MSPP 34TH SCIENTIFIC MEETING: PHARMACOLOGICAL PERSPECTIVES ON NATURAL PRODUCTS IN DRUG DISCOVERY

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MSPP 34TH SCIENTIFIC MEETING: PHARMACOLOGICAL PERSPECTIVES ON NATURAL PRODUCTS IN DRUG DISCOVERY

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Editorial: MSPP 34th scientific meeting: Pharmacological perspectives on natural products in drug discovery

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Editorial on the Research Topic
MSPP 34th scientific meeting: Pharmacological perspectives on natural products in drug discovery

The aim of this Research Topic was to highlight advances in the field of translational research utilises natural products as an intervention strategy for various conditions, especially in the area of cancer, cardiovascular, neurological, and metabolic diseases. This Research Topic seeks to provide opportunities to share and exchange evidence-based practices and scientific endeavours concerning therapeutic natural products among physiologists, pharmacologists, physicians, general practitioners, research scientists and other health care professionals.

This Research Topic compiles 9 articles, including 4 reviews and 5 original research articles from prominent scientists in the field. This compilation of papers comprehensively covers a number of natural products in drug discovery and provides insights into molecular mechanisms of a number of conditions. The content of each article is summarised below.

An original research article by Liu et al. demonstrated the inhibitory effects of gypenoside L (Gyp L) and gypenoside LI (Gyp LI) in the proliferation and induced apoptosis in clear cell renal cell carcinoma (ccRCC) cells *via in vitro* studies. The authors also elucidated the mechanism of action of gypenoside treatment using an *in vivo* model *via* the downregulation of cPLA2 levels, reduction of arachidonic acid content and inhibition of tumour growth. Although further research is necessary, this study provided preliminary data to support that Gyp L and Gyp LI could be promising drugs in ccRCC treatment.

A comprehensive review by Fuloria et al. on the therapeutic potential of *Curcuma longa* Linn. about curcumin, its primary active constituent. Curcumin is universally

known for its therapeutic effects in multiple disorders, but the same cannot be said for *C. longa*. The review provided extensive information about *C. longa* and discussed the knowledge gap in traditional and scientific evidence about *C. longa* and curcumin. Despite all the promising evidence about C. longa, the authors established that there is still inadequate supportive evidence, especially from clinical studies on the adjunct use of *C. longa* and curcumin. This calls for more clinical and pre-clinical studies on *C. longa* and curcumin.

Channa striatus (CS) is traditionally consumed by Malaysians, believed to promote wound healing and alleviate inflammation. Lee et al. presented a study to investigate the anticonvulsive potential of CS extract on neuroinflammation-induced seizures using zebrafish. The authors developed a zebrafish model of neuroinflammation using cerebroventricular microinjection of lipopolysaccharide. Zebrafish behaviour and swimming pattern were analysed together with gene expression to demonstrate the pharmacological property of CS. CS extract treatment exhibited some anticonvulsive and anti-inflammatory activity, which provides the basis for future research to discover new therapeutics for epileptic seizures potentially.

A review by Fuloria et al. discussed the 590 biologically active meroterpenoids from various sources such as fungus, plants and marine organisms. Meroterpenoids have been reported to have pharmacological properties such as anti-cholinesterase, COX-2 inhibitory, anti-leishmanial, anti-diabetic and many more.

In an original research article, George et al. demonstrated that caloric vestibular stimulation (CVS) is a safe and straightforward neuroprotective treatment against stress and qualifies as a non-invasive therapy for overcoming motor symptoms associated with Chronic Mild Stress. CVS was found to improve behavioural and immunohistochemical modifications, which are indicators of neurodegeneration. This study paves the way for more research to study the therapeutic potential of CVS as a neuroprotectant in stress-related disorders.

Rahman et al. presented an original research article which reported the anticancer effects of hydroxychavicol (HC) and epigallocatechin-3-gallate (EGCG) against cancer cells. Their individual therapeutic properties have been reported before. Therefore Rahman et al., hypothesized that combining these two compounds may enhance the therapeutic activity. It was proposed that HC + ECGC halted glioma cell proliferation and exerted apoptotic effects on glioma cells. The mechanisms of action were elucidated in the article as well. More research needs to be done to investigate this promising treatment against glioma cancer.

A narrative review by Hossain et al. summarises colorectal cancer (CRC) prevention and treatment using herbs and spices. Due to the protective effects of herbs and spices, they have been deemed a safer natural alternative which can be used as an adjuvant therapy against CRC. The six herbs and spices selected for the review were ginger, turmeric, garlic, fenugreek, sesame, and flaxseed. The authors provided evidence to show the potential roles of these herbs and spices in preventing and reducing CRC severity. The authors also identified several challenges in incorporating intestinal microbiota into the treatment of CRC and proposed several steps for the appropriate management of CRC.

The study by Xu et al. investigated the effects and underlying mechanisms of methyl 6-O-cinnamoyl-α-d-glucopyranoside (MCGP) on acute liver injury caused by acetaminophen (APAP) or carbon tetrachloride (CCl_4). MCGP is a modified compound from cinnamic acid, which has significant antioxidant, anti-inflammatory and anti-diabetic effects. The protective effects of MCGP in APAP-/CCl4-intoxicated acute liver injury were identified for the first time. The authors suggested the mechanism of action of MCGP, which paves the way for more research.

A review article by Lambuk et al. outlined the neuroprotective potential of brain-derived neurotrophic factor (BDNF) in Glaucoma. The authors highlighted the possibility of using BDNF as a biomarker in neurodegenerative diseases and future strategies. The review featured the various sources of BDNF, its transport mechanisms within neurons and its extensive functions. The highlighted therapeutic benefits help physicians to remain updated on recent discoveries.

The editorial team is grateful of all the authors and review editors for their contributions to the special Research Topic. We are confident that these diverse, interesting and important papers will guide researchers in their future research and will kindle advanced discussions in translational evidence based complementary and alternative medicine research.

Author contributions

MS took the initiative to draft the manuscript. SS and WW contributed to it. All the authors approved the final draft.

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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Gypenoside L and Gypenoside LI Inhibit Proliferation in Renal Cell Carcinoma *via* Regulation of the MAPK and Arachidonic Acid Metabolism Pathways

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Renal cell carcinoma (RCC) has the highest mortality rate of all urological malignancies. Clear cell renal cell carcinoma (ccRCC) accounts for approximately 80% of all RCC cases and is often accompanied by the accumulation of lipid droplets. Growing evidence indicates that ccRCC is a metabolism-related disease. Gypenosides are commonly used for the clinical treatment of hyperlipidemia, and their antitumor activity has also been recognized. However, the potential inhibitory effects and mechanisms of action of gypenoside L (Gyp L) and gypenoside LI (Gyp LI) in ccRCC remain unclear. In this study, we confirmed that Gyp L and Gyp LI significantly inhibited proliferation and induced apoptosis in ccRCC cells *in vitro*. We performed network pharmacology and RNA-seq, and verified the results by Western blotting, RT-qPCR, and immunofluorescence experiments. Our results demonstrated that Gyp L and Gyp LI upregulate the expression of *COX2* and downregulate the expression levels of cPLA2 and CYP1A1, resulting in reduced arachidonic acid and apoptosis. Gyp L and Gyp LI upregulated the protein levels of DUSP1, p-JUN, and p-JNK, and downregulated p-MEK1/2, p-ERK, and p-P38 levels. Moreover, gypenosides significantly inhibited tumor growth *in vivo*, and gypenosides significantly reduced cPLA2 and CYP1A1 expression. Furthermore, we performed absolute quantification of arachidonic acid (AA) content in ccRCC cells and tumor tissues by HPLC-MS, and found that the arachidonic acid content was significantly reduced after Gyp L, Gyp LI, and gypenoside intervention. In conclusion, our data suggest that Gyp L, Gyp LI, and gypenosides decrease the content of arachidonic acid in ccRCC cells and tumor tissues, but do not have cytotoxic effects on nude mice. Thus, Gyp L, Gyp LI, and total gypenosides extracted from *Gynostemma pentaphyllum* exhibited antitumor activities against ccRCC.

Keywords: gypenoside LI, ccRCC, arachidonic acid, MAPK pathway, gypenoside L

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INTRODUCTION

Renal cell carcinoma (RCC) is among the 10 most common cancers worldwide, accounting for 3.7% of all new cancer cases (Siegel et al., 2017). Clear cell renal cell carcinoma (ccRCC), an aggressive cancer originating from the proximal tubular epithelium, accounts for approximately 80% of all cancers (Choueiri and Motzer, 2017). Owing to lipid accumulation, ccRCC cells can be histologically classified by the appearance of clear cytoplasm (Rezende et al., 1999). In addition to the accumulation of intracellular lipid droplets, abnormal fatty acid (FA) metabolism is characteristic of ccRCC cells. Obesity is recognized as a strong risk factor for ccRCC (Renehan et al., 2008; Lowrance et al., 2010; Sawada et al., 2010; Keum et al., 2015).

Inflammation in the tumor microenvironment (TME) is a major factor driving tumor progression and is one of the hallmarks of cancer, while eicosanoids have been closely linked to inflammation and cancer (Greene et al., 2011). AA and eicosanoids play a central role in many diseases, including cancer and obesity (Wang and Dubois, 2010; Dennis and Norris, 2015; Sonnweber et al., 2018). Previous studies have shown that PLA2s is the initial enzyme of the AA metabolic pathway that converts membrane-bound arachidonyl phospholipids under several stimuli to form free fatty acids, predominantly AA and LPLs (Dessen et al., 1999; Gijón and Leslie, 1999; Yarla et al., 2016a). Key enzymes in the AA metabolic pathway include cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP). Furthermore, prostaglandins (PGs), leukotrienes (LTs), epoxy/hydroxy-eicosatrienoic acids, and other bioactive signaling oxylipids play key roles in the treatment of inflammation and cancer (Wang and Dubois, 2010; Yarla et al., 2016b). The relationship between AA and PI3K (Hughes-Fulford et al., 2006) and the MAPK signaling pathway has previously been reported (Alexander et al., 2006). However, the cancer-associated signaling pathways and the relationship between these bioactive lipids and cell proliferation remain largely unclear.

Gynostemma pentaphyllum (*G. pentaphyllum*) is commonly used as a source of medicine in China and Southeast Asia, in the treatment of various diseases, including hyperlipidemia and tumors. Gypenoside LVI is a monomer compound of *G. pentaphyllum*, which can be used to treat atherosclerotic cardiovascular disease (ASCVD) by increasing LDL-C uptake in hepatocytes by inhibiting PCSK9 expression (Wang et al., 2021). *G. pentaphyllum* exerts anti-hyperlipidemic effects by reducing triglycerides, cholesterol, and nitrite (Megalli et al., 2005). The antihyperlipidemic mechanisms of gypenosides may regulate lipid metabolism disorders and ameliorate hepatic function (Yang et al., 2013). Previous studies have reported that Gyp L and Gyp LI, dammarane-type saponins from *G. pentaphyllum*, induced apoptosis and inhibit proliferation in a variety of cancers, including esophageal cancer, hepatocellular carcinoma, breast cancer, melanoma, and lung cancer (Zheng et al., 2016; Xing et al., 2018; Ma et al., 2019; Zu et al., 2020; Zu et al., 2021). Importantly, our previous investigations have reported that the total saponins of *G. pentaphyllum* induced apoptosis in ccRCC by regulating the PI3K/AKT/mTOR pathway *in vitro*. However, the effects and mechanisms of the monomer compounds Gyp L and Gyp LI, individual dammarane-type saponins isolated from steamed *G. pentaphyllum* have not yet been explored in RCC. Based on previous findings, we hypothesized that Gyp L and Gyp LI could induce apoptosis in ccRCC, and thus, we investigated the effect of gypenoside L and gypenoside LI on apoptosis.

MATERIALS

Chemicals and Reagents

The extraction and identification of gypenoside L (Gyp L) and gypenoside LI (Gyp LI) were performed as previously described (Xing et al., 2018). The purity of Gyp L and Gyp LI was determined to be >99% using liquid chromatography-mass spectrometry (LC-MS). The antibodies used included Anti-Cytochrome C (ab13575); Bcl-2 (124) (15071S; Cell Signalling Technology, Inc.), Bax (2772S; Cell Signalling Technology, Inc.), cyclin A (sc-271682; SANTA), CDK2 (sc-6248; SANTA), CDK1 (ab245318; Abcam), cyclin B1 (sc-8396; SANTA), and JNK antibody (#9252; Cell Signalling Technology, Inc.); Phospho-JNK (Thr183/Tyr185) (81E11; Cell Signalling Technology, Inc.), c-Jun (60A8; #9165; Cell Signalling Technology, Inc.), Phospho-c-Jun (Ser73) (D47G9; #3270; Cell Signalling Technology, Inc.), P44/42MAPK (Erk1/2) (137F5; Cell Signalling Technology, Inc.), Phospho-p44/42 MAPK(Erk1/2) (20G11; Cell Signalling Technology, Inc.), P38 MAPK (#9212; Cell Signalling Technology, Inc.), p-p38 MAPK (D-8) (sc-7973; SANTA), MKP-1 (E-6) (sc-373841; SANTA), cPLA2 (#2832, Cell Signalling Technology, Inc.), LOX-1 (ab60178; Abcam), COX-2 (H-3; sc-376861; Santa Cruz Biotechnology), and CYP1A1(A-9) (sc-393979; SANTA Cruz).

Cell Culture

The human ccRCC cell line ACHN was cultured in DMEM (Gibco, China), while 769-P cells were cultured in RPMI 1640 medium (Gibco, China), supplemented with 10% fetal bovine serum (FBS, BioInd) and 1% penicillin–streptomycin (BasalMedia). ACHN and 769-P were maintained at 37°C and 5% CO_2.

CCK8 Assay

For the Gyp L and Gyp LI viability assays, 1×10^4 ACHN or 769-P cells were seeded in 96-well plates and allowed to adhere for 12 h. DMSO or 20–100 μM Gyp L/Gyp LI were added to the wells after washing twice with PBS. Cell viability was detected after 48 h by CCK8 assay (Dojindo Molecular Technologies, Inc.), and the absorbance was measured at 450 nm using a microplate reader (BMG Labtech FLUOstar Omega).

Colony Formation Assays

For colony formation assays, 5×10^2 ACHN and 769-P single cells were seeded in 12-well plates. Wells were washed twice with PBS after being allowed to adhere for 12 h and supplemented with either 0.1% DMSO, Gyp L, or Gyp LI. Colonies were stained with

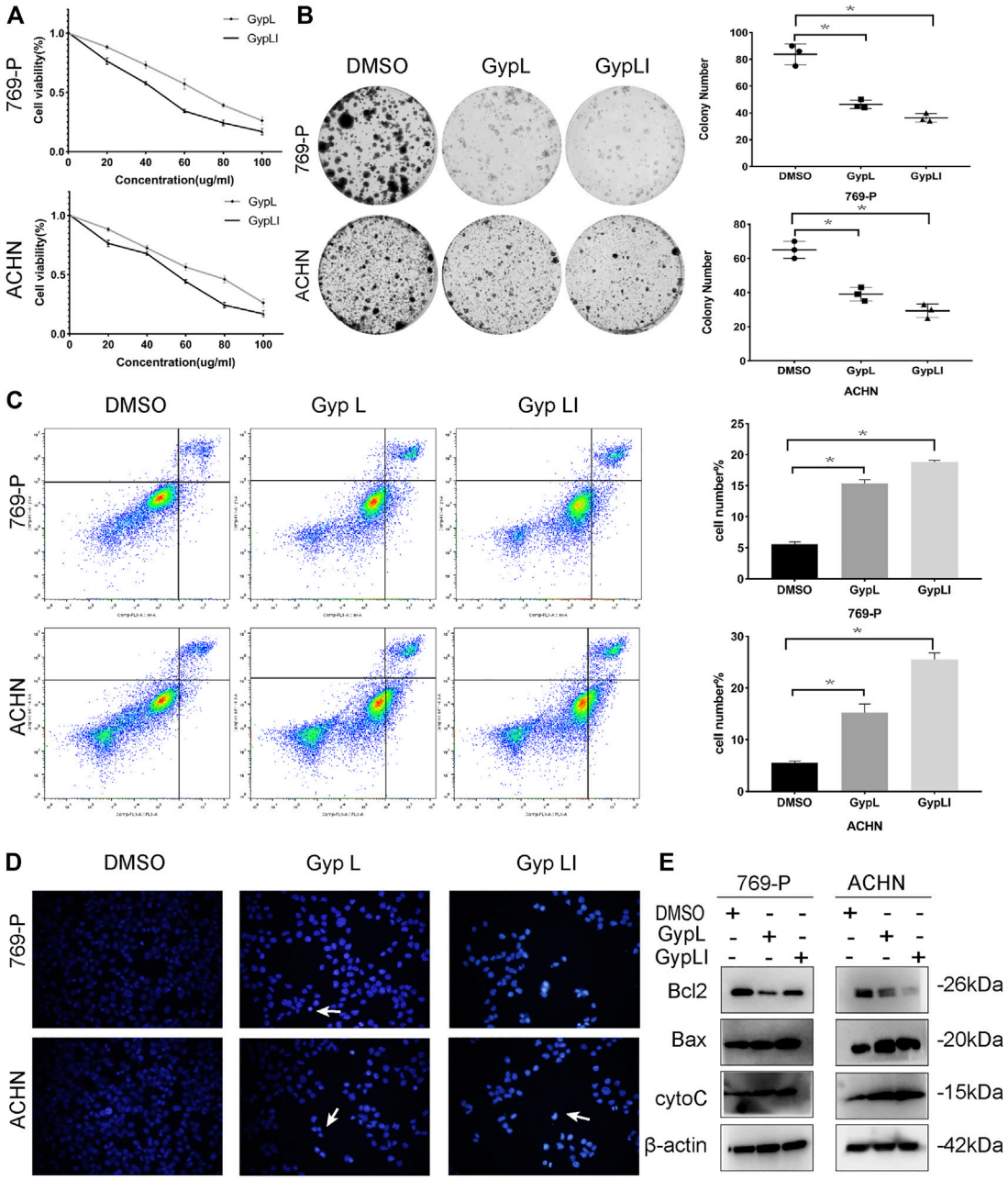

FIGURE 1 | Gypenoside L (Gyp L) and gypenoside LI (Gyp LI) inhibit proliferation and induce apoptosis of clear cell renal cell carcinoma (ccRCC) cells. **(A)** CCK8 assays were performed to detect the cell viability of 769-P and ACHN cells after different doses of Gyp L and Gyp LI treatment for 48 h. **(B)** The Colony formation assays were used to detect clonogenicity of 769-P and ACHN cells after Gyp L and Gyp LI treatment. **(C)** The apoptosis of 769-P and ACHN cells treated with Gyp L and Gyp LI was analyzed *via* flow cytometry. **(D)** Hoechst 33258 was used for ACHN and 769-P cell apoptosis detection, stained, and observed with a fluorescence microscope. **(E)** The protein levels of Bax, Bcl2, and cytochrome C were detected by Western blotting experiments. Data are presented as the mean ± SD of expression levels from three independent experiments (*$p < 0.05$, vs. control group).

0.1% crystal violet after 14 days and quantified using ImageJ software.

Cell Apoptosis Assays

To evaluate cell apoptosis, 1×10^5 ACHN or 769-P cells were seeded in 12-well plates and treated with 0.1% DMSO, Gyp L, or Gyp LI for 48 h. The cells were detached using trypsin, washed with cold PBS, and resuspended in 1× binding buffer. The cells were incubated for 20 min with 5 μl of FITC and 5 μl of PI for 5 min in the dark. Apoptosis was measured using a FACSCalibur flow cytometer (BD FACSDiva 8.0.1.1), and the data were analyzed using FlowJo_V10 software.

Cell Cycle Analysis

For cell cycle analysis, 2×10^5 ACHN or 769-P cells were seeded in six-well plates and treated with DMSO, Gyp L, or Gyp LI for 48 h. Wells were then washed with ice-cold PBS, the cells were trypsinized, and the cell suspensions were fixed in cold 70% ethanol at 4°C for 24 h. Furthermore, cells were stained with propidium iodide (PI) and incubated at 37°C for 30 min. The cell cycle distribution was subsequently analyzed using BD FACSDiva 8.0.1.1, and the data were analyzed using the ModfitLT 5 software.

Collation of Targets for Gypenoside L, Gypenoside LI, and Renal Cell Carcinoma

The targets of gypenoside L and gypenoside LI were retrieved from the Traditional Chinese Medicine Database and Analysis Platform (TCMSP) (https://tcmsp-e.com/), and RCC-related genes were searched from three databases: TTD (http://db.idrblab.net/ttd/) (Zhou et al., 2021), Online Mendelian Inheritance in Man (OMIM) (https://www.omim.org/), and The Human Gene Database (Genecards) (https://www.genecards.org/).

Gene Ontology and KEGG Analysis

Gene ontology (GO) and KEGG pathway analyses were performed using the DAVID Bioinformatics Resources ver. 6.8 (https://david.ncifcrf.gov/) (Huang da et al., 2009). Functional annotation clustering was used to select terms that met the cutoff limits of count ≥2, EASE scores ≤0.05, and $p < 0.05$.

RNA-Seq

To evaluate the effects of Gyp L and Gyp LI on the mRNA levels of 769-P and ACHN cells, we obtained RNA from cells after 48 h of treatment with Gyp L and Gyp LI. The concentration of Gyp LI was 45 and 55 μM for 769-P and ACHN, respectively, while the concentrations of Gyp L in 769-P and ACHN cells were 60 and 70 μM, respectively. TRIzol Total RNA Isolation Kit (Tiangen, Beijing, China) was used to isolate total RNA from ACHN and 769-P cells. RNA-seq was performed by Qinglian Biotech Corporation (Beijing, China). Briefly, RNA samples were sequenced on the Illumina HiseqX10 platform and analyzed using DAVID Bioinformatics Resources6.8 (https://david.ncifcrf.gov/) and Metascape (http://metascape.org/gp/index.html#/main/step1).

Immunofluorescence

For immunofluorescence assays, cells were fixed with 4% paraformaldehyde at room temperature for 15 min, permeabilized with 0.3% Triton X-100 for 20 min, and then blocked with 2% BSA/PBS for 1 h. After blocking, the cells and cPLA2 primary antibodies were incubated overnight at 4°C in a humidified box. The next day, cells were incubated with fluorescently labeled secondary antibodies for 1 h, the nuclei were stained with DAPI (Beyotime, China), and images were captured using an Olympus microscope.

Quantitative RT-PCR

RNA was extracted from cells using the TRIzol reagent kit (TIANGEN BIOTECH Co., Ltd.). cDNA was synthesized from the total RNA using PrimeScript™ RT Master Mix CFX96 Real-Time System (Bio-Rad). The primer sequences used in this study were as follows: PLA2G4, forward: 5′-AATACTGCACAATGCCCTT TACC-3′, reverse: 5′-GCTTCCAAATAAGTCGGGAGC-3′. COX7A, forward: 5′-CCAAATGCTTTACCGGACCAC-3′, reverse: 5′-GCTGCGAAGCCATG TAGAG G-3′. ALOX5, forward: 5′-CTCAAGCAACACCGACGTAAA-3′, reverse: 5′-CCT TGTGGCATTTGGCATCG-3′. CYP2E1, forward: 5′-GGGAAACAGGGCA ATGAGAG-3′, reverse: 5′-GGAAGGTGG GGTCGAAAGG-3′. c-FOS, forward: 5′-CAGGCGGAGACTGAC AA ACTG-3′, reverse: 5′-TCCTTCCGGGATTTTGC AGAT-3′. JUN, forward: 5′-GGATATTGGAT TCCGACTCGAC-3′, reverse: 5′-GGG ATCAAGTAGCTCAATCAGC-3′. DUSP1, forward: 5′-AGGTGGGTTTGCTGAG TTCTC-3′, reverse: 5′-CTCGGGGATAAAGTC AGGCTT-3′. GAPDH, forward: 5′-GGA GCGAGATCCCTCCAAAAT-3′, reverse: 5′-GGCTGTTGTCAT ACTTCTCA TGG-3′. ChamQ™ SYBR Color qPCR Master Mix (Vazyme) was used for the reaction. Briefly, the cycling conditions were as follows: predenaturation for 60 s at 95°C, followed by 45 cycles of 10 s at 95°C (denaturation) and 30 s at 60°C (annealing).

