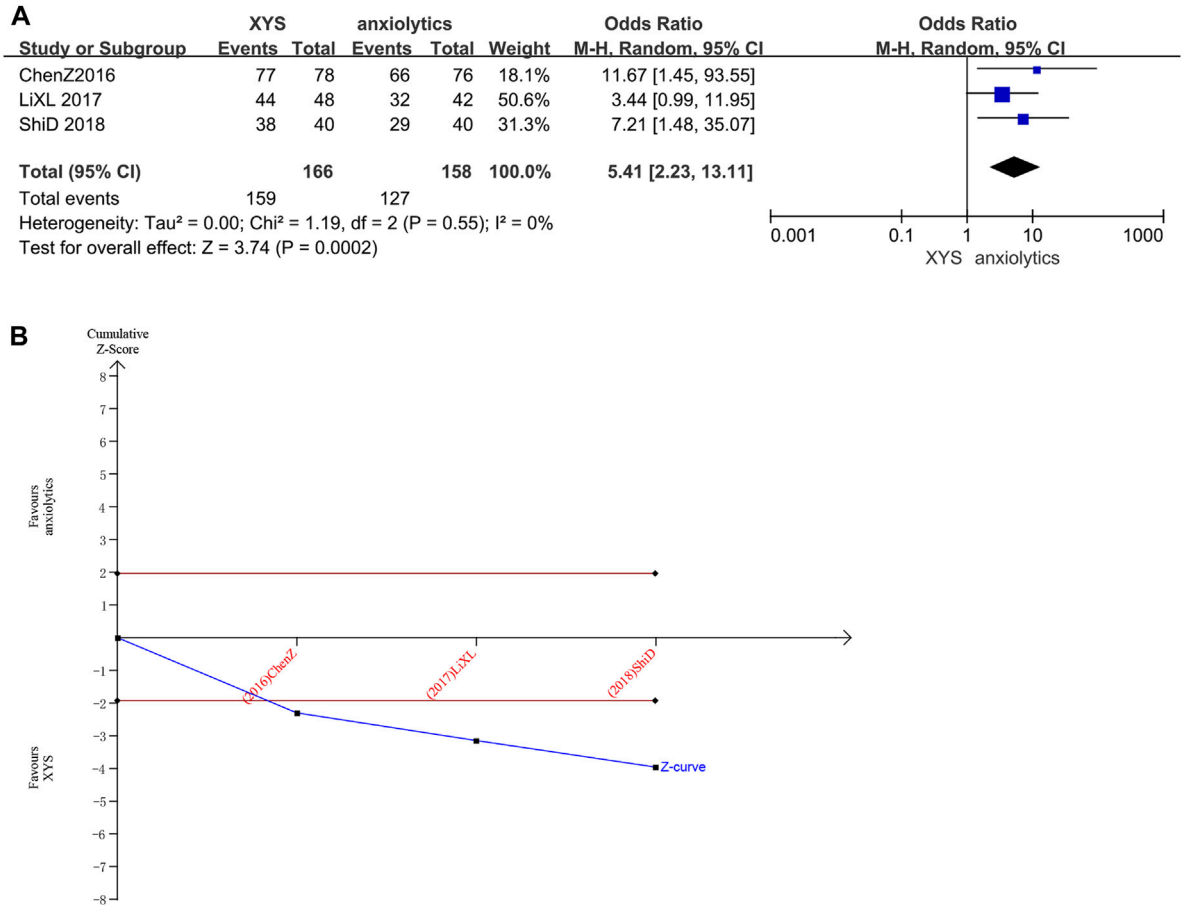

FIGURE 8
Data analysis of the clinical efficacy rates (XYS vs. anxiolytics). **(A)** Meta-analysis. **(B)** Trial sequential analysis. The annotations of the red line and blue line in TSA are the same as those in Figure 3.

those in the control group ([RR = 0.44, 95% CI: 0.27, 0.73, $p = 0.001$]; Figure 6A).

We confirmed the reliability of the meta-analysis through TSA. The cumulative Z-curve crossed the trial sequential boundary and the traditional boundary in the second study, indicating a low probability of a false positive. Although the accumulated information did not reach the expected value, no more tests were needed to reach a positive conclusion in advance. The results of TSA matched those of the meta-analysis, suggesting that compared to anxiolytics treatment, the XYS combined with anxiolytics treatment significantly decreased the adverse reactions of patients with anxiety disorder; the results were stable, and the evidence was reliable (Figure 6B).

3.3.1.6 TESS scores

Two studies reported the TESS scores (Zhu, 2015; LI and Qin, 2016). No heterogeneity was detected when these data were analyzed ($p = 0.89$, $I^2 = 0\%$). The results were thus analyzed using a fixed-effects model. The pooled analysis results indicated that the TESS scores in the treatment group were significantly lower than those in the control group ([MD = 3.64, 95% CI: −4.01, −3.26, $p < 0.00001$]; Figure 7A).

The TSA analysis for the TESS scores showed that the cumulative Z-curve exceeded the traditional significance boundary, but the sequential monitoring boundary could not be rendered because the first information fraction exceeded 100%, which indicated that the first study showed sufficient statistical power for a meta-analysis. The results of the TSA suggested that compared to treatment with anxiolytics, treatment with XYS combined with anxiolytics significantly decreased the TESS score of patients with anxiety symptoms; the results were stable, and the evidence was reliable (Figure 7B).

3.3.2 Comparison of treatment with XYS alone vs. anxiolytics alone

3.3.2.1 Clinical efficacy rates

Three studies ($n = 324$ patients) (Chen and Du, 2016; Li et al., 2017; Shi, 2018) reported clinical efficacy rates. Several studies did not significantly differ in clinical efficacy rates from one another ($Chi^2 = 1.19$, $p = 0.55$, $I^2 = 0\%$). The efficacy recorded in the treatment groups was substantially higher than that recorded in the control groups, as determined by the results of the random-effects model (RR = 5.41, 95% CI: [2.23, 13.11], $p < 0.0001$, Figure 8A).

The cumulative Z-curve for Clinical Efficacy Rates scores exceeded the conventional significance boundary, as determined by the results of the TSA analysis, but the sequential monitoring boundary could not be rendered because the first information

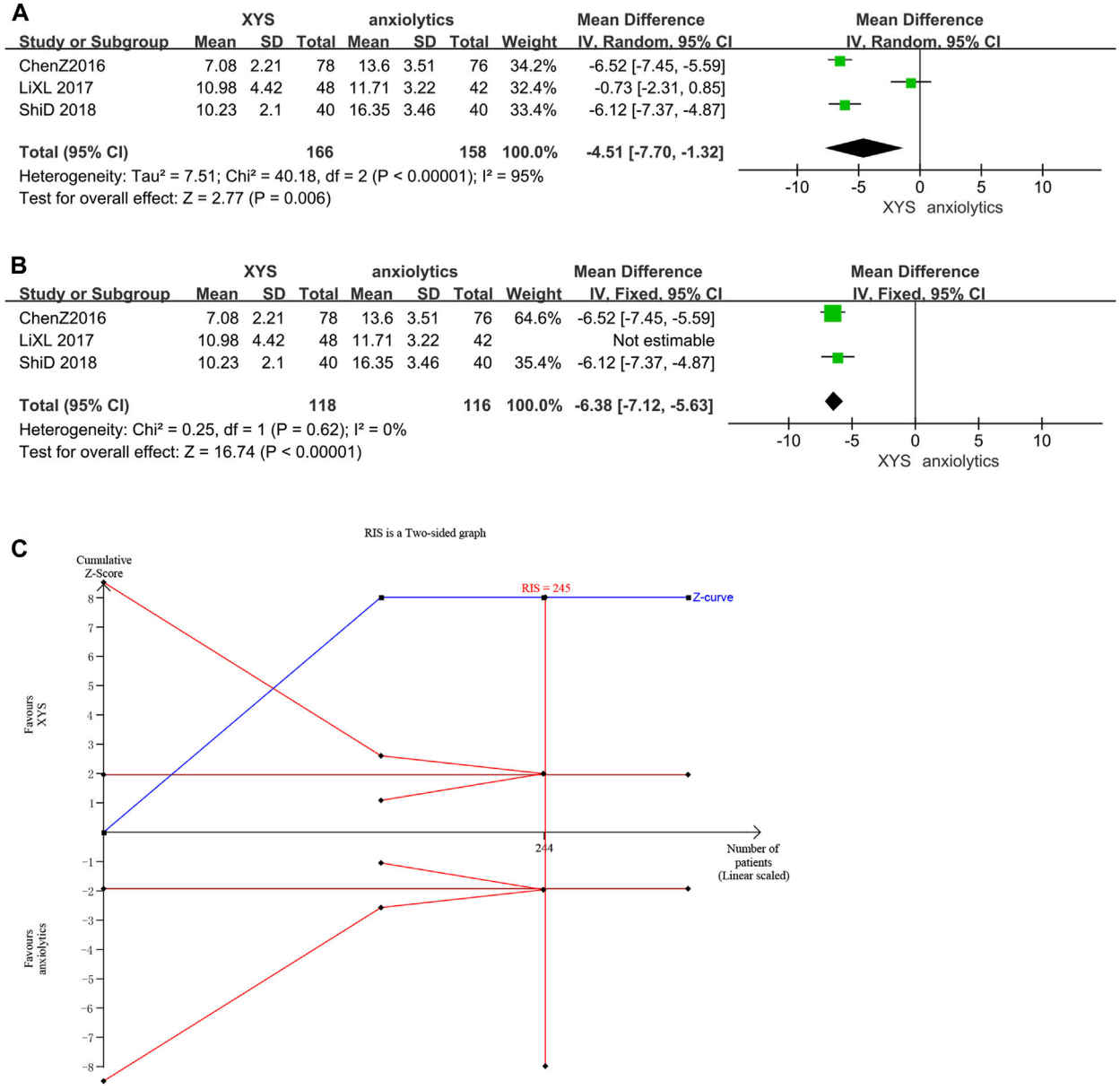

FIGURE 9
Data analysis of HAMA-14 scores (XYS vs. anxiolytics). **(A)** Before eliminating sources of heterogeneity. **(B)** After eliminating sources of heterogeneity. **(C)** Trial sequential analysis. The annotations of the red line and blue line in TSA are the same as those in Figure 3.

fraction was greater than 100%, which indicated that the initial study obtained enough statistical power for a meta-analysis. The results of the TSA matched those of the meta-analysis, suggesting that compared to treatment with anxiolytics, treatment with XYS can significantly improve the anxiety symptoms of patients with emotional disorders; the results were stable, and the evidence was reliable (Figure 8B).

3.3.2.2 HAMA scores

Three studies (Chen and Du, 2016; Li et al., 2017; Shi, 2018) reported the HAMA scores, and there was significant variation among these studies ($p < 0.00001$, $I^2 = 95\%$). The HAMA scores in the treatment group were considerably lower than those in the control group ([MD = −4.51, 95% CI: −7.70, −1.32, $p = 0.006$]; Figure 9A), as determined by the results of a random-effects meta-analysis. According to the analysis of the treatment course, the source of heterogeneity was one study (Li et al., 2017). In this study, Lorazepam was used in the anxiolytics group, whereas other studies used Deanxit. A meta-analysis of the other two studies showed that XYS therapy reduced the HAMA scores of AD patients with low heterogeneity and was more effective than anxiolytic therapy ([MD = −6.38, 95% CI: −7.12, −5.63]; $p < 0.00001$; $I^2 = 0\%$; Figure 9B).

The first information fraction was greater than 100%, which indicated that the initial study had sufficient statistical power for a meta-analysis, and the cumulative Z-curve for the HAMA scores exceeded the conventional significance boundary, as determined by

the TSA analysis. The TSA suggested that compared to anxiolytics, XYS significantly decreased the HAMA score of patients with anxiety symptoms; the results were stable, and the evidence was reliable (Figure 9C).

3.3.2.3 SAS scores

The SAS scores were reported in one study (Shi, 2018). As trial sequence analysis and meta-analysis could not be performed, descriptive analysis was conducted. The findings showed that the XYS group had significantly lower SAS scores than the anxiolytics group ([MD = −13.50, 95% CI: −15.26, −11.74]), $p < 0.00001$).

3.3.2.4 TESS scores and adverse events rates

The Adverse Events Rates and TESS scores were reported in one study (Li et al., 2017). As trial sequence analysis and meta-analysis could not be performed, descriptive analysis was conducted instead. Our findings showed that the XYS group had considerably lower TESS ratings than the anxiolytics group ([MD = −1.22, 95% CI: −1.82, −0.62]), $p < 0.0001$). The adverse events of the XYS group were not significantly different from those of the other group ([RR = 0.29, 95% CI: 0.06, 1.37, $p = 0.12$]).

4 Discussion

In this study, we provided new information for therapeutic decision-making by conducting a meta-analysis of the randomized controlled trial of XYS in treating anxiety disorder and by conducting a more impartial evaluation of this study through TSA. The TSA we performed on the efficacy of XYS for managing anxiety showed that the cumulative sample size was adequate to support the meta-analysis. The TSA, however, could not correct mistakes that occurred due to methodological flaws in the included RCTs. As the sometimes subpar quality of RCTs might have affected the reliability of TSA outcomes, the conclusions must be interpreted with caution.

In this study, 14 randomized controlled trials were included; 11 studies on XYS combined with anti-anxiety Western medicine and three studies on XYS compared to Western medicine. When XYS was compared to anti-anxiety medication and XYS alone for evaluating the effective rate and the HAMA score, the results were more favorable for the former, indicating that XYS can be used as an alternative or supplemental treatment for anxiety disorders.

The adverse event rates in the XYS combined with the anxiolytics group were significantly lower than those in the anxiolytics group, whereas no significant difference was found between the adverse events of the XYS group and the anxiolytics group, although the incidence of adverse events in the XYS group was lower than that in the anxiolytics group. TSA of the XYS combined with anxiolytics vs. anxiolytics showed that the adverse event rates reached the expected sample size.

We also analyzed secondary indicators, including the SAS score, PSQI score, and TESS score. The SAS score was reported in only one study on XYS combined with anxiolytics treatment and anxiolytics alone treatment. Two studies reported the PSQI scores for the XYS combined with the anxiolytics group and the anxiolytics group. Two studies reported the TESS scores for the XYS combined with the anxiolytics group vs. the anxiolytics group, and one study reported the TESS scores for the XYS group vs. the anxiolytics group. These findings led to more positive results. However, the results need to be interpreted with caution as the sample size was small.

Anxiety is an extremely common mental health problem, and it can adversely affect daily life and general wellbeing. Anxiety may occur with or contribute to the development or worsening of medical conditions, including cardiovascular diseases, gastrointestinal diseases, pulmonary diseases, cancer, chronic pain, and migraine headaches (Hackbarth et al., 1986). Oliver et al. speculated that the bed nucleus of the stria terminalis, the amygdala, and the hippocampus, and their connection to the cortical areas like the dorsal medial and lateral prefrontal/cingulate cortex and the insula, plays an important role in maintaining anxiety response (Robinson et al., 2019). The most commonly prescribed anti-anxiety medications are quetiazide, venlafaxine, paroxetine, escitalopram, and duloxetine. These medications have limited tolerance but good remission effects (Kong et al., 2020).

According to TCM, emotional elements directly affect the etiology of anxiety. Emotional disorders can lead to stagnation of the liver qi, dysfunction of spleen transport, and disorder of qi and blood, which is known as liver stagnation and spleen deficiency syndrome (LSSDS). Throughout treatment, the fundamental therapeutic effects of TCM include nourishing blood, tonifying the spleen, and soothing liver-qi stagnation. XYS can soothe the liver, relieve depression, nourish the blood, and invigorate the spleen. For over a century, XYS has been used in TCM clinics to treat various illnesses that share the symptoms of liver stagnation and spleen deficiency syndrome (LSSDS). A component of XYS known as Chai Hu is used to spread stagnant liver qi and treat depression. It also regulates liver qi, making it an essential monarch drug. Bai Shao, Dang Gui, and stewed ginger, which are used as ministerial drugs, can nourish the blood. Astringe yin can nourish the liver and reduce stress. As adjuvants, Bai Zhu, Fu Ling, and licorice can energize the spleen, remove moisture, facilitate transportation and transformation, and provide qi and blood to their source. A small amount of mint is used as a conductive medicine, which helps inhibit the spread of qi and reach the liver meridian to treat the syndrome of heat stagnation. Suitable medications can be administered to treat the liver and spleen while also nourishing the liver and promoting liver function.

The mechanism of the anti-anxiety effect of XYS was found in some animal experiments. Hao et al. found that XYS can reduce anxiety-like behavior by controlling the gut microbiota, reducing excessive LPS production, and preventing excessive activation of NLRP3 inflammasome in the colon (Hao et al., 2021). XYS can limit the expression of miR-200a/b-3p that is produced by CUMS, control miR-200a/b-3p/NR3C1 signaling in the prefrontal cortex that is induced by chronic stress, and decrease neuronal death and anxiety-like behavior (Yuan et al., 2020). In an animal study, it was found that XYS can promote the regeneration of hippocampal neurons by blocking the Notch signal pathway, which decreases anxiety-provoking behavior (Liu et al., 2021). Zhao et al. showed that XYS can maintain mitochondrial function by increasing the level of BDNF and phosphorylated AMPK, thus alleviating anxiety (Zhao et al., 2022). XYS can treat anxiety and depression caused by a high-fat diet by regulating metabolites derived from the intestinal microflora (Yang et al., 2022).

5 Limitations and recommendations

Our study had some limitations. First, poor quality of methodology is a common problem in studies on traditional Chinese medicine. The studies included also lacked a detailed description of random methods, random assignment concealment, blind method implementation, selective outcome reports, etc. The randomization method only describes the representation of random numbers and is ambiguous. In most cases, randomization is employed to balance known and unknown confounding factors between experimental and control groups. It is recommended that future studies provide detailed descriptions of the randomization methods used, such as simple randomization, block randomization, stratified randomization, and cluster randomization. To prevent the knowledge of the randomization results from biasing the researchers, it is preferable to implement concealed randomization using methods such as sealed envelopes or central randomization. Central randomization is suitable for large multicenter studies, while sealed envelopes are applicable to small-sample clinical studies conducted at a single center. Additionally, implementing blinding procedures is also crucial. It is hoped that future studies can achieve blinding of participants, observers, and outcome assessors/data analysts. Second, due to the limitations of traditional Chinese medicine, implementing a blind method is difficult. Double-blind and double-simulation designs may be used in future studies on traditional Chinese medicine. Most of the included studies were single-center, small-sample studies with low-level evidence. More high-quality evidence with more participants and from multiple centers is needed. Third, studies included in this manuscript lacked a long-term follow-up evaluation, and evaluating the long-term efficacy of XYS was difficult. Finally, all participants were Chinese, and the applicability of the conclusions of the study to individuals of other nationalities is limited. XYS has been registered in Denmark and is used for alternative self-care of European patients to relieve mental stress. This provides a basis for research on the applicability of XYS to individuals of other nationalities. We strongly recommend conducting multi-center, double-blind, and double-simulated high-quality clinical studies to obtain more detailed information and reliable results.

6 Conclusion

To assess the effectiveness and safety of administering XYS for treating anxiety disorder, we conducted a meta-analysis and trial sequential analysis. We found that XYS can be used safely and effectively to treat anxiety combined with Western medicine. However, although XYS alone was found to be effective in treating anxiety disorder, more large-scale studies are needed to determine adverse events. Additionally, considering that the methodology had low quality, the results should be interpreted with caution.

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

YW designed the study and drafted the article. YW and XC performed the literature database search and data collection. WW and YD performed the literature extraction and assessment of evidence quality. JX and JW performed data analysis and rationalization of the results. RG revised the manuscript critically and supervised all aspects of the study. All authors contributed to the article and approved the submitted version.

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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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Post-marketing safety surveillance and re-evaluaiton of Shu-Xue-Ning injection: a real-world study based on 30,122 cases

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Objective: This study aims to investigate the safety of Shu-Xue-Ning injection (SXNI) in real-world clinical applications.

Methods: A prospective, multi-center, large-sample intensive monitoring method was used to monitor the use of SXNI in several medical institutions across China while collecting patients' dosing and adverse event information. Patients who suspected as adverse reactions made comparisons with patients who did not report adverse reactions to calculate the correlation between relevant risk factors and suspected adverse reactions. Statistical analysis software SAS 9.1 was used for data analysis.

Results: A total of 48 hospitals participated in this intensive monitoring study of SXNI, and 30,122 patients were monitored from July 2015 to December 2018. A total of 1,908 adverse events were reported during the use of SXNI, with an adverse event rate of 6.33% and a 95% confidence interval (CI) of 6.06%–6.61%. Association assessment showed that 54 cases presented with SXNI-related adverse reactions with an incidence of 0.18% and a 95% CI of 0.13%–0.23%, thereby indicating that the incidence of SXNI-related adverse reactions was occasional. SXNI-related adverse reactions involved 9 systems-organs with 20 clinical manifestations, and the most common adverse reactions were rash, pruritus, and other damages of skin and its appendages. No serious adverse reactions were observed; 27.78% of the adverse reactions occurred within 30 min of drug administration and more than half of them occurred within 2 h of drug administration; 96.3% of the adverse reactions were cured or improved. Causal analysis showed that women, long dispensing time, and slow dripping speed rate were considered as risk factors.

Conclusion: The incidence of SXNI-related adverse reactions in real-world clinical applications is occasional and in a reasonable range with a good prognosis.

KEYWORDS

Shineway® Shu-Xue-Ning injection, TCM injection, adverse reactions, intensive hospital monitoring, real-world study

1 Introduction

Traditional Chinese medicine (TCM) injections are a landmark achievement in the modernization of TCM and have advantages of high bio availability and fast onset of action (Wang and Lai, 2005). TCM injections have gained wide applications, but its safety issue is of great concern (Gong et al., 2017). The *National Annual Monitoring Report of Adverse Drug Reactions* (2020) shows that the National Monitoring Network of Adverse Drug Reactions received 1,676,000 *Adverse Drug Reaction/Event Report Forms* in 2020, of which chemical drugs accounted for 83.0% and TCM accounted for 13.4%; injections accounted for 60.4% and 33.3% of the chemical drug-related and TCM-related adverse reaction/event reports, respectively (State Drug Administration Drug Assessment Center, 2020). It is evident that the safety of TCM injections is not necessarily lower than that of chemical injections, and the safety events in TCM injections may be attributed to drug properties, irrational drug use, and individual patient differences (Liang and Li, 2007; Zhang, 2014).

Systematic post-marketing pharmaco-epidemiological studies are lacking for some TCM injections, which results in a relative shortage of data on the incidence of adverse reactions, and a relatively poor understanding of clinical manifestations of adverse reactions. In order to further improve the safety of TCM injections, the State Food and Drug Administration of China issued the *Notice on the Re-assessment of the Safety of TCM injections* in 2009 to initiate re-assessment of the safety of TCM injections (Zhang, 2009).

The national 12th Five-Year Plan of drug safety also proposes to carry out safety risk analysis and assessment of TCM injections (State Council, 2012). Later, the State Council issued the *Opinions on Deepening the Review and Approval System Reform to Encourage Innovation in Drugs and Medical Devices* in 2017 proposing to re-evaluate the marketed drug injections as well. Currently, the clinical safety of several TCM injection products, such as Xuesaitong injections (lyophilized) and Xueshuantong injections (lyophilized), have been intensively monitored for more than 30,000 cases (Wang et al., 2015; Li et al., 2018; Jin et al., 2020). A systematic assessment based on an intensive, clinical safety monitoring study of TCM injections revealed that, as of February 2018, a total of 296,200 patients had been monitored for 14 TCM injection products with an overall incidence of adverse reactions of 1.57 per 1,000 (Li et al., 2019).

The Shu-Xue-Ning injection (SXNI) is a sterilized aqueous solution of Ginkgo biloba leaf extract made by Shineway Pharmaceutical Group Co., Ltd. It's a traditional Chinese patent medicine, according to the package inserts of drug, its specification is 5 mL per tube, equivalent to 17.5 mg of ginkgo biloba extract (containing 4.2 mg of total flavonoids; containing 0.70 mg of ginkgolides). It has been used in clinical for many years and can ensure uniform and stable composition between batches. SXNI used in the clinical treatment of ischemic cardiovascular, cerebrovascular diseases, angina pectoris, coronary heart disease, cerebral embolism, and cerebral vasospasm (Ge et al., 2012; Tao and Chen, 2012; Du et al., 2017; Wan et al., 2018). The main active constituents of SXNI are total flavonoids (Quercetin, Kaempferin, Isorhamnetin) and terpene lactones (Ginkgolide A, Ginkgolide B, Ginkgolide C, and Bilobalide) (Ou et al., 2010).

Since the start of the re-assessment work, quality control standards of SXNI has been established in all aspects from the production of raw materials to product processing, which has improved the stability and controllability of the product (Zhao et al., 2015). A post-marketing re-assessment of SXNI was conducted herein using an intensive hospital monitoring approach to systematically assess the safety of this injection for a wide range of populations in a real-world clinical setting.

SXNI is commonly used in clinical practice and has previously been completed reevaluation throng post-marketing safety based on real-world and evidence-based evaluations (Wang et al., 2018). Compared with previous study, this study were only includes pantients who used SXNI produced by Shineway and the data were completely based on real-world observations, with a much larger sample size than previous studies.

2 Data and methods

2.1 Study design

This study adopted a prospective, multicenter, large-sample intensive monitoring design. The clinical trial registration number was ChiCTR-OPC-15006649, which was approved by the Medical Ethics Committee of Tianjin University of Traditional Chinese Medicine (TJUTCM-EC20150005). This was a retrospective study without intervening in the diagnosis and treatment of patients, adhered to the Declaration of Helsinki and was approved by ethics committee to waive the patients' informed consent. This observational study was reported based on the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement (von Elm et al., 2014). Post-marketing safety re-evaluation of SXNI workflow was shown in Figure 1.

2.2 Monitoring institutions

Considering factors such as hospital type, hospital grade, and geographical location, 48 hospitals (secondary Grade-A or above) were selected nationwide as intensive monitoring institutions.

2.3 Inclusion and exclusion criteria

Inclusion criteria: In-patients who received at least 1 SXNI treatment in the monitoring institution (regardless of the departments) were included in this study, regardless of patient age, gender, disease type, or condition.

Exclusion criteria: patients who did not use SXNI.

2.4 Sample size calculation

The reported incidence of adverse reactions to TCM injections is mostly occasional (0.1%, 1%) and rare (0.01%, 0.1%) (Li et al., 2018). The number of subjects should be 30,000 when assuming a 95% probability of finding at least one rare adverse reaction (Zhang et al., 2017).

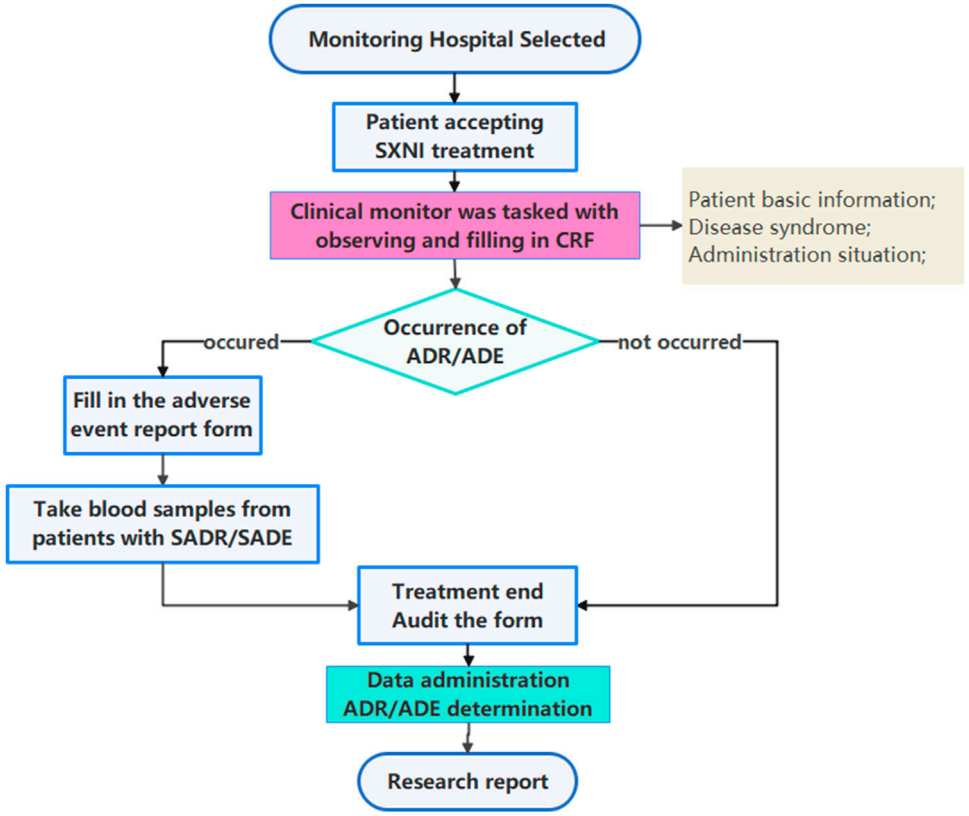

FIGURE 1
Post-marketing safety surveillance and re-evaluation of SXNI workflow.

2.5 Monitoring cycle

The monitoring cycle ranged from July 2015 to December 2018.

2.6 Monitoring content

In view of the prior research experiences of intensive hospital monitoring and the characteristics of SXNI, this study determined that the information to be collected was basic patient information, SXNI production and administration-related information, and SXNI safety-related information. Basic patient information comprised gender and age; SXNI production and administration-related information comprised production batch number, drug specification, usage, dosage, treatment course, combined medication, and change of treatment regimen. SXNI safety-related information comprised the incidence of adverse reactions/events, clinical manifestations of adverse reactions, types of adverse reactions, severity of adverse reactions, treatment measures of adverse reactions, and prognosis.

2.7 Data acquisition and management

In each participating hospital, data were collected using Case Reporting Form (CRF) and Adverse Event Reporting Form (AERF). Basic patient information and medication information were retrieved directly from the medical record of patients without interfering with the medication treatment conducted by the responsible clinical physician and without affecting the normal treatment of the patient, where a clinical monitor was tasked with observing and filling in CRF, as well as filling in AERF if an adverse event occurred. A clinical inspector was responsible for data verification and data transfer to ensure quality control of the intensive monitoring process.

After confirming that the CRF was completed in a timely, accurate, and complete manner, the clinical inspector signed with the clinical monitor and transmitted the CRF data to the online electronic data management system. The medical data manager revised the medical event names to standard terms with reference to WHO-ART and verified them with the monitor while standardizing the information of doctors' orders, such as the names of chemical and proprietary Chinese medicines. The database was locked upon verification of the information, followed by statistical analysis.

2.8 Quality control

The Institute of Clinical Assessment of Tianjin University of Traditional Chinese Medicine organized program training for the project managers of all participating hospitals before the start of monitoring in the participating hospitals. This included interpretation of study protocol, filling out CRF and AERF, and judgment and association assessment of adverse reactions/events. Beijing Excellence Future International Consulting Co., Ltd.

TABLE 1 Distribution of monitoring institutions and the patients.

Serial number	Province/ municipality	Institution name	Number of cases	Composition ratio (%)
1	Shanxi	Shanxi Cardiovascular Disease Hospital	2021	6.71
2	Henan	Luoyang First People's Hospital	1997	6.63
3	Liaoning	Panjin Central Hospital	1800	5.98
4	Hebei	Handan Central Hospital	1500	4.98
5	Henan	The First Affiliated Hospital of Xinxiang Medical College	1500	4.98
6	Jilin	Jilin City People's Hospital	1300	4.32
7	Liaoning	Shenyang Medical College Shenzhou Hospital	1290	4.28
8	Zhejiang	Zhejiang Xinhua Hospital	1144	3.8
9	Shaanxi	The Second People's Hospital of Shaanxi Province	1000	3.32
10	Heilongjiang	The First Hospital of Heilongjiang University of Traditional Chinese Medicine	996	3.31
11	Jilin	Jilin University Second Hospital	950	3.15
12	Sichuan	Zhongjiang County People's Hospital	900	2.99
13	Jilin	General Hospital of Jihua Group Corporation	883	2.93
14	Shaanxi	The Fourth People's Hospital of Shaanxi Province	800	2.66
15	Shanxi	Shanxi Provincial People's Hospital	793	2.63
16	Hebei	Shijiazhuang City Hospital of Traditional Chinese Medicine	761	2.53
17	Zhejiang	Zhejiang Hospital of Traditional Chinese Medicine	700	2.32
18	Shaanxi	Xi'an High-Tech Hospital	700	2.32
19	Hebei	Hebei Provincial Hospital of Traditional Chinese Medicine	615	2.04
20	Henan	Henan Provincial Hospital of Traditional Chinese Medicine	594	1.97
21	Shanghai	Shanghai Renji Hospital	525	1.74
22	Zhejiang	Yuhang District First People's Hospital	502	1.67
23	Hebei	Tangshan Hospital of Traditional Chinese Medicine	501	1.66
24	Shanxi	Taiyuan Second People's Hospital	497	1.65
25	Henan	Hebi First People's Hospital	452	1.5
26	Heilongjiang	The Second Hospital of Heilongjiang University of Traditional Chinese Medicine	450	1.49
27	Jilin	Jilin Provincial People's Hospital	435	1.44
28	Beijing	The Central Hospital of China Aerospace Corporation	413	1.37
29	Hebei	Handan City People's Hospital	400	1.33
30	Hebei	The Second Hospital of Hebei Medical University	388	1.29
31	Henan	Luoyang Oriental Hospital	355	1.18
32	Heilongjiang	Qiqihar First Hospital	350	1.16
33	Hebei	Yi County Hospital of Traditional Chinese Medicine	350	1.16
34	Beijing	Oriental Hospital of Beijing University of Traditional Chinese Medicine	338	1.12
35	Tianjin	The Second Affiliated Hospital of Tianjin University of Traditional Chinese Medicine	275	0.91
36	Chongqing	Chongqing Fifth People's Hospital	255	0.85

(Continued on following page)

TABLE 1 (*Continued*) Distribution of monitoring institutions and the patients.

Serial number	Province/ municipality	Institution name	Number of cases	Composition ratio (%)
37	Hebei	Baoding Hospital of Integrative Medicine	245	0.81
38	Hebei	Qinghe County Central Hospital	200	0.66
39	Liaoning	Liaoning University of Chinese Medicine Affiliated Hospital	200	0.66
40	Hebei	Hebei Provincial People's Hospital	182	0.6
41	Liaoning	The Second Hospital of Liaoning University of Traditional Chinese Medicine	128	0.42
42	Hebei	Jingmen First People's Hospital	105	0.35
43	Sichuan	Chengdu Armed Police Hospital	100	0.33
44	Chongqing	Chongqing Third People's Hospital	87	0.29
45	Hebei	Shijiazhuang Eighth Hospital	60	0.2
46	Shanxi	Shanxi Linfen Hospital	43	0.14
47	Hunan	The First Hospital of South China University	31	0.1
48	Shanxi	The First Hospital of Shanxi Medical University	11	0.04
Total			30122	100.00

assigned inspectors to train all monitors in the participating hospitals on the study protocol and the completion of CRF and AERF. Primary quality control was the responsibility of the participating hospitals, who were tasked with inspecting the quality of monitored cases, including the study progress, all original data verification, authenticity verification, and electronic data reporting. The project leader of the hospital should review and sign the quality checklist every week and take appropriate measures to overcome the existing quality problems. For secondary quality control, inspectors from Beijing Excellence Future International Consulting Co., Ltd. regularly monitored each monitoring hospital to ensure that the CRF data were true, accurate, and complete. They verified the same with the electronic data management system data, completed the monitoring report, and signed it. For tertiary quality control, auditors from the Institute of Clinical Assessment of Tianjin University of Traditional Chinese Medicine regularly audited the monitored cases in the participating hospitals, completed the audit report, and signed it.

2.9 Association assessment of adverse events

After the data were verified and the database was locked, the research team set up an expert committee of adverse event assessment with three to five experts selected from the assessment expert pool. The association was initially determined by the monitor and finally determined by the expert committee.

2.10 Statistical analysis

Data analysis was performed using software SAS 9.1. Measurement data such as age were expressed as mean, standard deviation, minimum, and maximum values. Categorical variables such as gender, drug allergy history, and the occurrence of adverse reactions were expressed as their frequencies and composition ratios. For hypothesis tests, differences were considered statistically significant at $p < 0.05$. The type and incidence of SXNI-related adverse reactions/events were expressed as percentages and 95% confidence intervals (CI), respectively. Pearson's chi-square test, Permutation test and Logistic regression was used to compare the classified data on correlation analysis.

3 Results

3.1 Monitoring units and patient inclusion

As detailed in Table 1, a total of 48 medical institutions participated in this study, monitoring 30,122 patients, with a sample size of 11 to 2,021 cases monitored at each institution.

There were 7 secondary hospitals and 41 tertiary hospitals monitoring 2,652 (8.8%) and 27,470 (91.2%) of the total number, respectively. There were 34 Western medicine hospitals monitoring 22,825 patients (75.8%) and 13 TCM hospitals monitoring 7,052 patients (23.4%), with 1 TCM and Western medicine-combined hospital monitoring 245 patients (0.8%). Of the 48 hospitals, 19 are in North China monitoring 9,593 patients (31.85%), 11 in Northeast China monitoring 8,782 patients (29.15%), 7 in Central China monitoring 5,034 patients (16.71%), 4 in East China monitoring 2,871 patients (9.53%), 3 in Northwest China monitoring 2,500 patients (8.30%), and 4 in Southwest China monitoring 1,342 patients (4.46%).

The mean age of the included patients was 62.63 ± 14.85 years, with the youngest and oldest ages being 3 and 103 years, respectively; the mean height and mean body weight were

TABLE 2 Clinical characteristics of all patients and patient groups according to adverse reactions.