Immunoblot Analysis

769-P and ACHN cells were lysed using RIPA buffer (Beyotime). Subsequently, the lysate was centrifuged at 12,000 × *g* for 30 min at 4°C, and concentrations were quantified using a BCA assay kit (Beyotime Biotechnology, Jiangsu, China). Lysates were boiled at 99°C for 5 min, separated by SDS-PAGE electrophoresis using 10% gels, and transferred to a polyvinylidene fluoride (PVDF) membrane at 250 mA for 2 h. The membrane was incubated with the primary antibody at 4°C overnight after blocking with 5% skim milk for 1 h. All primary antibodies were used in a 5% BSA/TBST solution at the dilutions indicated in the instructions of the manufacturers. The membranes were then incubated with a secondary antibody at a dilution of 1:5,000 in 5% milk.

Lipid Extraction

Total lipids were extracted based on the method of Koundouros et al. (2020). In brief, the media of 769-P and ACHN cells grown to 80% confluency were replaced with FBS-free medium, cells were cultured for 1 h, and then lipids were extracted. For extraction, cells were collected and resuspended in 1 ml of water, then 3.75 ml of chloroform/methanol mixture (1:2 v/v) was added, and the samples were vortexed and incubated on ice for at least 30 min. Afterward, 1.25 ml of chloroform was added followed by 1.25 ml of water. The samples were then vortexed and centrifuged at 1,000 rpm at 4°C for 10 min, and the organic bottom phase was separated and dried under a nitrogen flow for analysis.

Xenograft

ACHN cells (5×10^6) were subcutaneously inoculated into both flanks of 4- to 6-week-old male immunodeficient BALB/c nude mice weighing 14–16 g. Either a normal diet or 100 mg/kg of gypenosides was provided daily through oral gavage for 21 days when tumors reached a volume of 100–200 mm^3. After 3 weeks, the mice were sacrificed, and the tumor tissues were subjected to immunohistochemical detection and arachidonic acid metabolism. All animal experiments were approved by the Animal Research Ethics Committee of China Medical University.

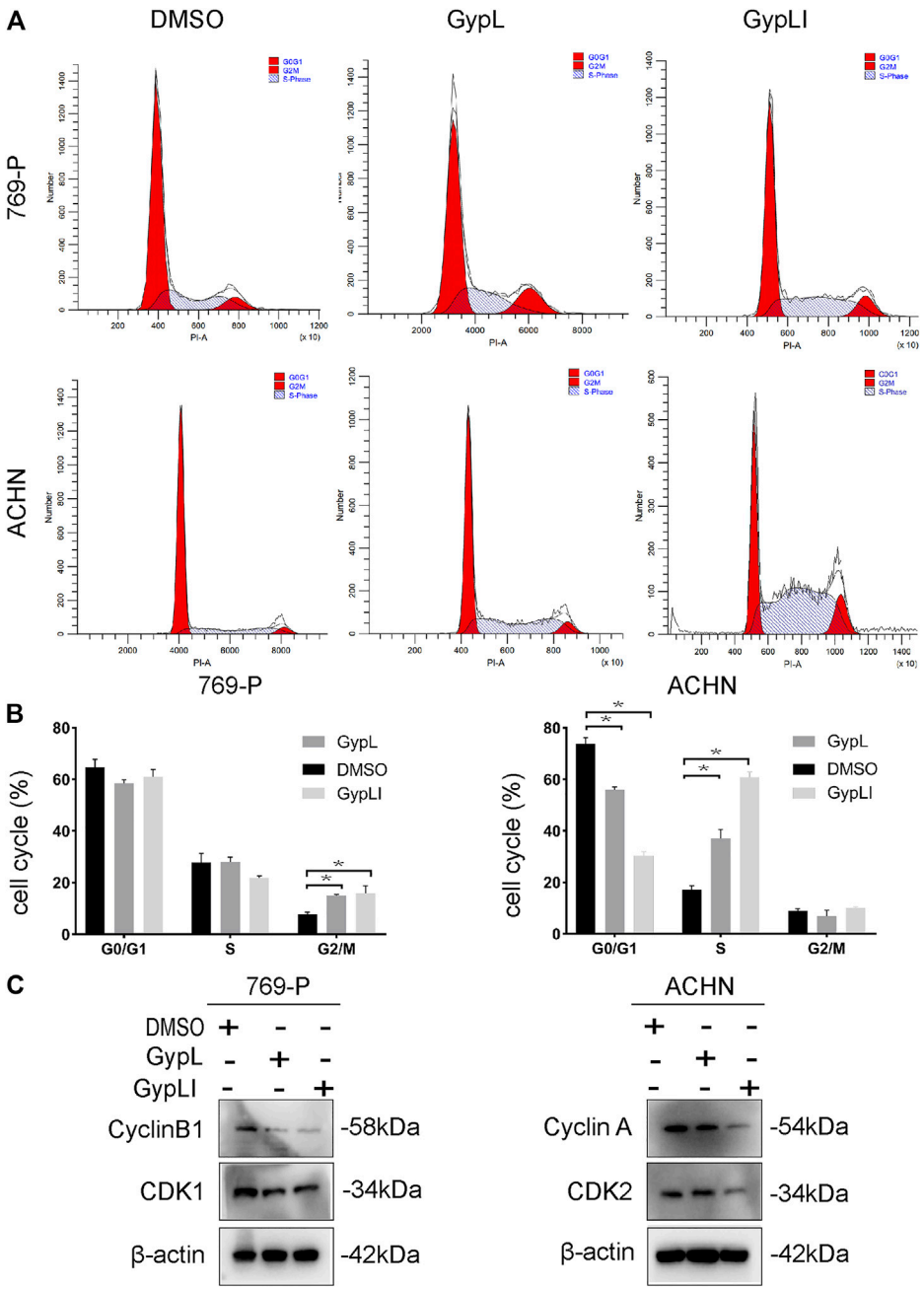

FIGURE 2 | Gyp L and Gyp LI induced cell cycle arrest in 769-P and ACHN cells. **(A)** Gyp L and Gyp LI arrest cell cycle at G2/M phase in 769-P, while G1/S phase arrest after Gyp L and Gyp LI treatment in ACHN, were determined by flow cytometry. **(B)** Statistical graph of three independent repeated experiments. **(C)** The protein levels of cyclin A and B1, CDK1, and CDK2 were detected by Western blotting. Data are expressed as the mean± SD of expression levels from three independent experiments (*$p < 0.05$, vs. control group).

Immunohistochemistry

To assess the expression of Ki-67 *in vivo*, fresh frozen tumor pieces were sectioned to a thickness of 10 mM and boiled in citrate unmasking solution for 35 min. After incubation in 3% hydrogen peroxide for 10 min, the sections were blocked with 5% BSA for 1 h at room temperature, and then with rabbit anti-human Ki-67 (1:100, Abcam, United States) antibody overnight at 4°C. Next, the slides were incubated with an appropriate secondary antibody (Zsbio, China), and stained with DAB and hematoxylin.

Statistics

All statistical analyses were performed using SPSS 25.0 and GraphPad Prism 8. All data are presented as the mean ± SD, and analyzed using a variance (ANOVA). Significance was

defined as $p < 0.05$, and all experiments were performed at least three times.

RESULTS

Antiproliferation Activity of Gypenoside L and Gypenoside LI in Clear Cell Renal Cell Carcinoma Cells

To ascertain the inhibitory effects of Gyp L and Gyp LI on renal cancer cells, we conducted CCK8 assays on ACHN and 769-P cells after 48 h of treatment with Gyp L and Gyp LI at different concentrations (0, 20, 40, 60, 80, and 100 μM). We observed that Gyp L can significantly inhibit the viability of ccRCC cells, with half-maximal inhibitory concentrations (IC_{50}) of 60 and 70 μM in 769-P and ACHN cells, respectively. The inhibitory effect of Gyp LI on ccRCC cells was stronger than that of Gyp L, with IC_{50} values of 45 and 55 μM for 769-P and ACHN, respectively. These results show that both Gyp L and Gyp LI significantly inhibit cell viability in a dose-dependent manner (**Figure 1A**).

In addition to assessing viability, we demonstrated that Gyp L and Gyp LI significantly reduced the clonogenicity of 769-P and ACHN cells (**Figure 1B**). Furthermore, we evaluated the effects of Gyp L and Gyp LI on apoptosis in the two cell lines by flow cytometry and Hoechst 33258 assays. As shown in **Figures 1C, D**, after Gyp L and Gyp LI treatment, the apoptosis rate of the two cell lines was significantly increased. Furthermore, we evaluated the expression of the apoptosis-related proteins Bax, Bcl2, and cytochrome C by Western blotting. Both Gyp L and Gyp LI downregulated the expression of the apoptosis-inhibiting protein Bcl2 and upregulated the expression of Bax and cytoC (**Figure 1E**). These results indicate that Gyp L and Gyp LI inhibit cell proliferation and induce apoptosis in ccRCC cells.

Gypenoside L and Gypenoside LI induced Cell cycle arrest in Clear Cell Renal Cell Carcinoma Cells

To detect the effect of Gyp L and Gyp LI on the cycle distribution of ccRCC cells, cell cycle analysis was performed for 769-P and ACHN cells using flow cytometry. The results showed that Gyp L and Gyp LI treatment blocked 769-P cells in the G2/M phase. In ACHN cells, after treatment with Gyp L and LI, the cells were arrested in the G1/S phase (**Figures 2A,B**). Furthermore, Western blotting revealed that the expression levels of cyclin A and B1, CDK1, and CDK2 were all reduced after treatment with Gyp L and Gyp LI (**Figure 2C**).

Network Pharmacology and Transcriptomics to Predict Key Targets and Pathways

Using network pharmacology to study the molecular mechanisms of actions of these drugs, we first examined whether the target genes of Gyp L and Gyp LI correlated with genes involved in RCC. For this, we screened 123 targets of Gyp L and Gyp LI using the Swiss target prediction platform, and further screened 1,195 renal cancer-related target genes using the TTD, OMIM, and Genecards databases. These targets were then crossed on the VENNY platform, and 49 common genes were obtained for further analyses (**Figure 3A**). Furthermore, we constructed a protein interaction network of 49 overlapping genes to predict the hub genes obtained using the Cytoscape platform (**Figure 3B**). To identify the biological characteristics of Gyp L and Gyp LI on RCC, GO and pathway enrichment analyses were performed using DAVID 6.8. Forty-one biological processes (BP), four cell components (CC), and 25 molecular function (MF) terms met the requirements of Count ≥ 2 and EASE score ≤ 0.05. Detailed GO information is shown in **Supplementary Table S2**. The first 22 significant terms in the BP, CC, and MF categories are shown in **Figure 3C**. Of note, GO enrichment analysis revealed that the related biological functions mainly include enzyme activation, cell proliferation, and other functions. To explore the target pathways of Gyp L and Gyp LI in RCC, KEGG analysis of common targets was performed. The pathway information of Gyp L and Gyp LI on RCC is shown in **Supplementary Table S1**. The top 20 significant pathways of Gyp L and Gyp LI on RCC are shown in **Figure 3D**, ranking hits according to the *p*-value, from most to least significant. KEGG pathway enrichment identified the PI3K/AKT and Ras/MAPK pathways as key. To shed light on the mechanism by which Gyp L and Gyp LI act on ccRCC cells, we performed transcriptome sequencing analysis of 769-P and ACHN cells treated with Gyp L and Gyp LI. We compared the transcriptomes of Gyp L-/Gyp LI-treated cells vs. Control, and found that genes related to the MAPK pathway, such as DUSP1, FOS, c-JUN, etc., were significantly upregulated (log2 fold change >1, $p < 0.05$), while the enzymes related to the arachidonic acid metabolism pathway, including PLA2G4, COX7A, ALOX12, and CYP2E1, were significantly downregulated (log2 fold change < −1, $p < 0.05$) (**Figures 3E, F**) (**Supplementary Tables S4, S5**). Arachidonic acid is an omega-6 polyunsaturated fatty acid. Its metabolism-related enzymes and metabolites participate in inflammation and regulate a variety of cellular processes, including cell proliferation, angiogenesis, tumor invasion, and metastasis (Wang and Dubois, 2010). Based on the results of our network pharmacology and RNA-seq analyses, we speculated that Gyp L and Gyp LI inhibit ccRCC cells by modulation of the MAPK pathway. Both *G. pentaphyllum* and RCC have previously been strongly linked to lipid metabolism; thus, in subsequent experiments, we focused on arachidonic acid metabolism.

Gypenoside L and Gypenoside LI Act on Tumor Cells *via* the Mitogen-Activated Protein Kinase and Arachidonic Acid Metabolism Regulatory Mechanisms

To further confirm the network pharmacology and RNA-seq results, and to ascertain the mechanisms by which Gyp L and Gyp LI inhibit tumorigenesis in ccRCC cells, we evaluated the

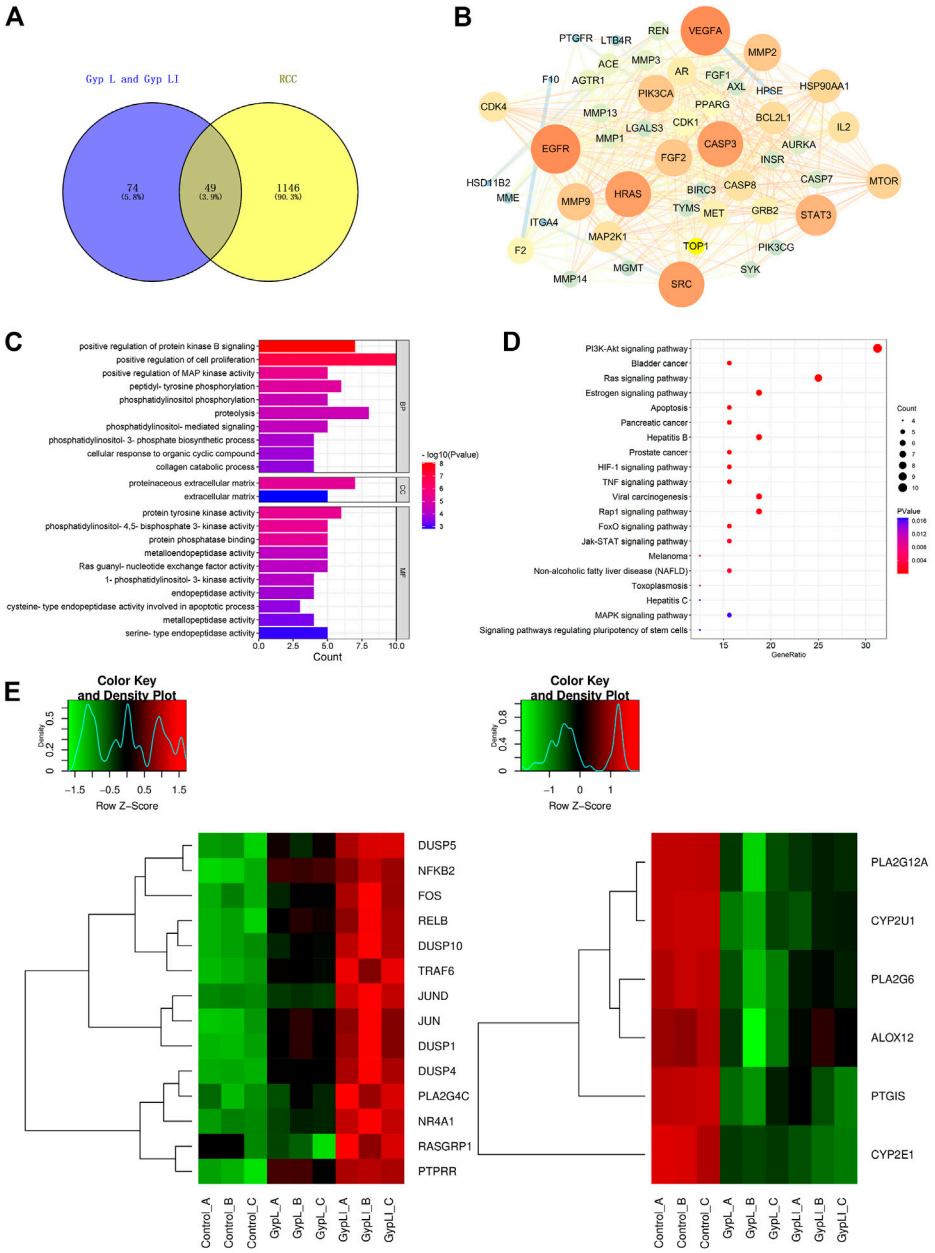

FIGURE 3 | Analyses of the key targets and pathways of Gyp L and Gyp LI inhibiting ccRCC cells through network pharmacology and RNA-seq. **(A)** Venn diagram summarizing the intersection targets between Gyp L, Gyp LI, and renal cell carcinoma (RCC). **(B)** Protein interaction network diagram of 49 intersection targets was constructed through the cytoscape platform. **(C)** Gene ontology (GO) analysis of 49 targets in terms of biological processes, cell components, and molecular functions. **(D)** The KEGG pathway that Gyp L and Gyp LI affect the RCC process analyzed by R language. **(E, F)** Unsupervised hierarchical clustering of mitogen-activated protein kinase (MAPK) **(E,F)** arachidonic acid.

effects of the two drugs on MAPK and arachidonic acid pathway-related genes in ACHN and 769-P lines. Furthermore, we performed RT-qPCR to detect key genes involved in the MAPK and arachidonic acid pathways. We observed that Gyp L and Gyp LI significantly upregulated the expression of DUSP1, FOS, JUN, and COX7A, while downregulating the expression of PLA2G4, ALOX5, and CYP2E1 (**Figures 4A,B**). Through Western blotting (WB) analysis, we found that the protein levels of DUSP1, p-JUN, and p-JNK were upregulated in 769-P and ACHN cells, while the p-MEK1/2, p-ERK, and p-P38 levels were downregulated after Gyp L and Gyp LI intervention (**Figure 4C**). The PI3K and MAPK pathways regulate arachidonic acid metabolism *via* the cPLA2 kinase (Koundouros et al., 2020). Therefore, we further detected the expression levels of the enzymes in the arachidonic acid metabolism pathway using WB, revealing that Gyp L and

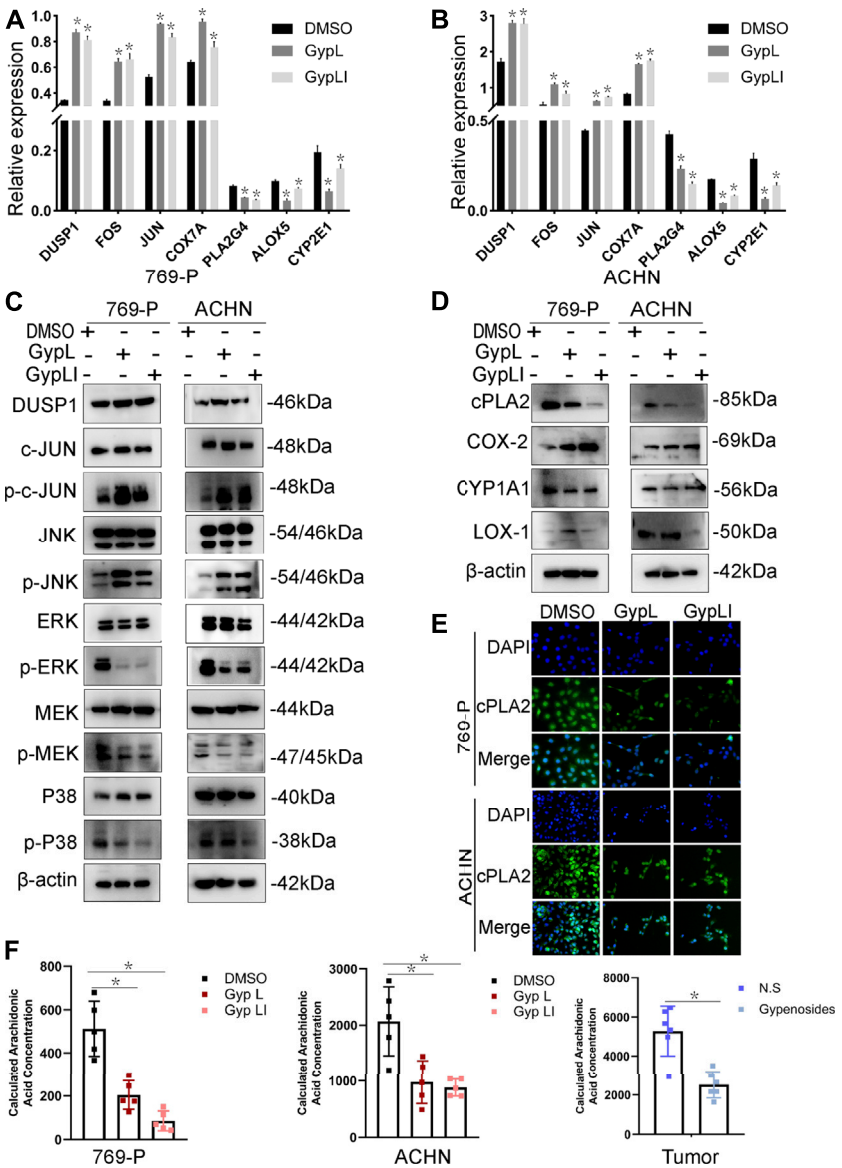

FIGURE 4 | Expression of MAPK and arachidonic acid metabolism signaling-related genes and the reduced content of AA after Gyp L and Gyp LI treatments. **(A, B)** The expressions of arachidonic acid metabolism signaling-related genes COX7A, PLA2G4, ALOX5, CYP2E1 and MAPK pathway-related genes DUSP1, FOS, JUN in 769-P (A) and ACHN (B) cells were detected by RT-qPCR. **(C)** The expression of MAPK-related proteins after Gyp L and Gyp LI treatments were detected *via* Western blotting. **(D)** The expression of arachidonic acid metabolism signaling-related proteins after Gyp L and Gyp LI treatments were detected *via* Western blotting. **(E)** The expression of cPLA2 after Gyp L and Gyp LI treatment was detected by immunofluorescence assays. **(F)** The levels of arachidonic acid in 769-P and ACHN treated with Gyp L and Gyp LI and in tumor tissues after gypenoside treatment were measured by liquid chromatography-mass spectrometry (LC/MS). Data are presented as the mean ± SD of expression levels from three independent experiments (*$p < 0.05$, vs. control group).

Gyp LI upregulated COX-2 and simultaneously downregulated the expression of CYP1A1 and cPLA2 (**Figure 4D**). The immunofluorescence results were consistent with the RT-qPCR and WB results. Gyp L and Gyp LI reduced the cPLA2 levels in 769-P and ACHN cells (**Figure 4E**). cPLA2 is the initial enzyme involved in arachidonic acid metabolism, which promotes the release of AA (Wang and Dubois, 2010). AA levels were significantly reduced when cPLA2 was inhibited. Interestingly, UHPLC-MS profiling analysis revealed that substantial AA reduction was observed in xenograft tumors and cells following Gyp L and Gyp LI treatment, compared with the control group (**Figure 4F**).

Gypenoside L and Gypenoside LI Inhibit Tumor Growth by Reducing the Arachidonic Acid Content

We designed a rescue experiment to further study the mechanism of action of Gyp L and Gyp LI on 769-P and ACHN cells. Cell medium was supplemented with AA and treated with Gyp L and LI. Through

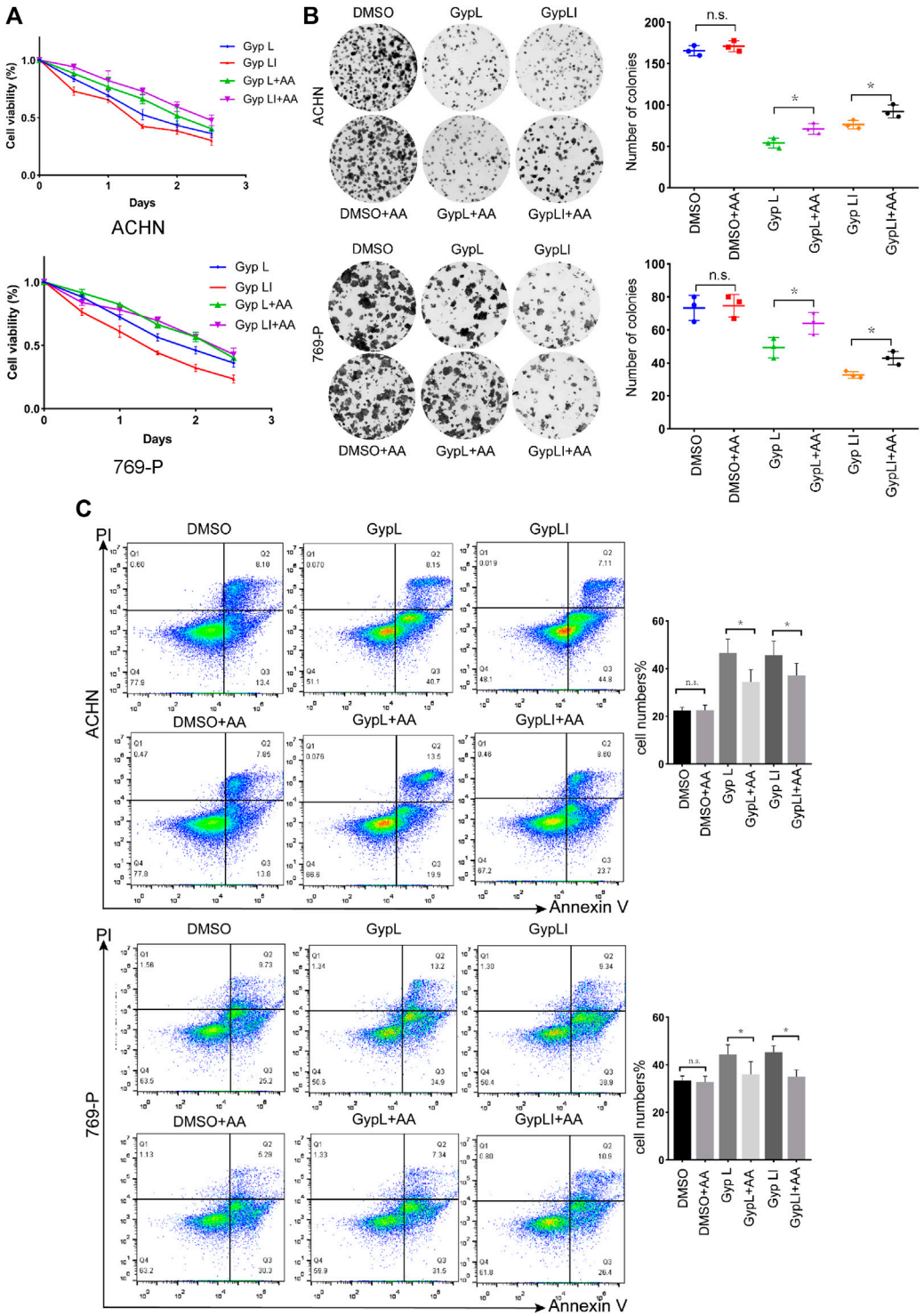

FIGURE 5 | The supplement of AA rescued the killing effect of Gyp L and LI. **(A)** Cell viability of 769-P and ACHN cells following treatment with different doses of Gyp L and Gyp LI for 48 h, supplemented with or without AA. **(B)** Clonogenic assays of 769-P and ACHN cells treated with different doses of Gyp L and Gyp LI under conditions, supplemented with or without AA. **(C)** Cell apoptosis of 769-P and ACHN after treatment with Gyp L and LI, with or without AA detected by flow cytometry. Data are presented as the mean ± SD of expression levels from three independent experiments (n.s., not significant; *$p < 0.05$, vs. control group).

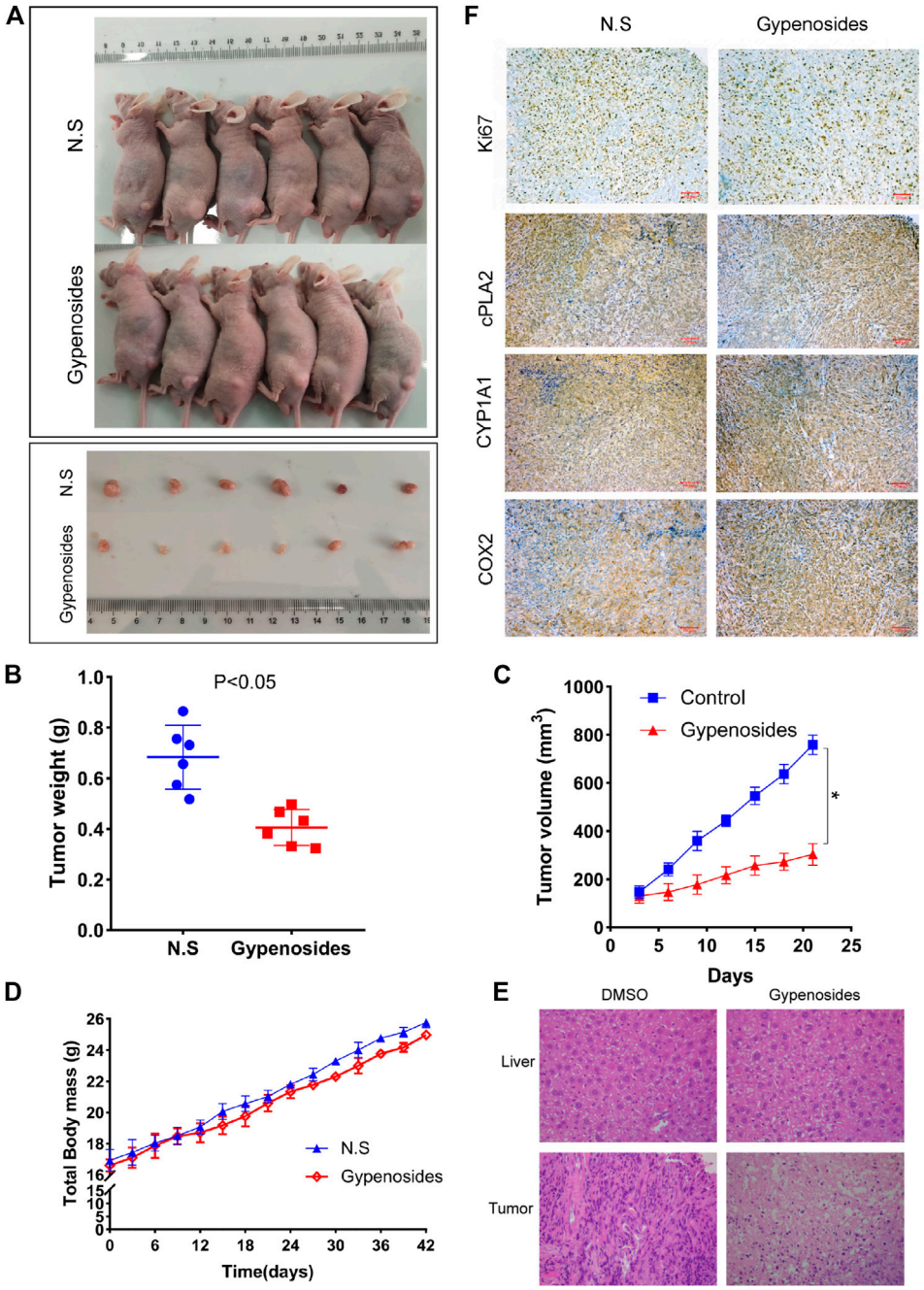

FIGURE 6 | Gypenosides suppressed the growth of RCC cell xenograft tumors *in vivo*. **(A)** Nude mice were treated with saline or gypenosides for 28 days, and pictures of nude mice and tumors were obtained. **(B)** Changes in tumor weight after gypenoside administration, compared with the control group. **(C)** Changes in tumor volume after administration of gypenosides, compared with the control group. **(D)** The body weight of the two groups of mice during the entire experiment. **(E)** HE staining of mice liver and tumors. **(F)** The differences in the protein expression levels of Ki67, cPLA2, CYP1A1, and COX2 between the two tumor groups were detected by immunohistochemistry.

the CCK8 assay, we observed that supplementation with AA rescued the killing effect of Gyp L and Gyp LI (**Figure 5A**). We further used clone formation experiments to confirm the importance of arachidonic acid supplementation on the cloning ability of Gyp L and Gyp LI in ccRCC cells. We observed a marked reduction in the number of colonies following Gyp L and Gyp LI treatment in 769-P and ACHN cells, which was restored in the presence of AA (**Figure 5B**). In addition, flow cytometry was used to detect the effect of arachidonic acid supplementation on the apoptosis of ccRCC cells induced by Gyp L and Gyp LI. The results revealed that supplementation with arachidonic acid reversed the effects of Gyp L and Gyp LI on the induction of ccRCC cell apoptosis (**Figure 5C**).

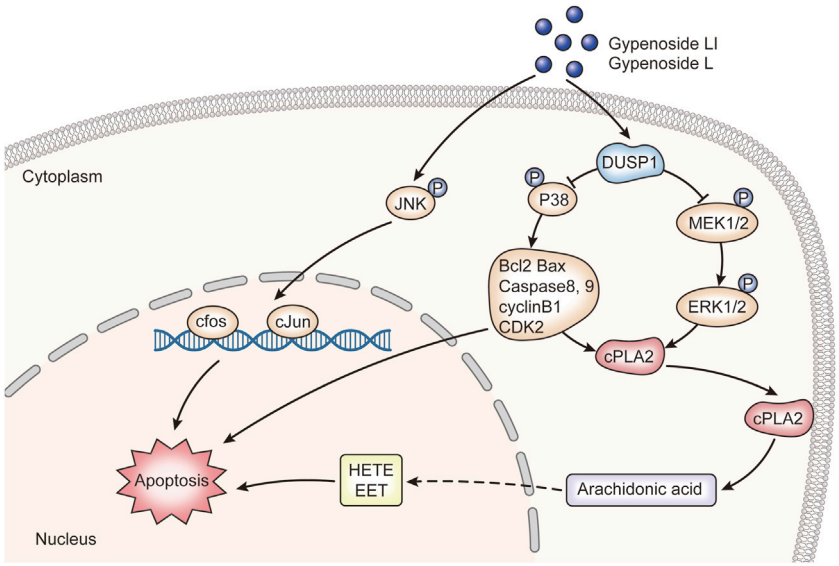

FIGURE 7 | The molecular mechanism of gypenoside L and gypenoside LI inducing apoptosis in renal cell carcinoma.

Antitumour Effects of Gypenosides on Tumor Growth in Vivo

It is known that Gyp L, Gyp LI, and gypenosides can inhibit the proliferation of ccRCC cells *in vitro* (Liu et al., 2021). We further confirmed whether gypenosides could inhibit tumor growth *in vivo*. As shown in **Figures 6**A, C, gypenoside-treated ACHN cell xenografts grew much slower than those of the control group. Consistently, the weight of tumors was 37% lower, on average, after treatment with gypenosides compared with tumors from control mice (**Figure 6B**). However, there was almost no difference in the body weights of the two groups of mice during the entire experiment (**Figure 6D**). HE staining analysis revealed that the livers of mice treated with gypenosides showed no significant difference compared with the control group, indicating that treatment with gypenosides did not cause significant hepatotoxicity. However, unlike control mice, gypenoside-treated mice showed signs of tumor necrosis (**Figure 6E**). Furthermore, immunohistochemistry results revealed that the levels of Ki67, cPLA2, and CYP1A1 were reduced compared with those in the control group. The expression level of COX2 was significantly higher than that in the control group (**Figure 6F**). The results showed that gypenosides could inhibit tumor growth without hepatotoxicity *in vivo*. This mechanism suggests that gypenosides affect tumor growth by regulating the expression of cPLA2, CYP1A1, and COX2 in the arachidonic acid pathway.

DISCUSSION

The development of ccRCC is strongly linked to lipid metabolism (Lowrance et al., 2010). Lipid accumulation is currently considered to be an important marker of the aggressiveness of RCC, indicating that reprogramming of lipid metabolism may occur during the development of renal cancer (Capitanio et al., 2019). Lipid metabolism plays an important role in tumor cell proliferation and metastasis. Among the related pathways, arachidonic acid metabolism, sphingolipid metabolism, and steroid biosynthesis play a central role in the development of many diseases (Wang and Dubois, 2010; Dennis and Norris, 2015; Sonnweber et al., 2018). Arachidonic acid is an omega-6 polyunsaturated fatty acid whose metabolism-related enzymes and products participate in inflammation and regulate various cellular processes, including cell proliferation, angiogenesis, tumor invasion, metastasis, etc. (Yarla et al., 2016). Phospholipase A2 (PLA2s) is the initial enzyme of the AA metabolic pathway, which converts cell membrane-bound phospholipids into free fatty acids under various stimuli, mainly arachidonic acid and lysophospholipids (Dessen et al., 1999; Gijón and Leslie, 1999; Yarla et al., 2016). Of note, COX, LOX, and CYP 450 enzymes and their inhibitors are widely used to treat inflammation and cancer (Fishbein et al., 2021). Several prior studies have found that COX-2, LOX-1, and their inhibitors can reduce resistance and enhance sensitivity to chemotherapeutic drugs (Apaya et al., 2016). COX-2 and PGD2 have previously been identified as potential targets for the prevention and treatment of colon cancer (Wang and Dubois, 2010). However, COX-2 and CYP450 are also key enzymes that can stimulate the resolution of inflammation and produce pro-resolving mediators (SPMs), such as lipoxins (LXA4) and EETs (Wallace, 2006). Arachidonic acid metabolism products, including prostaglandins (PGs), leukotrienes (LTs), EETs, and HETEs play a role in inhibiting tumor cell apoptosis, stimulating angiogenesis, and enhancing cell proliferation and metastasis (Schneider and Pozzi, 2011; Chen and Wang, 2013). Thus, targeting lipid metabolism may be an effective treatment strategy for renal cell carcinoma. However, previous studies have focused on the treatment of inflammatory diseases, such as hepatitis or hyperlipidemia by regulating lipid metabolism

(Li et al., 2020; Weng et al., 2021). Here, we demonstrate that the gypenosides Gyp L and Gyp LI could reduce the content of arachidonic acid in ccRCC cells by downregulating cPLA2, thereby inhibiting the growth of renal cancer. This observation promotes the possibility that gypenoside could significantly increase the sensitivity of cancers to cPLA2 inhibitors and could, thus, provide a new approach for the treatment of renal cancer. Although we demonstrated a decrease in AA following treatment, further work is needed to analyze the content of metabolites, such as EETs and PGE2 in cells and tumors treated with gypenosides.

Mitogen-activated protein kinase (MAPK) is an important transmitter, which functions to transmit signals from the cell surface to the nucleus *via* the phosphorylation of key protein targets following activation by different extracellular stimuli, including cytokines, cell stress, and cell adhesion. The continuous activation of the upstream MAPK kinase kinase (MAPKKK) and MAPK kinase (MAPKK) leads to the activation of MAPK (Donohoe et al., 2020). The MAPK pathway is also mediated by ERK, JNK, and p38 protein kinases (Johnson and Lapadat, 2002). The cascade of extracellular signal-regulated kinases ERK1 and ERK2 (ERK1/2) is closely related to cancer and is strongly involved in multiple tumor processes, including cell differentiation, cell senescence, and apoptosis *via* the phosphorylation of multiple target proteins (Deschênes-Simard et al., 2014). The dysregulation of the JNK pathway is also closely associated with cancer; this pathway is involved in various cellular processes, such as cell proliferation, survival, apoptosis, and inflammation (Hammouda et al., 2020). p38 plays a dual role in tumorigenesis, alternatively acting as a tumor suppressor and a tumor promoter (Martínez-Limón et al., 2020). Importantly, previous studies have demonstrated that p38 and p42/p44 MAPK are essential for ATPgammaS-induced COX-2 expression and PGE2 synthesis (Lin et al., 2009). Notably, ERK1/2 regulates PKC protein in a dependent and independent manner, and further mediates cPLA2 phosphorylation and AA release in astrocytes (Xu et al., 2002). We have demonstrated the efficacy of gypenosides in inducing apoptosis of renal cell carcinoma *via* activation of the PI3K/AKT/mTOR pathway (Liu et al., 2021). This fits with previous research, which has shown that gypenosides inhibit the proliferation of liver and esophageal cancer by regulating the MAPK pathway (Ma et al., 2019). However, the applications and mechanisms of action of gypenosides Gyp L and Gyp LI, which modulate the progression of renal cancer through the MAPK pathway, remain largely obscure.

In this study, the integration of network pharmacology and RNA-seq analysis revealed that gypenoside may inhibit the occurrence and development of renal cancer *via* action on the MAPK pathway. Here, we experimentally demonstrated that Gyp L and Gyp LI significantly inhibited the proliferation of 769-P and ACHN by upregulating DUSP1 and downregulating p-P38, p-MEK, and p-ERK. We also confirmed that Gyp L and Gyp LI induced apoptosis in ccRCC cells by upregulating p-JUN, p-c-Jun, and c-fos. We hypothesized that key genes in the MAPK pathway and in the metabolism of arachidonic acid regulate arachidonic acid levels in ccRCC cells and contribute to tumor growth (**Figure 7**). However, this hypothesis still requires experimental validation. How gypenosides regulate the metabolism of arachidonic acid through the MAPK pathway requires further investigation. Overall, the gypenosides, Gyp L, and Gyp LI may be safe and effective drugs for the treatment of ccRCC.

CONCLUSION

In conclusion, the present study demonstrates that Gyp L and Gyp LI can both cap inhibit the proliferation of ccRCC cells by regulating key genes in the MAPK pathway and the metabolism of arachidonic acid. Gypenosides reduce the content of arachidonic acid by downregulating cPLA2 levels *in vivo* to inhibit tumor growth without inducing hepatotoxicity. Although further research is necessary, this study provides preliminary results to indicate that Gyp L and Gyp LI are promising drugs in the treatment of renal cancers, specifically ccRCC.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA785289/.

ETHICS STATEMENT

The animal study was reviewed and approved by China Medical University Application for Laboratory Animal Welfare and Ethica Committee.

AUTHOR CONTRIBUTIONS

HL performed the main analysis and wrote the manuscript. XL performed the analysis. CL, FL, and SZ carried out the animal experiments and helped revise the manuscript. JX and YD extracted and isolated Gyp L, Gyp LI, and gypenosides from *G. pentaphyllum*. YZ conceived and designed the study. XP approved the finalized manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.820639/full#supplementary-material

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Inhibitory Mechanism of Combined Hydroxychavicol With Epigallocatechin-3-Gallate Against Glioma Cancer Cell Lines: A Transcriptomic Analysis

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Emerging reports have shown therapeutic potential of hydroxychavicol (HC) and epigallocatechin-3-gallate (EGCG) against cancer cells, however high concentrations are required to achieve the anticancer activity. We reported the synergy of low combination doses of EGCG+HC in glioma cell lines 1321N1, SW1783, and LN18 by assessing the effects of EGCG+HC through functional assays. Using high throughput RNA sequencing, the molecular mechanisms of EGCG+HC against glioma cell lines were revealed. EGCG/HC alone inhibited the proliferation of glioma cell lines, with IC50 values ranging from 82 to 302 µg/ml and 75 to 119 µg/ml, respectively. Sub-effective concentrations of combined EGCG+HC enhanced the suppression of glioma cell growth, with SW1783 showing strong synergism with a combination index (CI) of 0.55 and LN18 showing a CI of 0.51. A moderate synergistic interaction of EGCG+HC was detected in 1321N1 cells, with a CI value of 0.88. Exposure of 1321N1, SW1783, and LN18 cells to EGCG+HC for 24 h induces cell death, with caspase-3 activation rates of 52%, 57%, and 9.4%, respectively. However, the dose for SW1783 is cytotoxic to normal cells, thus this dose was excluded from other tests. EGCG+HC induced cell cycle arrest at S phase and reduced 1321N1 and LN18 cell migration and invasion. Combined EGCG+HC amplified its anticancer effect by downregulating the axon guidance process and metabolic pathways, while simultaneously interfering with endoplasmic reticulum unfolded protein response pathway. Furthermore, EGCG+HC exerted its apoptotic effect through the alteration of mitochondrial genes such as MT-CO3 and MT-RNR2 in 1321N1 and LN18 cells respectively. EGCG+HC dynamically altered DYNLL1 alternative splicing expression in 1321N1 and DLD splicing expression in LN18 cell lines. Our work indicated the pleiotropic effects of EGCG+HC treatment, as well as particular target genes that might be investigated for future glioma cancer therapeutic development.

Keywords: glioma, epigallocatechin-3-gallate, hydroxychavicol, synergism, gene expression, apoptosis, transcriptomic

1 INTRODUCTION

Gliomas are the most frequent primary intracranial tumour, accounting for 81% of all malignant brain tumours. Although relatively uncommon with an annual incidence of around 5 cases per 100000, the median survival of glioblastoma patients are around 15 months even after rigorous combination treatment of surgery, chemotherapy and radiotherapy, with and the median progression-free survival ranged from 6.2 to 7.5 months (Liang et al., 2020). Human cancer is a complicated disease, thus, alternative methods for cancer management and treatment and a better understanding of the treatments' mechanism of action are required to improve patients' quality of life (Pangal et al., 2021).

Dietary bioactives with high effectiveness and little side effects are preferred as alternatives to synthetic therapies, with a variety of negative side effects. At the moment, the search for combination therapy is of interest as this approach may reduce the development of drug resistance, as well as provides opportunity to discover potential cancer medicines (Maruca et al., 2019). Polyphenols are one of the major classes of phytochemical that is well-known for its disease-fighting effects.

The health benefits, the relatively low side effects and the origin from natural sources may provide added benefits and have resulted in continued interest for these bioactives. They are thought to play two roles: one that modulates chemopreventive benefits by improving antioxidant defences and the ability to scavenge ROS, hence lowering oxidative stress, and the other that targets chemotherapeutic effectiveness by inducing cellular stress (ROS levels) (Surh, 2011). At low concentrations and in normal cells, phenolic compounds may act as cancer preventive agents (Khurana et al., 2018). Some polyphenols species can act as prooxidants, enhancing its chemotherapeutic action, by generating high levels of ROS and eventually induces DNA damage and apoptosis (Kirtonia et al., 2020).

The cancer-preventive effects of (−)-epigallocatechin-gallate (EGCG) on cells *in vitro*, in animal models and within clinical studies have been previously reported (Peter et al., 2016). The anti-cancer effect of EGCG is proposed to originate from its antioxidant activity, through the induction of phase II enzymes, and manipulation of signal transduction pathways such as JAK/STAT (Xiao-Mei et al., 2016), MAPK, VEGF and PI3K/AKT (Liu et al., 2013). The mechanism of EGCG also includes epigenetic regulatory alteration, altering DNA methyltransferase (DNMT), histone deacetylase (HDAC), and miRNA expression (Zhang et al., 2015; Yamada et al., 2016).

Hydroxychavicol is a less studied phenolic compound derived from *Piper betle* leaf extract. Emerging reports including our own have shown the potent activity of HC in impeding cell proliferation in glioma cells (Abdul Rahman et al., 2014), and inhibiting prostate cancer cell cycle progression (Gundala et al., 2014). The efficacy of HC in inhibiting prostate tumour xenografts and chronic myeloid leukemia (CML) cells is suggested to be attributed to its selective prooxidant activity by reactive oxygen species (ROS) generation and induction of caspase-mediated apoptosis (Gundala et al., 2014) and/or by caspase-independent manner via apoptosis inducing factor (AIF) (Chowdhury et al., 2013) to eliminate cancer cells.

We hypothesize that the combination of these phenolic compounds may enhance the therapeutic activity as they impact on a number of pathways in tumour progression. Although studies have reported that EGCG or HC induce cell death without adversely affecting normal cells (Gundala et al., 2014; Meng et al., 2019), a high concentration of EGCG/HC is usually needed for the treatment to be effective on cancer cells. A high dose of EGCG or HC might result in cytotoxicity in normal cells. Therefore, a combination of low concentrations of EGCG+HC may be more effective in killing cancer cells, compared to a single high concentration treatment, as combination treatments may have pleotropic effects, targeting several pathways.