Clinical features	Overall (*N* = 30122)	Adverse reactions group (*N* = 54)	Non-adverse reactions group (*N* = 30068)
Age [years, M±S]	59.33 ± 14.52	56.28 ± 14.24	61.41 ± 14.34
Sex [n (%)]			
Female	14522(48.21)	38 (70.37)	14484 (48.17)
Male	15600(51.79)	16(29.63)	15584(51.83)
Storage period [min, M±S (%)]	17.44 ± 11.96	22.85 ± 13.71	17.43 ± 11.96
Treatment course [d, M±S (%)]	9.07 ± 5.56	5.00 ± 4.86	9.07 ± 5.56
Dripping speed [Drips per minute, M±S (%)]	46.71 ± 15.68	42.28 ± 13.42	46.72 ± 15.69

TABLE 3 Systems/organs involved in SXNI-related adverse reactions and their clinical manifestations.

Involved systems/organs	Frequency (composition ratio %)	Clinical manifestations (frequency)
Skin and its appendage damage	33 (42.31)	Pruritus (16), rash (14), erythematous rash (1), angioneurotic edema (1), purpuric rash (1)
Central and peripheral nervous system damage	21 (26.92)	Dizziness (10), headache (10), local numbness (1)
Systemic damage	10 (12.82)	Fever (4), malaise (2), weakness (2), chills (2)
Heart rate and rhythm disturbances	5 (6.41)	Heart palpitations (5)
Respiratory damage	3 (3.85)	Dyspnea (2), laryngeal edema (1)
Sympathetic parasympathetic nervous system damage	2 (2.56)	Flushing (1), wet and cold skin (1)
Gastrointestinal system damage	2 (2.56)	Nausea (2)
General cardiovascular system damage	1 (1.28)	Hypertension (1)
Medication site damage	1 (1.28)	Injection site rash (1)

165.29 ± 8.63 cm and 66.24 ± 11.78 kg, respectively. Of the patients, 15,600 (51.79%) were male and 14,522 (48.21%) were female. There were 5,326 patients (17.68%) with a habit of smoking and 3,676 patients (12.20%) with a habit of drinking. A total of 1,013 cases (3.36%) had a history of adverse reactions. Clinical characteristics of all patients and patient groups according to adverse reactions were showed in Table 2.

3.2 Adverse events and association assessment

A total of 1,908 adverse events were reported in the study, with an adverse event rate of 6.33% and 95% CI of 6.06%–6.61%. The incidence of SXNI-related adverse reactions was 0.18%, with 95% CI ranging from 0.13% to 0.23%.

3.3 Clinical manifestations of adverse reactions

There were 20 clinical manifestations of SXNI-related adverse reactions involving 9 systems/organs. Among them, 33 cases presented with skin and appendage damage, for which the most common manifestations were pruritus and rash, while 21 cases presented with central and nervous system damage, for which the common manifestations were dizziness and headache. The results are summarized in Table 3.

3.4 Time from drug administration to the onset of adverse reactions

In this study, SXNI-related adverse reactions were found to occur within 7 days of dosing, including 27.78% within 30 min and 83.33% within 24 h. The results are summarized in Table 4.

3.5 Types of adverse reactions and outcomes

According to the definition of adverse reactions in the *Administrative Measures for the Reporting and Monitoring of Adverse Drug Reactions*, no serious adverse reactions were found in this study. According to the description of clinical manifestations in SXNI instructions (General Office of the State Food and Drug

TABLE 4 Time from drug administration to the onset of adverse reactions.

Time	Frequency (number of cases)	Composition ratio (%)
0–30 min	15	27.78
31 min–2 h	16	29.63
2–24 h	14	25.92
1–7 d	9	16.67
Total	54	100

TABLE 5 Outcomes of adverse reactions.

Outcome	Frequency (number of cases)	Composition ratio (%)
Cured	39	72.22
Improved	13	24.08
Unknown	2	3.70
Total	54	100.00

TABLE 6 Age distribution of patients with adverse reactions.

Age (years)	Frequency (number of cases)	Composition ratio (%)
≤ 18	0	0.00
19–45	2	3.70
46–65	26	48.15
66–80	16	29.63
≥ 81	10	18.52
Total	54	100.00

Administration, 2013), three general adverse reactions were observed: discomfort, weakness, and local numbness.

We observed that 39 cases of adverse reactions were cured, which accounted for 72.22% of the total number of adverse reactions; 13 cases of adverse reactions were improved, which accounted for 24.08%, and no adverse reactions led to death. The results are summarized in Table 5.

3.6 Age and gender of patients with adverse reactions

Among the patients with adverse reactions, 38 were female accounting for 70.37%, and 16 were male accounting for 29.63%. Patients with adverse reactions were predominantly middle-aged and elderly, which was consistent with the age of onset of cardiovascular and cerebrovascular diseases. The results are summarized in Table 6.

3.7 History of allergy in patients with adverse reactions

Among the 54 patients with adverse reactions, 34 patients (62.96%) had no history of allergy, 4 (7.41%) had an unknown history of food/drug allergy, and 16 (29.63%) had a history of food/drug allergy. There were 12 patients with a history of drug allergy who were allergic to cephalosporin antibiotics, penicillin, and sulfonamides, and 2 patients with food allergy were allergic to seafood; 2 patients had a history of both food and drug allergies.

3.8 Dosing characteristics of SXNI

Fifty-four patients with adverse reactions were administered by intravenous drip, of whom 25 were first-time SXNI users, 1 had a history of SXNI treatment, and 28 had an unknown history of SXNI treatment. The single dose was 10 mL in 3 cases, 15 mL in 2 cases, 20 mL in 48 cases, and 25 mL in 1 case, with 88.89% of the patients qualifying for the intravenous drip dose of 20 mL per day as required in the SXNI instructions.

The solvent dose was 100 mL in 6 cases, 150 mL in 1 case, 200 mL in 2 cases, and 250 mL in 45 cases; the solvent type was 0.9% normal saline in 32 cases, 10% glucose in 1 case, 5% glucose in 20 cases, and 5% xylitol in 1 case. A total of 18 cases qualified for the intravenous drip dose of 250 mL or 500 mL in 5% glucose as required in the SXNI instructions, thereby accounting for 33.33%.

The drip rate ranged from as fast as 75 drops/min to as slow as 20 drops/min, with a mean of 42.28 ± 13.42 drops/min. After dispensing, the injection mix was allowed to settle for 0–60 min, with a mean of 22.85 ± 13.71 min.

TABLE 7 Causal analysis of ADRs for patients using SXNI.

Elements	ADR		Correlation with ADRs		
	Yes	No			
Sex [n (%)]	38(70.37)	14484(48.17)	c^2 test, $p = 0.016$	Regression coefficient, 0.8883	$p = 0.0030$
Famale [n (%)]					
Storage period [min, M±S (%)]	22.85 ± 13.71	17.43 ± 11.96	Permutation test, $p = 0.0015$	Regression coefficient, 0.0243	$p = 0.0032$
Treatment course [d, M±S (%)]	5.00 ± 4.86	9.07 ± 5.56	Permutation test, $p < 0.0001$	Regression coefficient, −0.2189	$p < 0.0001$
Dripping speed [Drips per minute, M±S (%)]	42.28 ± 13.42	46.72 ± 15.69	Permutation test, = 0.0380	Regression coefficient, −0.0246	$p = 0.0178$

3.9 Combined medications in patients with adverse reactions

The top 10 drugs in combined medications in 54 patients who experienced adverse reactions were aspirin (21), atorvastatin calcium tablets (11), oxiracetam injection (8), TCM decoction (7), metoprolol tartrate (7), insulin (6), deproteinized calf blood extract (6), dexamethasone injection (6), trimetazidine hydrochloride tablets (5), and isosorbide mononitrate (5).

The top 10 injections in combination with SXNI were oxiracetam injection (8), deproteinized calf blood extract (6), insulin (6), dexamethasone injection (6), L-carnitine injection (4), omeprazole injection (4), pantoprazole injection (4), sodium ozagrel (3), bozhi glycopeptide injection (3), and magnesium isoglycyrrhizinate injection (3).

3.10 Causal analysis

Age, sex, ethnicity, dosage and twenty-two potential factors contributing to adverse reactions were analysed. There were correlations betweenadverse reactions and sex ($p = 0.016$), storage period ($p = 0.0015$), treatment course ($p < 0.0001$), dripping speed ($p = 0.0380$). Logistic regression was used to analyze the above factors, and the results indicated that the combined significance of the four factors had a significant impact on the occurrence of adverse reactions. At 95% confidence, according to the regression coefficient, women, long dispensing time, short medication course and slow dripping speed rate were considered as risk factors. The causal analysis of ADRs is displayed in Table 7.

4 Discussion

4.1 The safety result of SXNI

A total of 30,122 patients were included in the prospective hospital-based intensive monitoring study of SXNI, and 54 adverse reactions were identified, with an incidence rate of 0.18%, thereby indicating that the adverse reactions were generally occasional. Most of the adverse reactions of SXNI occurred within 2 h of dosing, mainly manifesting as pruritus, rash and other damage to the skin and its appendages, and the prognosis was good. No serious adverse reactions were observed.

4.2 Characteristics of SXNI-related adverse reactions

SXNI-treated patients showing adverse reactions were mostly middle-aged and elderly (over 45 years old), and the proportion of female patients with adverse reactions was higher than that of male patients; 29.63% of patients with adverse reactions had a previous history of food/drug allergy, 27.78% of adverse reactions occurred within half an hour of dosing, and more than half of adverse reactions occurred within 2 h of dosing. Therefore, clinical application of SXNI should be monitored within half an hour of dosing such that adverse events can be handled promptly. Middle-aged and elderly women, especially patients with a history of food/drug allergy, should be under close monitoring to be alert to the occurrence of adverse reactions. Causal analysis showed that women, long dispensing time, short medication course and slow dripping speed rate were considered as risk factors. According to statistic analysis, the shorter the medication course, the more likely ADRs will occur. However, clinically, when patients experience ADRs with SXNI, they generally refuse to use it again, so the occurrence of ADRs may leads to a shortened course of treatment.

4.3 Strengths and limitations of the study

This intensive monitoring study of SXNI safety was a prospective observational study with a large sample. The prospective design and quality control process ensured the accuracy and reliability of the study results. This intensive monitoring study covered a period of time from the beginning of drug administration and monitored the incidence of adverse reactions and their clinical manifestations in real-world applications, and was aimed at providing data to support safe applications of SXNI in a clinical setting (Xie et al., 2010; Zheng, 2017). Intensive monitoring is an important supplement to the spontaneous reporting system of adverse drug reactions, and facilitates the detection of unknown or unanticipated adverse drug reactions, thereby allowing for calculation of the incidence of adverse reactions, and compensates for the limitations of insufficient safety assessment in pre-marketing studies (Xie et al., 2019).

However, there is a need to further improve the quality of the study: (1) Given the large number of participating patients and hospitals, the quality control of data collection should be further strengthened; (2) Program training and study implementation

should be strengthened to reduce the clinically irrational use of SXNI in healthcare institutions that participate in the intensive monitoring study; and (3) A more in-depth and comprehensive association analysis on the risk factors of adverse reactions is required.

4.4 Recommendations for clinical applications

Solvent type and dose are the most easily overlooked yet important factors to consider in a clinical setting. Thus, when applying SXNI in a clinical setting, care should be taken to avoid its application to off-label indications and beyond recommended dosage. Clinicians should be alert to the occurrence of adverse reactions within half an hour of dosing, especially for patients with a history of drug/food allergy. If rash or other discomfort appears, it is required to immediately stop using the drug and provide appropriate treatment. Feedback should be given to the clinician if there is any discomfort within 24 h of dosing.

4.5 Recommendations for research and regulation

The requirements for drug risk assessment and management are constantly changing as the methods for clinical safety assessment of drugs are being updated and developed. The new Drug Administration Law of China establishes that the country will implement a pharmacovigilance system to regulate the whole life cycle of drugs. Accordingly, there is an urgent need to upgrade the intensive monitoring research in hospitals by establishing a professional team with the collaboration of multiple participants including government regulators, enterprises, research institutions, and hospitals, to form a long-term, economical, and whole life-cycle monitoring-based safety monitoring model for TCM injections with the aim of achieving routine monitoring of the safety of TCM injections and taking quick actions in response to unexpected safety events. After the completion of this intensive hospital monitoring study of SXNI safety, it is desirable to choose a number of hospitals showing a high degree of cooperation, strict quality control, and high research quality as pilot sites to perform continuous monitoring of the clinical safety of SXNI. The results of an intensive hospital monitoring study should be combined with the results of safety assessment based on literature-reported case data and the results of overall clinical safety assessment based on spontaneous reporting systems. This strategy will provide a basis for pharmaceutical enterprises to revise drug instructions and develop risk control measures. Consequently, this will help incorporate the study results into drug instructions and relevant clinical practice guidelines, which will further guide the rational use of clinical drugs.

5 Conclusion

Post-marketing safety surveillance and re-evaluaiton of SXNI included 30,122 patients from 48 hospitals. The incidence of SXNI-related adverse reactions in real-world clinical applications is occasional and in a reasonable range with a good prognosis. Causal analysis showed that women, long dispensing time, and slow dripping speed rate were considered as risk factors. When administering SXNI, physicians should be careful to follow guidelines and package insert.

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 humans were approved by the Medical Ethics Committee of Tianjin University of Traditional Chinese Medicine. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The participants provided their written informed consent to participate in this study.

Author contributions

ZW and XY participated in the planning, conception and design of the study. JX, WH, and LC collected the data; JX and WK drafted the original manuscript; PW, WC, and ZW edited the manuscript; XY and ZY participated in the statistical analysis. All authors contributed to the article and approved the submitted version.

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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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Correlation of bioactive marker compounds of an orally applied *Morus alba* root bark extract with toxicity and efficacy in BALB/c mice

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Introduction: In traditional Chinese medicine, the root bark of *Morus alba* L. is used to treat respiratory infections. Recently, anti-inflammatory and multiple anti-infective activities (against influenza viruses, corona virus 2, *S. aureus*, and *S. pneumoniae*) were shown *in vitro* for a standardized root bark extract from *M. alba* (MA60). Sanggenons C and D were identified as major active constituents of MA60. The aim of the present preclinical study was to evaluate, whether these findings are transferable to an *in vivo* setting.

Methods: MA60 was orally administered to female BALB/c mice to determine 1) the maximum tolerated dose (MTD) in an acute toxicity study and 2) its anti-influenza virus and anti-inflammatory effects in an efficacy study. A further aim was to evaluate whether there is a correlation between the obtained results and the amount of sanggenons C and D in serum and tissues. For the quantitation of the marker compounds sanggenons C and D in serum and tissue samples an UPLC-ESI-MS method was developed and validated.

Results: In our study setting, the MTD was reached at 100 mg/kg. In the efficacy study, the treatment effects were moderate. Dose-dependent quantities of sanggenon C in serum and sanggenon D in liver samples were detected. Only very low concentrations of sanggenons C and D were determined in lung samples and none of these compounds was found in spleen samples. There was no compound accumulation when MA60 was administered repeatedly.

Discussion: The herein determined low serum concentration after oral application once daily encourages the use of an alternative application route like intravenous, inhalation or intranasal administration and/or multiple dosing in further trials. The established method for the quantitation of the marker sanggenon compounds in tissue samples serves as a basis to determine pharmacokinetic parameters such as their bioavailability in future studies.

KEYWORDS

natural product, Mulberry Diels-Alder-type adducts, sanggenon, quantitation method, bioavailability, *in vivo*, lung, influenza

1 Introduction

Besides the coronavirus 2 which emerged in 2019 (SARS-CoV-2), influenza viruses and *Streptococcus pneumoniae* (pneumococci) belong to the most common pathogens causing pneumonia (Cilloniz et al., 2022; Dhoubhadel et al., 2022). Influenza is a respiratory illness with complications ranging from mild to severe symptoms or even death (Duwe et al., 2021). About three million severe cases of illness causing hospitalization occur annually worldwide (Paget et al., 2023) and the World Health Organization (WHO)[1] estimates between 290,000 and 650,000 deaths resulting from seasonal influenza outbreaks. Factors that drastically increase mortality rates of influenza cases are bacterial co-infections with pathogens like *S. pneumoniae* (McCullers, 2014). The neuraminidases of influenza viruses and *S. pneumoniae* contribute to this lethal synergism (Walther et al., 2016). The dramatic economic and medical impact of this lethal interplay drives the intense search for new therapeutic interventions and curative drugs (Langeder et al., 2020).

Morus alba L., commonly called the white mulberry tree, belongs to the family of Moraceae. In traditional Chinese medicine and other Asian folk medicines, various parts of *M. alba* (leaves, fruits, twigs, and the root bark) are used for antiphlogistic, diuretic, expectorant, and antidiabetic properties (Batiha et al., 2023). Apart from these medicinal effects, white mulberry fruits are also used for their nutritive value since they are known to contain important proteins, carbohydrates, fiber, organic acids, vitamins, and minerals (Chen et al., 2021). Regarding respiratory infections, the root bark of *M. alba* (Sang Bai Pi in Chinese) is traditionally used for the treatment of cough, bronchitis, and other pulmonary diseases (Yadav et al., 2022).

Recently, *M. alba* root bark has been shown to constitute promising dual inhibitors to combat viral and bacterial respiratory infections: it contains mulberry Diels-Alder-type adducts (MDAAs) which are prenylated flavonoids resulting from a [4 + 2]-cycloaddition of dehydroprenylphenols and chalcones (Grienke et al., 2016; Luo et al., 2021). In addition to the anti-infective properties, the anti-inflammatory potential of MDAAs has been identified *in vitro* (Rollinger et al., 2005; Chang et al., 2019). Recently, a specialized extract of the white mulberry root bark, labelled as MA60, enriched in 29% MDAAs (6.9% of sanggenon C and 10.7% of sanggenon D) was shown to exhibit promising anti-infective properties against influenza viruses, SARS-CoV-2, *Staphylococcus aureus*, and *S. pneumoniae* (Langeder et al., 2023; Wasilewicz et al., 2023). The high MDAA content clearly correlated with a dual inhibitory activity against influenza viruses and *S. pneumoniae* (Langeder et al., 2023). The two major MDAAs, sanggenons C and D (Figure 1), contained in the root bark, show remarkable anti-influenza virus, anti-*S. pneumonia* and anti-*S. aureus* activities *in vitro* (Grienke et al., 2016). These results indicate sanggenons C and D as valuable bioactive marker compounds for preclinical studies.

In vitro and *ex vivo* models are approaches to identify bioactive lead compounds. However, the translatability to an *in vivo* setting or even clinical studies is challenging[2]. Aspects like toxicity or complex pathogen-host interactions like the cellular and humoral immune defense of mammals are completely neglected in an *in vitro* setting (Roosenhoff et al., 2018). Thus, preclinical studies are essential (Butterweck and Nahrstedt, 2012). Mouse models of influenza A virus infection are valuable to study therapeutic effects (Barnard, 2009).

To translate the promising *in vitro* data of MA60 to an *in vivo* setting, the present study aimed to determine the maximum tolerated dose (MTD) of MA60 in female BALB/c mice (acute toxicity study) to be then used in the subsequent influenza virus infection model (efficacy study). These preclinical studies aim i) to evaluate the potential toxicity and to identify the most suitable oral dosage for the antiviral *in vivo* study, ii) to study whether an oral application ensures anti-influenza and/or anti-inflammatory effects, and iii) to determine the concentration of two selected marker constituents (sanggenons C and D) in serum as well as tissue samples after oral application of MA60.

2 Material and methods

2.1 Plant material, standards and MA60 extract preparation

The plant material from *M. alba* (root bark, batch no. 460797) was purchased from Plantasia in 2018. A voucher specimen (JR-20190928-A1) is deposited at the Department of Pharmaceutical Sciences, Division of Pharmacognosy, University of Vienna, Austria.

Double distilled water, HPLC-grade acetonitrile (VWR) and formic acid (VWR) were used for the chromatographic separation. For extract preparations, petroleum ether and n-hexane were distilled according to the Austrian Pharmacopoeia. Isopropanol (VWR) was purchased in analytical grade. Prior to LC-MS analyses, samples were dissolved in HPLC-grade methanol (VWR).

The MA60 extract was prepared as previously reported (Langeder et al., 2023). Briefly, a two-step extraction method was performed on a Dionex ASE 350 accelerated solvent extraction (ASE) instrument from Thermo Fisher Scientific, United States. Approximately 15 g of ground *M. alba* root bark were filled in a 34 mL extraction cell. In a first defatting step, the root bark was extracted with n-hexane in the flow mode (120°C) to remove highly lipophilic compounds. The obtained n-hexane extract was discarded. The second and essential extraction step was carried out with a mixture of isopropanol and petroleum ether in the ratio 2: 1 in the flow mode (80°C). This procedure was repeated multiple times to obtain 2.8 g extract from 300 g plant material. MA60 consists of 10.7% sanggenon C and of 6.9% sanggenon D. Further, it contains other sanggenons, i.e., sanggenon B (1.2%), sanggenon G (1.2%), sanggenon O (0.8%) and sanggenon E (1.0%).

1 https://www.who.int/news-room/fact-sheets/detail/influenza-seasonal

2 https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m3r2-non-clinical-safety-studies-conduct-human-clinical-trials-marketing-authorisation_en.pdf

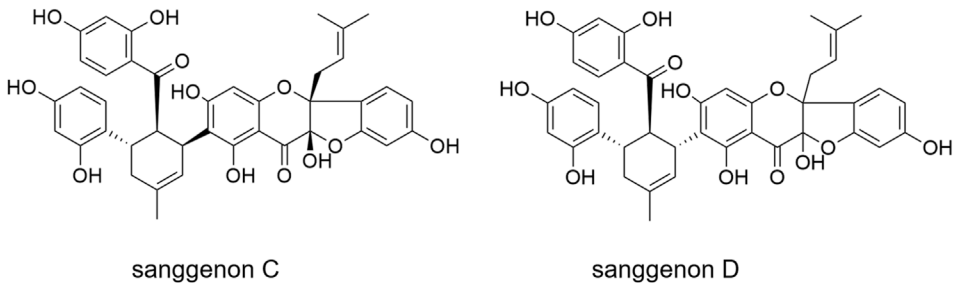

FIGURE 1
Major MDAAs contained in the *M. alba* root bark extract: sanggenon C and sanggenon D.

Sanggenons C and D were initially isolated from a methanolic *M. alba* root bark (2 kg) extract. Eleven fractions were obtained via flash chromatography (Interchim puriFlash® 4250) separated on a Puriflash 25 Silica HC 200G 25 µm column. Fractions 5 and 6 were further purified on a Gemini-Nx C_{18} phemomenex® column yielding 515 mg of sanggenon C (purity: 99%) and 730 mg of sanggenon D (purity: 99%), as previously described in detail (Langeder et al., 2023).

2.2 Animals, cells, and virus

Female mice are known to be more susceptible to influenza virus infection than male mice (Robinson et al., 2011). Therefore, female BALB/c mice (eight-weeks-old; 16–18 g; n = 66) were used in the present *in vivo* studies. Mice were purchased from Charles River (Bad Sulzfeld, Germany). They were housed in individually ventilated cages, at 22°C ± 2°C with a relative humidity of 55% ± 10% and a 14/10 h light/dark cycle. Mice were given food and water *ad libitum*.

Madin Darby canine kidney (MDCK) cells (Friedrich Loeffler Institute, Riems, Germany) were applied in Eagle's minimal essential medium supplemented with 2 µg/mL trypsin, 2 mM L-glutamine, and 1% nonessential amino acids.

Isolation and propagation of the working passage of influenza virus A(H1N1)pdm09 HA-G222-mpJena/5258 were published (Seidel et al., 2014).

2.3 Ethics statement

All trial procedures and animal care activities were conducted following the German Animal Protection Law. Experiments were approved by the Thüringer Landesamt für Verbraucherschutz (Reg.-Nr.: UKJ-18-015).

2.4 Dose finding study and acute toxicity of MA60 *in vivo* (experiment I)

For the development of the standard operating procedure (SOP) for the preparation of MA60 suspensions, the solubility of MA60 was evaluated. Based on previous studies from literature, 0.3% carboxymethyl cellulose (CMC) was used as a vehicle for preparing an MA60 extract suspension for oral application (Lim et al., 2013; Yimam et al., 2017). The 300 mg/kg dose was selected as maximum dose for the acute toxicity study. This was the maximum dose still resulting in a nearly homogenous solution (Supplementary Figure S1A) which is the prerequisite for oral gavage.

Aiming to define the dose and to exclude/reduce the risk of unacceptable side effects for experiment II (efficacy study), an acute toxicity study was performed. Twenty mice were randomly divided into four groups of five mice. One group was treated with the solvent (placebo: 0.3% CMC) and the other groups with 30, 100, and 300 mg/kg of solved MA60 (Supplementary Figure S1B). According to our previous results, influenza virus replication decreases until day 7 after infection (Seidel et al., 2014). The time schedule for MA60 administration was selected based on these observations. For the administration of MA60, oral gavage once daily was applied based on promising literature data reported for an ethanol extract from *M. alba* rootbark in 0.3% CMC applying 200 or 400 mg/bw/daily in a mouse model of LPS-induced airway inflammation (Lim et al., 2013).

Body weight and clinical score (weight change, physiognomy, and behavior) of mice were determined daily and considered for the determination of the MTD. Mice were sacrificed and organ tissue samples (lung, liver, heart, spleen) were aseptically removed and weighed 3 hours after the last treatment. In addition, serum was obtained from collected blood samples by centrifugation at 855 *g* for 15 min. The lung, liver, spleen, and serum samples of mock-infected mice were also used to establish and validate a UPLC-MS method for the quantitation of the marker compounds sanggenon C and D.

2.5 Efficacy study regarding anti-influenza and anti-inflammatory effects of MA60 *in vivo* (experiment II)

To determine the antiviral effect of MA60 *in vivo*, 46 mice were randomly divided into experimental groups as summarized in Supplementary Table S1. Mice were treated orally (per oral gavage) once daily for a maximum of 7 days with either the solvent (placebo: 0.3% CMC; n = 23) or 100 mg/kg of solved MA60 (n = 23). One hour after the first application of MA60, groups of mice were mock-infected (NaCl) or infected with the influenza virus in NaCl. For this procedure, mice under isoflurane

anesthesia were inoculated intranasally with 20 µL NaCl or 10^5 $TCID_{50}$/20 µL of HA-G222-mpJena/5258 diluted in NaCl. The selected number of mice as well as the statistical evaluation are based on literature data (Richardson and Overbaugh, 2005).

Body weight and clinical score (weight change, physiognomy, and behavior) of mice were determined at least once daily. Mice that lost more than 20% of their initial body weight over 48 h or 25% of their initial body weight and/or became severely ill were sacrificed for ethical reasons (n = 3; two placebo-treated, influenza virus-infected mice and one MA60-treated, influenza virus-infected mouse).

One hour after the second treatment (day 1 p.i.), as well as 24 h after the last treatment (day 7 p.i.), groups of mice were sacrificed and organ tissue samples (lung, liver, spleen) were aseptically removed. Serum was obtained from collected blood samples by centrifugation at 855 *g* for 15 min. The superior lobe of influenza virus-infected right lung was homogenized in test medium and used for virus titer determination (Reed and Muench, 1938). Another right lung lobe was frozen for analysis of cytokine mRNA levels as described in 2.6. The left lobe was fixed in 10% formalin and embedded in paraffin for histopathological evaluation. For that, sections of 4 µm were prepared and stained with hematoxylin and eosin. Further two right lung lobes, liver, spleen, and serum of mock-infected mice were used for the determination of the content of sanggenons C and D.

2.6 RNA isolation, reverse transcription and quantitative PCR

Lung samples were homogenized in lysis buffer and TissueLyzer beads (Qiagen, Hilden) in the TissueLyzer II (Qiagen, Hilden). Then RNA was isolated with the RNeasy® Mini Kit (Qiagen, Hilden) according to the producer's manual. NP-vRNA copy number was determined after reverse transcription of 1,000 ng RNA with uni12 primer (Hoffmann et al., 2001) by a quantitative PCR with two NP-gene targeting primers and a NP-plasmid standard. The expression profile of cytokines (IP10, IL-6, IFN-ß, CXCL9 and Eif2ak2) was determined after reverse transcription of 1,000 ng RNA with oligo dT primer by a semi quantitative PCR with cytokine mRNA-targeting primers. The expression of GAPDH mRNA was used as a baseline control. Results are calculated and expressed as Log2(2^-ΔΔCt)-fold change of mRNA expression related to control (Pfaffl et al., 2002). Primer sequences and PCR temperature profiles are summarized in Tables (Supplementary Tables S2–S4).

2.7 Statistical analysis of *in vivo* data

Semi-quantitative PCR results were analyzed using EXCEL 2016 software according to the method described by Pfaffl (Pfaffl, 2004). Data are presented as mean with standard deviation (SD) calculated with EXCEL 2016.

Statistical analysis of data is based on "basic statistical considerations in virological experiments" published by (Richardson and Overbaugh, 2005). The number of animals (n) per experimental group is given in Supplementary Figure S1 (acute toxicity study) and Supplementary Table S1 (efficacy study). Mice succumbing to infection before day 7 are mentioned in paragraph 2.5 and 3.2. After performing normality test (Shapiro-Wilk) and equal variance test (Brown-Forsythe), the significance of differences within multiple cardinal data sets (body weight, cytokine mRNAs) were analyzed with One-way ANOVA (normal distribution) or Kruskal–Wallis One Way Analysis of Variance on Ranks (non-normal distribution) with Sigmaplot 14.0. Virus titers and nucleoprotein gene copies of the mock-treated, influenza virus-infected and the MA60-treated influenza virus-infected group of mice were analyzed with the unpaired, two-sided Student's t-test by using EXCEL 2016. $p \leq 0.05$ was set as cut off for statistical significance.

2.8 Serum and tissue extraction protocol

The bio sample extraction was adapted according to the published workflow from Yuan and co-workers (Yuan et al., 2012). 50 µL of thawed serum sample was transferred to a 1.5 mL Eppendorf tube. 1,000 µL of MTBE was added and shaken for 1 h. After a centrifugation step at 13,400 rpm for 15 min, 900 µL of supernatant was removed and the solvent was evaporated until complete dryness.

To analyze tissue samples, an aliquot of approximately 200 mg of each mouse liver, the whole spleen and lung were homogenized in a Zentrimix 380R (Hettich) at 4°C for 15 min at 1,500 rpm in a separate tube by three zirconium oxide beads (Ø 3 mm) in 500 µL water. 900 µL cooled methanol (−70°C) was added and ultrasonicated for 5 min in an ice bath. After vortexing, the samples were incubated for 1.5 h at −70°C. Then, tissue homogenates were centrifuged at 15,000 rpm in a refrigerated centrifuge (4°C) for 10 min. Supernatants were collected and the incubation cycle was repeated for exhaustive extraction with 400 µL fresh methanol (−70°C) followed by ultrasonication (10 min on ice) and vortexing under cooled conditions (4°C). Samples were transferred to a refrigerator for a 30 min incubation at −70°C. After centrifugation, supernatants were combined and dried in a GeneVac EZ-2 plus using the program "Aqueous" set to 30°C. For analysis, serum, as well as tissue samples, were dissolved in 50 µL MeOH, centrifuged and 5 µL were injected in duplicates for analysis.

2.9 UHPLC-ESI-MS analysis and method validation for the quantitation of marker compounds sanggenon C and sanggenon D in serum and tissue samples

UHPLC-ESI-MS (Thermo Fisher Scientific, CA) analysis was performed on a Dionex UltiMate 3,000 system coupled to an LTQ XL ion trap mass spectrometer. MS detection was carried out using HESI source (300°C heater temperature, 40/10/1 arb. units for the sheath, aux and sweep gases, respectively and 3.5 kV spray voltage at 275°C capillary temperature) to achieve negative ion mode ionization. For quantitation, SIM (single ion monitoring) was applied in the negative mode. The $[M-H]^-$ adduct 707.21 Da was used as a center mass with a 1.00 Da window. For confirmation and method validity, selective MS/MS scans of the 3 most abundant ions

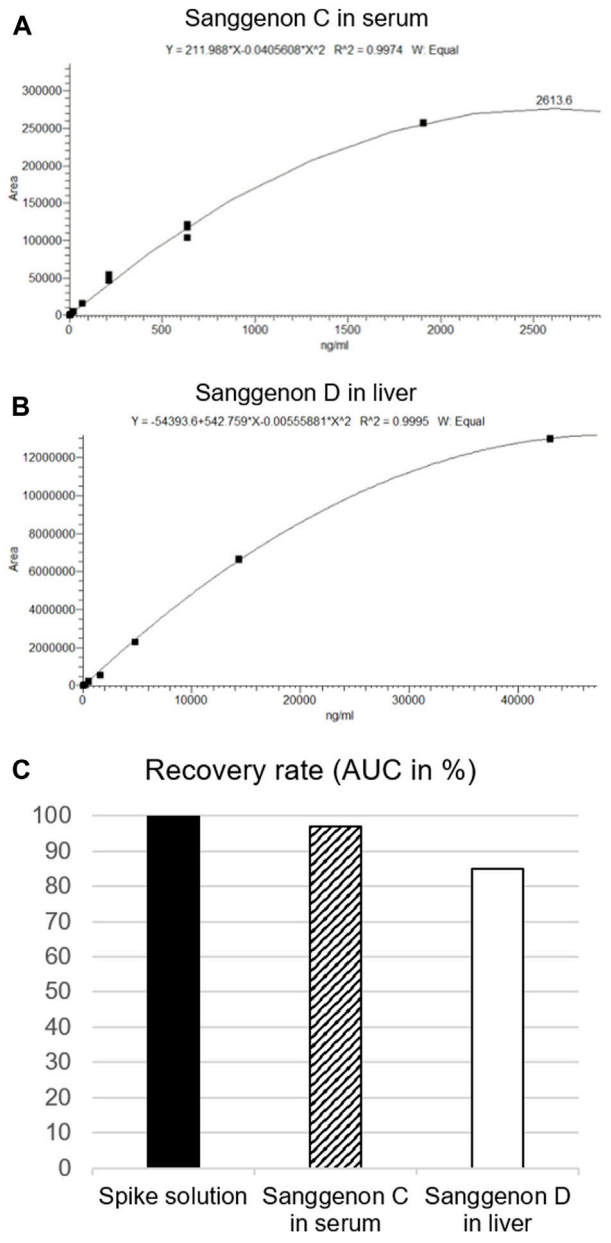

FIGURE 2
Calibration curves, fitting and dilutions of **(A)** sanggenon C for serum samples and **(B)** sanggenon D for liver samples. **(C)** Extraction recovery rates given as % of AUC of sanggenon C and D from the respective biomatrix in comparison to the AUC from the spike solution (set to 100%).

were achieved through collisional induced dissociation (CID) fragmentation at 30% normalized collision energy.

Separation was carried out on a Waters ACQUITY BEH phenyl column (2.1 × 100 mm, 1.7 µm) within 13 min. Solvent A: Water/formic acid (99.9:0.1), Solvent B: Acetonitril/formic acid (99.9:0.1). The following gradient was applied: 0–2 min: 30% B, 2–13 min: 30%–98% B, followed by a washing step: 13–17 min 98% B and an equilibration step for 3 min 5 µL of dissolved sample in methanol were injected. Data acquisition and evaluation was carried out using Xcalibur (version 4.27.0.19). The UHPLC-ESI-MS method was validated for specificity, linearity, precision, and extraction recovery according to ICH guidelines[3]. In terms of specificity, chromatograms of the spiked samples were compared to respective blank serum or liver samples. Calibration curves were established by serial dilution of standards of sanggenons C and D, respectively in a ratio of 1:3 in methanol (level 0: 1 mg/mL) followed by injection of each level in triplicate. The peaks were integrated using Thermo Xcalibur software using the integration algorithm ICIS. Limit of detection (LOD) and limit of quantitation (LOQ) were evaluated visually as a signal-to-noise ratio of more than 3 times (LOD) or 10 times (LOQ). Linearity was determined as the range of all included calibration levels in the regression equation. For precision, intraday (within 1 day, sample #64) and interday (over 3 days, sample #65) measurements were evaluated accordingly. To assess extraction recovery, spiking experiments were conducted as follows: Stock solutions of the extract MA60 were prepared at a concentration of 1 mg/mL. 10 µL of the stock solution was added to 50 µL of blank serum or blank liver homogenate and treated as described above. By integration of the AUC of the peak of sanggenon C (serum samples) and sanggenon D (tissue samples) and comparing it to the peak area of sanggenons C and D, respectively in a 1:5 dilution of the stock solution, the recovery rate was calculated accordingly.

MA60 contains a well-characterized amount of bioactive MDAAs of 29% (Langeder et al., 2023). Sanggenons C and D calibration curves were obtained by serial dilution down to 1.88 ng/mL (Figure 2). A quadratic fit of the calibration curves revealed correlation coefficients of $R^2 \geq 0.9974$ (Table 1). Validation parameters (specificity, linearity, precision, and extraction recovery) were evaluated in accordance with ICH guidelines.[4] The specificity was assessed by comparing the selected MS/MS spectra of serum and tissue samples to the fragment spectra of sanggenons C and D. As there was a precise matching of the standard fragment ions to the fragment ions found in serum and tissue samples, it can be concluded that the established method is specific for the marker compounds (Supplementary Figure S2).