Therefore, we aim to investigate the synergistic interaction of phytochemicals by examining the effect of EGCG or HC singly and its combination against 1321N1, SW1783 and LN18 cell proliferation, cell cycle progression, migration/invasion and colony formation. The molecular mechanisms of EGCG+HC against glioma cell lines were elucidated using high throughput RNA sequencing. Until now, no direct evidence has shown on the anticancer effect of ECGC+HC in different stages of glioma cells.

2 MATERIALS AND METHODS

2.1 Reagents and Chemicals

Hydroxychavicol (HC) was bought from Hangzhou Imaginechem Co. Ltd. (Hangzhou, China) and (−)-epigallocatechin-3-gallate (EGCG) was bought from Sigma-Aldrich (United States). CellTiter 96® AQueous Non-Radioactive Cell Proliferation kit (Promega, United States), FITC Active Caspase-3 and Annexin V-FITC Apoptosis Detection Kit, BD CycleTEST™ PLUS DNA Reagent kit from BD Biosciences (United States) and QCMTM 24-well Cell Invasion/Migration Assay kit (ECM550 and ECM508) (Millipore, United States). All of the other chemicals utilised were of analytical grade.

2.2 Cell Line and Culture Environment

The human glioblastoma cell line 1321N1 (Grade II) was bought from the European Collection of Cell Culture (ECACC), while the American Type Culture Collection (ATCC) supplied SW1783 (Grade III) and LN18 (Grade IV) cell lines (Manassas, United States). 1321N1 and LN18 were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with penicillin/streptomycin, 10% or 5% foetal bovine serum (FBS) respectively, in a humidified incubator at 37°C in an atmosphere of 95% air and 5% CO2. SW1783 was grown in Leibovitz, 10% FBS, under a 100% air atmosphere. The medium was replaced three times a week, and the cells were passaged with accutase.

2.3 Natural Compound Treatments

Fresh EGCG stock solutions were made in culture growth media, whereas HC stock solutions were prepared in 100% ethanol and kept at −20°C. Vehicle control was added with 0.1% ethanol.

2.4 Cell Viability Determination

Glioma cancer cell viability treated with combined EGCG+HC or EGCG/HC singly was determined using the Cell Proliferation Assay (Promega, United States), as previously described [11]. Cells were seeded at 1×10^4 cells per well in 96-well plates. After a 24-h incubation period, the media was withdrawn and 100 µl of medium were added, which contains a range of concentration for EGCG (50, 100, 150, 200, 300 µg/ml) or HC (50, 100, 150, 200 µg/ml). EGCG+HC compounds were titrated to a range of concentrations (1, 10, 50, 100 µg/ml). The treatments were incubated for 24 h. The media was then carefully removed, replaced with new medium, and 20 µl of [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt] (MTS) was added to each well and incubated for 2 h at 37°C. In a VersaMax ELISA microplate reader, absorbance was measured at 490 nm (Molecular Device, United States). At each concentration, the percentage of viable cells was estimated by dividing the absorbance (490 nm) of treated cells by that of control cells. The cell viability (%) versus concentrations graph was used to calculate the half maximum inhibitory concentration (IC50). All tests were carried out in three independent experiments.

2.5 Apoptosis Assay Using Active Caspase-3 and Annexin V-Propidium Iodide Staining

Cells were seeded at a density of 5×10^5 cells/dish in a 60 mm culture dish for all functional assays. EGCG was dissolved in culture medium, while HC was dissolved in ethanol and added to the culture media with stipulated concentration. Cells were collected after 24 h and washed twice with PBS. The assays were carried out as specified in the manufacturer's procedure. In brief, for active caspase-3 assay, cells were fixed in BD Cytofix/Cytoperm solution, incubated on ice for 20 min, washed with BD Perm/Wash buffer, and then incubated for 30 min at 25°C with FITC rabbit anti-active caspase-3 antibody. For annexin V-propidium iodide staining, cells were resuspended in 1X binding buffer. Annexin-V FITC and propidium iodide (PI) were added and incubated in the dark for 15 min at 25°C. The BD FACSCanto™ flow cytometer and CellQuest Pro (IVD) software (Becton Dickenson, United States) were used to detect fluorescence from a population of 1×10^5 cells. Three independent tests were carried out in triplicate.

2.6 Analysis of Cell Cycle Progression

The cells were prepared according to the manufacturers' protocol. Cells were trypsinized, washed, and fixed in Buffer Solution at 4°C. After 10 min of incubation with trypsin buffer at 25°C, 200 µl of trypsin inhibitor and RNase buffer solution were added and incubated for another 10 min. In a dark room, 200 µl of propidium iodide stain solution were added to the mixture and incubated on ice for 10 min. The analysis were done using the BD FACSAria™ flow cytometer, FACScan and ModFit software (Becton Dickenson, United States).

2.7 Wound Healing Test

The wound healing test was carried out as previously reported, with several modifications (Liang et al., 2007). After 24 h of plating in a 6-well plate, cells were scratched with a 200 µl sterile pipette tip, rinsed three times with PBS, and incubated with the treatments for another 24 h. The cells were rinsed twice with PBS before being examined and photographed using a Nikon Eclipse TS100 phase-contrast microscope. Using the NIS-Elements imaging programme, the migration percentage was estimated by comparing cells which migrated into scratched regions to 0 h cells.

2.8 Transwell Cell Invasion and Migration Assay

The assays were prepared according to the manufacturers' protocol (Millipore, United States). Both invasion and migration kits utilized an 8 µm pore size polycarbonated membrane insert. A thin layer of ECMatrix™ were pre-coated on the insert for invasion kit which function to seal the membrane pores and block the non-invasive cell migration. Briefly, 300 µl of cells re-suspended in a serum-free medium was added to the upper chamber, while the bottom well was filled with 500 µl of complete culture medium or treatment. Following 24 h incubation, unmigrated cells were removed from the upper chamber, and the migration insert containing migrated cells were transferred into a clean well containing 400 µl of staining buffer. After 20 min incubation at 25°C, the inserts were rinsed in water, and unmigrated cells were removed from the inside of the insert with a cotton-tipped swab. After drying, the stained inserts were placed to a clean well containing 200 µl of Extraction Buffer for 15 min at 25°C. 100 µl of the mixture solution was pipetted a 96-well plate and the absorbance was measured at 560 nm.

2.9 Colony Formation Assay

Following a 24 h treatment with combined EGCG+HC and EGCG/HC singly, approximately 400 cells were plated in a 21 cm^2 culture dish. The cells were grown in a complete media for 12 days and the medium were replaced every 3 days. On day-12, the colonies formed were washed with PBS, and then fixed for 30 min with a mixture of crystal violet solution and methanol (1:1). The plate were rinsed three times with distilled water to remove any excess staining. ChemiDocTM MP (Biorad, United States) was used to capture the images of the stained plates, and Cell Counter v0.2.1 (http://nghiaho.com/?page id=1011) was used to count the colonies. Each treatment was carried out in triplicate.

2.10 Statistical Evaluation

Isobologram analysis based on the Chou-Talalay technique (Chou and Talalay, 1984; Zhao et al., 2004) was used to determine the interaction between the two treatments, with the output represented as combination indexes (CI). The CI between two compounds A and B is as follows:

$$CI = \frac{d_1}{(D_m)1} + \frac{d_2}{(D_m)2}$$

Where; CI: combination index d_1: the IC_{50} of combination dose for compound 1 d_2: the IC_{50} of combination dose for compound 2 $(D_m)_1$: the IC_{50} dose for compound 1 $(D_m)_2$: the IC_{50} dose for compound 2.

The magnitude of synergism/antagonism were measured using CI values. CI values between 0.9 and 0.85 indicate mild synergy, those between 0.7 and 0.3 indicate strong synergistic interactions between the treatments. A near additive effect is shown by CI values ranging from 0.9 to 1.10. SPSS 16.0 software was used to analyse the two-tailed Student's t-test for comparison with vehicle control only (cell viability with single treatments), or two-way ANOVA for multiple comparisons of apoptosis, cell cycle, migration/invasion, and colony formation tests where $p < 0.05$ were considered statistically significant. The data were presented as mean ± standard deviation (SD).

2.11 RNA Library Preparation and Sequencing

After 24 h of glioma cells treatment, total RNA was using TRI Reagent® (Molecular Research Center, United States). RNase-free DNase treatment was used to remove contaminating DNA (QIAGEN), and RNA was purified using the RNeasy Mini Kit (QIAGEN). The quality of the RNA was then determined using an Agilent Bioanalyzer 2100 (Santa Clara, United States) and all readings have a minimum RIN score of 9.5. The Qubit 2.0 Fluorometer was used to measure the amount of RNA (Life Technologies, United States). The cDNA libraries were prepared according to the protocol outlined in the TruSeq RNA sample Preparation Kit v-2 (Illumina, San Diego, United States). The description of the procedure may be found elsewhere (Abdul Rahman et al., 2019). A total of 10–12 samples per lane were multiplexed and sequenced on an Illumina HiSeq 2500 using the paired-end cluster generation kit (Illumina).

2.12 RNA-Sequencing Data Processing and Pathway Analysis

The analysis of RNA-seq data was previously described elsewhere (Abdul Rahman et al., 2019). For quality control, RNA-seq raw data was trimmed at a PHRED score of <Q25, with a read length of at least 33 bp, and read quality was evaluated for each sample using FastQC. A total of 28–47 million paired end reads were aligned and mapped against the Human Genome version 37, GRCh37/hg19. Significant differences in gene/transcript expression were determined for pairwise comparisons between two sets of samples using the Empirical analysis of DGE (Robinson and Smyth, 2008) by Negative Binomial distribution followed by Bonferroni multiple testing correction (MTC) and Benjamini–Hochberg false discovery rate (FDR) using the CLC Genomics Workbench (7.0.6 version), and by Cuffdiff 2.0 in Tuxedo Suite pipeline (Trapnell et al., 2012). For each comparison, the intersection of the significant genes discovered using both algorithms were deemed differentially expressed.

Pathway Studio (Ariadne Genomics, United States) was used for network analysis, which included Gene Significant Enrichment Analysis (GSEA), Fisher Exact Test (FET), and subnetwork analysis of RNA-seq data, using the mean of RPKM values, and the gene expression log ratio of treatment to control cells were calculated. DAVID Bioinformatics Resources 6.7 was also used for gene ontology annotation. In the subnetwork analysis, genes were deemed key regulators of a network if they controlled five or more gene targets. These networks give a global picture of potentially important, interacting partners of genes that have undergone significant alterations. For the assessments of alternative splicing expression, two types of analyses were performed using the Partek® Genomics Suite (Partek Inc., United States) and the Tuxedo Suite (Tophat and Cuffdiff 2.0). Alternative splicing entities expressed from both platforms were overlapped (**Supplementary Tables S1–S3**). The expression of alternative splicing events in both analyses was then overlapped to the list of transcripts expression at $p \leq 0.05$ with FC ≥ 1.5, to get the list of alternative splicing events with a significant differential transcripts expression (**Supplementary Tables S1–S3**).

3.13 Validation of Gene Expression Data by Quantitative Real-Time PCR (qPCR)

The genes were chosen for validation because they either demonstrated the most significant increase or reduction in response to EGCG+HC therapy, are a central gene, or are important to cancer formation. 300 ng of total RNA were used for cDNA synthesis using the iScript™ cDNA Synthesis kit (BioRad, United States). TaqMan® Gene Expression Assays and TaqMan® Fast Advanced Master Mix (Applied Biosystem, United States) were used for the amplification of genes and transcripts. cDNA synthesis reactions were performed on Veriti® Thermal Cycler (Applied Biosystems) and qPCR were performed on CFX96 Touch™ C1000 Touch Thermal Cycler (BioRad, United States). The data were normalized to the expression of housekeeping genes TATA box binding protein (*TBP*) or glucuronidase, beta (*GUSB*) and analyzed using the standard $2^{-\Delta\Delta CT}$ method.

3 RESULTS

3.1 Treatment of EGCG or HC Singly and Its Combination Decreases Glioma Cell Viability

We have previously reported the effect EGCG and HC singly on glioma cell viability (Abdul Rahman et al., 2014;Rahman et al., 2014). The optimum IC50 doses for EGCG, HC and its combination for each cell lines were obtained by performing an initial dose response curve (**Figure 1**). The growth of 1321N1, SW1783 and LN18 cells was inhibited with the inhibitory concentration at 50% cell death (IC50) values for EGCG ranging from 82 to 302 μg/ml (Rahman et al., 2014) and HC (Abdul Rahman et al., 2014) with values of IC50 between 75–119 μg/ml (**Table 1**). The cytotoxicity induced by EGCG and HC was found to be dose dependent with 58–84% and

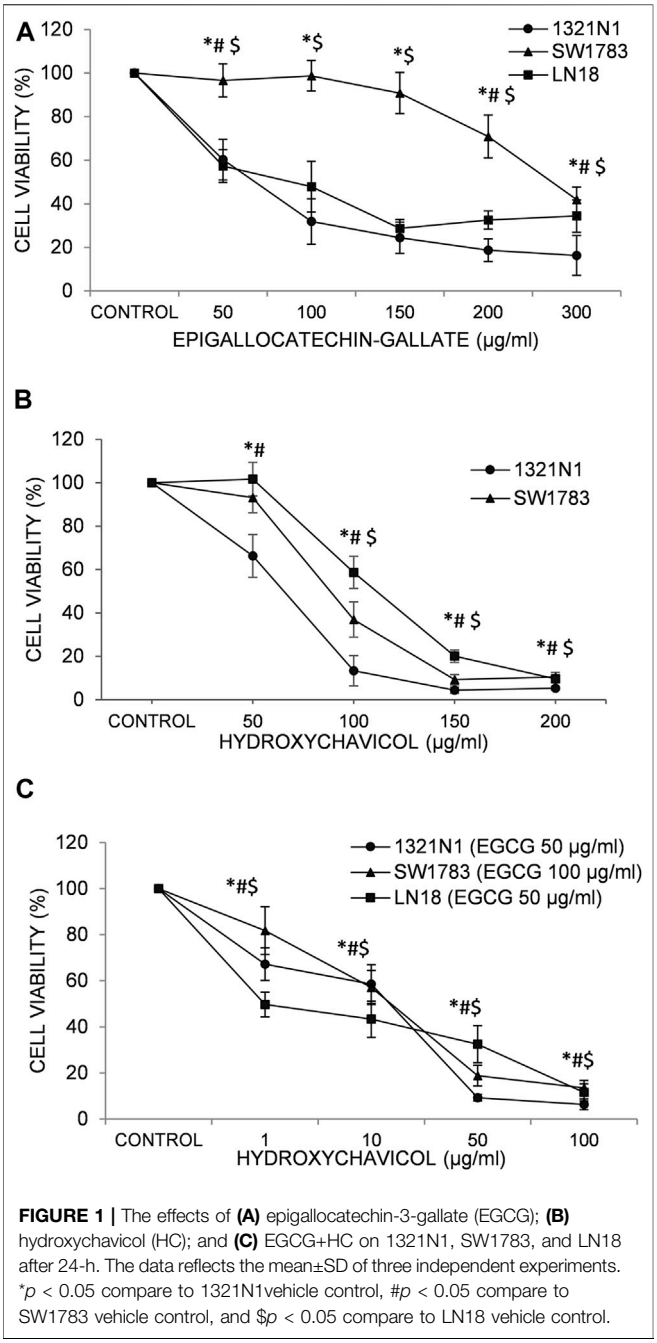


FIGURE 1 | The effects of **(A)** epigallocatechin-3-gallate (EGCG); **(B)** hydroxychavicol (HC); and **(C)** EGCG+HC on 1321N1, SW1783, and LN18 after 24-h. The data reflects the mean±SD of three independent experiments. *$p < 0.05$ compare to 1321N1vehicle control, #$p < 0.05$ compare to SW1783 vehicle control, and \$$p < 0.05$ compare to LN18 vehicle control.

90–95% inhibition respectively achieved after 24 h of treatment (**Figure 1**).

The lower IC50 values of EGCG (50/100 µg/ml; compared to the IC50 values of EGCG obtained, ranging from 82 to 302 µg/ml (**Table 1**)) were titrated on a range of HC concentrations (1–100 µg/ml). Results obtained showed lower IC50 values ranging from 10 to 25 µg/ml for HC when combined with EGCG (**Table 2**), compared to HC treatment alone on glioma cells (**Table 1**). Moreover, the combination dose of EGCG and HC were observed to induce morphological changes in the glioma cells by microscopic examination (**Figures 2A,B**).

3.2 Isobologram Analysis of Treatments

The suppression of cell viability was shown to be stronger in the combination of EGCG+HC at lower concentrations than either component alone (**Figure 1C**, **Table 2**). An isobologram plot was done for synergism analysis (**Supplementary Figure S1**) and to determine the combination index (CI). Synergistic interactions were seen in all glioma cells treated with combined sub-effective concentrations of EGCG and HC, as shown in **Table 2**, with CI = 0.88 for 1321N1, CI = 0.54 for SW1783, and CI = 0.43 for LN18 cells. The combined EGCG+HC treatment for 1321N1 is 50 µg/ml EGCG + 20 µg/ml HC, SW1783 is 100 µg/ml EGCG + 25 µg/ml HC and LN18 is 50 µg/ml EGCG + 10 µg/ml HC.

3.3 Combined EGCG+HC Induced Apoptosis by Triggering Caspase-3 Activation

The synergy effect of combined EGCG+HC on 1321N1 and SW1783 amount to multiplication of caspase-3 activation of 52 and 57% respectively than by either EGCG or HC alone (**Figure 3**). Induction of active caspase-3 in LN18 EGCG+HC treated cells was observed to be the lowest (9.4%) compared to both 1321N1 and SW1783 (**Figure 3**).

Figure 4B shows that the percentage of both early (29.4%) and late (8.3%) apoptotic cells for EGCG+HC treatment in 1321N1 cells increased significantly compared to vehicle control and EGCG, but no changes were observed when compared to HC treatment alone. An increase of early (17.1%) and late (32.5%) apoptosis in LN18 treated with combined EGCG+HC was observed when compared to vehicle control and HC alone, but no changes were observed for early apoptosis when compared to EGCG treatment alone (**Figure 4D**). While for SW1783, a significant increase was seen in the percentage of late apoptosis (64%) for cells treated with EGCG + HC compared to vehicle control, ECGC (32%) or HC (14.6%) treatment alone (**Figure 4C**).

3.4 The Effect of Combined EGCG+HC on Normal Cells

Figure 5 shows that no cytotoxicity was observed on normal cells (foreskin fibroblasts cells and WRL68) for combined EGCG+HC treatment using IC50 doses of 1321N1 (50 µg/ml EGCG + 20 µg/ml HC) and LN18 (50 µg/ml EGCG + 10 µg/ml HC) obtained from the MTS assay data. However, a significant reduction of cell proliferation was seen on normal cells using the combination dose of SW1783 (100 µg/ml EGCG + 25 µg/ml HC) (**Figure 5**). For this reason, the dose of 100 µg/ml EGCG + 25 µg/ml HC on SW1783 was excluded for further testing.

3.5 The Effect of Combined EGCG+HC on Cell Cycle Progression of Glioma Cells

The proportion of cells in G0/G1 phase reduced (35% for 1321N1 and 30.4% for LN18, respectively) in EGCG+HC treated cells when compared to vehicle control, while S phase was enhanced in 1321N1 (50.4%) and LN18 (49.9%) compared to the vehicle

TABLE 1 | 50% Inhibitory concentration (IC50) of EGCG and HC on 1321N1, SW1783 and LN18 cells. Viable cells (%) were expressed as the mean ± SD of three independent experiments.

Cell lines	Compound	IC50 value (µg/ml)	Viability (% cells)[a]
Grade II	Epigallocatechin-gallate (EGCG)	82 ± 12.31	16.3 ± 9.2
1321N1	Hydroxychavicol (HC)[b]	75 ± 7.51	5.2 ± 0.89
Grade III	Epigallocatechin-gallate (EGCG)	302 ± 9.10	41.9 ± 5.74
SW1783	Hydroxychavicol (HC)[b]	95 ± 5.83	10.5 ± 2.04
Grade II	Epigallocatechin-gallate (EGCG)	134 ± 11.36	34.4 ± 7.41
LN18	Hydroxychavicol (HC)[b]	119 ± 7.77	9.6 ± 1.66

[a]*Cell viability (%) following a 24 h treatment of the highest concentration value of each compound.*
[b]*The results for the treatment of EGCG and HC singly against the viability of glioma cells have been published previously (Abdul Rahman et al., 2014; Rahman et al., 2014).*

TABLE 2 | The ratio of combined EGCG and HC at 50% inhibitory concentration (IC50) of 1321N1, SW1783, LN18 cells and its combination index (CI).

Type of cell line	EGCG:HC	IC50[a] [µg/ml]	EGCG[b] [µg/ml]	HC[b] [µg/ml]	Combination Index[c] (CI)
1321N1	5: 2	20	82	75	0.88 ± 0.05
SW1783	4: 1	25	300	95	0.54 + 0.05
LN18	5: 1	10	134	119	0.43 + 0.06

[a]*IC50 of combined compounds.*
[b]*IC50 of compound A or B.*
[c]*CI < 1.0 indicates synergism; 0.9 < CI < 1.10, near additive; CI > 1.10 indicates antagonism.*

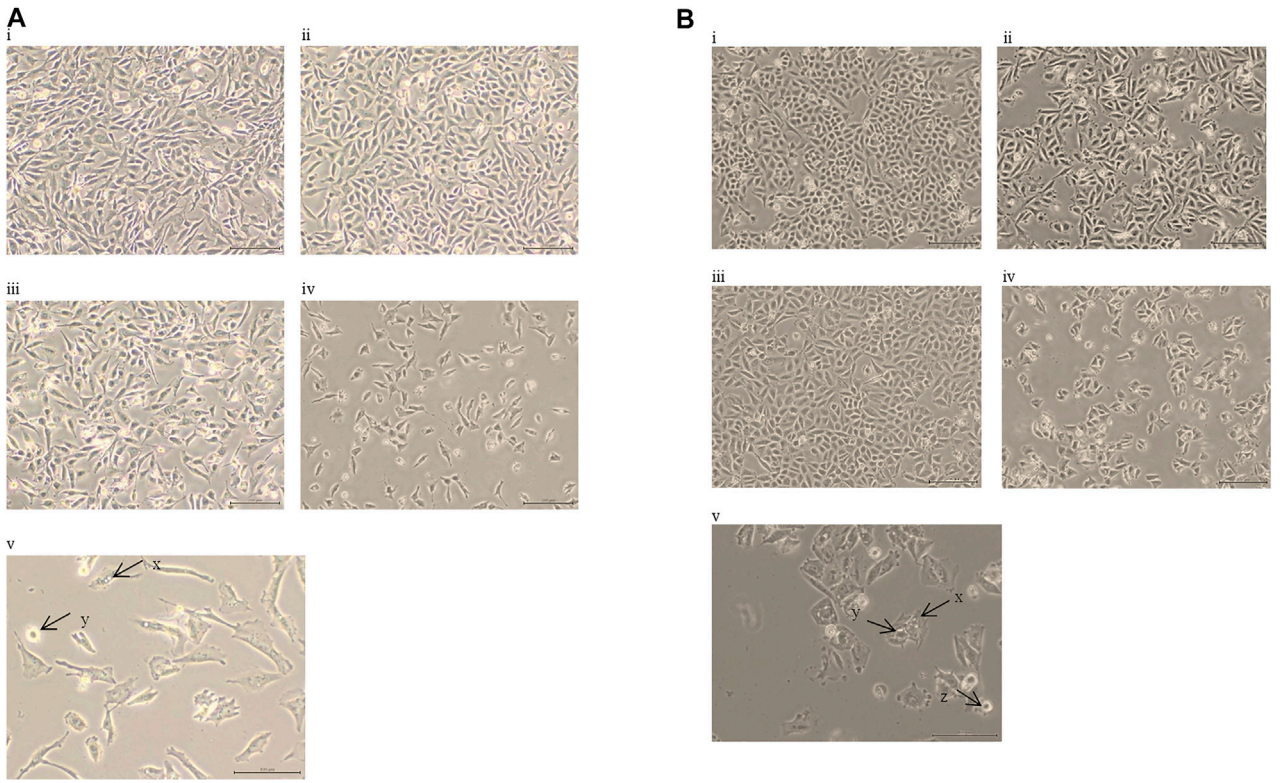


FIGURE 2 | (A) Morphological alterations in 1321N1 cells following treatment with i. vehicle control cells, ii. EGCG 50 µg/ml, iii. HC 20 µg/ml, iv. 50 µg/ml of EGCG + 20 µg/ml of HC, v. scale 100 µm; 50 µg/ml of EGCG + 20 µg/ml of HC; for 24 h (40× magnification). The arrows indicate; x. vacuolated cytoplasm and y. cell shrinkage; **(B)** Morphological alterations in LN18 cells following treatment with i. vehicle control cells, ii. EGCG 50 µg/ml, iii. HC 10 µg/ml, iv. 50 µg/ml of EGCG + 10 µg/ml of HC, v. scale 100 µm; 50 µg/ml of EGCG + 10 µg/ml of HC; for 24 h (40× magnification). The arrows indicate; x. vacuolated cytoplasm, y. the collapse of nucleus, and z. cell shrinkage.

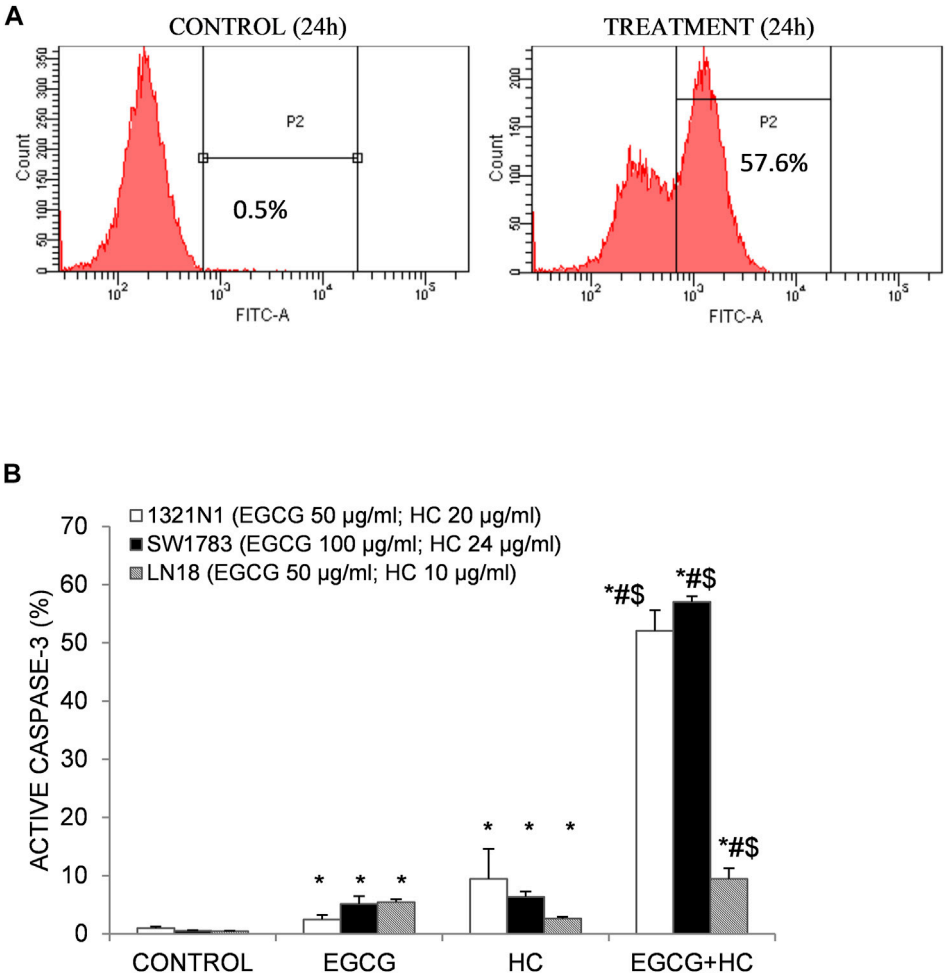


FIGURE 3 | (A) Flow cytometry detection of apoptosis following caspase-3 antibody staining. An example of an active caspase-3 detection diagram using 1321N1 cells: viable cells are indicated in the left quadrant, while active caspase-3 is displayed in the right quadrant (P2). **(B)** As evidenced by the presence of active caspase-3, combined EGCG+HC triggered stronger induction of apoptosis in 1321N1, SW1783, and LN18 cells than either EGCG/HC alone. Data represents mean±SD of three independent experiments. *p < 0.05 compare to vehicle control, #p < 0.05 compare to EGCG, $$p$ < 0.05 compare to HC.

control and EGCG alone (**Figure 6**). The G2M phase was marginally reduced (14.6%) compared to the vehicle control (**Figure 6A**).

3.6 The Effect of EGCG+HC on the Migration and Invasion of Glioma Cells

In the wound healing experiment, 1321N1 and LN18 cells treated with EGCG+HC had lower migratory potential, with only 10.9 and 14.8% of cells migrated, respectively, as compared to the vehicle control and cells treated with EGCG/HC alone (**Figure 7**). Transwell migration assays revealed a similar outcome, with less 1321N1 (34.8%) and LN18 (50.7%) EGCG+HC treated cells migrated across the membranes compared to the vehicle control and EGCG/HC alone (**Figure 8A**). A thin coating of ECM was utilised as an impediment to non-invasive cells *in vitro* in the transwell invasion experiment. As indicated in **Figure 8B**, EGCG+HC treated cells had lower percentage of 1321N1 and LN18 cell invasion (42.1 and 52.8% respectively) compared to vehicle control and EGCG/HC alone.

3.7 The Effect of EGCG+HC on Colony Formation of Glioma Cells

EGCG+HC treatment was more effective than vehicle control and EGCG/HC singly in preventing 1321N1 and LN18 cell colony formation with 10.5 and 11.8% of colonies survived, respectively (**Figure 9**).

3.8 Glioma Cell Lines Expression Profiles

Supplementary Table S1 illustrates a total of 2103 and 2442 differentially expressed genes (FDR $p \leq 0.05$ and fold change (FC) ≥ 1.5) in 1321N1 and LN18, respectively, treated with EGCG+HC compared to controls. The list of the most significantly expressed genes with the highest FC is shown in **Supplementary Tables S2A,B**. Approximately 52.8% (1321N1) and 61.5% (LN18) genes

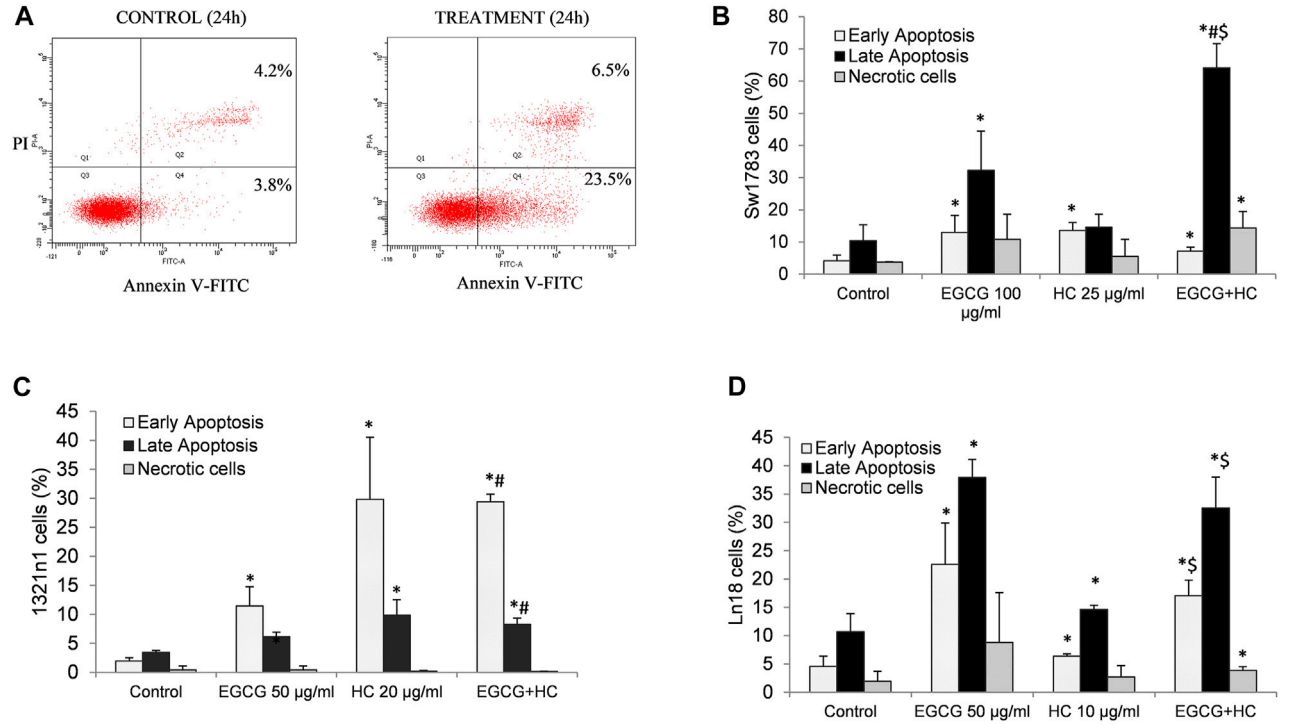


FIGURE 4 | (A) Flow cytometry detection of apoptosis following annexin V-FITC/propidium iodide (PI) labelling. The lower left quadrant (Q3) contains viable cells, the lower right quadrant (Q4) contains early apoptotic cells, the upper right quadrant contains late apoptotic cells, and the upper left quadrant contains non-viable necrotic cells (Q1). A bar graph displaying the mean values from three independent experiments for **(B)** 1321N1, **(C)** SW1783, and **(D)** LN18. *$p < 0.05$ compare to control early or late apoptosis respectively, #$p < 0.05$ compare to EGCG early or late apoptosis respectively, \$$p < 0.05$ compare to HC early or late apoptosis respectively.

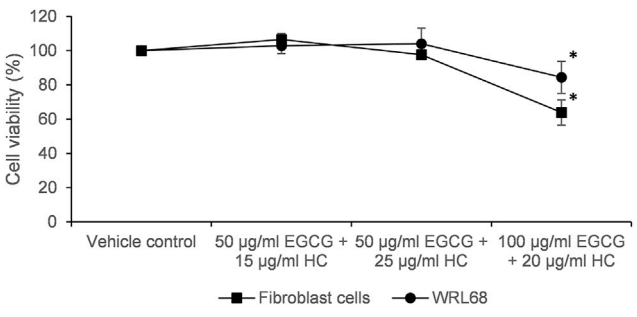
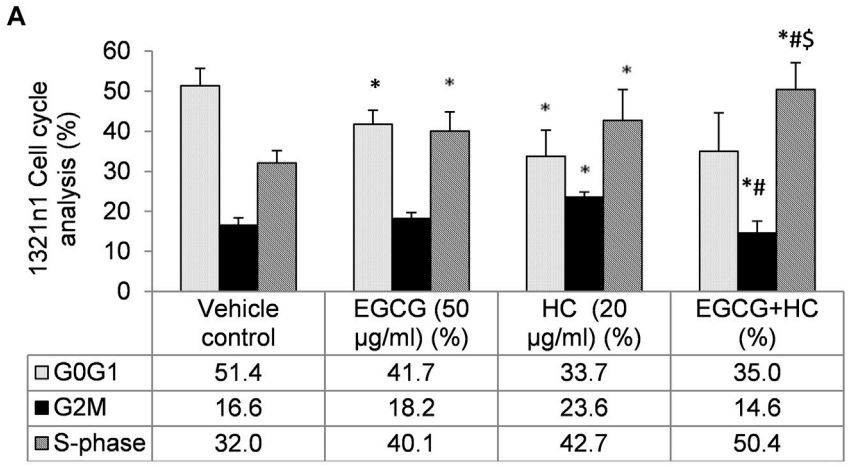


FIGURE 5 | Cytotoxicity of EGCG+HC on normal cells; foreskin fibroblast cells and WRL68 using the IC50 of combined EGCG+HC treatment: 50 µg/ml EGCG + 20 µg/ml HC (dose for 1321N1), 100 µg/ml EGCG + 25 µg/ml HC (dose for SW1783) and 50 µg/ml EGCG + 10 µg/ml HC (dose for LN18).

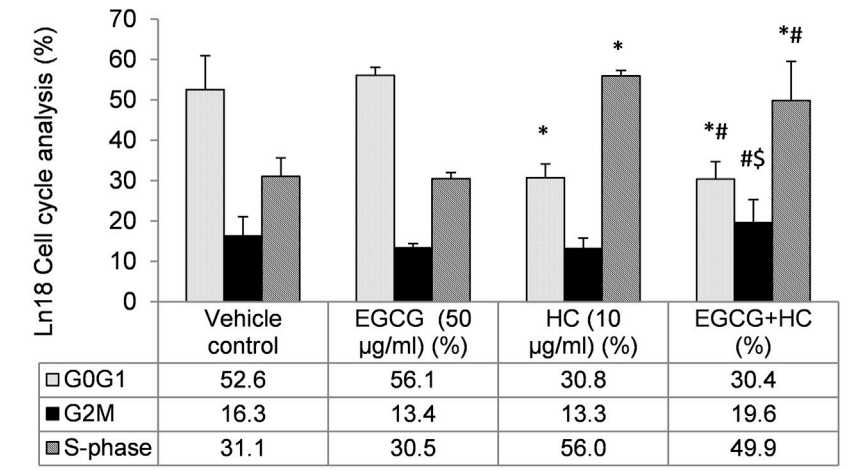
were downregulated in EGCG+HC treated cells compared to controls. According to the hierarchical clustering analysis, all of the control and treatment groups were well separated and grouped based on their expression similarity (**Supplementary Figure S2**). The red colour represented elevated genes, whereas the green colour represented downregulated genes.

3.9 Glioma Cell Lines Transcript and Alternative Splicing Expression Changes

Approximately 3782 (1321N1) and 4793 (LN18) transcripts were differently expressed in EGCG+HC treated cells when compared to controls (FDR $p \leq 0.05$ with fold change (FC) ≥ 1.5) (**Supplementary Table S3**). The list of the most significantly expressed genes with the highest FC is shown in **Supplementary Tables S4A,B**. The results revealed that around 27% of transcripts in both cell lines were selectively expressed, with no alterations seen in genes corresponding to the transcripts expressed (**Supplementary Table S5**). The Partek® Genomics Suite and Tuxedo were used to evaluate the transcripts implicated in alternative splicing events (**Supplementary Table S6**). The alternative splicing expression generated from Partek ∩ Tuxedo ∩ transcript (FC ≥ 1.5) is provided in the **Supplementary Tables S7A,B**. Alternative splicing expression of DYNLL1 (downregulated) and DDX39B (upregulated) transcript were altered in 1321N1, whereas RBMX (downregulated) and



	Vehicle control	EGCG (50 μg/ml) (%)	HC (20 μg/ml) (%)	EGCG+HC (%)
G0G1	51.4	41.7	33.7	35.0
G2M	16.6	18.2	23.6	14.6
S-phase	32.0	40.1	42.7	50.4



	Vehicle control	EGCG (50 μg/ml) (%)	HC (10 μg/ml) (%)	EGCG+HC (%)
G0G1	52.6	56.1	30.8	30.4
G2M	16.3	13.4	13.3	19.6
S-phase	31.1	30.5	56.0	49.9

FIGURE 6 | The distribution of **(A)** 1321N1 and **(B)** LN18 cells in the cell cycle phases. The data indicate the mean±SD ($n = 3$). *$p < 0.05$ compare to vehicle control, #$p < 0.05$ compare to EGCG, \$$p < 0.05$ compare to HC.

SEC31A (downregulated) transcript were altered in LN18 treated with EGCG+HC.

3.10 Pathway Analysis and Functional Enrichment

Table 3 (GSEA)summarised the biological pathways and the number of genes implicated (control vs treatment). The most significant biological pathway that is upregulated in EGCG+HC treated cells is the endoplasmic reticulum (ER) unfolded protein response (UPR), followed by the activation of the inflammatory response pathway. EGCG+HC downregulated pathways such as mitotic cell cycle control, telomere maintenance, and DNA repair. FET analysis (gene dataset (*$p < 0.05$, FDR)*) reveals that the apoptotic process, axon guidance and cell cycle arrest were the most substantially enriched BP in EGCG+HC treated groups (**Table 4**). The selective effects of EGCG+HC on various gene targets in each cell line are most likely responsible for the differences in cellular response seen between the glioma cell lines. Subnetwork studies of gene lists, for example, revealed that major regulator genes such as *MYC, TGFB1, EGR1,* and *KLF4* were present in 1321N1 cell lines treated with EGCG+HC *($p < 0.05$, FDR). E2F4, MTOR, E2F1*, and *BRCA* were among the LN18-specific central regulator genes (**Table 5**).

3.11 RNA-Seq Data Validation

qPCR was used to validate 14 genes from lists of biological processes provided by GSEA, FET, and analysis of gene regulatory subnetworks, as well as three transcripts

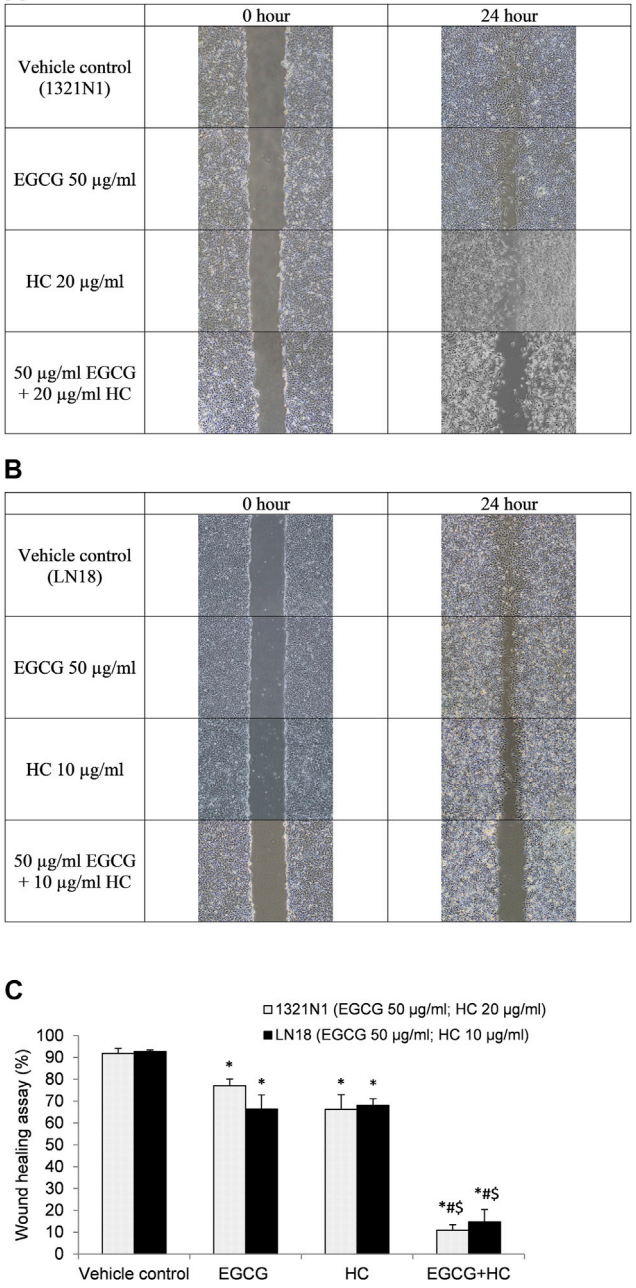


FIGURE 7 | Wound healing assay. The photo was taken at 0- and 24-h following the scratch test. **(A)** 1321N1 cells were treated for 24-h 50 μg/ml EGCG, 20 μg/ml HC, or EGCG+HC, and **(B)** LN18 cells were treated for 24-h with 50 μg/ml EGCG, 10 μg/ml HC, or EGCG+HC. **(C)** The percentage of wound healing closure (%) of 1321N1 and LN18 treated with EGCG, HC, and EGCG+HC. *$p < 0.05$ compare to control, #$p < 0.05$ compare to EGCG, \$$p < 0.05$ compare to HC.

implicated in alternative splicing events in LN18. Additionally, 13 genes were validated which included three transcripts implicated in alternative splicing events in 1321N1. All the genes and conditions tested were parallel with RNA-seq results (**Figure 10**).

4 DISCUSSION

The purpose of combination therapy is to achieve a synergistic therapeutic effect using lower doses to lessen the toxicity of each agent, and to slow down the induction of drug resistance (Chou, 2010; Zhao et al., 2020) since multiple signal pathways are targeted during the treatment. Previous research found that EGCG increased the efficacy of temozolomide and metformin in U87MG cells and rat C6 glioma cell lines, indicating that EGCG might be a useful adjuvant for cancer chemoprevention (Kuduvalli et al., 2021). Recent literature suggests that HC synergizes with buthionine sulfoximine (BSO), a glutathione synthesis inhibitor to eliminate chronic myeloid leukemic (CML) cells through the GSH-ROS-JNK-ERK-iNOS mediated pathway (Chowdhury et al., 2013) and reports have suggested that HC may be developed as a single-agent chemotherapeutic drug or as an adjuvant (Gundala et al., 2014). However, the effects of EGCG or HC on different stages of glioma cancer cell growth inhibition have not been compared. Furthermore, there has been little research on the interactions of these bioactives, as well as their mode of action on glioma cell death and other inhibitory pathways.

While the concentration of EGCG+HC utilised for 1321N1 and LN18 cells was not harmful to normal WRL68 and normal foreskin fibroblast cells, normal cell proliferation was significantly reduced when treated with the combination dose of SW1783 (100 μg/ml EGCG + 25 μg/ml HC). Different responses to EGCG+HC treatment suggested that the mechanisms by which the combined EGCG+HC act differ in 1321N1, SW1783, and LN18 due to each cell line's unique mutation. The grade II 1321N1 and grade III SW1783 cell lines both had mtDNA mutations in the coding region, which controls the expression of respiratory complex genes (Soon et al., 2017). They were shown to have decreased mitochondrial activity, and 1321N1 cells were found to have high oxidative stress level. Interestingly, Grade IV LN18 cells do not have any non-synonymous mtDNA mutations and possess high antioxidant capability (Soon et al., 2017). As cancer cells have higher ROS concentrations than normal cells, a high polyphenol concentration is required to enhance the baseline level ROS formation and tilt the redox balance in cancer cells to induce cell death (Harris and Denicola, 2020). The combined dose of 100 μg/ml EGCG + 25 μg/ml HC for SW1783 may have elevated ROS generation over the baseline level of normal cells, disrupting the homeostatic balance of ROS and ultimately resulting in cytotoxicity in normal cells.