The extraction efficiency for serum and tissue samples was probed by performing spiking experiments. 10 µL of a stock solution of MA60 (1 mg/mL) was spiked to samples of blank serum and liver. Sanggenons C and D were extracted according to the given protocol. Their peak areas were compared to the extract solution (diluted 1:5), respectively. From the spiked samples, 97% of sanggenon C in serum samples and 85% of sanggenon D in liver samples were retrieved. These results confirmed the suitability of the extraction protocol for sanggenon C in serum samples and sanggenon D in liver samples (Supplementary Figure S2). Interestingly, the extraction recoveries for sanggenon D in serum samples (84%) and sanggenon C in liver samples were significantly lower (26%), and therefore not applied as marker compounds in the respective matrices.

3 https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf

4 https://www.ema.europa.eu/en/documents/scientific-guideline/ich-s-7-b-nonclinical-evaluation-potential-delayed-ventricular-repolarization-qt-interval_en.pdf

TABLE 1 Validation parameters for the quantitation of sanggenon C in serum samples and sanggenon D in liver samples.

Compounds	Sanggenon C	Sanggenon D
Regression equation (y =)	211.988x-0.0405608x^2	−54393.6 + 542.759x-0.00555881x^2
Correlation coefficient (R^2)	0.9974	0.9995
Linearity range (ng/mL)	2.62–1,906.72	2.1–42,962
LOD (ng/mL)	0.87	3.92
LOQ (ng/mL)	2.91	13.05
Intraday precision (%)	n.d.	6.20
Interday precision (%)	n.d.	7.71
Extraction recovery (%)	95	85

n.d., not determined.

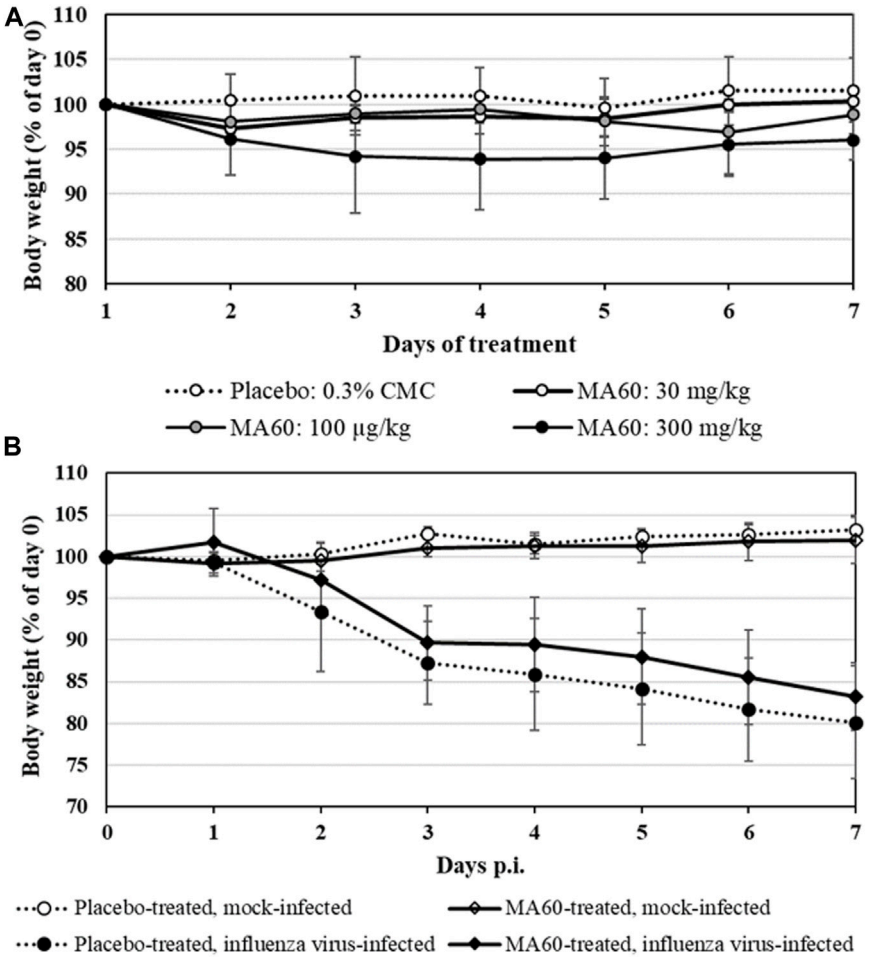

FIGURE 3
Mean body weight changes of 8-week-old female BALB/c mice which were treated **(A)** once daily for 7 days with 30, 100 or 300 mg/kg MA60 or placebo (solvent: 0.03% CMC) orally in the toxicity study or **(B)** once daily for 7 days with 100 mg/kg MA60 or placebo (solvent: 0.03% CMC) orally in the efficacy study. Treatment started 1 h before intranasal mock-infection (NaCl) or infection with influenza virus A(H1N1)pdm09 (10^5 $TCID_{50}$/mouse in 20 µL NaCl).

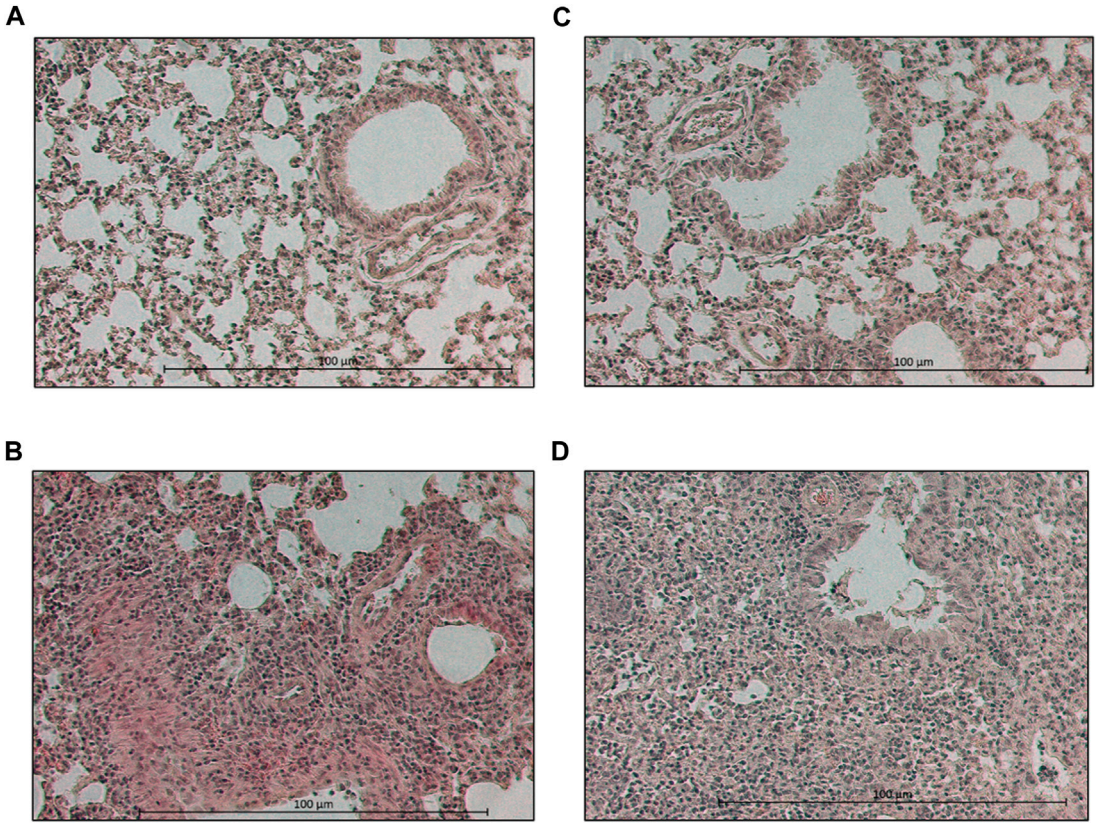

FIGURE 4
Photographs of hematoxylin-eosin-stained lung sections of day 7 p.i. Lung section of a **(A)** placebo-treated, mock-infected, **(B)** placebo-treated, virus-infected, **(C)** MA60-treated, mock-infected, and **(D)** MA60-treated, influenza virus-infected BALB/c mouse is shown, for example,.

3 Results

3.1 Dose finding study and acute toxicity of MA60 *in vivo* (experiment I)

BALB/c mice were treated per gavage feeding with 30, 100, and 300 mg/kg of MA60 in 0.3% CMC or 0.3% CMC (placebo) once daily for 7 days to determine the MTD for the subsequent *in vivo* efficacy study. Daily monitoring of body weight revealed that neither treatment with 30 nor with 100 mg/kg of MA60 caused a significant body weight change compared to placebo (Figure 3A; Supplementary Table S5). In contrast, mice treated with 300 mg/kg of MA60 continuously lost body weight until day 4 of treatment ($p = 0.03$ at day 4 of treatment compared to the placebo-treated group). The 300 mg/kg dose of MA60 did not affect the weight of liver, lung, heart, and spleen (Supplementary Table S6). The applied tests and the results of statistical analysis of the percentage of daily body weight (% of day 0) are summarized in Supplementary Table S5. After day 4, a slight amelioration was observed.

In summary, body weight loss was the only adverse effect observed after treatment (clinical score not shown). The 300 mg/kg dose of MA60 causes more than a 10% depression in body weight gain. Thus, the 100 mg/kg dose of MA60 was identified as MTD in the acute toxicity study.

3.2 Efficacy study regarding anti-influenza and anti-inflammatory effects of MA60 *in vivo* (experiment II)

Based on the dose-finding experiment, the 100 mg/kg dose of MA60 was well tolerated when administered orally once a day for 7 days to 8-weeks-old female BALB/c mice (MA60-treated, mock-infected). Neither body weight (Figure 3B) nor lung histology (Figures 4A,C and Supplementary Figure S3A, S3C) and cytokine mRNA expression (Figure 6) were significantly affected when applying this MA60 dose (Supplementary Tables S7, S8).

In contrast, placebo-treated, influenza virus-infected mice lost body weight starting on day 2 after infection (Figure 3B). Due to critical body weight loss, one mouse of this experimental group had to be sacrificed for ethical reasons on day 5 p.i. and another one on day 6 p.i. As of day 2 p.i. or day 3 p.i., the difference in body weight was statistically significant compared to placebo-treated, mock-infected mice (p values between <0.001 and 0.020) or MA60-treated, mock-infected mice (p values between <0.001 and 0.027), respectively. The applied statistical tests and the calculated p values are summarized in Supplementary Table S7. High virus titers and nucleoprotein gene copy numbers were detected on day 1 p.i. (Figures 5A, B). Infectious virus was nearly eliminated on day 7 after virus infection (Figure 5A) whereas nucleoprotein gene copies declined more slowly (Figure 5B). The expression of cytokines was enhanced, however not significantly

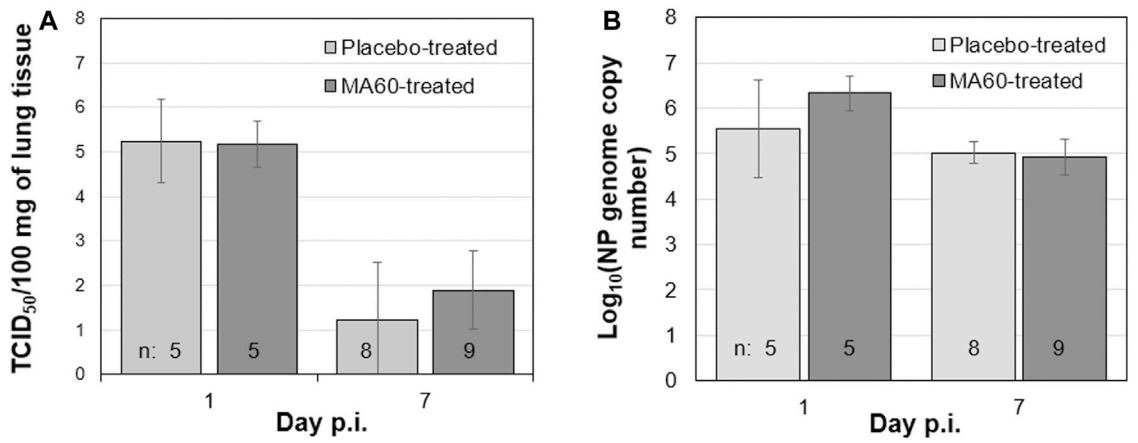

FIGURE 5
Oral treatment with 100 mg/kg MA60 (7 days, once per day) did not inhibit influenza virus replication in the lung. To study the effect of MA60 on viral replication, **(A)** virus titers and **(B)** nucleoprotein genome copies were determined in lung tissue samples.

on the day after influenza virus infection when comparing placebo-treated, mock-infected mice with placebo-treated, influenza virus-infected mice (Figure 6; Supplementary Table S8). In contrast, a significant difference was found between both experimental groups for the expression of IL-6, IP10, and CXCL9 7 days after virus infection (Figure 6; Supplementary Table S8).

The loss of body weight occurred more slowly and was less pronounced in MA60-treated, influenza virus-infected mice compared to placebo-treated, influenza virus-infected mice (Figure 3B). The body weight differences were statistically different between day 3 and 5 p.i. (p values between 0.022 and 0.004) or day 4 p.i. (0.004) when comparing MA60-treated, influenza virus-infected mice with placebo- or MA60-treated, mock-infected mice, respectively. However, the observed difference between MA60-treated, influenza virus-infected mice and placebo-treated, influenza virus-infected mice was not significant (Supplementary Table S7). Only one mouse of this experimental group had to be sacrificed for ethical reasons on day 6 p.i. The treatment effect of 100 mg/kg MA60 administered orally was not strong enough to significantly reduce virus titers (Figure 5A) and the number of viral nucleoprotein genome copies (Figure 5B). At day 1 after infection, the cytokine expression (IL-6, CXCL-9, IP10, and IFN-ß) in lung samples of MA60-treated, influenza virus-infected mice was stronger than in all other experimental groups. The difference was not significant in comparison to the placebo-treated, influenza virus-infected group but it was significant in comparison to the placebo-treated, mock-infected and/or MA60-treated, mock-infected groups (Figure 6; Supplementary Table S8). The comparison of lung histopathology did not reveal differences between placebo-treated and MA60-treated influenza virus-infected mice (Figures 4B, D; Supplementary Figures S3B, S3D).

3.3 Quantitation of sanggenons C and D in serum and tissue samples using UPLC-ESI-MS

In the samples from the acute toxicity trial, a dose-dependency could be observed for the content of sanggenon C in serum samples (Figure 7A). In the lowest dose group (30 mg/kg) an amount of sanggenon C below 9.10 ng/mL was found; in the 100 mg/kg dose group between 7.94 ng/mL and 31.53 ng/mL, and in the group of mice treated with 300 mg/kg of MA60 between 21.21 ng/mL and 227.26 ng/mL of sanggenon C were determined (Supplementary Table S9).

In the efficacy trial (100 mg/kg dose), serum concentrations of sanggenon C were found to be between 8.00 ng/mL and 124.22 ng/mL (#64-#67) after 1 h. After 24 h, the concentrations were below 2.77 ng/mL or not detectable (#68-#71).

The amount of sanggenon D was determined in both the acute toxicity as well as the efficacy experiment in lung, liver, and spleen samples (see Figure 7B; Supplementary Table S9). In lung samples, sanggenon D was detected in relatively high amounts between 343 and 10,306 ng/mL in four samples (#29, #32, #33 and #35). In liver samples of the low dose group sanggenon D was not detected. Only in two (#32 and #35) out of five samples, a sanggenon D concentration of 34.18 ng/mL and 26.97 ng/mL was detected, respectively. However, in the high dose group (300 mg/kg) especially in liver tissues derived from #38 and #39, a concentration of almost 1 µg/mL was determined. In general, samples from mice #38-#40 resulted in high concentrations of sanggenon C (serum) and sanggenon D (liver). Comparable to the serum concentrations of sanggenon C in the infection experiment, a high amount of sanggenon D was detected when mice were sacrificed after 1 h. In contrast, sanggenon D could not be determined after 24 h in the liver. None of the marker compounds was found in spleen samples (data not shown).

4 Discussion

The presented *in vivo* studies i) enabled the determination of the MTD of MA60, ii) gave first insights into the potential efficacy of MA60 against influenza virus infection *in vivo*, and iii) allowed for the correlation of marker compound concentrations in serum and tissue samples with the doses of MA60 applied orally.

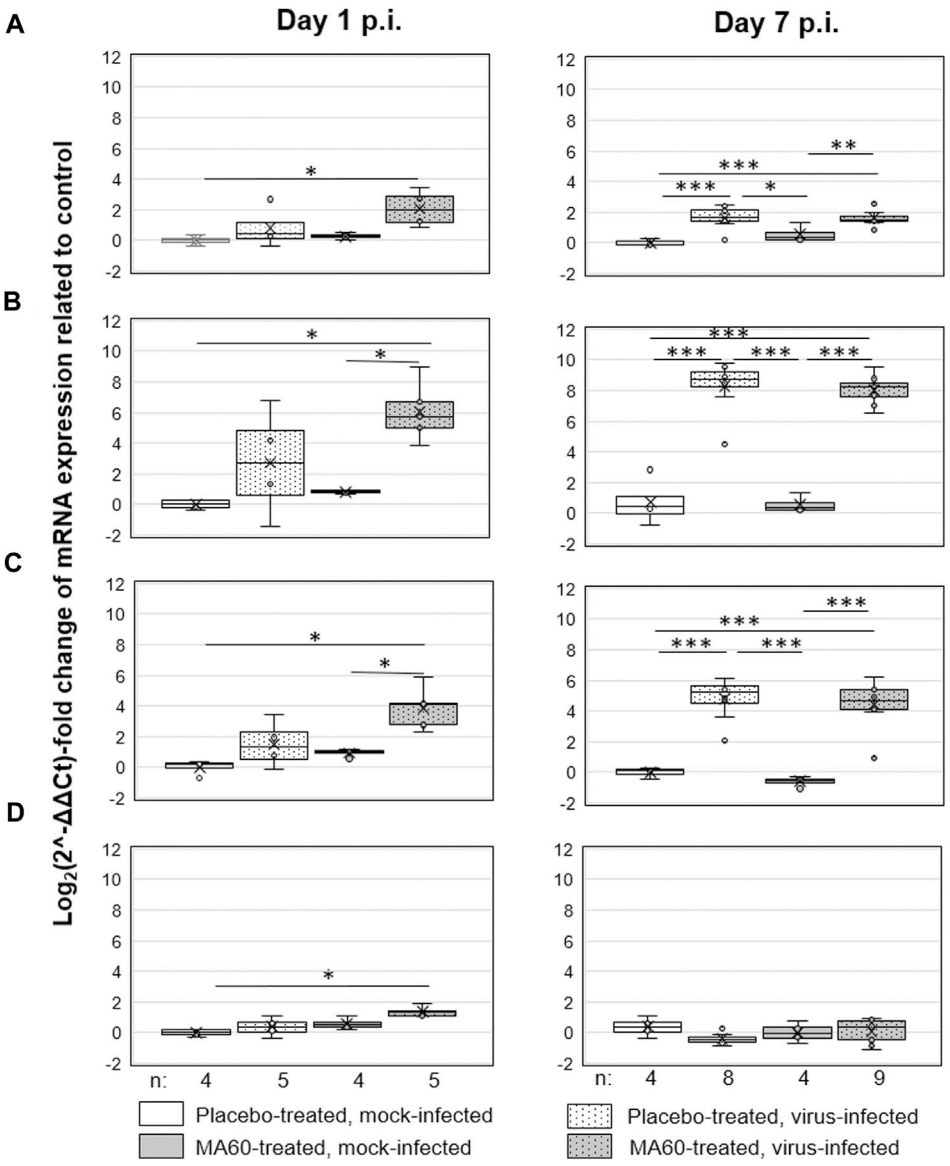

FIGURE 6
Oral treatment with 100 mg/kg MA60 (7 days, once per day) had no significant effect on cytokine expression in the lung of influenza virus-infected BALB/c mice. The mRNA copies of **(A)** IL-6, **(B)** IP10, **(C)** CXCL9, and **(D)** IFN-ß were determined by qPCR.

The MTD of MA60 was determined in female BALB/c mice by oral application of 30, 100, and 300 mg/kg MA60 in the acute toxicity study using the following parameters: body weight, clinical score, and organ weight (lung, liver, heart, and spleen). Higher doses, published in two mice studies with ethanolic root bark extracts of *M. alba* (Lim et al., 2013; Yimam et al., 2019), did not reveal homogeneous solutions of MA60 in 0.3% CMC. Thus, the dose limit for acute toxicity studies of 1,000 mg/kg/day for rodents as recommended in the ICH guideline M3(R2)[5] could not be applied for practical reasons.

The 30 and 100 mg/kg/day doses did not affect the selected study parameters. In contrast, the 300 mg/kg dose of MA60 caused a body weight loss of more than 10%. This is in agreement with a study performed with an ethanolic root bark extract of *M. alba* (Yimam et al., 2019). The authors describe an appetite suppression and body weight loss in mice treated orally with daily dosages of 250 and 500 mg/kg. Thus, the 100 mg/kg dose of MA60 fulfills the MTD definition as "The highest dose of a drug or treatment that does not cause unacceptable side effects."[6] The MTD for MA60 determined in this study is also in line with previous literature (Dorato and

5 https://www.ema.europa.eu/en/ich-m3-r2-non-clinical-safety-studies-conduct-human-clinical-trials-pharmaceuticals-scientific

6 https://www.cancer.gov/publications/dictionaries/cancer-terms/def/maximum-tolerated-dose

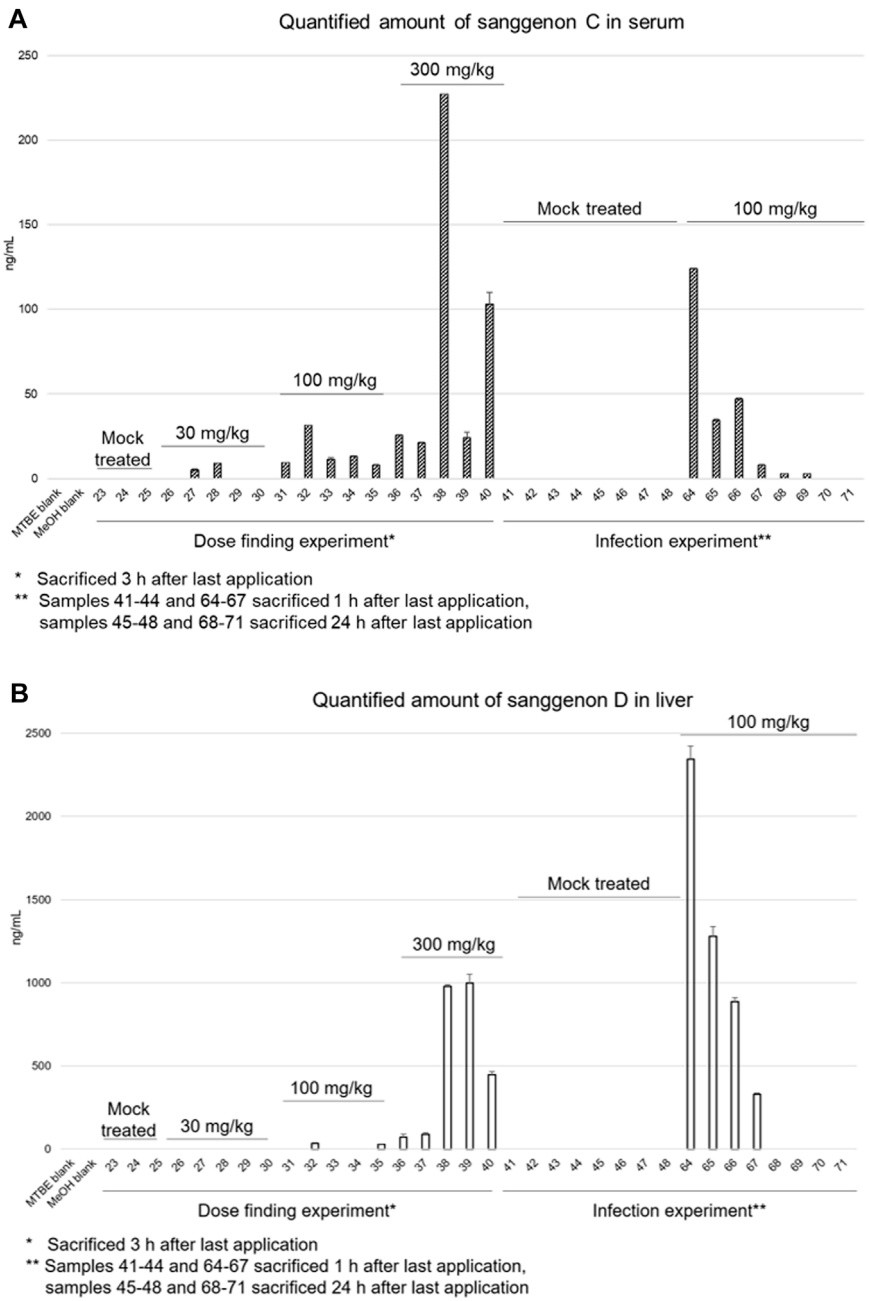

FIGURE 7
Quantitative results of sanggenon C in serum samples **(A)** and sanggenon D in liver samples **(B)** for the dose finding trial where 30 mg/kg, 100 mg/kg and 300 mg/kg extract (MA60) were applied orally and the infection trial with 100 mg/kg body weight, respectively.

Buckley, 2006) stating that experimentally, the MTD should cause no more than a 10% depression in body weight gain.

Accordingly, 100 mg/kg was applied to get first insights into the potential efficacy of MA60 against influenza virus infection *in vivo*. The body weight loss caused by influenza virus infection, virus replication (virus titer and viral RNA load in lung tissue), and cytokine mRNA induction (IFN-ß, IL-6, IP10, CXCL9 in lung tissue) were selected as readout parameters.

In agreement with the acute toxicity study, the 100 mg/kg dose of MA60 in the efficacy study did not affect body weight confirming the MTD selection. A delayed loss of body weight was observed in MA60-treated, influenza virus-infected mice pointing towards a moderate protective effect. In the presented *in vivo* setting, however, no significant efficacy was obtained. One reason might be an inefficient inhibition of viral replication. Indeed, there was no effect on virus replication (influenza virus titers and number of viral nucleoprotein genome copies) in the lung. This might be based on the antiviral mechanism of action of sanggenons which target the neuraminidase (Grienke et al., 2016; Langeder et al., 2023). The activity of neuraminidase inhibitors in

cell-based assays is dependent on a balanced function of the viral hemagglutinin and neuraminidase (Grienke et al., 2012). It could be demonstrated previously that sanggenons dose-dependently inhibited the influenza virus replication in MDCK cells but not in lung bronchial cells (Langeder et al., 2023). A further explanation for the lack of inhibition of influenza virus replication might be an ineffective concentration of bioactive sanggenons in lung tissue.

The analysis of cytokine expression revealed higher amounts of the antiviral IFN-β in MA60-treated, influenza virus-infected mice, compared to placebo-treated, influenza virus-infected mice (p = 0.055) at day one after infection. In addition, a highly significant difference (p = 0.006) was identified between MA60-treated, influenza virus-infected mice and the placebo-treated, mock-infected group. The more pronounced induction of IFN-β in the presence of MA60 might have contributed to a delayed induction of influenza symptoms as previously reported (Chen et al., 2018). This hypothesis is supported by the delayed body weight loss observed in our efficacy study. Intriguingly, protective effects were recently observed for a white mulberry fruit extract in a rat model, showing protective effects of the applied extract on the fertility of rats treated with the alkylating agent carmustine (Inanc et al., 2022; Ipek et al., 2022). Whether higher amounts of the bioactive sanggenons in lung tissue would reveal a stronger IFN-ß response and whether this is associated with stronger protection against influenza remains to be studied.

In the present study, concentration of bioactive sanggenons C and D was determined in serum and tissue samples and correlated with the administered the doses of MA60. UHPLC-ESI-MS analyses of the *in vitro* bioactive sanggenons C and D revealed serum concentration of less than 1% after one (efficacy study) and three (acute toxicity study) hours of the last oral MA60 administration. Already during dose finding and acute toxicity studies, a high variation in serum concentrations of the selected marker compounds was detected within all three dose groups (30, 100, and 300 mg/kg). No dose dependency could be observed regarding the serum concentrations. This might be due to i) inhomogeneities in the CMC suspension of the MA60 extract, ii) the inter-individual differences in the resorption of sanggenons, or iii) the formation of sanggenon metabolites:

(i) For oral gavage administration, MA60 was suspended in 0.3% carboxymethyl cellulose (CMC) as vehicle. This is based on literature data, where even a higher dose of up to 400 mg/kg of a 70% EtOH extract of *M. alba* root bark (Lim et al., 2013) was previously administered to mice per gavage feeding. In the present study, poor solubility of MA60 prevented the use of higher doses. Thus, the applicability of 0.3% CMC as vehicle could only partly be confirmed for MA60, which could have impacted the homogenous distribution of sanggenons in the oral gavage.

(ii) Inter-individual differences in absorption, distribution, metabolism, and excretions might further affect drug metabolism and contribute to the high variation in serum concentrations of the selected marker compounds.

(ii) Regarding the potential formation of MDAA metabolites, Liu and coworkers found mono- and di-glucuronides as well as hydroxylated derivatives of the *M. alba* constituent kuraridin (prenylated flavonoid) in rat serum (Liu et al., 2018). In the present study, an MS/MS analysis was conducted to also search for metabolites of the selected marker compounds sanggenon C and sanggenon D. The search included (di)glucuronides and hydroxylated metabolites as well as further conjugates such as sulfated metabolites. Interestingly, neither the corresponding masses nor the phase-II-conjugates could be detected in the different serum samples.

A rapid metabolism or excretion of sanggenon C is highly plausible when comparing the quantities determined in mice that were sacrificed 1 hour versus 3 hours after the last application. Here, the average serum concentration decreased from 15 ng/mL to below 2 ng/mL. Hence, no accumulation of MDAAs was observed after oral application.

Intriguingly, in this study, a pronounced difference was observed for extraction recoveries of the isomers sanggenons C and D from the investigated samples: Recovery rates were superior for sanggenon C in serum and in liver samples for sanggenon D. Their difference in stereochemical configuration obviously results in a deviating behavior in the chiral environment of biofluids or tissue samples.

Although for the present study female mice were selected because of their higher susceptibility to influenza virus infection (Robinson et al., 2011), it would be highly worth to analyze the acute toxicity of MA60 also in male mice. Moreover, safety and efficacy are important preclinical parameters (Buckley and Dorato, 2009) which could be more focused on, e.g., by determining the maximum feasible dose (MFD) of MA60.

Overall, the non-significant treatment effect and the determined low serum concentrations of sanggenon C and sanggenon D raise the question about the bioavailability of MA60 after oral application. In future studies, oral administration of MA60 twice a day (BID) or three times a day (TID), intravenous administration, or inhalation administration should be conducted to fully assess the *in vivo* potential of the bioactive sanggenons and to further evaluate their pharmacokinetic parameters.

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 approved by the Thüringer Landesamt für Verbraucherschutz (Reg.-Nr.: UKJ-18-015). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

MS, JR, and UG designed and supervised the study. JL and MS wrote the manuscript; MK and MS conducted the *in vivo* trial. HS

investigated the antiviral and anti-inflammatory effects. AT helped in setting up the parameters for MS detection. JL developed the analytical protocol and performed the processing of *in vivo* samples including the MS-based quantitation of marker compounds sanggenons C and D. UG and JR revised the manuscript. All authors contributed to the article and approved the submitted version.

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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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The application and sustainable development of coral in traditional medicine and its chemical composition, pharmacology, toxicology, and clinical research

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This review discusses the variety, chemical composition, pharmacological effects, toxicology, and clinical research of corals used in traditional medicine in the past two decades. At present, several types of medicinal coral resources are identified, which are used in 56 formulas such as traditional Chinese medicine, Tibetan medicine, Mongolian medicine, and Uyghur medicine. A total of 34 families and 99 genera of corals are involved in medical research, with the Alcyoniidae family and *Sarcophyton* genus being the main research objects. Based on the structural types of compounds and the families and genera of corals, this review summarizes the compounds primarily reported during the period, including terpenoids, steroids, nitrogen-containing compounds, and other terpenoids dominated by sesquiterpene and diterpenes. The biological activities of coral include cytotoxicity (antitumor and anticancer), anti-inflammatory, analgesic, antibacterial, antiviral, immunosuppressive, antioxidant, and neurological properties, and a detailed summary of the mechanisms underlying these activities or related targets is provided. Coral toxicity mostly occurs in the marine ornamental soft coral Zoanthidae family, with palytoxin as the main toxic compound. In addition, nonpeptide neurotoxins are extracted from aquatic corals. The compatibility of coral-related preparations did not show significant acute toxicity, but if used for a long time, it will still cause toxicity to the liver, kidneys, lungs, and other internal organs in a dose-dependent manner. In clinical applications, individual application of coral is often used as a substitute for orthopedic materials to treat diseases such as bone defects and bone hyperplasia. Second, coral is primarily available in the form of compound preparations, such as Ershiwuwei Shanhu pills and Shanhu Qishiwei pills, which are widely used in the treatment of neurological diseases such as migraine, primary headache, epilepsy, cerebral infarction, hypertension, and other cardiovascular and cerebrovascular diseases. It is undeniable that the effectiveness of coral research has exacerbated the endangered status of corals. Therefore, there should be no distinction between the advantages and disadvantages of listed endangered species, and it is imperative to completely prohibit their use and provide equal protection to help them recover to their normal numbers. This article can provide some reference for research on coral chemical composition, biological activity,

chemical ecology, and the discovery of marine drug lead compounds. At the same time, it calls for people to protect endangered corals from the perspectives of prohibition, substitution, and synthesis.

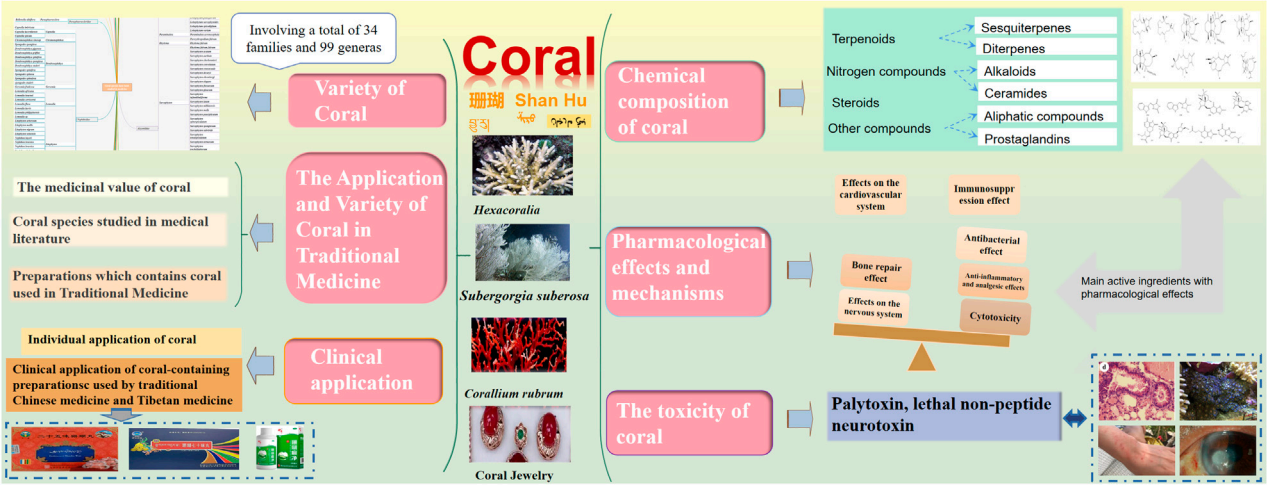
KEYWORDS

coral, traditional medicine of China, chemical constituents, pharmacology, toxicology, clinical application

1 Introduction

Marine biological resources are abundant, and coral is a common organism in the ocean. It is a low-level invertebrate of the ocean, belonging to the phylum Coelenterata and the class Coralis. Coral mainly lives in tropical oceans and has a wide variety and distribution. There are over 6,100 species of coral worldwide and 719 species in China (Li T. T., 2010). Corals can be divided into Hexacorallia and Octocorallia (Xu, 2016). Corals are known as "sea flowers" and are a type of aquatic coelenterate. Their population is dendritic, branching and spreading like fans, with fine branches. Their surface contains many hydra bodies called anthozoan polyps. Their body is hemispherical in shape, with eight feathered tentacles on top. The tentacles have a mouth in the center, and the insect body can secrete limestone to form bones. White is better than snow; red is similar to blood; green is similar to jade, and yellow is similar to gold. Coral naturally grows in the sea, with strange shapes and unparalleled beauty (National Compilation of Chinese Herbal Medicine, 1996). The main compound of coral is calcium carbonate, which also contains a series of elements such as iron, manganese, copper, and strontium, as well as chitin and organic acids. Corals are commonly white, whereas gemstone-grade corals are red, pink, and orangey red, with a small amount of black and blue. The color of coral is due to its content of approximately 1% iron oxide and organic matter. With red as the top grade, red coral is as red as fire, known as the "fire tree" in ancient times. Its origin is in the deep sea of the Mediterranean and Atlantic oceans, and it is primarily used for jewelry, with the largest being used for carving figures, flowers, and birds, and other handicrafts (Wen, 2007). In India and Tibet of China, people use coral as a mascot for worship, often used to make Buddhist beads and decorate deities. In the West, coral is one of the three major organic gemstones, whereas in the East, coral symbolizes auspiciousness and happiness since ancient times. It also represents nobility and power and symbolizes happiness and eternity (Wen et al., 2007). The ancient Romans believed that coral played roles in disaster prevention, intelligence, hemostasis, and heat dissipation, which continued until this century (Hong, 2009).