Phenolic compounds with pyrogallol groups (EGCG) and/or catechol (HC) are known for their antioxidative effects (Almatroodi et al., 2020; Zamakshshari et al., 2021) as well as their pro-oxidative properties (Gundala et al., 2014; Eghbaliferiz and Iranshahi, 2016; Chen et al., 2020). The utility of antioxidants as an adjuvant with conventional chemotherapy in cancer patients is debatable (Saeidnia and Abdollahi, 2013), due to research indicating that antioxidants may protect cancer cells and impair the efficiency of cytotoxic treatment (Khurana et al., 2018). For example, excessive dosages of beta carotene or vitamin E activity can hasten the progression of lung cancer in smokers

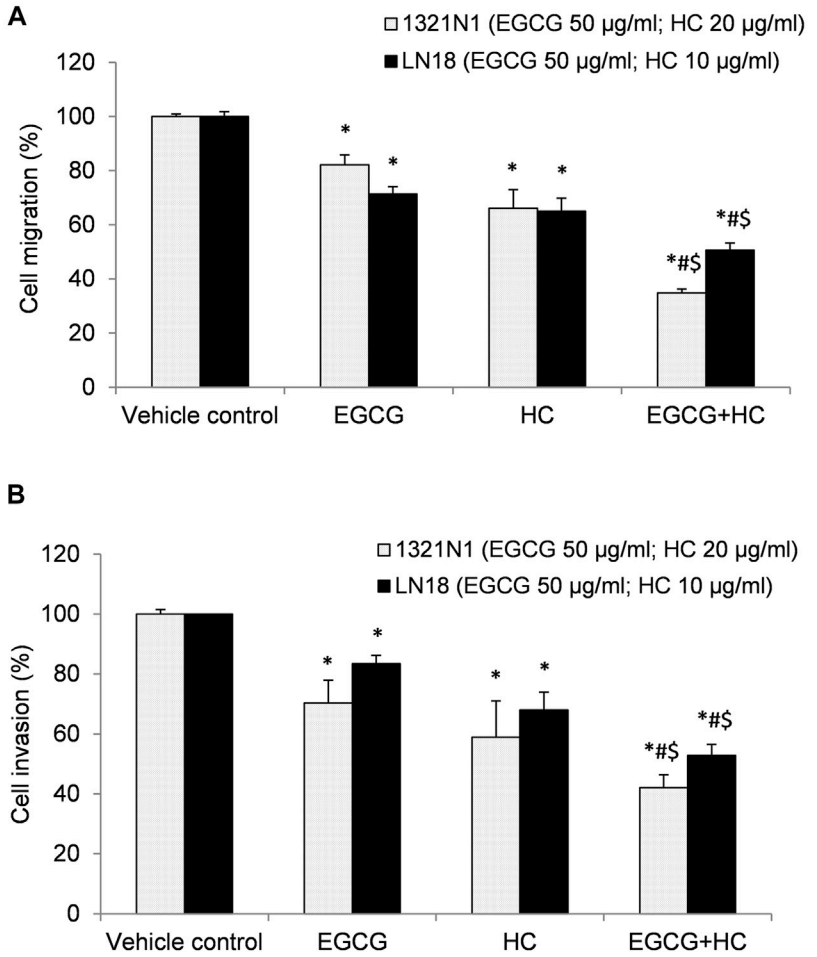


FIGURE 8 | (A) Cell migration, and **(B)** cell invasion of 1321N1 and LN18 treated with EGCG+HC. The proportion of cell migration/invasion was represented as a percentage of the vehicle control. Each bar reflects the mean ± SD determined from three independent experiments. *$p < 0.05$ compare to control, #$p < 0.05$ compare to EGCG, \$$p < 0.05$ compare to HC.

(Middha et al., 2019). In contrast, vitamin C and E supplementation may be beneficial in preventing against chemotherapy-related adverse effects (Suhail et al., 2012; Roa et al., 2020). On the other hand, besides their dual roles in scavenging and/or utilizing reactive oxygen species (ROS) to kill cancer cells (Kirtonia et al., 2020), dietary antioxidants possess other anticancer effects as shown by EGCG inhibiting human lymphoma cell proliferation by modulating the epigenetic modification of p16INK4a (Wu et al., 2013), and halting the proliferation of triple-negative breast cancer cells via epigenetic changes of cIAP2 gene (Steed et al., 2020).

In the present study, EGCG+HC treatment inhibited the proliferation of glioma cells, by arresting these cells in the S phase and decreasing the G0/G1 phase to a greater extent than either agent alone. Shen et al. (2014) showed that EGCG (<60 μg/ml) induced apoptosis and cause S phase arrest in hepatocellular carcinoma via the supression of Akt pathway. Similarly, the induction of apoptosis in EGCG treated HT-29 colon cancer cells was reported to involve the p38MAPK activity and Akt pathways (Cerezo-Guisado et al., 2015). Meanwhile, HC was shown to be effective in halting the cell cycle progression of prostate cancer and oral KB carcinoma cells (Gundala et al., 2014). Our results further reveal that EGCG+HC inhibit the migration, invasion, and colony formation of 1321N1 and LN18. Consisent with our findings, EGCG has been shown to suppress A549 lung cancer cell growth and reduce vascular endothelial growth factor (VEGF) expression suggesting its role in the suppression of angiogenesis (Sakamoto et al., 2013). The invasion inhibitory properties of EGCG on thyroid carcinoma 8505C cells was reported via the TGF-β1/Smad signaling through the decrease of epithelial to mesenchymal transition (EMT) markers (Li et al., 2019), while the inhibition of invasion and migration of HeLa, cervical cancer cells were through the modulation of MMP-9 and TIMP-1 (Sharma et al., 2012). Moreover, HC was reported to inhibit the colony formation of prostate cancer cells (Gundala et al., 2014). Limited information is available on the ability of HC to halt the migration/invasion of cancer cells.

Our transcriptomic analysis demonstrated that the molecular mechanism of EGCG+HC against glioma cells is via the down

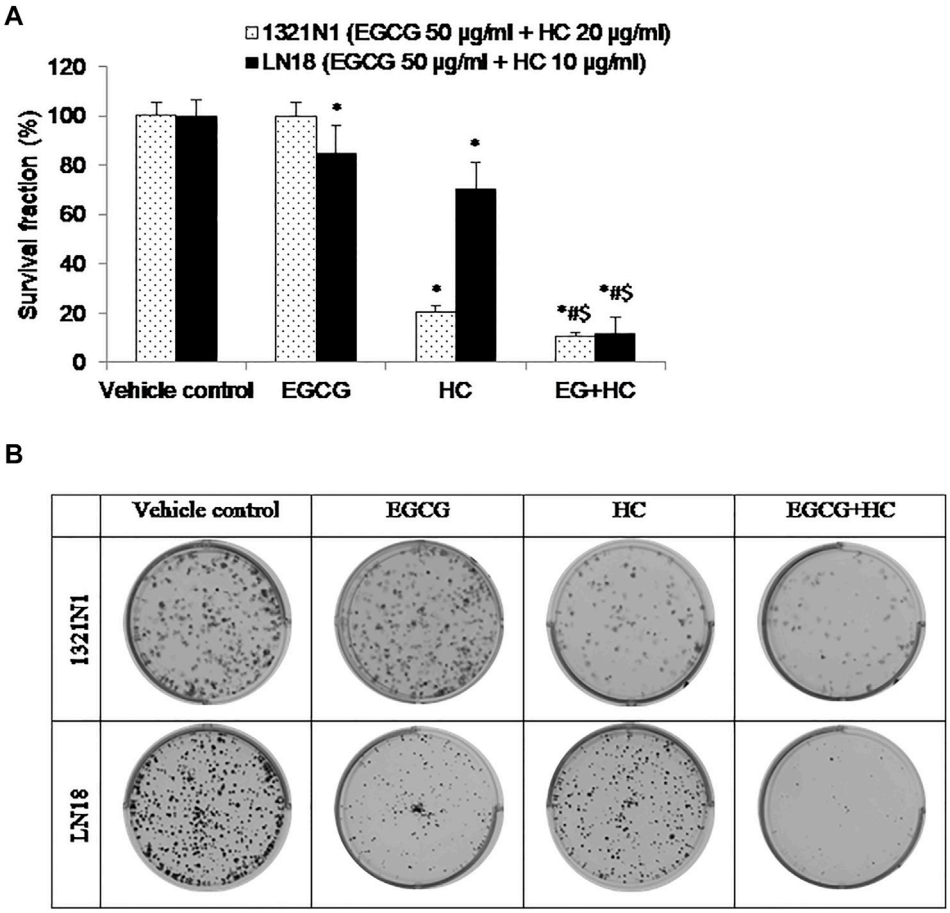


FIGURE 9 | (A) The bar graph depicts the suppression of glioma cell colony formation by EGCG, HC, and EGCG+HC in 1321N1 and LN18 cells. **(B)** 1321N1 and LN18 cell colonies treated with EGCG, HC, and EGCG+HC. Each bar reflects the mean±SD determined from three independent experiments. *$p < 0.05$ compare to control, #$p < 0.05$ compare to EGCG, \$$p < 0.05$ compare to HC.

TABLE 3 | Gene set enrichment analysis (GSEA). Positive median changes imply increased biological process regulation, whereas negative median changes indicate decreased biological process regulation in cells treated with EGCG+HC vs vehicle control cells.

Gene Significant Enrichment Analysis (GSEA)	1321N1 (no. entities)	1321N1 Median Change	1321N1 *p*-value	LN18 (no. entities)	LN18 Median Change	LN18 *p*-value
endoplasmic reticulum unfolded protein response	80	125.41	0.00E+00	81	127.65	0.00E+00
inflammatory response	253	104.68	9.00E-04	n/a	n/a	n/a
activation of signaling protein activity involved in unfolded protein response	62	103.14	5.00E-04	63	105.67	0.00E+00
immune response	252	102.46	1.40E-03	n/a	n/a	n/a
intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress	25	102.11	6.00E-04	25	65.48	1.20E-03
cellular metabolic process	132	−47.04	0.00E+00	843	−123.598	0
axon guidance	316	−28.946	0	320	−77.104	0
mitotic cell cycle	387	−108.81	0.00E+00	388	−225.07	0.00E+00
telomere maintenance	54	−53.25	0.00E+00	54	−94.39	0.00E+00
cell division/cytokinesis	88	−51.06	0.00E+00	52	−86.52	0.00E+00
DNA repair	270	−49.21	0.00E+00	270	−154.39	0.00E+00

TABLE 4 | Fisher exact test (FET) analysis on gene dataset of $p < 0.05$, FDR.

Biological process/signalling pathway	1321N1 No. of overlapping entities	1321N1 *p*-value	LN18 o. of overlapping entities	LN18 *p*-value
positive regulation of apoptotic process/apoptotic process	50	2.95E-10	137	5.68902E-14
axon guidance	57	3.20E-10	60	4.47362E-08
cell cycle arrest	29	1.31E-07	33	1.07385E-07
cellular protein metabolic process	78	4.30E-16	119	2.15597E-33
endoplasmic reticulum unfolded protein response	23	8.59E-09	32	1.24068E-13
mitotic cell cycle	91	4.1441E-31	128	7.19555E-52
mRNA metabolic process	48	5.29E-12	90	5.3289E-37
mRNA splicing, via spliceosome	47	5.10E-16	64	1.57876E-24
negative regulation of cell growth	34	7.11E-11	22	0.003878426
nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	26	4.4963E-07	58	7.18064E-29
response to DNA damage stimulus	46	8.6222E-05	78	6.22536E-14
response to drug	71	1.36E-08	76	1.41321E-06
small molecule metabolic process	155	1.46E-11	202	2.82555E-17
SRP-dependent co-translational protein targeting to membrane	20	6.1778E-05	58	4.58081E-33
Actin Cytoskeleton Regulation	86	2.78E-03	100	0.013074903
Focal Adhesion Regulation	50	2.94E-02	n/a	n/a
Hedgehog Pathway	92	1.24E-02	120	0.001067468
Insulin Action	n/a	n/a	199	2.90484E-10
Notch Pathway	n/a	n/a	248	0.010106517

TABLE 5 | Subnetwork enrichment analysis on gene dataset of $p < 0.05$, FDR.

Expression target	1321N1 No. of overlapping entities	1321N1 *p*-value	LN18 No. of overlapping entities	LN18 *p*-value
MYC	113	2.93E-17	n/a	n/a
AKT1	137	2.35E-16	136	5.16E-10
TGFB1	228	3.31E-13	n/a	n/a
HIF1A	117	1.98E-12	117	1.28E-07
EGR1	77	4.94E-12	n/a	n/a
JUN	105	5.20E-12	n/a	n/a
NFE2L2	72	7.90E-12	75	2.05E-09
KLF4	53	9.72E-11	n/a	n/a
MAP2K1	54	1.95E-10	n/a	n/a
FGF2	107	3.23E-09	n/a	n/a
ATF4	37	3.36E-09	37	3.96E-07
PARP1	41	4.13E-09	n/a	n/a
FOXM1	46	2.05E-08	n/a	n/a
EIF2AK3	27	2.60E-08	25	1.19E-05
NFYA	26	3.20E-08	n/a	n/a
EDN1	60	4.08E-08	n/a	n/a
ATF3	32	4.44E-08	n/a	n/a
VEGFA	76	6.04E-08	n/a	n/a
E2F4	n/a	n/a	27	1.04E-08
MTOR	n/a	n/a	88	1.24E-08
E2F1	n/a	n/a	74	5.72E-08
BRCA1	n/a	n/a	34	8.09E-08
SRSF3	n/a	n/a	13	6.18E-07
HSP90AA1	n/a	n/a	38	3.00E-06
CUL4A	n/a	n/a	11	8.03E-06
VCP	n/a	n/a	13	1.66E-05
CAPN2	n/a	n/a	16	2.17E-05
CDC20	n/a	n/a	12	2.93E-05
CUL1	n/a	n/a	12	2.93E-05
EGFR	n/a	n/a	70	3.17E-05
DDIT3	n/a	n/a	28	3.68E-05
NRG1	n/a	n/a	40	4.68E-05
SOD1	n/a	n/a	20	6.78E-05
SREBF1	n/a	n/a	41	6.83E-05

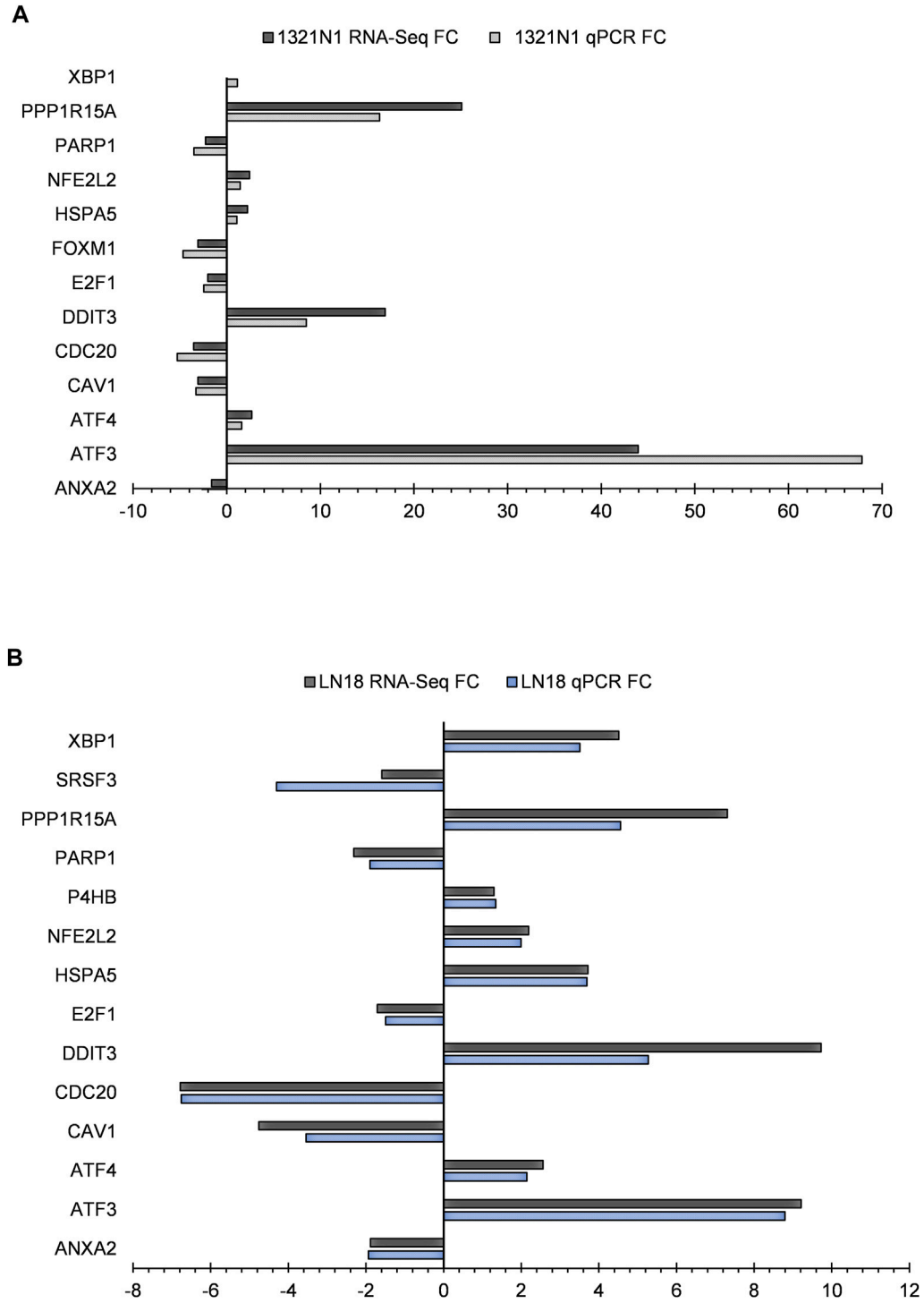


FIGURE 10 | RNA-seq gene expression and genes confirmed by qPCR in EGCG+HC treated cells: **(A)** 1321N1 and **(B)** LN18.

regulation of axon guidance and metabolic pathways. The mechanism of EGCG+HC may be through the downregulation of SEMA3A and SEMA3F transcript expression which may play some roles in inhibiting the glioma proliferation and halts invasion via Plexin A1 (PLXNA1) and B2 (PLXNB2) receptors. Prior research has shown that Semaphorin 3A (SEMA3A), which is known for its axon guidance and antiangiogenic properties, has been implicated in glioblastoma development. Interestingly, SEMA3A was reported to inhibit BTSC proliferation, while inducing invasion where its action is

dependent on NRP1 or PLXNA1 receptors. On the other hand, a decrease in SEMA3A receptor expression is enough to stop proliferation and enhance invasion (Higgins et al., 2020). High SEMA2A and PLXNA1 expression are all associated with poorer overall survival in GBM. Similarly, PLXNB2 which was found to be downregulated with EGCG+HC in this study, is recognized a potential biomarker for high-grade glioma. Its knockdown was reported to halt malignant glioma invasion and perivascular diffusion (Le et al., 2015; Huang et al., 2021). Besides the family of semaphorins mentioned above, SEMA7A, downregulated in both 1321N1 and LN18 cells in this study, plays a significant role in mediating the cross-talk between exosomes produced by glioma stem cells (GSC) and the glioma microenvironment (Manini et al., 2019). This further emphasize the axon guidance pathway as an interesting new therapeutic target to curb glioma progression.

Exosomes have been shown to act as signaling mediators of the tumor microenvironment (TME) regulation. Studies indicated that exosomes may transport functional molecules to the recipient cells and aid cancer growth by altering the metabolism of cancer cells and nearby stromal cells (Yang et al., 2020). We postulate that EGCG+HC treatment may inhibit the glioma cancer cells by obstructing the cells' metabolic reprogramming hence depriving these fast-growing cells of their energy demands. On a similar note, MT-CO3 (down regulated in this study), also involved in the metabolism process specifically the oxidative phosphorylation, influence abnormal energy metabolism and facilitate the growth of tumor cells. Levodopa was shown to inhibit the proliferation of esophageal squamous cell carcinoma (ESCC) via down-regulating the levels of oxidative phosphorylation proteins which includes MT-CO3, SDHD and NDUFS4 (Li et al., 2020). This inhibition is related to mitochondrial dysfunction. Interestingly, miR-5787 was suggested to regulate cisplatin chemoresistance of tongue squamous cell carcinoma (TSCC) by downregulating MT-CO3 which in turn disrupted glucose metabolism (Chen et al., 2019). MT-RNR2, upregulated in both 1321N1 and LN18 cells, is linked to anti-apoptotic activities in bladder cancer (Omar et al., 2017). Although the Warburg theory indicated decreased reliance on mitochondrial function may enhance resistance to apoptosis, studies on the association of mitochondrial genes in cancer progression is limited and this warrants further research (Beadnell et al., 2018).

Genes related to endoplasmic reticulum unfolded protein response (ER UPR) (DDIT3, ATF4, EIF2AK3, XBP1) were mostly found to be upregulated in response to EGCG+HC treatment. UPR plays a vital role in malignant transformation, as well as the regulation of cancer migration and invasion (Limia et al., 2019). Emerging reports have shown the importance of inducing ER stress pathway in cancer treatments, for example, the combination of lopinavir and ritonavir, a protease inhibitor, promotes urological cancer cell death (Okubo et al., 2019). Our previous study has also shown that ER UPR was induced in 1321N1, SW1783 and LN18 cells treated with combined gamma-tocotrienol and hydroxychavicol (Abdul Rahman et al., 2019). Although ER stress seemed to have pertinent role in the anticancer properties of EGCG+HC, further investigation is needed to elucidate whether this induction crosstalk with ROS production or autophagy to unveil the potential regulatory mechanisms of ER-UPR for therapeutic purposes.

Transcriptomic data provides an enormous set of data that can be analysed simultaneously. Our findings are far from exhaustive and may be further explored in terms of the long noncoding genes and alternative splicing expression patterns. For instance, DYNLL1 alternative splicing expression, which is significantly decreased in EGCG+HC treated glioma cells, is reported to be upregulated in gastric cancer high-risk group patients and hepatocellular cancer (Berkel and Cacan, 2020) (Li et al., 2021). How DYNLL1 promotes aberrant transcription in cancers are still unknown.

Despite the fact that only three types of cell lines were examined in this study, the mutations in these cell lines are diverse and reflect different grades of glioma malignancy. Our *in vitro* findings highlighted important points concerning personalised medicine; different dose combinations are required for different grades/mutations, and an increase in grade does not always necessarily require an increase in treatment concentration (50 ug/ml EGCG + 20 ug/ml HC for grade II 1321N1; 50 ug/ml EGCG + 10 ug/ml HC for grade IV LN18). However, insufficient drug exposure was suggested to be one of the contributing factors to the development of resistance to RTK-targeted therapies in glioblastoma due to the heterogeneous expression of the epidermal growth factor receptor (EGFR) (Furnari et al., 2015). Our findings warrant further elucidation on the significance of having specific treatment doses for different glioma grades.

5 CONCLUSION

EGCG+HC can potentiate the S phase arrest and induce the activation of caspase-3 to initiate apoptosis and inhibit the cell proliferation of 1321N1 and LN18 glioma cells. Furthermore, the strong inhibition of migration, invasion and colony formation in EGCG+HC treated cells indicated enhanced efficacy of combined EGCG and HC compared to single treatments. EGCG+HC exerted its apoptotic effect through the alteration of mitochondrial genes and metabolic pathways, while simultaneously interfering with endoplasmic reticulum unfolded protein response and axon guidance pathway. Crosstalk between activated pathways might be a significant regulator of glioma cell response to EGCG+HC treatment, making it a promising therapeutic target. Further research on the possible interactions between metabolic pathway, endoplasmic reticulum unfolded protein response, and axon guidance signalling in glioma is required.

DATA AVAILABILITY STATEMENT

The RNA-seq dataset presented in this study have been deposited in the NCBI Gene expression omnibus (GEO) database and are accessible through the accession number GSE193838 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE193838).

AUTHOR CONTRIBUTIONS

AR is the first author and performed all the experiments, drafted and revised the manuscript and manuscript correction. WN conceived the idea, designed the experimental plan, revised the whole manuscript and provided supervision. RH and SM provided technical support and data analysis assistance. RJ and NM contributed toward study design and general supervision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.844199/full#supplementary-material

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frontiers in Pharmacology

REVIEW
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A Comprehensive Review on the Therapeutic Potential of *Curcuma longa* Linn. in Relation to its Major Active Constituent Curcumin

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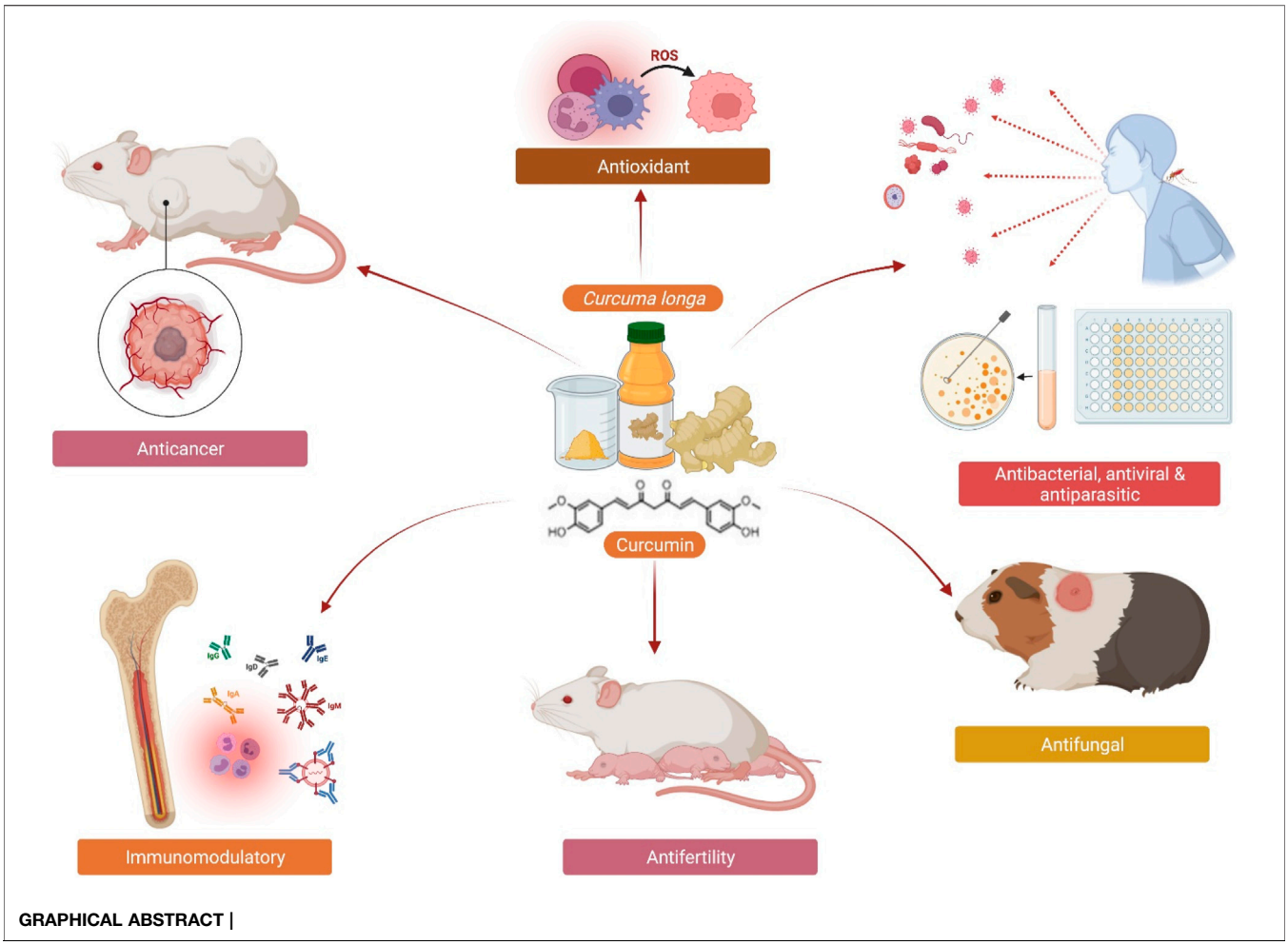
Curcuma longa Linn. (*C. longa*), popularly known as turmeric, belongs to the Zingiberaceae family and has a long historical background of having healing properties against many diseases. In Unani and Ayurveda medicine, *C. longa* has been used for liver obstruction and jaundice, and has been applied externally for ulcers and inflammation. Additionally, it is employed in several other ailments such as cough, cold, dental issues, indigestion, skin infections, blood purification, asthma, piles, bronchitis, tumor, wounds, and hepatic disorders, and is used as an antiseptic. Curcumin, a major constituent of *C. longa*, is well known for its therapeutic potential in numerous disorders. However, there is a lack of literature on the therapeutic potential of *C. longa* in contrast to curcumin. Hence, the present review aimed to provide in-depth information by highlighting knowledge gaps in traditional and scientific evidence about *C. longa* in relation to curcumin. The relationship to one another in terms of biological action includes their antioxidant, anti-inflammatory, neuroprotective, anticancer, hepatoprotective, cardioprotective, immunomodulatory, antifertility, antimicrobial, antiallergic, antidermatophytic, and antidepressant properties. Furthermore, in-depth discussion of *C. longa* on its taxonomic categorization, traditional uses, botanical description, phytochemical ingredients, pharmacology, toxicity, and safety aspects in relation to its major compound curcumin is needed to explore the trends and perspectives for future research. Considering all of the promising evidence to date, there is still a lack of supportive evidence especially from clinical trials on the adjunct use of *C. longa* and curcumin. This prompts further preclinical and clinical investigations on curcumin.

Keywords: *Curcuma longa*, curcumin, phytochemical, pharmacology, toxicology



GRAPHICAL ABSTRACT |

INTRODUCTION

Herbs and natural products have long been exploited by humans to combat numerous diseases since the dawn of time. The Indian subcontinent boasts diverse flora, including both aromatic and therapeutic species. After all, contributions should be made to analyze, standardize, and confirm Unani and Ayurvedic medication for potential, safety, and effectiveness prior to actually introducing them to the market as first-line drug delivery. Plant-based therapies are being used across all civilizations. Plant-based medicines are already extensively utilized, and several countries invest 40%–50% of their total health budget to produce novel drugs. Herbal medicines are assumed to have a beneficial effect on health without any side effects.

The genus *Curcuma* has been employed from many years back due to its medicinal applications; it is composed of approximately 133 species worldwide. *C. longa* (Haridra), *C. aromatica* Salisb (Vana Haridra), *C. angustifolia* Roxb., *C. zanthorrhiza* Roxb., *C. amada* Roxb (Amaragandhi Haridra), *C. caesia* Roxb (Kali Haridra), and *C. zedoaria* Rosc (Zedoary) are common species of genus *Curcuma* found in several regions of the globe. *Curcuma longa* Linn. (*C. longa*) is the common tall herb that flourishes in tropical as well as in other Indian regions and is referred to as "Indian saffron or The Golden Spice of India" because of its use in a broad range of diseases in Indian homes as a spice, food preservative, and coloring source. *C. longa* belonging to the Zingiberaceae (ginger) family is a perennial plant commonly planted in Asian nations. It is among the oldest spices of India that have been used in Western and Southern parts for centuries, and is a significant part of Ayurvedic medicine. In Ayurveda, the therapeutic effects of *C. longa* have been well established and are discussed in Dashemani Lekhaniya (emaciating), Kusthagna (anti-dermatosis), and Visaghna (anti-poisonous) texts. It is known by many distinct names such as Haridra in Sanskrit, Haldi in Hindi, Jianghuang (yellow ginger in Chinese), manjal in South India, and Kyoo or Ukon in Japanese, which means an effective medication for jaundice (Sharma, 2000). *C. longa* is also extensively described in Indian material medica (Dravyaguna Shastra) and is used in the beauty regimen of Hindu girls where it is applied daily on their foreheads. The bride is smeared with a *C. longa* paste, which is a key aspect of

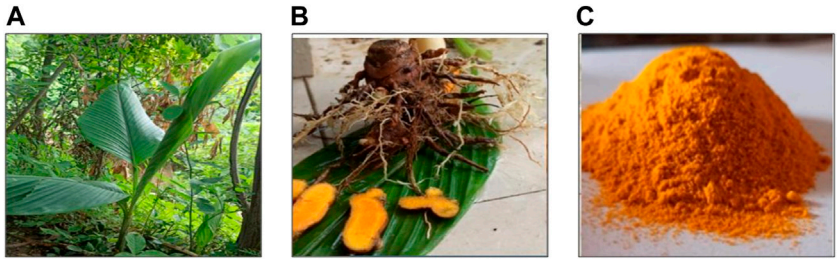


FIGURE 1 | Important parts of *C. longa*. **(A)** *C. longa* in natural habitat, **(B)** medicinally important part of *C. longa* (rhizome), and **(C)** powder of dried rhizome of *C. longa* (used as a coloring agent in food).

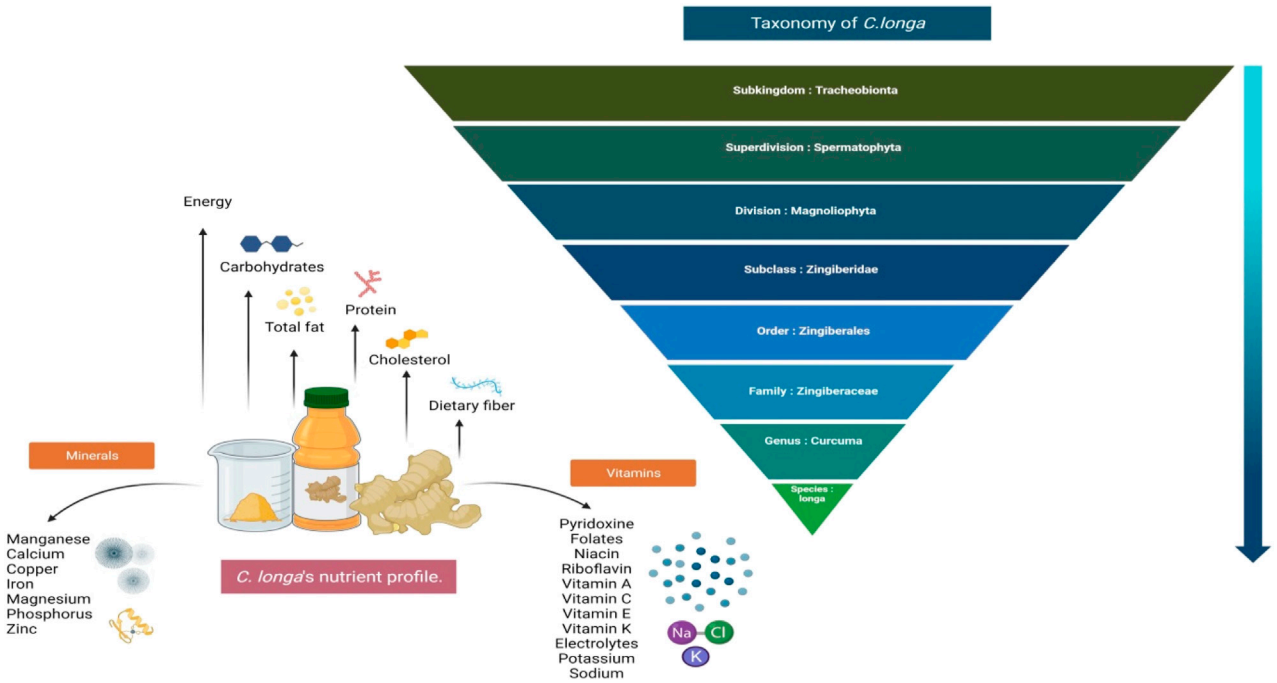


FIGURE 2 | The taxonomic classification and nutritional profile of *C. longa*.

Hindu tradition (Paranjpe and Pranjpe, 2001). It is well recognized by the Chinese, Japanese, and Korean Pharmacopoeias, and its application spans a broad range of medical conditions. In China, it is being used to relieve urticaria, dermatitis, hepatitis infection, inflammatory joints, sore throat, and wounds. It was mentioned in Hindu Mythology manuscripts as an aromatic stimulant and carminative. Turmeric powder combined with calcium hydroxide is indeed a popular home remedy for treating sprains and swelling induced by wounds or might be applied directly over the injury site. Traditional medicine has exploited dried curcumin powder to treat illnesses in history. *C. longa* is said to have antitoxic, anticancer, antibacterial, anti-inflammatory, and antioxidant effects (Ghotaslou et al., 2017). The tuberous rhizome from which *C. longa* is formed has a coarse and segmented skin. In the ground soil, the rhizomes mature underneath the foliage. The matured rhizomes have a yellowish-brown color with a dull orange from inside. Small pointed or tapered tubers sprout off the main rhizome measuring 2.5–7.0 cm (1–3 inches) in length and 2.5 cm (1 inch) in diameter (Prasad and Aggarwal, 2011). The dry rhizome is ground into a yellow powder form that has a bitter, yet sweet taste. A yellow-colored substance derived from the rhizome is curcumin (1,7-bis[4-hydroxy-3- methoxyphenyl]-1,6-heptadiene-3,5-dione), a combined form of resin and oil. Rhizome powder is supposed to flavor various cuisines and treat numerous disorders, including inflammation, flatulence, jaundice, menstrual troubles, hematuria, and hemorrhage. It is also a useful ointment to treat several skin disorders. Curcumin or

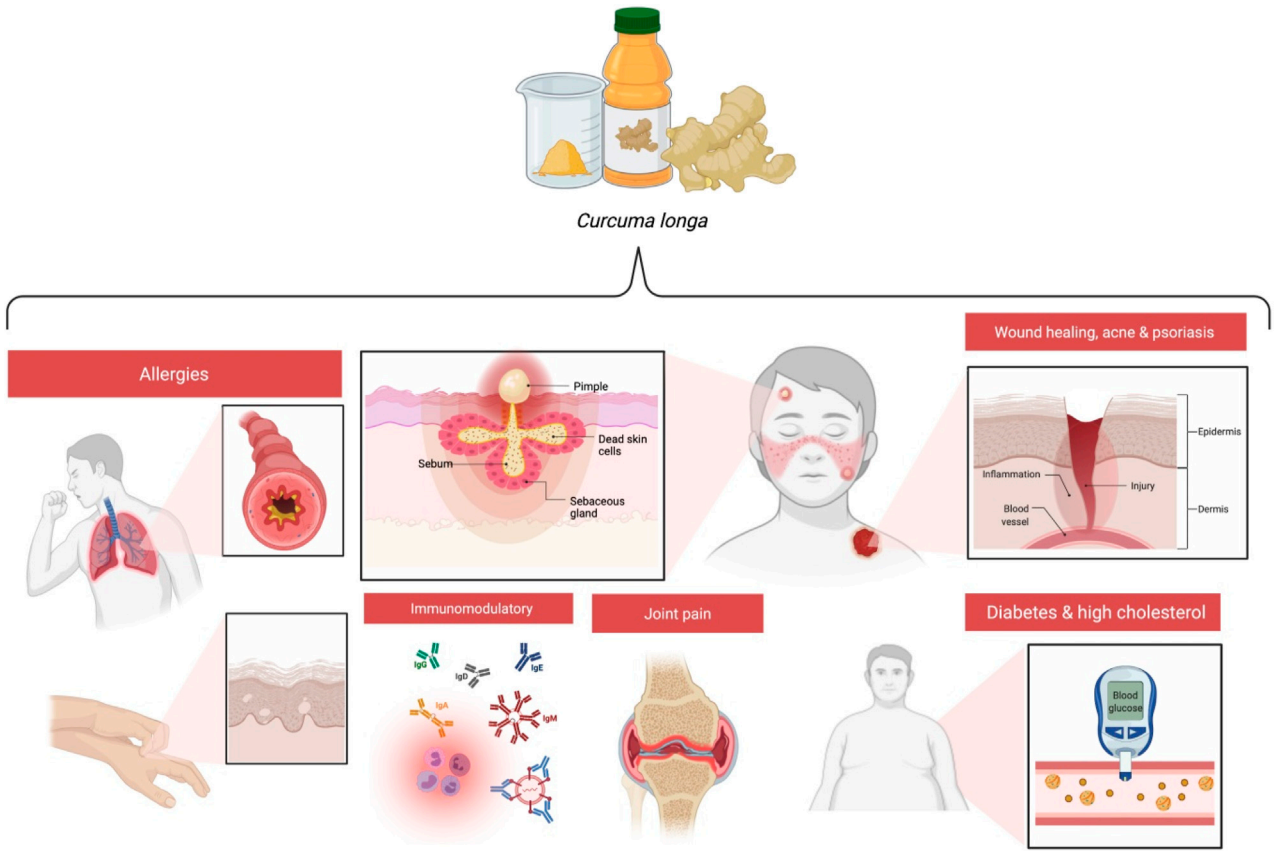


FIGURE 3 | Benefits of curcumin in various conditions.

diferuloylmethane and numerous volatile oils. *C. longa* of India is particularly popular when compared with those from other countries due to its high curcumin concentration, which is the most essential and active biological ingredient responsible for its therapeutic potential (Verma et al., 2018). Curcumin is a flavonoid having a lipophilic affinity that is practically water-insoluble (Dave et al., 2017) yet quite stable at the stomach's acidic pH. *C. longa* and curcumin show antioxidant features close to vitamins C and E in both aqueous and fat-soluble extracts.

Due to shortcomings in the earlier published review articles, such as a lack of information on the therapeutic potential of *C. longa* in relation to its major compound curcumin, we have attempted to provide in-depth information by highlighting knowledge gaps in traditional and scientific evidence about *C. longa* in relation to the therapeutic potential of curcumin against numerous disorders. This review mainly focuses on the distribution, cultivation, botany, nutritional composition, phytochemistry, toxicology, traditional and medicinal properties, and safety aspects including the pharmacological activities of *C. longa* in relation to its major compound curcumin. This review will further discuss the current advances in *C. longa* and curcumin, such as the utilization of nanocarriers to increase curcumin bioavailability and overcome all the disadvantages in relation to drug delivery.

Botanical Description, Geographical Distribution, and Cultivation of *C. longa*

C. longa is a perennial herb with no stem and rootstock. Their leaves are 1 m long, lanceolate or oblong, dark green from the upper surface and pale green from beneath. The petiole and sheath are about the same length as the blade. Spike makes its appearance before the leaves. Flowers are sterile, pale yellow with a reddish covering, and flowering bract is green with a deep ferruginous purplish color. It has a 2-m-long, erect leafy shoot (pseudostems) (Rajkumari and Sanatombi, 2017) bearing 8–12 leaves and is commonly grown in rural backyard gardens. Its medicinally important parts are presented in **Figure 1**. The rhizomes have a balmy smell and bitter in taste (Puteri et al., 2020). The taxonomic classification of *C. longa* is shown in **Figure 2**.

Turmeric is believed to have originated from South or Southeast Asia, more likely in Vietnam, China, or western India. It is only identified as a domesticated plant and has not been found in the wild. India is the biggest producer, consumer, and supplier, but it is also cultivated extensively in Cambodia,

TABLE 1 | The main products of *C. longa*, their appearance, chemical constituents, and use.

Product name	Appearance	Chemical constituents	Uses
Whole rhizome (dried form)	Orange-brown, red-yellow, or pale yellow	3%–15% curcuminoids, and 1.5%–5% essential oils	Medicinal purposes
Ground *C. longa*	Yellow or red-yellow	Curcuminoids and essential oils may be reduced during the processing, as well as by light exposure. The powder must be stored in a UV-resistant container	Used as a condiment, dye, medicine, and dietary supplement
C. longa oil	Yellow to brown oil	Monoterpenes and sesquiterpenes are predominated in essential oils of leaves and rhizomes, respectively	Used as a spice, medicine, and dietary supplement
C. longa oleoresins	Dark yellow, reddish-brown viscous fluid	25% of essential oil and 37%–55% of curcuminoids	Used as a food dye, medicine, and dietary supplement
Curcumin	Yellow to orange-red colored crystalline powder	Curcumin and its bisdemethoxy and demethoxy derivatives. The three primary curcuminoids may account for up to 90% of the total curcuminoids. Oils and resins may make up a small percentage of the total composition.	Used as medicine and dietary supplement

Bangladesh, Nepal, Indonesia, Thailand, Cambodia, Malaysia, West Bengal, Madagascar, Tamil Nadu, Maharashtra, Madras Indonesia, and Philippines (Royal Botanic Gardens Kew, 2021). The turmeric plant requires an average temperature from 20 to 30°C and a good annual rainfall to grow. Plant species can reach 1 m in height and have long, oblong leaves. Turmeric can be found in both tropical and subtropical regions. This will thrive best in the dark if not overcrowded, but somehow it also develops larger and better rhizomes when exposed to sunlight. Turmeric usually grows in a humid environment. Harvest time usually lasts from January to March–April. Early types are ready in 7–8 months, while medium types mature in 8–9 months. After the formation of yellow colored leaves, they begin to dry and the crop is ready to be harvested (Soudamini and Kuttan, 1989). Upon ripening, cutting of the leaves are done nearer to the soil surface, the ground is ploughed, and the rhizomes are collected by hand picking or carefully lifting the clusters with a spade. The turmeric plant needs a rich and friable soil with just a little sand content. It grows in irrigated and rain-fed areas on light black, ashy loam, and red soils to stiff loams. Total irrigation for turmeric will be defined by the climatic and soil conditions. Based on the soil types and rainfall, 15–25 irrigations are provided in medium heavy soils, and 35–40 irrigations are needed in light texture red soils. Rhizomes are typically piled under trees for shade or in well-ventilated shelters and finally wrapping is done with turmeric leaves. Matured rhizomes as a seed could be kept in sawdust pits (Aggarwal et al., 2004).

Traditional and Medicinal Properties of *C. longa*

C. longa rhizome is also consumed as an herbal infusion with conventionally produced gin called "ogogoro" by the native individuals of Delta State, Nigeria, with the notion that it heals various ailments. It is used to preserve and flavor food and is also used as a condiment in Nigeria. It is used for wound-healing and pimples in Pakistan and is widely consumed as folk medicine (Wahono et al., 2017). In Bhutanese traditional medicine, it is named Yung-ba, and it is employed as a tonic, an antidote, an antiseptic, an anti-inflammatory agent, and a preservative (Ayati et al., 2019).

C. longa is also developed in Thailand, Philippines, Sri Lanka, and Malaysia and is considered an ethnomedicinally important plant in Indonesia and Malaysia. Its poultice, when rubbed to the perineum, ensures the healing of any birth canal lesions. *C. longa* is also used to relieve dental issues and digestive troubles like discomfort or pain in the upper abdomen and acidity, indigestion, gas, and ulcers, as well as mitigate the hallucinogenic effects of hashish and other psychoactive drugs. The tribes of Jhalda, Parulia District of West Bengal, apply rhizome paste to the body to relieve physical pain. Assamese tribal women apply a fresh rhizome paste for skin infection and also to improve their complexion. Rhizome in addition to other ingredients cures loose stools in cattle. It is considered as a source of various problems such as blood purification, brain and heart tonic, asthma, leucoderma, piles, bronchitis, spleen enlargement, tumor, biliary disorders, anorexia, cough, rheumatism, sinusitis, tuberculous glands in the neck, diabetic wounds, hepatic disorders, leucorrhea, and gonorrheal secretion. It helps to lower blood clotting and blood sugar level (Zhang et al., 2013). Curcumin is now regarded as a promising "new medicine" that is being utilized as a supplement in a number of countries, namely, India, Japan, Thailand, Korea, China, Malaysia, and Pakistan; it is added to curry, tea, cosmetics, and drinks, and used as a colorant, antiseptic, and anti-inflammatory agent to treat gastrointestinal discomfort. It is also used as a component in cheese, butter, mustard sauce, and chips, and as a preservative and a dyeing agent in the United States. Curcumin is commercially available in multiple forms, including capsules, energy drinks, soaps, tablets, ointments, and cosmetics. Goud et al. (1993) found out that *C. longa* is the best source of ω-3 fatty acid and α-linolenic acid (2.5%).

Rhizomes are being added to other plants to develop traditional remedies for a range of infections, such as tonsillitis, snake bites and stings, headaches, wounds, sprains, and fractured bones. Turmeric has been applied as a home remedy to heal wounds and also facilitates the treatment for

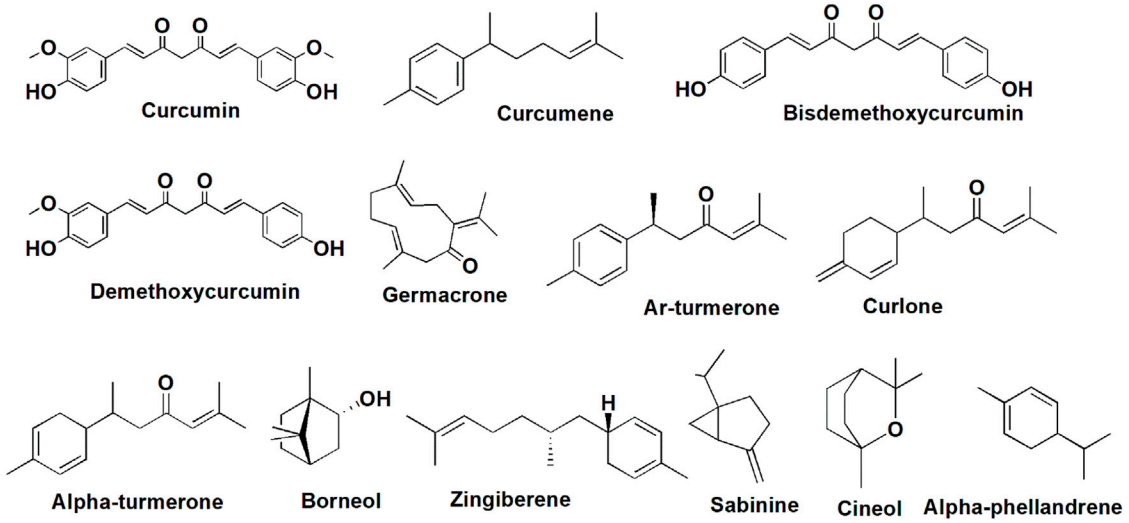


FIGURE 4 | Chemical structure of phytoconstituents present in *C. longa*.

digestive dysfunction, hepatic problems, leukemia, atherosclerosis, osteoarthritis, menstrual problems, bacterial infections, and eye problems. Turmeric has a role in preventing inflammation in the mucous membranes that line the throat, stomach, intestine, and lungs (**Figure 3**).