Corals are distributed in the South China Sea, North China Sea, and East China Sea, among which the South China Sea is located in a tropical and subtropical zone and contains abundant coral biological resources. Since the 1980s, chemists have conducted in-depth research on corals in the South China Sea. At the beginning of the 20th century, the utilization of coral resources includes human bone substitutes, feed calcium filler, and a good source of calcium supply for the human body (Huang et al., 1997). With the rapid development of modern separation and identification methods and the increasing maturity of biotechnology, a large number of active substances have been isolated from marine organisms (Zhang G. et al., 2013), such as salicin with antibacterial activity and alkaloids with cytotoxic activity, which have been isolated from *Sinularia suberosa* on the side of the South China Sea (Qi et al., 2005). In addition, antitumor alkaloids have been obtained from *Ellisella curvata* on the side of the South China Sea (Zhang J. R., 2012). With the deepening of chemical research on natural products of soft coral and gorgonians※, thousands of compounds with dozens of structural skeletons have been discovered, including steroids,



GRAPHICAL ABSTRACT

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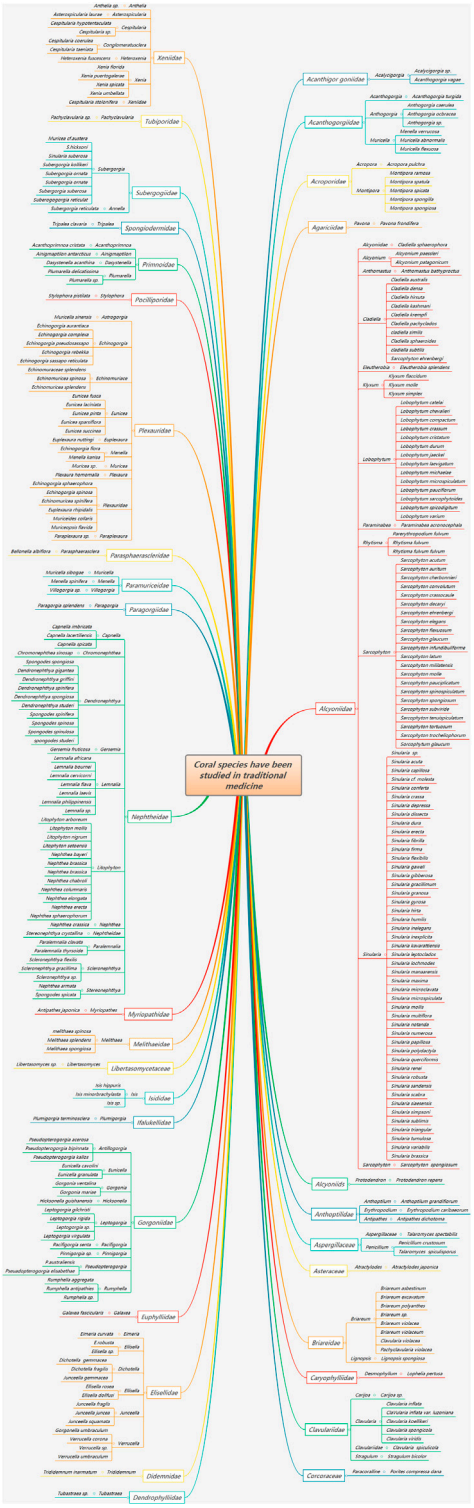

FIGURE 1
Coral species studied in the medical literature.

terpenoids, nitrogen-containing compounds, long-chain fatty acids, and long-chain alcohols. The diverse structures, unique molecular frameworks, and significant pharmacological activities of coral secondary metabolites fully demonstrate their potential medicinal value (Zhang W. et al., 2006; Shao et al., 2009a).

In the late 1960s, scholars and others discovered prostaglandin precursors with unique structures and strong physiological activity from gorgonian※, which further promoted coral chemistry research. The pharmacological activity of coral is also gradually being explored, which is primarily manifested in various aspects such as antitumor, anticancer, antioxidant, and anti-cardiovascular and in cerebrovascular system diseases. These pharmacological effects are mostly exerted by a single active substance extracted from coral bodies, while the bones of corals are mostly used as materials for bone transplantation and other applications (Wang et al., 2002b). Coral, as a medicinal material, has been recorded in detail in *The Compendium of Materia Medica* (1578 AD). It tastes sweet, and the property of the medicine is flat. It can improve eyesight, tranquilize the mind, and stop epilepsy. Coral is primarily used to treat corneal opacity. It also dissipates blood stasis. Coral powder can stop epistaxis. In the clinic, coral is also used in various compound preparations, such as Ershiwuwei Shanhu pills and Shanhu Qishiwei pills, which can restore nerve function and relieve pain. It is used in the treatment of albichoriasis, unconsciousness, body numbness, dizziness, brain pain, irregular blood pressure, headache, epilepsy, and various types of neuropathic pain. *The Compendium of Materia Medica* (1578 AD) records that corals are nontoxic, but according to literature reports, corals can release toxins, which are the second largest known deadly gas in the world, ultimately leading to toxic reactions such as muscle pain, four-limb weakness, and fainting. By contrast, in compound use, short-term use does not produce acute toxic reactions, but long-term use can cause damage to the liver, kidneys, and other organs.

In this review, we first conducted keyword searches on coral on academic websites such as PubMed, ScienceDirect, and CNKI and screened thousands of literature works related to medicine. Second, we conducted data mining to establish a database and finally extracted effective information for organization and analysis. This review discusses the use of coral in traditional medicine and its application in chemical composition, pharmacology, toxicology, and clinical research in the past two decades to provide important research data for the comprehensive development of marine biological resources, the discovery of drug lead compounds, the chemical ecological research of marine invertebrates, and the determination of organic synthetic chemical target compounds.

2 The application and variety of coral in traditional medicine

2.1 Coral species studied in the medical literature

Tang Sujing mentioned in the *Newly Revised Materia Medica* in 659 AD that *Corallium rubrum* (Linnaeus), also known as red coral, Hong shan, Huo shu, and *Corallium japonicum*※, belongs to the genus *Corallium* in the family Coralliidae. In addition, *Corallium japonicum* Kishinouye※ was included in the genus *Corallium* in the family Coralliidae in the *National Compilation of Chinese Herbal Medicine* (Second Edition). Zhuru, Ulan Shuru, and Shuru are recorded as Mongolian medicines. *Fossilia corrallium* is recorded as a Uyghur medicine in the *Dictionary of Chinese Ethnic Medicine*, which is mostly distributed in the Baihe Mahle River. It is commonly used to treat diarrhea, gastrointestinal bleeding, and neurasthenia. The

TABLE 1 Preparations that contain coral used in traditional medicine (Lai et al., 2016).

Name of the preparation	Systems of traditional medicine	Indication	Source
Teling eye ointment	Traditional Chinese medicine	Swelling and pain of eyes, epidemic hemorrhagic conjunctivitis, marginal blepharitis, trachoma, and corneal opacity	Ministry of Health of the People's Republic of China Drug standards Volume 14 of traditional Chinese medicine preparations
Jinniu eye ointment	Traditional Chinese medicine	Epidemic hemorrhagic conjunctivitis, marginal blepharitis, trachoma, eyes tear up in the wind, and external eye diseases such as Suyi	Ministry of Health of the People's Republic of China Drug standards Volume 20 of traditional Chinese medicine preparations
Jinniu eye ointment	Traditional Chinese medicine	Epidemic hemorrhagic conjunctivitis, marginal blepharitis, trachoma, tears in wind and external eye diseases such as Suyi	New National traditional Chinese patent medicines and simple preparations 2nd Edition
Babao Boyun powder	Traditional Chinese medicine	Swelling and pain of eyes and pterygium	National Prescription Collection of Traditional Chinese Medicine (Nanjing Formula)
Babao Guangming powder	Traditional Chinese medicine	Swelling and pain of eyes, inflammation of the conjunctiva, photophobia and tears, and wind–heat congestion	National Prescription Collection of Traditional Chinese Medicine (Sha shi Formula)
Babao Ruiren plaster	Traditional Chinese medicine	Corneal opacity and xerophthalmia	System of Ophthalmology Volume 6
Babao eye ointment	Traditional Chinese medicine	Epidemic hemorrhagic conjunctivitis, swelling and pain of eyes, corneal opacity, pterygium, photophobia, and marginal blepharitis	National Prescription Collection of Traditional Chinese Medicine (Tianjin Formula)
Babao eye ointment	Traditional Chinese medicine	Epidemic hemorrhagic conjunctivitis, swelling, pain, stickiness, corneal opacity, photophobia, and tears	Traditional Chinese Medicine Formula Preparation
Bo feng yun plaster	Traditional Chinese medicine	Corneal opacity, epidemic hemorrhagic conjunctivitis, pterygium, and bloodshot eyes	Yixue Rumen Roll 7
Boyi Zijinplastter	Traditional Chinese medicine	All kinds of acute conjunctivitis and blood–membrane barriers	Outline for Men's Diseases Roll 101
Dajin pill	Traditional Chinese medicine	Sputum fire-burnt diaphragm, wind damp phlegm, asthenic disease, and timidity syndrome	Zunsheng Bajian Volume 18
Dianyan Qibao powder	Traditional Chinese medicine	Wind heat rush up and acute conjunctivitis	General Medical Collection of Royal Benevolence Roll 105
Fo Bao Dan (Saizhen powder)	Traditional Chinese medicine	Throat poisoning and throat ulcers	Guide Book for Laryngology Roll 1
Gengong Chuhai pills	Traditional Chinese medicine	All symptoms of diphtheria	Complete Collection of Diphtheria
Hongding eye ointment	Traditional Chinese medicine	Hyperemia of bulbar conjunctiva, marginal blepharitis, and epidemic hemorrhagic conjunctivitis	Prescriptions for Universal Relief Roll 77
Wiping teeth white quartz powder (white quartz powder)	Traditional Chinese medicine	Tooth whitening	General Records of Holy Universal Relief Roll 121
Keming Liangyan ointment	Traditional Chinese medicine	Swelling and pain of eyes, bloodshot eye, and obstruction	National Prescription Collection of Traditional Chinese Medicine (JiNan Formula)
Luma Baoyuan pill	Traditional Chinese medicine	Supporting Yang and suppressing Yin and supplementing benefits and prolonging Years	Prescriptions for Universal Relief Roll 223
Qibao powder	Traditional Chinese medicine	Corneal opacity	A Profound Treatise on Eye Diseases
Zhenzhu powder	Traditional Chinese medicine	Corneal opacity	Zhenzhu Shibaosan (Surgical Prescription and Extraordinary Prescription roll 2)
Qishiwei Songshi pills	Tibetan medicine	Chest and hypochondriac pain, vomiting, hiccup, and loss of appetite caused by liver stagnation and stagnation and heat stasis	National Standard Compilation of Proprietary Chinese Medicines Internal Medicine Hepatobiliary Volume
Sanshiyiwei Songshi pills	Tibetan medicine	Acute and chronic hepatitis caused by diseases and heat in the liver and gallbladder	National Standard Compilation of Proprietary Chinese Medicines Internal Medicine Hepatobiliary Volume

(Continued on following page)

TABLE 1 (*Continued*) Preparations that contain coral used in traditional medicine (Lai et al., 2016).

Name of the preparation	Systems of traditional medicine	Indication	Source
Sishierwei Shugan capsules	Tibetan medicine	Damp heat in the liver and gallbladder, hypochondriac pain caused by stagnation and blood stasis, and abdominal distension; acute and chronic hepatitis B with the above symptoms	National Standard Compilation of Proprietary Chinese Medicines Internal Medicine Hepatobiliary Volume
SareShisanweiPengniao Pills	Tibetan medicine	Apoplexy, oral and eye deviation, numbness and paralysis, vasculitis, tenosynovitis, limb joint dysfunction, and leprosy caused by albichoriasis	Tibetan medicine in the Drug Standards of the Ministry of Health Volume I
Ershiwuwei Songshi Pills	Tibetan medicine	Liver depression and stagnation, blood stasis, liver poisoning, liver pain, liver cirrhosis, liver effusion, and various acute and chronic hepatitis and cholecystitis	Pharmacopoeia of the People's Republic of China 2020 Volume I
Ershiwuwei Shanhu pills	Tibetan medicine	Albichoriasis, unconsciousness, body numbness, dizziness, brain pain, irregular blood pressure, headache, epilepsy, and various neuropathic pain conditions	Pharmacopoeia of the People's Republic of China 2020 Volume I
Ershiwuwei Shanhu capsules	Tibetan medicine	Albichoriasis, unconsciousness, body numbness, dizziness, brain pain, irregular blood pressure, headache, epilepsy, and various neuropathic pain conditions	New Drug Regularization Criteria Volume 83
Hupo powder	Tibetan medicine	Weary eyes and corneal opacity	Precious Book of Ophthalmology Roll 3
Ruyi Zhenzhu powder	Tibetan medicine	Plague, heat enters the choroid and cannot be cured for a long time, rheumatoid arthritis, scrofula, contractures, renal vein damage, and albichoriasis	Lantab
Ershisanwei chen powder	Tibetan medicine	Cough with gray phlegm, red phlegm, yellow phlegm, and other symptoms	Shanhu Zan
Shibawei Jiangjun powder	Tibetan medicine	Albichoriasis	Shanhu Zan
Jing ying pills	Tibetan medicine	Albichoriasis, xila wusu, cerebral hemorrhage, muscle and tendon pain, and other symptoms	Linzheng Zhaji
Sishiwei Jiangjun powder	Tibetan medicine	Various poisoning symptoms	Ganlu Baijing
Mingmu pills	Tibetan medicine	Various febrile liver diseases and various eye diseases	Linzheng Zhaji
SareShisanweiPengniao pills	Tibetan medicine	Ocular deviation, numbness and paralysis caused by albichoriasis as well as vasculitis, tenosynovitis, and disadvantageous limb joints	Tibetan Medicine Standards
Sishibawei Jiedu powder	Tibetan medicine	Poisoning attacks such as self-poisoning, solid poisoning, visible poisoning, contact poisoning, sunlight poisoning, and oral poisoning	Summary of Ganlu Prescription
Shibawei Xijiao powder	Tibetan medicine	Albichoriasis	Summary of Ganlu Prescription
Coral Bone Joining pill (Sunrise Tu Uril)	Mongolian medicine	Various new and old fractures, soft tissue injuries, and femoral head necrosis	Essence of Hundred Therapeutic Prescriptions
Jiuwei Hailuo powder	Mongolian medicine	Panic, palpitations, fever, heart adhesion, dry mouth and tongue, and other symptoms	Yiyao yuedi
Shisanwei Ying pill	Mongolian medicine	Albichoriasis, cerebral hemorrhage, hemiplegia, and poisoning	Selected Compilation of Mongolian Medicine
Zhachong Shisanwei pill	Mongolian medicine	Hemiplegia, left paralysis and right paralysis, distorted mouth and eyes, numbness in limbs, unfavorable waist and legs, unclear speech, muscle and bone pain, nerve paralysis, rheumatism, and joint pain	Mongolian Medicine in the Drug Standards of the Ministry of Health Volume
Ershiwei Huangjin powder	Mongolian medicine	Albichoriasis	Mongolian Medicine Golden Chamber
Bianbao pills	Mongolian medicine	Various edema syndromes	Mongolian Medicine Golden Chamber
Lianchuang powder	Mongolian medicine	All kinds of long-term sores do not heal	Guanzhe Zhixi
Shiwei Baohui powder	Mongolian medicine	Various edema syndromes	Mongolian Medicine Golden Chamber

(Continued on following page)

TABLE 1 (*Continued*) **Preparations that contain coral used in traditional medicine (Lai et al., 2016).**

Name of the preparation	Systems of traditional medicine	Indication	Source
Shiqiwei Jinhui pills	Mongolian medicine	Scrofula and black Hiraousu disease	Selected Prescriptions for Mongolian Medicine
Shibaweiguan pills	Mongolian medicine	Wind cold, muscle pain, numbness in limbs, choroidal diseases	Selected Prescriptions for Mongolian Medicine
Jiuwei Xionghuang powder	Mongolian medicine	Seasonal heat, plague and toxin, acute fire and convulsive wind, and various fever symptoms	Mongolian Medicine Prescription
Sishiwei Chenxiang powder	Mongolian medicine	Spermatorrhea	Mengyi Miaofang
Ershiwuwei Songshi pills	Mongolian medicine/ Tibetan medicine	Various liver diseases	Clinical Experience in Mongolian Medicine
Shiwuwei Zhenzhu powder	Mongolian medicine/ Tibetan medicine	In an abject state of mental confusion and forgetfulness	Clinical Experience in Mongolian Medicine
Shiwuweirupeng pills	Mongolian medicine/ Tibetan medicine	Rheumatoid disease	Mongolian Medicine Golden Chamber
Shanhu qishiwei pill	Mongolian medicine/ Tibetan medicine	Cerebral thrombosis, cerebral hemorrhage, coronary heart disease, limb paralysis, tachycardia or bradycardia, hypertension, poliomyelitis, epilepsy, and various types of neuritis. Particularly, effective for brain, nervous, and heart diseases	Mongolian Medicine in the Drug Standards of the Ministry of Health Volume
A'naer Vigills	Uyghur medicine	Various bacterial, fungal, trichomonal vulvitis, and vaginitis can cause itching, redness, and swelling of the genital area in women, as well as excessive vaginal discharge	Uyghur Medicine in the Drug Standards of the Ministry of Health Volume
GangKangMuKuLi tablets	Uyghur medicine	Hemorrhoids, cluneal cleft, and hematochezia	Uyghur Medicine in the Drug Standards of the Ministry of Health Volume
Poison symptom drug powder	—	Various poison formulations	Jin Yaoshi

Dictionary of Traditional Chinese Medicine also records *Corallium japonicum* Kishinouye※, which is recorded as *Corallium konojoi*※ with the same name as that recorded in the *Chinese Traditional Chinese Medicine Resources*. *Corallium secundum* Dana※ and *Corallium elatius* Ridley are also recorded. The *Records of Chinese Traditional Chinese Medicine Resources* (*Part 2*) also records six species of coral, namely, *Porites nigrescens* Dana in the Poritidae family, *Porites* genus; *Antipathes* sp.; and the national first-class protected wild animals *Corallium japonicum* Kishinouye※, *Corallium elatius* Ridley, and *Corallium konojoi* Kishinouye※. In the past two decades, most of the coral species that have been studied in medicine belong to Alcyoniidae, Gorgonacea※, and Scleractinia※. After sorting, red coral is mostly used in medical records. Modern research on coral species is diverse, involving a total of 34 families and 99 genera. Corals in the Alcyoniidae, Nephtheidae, Plexauridae, Gorgoniidae※, Xeniidae, Elisellidae, Briareidae, Subergorgiidae, and Clavulariidae families are more common. *Sarcophyton* and *Sinularia* are research hotspots in Alcyconiidae, followed by *Dendronephthya*, *Litophyton*, and *Lemnalia* corals in the Nephtheidae family and by *Echinogorgia*, *Plexauridae*, and *Eunicea* corals in the Plexauridae family (Figure 1).

2.2 The medicinal value of coral

The records of coral can be traced back to the Three Kingdoms period (226–231 AD). Kangtai and Zhu Ying of the Eastern Wu Dynasty mentioned in their *Biography of Fu Nan* that "In the rising sea, the coral reef falls, and there is a rock at the bottom of the reef, and the coral grows on it" (Cao, 2012). Coral is used as a medicinal material, which was first recorded in the *Newly Revised Materia Medica* (659 AD) as "sweet, flat, and nontoxic" and primarily used for blood retention and corneal opacity. In addition, coral is ground to a powder and used to stop epistaxis. It grew in the South China Sea, resembling jade red, with many pores in the middle and some without pores. It can also be found in Persia and Sri Lanka. "*The General Introduction to the Essential Prescriptions of Zengguang and Zhiju*" (1208 AD) records that coral is effective in the removal of corneal opacity and cessation of bleeding in epistaxis. *Yue Hau zi* (908–923 AD) describes that coral can tranquilize the mind and stop epilepsy. *Oversea Materia Medica* (907–960 AD) records that coral is the main cause of blood stasis and wind epilepsy. The classic work *Compendium of Materia Medica* (1578 AD) points out that coral can treat corneal opacity. *Materia Medica Yanyi* (1116 AD) records that coral can be used to remove corneal opacity. *Compendium of Selected Essentials of Materia Medica* (1,644–1911 AD) describes that coral is primarily used for corneal opacity, blood stasis, and epistaxis. It can also improve eyesight, tranquilize mind, stop epilepsy, and drop and remove flying silk. In the *Second Edition of the National Compilation of Chinese Herbal Medicine* (*Volume 2*; compiled by the Compilation Team of the National Compilation of Chinese Herbal Medicine, 1996), coral is mentioned as red coral, with sweet and flat properties; it can tranquilize the mind, stop epilepsy, and improve eyesight, and it is primarily used in treating convulsions, stopping

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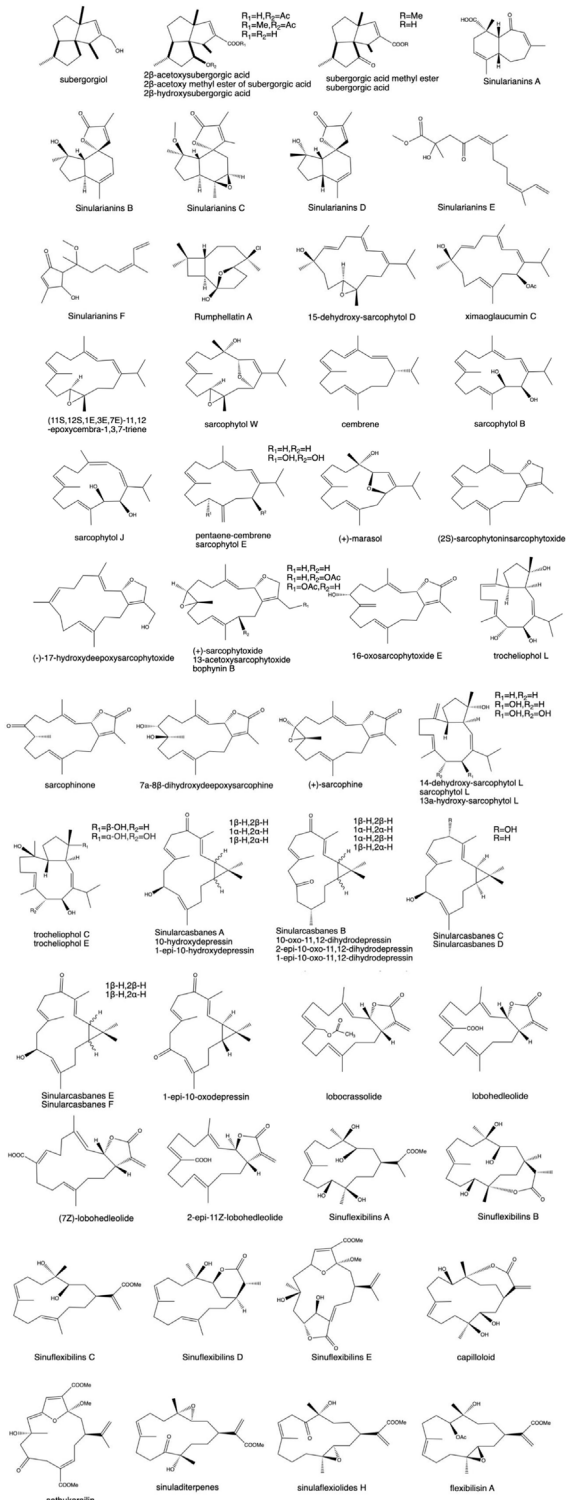

FIGURE 2
(Continued).

epilepsy, and removing corneal opacity. Traditional Chinese medicine books such as *Taiping Holy Prescriptions for Universal Relief* (992 AD), *Fangmai Zhengzong* (1749 AD), *Peng Family Miao Prescription*, and *Aquatic Product Nutrition and Medicinal Manual* all contain prescriptions made from red coral, which can remove corneal opacity in children, dizziness, epilepsy or palpitations, heart and lung congestion, persistent vomiting and bleeding, and water and fire burns (Lai et al., 2016).

Tibetans, Mongols, and Uyghurs also often use coral as a medicinal material for compatibility treatment. Coral Tibetan

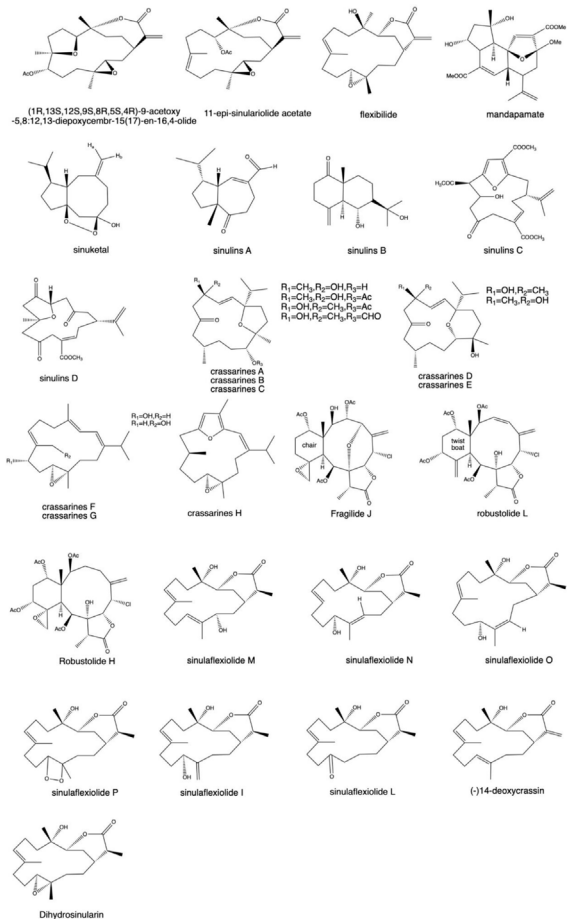

FIGURE 2
(Continued). Some pharmacologically active terpenoids extracted from coral.

medicine, namely, Qiwuru, also known as Pazhuma, can clear liver heat and detoxify various toxins. It is primarily used to treat encephalopathy, liver disease, various fevers, and poisoning. The Mongolian medicine, namely, Shuru, which is also known as Zhuru and Ulan Shuru, can clear heat, detoxify toxins, and tranquilize the mind. It is primarily used to treat liver heat, lung heat, detoxify, toxic heat, stroke, and brain disease. The Uyghur medicine, namely, Bihe Marjiang, which is also known as Busai, can restore function and astringing sores, clear heat and inflammation, traete loose teeth, refresh the heart, please the mind, and stop bleeding and diarrhea. It is primarily used to treat damp heat or blood-related diseases. Li et al. (2015) found through experimental research that Mongolian Jiegu Medicine Water Pills have good therapeutic effects on fractures. A'naer Vigills can clear heat, restore function, and relieve itching. It has been used for various symptoms, such as itching, redness, swelling, and excessive vaginal discharge, caused by bacterial and fungal vaginitis in women. It is a commonly used Uyghur medicine preparation in clinical practice (Chen, 2011). Ershiwuwei Shanhu pills can intervene in the treatment of neurological diseases such as Alzheimer's disease, cerebral infarction, and migraine (Zhou et al., 2019; Zhu et al., 2020; Jiaojia et al., 2022).

The use of coral in modern medicine is no longer limited to red coral. Jiang (2013) conducted an extraction experiment on the active ingredients of *Dichotella gemmacea*※ and found that some of its diterpenoid compounds showed cytotoxicity to human lung pancreatic cancer cells (A549) and human osteosarcoma cells (MG63), and some of the compounds had antibacterial activity. Wu (2013) conducted a study on *Echinogorgia flora* and found that its sesquiterpene active ingredients showed a weak antiviral activity against influenza virus. Mahmoud et al. (2022) showed that the steroids and sesquiterpene of Red Sea soft corals showed evident activity on A549, MCF-7, and HepG2 cell lines. The chemical compounds in Scleractinia※ (Zhao et al., 2016) exhibit good biological activities, such as cytotoxic, antibacterial, insecticidal, and toxic effects on fish. At present, the corals used as medicinal materials include soft corals, gorgonians※, Scleractinia※, and red corals (Ai et al., 2006). Scleractinia※ have received little attention from chemists because they are primarily composed of calcareous bones, and the scarcity of red coral resources also limits their utilization. Therefore, active soft corals and gorgonians※ have become the first option for coral reef benthic research, and they are increasingly becoming popular biological species in modern marine natural product research (Xue, 2014).

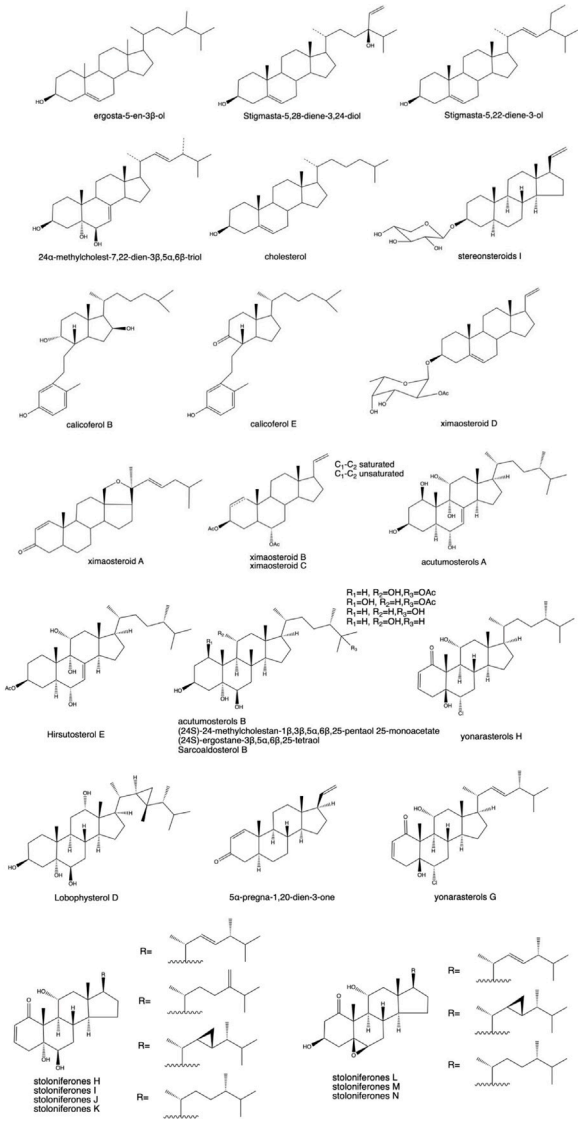

FIGURE 3
(Continued).

2.3 Preparations that contain coral used in traditional medicine

Coral is used as a medicinal material, which has a long history in China. Ancient Chinese ancestors recognized the medicinal value of coral. Coral is primarily used in traditional Chinese medicine, Tibetan medicine, and Mongolian medicine, but the specific variety of coral is not clearly specified in the prescription. Red coral is primarily used as medicine, and the method of medicine includes the following steps: take the original medicinal material, remove impurities, wash and grind it into a fine powder, sieve to obtain an extremely fine powder, and dry it. The compatibility of its medication is shown in Table 1. It is primarily used to treat nervous system disease, chronic ulcers, and various heat syndromes. Traditional Chinese herbs and formulas often play a role in clearing heat, treating eye diseases, relieving chest and hypochondriac swelling and pain caused by diseases, and dissipating heat in the liver and gallbladder. Tibetan medicine is used to treat headache, epilepsy, and various types of neuropathic pain caused by albichoriasis. Apart from traditional Chinese medicine and Tibetan medicine, Mongolian medicine has a wide range of treatments, including various new and old fractures, soft tissue injuries, femoral head necrosis, and various edemas. Records in Uyghur medicine provide evidence for the treatment of various bacterial and fungal infections and trichomonal vulvovaginitis, causing itching, redness, and swelling of the genital area, as well as excessive vaginal discharge, in women.

3 Chemical composition of coral

In recent years, Chinese scholars have made important contributions to the research of international marine natural products. In 1980, Su Jingyu first isolated two new types of

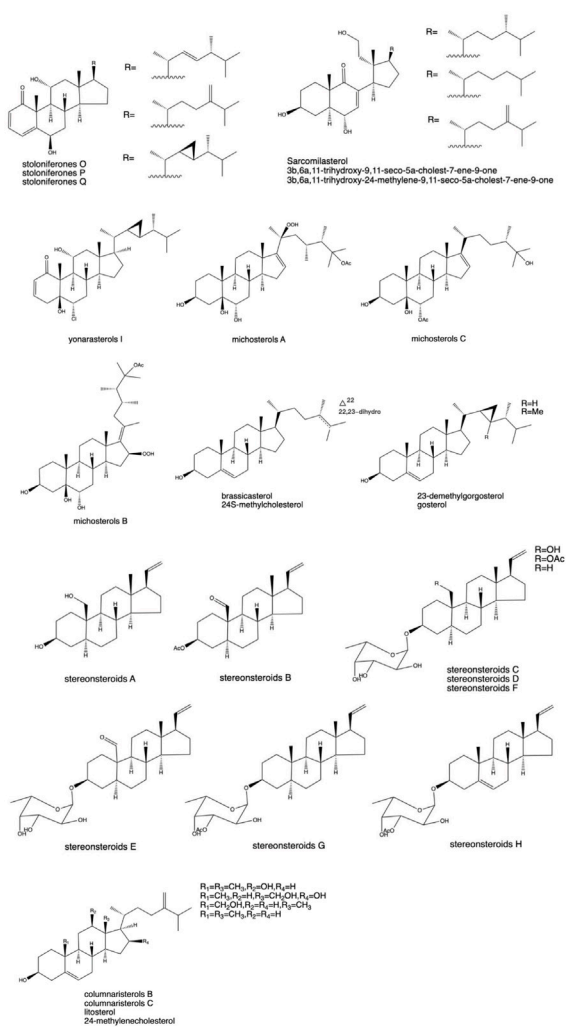

FIGURE 3
(Continued). Some pharmacologically active steroids extracted from coral.

diterpenoid dimers with double fourteen-membered cyclic carbon frameworks from soft corals (Xue, 2014). Weinheimer and Washecheck (1969) first discovered abundant and highly active prostaglandin-like compounds from gorgonians※. These research results have aroused great interest in the study of coral chemical composition. After decades of research exploration and development, a large number of structurally novel and biologically active compounds have been discovered and determined from corals. Each type of compound contains many compounds with different structures, such as terpenoids, alkaloids, steroids, macrolides, quinones, polyethers, flavonoids, and peptides (Li R., 2012). The following sections provide an explanation of the chemical composition of corals based on different structural types.

3.1 Terpenoids

Terpenoids are the most abundant and diverse class of compounds in coral, and terpenoids with a new skeleton are constantly being discovered. Its pharmacological screening shows strong biological activity (Zhang and Guo, 2003; Liu, 2017). Therefore, the isolation and identification of terpenoids have always been the focus and hotspot of coral chemistry research. After sorting out and analyzing the literature, the primary terpenoid compounds are sesquiterpene and diterpenes, in addition to semiterpenoids and triterpenes.

3.1.1 Sesquiterpenes

Sesquiterpenes are an important class of terpenoids that are widely distributed in terrestrial fungi, higher plants, insects, and marine organisms such as soft corals. In addition to the earlier discovery of guaiacane and furan sesquiterpenes, sesquiterpene also contains africanne, capnellane, and illudalane (He, 2013). Wang et al. (2002a) isolated subergorgiol and 2β-acetyl subergorgic acid with a unique angular triquetane structure from the Taiwanese soft coral *S. suberosa*, in which subergorgiol exhibited moderate cytotoxicity against HeLa tumor cells. Menecubebane B, the known compound analog isolated from gorgonian※ *Menella* sp., showed moderate cytotoxicity against Eca9706 and HeLa cell lines with semi-inhibitory concentration values of 20.8 and 30.6 μM, respectively. In the coming year, Ngoc et al. (2017a) extracted and identified four sesquiterpenes, namely, nanolobatols A and B and sinularianins B and D, in the Vietnamese soft coral *Sinularia nanolobata*. Sinularianins B and D were similarly extracted from *Sinularia* sp. (Chao et al., 2006; Yang et al., 2013). A novel chlorine-containing carbon-deficient sesquiterpene was isolated from Taiwan gorgonian※, and this compound showed inhibitory effects on Gram-negative bacteria (Figure 2) (Sung et al., 2007).

3.1.2 Diterpenes

Many diterpenes show strong biological activities, so diterpenoids have remained a focus and hotspot for research in the past few years. Diterpenes are the most abundant and diverse structural types in corals, and the most common and diverse diterpene is cembrane, which is characterized by an isopropyl and three methyl substitutions in the tetradecane ring. Other diterpenes include eunicellin, casbane, biflorane, briarellin, dolabellane, lobane, sarcodictyins, and xenia (Shao et al., 2009b). Li J. F. et al. (2022) extracted 20 sissonane-type diterpenes from *Sarcophyton glaucum*. The *Sinularia* genus is rich in diterpenes. As isolated from the extract of CH_2Cl_2/EtOH in it, 18 sesquiterpenes such as sinoflexibilins A–F were identified (Yin et al., 2013; Jiang et al., 2019a), and two sinulins C and D (Qin et al., 2018) were isolated from the CH_2Cl_2/C_2H_5OH extract of *Sinularia* sp. Some of the compounds exhibit some degree of cytotoxicity against A549 and HL-60 cells or exert anti-inflammatory effects through inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) expression (Chao et al., 2011a). Wang et al. (2010) first discovered a new chlorinated briarane (fragilide J) and two chlorinated briaranes (robustolide L and robustolide H) from *Junceella fragilis*※ and *Ellisella robusta*※. Jiao-Jiao Xu isolated 10 sissonane diterpenes from soft coral *Sinularia flexibilis* samples, and the inhibitory effects of each monomer compound on LPS-induced NO release from RAW 264.7 cells were examined using the Griess method at noncytotoxic doses. The results showed that the compounds had some inhibitory effects on NO production (Xu, 2016).

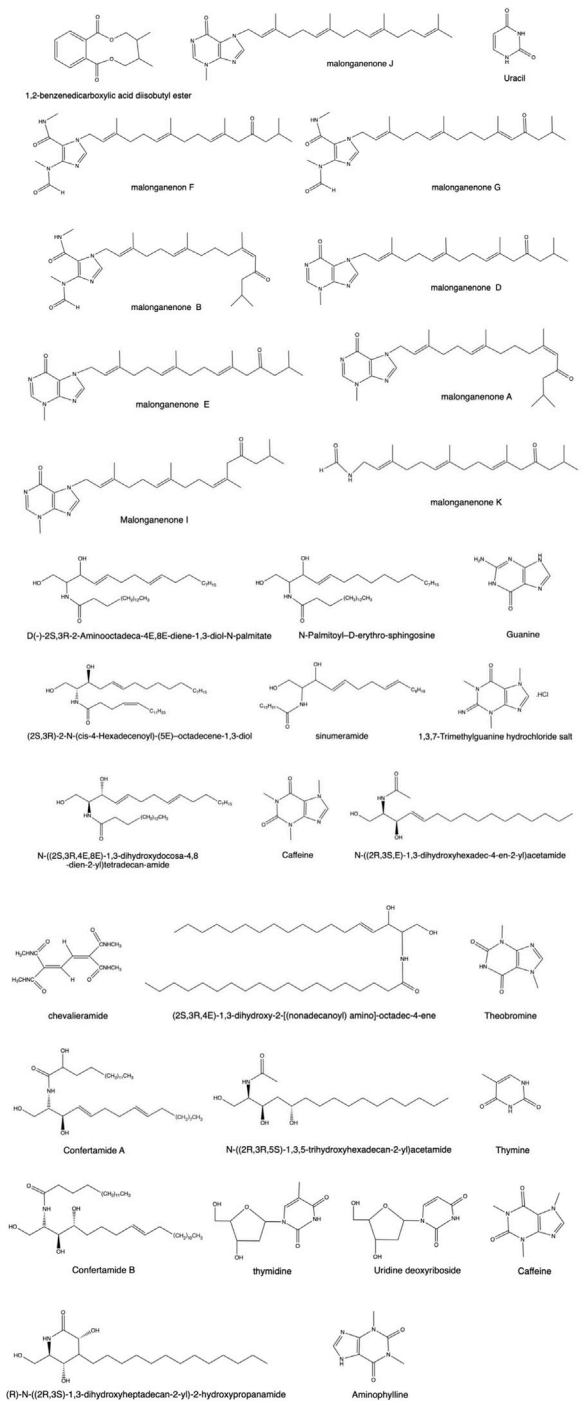

FIGURE 4
Some pharmacologically active nitrogen compounds extracted from coral.

3.2 Steroids

As shown in Figure 3, steroids are a class of biologically active compounds in corals, particularly pregnane, cholestane, and ergosterone. It has received considerable attention because of its structural diversity and remarkable biological activity (Liu, 2017). Sterols are abundant in corals, and the structure is more complex because of the diversification of the sterol side chain structure and the different degrees of oxidation (Ai et al., 2006). Seven new cleaved ring sterols with $C_{9,11}$ breaks and C_{22} hydroxylation were isolated for the first time from *Tripalea clavaria* collected from the South Atlantic Ocean in 2006, as determined by wave spectroscopy and the Mosher method, and further studies revealed that some of their substances showed some inhibitory activity against *Staphylococcus aureus*. Four bioactive sterols with anti-inflammatory, antibacterial, antioxidant, antitumor, and antitubercular properties were isolated from *J. fragilis*※ from Sanya, Hainan (Wen et al., 2007). Subsequently, two sterols were isolated from the CH_2Cl_2/C_2H_5OH extract of this coral (Qi et al., 2004). For the first time, two B-ring open-loop sterols were isolated from the Chinese small pointed gorgonian *Muricella sinensis* (Verril1) ※ from the South China Sea. During bioactivity screening, calicoferol E was found to show inhibitory activity against protein tyrosine phospholipase 1B (PTP1B), with an IC_{50} value of 27.28 μM (Yan et al., 2005).

3.3 Nitrogen compounds

The nitrogenous compounds in corals primarily include ceramides and alkaloids (deoxythymidine, thymine, methyluracil, and urea). They generally exhibit antifungal, antibacterial, and cytotoxic activities. Such compounds can also inhibit acetylcholestan-converting protease, thereby providing an alternative lead compound for the development of therapeutic drugs for atherosclerosis and other cardiovascular diseases (Ai et al., 2006). Zhang J. R. (2012) isolated 16 alkaloids (nine diterpene alkaloids, including three new diterpene alkaloid compounds) and five ceramides from *E. robusta* and *E. curvata* of gorgonian※. A preliminary evaluation of the antitumor activity at the cellular level was carried out, from which four diterpene alkaloids were screened to show strong cytotoxicity against HeLa and K562 cancer cells, and the enzymatic activity inhibition was evaluated by enzyme-linked immunosorbent assay (ELISA). The activity results showed that diterpene alkaloid malonganenone D had a strong inhibitory effect on the enzymatic activity of c-Met. The ceramide N-1-hydroxymethyl-2-hydroxy-(E, E)-3,7-heptadecadienylhexadecanoamide (Liu et al., 2001), thymine, and uracil were isolated from *Acropora pulchra*※ (Brook; Xu et al. (2003)). In addition to different corals, such as *Litophyton arboreum* (Abou El-Kassem et al., 2018) and *Junceella juncea*※ (*Pallas*; Krishna et al. (2004)), *Lobophytum chevalieri* (Li et al., 1989) has bioactive ceramides. The structure diagram is shown in Figure 4.

3.4 Other compounds

As shown in Figure 5, aliphatic compounds (long-chain fatty acids, long-chain aliphatic alcohols, and the aldehydes and esters they form) and prostaglandins were also extracted from different corals (Watanabe et al., 2003; Reina et al., 2013; Hurtado et al., 2020). According to the literature, a large amount of batyl alcohol has a pharmacological effect of raising leukocytes, which is extracted from coral and has been widely used in clinical practice (Ma, 2008; Zhao et al., 2011; Sun, 2012; Xue et al., 2014). Watanabe et al. (2003) tested 15 new halogenated prostaglandins isolated from the Okinawan soft coral *Clavularia viridis*. Among these prostaglandins, three belong to iodovulone; seven belong to 12-*O*-acetyliodovulones, 12-*O*-

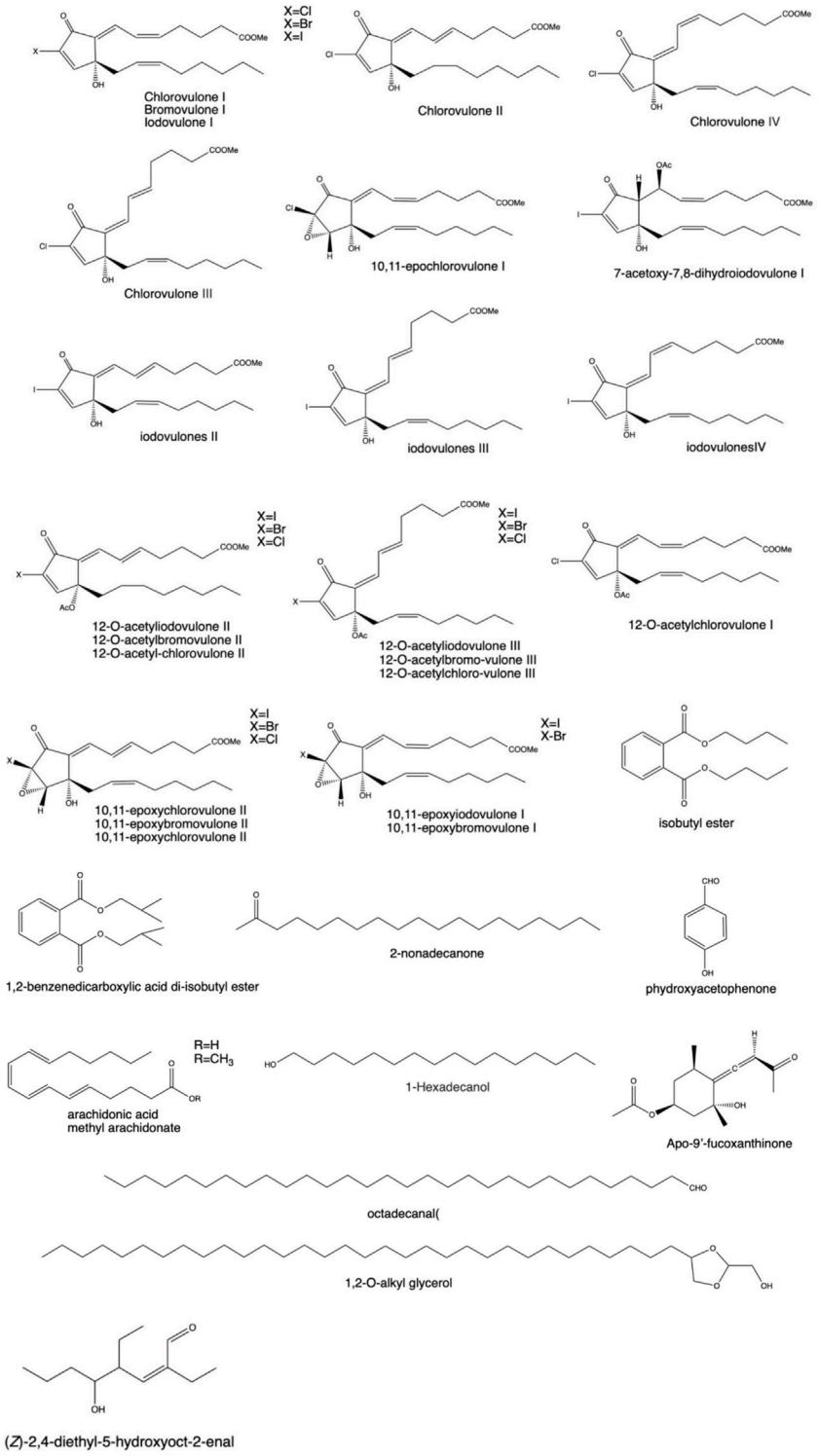

FIGURE 5
Some pharmacologically active compounds extracted from coral.

acetylbromovulones, and 12-*O*-acetylchlorovulones; and the rest belong to 10,11-epoxy congeners of iodovulone, bromovulone, and chlorovulone. A simple compound, *p*-hydroxybenzaldehyde, was obtained from crude extracts of *Sinularia dissecta* (Jin, 2005) and *Muriceides collaris*※ (Zhu et al., 2013). In addition, some simple aldehydes were isolated from *Antipathes dichotoma* Pallas※ (Ge et al., 2010), *Sinularia notanda* (Xu et al., 2017), *Scleronephthya* sp. (Huo et al., 2011), *D. gemmacea*※ (*valenciennes*; Liu (2008)), *Dendronephthya* sp. (Li, 2004), and *Hicksonella guishanensis* Zou※ (Yu et al., 2004). *p*-Hydroxybenzoic acid can be extracted from

TABLE 2 Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
Sesquiterpenoids	*Muriceides collaris*※	—	50 μg/mL (Y)	P388 and BEL-7402	Shi (2009)
Sesquiterpenoids	*Litophyton arboreum*	—	4.32 ± 0.13–44.52 ± 0.5 μM (IC_{50})	MCF-7	Abou El-Kassem et al. (2018)
Sesquiterpenoids	*Xenia* sp.	—	5.89–6.45 μM (IC_{50})	STI	Phan et al. (2019)
Sesquiterpenoids	*Lemnalia* sp.	—	15.9 μM (IC_{50})	CCRF-CEM	Yan et al. (2021)
Sesquiterpenoids	*Sarcophyton glaucum*	—	18.8 ± 0.07, 19.9 ± 0.02 (HEPG2), 9.9 ± 0.03, 2.4 ± 0.04, 3.2 ± 0.02 (MCF-7), 29.4 ± 0.03, 19.4 ± 0.02, and 25.8 ± 0.03 (HCT116) μM (IC_{50})	HepG2, MCF-7, and HCT116	Abdel-Lateff et al. (2015)
Sesquiterpenoids	*Muriceides collaris*※	—	50 μmol/L (Y)	HL-60 and HeLa	Zhu et al. (2013)
Sesquiterpenoids	*Sinularia kavarattiensis*	Antiproliferation	17.5 and 16.8 μM (IC_{50})	Leukemia and prostate cancer	Rajaram et al. (2013)
Sesquiterpenoids	*Sinularia scabra*	—	9.6–13.8 μg/mL (ED_{50})	MCF-7, WiDr, Daoy, and HEp-2	Su et al. (2012)
Sesquiterpenoids	*Sinularia* cf. *molesta*	—	5.26 and 8.37 μM (IC_{50})	HeLa and HCT116	Chu et al. (2018)
Sesquiterpenoids	*Sinularia* sp.	Cells that inhibit apoptotic proteins and trigger apoptosis by regulating Nrf2-ARE signaling	61.22 and 43.73 μM (Y)	HCT116	Taira et al. (2018)
Sesquiterpenoids and Steroids	*A. ochracea*	—	3.70–29.03 μg/mL (IC_{50})	HepG2, Hep3B, MCF-7/ADR, PC-3, HT-116, and Caski	Sun (2012)
Sesquiterpenoids and lactone	*Melithaea* sp.	—	50 μg/mL (Y)	K562, P388, and HeLa	Su (2011)
Diterpene	*Nephthea* sp.	—	37 μg/mL (IC_{50})	MCF-7	Hegazy et al. (2016)
Diterpene	*Lobophytum* sp.	—	4.52–6.62 μM (IC_{50})	HT-29, Capan-1, A549, and SNU-398	Li et al. (2020b)
Diterpene	*Sinularia flexibilis*	—	6.9–26.7 μM (IC_{50})	P-388, K-562, and HT-29	Wu et al. (2018)
Diterpene	*Lobophytum* sp.	—	1.8–8.2 μM (IC_{50})	A549 and HT-29	Nguyen et al. (2010)
Diterpene	*Cladiella* sp.	—	4.7and 10.2 μM (IC_{50})	CCRF-CEM	Chen et al. (2010b)
Diterpene	*Cladiella* sp.	—	2.0–31.1 μg/mL (IC_{50})	DLD-1 and HL-60	Chen et al. (2011b)
Diterpene	*Lobophytum laevigatum*	Inhibition of transcriptional activity	9.0 ± 0.8–38.8 ± 3.8 μM (IC_{50})	HL-60, A549, HCT116, and MCF-7	Quang et al. (2011a)
Diterpene	*Asterospicularia laurae*	—	1.3–19.41 μM (IC_{50})	Molt 4, K562, Sup-T1, and U937	Su et al. (2021)
Diterpene	*Dichotella gemmacea*※	—	11.4–72.0 μM (IC_{50})	A549 and MG63	Li et al. (2016)
Diterpene	*Cladiella krempfi*	—	8.5 ± 1.0–18.1 ± 1.5 μg/mL (ED_{50})	H1299 and BT483	Tai et al. (2011)
Diterpene	*Sinularia triangular*	Antiproliferation	26.0–37.1 μM (ED_{50})	CCRF-CEM and DLD-1	Su, J.H. et al. (2011)
Diterpene	*Cespitularia taeniata*	—	0.3, 6.7, and 8.7 μM (IC_{50})	Medulloblastoma and colon adenocarcinoma cancer cells	Lin et al. (2014)

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TABLE 2 (*Continued*) Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
Diterpene	*Sinularia gibberosa*	Anti-invasion and anti-metastasis	4–8 μM (Y)	HA22T, RT4, and T24 human bladder cancer and HCC	Wu et al. (2020c)
Diterpene	*Nephthea* sp.	—	25, 70, 40, and 125 μg/mL (IC_{50})	HeLa/MCF-7	Ishii et al. (2016)
Diterpene	*Lobophytum* sp.	—	5.99–10.83 μM (IC_{50})	HeLa, A459, B16-F10, and RAW 264.7	Roy et al. (2019)
Diterpene	*Klyxum flaccidum*	—	16.5–49.4 μM (IC_{50})	HT-29, A549, K562, and P388	Ahmed et al. (2017a)
Diterpene	*Lobophytum crassum*	—	1.2–2.5 μg/mL (IC_{50})	Ca9-22	Chao et al. (2008a)
Diterpene	*Sinularia humilis*	—	12.5 μM (IC_{50})	HT-29	Li et al. (2022a)
Diterpene	*Lobophytum* sp.	—	1.2–8.6 μg/mL (IC_{50})	SGC7901, A549, MCF-7, HCT116, and B16	Zhao et al. (2013a)
Diterpene	*Sarcophyton elegans*	—	10 μM (Y)	MDA-MB-231	Liu et al. (2015)
Diterpene	*Sinularia microclavata*	—	5.0, 20.0 (KB, MCF), and 0.5 (A-549) μg/mL (IC_{50})	KB, MCF, and A-549	Zhang et al. (2005a)
Diterpene	*Lobophytum michaelae*	—	0.3–61.5 μM/mL (ED_{50})	HT-29 and P-388	Wang and Duh (2012)
Diterpene	*Nephthea* sp. *and Sarcophyton cherbonnieri*	Apoptosis	0.15–8.6 μg/mL (GI_{50})	HM02, HepG2, and MCF-7	Gross et al. (2003)
Diterpene	*Sinularia flexibilis*	—	0.16–32.4 μg/mL (ED_{50})	A549, HT-29, KB, and P-388	Duh et al. (1998)
Diterpene	*Pseudopterogorgia acerosa*※	—	1.25–>8.10 μM (GI_{50})	DU-145, LNCaP, IGROV, IGROV-ET, SK-BR-3, SK-MEL-28, A549, PANC1, HT29, HT29-KF, LoVo, LoVo-DOX, HeLa, and HeLa-APL	Montalvo et al. (2006)
Diterpene	*Sinularia gibberosa*	—	18.7, 19.5, and 11.0 μg/mL (IC_{50})	HepG2 and A549	Chen et al. (2009)
Diterpene	*Sinularia flexibilis*	—	0.7–16.0 μg/mL (ED_{50})	KB, A-549, HT-29, and P388	Hsieh et al. (2003)
Diterpene	*Clavularia inflata*	—	0.052–27.3 μg/mL (ED_{50})	A549, HT-29, and P-388	Duh et al. (2001)
Diterpene	*Lobophytum* sp.	Apoptosis	3.7 (HT-29), 5.1 (A549), and 6.6 (SNU-C5) μM (IC_{50})	HT-29, A549, and SNU-C5	Hong et al. (2012)
Diterpene	*Sinularia* sp.	—	7.98–17.23 μM (IC_{50})	HCT116	Xu (2013)
Diterpene	*Dichotella gemmacea*※	—	3.8–112.3 μg/mL (IC_{50})	A549 and MG63	Jiang (2013)
Diterpene	*Sarcophyton latum*	—	50 μg/mL (Y)	P388, A549, and BEL-7402	Wang (2008)
Diterpene	*Sinularia dura*	Antiproliferation and anti-invasion	20–30	Highly malignant + - SA breast epithelial cells, PC-3 M-CT+	Radwan et al. (2008)
Diterpene	*Sarcophyton trocheliophorum*	—	10 μmol/L (Y)	A-549 and HL-60	He (2013)
Diterpene	*Lobophytum* sp.	—	1.83–44.69 μg/mL (IC_{50})	B16F10, HeLa, and HepG2	Lang (2013)
Diterpene	*Lobophytum* sp.	—	50 μg/mL (Y)	P388 and HeLa	Fernando et al. (2017)
Diterpene	*Cladiella krempfi.*	—	6.7 ± 0.7–19.2 ± 4.0 μg/mL (IC_{50})	A549, BT483, H1299, HepG2, and SAS	Tai et al. (2013)

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TABLE 2 (*Continued*) Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
Diterpene	*Sinularia* sp.	Apoptosis	—	HL-60	Kamada et al. (2018)
Diterpene	*Sinularia* sp.	—	0.0039 μg/mL	HL-60, PC-3MIE8, and BGC-823	Li (2004)
Diterpene	*Dichotella gemmacea*※	—	10.6–70.0 μM (IC_{50})	A549, HL-60, and K562	Sun (2012)
Diterpene	*Cladiella*	Directly affecting tumor growth and angiogenesis	1.6 (MDA-MB-231 cell)/>10 μM (IC_{50})	EGF-dependent cancers	Mohyeldin et al. (2017)
Diterpene	*Sarcophyton mililatensis*	—	0.78–1.26 μM (IC_{50})	HL-60 and A549	Li (2018)
Diterpene	*Clavularia* sp.	—	50 μM (Y)	K562, HL-60, HeLa, and A549	Xue (2014)
Diterpene	*Sinularia* sp.	—	2.32–8.97 μM (IC_{50})	K563	Zou (2015)
Diterpene	*Anthoptilum grandiflorum*	Killed the NT2 cells and antiproliferation	—	NT2	Thomas et al. (2019)
Diterpene	*Sarcophyton crassocaule*	—	2.0, 1.2, 2.6, and 3.2 μM (ED_{50})	MCF-7, WiDr, HEp-2, and Daoy cancer cell lines	Lin et al. (2010)
Diterpene	*Briareum* sp.	Reduced the expression of COX-2	5–30 μM (IC_{50})	Caco-2 cells	Joyner et al. (2011)
Diterpene	*Dichotella gemmacea*※	Antiproliferation	5.0–78.5 μM (IC_{50})	A-549 and MG63	Li et al. (2013a)
Diterpene	*Pseudopterogorgia kallos*※	—	<0.01, 0.51 μM (GI_{50})	EKVX non-small-cell lung cancer and Caki-1 renal cancer	Marrero et al. (2004)
Diterpene	*Lobophytum crassum*	Inhibition of transcriptional activity	6.30 ± 0.42–6.63 ± 0.11 μM (IC_{50})	HepG2	Thao et al. (2014a)
Xenicane	*Protodendron repens*	—	0.2–6.3 μM (GI_{50})	MDAMB-231, HT-29, and NSLC A-549	Urda et al. (2017)
Terpenoids	*Sarcophyton* sp.	—	6.4–33.7 μM (IC_{50})	P338, A549, HL-60, and K562	Gong (2014)
Terpenoids	*Sarcophyton tortuosum*	—	3.5–24.7 μg/mL (IC_{50})	Human nasopharyngeal carcinoma CNE-2 cell line and P-388	Zeng et al. (2004)
Terpenoids	*Sinularia* sp.	Inhibitory activity	6.5–33 μM (IC_{50})	E3-ubiquitin ligase casitas B-lineage lymphoma proto-oncogene B (Cbl-b)	Jiang et al. (2021)
Terpenoids	*Sarcophyton* sp.	—	6.03 ± 1.93, 6.70 ± 1.06 μM (IC_{50})	Canpan-1	Lu (2020)
Diterpene and steroids	*Sinularia dissecta*	—	2.54–100 μg/mL (IC_{50})	PC-3MIE8 and A549	Jin (2005)
Diterpene and steroids	*Lobophytum compactum*	—	17.80 ± 1.43–59.06 ± 2.31 μM (IC_{50})	A549 and HL-60	Chau et al. (2011)
Diterpenoid lactone and steroids	*Sinularia polydactyla*	—	1.0, 6.1, and 8.2 μg/mL (IC_{50})	HepG2, HEp2, and HCT	Aboutabl el et al. (2013)
Steroids	*Sinularia gibberosa*	Antiproliferation	6.8–10.0 μM (ED_{50})	Hepa59T/VGH	Ahmed et al. (2003)
Steroids	*Sarcophyton glaucum*	Antiproliferation	0.62 and 2.3 μM (IC_{50})	Caco-2 and MCF-7	Shaaban et al. (2021)
Steroids	*Sinularia erecta*	—	15.57 ± 5.26–40.55 ± 7.51 μM (IC_{50})	A549, HT-29, SNU-398, and Capan-1	Liu et al. (2020)
Steroids	*Verrucella corona*	—	12.32 ± 1.47–33.77 ± 1.28 μM (IC_{50})	LNCaP, HepG2, KB, MCF-7, SK-Mel2, HL-60, LU-1, and SW480	Nam et al. (2018)
Steroids	*Sinularia leptoclados*	—	13.45 ± 1.81–29.01 ± 3.21 μM (IC_{50})	HepG2, SW480, HL-60, MCF-7 LU-1, SK-Mel2, and LNCaP	Ngoc et al. (2017b)

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TABLE 2 (*Continued*) Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
Steroids	*Heteroxenia fuscescens*	—	33.2 and 25.1 μM (IC_{50})	MCF-7	Abdelkarem et al. (2021)
Steroids	*Nephthea erecta*	Apoptosis and increases caspases activity	20 and 40 μM (Y)	H1688 and H146 lung cancer	Chung et al. (2017a)
Steroids	*Sinularia suberosa*	—	5.5–6.5 μM (IC_{50})	K562 and MDA-MB-231	Zhang (2013a)
Steroids	—	—	21.56–40.04 μM (IC_{50})	HT-29, SNU-398, and Capan-1	Zhang (2019)
Steroids	*Rumphella aggregata*※	—	10 μg/mL (Y)	K562	Liu et al. (2012)
Steroids	*Nephthea* sp.	—	7.51 ± 0.22–18.72 ± 0.78 μg/mL (IC_{50})	HeLa	Zhang et al. (2013b)
Steroids	*Pacifigorgia senta*※	—	7.0–29.7 μM (IC_{50})	HepG2, Hep3B, MCF-7/ADR, PC-3, and HCT116	Chen et al. (2016)
Steroids	*Paragorgia* sp.	Antiproliferation	3.0–90 μM (GI_{50})	A-549, HT-29, and MDA-MB 231	Poza et al. (2008)
Steroids	*Clavularia viridis*	—	0.1–6.8 μg/mL (IC_{50})	HT-29 and P-388	Duh et al. (2007)
Steroids	*Stereonephthya crystalliana*	—	1.6–13.3 μg/mL (ED_{50})	HT-29 and P-388	Wang et al. (2006)
Steroids	*Sinularia* sp.	—	0.69, 4.03, and 1.79 μM (IC_{50})	HL-60	Li et al. (2018a)
Steroids	*Menella kanisa*※	Antiproliferation	11.0 ± 4.2–257.2 ± 20.7 μM (IC_{50})	A549 and MG-63	Wang, P. et al. (2013)
Steroids	*Subergorgia suberosa*	—	15.1 μM (IC_{50})	HeLa	Zhang et al. (2015a)
Steroids	*Sinularia polydactyla*	Anti-migration and neuroprotective activity on nerve cells	10,20	HeLa, MCF-7, and SH-SY5Y	Tammam et al. (2020)
Steroids	*Sinularia brassica*	—	1.17 ± 0.42–92.53 ± 1.68 μM (IC_{50})	A-549, HeLa, and PANC-1	Tran et al. (2017)
Steroids	*Scleronephthya gracillimum*	—	23.3, 21.9, and 24.3 μM (IC_{50})	HepG2, A549, and MDA-MB-231	Fang et al. (2013)
Steroids	*Carijoa* sp.	—	9.33, 11.02, and 18.68 μM (IC_{50})	Bel-7402	Zhao et al. (2013c)
Steroids	*Sarcophyton* sp.	—	6.4–10.3 μM (IC_{50})	HL-60, HeLa, and K562	Gong et al. (2013)
Steroids	*Sinularia* sp.	—	8.36–37.30 μM (IC_{50})	HepG2 and HeLa	Sun et al. (2016)
Steroids	*Sarcophyton* sp.	—	5.25, 12.30, 4.95, 4.10 (K562), 7.30, and 6.20 (A549) μg/mL (IC_{50})	K562 and A549	Sun et al. (2013)
Steroids	*Subergorgia suberosa*	Inhibiting activity	5.5, 6.2, and 6.5 μM (IC_{50})	K562 and MDA-MB-231	Zhang et al. (2013a)
Steroids	*Klyxum flaccidum*	—	12.7–15.5 μM (IC_{50})	HT-29, P388, and K562	Tseng et al. (2016)
Steroids	*Nephthea chabrolii*	—	1.1, 1.2, and 1.0 μg/mL (ED_{50})	P-388, A-549, and HT-29	Shang-Kwei et al. (2013)
Steroids	*Lobophytum laevigatum*	Apoptosis and antiproliferation	3.2–18.1 μM (IC_{50})	HCT-116, A549, and HL-60	Quang et al. (2011b)
Steroids	*Nephthea* sp.	—	2.3*(10^{-7})–98.5*(10^{-4})	HL-60 and A-549	Ma (2008)
Steroids	*Lobophytum* sp.	—	21.56–38.83 and 40.04 μM (IC_{50})	HT-29, SNU-398, and Capan-1	Zhang et al. (2019)

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TABLE 2 (*Continued*) Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
Steroids	*Litophyton mollis*	—	10 μM (IC_{50})	K562 and PBMCs	Zovko Končić et al. (2016)
Steroids	*Nephthea erecta*	—	6.5–14.0 μM (IC_{50})	K562, Molt-4, Sup-T1, and U937	Tsai et al. (2016)
Steroids	*Lobophytum michaelae*	—	14.9 ± 5.7 μg/mL (IC_{50})	A549	Huang et al. (2018)
Steroids	*Verrucella corona*	—	12.32 ± 1.47–33.77 ± 1.28 μM (IC_{50})	LNCaP, HepG2, KB, MCF-7, SK-Mel2, HL-60, LU-1, and SW480	Nam et al. (2018)
Steroids	*Sinularia microspiculata*	—	72.32 ± 1.30–89.02 ± 9.93 μM (IC_{50})	HL-60 and SK-Mel2	Thanh et al. (2016)
Steroids	*Sarcophyton acutum*	—	17.2 ± 1.5 and 24.8 ± 2.8–57.2 ± 5.2 μg/mL (IC_{50})	HepG2, MCF-7, and A549	Zidan et al. (2020)
Steroids		Ability to induce autophagy	20 μM (Y)	MCF-7	Weng et al. (2018)
Steroids	*Cladiella hirsuta*	—	8.2–42.0 μM (IC_{50})	HepG2, HepG3B, MDA-MB-23, and Ca9-22	Chen et al. (2011a)
Steroids	*Sinularia variabilis*	Apoptosis	—	MCF-7 and MDA-MB-231	Mohammadi Pour et al. (2022)
Steroids	*Spongodes* sp.	—	0.14, 5, and 3.8 μg/mL (IC_{50})	BEL-7402, A-549, HT-29, and P388	Yan et al. (2007)
Steroids	*Sinularia acuta*	—	7.28–44.82 μM (IC_{50})	HL-60, K562, and HeLa	Zhang (2014)
Steroids	*Carijoa* sp.	—	9.33–18.68 μM (IC_{50})	Bel-7404	Zhao (2013)
Steroids	*Sarcophyton* sp.	—	—	K562	Sun (2012)
Steroids	*Sinularia* sp.	—	1.79 and 4.03 μM (IC_{50})	HL-60	Li (2018)
Steroids	*Sinularia* sp.	Antiproliferation	1.61 and 3.26 μmol/L (IC_{50})	HL-60	Li et al. (2018b)
Steroids	*Sinularia* sp.	Apoptosis	10.14–41.71 μM (IC_{50})	MDA-MB-436, A549, Hep3B, HT-29 and H157	Jiang et al. (2019b)
Steroids	*Subergorgia suberosa*	—	1.09–6.22 μM (IC_{50})	K562	Liu (2014)
Steroids and ceramide	*Cespitularia stolonifera*	—	23.0–1,574.0 μg/mL (IC_{50})	A549 and MCF-7	Elshamy et al. (2017)
Alkaloid	*Ellisella robusta*※	—	0.35–58.01 μM (IC_{50})	HeLa and K562	Zhang (2012b)
	Ellisella curvata※				
Alkaloid	*Menella kanisa*※	Inhibiting activity and antiproliferation	13.3, 55.0 μg/mol (IC_{50})	Osteosarcoma cells	Yao et al. (2015)
Alkaloid	*Muriceides collaris*※	—	5.08–8.37 μM (IC_{50})	K562 and HeLa	Zhu (2013)
Alkaloid	*Scleronephthya* sp.	Anti-metastasis	5.3 ± 0.2–12.4 ± 0.2 μM (IC_{50})	A549 and B16	Cheng et al. (2017)
Prostanoids	*Clavularia viridis*	Apoptosis	0.12–11.7 μM (IC_{50})	Prostate cancer PC-3 cells	Chiang et al. (2006)
Prostanoids	*Clavularia viridis*	Antiproliferation	0.5–7.9 μM (IC_{50})	PC-3 and HT29	Shen et al. (2004)
Prostanoids	*Plexaura homomalla*※	Inhibiting the expression of related enzymes	16.46, 25.20 μg/mol (IC_{50})	MDA-MB-213 and A549	Hurtado et al. (2020)
Ester	*Sinularia flexibilis*	Antiproliferation	10 mg/kg (Y)	Small cell lung cancer	Lin et al. (2013a)

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TABLE 2 (*Continued*) Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
Ester	*Cladiella kashmani*	Anti-invasion and anti-metastasis	1, 2.5, 5, and 10 μM (Y)	T24 human bladder cancer cells	Wu et al. (2019a)
Ester	*Paraminabea acronocephala*	—	0.5–2.2 μM (IC_{50})	HepG2, Hep3B, MDA-MB-231, MCF-7, and A-549	Chao et al. (2011b)
Ester	*Lobophytum durum*	—	3.8 μg/mL (ED_{50})	P-388	Cheng et al. (2011)
Ester	*Sinularia flexibilis*	Anti-invasion and anti-metastasis	—	Gastric cancer	Wu et al. (2019b)
Ester	*Stragulum bicolor*	Apoptosis	0.18 and 4.3 μM (IC_{50})	A2058	Nuzzo et al. (2019)
Sinulariolide	*Sinularia flexibilis*	Antiproliferation and apoptosis	15 μM (Y)	Bladder carcinoma cell and TSGH cells	Neoh et al. (2012)
Alkane	*Montipora* sp.	—	1.40–29.16 μg/mL (ED_{50})	A549, SK-OV-3, SK-MEL-2, XF498, and HCT15	Alam et al. (2001)
Aromatic compounds	*Scleronephthya gracillimum*	—	2.86–7.51 μg/mL μM (IC_{50})	HeLa and P388	Han (2011)
				HepG2, Hep3B, and HT116	
Oligopeptides	*Sarcophyton glaucum*	—	8.6, 4.9, and 5.6 mmol/L (EC_{50})	HeLa	Quah et al. (2019)
EPA	*Eunicea succinea*※	—	5.1–6.9 μmol/L (IC_{50})	Malignant glioma U87-MG and U373-MG cells	Iwamaru et al. (2007)
Lobophorin	*Lophelia pertusa*	—	6.3 ± 8.2, 23.0 ± 8.9, and 34.0 ± 85.1 μM (IC_{50})	MiaPaca-2, MCF-7, and THLE-2	Braña et al. (2017)
Tetraphenylbenzoquinone	*Sinularia capillosa*	—	9.8 and 12.7 μM (ED_{50})	P-388	Cheng et al. (2010a)
Durumolide	*Sinularia polydactyla*	—	1.0–8.2 μg/mL (IC_{50})	HepG2, HEp2, and HCT	Aboutabl el et al. (2013)
Biscembranoids	*Sarcophyton pauciplicatum*	—	7.93 ± 2.08–94.18 ± 3.02 μM (IC_{50})	LNCaP MCF-7 KB HepG2, SK-Mel2, HL-60, SW480, and LU-1	Nam et al. (2015)
Tryptamine derivatives	*Eunicella granulata*※	—	1.7–12.7 μM (GI_{50})	DU-145, LNCaP, SK-OV-3, IGROV, IGROV-ET, SK-BR3, SK-MEL-28, A549, K-562, PANC1, HT29, LoVo LoVo-DOX, HeLa, and HeLa-APL	Reyes et al. (2006)
Tetracyclic biscembranes	*Sarcophyton glaucum*	—	13.3–58.0 μM (IC_{50})	HL-60	Iwagawa et al. (2009)
Sinularin	*Sinularia flexibilis*	Increasing G2/M cell cycle arrest, inducing apoptosis, and activating DNA damage responses	17.5 ± 6.7, 9.4 ± 2.3 (HEPG2), 43.2 ± 8.1, and 33.9 ± 8.6 μM (Hep3B) μM (IC_{50})	HepG2 and Hep3B	Chung et al. (2017b)
13-Acetoxysarcocrassolide	*Sarcophyton crassocaule*	Apoptosis	1 and 1.5 μg/mL (Y)	BFTC	Su, C.C. et al. (2011)
Flaccidoxide-13-acetate	*Sinularia gibberosa*	Apoptosis	20 μM (Y)	RT4 and T24 human bladder cancer cells	Wu et al. (2019a)
Glycolipids	*Lobophytum crassum*	—	9.2–15.0 μg/mL (IC_{50})	HepG2, Hep3B, MDA-MB-231, and Ca9-22	Chao et al. (2007)
Crude extract	*Sinularia* cf. *molesta*	—	50 μg/mL (Y)	K562 and HL-60	Jiang (2015)
—	*Muricella sibogae*	—	1, 10, and 50 μg/mL (Y)	P388 and BEL-7402	Li (2010b)
—	*Cladiella australis, Clavularia viridis, and Klyxum simplex*	Apoptosis	31.5 ± 1.5–53.8 ± 2.1 μg/mL (IC_{50})	Squamous cell carcinoma cells	Liang et al. (2008)

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TABLE 2 (*Continued*) Classification statistics for cytotoxicity of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration*	Target cell	Reference
—	*Carotalcyon* sp.※	Antiproliferation and apoptosis	0.7 ± 0.4–250.9 ± 92.1 μg/mL (IC_{50})	HGUE-C-1, HT-29, and SW-480	Ruiz-Torres et al. (2019)
—	*Euplexaura rhipidalis*※	Apoptosis	<10 μg/mL (IC_{50})	A549 and HepG2	Gong et al. (2017)
—	*Sinularia maxima*	Inhibition of transcriptional activity	15.81 ± 2.29–29.10 ± 1.54 μM (IC_{50})	HepG2	Thao et al. (2014b)

*Y refers to the medication.

Subergorgia reticulata (Xie et al., 2013) and red coral (Lai, 2017). Zou (2015) sorted out olefins from the crude extract of *Sinularia* sp. Subsequently, Li R. (2012) extracted ketones and alcohols from this coral. Esters such as methyl arachidonic acid (Liang et al., 2017), dibutyl phthalate, diisobutyl (Wang et al., 2009), and 1,2-benzenedicarboxylate (Lv et al., 2012) are also found in this coral.

4 Pharmacological effects and mechanisms

Many structurally active unique secondary metabolites, such as terpenoids, steroids, ceramides, and prostaglandins, have been extracted from corals, and their significant pharmacological activities, such as cytotoxic and antiviral activities, have been widely noticed and studied by natural product chemists and other researchers. Meanwhile, the pharmacological activities of coral bone powder and various coral preparations in the cardiovascular system have been explored. This article focuses on their cytotoxic effects on a variety of tumor cells and cancer cells as well as their restorative effects on bone injury diseases and their biological activities, such as antioxidant, anti-inflammatory, analgesic, and antiviral activities, on tissues of the nervous system and respiratory system. Accumulating evidence suggests that they have significant therapeutic effects on diseases of the nervous system.

4.1 Bone repair effect

The key to the treatment of bone defects is the suitability of the repair material. Autologous bone grafts cannot meet clinical needs for various reasons, and allogeneic and xenogeneic bones are limited in clinical application because of their antigenic nature (Zhou et al., 1993). The microstructure of coral and skeleton is also very close, specifically in its internal structure. Corals are divided into pinnate, laminate, branching and pith-like structures depending on the arrangement of the calcification centers. According to the skeleton body, the tiny tube traffic is divided into interlocking and interoperable traffic. Depending on whether the microscopic tubes in the skeleton are in traffic or not, they are divided into interlocking and interoperable. The interconnected coral skeleton has longitudinally and horizontally arranged tiny tubes, with pore diameters of 0.05–2.0 mm. Regardless of the section, these pores are interconnected. The coral artificial bone is widely valued as a promising material for bone repair (Roux et al., 1988). Animal experiments have shown that artificial bones made from horned honeycomb coral (favites) have good biocompatibility and osteocompatibility. When it is implanted in the mandible, the femoral cortical defect site after 8 months can be repaired, resulting in complete restoration (Zeng et al., 1997). Zhou et al. (1993) used Hainan Cheng Huang Bin Coral (Hainan Coral, Porites Iutea; HNC) composite implant material as the material graft in the side mandibular defect model and affirmed that the coral group led to the formation of new bone tissue, wrapped with phenanthrene fibrous tissue, followed by its better bone repair effect when used with BMG. In addition, it led to osseous healing, bone marrow cavity formation, and clear visualization of new bone tissue. Lai (2017) concluded that red coral can promote fracture healing and reduce the fracture healing period. According to the literature (Souyris et al., 1985; Dagli et al., 1997; Zhou, 2014), coral can also be used to correct saddle nose deformities, oral implants, skull injuries or postoperative repairs, and other orthopedic disorders.

In addition, coral transplants in the human body do not cause rejection; countless fine pores in the coral facilitate the gradual growth of microscopic blood vessels and synthesis of living cells of bone (Ma, 1994). Guillemin et al. showed that the resorption of corals starts with the growth of granulation tissue and blood vessels from the bone marrow into the coral. Then, the coral is progressively resorbed by many osteoclasts near its edges, while the woven bone formed with osteoclasts gradually grows into the resorbed void; finally, bone marrow cavity is formed, and the newly formed bone tissue system is clear and visible (Guillemin et al., 1989; Lu and Chen, 1994).

Chemical compounds extracted from corals also play a role in bone injury diseases. Lin Y. F. et al. (2013) isolated Ya-s11 (9 mg/kg) from the Taiwanese soft coral *Sinularia querciformis*, which not only attenuated AIA-induced ankle joint pathological changes but also significantly reduced the expression of osteoclast-related proteins.

4.2 Cytotoxicity

As shown in Table 2, studies in the literature in the last two decades have found that compounds extracted from coral have good cytotoxicity, particularly diterpenes, sesquiterpenes, sterols, and a small number of alkaloids, prostaglandins, and esters as active substances that also have some biological activity. These compounds are mostly extracted from corals of the genera *Sinularia*, *Lobophytum*, and *Sarcophyton* all belonging to the family Alcyoniidae. Corals of the family Gorgoniidae※ are also used as a source of active natural substances. The evaluation of their cytotoxic activity against tumor cells such as A549, HL-60, MCF-7, colon cancer cells, K562, and HeLa, followed by HepG2, Hep3B, MDA-MB-231, P-388, HT-29, MCF-7, Sup-T1, U937 and other cells, has

TABLE 3 Classification statistics for anti-inflammatory and analgesic effects of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration[a]	Reference
Sesquiterpenoids	*Sinularia tumulosa*	I	2.6–7.5 μM (IC_{50})	Cai et al. (2020)
Sesquiterpenoids	*Anthogorgia* sp.	N and A	27.81 μg/mL (IC_{50})	Ji and Liu (2018)
Sesquiterpenoids	*Sinularia scabra*	I	10 μM (Y)	Su et al. (2012)
Diterpene	*Lobophytum crassum*	I and C	10 μM (Y)	Chao et al. (2008a)
Diterpene	*Cladiella krempfi*	I and C	10 μM (Y)	Tai et al. (2013)
Diterpene	*Briareum* sp.	C	5–30 μM (Y)	Joyner et al. (2011)
Diterpene	*Lobophytum* sp.	N	5, 10, and 25 μM (Y)	Roy et al. (2019)
Diterpene	*Klyxum flaccidum*	N	50, 46.7, and 47.0 (IC_{50})	Ahmed et al. (2017b)
Diterpene	*Sinularia flexibilis*	S and E	10.8 ± 0.38 and 11.0 ± 1.52 μM (IC_{50})	Wu et al. (2018)
Diterpene	*Cladiella krempfi*	I	10 μM (Y)	Tai et al. (2011)
Diterpene	*Sinularia triangular*	I, C	10 μM (Y)	Su and Wen (2011)
Diterpene	*Lobophytum laevigatum*	I and C	0.1–10 Y	Quang et al. (2011a)
Diterpene	*Sarcophyton glaucum*	A	20 μmol/L (Y)	Li et al. (2022b)
Diterpene	*Sinularia flexibilis*	N	10 μM (Y)	Xu (2016)
Diterpene	*Briareum excavatum*※	I and C	10 μM (Y)	Huynh et al. (2020)
Diterpene	*Briareum* sp.	I	10 μM (Y)	Su et al. (2015a)
Diterpene	*Briareum* sp.	I and C	10 μM (Y)	Su et al. (2015b)
Diterpene	*Lobophytum crassum*	N	2.4 ± 0.21–16.6_x0007_ 1.70 (IC_{50})	Wanzola et al. (2010)
Diterpene	*Lobophytum varium*	S and E	10 μM (Y)	Ahmed et al. (2017a)
Diterpene	*Lobophytum crassum*	N	50 μg/mL (Y)	Chao et al. (2008b)
Diterpene	*Sinularia gyrosa*	C	10 μM (Y)	Cheng et al. (2010a)
Diterpene	*Lobophytum durum*	I and C	10 μM (Y)	Cheng et al. (2009a)
Diterpene	*Sinularia querciformis and Sinularia granosa*	I and C	10 μM (Y)	Lu et al. (2008)
Diterpene	*Cladiella* sp.	S and E	10 μM (Y)	Chen et al. (2010b)
Diterpene	*Cladiella* sp.	S and E	8.1 ± 0.3–49.4 ± 0.2 (IC_{50}/Inh)	Chen et al. (2011b)
Diterpene	*Klyxum simplex*	I and C	10 μM (Y)	Chen et al. (2010a)
Diterpene	*Lobophytum* sp.	N	3.2–9.4 μM (IC_{50})	Zhao et al. (2013b)
Diterpene	*Sinularia gyrosa*	C	10 μM (Y)	Cheng et al. (2010b)
Diterpene	*Sarcophyton cherbonnieri*	S and E	30 μM (Y)	Peng et al. (2020)
Diterpene	*Lobophytum crassum*	I and C	6.30 ± 0.42–6.63 ± 0.11 μM (IC_{50})	Thao et al. (2014a)
Diterpene	*Sarcophyton glaucum*	A	10 μM (Y)	Shen et al. (2021)
Diterpene	*Junceella fragilis*※	I	10 μM (Y)	Su et al. (2019)
Diterpene	*Nephthea columnaris*	I and C	9.80 μg/mL (IC_{50})	Hsiao et al. (2015)
Diterpene	*Lobophytum durum*	I	10 μM (Y)	Cheng et al. (2009b)
Diterpene	*Sinularia maxima*	I	4.35 ± 0.12–59.77 ± 2.34 μM (IC_{50})	Thao et al. (2012)
Diterpene	*Sinularia maxima*	I	0.1, 1.0, and 10 μM Y	Thao et al. (2014b)
Diterpene	*Lobophytum pauciflorum*	N	2.8 μM (IC_{50})	Yan et al. (2010)
Diterpene	*Sinularia crassa*	I and C	10 μM (Y)	Chao et al. (2011a)

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TABLE 3 (*Continued*) Classification statistics for anti-inflammatory and analgesic effects of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration[a]	Reference
Diterpene	*Lobophytum sarcophytoides*	N	7.1–32.1 μM (IC_{50})	Shen et al. (2019)
Diterpene	*Klyxum molle*	I and C	10 μM (Y)	Hsu et al. (2011)
Diterpene	*Sarcophyton ehrenbergi*	I	7.2–38.6 μM (IC_{50})	Li et al. (2020a)
Diterpene	*Briareum excavatum*※	I and C	1–50 μM (Y)	Lin et al. (2015)
Diterpene	*Sinularia crassa and Lobophytum* sp.	—	10 mg/kg (Y)	Radhika et al. (2005)
Diterpene	*Sinularia nanolobata*	N and A	20 μM (Y)	Zeng et al. (2021)
Diterpene	*Cladiella* sp.	S and E	1.97 ± 2.44–41.08 ± 3.26 μg/mL (IC_{50})	Chen et al. (2012)
Cembranoid	*Sarcophyton crassocaule*	I and C	10 μM (Y)	Lin et al. (2010)
Cembranoid	*Sinularia* sp.	I	<6.25 μg/mL (Y)	Kamada et al. (2018)
Norditerpenoids	*Sinularia maxima*	I	5.30 ± 0.21–69.85 ± 4.11 μM (IC_{50})	Thao et al. (2013)
Norditerpenoids	*Sinularia numerosa*	I	10 μM (Y)	Yin et al. (2015)
Norditerpenoids	*Sinularia siaesensis*	A	20 μM (Y)	Chen et al. (2021)
Norditerpenoids	*Sinularia maxima*	I	23.52 ± 1.37 and 69.85 ± 4.11 μM (IC_{50})	Thao et al. (2013)
Norditerpenoids	*Sinularia* sp.	N and I	33 μg/mL (Y)	Hiroko et al. (2003)
Norditerpene	*Sinularia gyrosa*	I	10 μM (Y)	Cheng et al. (2010c)
Nanolobatolide	*Sinularia nanolobata*	I	10 μM (Y)	Tseng et al. (2009)
Diterpene and sesquiterpenoids	*Cespitularia* sp.	I, C, and N	100 μM (Y)	Lin et al. (2021)
Steroids	*Nephthea chabroli*	I, C	10 μM (Y)	Huang et al. (2008)
Steroids	*Sinularia crassa*	I, C	10 μM (Y)	Chao et al. (2012)
Steroids	*Klyxum flaccidum*	S, E	4.40 ± 0.19, 5.64 ± 0.41 (IC_{50})	Tseng et al. (2016)
Steroids	*Nephthea chabroli*	I and C	10 μM (Y)	Huang et al. (2008)
Steroids	*Scleronephthya gracillimum*	I and C	10 μM (Y)	Fang et al. (2013)
Steroids	*Clavularia viridis*	I and C	10 μM (Y)	Chang et al. (2008)
Steroids	*Dendronephthya griffini*	I and C	10 μM (Y)	Chao et al. (2008a)
Steroids	*Echinomuricea spinosa*※	S and E	1.13 ± 0.55–95.54 ± 6.17 μM (IC_{50})	Chung et al. (2012)
Steroids	*Dendronephthya gigantea*	I, C, S, E, and N	4.33 ± 0.50 μg/mL (IC_{50})	Fernando et al. (2017)
Steroids	*Pinnigorgia* sp.※	I and C	10 μM (Y)	Su et al. (2016)
Crude extract	*Nephthea* sp.	C	33.72–46.75 μg/mL (IC_{50})	Abdelhafez et al. (2020)
Flexibilisquinone	*Sinularia flexibilis*	I and C	5–20 μM (Y)	Lin et al. (2013b)
Tocopherol-derived	*Cladiella hirsuta*	S and E	3.7 ± 0.3–4.1 ± 1.1 μM (IC_{50})	Chen et al. (2015)
EGFR	—	C and I	10 μM (Y)	Lin et al. (2013a)
Lemnalol	—	I and C	30 mg/kg (Y)	Lee et al. (2013)
Lemnalol	*Lemnalia cervicornis*	A	0.05–10 μg (Y)	Lin et al. (2011)
Lemnalol	*Lemnalia cervicornis*	I and C	15 mg/kg (Y)	Jean et al. (2008)
Quinones	*Sinularia flexibilis*	I and C	5–20 μM (Y)	Lin et al. (2013b)
Glycoside	*Pseudopterogorgia elisabethae*※	—	1–4 μM (IC_{50})	Mayer et al. (1998)
Briarane	*Junceella fragilis*※	E	10 μg/mL (Y)	Sheu et al. (2006)
Isosarcophine	*Sarcophyton cherbonnieri*	S and E	30 μM (Y)	Peng et al. (2021)

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TABLE 3 (*Continued*) Classification statistics for anti-inflammatory and analgesic effects of active substances extracted from the coral.

Active ingredient	Source	Activity	Concentration[a]	Reference
Tetraphenylbenzoquinone	*Sinularia capillosa*	I and C	10 μM (Y)	Cheng et al. (2010a)
Withanolide	*Paraminabea acronocephala*	I and C	10 μM (Y)	Chao et al. (2011b)
Capnellene	*Capnella imbricate*	C	6.21 ± 2.5 and 17.9 ± 2.9 μM (IC_{50})	Jean et al. (2009)
Bicyclogermacrenes	*Capnella* sp.	I and N	10 and 20 μM (Y)	Phan et al. (2015)
Isoprenoids	*Sinularia erecta*	S and E	0.9 ± 0.1–8.5 ± 0.3 μM (IC_{50})	Lin et al. (2014)
Prostaglandin	*Plexaura homomalla*※	V and E	100 μM (Y)	Huang et al. (2016)

[a]Inhibition of iNOS (I), COX-2 (C), superoxide anion (S), N (N0), astrocytes (A), and elastase (E); Y refers to the medication.

become the hotspots of research. Shaaban et al. (2021) evaluated the *in vitro* anticancer effects of hydroazulenes, an extract of the soft coral *S. glaucum*, on colon (Caco-2) and breast (MCF-7) cell lines by MTT assays and showed that its antiproliferative or antiangiogenic effects were ultimately achieved by inhibiting the migration of MCF-7 cells and significant inactivation of VEGFR2 enzymes. Interestingly, the growth inhibitory concentrations of 5α-3β,6α,11-trihydroxy-24-methyl-9,11-seco-5a-cholest-7-en-9-one on colon (Caco-2) and breast (MCF-7) cell lines were 0.62 and 2.3 mM, respectively, but no toxicity was recorded against RPE-1 cells at a high concentration of 10 mM. The team also studied for the first time the anticancer properties of the sterol 10-epicatechin methyl ether. The first study of *Sarcophyton acutum* extract activity by Sabry A. H. Zidan studied for the first time the cytotoxic activity of *Sarcophyton acutum* extract and showed that polyhydroxylated steroid compounds had significant cytotoxicity to the HepG2 cell line (semi-inhibitory concentration 17.2 ± 1.5 μg/mL) and MCF-7 (semi-inhibitory concentration 33.2 and 25.1 mM) (Zidan et al., 2020; Abdelkarem et al., 2021) and that the side chains of polyhydroxylated sterols play an important role in the cytotoxic activity of such sterols. The researchers also demonstrated using the SRB method that the gorgonian of *Euplexaura rhipidalis*※ has a significant apoptosis-inducing effect on A549 and HepG2 cells (Gong et al., 2017); in other words, prostaglandins with hydroxyl and carboxylic acids possess good cytotoxic properties and that they may have potential inhibitory effects on certain types of cancer (Hurtado et al., 2020). In fact, more than a decade ago, studies showed that the structure of compounds could influence cytotoxicity. A free hydroxyl group at C-12 or C-22 is important for enhancing the cytotoxic activity of a sterol against HeLa cell lines. In addition, the introduction of hydroxyl groups at C-20 decreased the inhibitory potency against HeLa cell lines, while the presence of acetoxy groups at C-18 seemed to enhance the cytotoxic activity (Zhang J. et al., 2013).

4.3 Anti-inflammatory and analgesic effects

Inflammatory processes usually constitute the initial activation of the mammalian immune system and the body's normal defense or protective mechanisms against microbial infections or stimuli, tissue, or organ damage. Accumulating evidence shows a critical link between inflammation and the chronic promotion/progression of various human diseases, including atherosclerosis, diabetes, arthritis, inflammatory bowel disease, cancer, and Alzheimer's disease (Wei et al., 2013). Different types of cells, such as monocytes/macrophages, neutrophils, and lymphocytes, are involved in the inflammatory process (Serhan and Savill, 2005). Several marine biology and chemistry researchers have systematically screened the *in vitro* anti-inflammatory activity of several marine natural products isolated from corals, and lipopolysaccharide-stimulated mouse macrophage models have been widely used as a system for assessing the anti-inflammatory activity of secondary metabolites of marine and terrestrial origin (Lin et al., 2015). Yen-You Lin's study showed that the diterpene compound excavatolide B from the gorgonian of *Briareum excavatum*※ produced potent anti-inflammatory activity *in vitro* and *in vivo* and inhibited the expression of iNOS and COX-2 mRNA. Gyrosanols A and B show significant anti-inflammatory activity by reducing COX-2 protein levels in RAW 264.7 macrophages (Cheng et al., 2010a). Lee et al. (2013) found that soft coral-derived leminalol attenuated monosodium urate-induced gouty arthritis in rats by inhibiting leukocyte infiltration and the expression of iNOS and COX-2 proteins, among others.

The inflammatory process also involves the peripheral and central nervous system (CNS) and is thought to be involved in the pathogenesis of neuropathic pain (Ellis and Bennett, 2013s). Chen N. F. et al. (2014) investigated flexibilide, extracted from cultured soft corals, as a possible drug for neuropathic pain, and its anti-neuritis and analgesic mechanisms of action may be related to spinal TGF-β1 inhibition. The sphingosine derivative obtained from soft corals also has anti-inflammatory and analgesic effects (Radhika et al., 2005). After compiling nearly 100 studies, it was found that the anti-inflammatory activity of coral extracts is mainly attributed to diterpene compounds, followed by sterols, prostaglandins, and alkaloids. Its anti-inflammatory activity is mainly mediated by the inhibition of lipopolysaccharide-induced expression of iNOS and COX-2 in mouse macrophages (RAW 264.7) or by the inhibition of superoxide anion release from human neutrophils FMLP/CB and elastin. The specific functions of anti-inflammatory and analgesic effects in corals are shown in Table 3.

4.4 Antiviral

Viruses are infectious entities that use the cellular biosynthetic machinery to replicate their own nucleic acids, synthesize the proteins encoded by their nucleic acids, and finally assemble into

TABLE 4 Classification statistics for antiviral effects of active substances extracted from the coral.

Active ingredient	Source	Virus	Concentration[a]	Activity	Reference
Sesquiterpenoids	*Muriceides collaris*※	H1N1	50 μM (Y)	—	Zhu (2013)
Sesquiterpenoids	*Lemnalia sp.*	H1N1	1.1 and 7.1 μM (IC_{50})	—	Liu et al. (2022)
Sesquiterpenoids	*Lemnalia sp.*	H1N1	5.9 μM (IC_{50})	—	Yan et al. (2021)
Sesquiterpenoids	*Echinogorgia flora*※	H1N1	50 μM (Y)	—	Wu (2013)
Diterpene	*Sinularia gyrosa*	HCMV	2.6 and 3.7 μM (IC_{50})	—	Cheng et al. (2010b)
Diterpene	*Junceella fragilis*※	HBeAg	0.89–6.47 μM (IC_{50})	Inhibition of HBeAg antigen expression	Wei et al. (2017)
Diterpene	*Ellisella sp.*	HBV and HBeAg	10 μM (Y)	Suppression of virus replication	Wu et al. (2020a)
Diterpene	*Clavularia sp.*	H1N1	50 μM (Y)	—	Xue (2014)
Diterpene	*Lobophytum durum*	HCMV	5.2 μg/mL (IC_{50})	Inhibition of viral transcription	Cheng et al. (2011)
Norditerpenoids	*Sinularia gyrosa*	HCMV	1.9 μg/mL (IC_{50})	—	Cheng et al. (2010c)
Steroids	*Echinogorgia rebekka*※	Respiratory syncytial virus	0.19 μM (IC_{50})	—	Cao et al. (2014)
Steroids	*Sarcophyton sp.*	H1N1	19.6–36.7 μg/mL (IC_{50})	—	Gong (2014)
Steroids	*Sarcophyton sp.*	H1N1-IAV	19.6 and 36.7 μM (IC_{50})	Suppression of virus replication	Gong et al. (2013)
Steroids	*Subergorgia suberosa*	H1N1	35.64–50.95 μM (IC_{50})	—	Cheng et al. (2016)
Streptomycetes	*Sarcophyton convolutum*	H1N1 and HCV	—	Suppression of virus replication	El-Gendy et al. (2022)
Lobohedleolide	*Lobophytum crassum*	HCV	10±0.56–22±0.75 μM (EC_{50})	Inhibition of HCV-induced cyclooxygenase-2 (COX-2) expression	Lin et al. (2018)
Tetraphenylbenzoquinone	*Sinularia capillosa*	HCMV	—	—	Cheng et al. (2010a)

[a]Y refers to the medication

complete, infectious viral particles. In most cases, viruses can cause disease and even death in infected hosts (Li W. et al., 2022). Almost all clinical and public health outbreaks over the decades have been due to emerging viruses, including coronavirus (SARS), which causes severe acute respiratory distress syndrome, influenza A virus subtype H1N1 (IAV-H1N1), which caused an influenza pandemic in 2009, human cytomegalovirus (HCMV), which can cause visceral disease, and the SARS CoV-2, which caused a widespread outbreak worldwide in 2019 (Chen et al., 2023). The widespread outbreak of the virus not only poses a great threat to the lives and health of people across the country but also severely hinders global economic development. Marine organisms have been shown to be a rich source of antiviral drugs (Cao et al., 2014). Chun-Kuang demonstrated that lobohedleolide isolated from the Taiwanese soft coral *Lobophytum crassum* significantly reduced HCV replication in replicon cells and JFH-1-infected systems with EC_{50} values of 10 ± 0.56 and 22 ± 0.75 μM at nontoxic concentrations, respectively. Their study also concluded that the inhibitory effect on HCV replication was due to the inhibition of HCV-induced COX-2 expression (Lin et al., 2018). Gong et al. (2013) showed for the first time thatspecific types of steroids were active against influenza viruses. The antiviral effect of coral is mainly achieved through the inhibition of viral replication and expression of antigens. As summarized, coral mainly has antiviral activity against pathogens such as HCMV and H1N1, and some studies have also found antiviral activity against pathogens such as HBV and HCV, as shown in Table 4.

4.5 Antibacterial

As shown in Table 5, according to the literature, the antimicrobial activity of coral is mainly exhibited in terms of activity against bacteria (Gram-negative and Gram-positive bacteria, etc.) and fungi. Its antibacterial activity is mainly attributed to terpene compounds extracted from coral, particularly sesquiterpenes and diterpenes, followed by steroidal active substances. In 1997, Badria's team demonstrated the antibacterial activity of sarcophytolide extracted from soft corals using reagents such as dimethyl sulfoxide and showed that the compound had broad activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *oenococcus oeni*. Mohamed N. Gomaa not only tested the antibacterial activity of the soft coral of the *Sarcophyton* genus but also compared the differences in the antibacterial activity of different extracts. The

TABLE 5 Classification statistics for antibacterial effects of active substances extracted from the coral.

Active ingredient	Source	Strain	Concentration[a]	Reference
Sesquiterpenoids	*Anthogorgia* sp.	*Staphylococcus aureus*	100 μg/mL (Y)	JI and Liu (2018)
Sesquiterpenoids	*Muriceides collaris*※	*Vibrio anguillarum*, *Vibrio harveyi*, and *Vibrio alginolyticus*	0.1, 1, 10, and 100 μg/mL (Y)	Shi (2009)
Sesquiterpenoids	*Litophyton arboreum*	*Bacillus cereus*	1.8 μmol (Y)	Abou El-Kassem et al. (2018)
Sesquiterpenoids	*Paralemnalia thyrsoide*	*Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*	0.221–2.248 μmol (MIC)	Elshamy et al. (2021)
Sesquiterpenoids	*Lemnalia* sp.	*Bacillus subtilis*	4–8 μg/mL (MIC)	Liu et al. (2022)
Sesquiterpenoids	*Xenia* sp.	*Lagenidium thermophilum*	25 μg/mL (MIC)	Phan et al. (2019)
Diterpene	*Junceella juncea*※	Fungi: *Aspergillus niger*, *Candida albicans, and Penicillium notatum*	200 μg/mL (Y)	Murthy et al. (2011)
Diterpene	*Lobophytum pauciflorum*	*Staphylococcus aureus* and *Streptococcus pneumoniae*	20 μg/mL (Y)	Yan et al. (2010)
Diterpene	*Dichotella gemmacea*※	*Staphylococcus albus* and *Staphylococcus aureus*	10–20 μmol/mL (MIC)	Sun (2012)
Diterpene	*Lobophytum* sp.	*Staphylococcus aureus* and *Streptococcus pneumoniae*	—	Zhao et al. (2013a)
Diterpene	*Lemnalia* sp.	*Bacillus subtilis and Staphylococcus aureus*	4–64 μg/mL (MIC)	Yan et al. (2021)
Diterpene	*Dichotella gemmacea*※	Gram-positive bacterium *Bacillus megaterium and* Gram-negative bacterium *Escherichia coli*	0.05 mg (Y)	Li et al. (2016)
Diterpene	—	*Trypanosoma brucei* and *Leishmania donovani*	≤1 μM and <0.2 μM (IC_{50})	Thao et al. (2015)
Diterpene	*Nephthea* sp.	*Staphylococcus aureus* and *Escherichia coli*	2.4, 3.0, and 6.0 μg/mL (MIC/MBC)	Ishii et al. (2016)
Terpenoids	*Sarcophyton trocheliophorum*	Gram-positive and Gram-negative bacteria	0.5, 1, 2.5, and 5 mg/mL (Y)	Gomaa et al. (2016)
Steroids	*Sarcophyton* sp.	*Escherichia coli*, *Bacillus megaterium*, *Microbotryum violaceum*, and *Septoria tritici*	—	Wang et al. (2013a)
Steroids	*Carijoa* sp.	*Pseudomonas putida*, *Bacillus cereus*, and *Tetragenococcus halophilus*	31 nM (Y)	Zhao (2013)
Steroids	*Sarcophyton* sp.	*Staphylococcus albus*	20 μmol (Y)	Sun et al. (2013)
Steroids	*Carijoa* sp.	Pseudomonas putida	31 nM (Y)	Zhao et al. (2013c)
Diterpene and steroidal saponin	*Dichotella gemmacea*※	*Bacillus megaterium* and *Botrytis cinerea*	—	Jiang (2013)
Polyphenol	*Talaromyces* sp.	*Escherichia coli*, *MRSA*, *Staphylococcus. aureus*, and *Enterococcus faecalis*	0.45–15.6 μg/mL (MIC)	Li et al. (2021b)
Lobophorin	*Lophelia pertusa*	Pathogenic Gram-positive bacteria such as *Staphylococcus aureus*	40–80 μg/mL (MIC)	Braña et al. (2017)
BCE	*Sarcophyton sp.*	Pathogenic Gram-positive bacteria such as *Staphylococcus aureus* and *Staphylococcus epidermidis*	37 and 73 μg/μL (MIC)	Bai (2011)
—	*Xenia* sp.	*Lagenidium thermophilum*	25 μg/mL (MIC)	Phan et al. (2019)
—	*Nephthea* sp.	*Lagenidium thermophilum*	12.5 μg/mL (MIC)	Tani et al. (2019)
—	*Muricella sibogae*	*Vibrio anguillarum*	0.1, 1, 10, and 100 μg/mL (Y)	Li (2010b)
—	*Sinularia polydactyla*	*Gram-positive bacteria: Bacillus subtilis* and *Bacillus megaterium*	3.9–62.5 μg/mL (MIC)	Aboutabl el et al. (2013)

[a]Y refers to the medication

results showed that the hexane extract had a strong antibacterial effect. The antibacterial activity of nerve sphingolipids and sterols extracted from *A. dichotoma*※ was also demonstrated using the disc diffusion technique (Al-Lihaibi et al., 2010). The diterpenoids isolated from *Lemnalia* sp. also showed antibacterial activity with MICs of 4–64 μg/mL for *Bacillus subtilis* and *Staphylococcus aureus* (Yan et al., 2021). The antibacterial mechanism has not been specifically reported.

4.6 Antioxidant activity

Altered oxidative status may have peroxidative effects on lipids, proteins, and RNA and regulate cellular responses, signal transduction, and metabolism, thereby impairing their biological functions. At present, few reports on the antioxidant effect of coral can be retrieved, and the antioxidant effect mostly works through free radical scavenging, oxidative free radicals, and lipid peroxidation. In general, common free radicals include $^{-}$OH, O^{2-}, DPPH, and $ABTS^{-/+}$. The coral derivatives sinularin and dihydrosinularin showed general radical scavenging activity against the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), and hydroxyl (-OH), as well as the induction of Fe^{+3} reduction and Fe^{+2}-chelating ability, all of which enhanced their antioxidant activity. Sinularin exhibited higher antioxidant properties than dihydrosinularin. Further ATP assays showed that the different antioxidant properties contributed to the antiproliferative effect on different cancer cells as well (Wang et al., 2021). The *in vitro* antioxidant results of the active ingredients BCE (alkanes, terpenoids, esters, fatty acids, and aromatic compounds) extracted from black horn coral※ indicated that some of them have scavenging effects on DPPH- and OH-. The *in vivo* antioxidant effect not only induces a morphological protective effect on lung tissue but also effectively increases SOD activity *in vivo* and reduces the MDA content, thereby reducing the damage to lung tissue caused by the large amount of oxygen free radicals in tobacco (Bai, 2011).

4.7 Antimalarial

Malaria, caused by *Plasmodium vivax*, poses a major health threat to the majority of the world's population (Thao et al., 2015). Various marine natural products with anti-protozoal activity have been reported in the literature (Watts et al., 2010; Sanchez et al., 2013; Mohyeldin et al., 2017). Thao et al. (2015) identified laevigatol A in Vietnamese soft corals, which showed inhibition of the *Plasmodium falciparum* (Pf) NF54 strain with IC_{50} < 5.0 μM. The antimalarial activity of sesquiterpene extracts of the octocoral coral *Eunicea* sp.※ (Plexauridae: *Octocorallia*: Cnidaria) was demonstrated against chloroquine-resistant strains of *Plasmodium falciparum* by inserting fluorochromes into the parasite DNA. The results revealed that compounds showed a significant inhibition of *Plasmodium falciparum* growth (Garzón et al., 2005). Ospina et al. (2005) conducted an experiment and showed that caucanolide A, a diterpene compound extracted from anise coral, exhibited significant *in vitro* antiplasmodial activity against *Plasmodium falciparum* W2 at an IC_{50} of 17 μg/mL, and caucanolide D was equally effective at an IC_{50} of 15 μg/mL. Please refer to Table 6 for details.

4.8 Immunosuppressive effect

According to incomplete statistics, terpenoid and sterol active substances extracted mainly from the soft coral *Sinularia scabra*, *Sinularia polydactyla*, *Sinularia* sp., *Libertasomyces* sp., and gorgonian※ *Verrucella umbraculum*※ have immunosuppressive effects *in vitro*. Sun et al. (2017) reported for the first time the immunomodulatory activity of new polyketide and trans-fused decane ring system-like metabolites by inducing the proliferation of $CD3^+$ T cells. Further structure–activity analysis revealed a key role of the Δ7 and terminal OH groups in the regulation of $CD3^+$ T-cell proliferation. Yang et al. (2020) revealed that the sterol compound yalongsterol A, 5α,8α-epidioxy-24-methyl-cholesta-6,24 (28)-dien-3β-ol and (22E,24S)-5α,8α-epidioxy-24-methylcholesta-6,22 -dien-3β-ol,

TABLE 6 Classification statistics for antioxidant effects of active substances extracted from the coral.

Active ingredient	Source	Mechanism	Concentration	Reference
Sesquiterpenoids	*Sinularia sp.*	Oxidative free radical absorption	5.36 units (1 mM of Trolox equivalent) per 0.31 mg/mL (IC_{50})	Zhang et al. (2006a)
Steroids	—	Lipid peroxidation (Vit C/Fe^{2+} excited)	7.6, 30.6, and 122.2 μmol/L (IC_{50})	Xu et al. (1997)
pseudopterosin I	*Sinularia suberosa*	Free radical scavenging:·OH, O^{2-}, DPPH	0.1006, 0.1001, and 0.021 mg/mL (IC_{50})	Xiang (2016)
pseudopterosin II	*Sinularia suberosa*	Free radical scavenging:·OH, O^{2-}, DPPH	0.2509, 0.2519, and 0.053 mg/mL (IC_{50})	Xiang (2016)
Cladiellin A	*Cladiella sp.*	Oxidative free radical absorption	3.151, 4.781, and 5.171 μM (IC_{50})	Zhang et al. (2005b)
Sinularin	—	Free radical scavenging: DPPH, $ABTS^{\bullet+}$, and •OH	250–400 μM (Y)	Wang et al. (2021)
Dihydrosinularin	—	Free radical scavenging: DPPH, $ABTS^{\bullet+}$, and •OH	200–400 μM (Y)	Wang et al. (2021)
Lobocompactols A	*Lobophytum compactum*	Oxidative free radical absorption	1.4 and 1.3 μM Trolox equivalents, respectively, at a concentration of 5 μM (IC_{50})	Chau et al. (2011)
Lobocompactols B	*Lobophytum compactum*	Oxidative free radical absorption	1.4 and 1.3 μM Trolox equivalents, respectively, at a concentration of 5 μM (IC_{50})	Chau et al. (2011)
BCE	—	Free radical scavenging: DPPH, •OH, and Lipid peroxidation	—	Bai (2011)

TABLE 7 Classification statistics for enzymatic activity of active substances extracted from the coral.

Active ingredient	Source	Related active substance	Concentration[a]	Effect	Reference
Sesquiterpenoids	*Sinularia* cf. *molesta*	PTP1B	1.24 μmol/L (IC_{50})	Inhibitor	Chu et al. (2018)
Diterpene	*Sarcophyton trocheliophorum*	PTP1B	6.97 μmol/L (IC_{50})	Inhibitor	Liang et al. (2013)
Diterpene	*Sinularia crassa*	α-Glucosidase	10.65±0.16, 30.31±1.22 μmol/L (IC_{50})	Inhibitor	Wu et al. (2020b)
Diterpene	*Sinularia polydactyla*	PTP1B	51.8–72.4 μmol/L (IC_{50})	Inhibitor	Ye et al. (2018)
Diterpene	*Sarcophyton glaucum*	Cytochrome P450 1A	1 μg/mL (Y)	Inhibitor	Hegazy et al. (2012)
Diterpene	*Sarcophyton glaucum*	Glutathione *S*-transferases (GST), quinone reductase (QR), and epoxide hydrolase (mEH)	10 μg/mL (Y)	Inducer	Hegazy et al. (2012)
Diterpene	*Sarcophyton trocheliophorum*	Acetylcholinesterase	40 μmol/L (Y)	Inhibitor	He (2013)
Diterpenoid alkaloids	*Ellisella robusta*※ and *Ellisella curvata*※	Tyrosine kinase c-Met	10 μmol/L (Y)	Inhibitor	Zhang (2012b)
Steroids	*Sinularia dissecta*	COX-2 (cyclooxygenase-2)	7.04 ± 1.03 μmol/L (IC_{50})	Inhibitor	Jin (2005)
Prostaglandin	*Plexaura homomalla*	P38α-kinase, Src-kinase, and topoisomerase IIα	2.5 and 10 μmol/L (Y)	Inhibitor	Hurtado et al. (2020)

[a]Y refers to the medication.

exhibited moderate immunosuppressive activity against T and/or B lymphocytes with semi-inhibitory concentration values of 19.30–59.49 μM. Subsequently, Cui et al. (2020) showed that polycyclic furanobutenolide-derived norditerpenoids exhibited strong inhibitory effects on ConA-induced T lymphocyte and/or LPS-induced B lymphocyte proliferation. Diterpenoids of different membrane types isolated from the South China Sea soft coral *S. scabra* have the same biological activity (Yang et al., 2019). A recent report revealed that metabolites containing the 9,10-secosteroid structure extracted from the South China Sea gorgonian *V. umbraculum*※ showed immunomodulatory activity by inhibiting the differentiation of $CD4^+$ T lymphocytes (Li J. et al., 2021).

4.9 Enzymatic activity

As summarized in Table 7, reports on coral enzyme activity are rare, but from the collected literature, it can be seen that some terpene masses isolated from coral have enzyme inhibitory activity. In addition, some steroid, polyketide, and alkaloid active substances may also have enzyme activity. In-depth research has led to the understanding of the significant role of enzymes in the regulation of diseases, not only for the adjuvant treatment of important organs such as the brain, heart, liver, and kidneys but also in the selective treatment of tumors with remarkable results. The diterpenes sinupol and sinulacetate exhibit good inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), which in turn is a potential drug target for the treatment of type II diabetes and obesity (Ye et al., 2018). Cespine diterpenes isolated from the soft coral *Sinularia crassa* in the South China Sea are used as alpha-glucosidase inhibitors for antidiabetic treatment. This provides a different way of thinking for developing new drugs (Wu et al., 2020b).

4.10 Effects on the nervous system

The neuroprotective effects of coral are manifested in two ways. On the one hand, they exhibit anticonvulsant and antiepileptic effects. As early as 1984, preliminary pharmacological experimental studies on the soft coral *Lemnalia exilis* showed that its extract had a significant antispasmodic effect on the isolated ileum of guinea pigs (Fang and Zhang, 1984). Eltahawy et al. (2015) measured the anticonvulsant activity of ceramide isolated from the Red Sea soft coral *Sarcophyton auritum* using a pentylenetetrazol (PTZ)-induced seizure model, and the mechanism may be through the modulation of CNS inhibitory activity through GABA and serotonin receptors. Some sterols also exhibited neuroprotective activity against neuron-like SH-SY5Y cells (Tammam et al., 2020). On the contrary, it has a sedative–hypnotic effect (Liao et al., 1992). Finally, the coral derivative excavatolide B can enhance long-term induction by suppressing the delayed rectifier potassium current, which lowers the action potential onset threshold and ultimately enhances situational memory retrieval in mice, resulting in enhanced memory extraction.

The effects of formulated preparations of coral on the nervous system have also been documented. First, Ershiwuwei Shanhu pills can prolong the latency period of epileptic seizures, shorten the duration of epileptic seizures, reduce the level of epileptic seizures, decrease the number of clonic seizures, and suppress epileptic discharges. At a certain dose, its effect was significantly better than that of the positive control drug sodium valproate (Luo, 2012; Luo et al., 2013). Second, Li et al. (2014) explored the protective effects of Ershiwuwei Shanhu pills on senescent hippocampal cells. The drug inhibited D-lactose-induced neuronal degeneration and excessive activation of astrocytes, thereby

TABLE 8 Classification statistics for individual application of coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
Bone defects and non-union	Deer horn coral skeleton	35 cases: 32 males and 2 females	Randomized controlled trials	35 cases: 32 males and 2 females	—	Coral bone particles are disinfected under high pressure and placed at the desired bone graft site in the human body	—	—	—	Wang et al. (2002b)
Avascular necrosis of the femoral head, bone hyperplasia, and spinal and lumbar lesions	Black horned coral skeleton	23 cases: 14 males and 9 females	Randomized controlled trials	23 cases: 14 males and 9 females	—	Crush the black horn coral and add softener to form a powder and take it in the herbal soup	—	1 dose per day for 20–30 days	One case died of cerebrovascular accidental death, and the other 22 cases were examined 1~2 months after taking the medicine. The clinical symptoms basically disappeared or relieved; particularly, the osteoporosis was basically corrected or stabilized	Xiao et al. (2005)
Patients with residual roots of anterior teeth and premolars	Coral bone powder	34 cases: 19 males and 15 females	Randomized controlled trials	34 cases: 19 males and 15 females	—	Artificial coral bone powder particles are implanted and undergo secondary repair surgery through porcelain crowns 6 months later	—	1 year	34 patients had significant bone formation in their alveolar ridges before the second-stage surgery. After the second-stage repair, they recovered normally, and the texture and color of the gums were better than before the repair	Zhou (2014)
Extraction of molars	Coral bone powder	45 cases: 23 males and 22 females	Randomized controlled trials	20 cases: 10 males and 10 females	25 cases: 13 males and 12 females	Fill the extraction socket with coral bone powder, and perform restoration operations such as filling the amount flush with the top of the adjacent alveolar ridge	Conventional biting gauze roll for approximately 30 minutes	6 months	The gingiva on the buccal and lingual sides of the experimental group was smooth and continuous, forming a plateau shape, and the alveolar bone was plump; the height and width of the alveolar bone in the control group were significantly reduced, and the buccal lingual side of the occlusal surface was significantly sunken, resulting in a narrow and elongated alveolar bone. After 6 months of tooth extraction, the degree of alveolar ridge atrophy in the experimental group was lower than that in the control group	Liu and Wang, (2014s)
Nasal deformity	Black horned coral skeleton	20 cases: 12 males and 8 females	Randomized controlled trials	20 cases: 12 males and 8 females	—	External nose shaping technology and implanting appropriately carved coral blocks into the nasal cone	—	—	18 patients recovered smoothly, 1 had an unexpected fracture, and 1 had an infection	Dagli et al. (1997)
Delayed sternal closure	Coral hydroxyapatite	1 male	Randomized controlled trials	1 case: 1 male	—	—	—	—	—	Zacharias et al. (2004)
Cranial injury or postoperative repair	Coral fragments	72 cases	Randomized controlled trials	72 cases	—	—	—	17 months	50% of cases have coral skeletons almost completely absorbed; another 50% of cases have partial absorption. The absorption of coral structures in larger implants does not	Roux et al. (1988)

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TABLE 8 (*Continued*) Classification statistics for individual application of coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
									exceed 40% of their volume, and no infectious complications have been found	
Craniofacial bone contour defect	Coral fragments	36 cases: 13.39% males and 22.61% females	Randomized controlled trials	—	—	—	—	12–36 months	Except for five clinically significant material absorption sites (incomplete absorption), the enhancement effect of other patients is very stable	Marchac and Sandor (1994)
Cervical adjacent segment degenerative disease	—	52 cases: 37 males and 15 females	Randomized controlled trials	52 cases: 37 males and 15 females	—	Eleven patients underwent anterior cervical discectomy and fusion (ACDF), 24 patients underwent anterior cervical discectomy and fusion (ACDF), and four patients underwent cervical disc replacement (CDA). The median time interval between the first and second surgeries was 74 months	Thirteen patients underwent their first SLAC surgery. The median time interval between the first and second surgeries was 33 months (21–59 months)	—	—	He et al. (2022)
Cerebrovascular sclerosis, coronary arteriosclerosis, and heart disease	Cockscomb coral extract	—	Randomized controlled trials	—	—	One part per million of this substance is refined and mixed with 1,000 ml of compounded saline for injection or infusion to patients with significant therapeutic effects	—	—	—	Yuan (1991)

reducing neuronal and astrocyte damage. Finally, Ershiwuwei Shanhu capsules can increase adenosine levels in secondary spinal cord injury, thereby increasing the ability of nerve cells to repair themselves (Jiao et al., 2013).

4.11 Effects on the cardiovascular system

Fang and Zhang (1984) found that soft coral extract has high physiological activity on the cardiovascular system. The extract of soft coral can not only delay the time of arrhythmia in isolated hearts of rats and shorten the duration of arrhythmia but also increase rabbit's heart coronary flow and slow down the heart rate. Lai (2017) also pointed out that red coral could regulate TXB_2/6-keto-$PGF_{1\alpha}$ levels, reduce plasma PF_4/β-TG levels, and lower plasma ET-1 levels in a blood stasis rat model, ultimately reducing vascular injury in rats. 15-Hydroxy-tetracosa-6,9,12,16,18-pentaenoic acid and sesquiterpenes isolated from the soft coral *S numerosa* and *Lemnalia* sp. exhibit anti-tubulinogenic and pro-angiogenic activities, respectively, in a dose-dependent manner (Yao et al., 2007; Yamashita et al., 2009; Wang et al., 2020).

4.12 Other effects

Other effects of corals include antihypertensive, hypolipidemic, and antiulcer activities. The diterpene glucoside isolated from the soft coral *Cespitularia turgida* in the South China Sea has a significant acute antihypertensive effect, and it has an obvious quantity–effect relationship; its antihypertensive effect has no rapid tolerance phenomenon, and at the same time, it has little effect on the heart rate when used as antihypertensives. The formulated preparation of coral, Shanhu Qishiwei pill, may reduce blood lipid levels in HLP model rats by inhibiting the LKB1/AMPK signaling pathway (Chun et al., 2022). Elshamy et al. (2017) demonstrated the antiulcer activity in a rat ulcer model induced by ethanol and acetic acid.

5 The toxicity of coral

Many corals, such as animal corals, also known as soft corals, are very popular in aquariums (home or public) because of their appreciation value and low maintenance costs. The soft corals of genera *Palythoa*, *Protopalythoa*, *Zoanthus*, and *Parazoanthus* in the Zoanthidae family contain a highly toxic and potentially lethal compound, palytoxin (Hoffmann et al., 2008). Therefore, the toxic compound of coral is mainly palytoxin. Ciminiello et al. (2011) extracted palytoxin and 42-hydroxy palytoxin at levels up to 25–450 ng per kg of Zoanthid. Palytoxin is a potent vasoconstrictor, and its neurotoxicity and cardiotoxicity are primarily due to dysregulation of the transmembrane pump Na/K-ATP enzyme, which can lead to serious human disease, causing gastrointestinal symptoms, myalgia, muscle spasms, respiratory and cardiac problems, and even death (Wieringa et al., 2014). The toxin is heat-resistant, and conventional boiling inactivation operations are not effective against it. Reports of human exposure to palytoxin consumption have described significant morbidity and mortality (Sud et al., 2013).

Palytoxin exposure and the production of toxic compounds through corals are primarily associated with toxin poisoning from inhalation of toxin-dissolved water aerosols during cleaning, scrubbing, or eradication of corals in home/public aquariums. Thus, aquarium store staff and home aquarium hobbyists face a consequent elevated risk of exposure. The data we collected showed that people aged less than 80 years and children exposed to palytoxin nebulized from coral had immediate symptoms such as cough, dyspnea, chest pain, myalgia, tachycardia, and gastrointestinal symptoms, and in severe cases, acute reactions such as burning or stinging and erythema also occur. Coral injuries may also have complications such as foreign body reactions, bacterial infections, or local eczema reactions (Na et al., 2008). Examples of poisoning due to prolonged and unprotected exposure to corals have also been reported (Smith et al., 2003; Hoffmann et al., 2008). A patient who placed his right hand on a Zoanthid colony while cleaning a seawater aquarium at home developed myalgia, symptoms of general weakness in limbs, and, subsequently, signs of poisoning such as speech impairment, dull eyes, and fainting. The degree of poisoning is closely related to the contact time, contact distance, and contact method. Subsequently, corneal toxicity due to exposure to Zoanthid corals has been documented. Seven patients presented with corneal manifestations ranging from superficial punctate epithelial lesions to bilateral corneal melting and subsequent perforation, with some patients presenting with progressive corneal melting even requiring therapeutic penetrating corneal transplantation. Fortunately, more than half of these case reports show that short-term minor injuries are reversible with medication or emergency measures, with only a few disabilities or a significant reduction in quality of life due to sequelae (Chang et al., 2020).

In 2014, water extracts from water corals were first reported to contain a lethal nonpeptide neurotoxin (García-Arredondo et al., 2015). The investigators administered 5.3 μg protein/g body weight of the extract to mice intravenously, which caused violent convulsions and death in the range of 1 min and histopathological damage to the kidneys and lungs at doses below the LD_{50} (LD_{50} = 4.62 μg protein/g body weight). After incubation under heat denaturing conditions, this histopathological damage was completely eliminated. However, the denatured extracts maintained their lethal effect. Second, in the process of researching the anti-neurotoxic active ingredients of the side flat soft willow coral, it was found that water-insoluble parts of alkali extracts of *S. suberosa* can make the animal produce a whole body soft, heavy limb tremor, turn positive reflex disappear, and cause other reactions (Liao et al., 1992).

Coral is often used as medicine in combination. Ershiwuwei Shanhu pills and others are classic Tibetan remedies consisting coral preparations. In the acute toxicity test of Ershiwuwei Shanhu pills, there were no obvious acute toxic reactions, but in the subacute toxicity test, toxic damage to liver, kidney, and lung pathological sections was observed (Liu F. L. et al., 2016). Long-term doses of Ershiwuwei Shanhu pills lead to accumulation of copper, mercury, and lead in the internal organs of the rats, with few rats developing symptoms of the vegetative nervous system, such as increased salivary gland secretion (LI, 2011). It can cause toxic reactions, manifested in immune function, and liver, kidney, and lung tissues are affected and damaged to varying degrees. The main toxic target organs are the liver, kidney, and lung, and damage due to toxicity occurs in a dose-dependent manner (Liu F. L. et al., 2016). However, given the complexity of its compounds, specific toxic substances remain to be investigated.

6 Clinical application

6.1 Individual application of coral

Coral's good stability, ease of use, and low cost contribute to its use as main material in the treatment of orthopedic diseases. In addition, coral contains 11 kinds of trace elements, namely, Zn0.05, Cu0.6, Pb0.0025, Ni0.004, Ti0.005, Mn0.004, Fe0.7, Al0.35, Mg3, Si > 1.0, and Sr0.1, and most of these trace elements are indispensable to the human body (Wang et al., 2002b). Xiao et al. (2005) systematically reported on black horn coral for the treatment of bone injury diseases. After taking the medicine for 5–7 days in mild cases and 1–2 months in severe cases, patients' clinical symptoms were basically relieved, and X-ray films showed that the bone changes were basically corrected or in a stable state. In the clinical method of immediate implant placement, artificial coral bone powder particles were placed in the bone defect area near the crest of the alveolar fossa, where significant osteogenesis was observed after 6 months. The gingival texture and color were better than before the restoration (Zhou, 2014).

Coral clinical applications are detailed in Table 8. It is often processed into powder for punching or used directly to treat bone injury diseases. It is also very effective in the treatment of cerebral vascular sclerosis and coronary artery sclerosis (Yuan, 1991). In 1990, the School of Medicine of Kyoto University in Japan extracted a substance from the coral of the cockle and used one 100th of a gram of it to mix into 1,000 mL of compound saline for injection or infusion. In difficult cases, it is also often used in combination with restorative dental tablets. However, the mechanism of action of coral is still unknown to us. In the available literature, it has been reported that it may be related to the absorption of coral by osteoclast-associated proteins (Lin Y. Y. et al., 2013) and bone marrow granulation tissue and blood vessels (Guillemin et al., 1989). However, it is also only a vague term, and a clearer and more explicit mechanism has to be studied.

6.2 Clinical application of preparations that contain coral

In clinical practice, the compound prescription of coral is mainly composed of Ershiwuwei Shanhu pills, Ershiwuwei Shanhu capsules, and Shanhu Qishiwei pills. Ershiwuwei Shanhu pills are a traditional, famous prescription and proven recipe for Tibetan medicine to treat albichoriasis and epilepsy. It uses coral as the monarch drug, together with pearl, *Terminalia chebula* and so on. It restores nerve function and relieves pain. It is mainly used to treat albichoriasis, unconsciousness, body numbness, dizziness, brain pain, irregular blood pressure, headache, epilepsy, and various types of neuropathic pain. Based on the collected literature, Ershiwuwei Shanhu pills has satisfactory clinical efficacy in the treatment of neurological diseases (epilepsy, primary headache, etc.), cardiovascular diseases (cerebral infarction, hypertension,

etc.), and orthopedic system (neurogenic cervical spondylosis, lumbar myofasciitis, etc.). In acute and severe cases, the combination of drugs is often used clinically to promote a synergistic effect and relief (Table 9).

6.2.1 Clinical application of preparations that contain coral for nervous system disease

Neurological disorders consist of two main areas. First, it is manifested in the treatment of epilepsy disorders. Epilepsy is a chronic disease of sudden, transient, recurrent central nervous system malfunction caused by abnormal over discharge of neurons in the brain (Xu et al., 2009). Ershiwuwei Shanhu pills can cause a significant reduction in the number of seizures, shorten the duration of seizures, improve the type of seizures, reduce the symptoms of headache after seizures, and reduce the degree of cognitive impairment, with significant anti-seizure and anticonvulsant effects. Clinically, 112 patients were randomly divided into a treatment group and a control group, and the treatment group was given 25 coral pills, whereas the control group was treated with Western standardized AEDs. The results showed that the total effective rate of the treatment group was 91.07%, whereas that of the control group was only 67.86% (Wang et al., 2014a). In the treatment of patients with epileptic tonic–clonic seizures, the total effective rate of the treatment group (taking Ershiwuwei Shanhu pills alone) was 88.23% (Wang et al., 2013b). The effects of combination drug treatment regimens have also been reported. Patients were treated orally with Ershiwuwei Shanhu pills in combination with oral levetiracetam tablets or carbamazepine, and the results showed that the therapeutic effect was higher than that of conventional Western medical treatment, reducing the levels of serum IL-2, TNF-α, sICAM-1, IL-6, and CRP. The combination of drugs has better clinical efficacy in the treatment of epilepsy, while improving the immune function of patients and reducing the inflammatory response (Huang and Zhao, 2017; Yuan et al., 2018).

Migraine, tension headache, and intractable headache are common clinical primary headache disorders. Sixty-three patients with migraine were randomly divided and treated with either Ershiwuwei Shanhu capsules or Nao Zhen Ning. After 30 days, 30 out of 33 patients taking Ershiwuwei Shanhu capsules were effectively treated, with a total effective rate of 90.9%, and 22 out of 30 patients taking Nao Zhen Ning were effectively treated, with a total effective rate of only 73.3% (Renwang and Renqing, 2010). A total of 110 patients were selected for the study, and the efficiency of the treatment group (taking Ershiwuwei Shanhu pills alone) was 94.55%, which was significantly higher than the total efficiency of the control group (taking flunarizine hydrochloride capsules combined with amitriptyline hydrochloride tablets), which was 74.55%. Meanwhile, clinical efficacy observation shows that Ershiwuwei Shanhu pills can improve the clinical outcomes of headache by reducing the abnormal blood flow condition (Wang et al., 2013c). In addition to medication, acupuncture can also be combined with treatment. A total of 110 patients were randomly divided into two groups: the control group was treated with acupuncture, and the observation group was treated with Ershiwuwei Shanhu pills. The results showed that the total effective rate was 80% in the acupuncture group but 94.5% in the observation group. Further study found that β-EP, NO, and 5-HT levels in the observation group were higher than those in the acupuncture group, and ET levels in the observation group were lower than those in the acupuncture group, suggesting that Ershiwuwei Shanhu pills can improve neuro-endocrine factors and regulate cerebral blood flow rate in patients with migraine, thereby contributing to the improvement of migraine symptoms (Gu, 2014). As early as 2000, a study found that Ershiwuwei Shanhu pills combined with acupuncture could treat intractable headaches (Bai and You, 2000). Modern research has shown that Ershiwuwei Shanhu pills not only dilate blood vessels and improve the effect of microcirculation in the brain but also alleviate the symptoms of vascular smooth muscle spasm to restore local central cerebral area blood perfusion, thereby relieving headache symptoms (Li, 2007; Yang, 2010).

6.2.2 Clinical application of preparations that contain coral for cardiovascular and cerebrovascular diseases

In cardiovascular system diseases, it is effective in treating poststroke headache and cerebral infarction-related conditions. Sixty-four patients with poststroke headache were studied, and after 4 weeks of treatment, the efficiency of the treatment group who underwent conventional medical treatment in combination with Ershiwuwei Shanhu capsules was 93.75%, which was significantly higher than that of the control group who underwent only conventional medical treatment (56.25%). The patient's headache level is reduced; the number of attacks is significantly reduced, and the duration of headache is significantly shortened during the treatment period (Shi and Zheng, 2018). In a study by Dongmei Guan, the clinical efficacy of Ershiwuwei Shanhu capsules given to patients with poststroke headache was higher than that of the reference group. Pharmacological analysis showed that the mechanism was similar to that of primary headache, which acted by dilating blood vessels, regulating cerebral blood flow, and improving neurological function (Wang and Li, 2014; Yang et al., 2015).

Regarding cerebral infarction disease, 90 patients were randomly divided into the control group and the observation group and were given Huoxue Tongmai Pian and Ershiwuwei Shanhu pills, respectively. The results showed that the efficacy of Erxuoyi Coral Pill was better, and its clinical application was more valuable (Zeng, 2019). On the basis of the study that Ershiwuwei Shanhu pills can significantly reduce infarct foci in rats with focal cerebral ischemia, researchers randomly selected 60 patients and tested their blood lipid, uric acid, homocysteine, and other levels. The results showed that the treatment group had elevated levels of glutamate transaminase, glutamic oxaloacetic transaminase, and other enzymes, which clearly demonstrated the efficacy of Ershiwuwei Shanhu pills in treating cerebral infarction, but such pills have a certain effect on heart, liver, and kidney function, and the mechanism may be related to the regulation of blood lipids (Zhu et al., 2020). Although aspirin can improve the hypercoagulable state of blood, the drug alone is not effective. Patients with acute cerebral infarction were observed after using Ershiwuwei Shanhu pills combined with aspirin, and the control group used aspirin combined with atorvastatin. The results showed that MMSE scores increased; NIHSS scores, FIB, D-dimer, and platelet aggregation index decreased, and the changes were large in the observation group (Tao et al., 2022). Pharmacological studies have further shown that Ershiwuwei Shanhu pills can inhibit cerebral

TABLE 9 Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
Cervical spondylosis of vertebral artery type	Ershiwuwei Shanhu capsules combined with Western medicine	84 cases: 54 males and 30 females	Randomized controlled trials	42 cases: 28 males and 14 females	42 cases: 26 males and 16 females	On the basis of the control group, add 2 capsules of Ershiwuwei Shanhu that contains coral each time, with a specification of 0.5 g per capsule, once a day	Routine treatment of Western medicine: oral flunarizine hydrochloride capsules every 10 mg, once a day, before sleep, and chiropractic treatment	30 days	Observation group: 22 cases were cured, 10 cases were significantly improved, six cases were effective, and four cases were ineffective, with a total effective rate of 90.48%. Control group: 14 cases were cured, six cases were significantly improved, nine cases were effective, and 13 cases were ineffective, with a total effective rate of 69.05%. The pain score decreased after treatment, and the decrease in the observation group was better than that in the control group	Ren et al. (2015)
Cervical spondylosis of vertebral artery type	Ershiwuwei Shanhu capsules	90 cases	Randomized controlled trials	45 cases	45 cases	On the basis of the control group, orally take 2 capsules of Ershiwuwei Shanhu that contains coral per day for 20 days as a course of treatment	Chiropractic treatment [spinal neurobiomechanical reduction method (found by Luo Xiaoyang)], once every 3–4 days, 5 times as a course of treatment	—	After treatment, the two subgroups showed improvements in relieving neck and arm pain, neck tenderness, cervical mobility, and upper limb numbness compared to before treatment, with the combined treatment group showing more significant improvements	Li et al. (2013b)
Cervical spondylosis	Ershiwuwei Shanhu pills	65 cases: 23 males and 42 females	Randomized controlled trials	65 cases: 23 males and 42 females	—	Twenty-five flavor coral pills, taken orally, at the same time, according to the condition and combined with acupuncture and moxibustion treatment, a course of 10 days, generally 2–3 courses	—	20–30 days	444 cases recovered without any clinical symptoms, 15 cases were effective, and six cases were ineffective. The total effective rate is above 90%	Zhang and Zhang (2011)
Lumbar fasciitis	Ershiwuwei Shanhu pills	150 cases: 98 males and 52 females	Randomized controlled trials	150 cases: 98 males and 52 females	—	Take Ershiwuwei Shanhu pills, 4 pills per dose, 1 dose per day, with warm water-soaked powder and medication residue on an empty stomach.	—	21 days	Cured 30 people by 20%; improved 108 people by 72%; 11 people had no significant changes, accounting for 7.3%; one person has not recovered, accounting for 0.6%, indicating aggravation	Li (2006)
Epilepsy	Ershiwuwei Shanhu pills	136 cases: 62 males and 74 females	Randomized controlled trials	68 cases: 32 males and 36 females	68 cases: 30 males and 38 females	Oral administration of Ershiwuwei Shanhu pills, 1 g each time, once a day, with warm water	Taking anti-epileptic drugs, including 20 cases treated with single drug, 38 cases treated with dual drug, and 10 cases treated with combination of three drugs	2 months	The total effective rate of the treatment group who only took Ershiwuwei Shanhu pills was significantly higher than that of the control group	Wang et al. (2014a)
Epilepsy	Ershiwuwei Shanhu pills	112 cases: 65 males and 47 females	Randomized controlled trials	56 cases: 34 males and 22 females	56 cases: 31 males and 25 females	Oral Tibetan medicine Ershiwuwei Shanhu pills for treatment, 1 g/time, 1 time/day, taken with warm water	Oral administration of sodium valproate tablets, 0.2–0.4 g/time, 3 times/day, or additional administration of topiramate tablets (25–200 mg/time, 2 times/day) or phenytoin sodium tablets (50–100 mg/time, 2–3 times/day)	2 months	The total effective rate of the Ershiwuwei Shanhu pills group was 91.07%, while the total effective rate of the control group was 67.86%	Wang et al. (2014b)
Epileptic tonic–clonic seizures	Ershiwuwei Shanhu pills	102 cases: 62 males and 40 females	Randomized controlled trials	51 cases: 32 males and 19 females	51 cases: 30 males and 21 females	Oral administration of Ershiwuwei Shanhu pills, 1 g each time, once a day, with warm water	Sodium valproate tablets, 0.2–0.4 g/time, 3 times/day, or topiramate tablets (25–200 mg/time, 2 times/day) or phenytoin tablets (50–100 mg/time, 2–3 times/day)	2 months	The total effective rate of the treatment group was 88.23%, while the total effective rate of the control group was 68.62%. Compared with before treatment, the epilepsy symptom scores of both groups were significantly reduced after treatment. Compared with the control group, the symptom scores of the treatment group were significantly reduced	Wang, Z.S. et al. (2013)

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TABLE 9 (*Continued*) Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
Epilepsy	Ershiwuwei Shanhu pills combined with carbamazepine	82 cases	Randomized controlled trials	41 cases	41 cases	On the basis of the control group, oral administration of Ershiwuwei Shanhu pills, 1 pill/time, 1 dose/day	Take orally carbamazepine tablet, the initial dose is 0.2 g/time, twice a day. After one week of continuous treatment, adjust the dose, increase by 0.1 g per week, to 0.4 g per time, twice a day	2 months	After treatment, the total effective rates of the control group and the treatment group were 80.95% and 95.24%, respectively. The HAD scores of both groups were significantly reduced, while MoCA was significantly increased. The number of epileptic seizures in both groups of patients was significantly lower than before treatment, and the serum levels of IL-2 and TNF-α in both groups of patients were significantly lower; the reduction of the above indicators in the treatment group was better than that in the control group	Huang and Zhao (2017)
Epilepsy	Ershiwuwei Shanhu pills combined with levofloxacin tablets	60 cases	Randomized controlled trials	30 cases	30 cases	On the basis of treatment in the control group, oral administration of Ershiwuwei Shanhu pills, 1 g/time, 1 time/day	Oral administration of levetiracetam tablets after meals, starting at a dose of 500 mg/time, twice a day, and adding to 1,000 mg/time, twice a day after one week	3 months	The total effective rates of the control group and the treatment group were 73.33% and 93.33%, respectively, and the levels of inflammatory factors in the treatment group were significantly lower than those in the control group. After treatment, the frequency of seizures in both groups was significantly reduced, and the frequency of seizures in the treatment group was significantly lower than that in the control group	Yuan et al. (2018)
Epilepsy	Combined use of Ershiwuwei Shanhu pills	176 cases: males: females=2:1	Randomized controlled trials	—	—	The addition group of Wuwei Coral pills was composed of carbamazepine, valproic acid, and Xilishu	Valproic acid added with Shunqi Anshen Wan	1 year	Three compatibility schemes of Ershiwuwei Shanhu pills (three groups of Ershiwuwei Shanhu pills addition group) have a significant effect on reducing the frequency of seizures, alleviating the degree of epileptic discharge, and improving the degree of headache and cognitive impairment after seizures in symptomatic epilepsy. Among them, the combination of Ershiwuwei Shanhu pills and sodium valproate group and the combination of Xilishu group both have the effect of improving the type of epileptic seizures. The combination of Xilishu group can also significantly shorten the duration of epileptic seizures	Luo (2012)
Primary headache	Ershiwuwei Shanhu capsules combined with cowpox vaccine	67 cases: 26 males and 41 females	Randomized controlled trials	30 cases: 12 males and 18 females	37 cases: 14 males and 23 females	Ershiwuwei Shanhu capsules, 4 capsules per day, intravenously administered with a dose of 3 ml of rabbit skin extract induced by cowpox vaccine, added to 5% glucose injection (250 ml) once a day	Conventional Western medicine treatment, oral fluranolol cinnarizine 1 capsule per night, intravenous drip of Venoruton 250 ml, once a day	2 weeks	Observation group: 18 cases showed significant effect, 10 cases were effective, and two cases were ineffective, with a total effective rate of 93.3%; Control group: 16 cases showed significant effect, 14 cases were effective, and seven cases were ineffective, with a total effective rate of 81.1%; The headache relief rate in the study group was higher than that in the control group	Bian et al. (2016)
Migraine	Ershiwuwei Shanhu pills	50 cases: 13 males and 37 females	Randomized controlled trials	30 cases: 8 males and 22 females	20 cases: 5 males and 15 females	Ershiwuwei Shanhu pills, 4 pills per time, once a day	Sibeline 10 mg, once a day; 10 mg of oryzanol, three times a day; Qiye Shen'an tablets 100 mg, three times a day	4 weeks	The total effective rate of the treatment group was 93.33%. The total effective rate of the control group was 75%	Huang (2008)

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TABLE 9 (*Continued*) Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
Migraine	Ershiwuwei Shanhu pills	40 cases: 12 males and 28 females	Randomized controlled trials	40 cases: 12 males and 28 females	—	Ershiwuwei Shanhu pills, 4 at a time, once a day	—	1 month	Twelve cases were cured, accounting for 30.0%; 17 cases showed significant effect, accounting for 42.5%; eight cases were effective, accounting for 20.0%; three cases were ineffective, accounting for 7.5% of the total. Total effective rate was 92.5%	Yang (2010)
Migraine	Ershiwuwei Shanhu pills	480 cases: 211 males, 269 females	Randomized controlled trials	235 cases: 111 males, 124 females	245 cases: 100 males, 145 females	Ershiwuwei Shanhu pills 3 tablets/1, 2 times/d, swallowed in installments	Ershiwuwei Shanhu pills 4 tablets/1, 1 time/day, taken by soaking in hot water	4 weeks	The cure rate in the conventional dose group was 115/245 cases, while the cure rate in the high-dose group was 148/235 cases	Zhou (2009)
Migraine	Ershiwuwei Shanhu pills in combination with flunarizine	112 cases: 49 males and 63 females	Randomized controlled trials	56 cases: 26 males and 30 females	56 cases: 23 males and 33 females	Twenty-five flavor coral pills, 4 pills each time (0.25 g each), once a day, fluranine cinnarizine capsules 5 mg, taken daily before sleep	Flunarizine 5 mg, taken daily before bed	4 weeks	After treatment, the peak systolic period in the treatment group improved significantly compared to before treatment	Zhao (2011)
Migraine	Ershiwuwei Shanhu pills combined with sibeline	158 cases: 56 males and 102 females	Randomized controlled trials	84 cases: 27 males and 57 females	74 cases: 29 males and 45 females	Ershiwuwei Shanhu pills, taken orally in warm water every morning, 4 capsules per dose; take 1 sibeline capsule before bedtime every night	On the basis of conventional medication treatment, sibeline is administered orally, taking 1 capsule before bedtime every night	4 weeks	The observation group significantly alleviated the level of anxiety or depression in patients, with better results than the control group	Chen et al. (2014a)
Migraine	Ershiwuwei Shanhu pills combined with acupuncture and moxibustion	110 cases: 37 males and 73 females	Randomized controlled trials	55 cases: 20 males and 35 females	55 cases: 17 males and 38 females	Acupuncture and moxibustion treatment and taking Ershiwuwei Shanhu pills, 1 g/time, once a day.	Acupoint selection: Select the acupoints on the patient's diseased side, such as Baihui, Shenting, Benshen and Lugu, as well as other acupoints such as Waiguan, Fengchi, and Jiaosun Qiuxu. Acupuncture at different acupoints for different diseases. Patients with liver disease may experience symptoms by needling the Taichong and Xingjian acupoints. For patients with blood deficiency, the Xuehai and Sanyinjiao acupoints should be added. For patients with kidney deficiency, the Guanyuan and Taixi acupoints should be added. For patients with blood stasis, the Quchi and Hegu acupoints should be added. After obtaining qi, use the technique of calming and tonifying and reducing the symptoms, and leave the needle for 30 minutes, once per day	5 weeks	The total effective rate of clinical efficacy was 94% in the observation group and 80% in the acupuncture and moxibustion group, and the observation group is superior to the acupuncture and moxibustion group	Gu (2014)
Stubborn headache	Ershiwuwei Shanhu pills	128 cases: 78 males and 50 females	Randomized controlled trials	64 cases: 40 males and 24 females	64 cases: 38 males and 26 females	Ershiwuwei Shanhu pills, 1 g each time, once a day, taken with warm water	Oral Zhengtian pills, 1 bag (6 g) each time, 3 times a day, discontinue other medications and painkillers 1 week before and during treatment	8 weeks	The frequency, intensity and duration of pain in the treatment group were significantly lower than those in the control group; the total effective rate of the treatment group was 93.75%. The total effective rate of the control group was 81.25%	Wang et al. (2014c)
Stubborn headache	Ershiwuwei Shanhu pills	80 cases: 47 males and 33 females	Randomized controlled trials	40 cases: 26 males and 14 females	40 cases: 21 males and 19 females	Ershiwuwei Shanhu pills, taken in boiling water, 1 g once, twice a day	Take Zhengtian pills orally, once in the morning, once in the afternoon, once in the evening, and take one bag each time. Take amitriptyline hydrochloride tablets in combination, once in the morning and once in the evening, taking 2 tablets each time	1 month	After treatment, the pain intensity and duration of the control group patients were higher than those of the observation group, with a total effective rate of 72.5% in the control group and 92.5% in the observation group	Wang (2016)
Stubborn headache	Ershiwuwei Shanhu pills combined with acupuncture and moxibustion	8 cases: 2 males and 6 females	Randomized controlled trials	8 cases: 2 males and 6 females	—	0.6 g per pill, once a day, one pill per time. Take one pill at night and soak it overnight with a little saffron and bear bile, then take it	—	—	—	Bai and You (2000)

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TABLE 9 (*Continued*) Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
						at dawn the next day. Acupuncture should be done once a day for the initial treatment, which can be combined with moxibustion. Change to acupuncture and moxibustion every other day after pain relief				
Tension headache	Ershiwuwei Shanhu pills	120 cases: 43 males and 67 females	Randomized controlled trials	55 cases: 22 males and 33 females	55 cases: 21 males and 34 females	4 pills (1 g) each time, once a day, ground and taken with warm water	Flunarizine hydrochloride capsules, 5 mg each time, twice a day	4 weeks	The total efficacy of the treatment group was 54.55%, while that of the control group was 29.09%	Wang, Z.S. et al. (2013)
Tension headache	Ershiwuwei Shanhu pills	70 cases	Randomized controlled trials	35 cases	35 cases	Ershiwuwei Shanhu pills 1 g, oral once a day	Amitriptyline tablets, 25 mg, taken orally 3 times a day	28 days	The total effective rate of the Ershiwuwei Shanhu pill treatment group was 82.86%, while the total effective rate of the amitriptyline control group was 80.00%; the total effective rate of traditional Chinese medicine syndrome in the treatment group was 88.57%, while that in the control group was 82.86%; the effect of the treatment group is better than that of the control group	Dai (2010)
Tension headache	Delixin combined with Ershiwuwei Shanhu pills	160 cases: 58 males and 102 females	Randomized controlled trials	80 cases: 31 males and 49 females	80 cases: 27 males and 53 females	Takes 1 tablet of dailixin orally in the morning and 1 tablet orally in the middle of the day, and 4 capsules of Jinzhu Yalong Ershiwuwei Shanhu pills are taken orally once in the morning	Take 1 tablet of Xi bi ling every night before going to bed, and add symptomatic medications (such as general painkillers, nourishing blood and clearing brain granules, and Tongtian oral liquid)	2 weeks	Among the 80 cases in the treatment group, 80 cases were effective with a total effective rate of 100%, while in the control group, 22 cases were effective with a total effective rate of 85%	Li (2007)
Chronic tension-type headache	Ershiwuwei Shanhu pills combined with low-dose trazodone hydrochloride tablets	120 cases: 26 males and 94 females	Randomized controlled trials	60 cases: 11 males and 49 females	60 cases: 15 males and 45 females	Ershiwuwei Shanhu pills 1 g, once a day (taken in hot water), trazodone hydrochloride tablets 25 mg, once a night	Amitriptyline hydrochloride tablets 25 mg, once per night, gradually increased according to patient tolerance (≤75 mg per day)	3 months	The total effective rate of the treatment group was 81.67%, which was better than the control group's total effective rate of 73.33%; VAS: The observation group showed a better decrease in scores than the control group; HAMD and HAMA: After treatment, the scores of both groups decreased significantly, and the observation group was better than the control group	Zhou et al. (2019)
Frequent episodes of tension-type headache	Ershiwuwei Shanhu pills combined with low-dose amitriptyline	240 cases: 92 males and 148 females	Randomized controlled trials	120 cases: 47 males and 73 females	120 cases, 45 males, 75 females	Take 4 capsules (1.0 g) of Ershiwuwei Shanhu pills orally and soak them in water once a day; amitriptyline tablets 12.5 mg. Twice daily	Amitriptyline tablets 25 mg, twice daily	12 weeks	The total effective rate of the treatment group was 93.33%. The total effective rate of the control group was 73.33%. The therapeutic effect of the treatment group was better than that of the control group	Li (2012b)
Angioneurotic headache	Combination of Ershiwuwei Shanhu pills and nursing intervention	60 cases: 37 males and 23 females	Randomized controlled trials	30 cases: 19 males and 11 females	30 cases: 18 males and 12 females	Take 2 Tibetan medicine Ershiwuwei Shanhu pills once a day, orally before meals; nursing interventions	Zhennaoling treatment: 4 capsules of Zhennaoling each time, three times a day, in the morning, mid-day, and evening, taken orally	30 days	Among the study group of patients, there were 12 controlled cases, seven significantly effective cases, eight effective cases, and three ineffective cases, with a total effective rate of 90.00%; in the control group, eight patients were under control, six were significantly effective, five were effective, and 11 were ineffective, with a total effective rate of 63.33%	He (2017)

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TABLE 9 (*Continued*) Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
Angioneurotic headache	Ershiwuwei Shanhu pills combined with nursing intervention	80 cases: 43 males and 37 females	Randomized controlled trials	40 cases: 22 males and 18 females	40 cases: 21 males and 19 females	Ershiwuwei Shanhu pills, combined with nursing interventions for treatment, dosage is 2 capsules, once a day, administered orally before meals	The dosage of aspirin enteric-coated tablets is 30 mg, 3 times a day, administered orally; the dosage of nimodipine is 30 mg, 3 times a day, administered orally	30 days	Observation group: Among the 40 cases, 28 were significantly effective, 11 were effective, and one was ineffective, with a total effective rate of 97.5%. Control group: Among the 40 cases, 21 were significantly effective, 10 were effective, and 9 were ineffective, with a total effective rate of 77.5%	Li (2021)
Angioneurotic headache	Ershiwuwei Shanhu pills	63 cases	Randomized controlled trials	33 cases	30 cases	Ershiwuwei Shanhu capsules, 2 capsules (0.5 g/capsule), once a day	Zhennaoling, 4 capsules (0.3 g/capsule), 3 times daily	30 days	Thirty cases in the treatment group were effective, with a total effective rate of 90.9%, and 22 cases in the control group were effective, with a total effective rate of 73.3%	Renwang and Renqing (2010)
Poststroke headache	Ershiwuwei Shanhu capsules	70 cases: 47 males and 23 females	Randomized controlled trials	35 cases: 25 males and 10 females	35 cases: 22 males and 13 females	Tibetan medicine Ershiwuwei Shanhu capsules, 2 capsules/time	Routine medical symptomatic treatment should be carried out with antiplatelet aggregation, analgesia, nutritional nerve, and softening vascular drugs	8 weeks	The frequency of headaches in both groups was lower than before treatment, and the duration of pain was shorter than before treatment. The frequency of headaches in the Tibetan medicine group was lower than that in the reference group, and the duration of pain was shorter than that in the reference group	Guan (2020)
Poststroke headache	Ershiwuwei Shanhu capsules	64 cases: 33 males and 31 females	Randomized controlled trials	32 cases: 18 males and 14 females	32 cases: 15 males and 17 females	Twenty-five coral capsules, 0.5 g pellets at 1 time/day and 2 pellets/time, were given orally on the basis of routine internal medicine treatment	Routine medical treatment is given, which includes nourishment of nerves, invigorating blood circulation and eliminating stasis, anti-platelet aggregation, anti-arteriosclerosis effect, and pain relief	4 weeks	The effective rate of the treatment group was 93.75%, while the control group was 56.25%	Shi and Zheng (2018)
Headache	Ershiwuwei Shanhu capsules combined with Danzhen headache capsules	76 cases: 35 males and 41 females	Randomized controlled trials	38 cases: 17 males and 21 females	38 cases: 18 males and 20 females	Combination therapy of Tibetan medicine, oral administration of 25 coral capsules and Danzhen headache capsules; the former 4 capsules once daily and the latter 2 capsules three times daily	To be treated with conventional Western medicine, take flunarizine or thagrelate orally, the former takes one capsule every night, the latter three times a day, the dose is 100 mg	1 month	In the treatment group of 38 patients, 10 were cured, 19 were significantly improved, seven were effective, and two were ineffective. The total effective rate of treatment was 94.7%; in the control group of 38 patients, six were cured, 15 were significantly improved, 10 were effective, and seven were ineffective. The total effective rate of treatment was 81.6%	Liu et al. (2016a)
Vertigo	Ershiwuwei Shanhu pills	160 cases: 88 males and 72 females	Randomized controlled trials	100 cases: 56 males and 44 females	60 cases: 32 males and 28 females	Boiled blister suit, 1 g once, 1 day	*Gastrodia elata* Blume capsules, 4 capsules at 1 time, 3 times at 1 day	1 month	The total effective rate of the treatment group was 85.00%. After ridit analysis of the comparison results between the two groups, the therapeutic effect of the treatment group was significantly better than that of the control group	Liu et al. (2004)
Acute cerebral infarction	Ershiwuwei Shanhu pills	60 cases: 32 males and 28 males	Randomized controlled trials	30 cases: 19 males and 11 females	30 cases: 13 males and 17 females	On the basis of the control group, add 1 g of Ershiwuwei Shanhu pills once a day	Aspirin 100 mg, once daily; clopidogrel hydrogen sulfate tablets 75 mg, once daily; atorvastatin calcium tablets 40 mg, once daily; ligustrazine 120 mg, once daily; cytidine sodium 0.5 g, once daily; and edaravone 30 mg, twice daily	—	In the control group, there were nine cases with a decrease in NIHSS score, four cases with an increase in NIHSS score, and 17 cases with no change in NIHSS score; in the treatment group, 21 cases showed a decrease in NIHSS score, two cases showed an increase in NIHSS score, and seven cases remained unchanged in the NIHSS score; compared with the control group, the NIHSS score significantly decreased after treatment	Zhu et al. (2020)

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TABLE 9 (*Continued*) Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
Acute cerebral infarction	Ershiwuwei Shanhu pills combined with aspirin	80 cases: 43 males and 37 females	Randomized controlled trials	40 cases: 22 males and 18 females	40 cases: 21 males and 19 females	On the basis of the control group, Ershiwuwei Shanhu pills were given 1 g/time, 1 time/day	Take atorvastatin tablets orally before bedtime, 20 mg/dose, once a day; oral aspirin enteric-coated tablets 100 mg/time, once a day.	60 days	Both groups showed an increase in MMSE scores, a decrease in NIHSS scores, FIB, D-dimer, and platelet aggregation index, with significant changes observed in the observation group	Tan (2020)
Cerebral infarction	Ershiwuwei Shanhu pills	60 cases: 38 males and 22 females	Randomized controlled trials	30 cases	30 cases	Ershiwuwei Shanhu pills, 2 capsules each time, 2 times a day, taken orally, or once a day, 4 capsules each time, taken orally	Huoxue Tongmai tablets, 4 tablets each time, 3 times a day, taken orally.	20 days	Among the 30 cases in the treatment group, six were basically cured, 13 were significantly improved, 10 were improved, and one was ineffective, with a total effective rate of 96.7%; among the 30 cases in the control group, one case was basically cured, eight cases were significantly improved, 12 cases were improved, and nine cases were ineffective. The total effective rate was 70.0%	Wang (2003)
Cerebral infarction	Ershiwuwei Shanhu pills	90 cases: 52 males and 38 females	Randomized controlled trials	45 cases: 27 males and 18 females	45 cases: 25 males and 20 females	Cerebral infarction Tibetan medicine Ershiwuwei Shanhu pills, 2 capsules each time, 2 times a day, taken orally	Huoxue Tongmai tablets, 2 capsules each time, 2 times a day, taken orally.	20 days	Observation group: Among 45 cases, 30 were significantly effective, 13 were effective, and two were ineffective, with a total effective rate of 95.56%; Control group: Among 45 cases, 13 were significantly effective, 19 were effective, and 13 were ineffective, with a total effective rate of 71.11%; after treatment, the NIHSS scores of both groups decreased, and the NIHSS scores of the observation group were significantly lower than those of the control group	Zeng (2019)
Cerebral hemorrhage	Shanhu Qishiwei pills	4 cases	Randomized controlled trials	—	—	Seventy flavored pills of coral, 1 pill per day	—	20 days	The patient's symptoms have decreased. CT scan shows circular low-density lesions visible in the intracranial region. After being discharged from the hospital, the patient took 70 flavored pills of coral under guidance, and their symptoms have improved significantly thus far without any other adverse reactions	Bian (2012)
Refractory heart failure	Heart failure mixture combined with Shanhu Qishiwei pills	150 cases: 90 males and 60 females	Randomized controlled trials	100 cases	50 cases	Conventional anti-heart failure treatment should, in principle, discontinue the use of Western medicine to dilate the coronary artery and improve myocardial ischemia. In severe cases, basic Western medicine treatment such as cardiotonic, diuretic, and vasodilation should be given. At the same time, one pair of heart failure mixture was given daily, after boiling twice, take 500 ml of the medicinal solution and take it warm in two separate doses. Take Coral 70, once a day in the morning, one pill each time, and take it with warm water	Routine anti-heart failure treatment	1 month	Among the 100 cases in the treatment group, 56 were significantly effective, 32 were effective, and 12 were ineffective, with a total effective rate of 88%; among the 50 cases in the control group, 20 were significantly effective, 18 were effective, and 12 were ineffective, with a total effective rate of 76%	He et al. (2007)
Hypertension	Ershiwuwei Shanhu pills	30 cases: 16 males and 14 females	Randomized controlled trials	30 cases: 16 males and 14 females	—	Soak in water in the morning and take it every night while sleeping, once a day	—	1 month	26 cases were cured, accounting for 86.7%; three cases showed significant effect, accounting for 10.0%; one case was ineffective, accounting for 3.3%; total effective rate was 96.7%	Li (2010a)
Hypertension	Ershiwuwei Shanhu pills in combination with dipine drugs	90 cases	Randomized controlled trials	45 cases	45 cases	Combined Tibetan medicine Ershiwuwei Shanhu pills, taken orally with warm water	Treatment with dipines	3 days	The effective rate of the treatment group (95.56%, 43/45) is	LI and Liu (2021)

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TABLE 9 (*Continued*) Classification statistics for clinical application of preparations that contain coral.

Disease	Pharmaceutical preparation	Experimental subject	Research design	Groups and number of people		Therapeutic method		Course of treatment	Curative effect	Reference
				Treatment group	Control group	Treatment group	Control group			
						on an empty stomach, 1 capsule/time, 1 dose/day, 30 days as a course of treatment			higher than that of the control group (77.78%, 35/45), and the systolic and diastolic blood pressure after treatment in both groups are lower than before treatment, and the reduction in the treatment group is more significant	
Cough with lung heat	Ershiwuwei Shanhu pills	54 cases: 33 males and 21 females	Randomized controlled trials	—	—	Coral Ershiwuwei Shanhu pills 1/2, 3 times before meals, powder, 3 times after meals	—	15 days	15 days of recovery	Yang (2003)
Waist, hand, and foot injuries	Ershiwuwei Shanhu pills	17 cases: 11 males and 6 females	Randomized controlled trials	—	—	Take Coral Ershiwuwei Shanhu pills orally, 3 times a day, 1 pill each time, chew carefully and take with boiling water. Take the medicine half an hour before meals. For external use, use 6 pills of Ershiwuwei Shanhu pills at the end, soak in 3 liang of Baijiu, half an hour later, apply externally to the affected part, 3–4 times a day, with moderate force	—	7 days	All 17 cases recovered	Yang (2003)

thrombosis, reduce the area of cerebral infarction, reduce brain tissue edema, dilate cerebral blood vessels, and improve cerebral blood circulation and brain tissue metabolism, which coexist with the antithrombotic effect of aspirin to improve the therapeutic effect and have higher clinical application value (Tan, 2020). The total effective rate of Shanhu Qishiwei pills for the treatment of persistent heart failure also reached 88%, whereas no significant toxic side effects were found (He et al., 2007).

Ershiwuwei Shanhu pills cured 26 out of 30 cases of hypertension, with a total efficiency of 96.7%. The pharmacological study proved that the whole formula lowered blood viscosity, reduced water retention in the body, and changed blood rheology. It has a long-lasting and stable effect on lowering blood pressure level, which is more effective for unstable hypertension (Li J. G., 2010). In addition, combination treatment regimens not only improve treatment efficiency but also ensure treatment safety. The total effective rate of Ershiwuwei Shanhu pills combined with diphenhydramine drugs in the treatment of hypertensive patients was as high as 95.56%, which was higher than that of patients taking only diphenhydramine drugs, whose effective rate was only 77.78% (LI and Liu, 2021).

6.2.3 Clinical application of preparations that contain coral for orthopedic system diseases

Similar to the application of coral single medicine, compound prescription is also effective in orthopedic system diseases, and it has better efficacy in the clinical treatment of neurogenic cervical spondylosis, lumbar myofasciitis, and traumatic synovitis of the knee joint (Li W. H. et al., 2013; Jiao et al., 2013). In 65 clinical cases of neurogenic cervical spondylosis, after taking Ershiwuwei Shanhu pills orally combined with acupuncture based on the condition for one course of treatment, the pain symptoms were significantly reduced, and after two courses, the symptoms disappeared completely, and no recurrence was seen thus far (Zhang and Zhang, 2011). Ershiwuwei Shanhu pills have also been used in combination with conventional Western medical treatment. The researchers randomly assigned 84 patients to the control group who received flunarizine hydrochloride capsules orally and the observation group received Ershiwuwei Shanhu capsules in combination with flunarizine hydrochloride capsules. The results showed that the observation group could increase patients' plasma neurohypophyseal hormone concentration, reduce pain, and improve blood flow velocity in the vertebral and basilar arteries, with a final total effective rate of 90.48%, which is significantly higher than the 69.05% of the control group (Ren et al., 2015). A patient with lumbar myofasciitis was treated with oral and external application of Ershiwuwei Shanhu pills for 20 days; all the symptoms were removed, and no recurrence was observed after 1 year of follow-up (Li, 2006).

6.2.4 Clinical applications of preparations that contain coral for other diseases

In addition, 25 flavored coral pills have shown clinical return in trauma, herpes zoster, and respiratory system. Seventeen patients with lumbar, hand, and foot sprains and smash injuries were cured within 7 days by using coral Ershiwuwei Shanhu pills alone, internally and externally on the affected area (Yang, 2003). In clinical practice, the efficacy of taking Ershiwuwei Shanhu pills as

the monarch drug, together with Chouluo Gengsheng powder, in clearing heat and detoxifying, clearing, and moistening the lung in 54 cases of patients with lung fever obtained satisfactory results (Yang, 2003). Acyclovir is also used clinically in combination with Ershiwuwei Shanhu pills to treat herpes zoster, a neuropathic pain caused by damage after the activation of the herpes zoster virus, which belongs to the Tibetan medical term "albichoriasis" (Zhang H. Y., 2012). Therefore, the treatment of neuralgia of herpes zoster with Ershiwuwei Shanhu pills has unique effects and efficacy. Shanhu Qishiwei pills is also a common classical compound prescription containing coral and is used to treat cerebral hemorrhage, limb paralysis, epilepsy, and various neuritis. In four patients with cerebral hemorrhage, headache and vomiting were relieved after taking Shanhu Qishiwei pills once a day for 20 days, and round-like hypodense foci were observed in the skull. In addition, no other adverse effects were observed (Bian, 2012). Although the compound prescriptions are diverse and the ingredients that exert their medicinal effects may be multiple, the synergistic effect of the coral in treating the symptoms of the disease and improving the efficacy of the treatment is evident.

In clinical practice, we use one side to treat multiple diseases, identify the syndrome accurately, and use the right medicine for the syndrome. The conventional Western medical treatment package includes symptomatic treatment, such as improving the patient's hemodynamics and pain relief, but the efficacy is not significant (Ren et al., 2015). The therapeutic rate of combined drugs is higher than that of single or compound drugs, and it can even produce additional therapeutic effects. Therefore, it has a higher promotion value and is an effective solution worth promoting in the clinic.

7 Discussion

Coral is an important marine biological resource, and species resources are extremely confusing and complex. In the Qing dynasty (1,616–1912 AD), red coral is a symbol of official status. In India and Tibet of China, people use coral as an auspicious object to worship Buddha, mostly to make Buddhist beads and decorate the statue of the deity in the temple (Hong, 2009). In ancient records, coral applications in medicine have also long been recorded, but only a few have pointed out that coral in medicine is a combination of coral species for the use of red coral. However, red coral has a broad range and many species, such as *Corallium japonicum* Kishinouye※, *Corallium secundum* Dana※, and *Corallium elatius* Ridley. *Corallium japonicum* Kishinouye※ (trade name: Aka) is mostly used in compounding. Arca is expensive, but no reports have been retrieved on whether other red corals can be substituted. In addition, the corals studied in modern pharmaceutical research involve a total of 34 families and 99 genera of corals, dominated by the families Alcyoniidae, Nephtheidae, and Plexauridae※. Coral species are confusing and complex, and sorting out their resource species not only helps us distinguish corals but also lays the foundation for developing new drugs and further research on corals.

Coral has a long history of medicinal value, which can remove corneal opacity, improve eyesight, tranquilize the mind, promote wound healing, and stop bleeding. Modern pharmacological studies have also gradually verified the medicinal value of coral and its mechanism of action. First, coral transplantation in the human body does not cause rejection; in coral, countless fine pores will gradually grow microscopic blood vessels and synthesize living cells of the bone. Numerous studies have reported that coral has become an alternative material to bone, and coral is often used in the fields of maxillofacial surgery and orthopedics (Guillemin et al., 1989; Zhu, 2001; Lai, 2017). Second, active ingredients such as terpenoids (diterpenes and sesquiterpenes) and steroids extracted from coral have evident pharmacological properties such as antiviral, antibacterial, antioxidant, and antimalarial activities. In addition, some of the active ingredients show not only good enzyme inhibition activity but also evident anticonvulsant, antiepileptic, and sedative–hypnotic effects in the nervous system; in the cardiovascular system, they show anti-tubular formation activity and proangiogenic activity as well as a certain amount–effect relationship. The antihypertensive, hypolipidemic (Chun et al., 2022), and antiulcer (Elshamy et al., 2017) activities have also been relevantly verified. Most of these chemical compounds were extracted from corals of Alcyoniidae and Gorgonidae※, and the compounds extracted from a particular coral may have multiple uses. Therefore, the study of active ingredients in corals has become the cornerstone of subsequent pharmacological studies, and exploring the mechanism of action of active substances can be a research direction to provide a basis for the elucidation of pharmacological effects and the design of clinical experiments (Wang, 2015).

Coral has various pharmacological activities, among which cytotoxic, anti-inflammatory, and analgesic pharmacological effects are more prominent. A549, HL-60, MCF-7, colon cancer cells, K562, HeLa, and other tumor cells are research hotspots. Scholars have mostly evaluated the inhibitory and apoptotic effects of different concentrations of active ingredients on different cells by MTT assay and SRB method. Studies have also shown that cytotoxicity can be influenced by compound structure. For example, prostaglandins with hydroxyl groups have good inhibitory properties (Hurtado et al., 2020); sterols introduced with hydroxyl groups decrease the inhibitory potency against HeLa cell lines; and acetyl groups increase the cytotoxic activity. Pro-inflammatory enzymes, particularly iNOS for nitric oxide production and prostaglandin-producing COX-2, play a central role in inflammatory mechanisms (Wei et al., 2013). In addition, glial cells and elastin are also important components of the anti-inflammatory mechanism. At present, pharmacological experiments of coral have identified its active ingredients. However, most of the results of pharmacological studies are derived from cellular or animal models, and they do not fully prove their effectiveness, so more clinical trials are needed to confirm these findings (Zhang X. L. et al., 2015).

Clinically, coral is often processed into powder for punching or used directly to treat bone diseases, in addition to showing good therapeutic effects in the treatment of epilepsy, primary headache, migraine, cerebral infarction, hypertension, neurogenic cervical spondylosis, and lumbar myofasciitis. In the face of complex diseases, obtaining the desired effect of a single drug is difficult, so coral is often used in combination with other drugs to treat the disease, which has satisfactory results in clinical applications. At a certain efficacy, compound prescriptions containing coral exhibit the same effects as when coral used alone. However, given the large number of herbs contained in the compound, the role played by

coral remains unclear; the effect may be weakened; the effect may be synergistically enhanced; or another effect may be stimulated. Moreover, the mechanism of action of coral remains unknown; thus, further research is needed.

Although coral toxicity is not included in the *Pharmacopoeia of the People's Republic of China*, studies have found that coral toxicity is mostly found in marine ornamental soft corals of the Zoanthidae family. Palytoxin is the main toxic compound. Nonpeptide neurotoxins were extracted from water coral all of which have toxic effects on the skin, cornea, etc. Short-term minor injuries are reversible with medication or emergency measures, with only a few disabilities or a significant decrease in quality of life because of sequelae. No significant acute toxicity was observed in coral-related compound preparations, but if applied for a long time, toxicity to the liver, kidneys, lungs, and other internal organs can still occur in a dose-dependent manner. The toxicity of coral is not yet generalized because of the complexity and diversity of its species. Coral insects are toxic, but whether coral is toxic after calcification is yet to be studied because of the special nature of coral.

The organic compounds in corals are remarkably studied, whereas other compounds, such as trace elements, are less studied. Coral as mineral medicine should strengthen the exploration and development of other compounds, such as trace elements, to pave the way for improving its quality standards and research on the basis of medicinal substances. Toxicological studies have also come to the forefront. The limited clinical trials are not perfect in quality, but they still have some reference value, and more scientific and representative clinical trials are needed in the future. At present, coral is used in several different fields, such as medical and apparel (Zhang Q. Y., 2013), with more areas still under development. Its value in medical care is particularly significant, which needs more attention and extensive research.

Coral reefs are one of the most diverse ecosystems on Earth, sensitive and fragile marine ecosystems, and one of the most sensitive environmental indicators of global climate change (Huang et al., 2023). Coral reefs not only provide a place for many fish and marine invertebrates to lay eggs, reproduce, and avoid predators, but also have extremely high biodiversity. It also plays a crucial role in homeland security. At present, habitat loss, diseases, bleach accidents, and species invasion are the main causes of coral death (Ma et al., 2018). *Antipatharia*, *Scleractinia*, *Helioporacea*, *Gorgonaceae*, *Tubiporidae*, *Corallium*, *Corallium elatius, Corallium japonicum*, *Corallium konjou*, and *Corallium secundum* are officially listed as endangered. Fishing for any coral species may have some impact on the ecosystem, as coral reefs are a complex ecosystem that is interdependent on many other organisms. So wild acquisition is strictly prohibited for species that have already been listed as extinct in the wild, regionally extinct, and critically endangered. Strengthening the protection and management of rare and endangered wild plants of vulnerable, near-endangered, and non-endangered species by building dynamic monitoring databases and strictly implementing on-site conservation measures is essential to maintain ecological balance and biodiversity (Wang, 2023). The most fundamental thing is that the coral species (extinct in the wild, regionally extinct, critically endangered, and rare and endangered wild plants of vulnerable, near-endangered, and non-endangered species) included in the list have no distinction between beneficial and insignificant and should receive equal protection. Strict implementation of the Endangered Species Law should help them restore to their normal numbers.

It is worth noting that some corals are currently listed as endangered on CITES and IUCN lists, such as *Antipatharia*※, *Tubiporidae*※, *Corallium elatius*※, *Corallium japonicum*※, *Corallium konjou*※, and *Corallium secundum*※, ensuring that a balance between the sustainability of scientific research and the protection of endangered species is a complex but crucial task. The development and research on the effectiveness of coral is likely to lead to the indiscriminate capture of coral, thereby exacerbating the endangered situation of coral. Ethically speaking, the application and development of coral should be prohibited. This article mainly discusses the endangerment caused by coral medicinal use. Therefore, we encourage scientists to use new technologies and methods, such as remote sensors, remote sensing technology, and genetic analysis, to reduce interference with endangered species while providing more valuable data (Wang et al.). The most effective measure is to avoid and prohibit the use of coral. We call on scholars to devise strategies for replacing coral in treatments to alleviate and solve the problems of endangerment of corals. First, search for alternatives based on similar biological species relationships. Species that are closely related often have similar physiological structures, as well as their chemical composition and pharmacological activities (Tian et al., 2023). *Naemorhedus goral* and *Saiga tatarica* have similar chemical components such as proteins, peptides, and amino acids (Liu et al., 2018), and as a substitute, *Naemorhedus goral* has a better sedative effect (Jiang and Zhai, 2006). Therefore, soft corals, sea fans, and sea yellows with similar chemical components have become one of the ways to replace endangered corals. Second, search for alternatives based on similar pharmacological effects. On the one hand, coral is usually used as an orthopedic material for the treatment of bone diseases. Therefore, we can achieve the same therapeutic effect by using other composite materials such as composite resin, ceramic, rubber, organic glass resin, and metal alloy instead of coral. This measure can not only achieve the effect of treating diseases but also reduce the use of coral. On the other hand, based on the bioactive substances extracted from coral, search for other organisms contain similar bioactive substances. For example, the pharmacological activity of cetosane diterpenoids can be extracted from *Boswellia carterii* (Xu et al., 2023). Medicinal compounds such as terpenoids and other substances can be extracted from *Croton tiglium* and *Panax notoginseng* (Wei et al., 2022). In modern research, replacing bile powder of *Rhinoceros unicornis* with that of *Bubalus bubalis* and using other animal bile powder instead of that of *Selenarctos thibetanus* as medicine have alleviated the endangerment problem of animals to a certain extent (Bai et al., 2018; Chen et al., 2022; Ye et al., 2022). Third, search for alternatives based on artificial domestication and breeding. Artificial cultivation of coral has become a feasible method (Wang, 2020). The artificial breeding technology of coral can be divided into sexual reproduction and asexual reproduction and can be classified into *in situ* cultivation technology and off-site cultivation technology according to the cultivation environment. The South China Sea Institute of Oceanography, Chinese Academy of Sciences, has carried out experiments on coral sexual reproduction and coral larva cultivation and proliferation in Sanya Bay and Yongxing Island

in Xisha, Hainan Province. At present, it has mastered the reproductive law and larva development process of species such as *Acropora gemmifera*※ and *Platygyra sinensis* (Yu et al., 2022)※. Fourth, look for alternatives based on synthetic methods. The active ingredients with pharmacological activities can be directly synthesized by chemical synthesis and enzyme engineering technology. The development of artificial *Moschus berezovskii* can be described as a sword sharpened for decades, which has completely solved the problem of long-term shortage of *Moschus berezovskii* supply (Fu et al., 2023). Artificial *Panthera tigris* is similar to natural *Panthera tigris* in fingerprint, pharmacological and pharmacodynamic indexes, and clinical efficacy (Liu and Han, 2006). They are all used in a variety of Chinese patent medicines (Yan et al., 2023). Finally, find the alternative mode of biotechnology based on industrialization. The use of coral stem cells to cultivate medicinal parts and secrete metabolites is a biotechnology method that can meet the requirements of syngeneic and homogeneous substitutes. Animal stem cell research mainly focuses on the direct use of stem cells for disease treatment, repair of organ damage, and establishment of the drug screening platform (Shi, 2020). *Taxus chinensis*'s stem cells have been used to produce paclitaxel and its corresponding active substance (Lee et al., 2010), and *Panax ginseng* stem cells have been used to produce ginseng (Jang et al., 2023).

Although some developments have not been broken yet through due to incomplete research on the material foundation and mechanism of action, as well as immature artificial farming techniques, which have prevented the formation of large-scale farming, modern biotechnology and multi-omics detection methods have brought new avenues for the development and evaluation of animal drugs, using genomics, proteomics, transcriptomics, metabolomics, and other detection methods at the molecular and cellular levels. The balanced and comprehensive approaches such as implementing multidimensional and multi-level systematic evaluation at the animal level, establishing new approaches for alternative research, and providing support for the protection, development, and utilization of endangered medicinal animals help ensure the survival of endangered species while providing valuable knowledge for the scientific community (Chun et al.).

It is undeniable that effective scientific research can alleviate the problem of endangerment of species, but the decision to "protect" and/or "establish a recovery plan" does not depend only on science. At the same time, there is ambiguity among managers regarding governance issues, and the institutional management plan seems to have failed to address the vulnerability of endangered species. Therefore, a statutory plan should be established to fundamentally alert people. Unfortunately, only relatively few countries have enacted national legislation on endangered and threatened species. Internationally, the Endangered Species Act of 1973 is a legislative model. Its implementation has achieved significant results, and it is said that 90% of the species on the bill's protected list have been restored (Teng and Zhang, 2022). So the government and relevant departments should strengthen regulations and policies to ensure that research on endangered coral species does not lead to abuse or overfishing, such as restrict or prohibit fishing and destructive activities and adopt sustainable fishing practices, including limiting fishing quantities, using selective fishing nets, and monitoring fishing activities, to protect marine ecosystems. Obviously, effective protection of endangered and threatened species in the ocean depends on appropriate legislation developed to protect them, and similarly, achieving the goals of these legislative tools depends on the political and social factors that affect their implementation. Since the 21st century, the debate between the protection of endangered species and economic benefits and ecological values has never stopped. Congress and the government always try to weaken the effectiveness of bills and tend to choose economic development. Opponents believe that some measures have harmed their own interests, and even more so, some are testing the "red line" for potential benefits. However, the public is more inclined to support the protection of endangered species, and the public support rate for the bill remains high. Obviously, the importance of protecting species is higher than economic growth and protecting private property, and maintaining biodiversity is a global cause (Jeffrey, 2016). Wildlife managers have a greater responsibility to ensure that their management actions reflect public values and attitudes. Third, the negative impact of social media comments on the public is very strong, which leads to significant differences in public beliefs in participating in endangered species management (Rodgers and Willcox, 2018). Some people, even with an urgent desire to protect endangered species, have low mobility. Therefore, it is important to raise awareness among the public, governments, and businesses about the importance of protecting coral reefs, encourage environmental action, and alleviate the pressure faced by these fragile ecosystems. In addition, with the development of industrialization and technology, the marine ecosystem is increasingly deteriorating, the ability to accommodate and rescue wildlife is weak, and habitats are gradually lost. Scientific research and monitoring are necessary. This includes ecosystem monitoring, which regularly monitors the health status of coral reef ecosystems and changes in environmental parameters such as temperature, salinity, and acidity, as well as research on coral diseases. Studying the pathogenesis of coral diseases will help develop prevention and control strategies (Yang, 2021). It is also necessary to reduce the flow of land pollution into the ocean, including agricultural and urban sewage discharge, as well as pollutants from rivers and streams (Gao, 2023; Zhu and Hu, 2023).

There is relatively little research on linking wildlife value orientations with attitudes toward T&E species and non-charismatic species, and even to a large extent, it has been overlooked (George et al., 2016). We encourage cooperation among scholars, conservation organizations, and governments to work together to achieve the common goals of scientific research and conservation (Lv et al., 2020; Zou and Jiang, 2023).

Coral is one of the marine species. The imminent extinction of coral reminds people to take immediate measures to protect endangered species and the ecological environment. The substitution principles are as follows: search for alternatives based on similar biological species relationships. Search for alternatives based on similar pharmacological effects. Search for alternatives based on artificial domestication and breeding. Look for alternatives based on synthetic methods. Search for the alternative mode of

biotechnology based on industrialization. These principles should be applied to protect all organisms from natural sources and not restricted to corals. Among them, the alternative strategy of artificial domestication is one of the most fundamental and effective measures. For example, treatments using pearls, centipedes, and others should be supplemented and replaced by other resources to prevent their endangerment or even extinction. In addition, reducing pollution, which means taking measures to reduce marine pollution, especially plastic waste, agricultural and industrial emissions, oil pollution, and sustainable economic management methods including fisheries, tourism, and marine technology, can promote economic growth and create decent employment opportunities, thereby reducing people's hunting of certain species, which is also one of the effective measures to protect ecosystems (Chinese and Foreign Experts and Scholars Talk Together on Marine Ecological Protection in the Process of Modernization, 2023). Protecting endangered species and protecting ecosystems is our mission and responsibility. These measures can balance the goal of protecting ecosystems and meeting human needs.

Recently, the signing of the High Seas Treaty in September 2023 and the Nagoya Protocol in October 2010 has pushed the protection of endangered species and ecological environmental issues onto the international stage, marking a significant shift in ecological governance from disorder to order. The Chinese government must strictly abide by the agreement, and in addition, it can educate the Chinese people, enterprises, and other relevant departments through media and policies to reduce and prohibit the use of coral. China's participation should make good use of the platform for treaty and agreement consultations and be more proactive in clarifying the content and value of this theory to the international community, injecting new vitality into the protection of ecology and subsequent consultations. The feasibility of global action is low, and its effectiveness is difficult to assess and regulate. However, the ability of countries or regional international organizations to respond more quickly to environmental changes greatly contributes to the improvement of the marine environment. International treaties can not only regulate the behavior of contracting parties but also promote the practice of non-contracting parties. Conducting this work under international cooperation undoubtedly has the most legitimacy and credibility. This not only strengthens cooperation among countries and encourages them to jointly address high seas protection issues but also enhances people's awareness of coral protection and ecosystem protection through universal participation. At the same time, these agreements also limit people's indiscriminate killing of corals and causing damage to the environment, pointing the way for protecting the ecological environment. To a large extent, it is urgent to alert and call on people to protect corals and endangered species, but how to achieve from regional practice to universal participation is still a difficult implementation dilemma.

Warning: The coral species marked with "※" have been included in the rare species list of CITES and IUCN. We should respect life and nature. We should protect wild animals and create a safe home for them. In addition, we call on researchers to increase their attention and importance to the protection of endangered corals and to conduct limited and valuable coral-related research within the framework of domestic and foreign laws and regulations. We also call on non-researchers to refrain from illegally collecting and using endangered corals after reading this review article and to carry out collection and utilization activities within the framework of domestic and foreign laws and regulations.

8 Conclusion

Marine invertebrates, a rich potential source of drug precursors, have been a popular avenue for the international search for drugs or drug precursors in recent decades. In the last two decades, coral chemistry and pharmacology research has made some achievements and discovered some new compounds with unique structures and strong physiological activities, but the utilization of corals is limited to only a small number and species of families, such as Alcyoniidae, Nephtheidae, Plexauridae, and Gorgoniidae※. This article provides the first comprehensive account of six aspects of the medicinal history, species, chemical composition, pharmacological activity, toxicology, and clinical application of coral in China. Coral is a natural mineral medicine, and its active ingredients are mixed and difficult to extract and identify. At present, the effective compounds extracted from coral are terpenoids, steroids, and nitrogen-containing compounds, with sesquiterpenes and diterpenes being the main compounds of terpenoids. However, during extraction, extraction conditions, and the joint use of related techniques, such as ICP-MS and LC-MS, have not been reported. Exploring the best extraction of the active ingredients of coral is a breakthrough for future experiments. The pharmacological effects of most of the compounds isolated from coral have been developed. The pharmacological activities of terpenoids are relatively rich, including cytotoxicity, anti-inflammatory, antibacterial, and antiviral. Second, steroid compounds also play important roles in antitumor, anticancer, and anti-inflammatory activities. Finally, other compounds such as lipids and aromatic compounds play important roles in their pharmacological activities such as antioxidant and immunosuppressive effects. However, they mostly reside in superficial areas, which shows a long way to go in the study of the mechanism. In addition, scientists are encouraged to use new technologies and methods, such as remote sensors and gene analysis, to reduce interference with endangered species while providing more valuable data. Toxicological studies have shown that corals of the family Zoanthidae cause toxic reactions in people through contact and inhalation, but they can be treated with pharmacological relief. With regard to clinical application, coral is mostly used in combination with other drugs to treat diseases, with limited cases of coral alone, which may lead to the inability to prove the effectiveness of coral but is still informative. Pharmacological studies of coral are mostly about the monomer extracted from coral, whereas clinical studies are more about the compound prescription application of coral. Studies show that coral is often used as a substitute for orthopedic materials to treat diseases such as bone defects and bone hyperplasia. Compound preparations that contain coral are widely used in the treatment of neurological diseases such as migraine, primary headache, epilepsy, cerebral infarction, hypertension, and other cardiovascular and cerebrovascular diseases.

More extensive and in-depth research on the active ingredients of coral and its mechanism of action should be focused on deepening the

understanding at genetic and molecular levels in the future to make it better applied in practice. In addition, whether the absorption, distribution, metabolism, and excretion as well as the blood concentration of coral change over time after administration in the body remains unclear. Coral is often used in the form of powder into the body, but whether coral powder can be taken orally as well as differences and similarities between its oral and external therapeutic effects remains unknown. Finally, diluted coral extracts have been derived as new drugs for the treatment of diseases. Therefore, the form of coral intake should not be limited to powder or as an orthopedic material. However, the development of its active ingredients is not a research strategy and prospect. It provides different ideas for the development of new drugs. We experienced pressure and challenge during the study of the clinical application of coral but offered us a good opportunity (Ai et al., 2006).

Author contributions

MH is responsible for collecting data and writing this article, ZoW is responsible for improving the chemical composition structure diagram and pharmacological activity content, and YL is responsible for the translation of the clinical application section. YS put forward relevant suggestions for the article, and ZaW directs the writing of the article and functions as our corresponding author. All authors contributed to the article and approved the submitted version.

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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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