GENERAL HEALTH BENEFITS

Evidence suggests the benefits of turmeric in relieving acne, inflammation, joint pain, asthma, eczema, and tonic and acute allergies; in wound healing; in maintaining a balanced mood and blood sugar levels; and in immunomodulation (Ammon and Wahl, 1991; Reddy and Rao, 2002).

PHYTOCHEMISTRY OF *C. LONGA*

C. longa contains carbohydrates, fiber, certain proteins and lipids (no cholesterol), vitamin C, pyridoxine, magnesium, phosphorus, potassium, and calcium, which makes it a nutritionally rich natural food ingredient. The nutritional profile of *C. longa* is shown in **Figure 2** (Pradeep et al., 1993). To date, 719 constituents have been isolated and recognized from 32 *Curcuma* species, including terpenoids, flavonoids, phenylpropene derivatives, alkaloids, diphenylalkanoids, steroids, and other compounds (Sun et al., 2017). The rhizome was found to contain over 235 phytoconstituents, the majority of which are polyphenols and terpenoids. Curcuminoids are made up of 80% curcumin and are the most common polyphenols. There are 109 sesquiterpenes, 68 monoterpenes, 22 diarylheptanoids and diarylpentanoids, eight phenolics, five diterpenes, four sterols, three triterpenoids, two alkaloids, and 14 remaining constituents. In a standard form, *C. longa* consists of moisture (>9%), curcumin (5–6.6%), extraneous matter (<0.5%), mold (<3%), and volatile oils (<3.5%). Monoterpenes dominate the essential oils of flowers and leaves, while sesquiterpenes dominate the oils of roots and rhizomes. The oil constituents contain 25% tumerone, 11.5% curdine, and 8.55% ar-turmerone (Nisar et al., 2015). *C. longa* oil contains anti-mutagenic qualities and is also capable of preventing the development and excretion of urinary mutagens in those who smoke cigarettes. According to the latest analysis, essential oil content in the rhizome was approximately 3.97%, with ar-turmerone (40%), α-turmerone (10%), and curlone (23%) being the major components analyzed by gas chromatography (Guimarães et al., 2020). The ar-turmerone has been employed as a repellant for insects, and its mosquitocidal ability has been revealed in the leaf extract. *C. longa* is a rich source of polyphenolic curcuminoids like curcumin (about 80%), demethoxycurcumin (about 12%), and bisdemethoxycurcumin (Ashraf, 2018), as well as proteins, volatile oils (tumerone, atlantone, and zingiberone), sugars, and resins. Curcumin, which makes up 0.3%–5.4% of raw *C. longa*, is the well-studied active ingredient. **Table 1** illustrates the principal *C. longa* products, their appearance, chemical contents, and use. The *C. longa* plant is known to possess acidic polysaccharides (which include ukonan A, B, C, and D), 4.2% volatile oils (which include turmerone, ar-turmerone, curcumene, germacrone, and ar-curcumene as main constituents), and 5.8% essential oils (which include 0.6% sabinene, 0.5% borneol, 1% α-phellandrene, 1% cineole, 53% sesquiterpines, 25% zingiberene, and 3%–4% curcumin) (Chattopadhyay et al., 2004). Phenolic diketone curcumin provides yellow color, and consists of curcumin I (94%), curcumin II (6%), and curcumin III (0.3%). Protein (6.3%), fat (5.1%), minerals (3.5%), carbohydrates (69.4%), and moisture (13.1%) were reported by Liao et al. (2011). The main phytocompounds are presented in **Figure 4**.

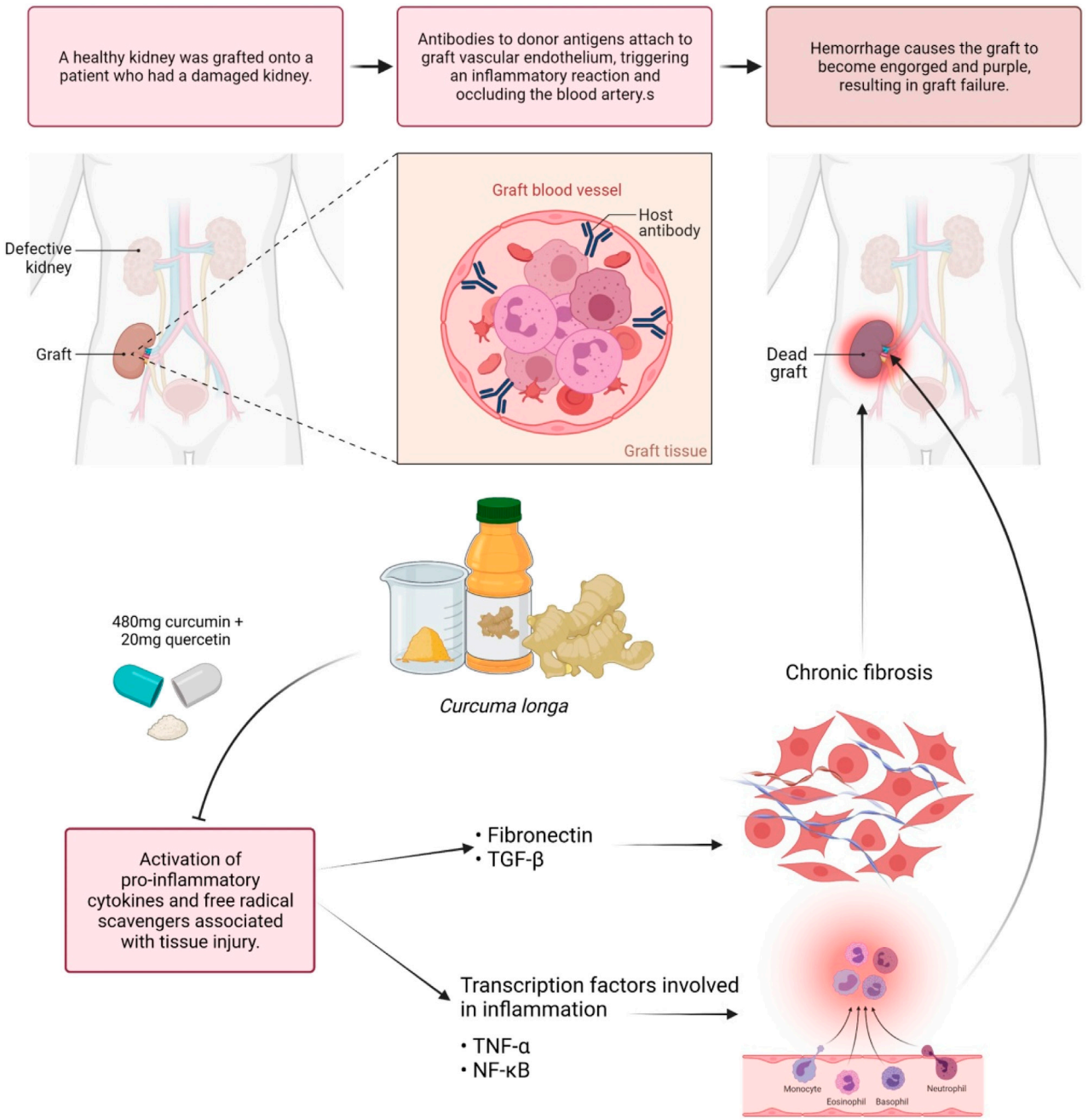


FIGURE 5 | Modulation of antibodies by curcumin. Curcumin assists in the modulation of antibodies that react with endothelium and causes hyperacute graft rejection. Blocking the expression of pro-inflammatory cytokines and transcription factors linked to inflammation and fibrosis may help to prevent dead grafts.

PHARMACOLOGICAL PROPERTIES OF *C. LONGA*

C. longa claims to offer a wide range of therapeutic properties. *C. longa* was reported to contain curcuminoids, glycosides, terpenoids, and flavonoids. Haridra rhizome has been employed by healthcare professionals for diabetes, cholesterol, inflammation, diarrhea, liver problems, asthma, and cancer with minimal cytotoxicity to normal cells, and has been used as a cosmetic ingredient (Paranjpe and Pranjpe, 2001). In human trials, curcumin is suggested to be effective and safe, and the U.S. Food and Drug Administration has certified it as "generally regarded as safe".

Gastrointestinal Disorders

C. longa has long been used to treat digestive problems, and clinical investigations have verified its therapeutic advantages. Its anti-inflammatory action has been established in preclinical

research to potentially protect the gastrointestinal tract. It has also been shown to increase gastrin, secretin, and bicarbonate secretion, as well as gastric wall mucus and pancreatic enzyme (Ammon and Wahl, 1991), as well as inhibit intestinal spasms and ulcer formation caused by stress, alcohol, indomethacin, pyloric ligation, and reserpine (Rafatullah et al., 1990) and improve dyspeptic patients' condition. The activity of curcumin against inflammation and its therapeutic effect on gastrointestinal illnesses such as dyspepsia, *Helicobacter pylori* infection, Crohn's disease, gastric ulcer, acidity, and ulcerative colitis in the form of fresh juice are thought to be antihelmintic. Curcumin inhibits nuclear factor (NF)-κB and reduces gastric mucosal damage in rats suffering from NSAID-induced gastropathy, leukocyte adhesions, intercellular adhesion molecule 1, and tumor necrosis factor (TNF)-α (Thong-Ngam et al., 2012). Between baseline and 8 weeks of treatment, *C. longa* tablet dramatically reduced irritable bowel syndrome (IBS) prevalence and abdominal pain/discomfort score, and IBS quality of life scores showed considerable improvement (Rahimi and Abdollahi, 2012). In male mice with liver damage, curcumin protects against acetaminophen-induced hepatitis by lowering oxidative stress and liver injury, and also restores glutathione levels (Somanawat et al., 2013).

Respiratory Disorders

C. longa and its constituents have a relaxing impact on tracheal smooth muscles, suggesting a possible bronchodilatory influence in individuals with obstructive lung disease. They also have a protective benefit in an animal model of respiratory disorders, involving effects on inflammatory cells and mediators, lung pathological alterations, airway responsiveness, and immunomodulatory responses (Boskabady et al., 2020). Curcumin has been shown in both *in vivo* and *in vitro* investigations to have antiasthmatic properties. Curcumin therapy during OVA sensitization exhibited significant protective effects in an OVA-induced asthma paradigm in guinea pigs, attenuating bronchial constriction and hyperreactivity (Ram et al., 2003). Bronchitis is treated with fresh rhizome juice. *C. longa* is boiled in milk and combined with jiggery and used internally for rhinitis and cough. In cases of catarrhal cough and painful throat with infection, a rhizome decoction is gargled, and a piece of the rhizome is slightly burned and chewed. Turmerones, curcuminoids, curcumin, and tetrahydrocurcumin are chemical compounds of *C. longa* that have anti-asthmatic properties, and Haridradhumvarti (fumes wick) fumes are used in asthma and congestion.

Inflammatory Disorders

Inflammatory markers include C-reactive protein (CRP), complements, and fibrinogen, all of which are induced by inflammatory cytokines in response to stimulation. According to Sandur et al. (2007), curcumin, demethoxycurcumin, and bisdemethoxycurcumin are the active compounds in *C. longa* that inhibit TNF-induced NF-κB activation. The methoxy groups on the phenyl ring were discovered to be responsible for their actions. *C. longa* extract was examined to improve serum inflammatory markers and mental health and mood disturbance in healthy participants who are overweight (Uchio et al., 2021). Researchers discovered that curcumin has anti-inflammatory properties by inhibiting the pro-inflammatory transcription factor (NF-κB) in 1995. They also discovered the molecular mechanism that underlies this inhibition (Singh and Aggarwal, 1995). TNF-α quickly activates NF-κB, which consists of the p50 and p65 subunits in human myeloid ML-1 cells, while curcumin prevented this activation. Curcumin also inhibits the binding of activator protein 1 (AP-1) binding factors, but the Sp1 binding factor remained unaffected. Curcumin inhibits the activation of NF-κB by phorbol ester and hydrogen peroxide, in addition to TNF-α. Furthermore, curcumin suppresses the NF-κB activation pathway after the convergence of multiple stimuli but before human I kappa B alpha phosphorylation. The capacity of *C. longa* to suppress both inflammatory prostaglandin derivative of arachidonic acid and neutrophil activity during inflammation may also indicate its anti-inflammatory activities. Curcumin is frequently used with bromelain to improve absorption and anti-inflammatory activity. Curcumin is equally efficacious as cortisone or phenylbutazone when given orally in acute inflammation. *C. longa* given orally to reduce inflammatory edema considerably. Curcumin's therapeutic effect in sepsis appears to be achieved by activation of peroxisome proliferator-activated receptor gamma (PPAR-γ), which leads to inhibition of pro-inflammatory cytokine along with expression and release of TNF-α (Jacob et al., 2008). One trial evaluated 43 kidney transplant patients; 480 mg of curcumin and 20 mg of quercetin per capsule were observed to be potent during delayed graft rejection. Significant lower serum creatinine after transplant was attained in 43% of control patients and 71% of low-dose-treated participants. Induction of the hemeoxygenase enzyme, proinflammatory cytokines, and free radical scavenger associated with tissue injury possibly caused the enhanced early performance of transplanted kidneys (**Figure 5**) (Shoskes et al., 2006). Majority of the benefits seemed to be due to the anti-inflammatory and antioxidant properties of curcumin, while the quercetin in the molecule was negligible.

Diabetes Mellitus

In diabetes mellitus, *C. longa* rhizome powder is particularly beneficial when added to amla juice and honey. Curcuminoids, the active component in the rhizome, reduce lipid peroxidation by keeping superoxide dismutase, catalase, and glutathione peroxidase active at higher levels. Curcuminoids have been demonstrated in diabetes mellitus type 2 patients to improve insulin resistance, reduce glucose and insulin levels, enhance adiponectin secretion, and lower levels of leptin, resistin, interleukin (IL-6, IL-1β), and TNF-α (Hajavi et al., 2017). According to the findings, *C. longa* ethanolic extract containing both curcuminoids and sesquiterpenoids is more hypoglycemic than curcuminoids or sesquiterpenoids alone (Nishiyama et al., 2005). *C. longa* extracts examined under *in vivo* conditions towards type 2 diabetes in mice models predict that it has a hypoglycemic impact by reducing blood glucose levels (Ponnusamy et al., 2012). *C. longa* has a low impact on postprandial plasma glucose and insulin in healthy individuals; it was found that consumption of 6 g of *C. longa* had no noticeable effect on the glycemic level. The change in insulin was substantially greater 30 and

60 min after the oral glucose tolerance test (OGTT) with *C. longa*. Following the OGTT, the insulin area under the curve was likewise considerably greater after consuming *C. longa*. Curcumin and its three derivatives (dimethoxy curcumin, bisdemethoxycurcumin, and diacetyl curcumin) were reported for their antioxidant capabilities (Faizal et al., 2009). *C. longa* dried rhizome powder diluted in milk has antidiabetic, hypolipidemic, and hepatoprotective properties, according to the scientific and systemic investigation, and could be used as an efficient and safe antidiabetic dietary supplement with great potential (Rai et al., 2010). Both isopropanol and acetone extract of *C. longa* inhibited human pancreatic amylase enzyme, which reduces starch hydrolysis, resulting in lower glucose levels (Ponnusamy et al., 2010).

Cardiovascular Diseases

Cardiovascular diseases (CVDs) seem to be a global health issue that is linked to high disease and death rates. Anti-hypercholesterolemic, anti-atherosclerotic (Gao et al., 2019), and protective capabilities against cardiac ischemia and reperfusion (Wang et al., 2018) of curcumin have been proven in preclinical and clinical trials. Curcumin has anti-CVD potential by improving the lipid profile of patients, and it might be administered alone or as a dietary supplement to traditional CV medicines (Qin et al., 2017). Curcumin is also seen in many studies to protect against coronary heart disease (Li H. et al., 2020) and also possesses anticoagulant properties. CV preventive characteristics of *C. longa* include reduction in the level of cholesterol and triglycerides, decrease in the vulnerability of low-density lipoprotein (LDL) to lipid peroxidation, and platelet aggregation prevention, which helps to defend against atherosclerosis according to animal studies and also inhibits thromboxane formation. Curcumin increases VLDL cholesterol *trans*-protein plasma, causing increased levels and mobilization of α-tocopherol from adipose tissue that protects against oxidative stress that occurs during atherosclerosis. However, the fatty acids in the animals were less susceptible to oxidation in the blood vessel. It was suggested that oral intake of 500 mg/day curcumin for a week leads to a significant reduction in serum lipid peroxide (33%) and total serum cholesterol (12%) levels while increasing HDL cholesterol (29%). Curcumin may reduce chronic heart failure by boosting p38 MAPK, JNK, and ASK1, according to Cao et al. (2018). Curcumin and its components were used in recent research to determine the utility of nanotechnology-based medication delivery systems in CVD patients (Salehi et al., 2020).

Hepatoprotective

In jaundice, rhizome powder added to amla juice is utilized. Jaundice is also cured by combining corriliyum (Anjana) with Haridra, Red ochre (Gairika), and Amalaki (*Emblica officinalis* Gaertn.) (Tripathi, 2009). *C. longa*'s hepatoprotective abilities have been proven in studies against several hepatotoxic ailments, including carbon tetrachloride, galactosamine, and acetaminophen (paracetamol) (Rao et al., 1995). The ethanolic crude extract of rhizomes was detected with curcumin, tumerone, atlantone, and zingiberene, which had substantial hepatoprotective ability at an oral dose of 250 and 500 mg/kg (Park et al., 2000). Karamalakova et al. (2019) investigated the decrease in the level of plasma bilirubin and gamma glutamyl transpeptidase, and the decrease in lipid peroxidation and provided significant hepatic protection against bleomycin toxicity by decreasing reactive oxygen species (ROS), which improves superoxide dismutase, catalase, and malondialdehyde levels. Curcumin is said to increase apoptosis in injured hepatocytes while also reducing inflammatory effects, hepatic fibrogenesis, and substantially liver injury. The hepatoprotective attribute of *C. longa* and curcumin might be due to direct free radical scavenging mechanisms, boosting glutathione levels, and assisting in liver detoxification. Aflatoxin-induced biliary hyperplasia, lipid alterations, and necrosis were likewise cured by *C. longa* and curcumin. Sodium curcuminate is a salt of curcumin that has choleretic effects, boosting biliary excretion of bile salts, cholesterol, bilirubin, and bile solubility, thus helping to prevent and treat cholelithiasis. This could be related to the antioxidant capacity of curcumin's phenolic groups. Tacrine is well-known for its hepatotoxic and T-cell-destructive properties. Curcumin was over ten times more efficient than standard therapy, ascorbic acid, in research involving human hepatocytes cells that had been disrupted by tacrine (Song et al., 2001).

Neuroprotective Activity

Curcuma oil lowers ischemia's negative effect by decreasing nitrosative and oxidative stress. Ischemia collapses the membrane potential of the mitochondria, cytochrome c releases, Bax:Bcl-2 protein ratio changes, and caspase-activated, which leads to the apoptotic initiation in a sequential manner, which was considerably inhibited by *Curcuma* oil. As a result, there is evidence for the action of *Curcuma* oil in neuroprotection with a wide therapeutic window for the reduction in ischemic brain injury (Dohare et al., 2008).

In an Alzheimer's disease transgenic mouse, curcumin decreased oxidative stress and repaired amyloid pathology. Antioxidant and anti-inflammatory features of curcumin helped to minimize the manifestation of Alzheimer's disease, which is characterized by inflammation and oxidation. Parkinson's disease (PD) is found to be the second most common neurodegenerative disease following Alzheimer's disease, which affects dopaminergic neurons of the substantia nigra pars compacta (SNpc) and decreases dopamine (DA) in their striatal terminals. Curcumin is suggested to be an effective therapeutic and nutraceutical agent for PD treatment. Interestingly, curcumin was found to inhibit the synthesis of MOA-B enzyme (Khatri and Juvekar, 2016), which would lead to an increase in the level and availability of DA in the brain. Neuroprotective effects of curcumin in a 6-hydroxydopmine animal model of PD (El Nebrisi et al., 2020) indicated an increase in the survival of striatal TH fibers and SNpc neurons, decreased abnormal turning behavior, and exerted neuroprotective properties. These findings provide evidence that α7-nicotinic acetylcholine receptors could be a potential therapeutic target and curcumin would be the first natural source that is found to modulate nicotinic receptors in PD. Curcumin can be a future therapy for various neurological

illnesses including major depression, involuntary movement, as well as diabetic neuropathy (Kulkarni and Dhir, 2010). Ethanol extract of *C. longa* was found to show neuroprotective effects on neuronal loss induced by dexamethasone treatment in rat hippocampus (Issuriya et al., 2014). In 2018, an *in vivo* study revealed that administration of *C. longa* extract at a dose of 200 mg/kg in trimethyltin (TMT)-treated Sprague–Dawley rats with neurotoxic damage seems to prevent the deficits in the spatial memory performance and partially inhibit the decrease in the number of CA2–CA3 region pyramidal neurons. Therefore, the anti-inflammatory as well as antioxidant effects of *C. longa* were observed (Yuliani and Mustofa, 2018) Furthermore, Yuliani and Mustofa (2019) examined the neuroprotective effects of ethanolic *C. longa* extract at 200 mg/kg in an *in vivo* analysis *via* preventing oxidative stress by decreasing the plasma and brain malondialdehyde levels and increasing the superoxide dismutase, catalase, and glutathione peroxidase enzyme activities and glutathione levels in the brain on TMT-exposed Sprague–Dawley rats. Terrestrial animals and aquatic animals are also required to be used for research purposes. An aquatic environment serves as a sink for environmental contaminants including Benzo[a]pyrene (B[a]P), and research on the fish model is also needed to understand the influence of B[a]P on oxidative stress-induced neurotoxicity and anxiety-like behavioral responses in aquatic animals (Billiard et al., 2006; Satpathy L. and Parida S. P., 2021). B[a]P is important in the mechanical aspects of oxidative stress to lipid membranes, nucleic acids, and proteins, as well as changes in antioxidant capacity. Curcumin has a potential to act as a co-supplement by reducing anti-anxiety behavioral response and altering antioxidant activity with a significant increase in pyknotic neuronal counts in the periventricular gray zone of the optic tectum that regulates anxiety against B[a]P-induced neurotoxicity in adult zebrafish (Satpathy L. and Parida S., 2021).

Banji et al. (2021) demonstrated the neuroprotective and antioxidant activity of *C. longa* extract in synergy with essential oil against neurotoxicity mediated by aluminum. Detection of free curcumin and its metabolites in the brain and plasma has increased bioavailability and tissue distribution, implying that it could be used in neurodegenerative illnesses.

Another neurodegenerative disease, amyotrophic lateral sclerosis (ALS), causes a selective loss of motor neurons in the spinal cord, brainstem, and motor cortex. Curcumin was studied to determine if it could help ALS patients, particularly those with bulbar involvement, survive longer (Ahmadi et al., 2018). Curcumin therapy reduced the development of ALS and oxidative damage in a double-blind therapeutic trial (Chico et al., 2018). Curcumin-based drug delivery systems are beneficial for the treatment of ALS, according to a study (Tripodo et al., 2015), although Rakotoarisoa and co-workers pointed out that curcumin has chemical instability, low oral bioavailability, and low water solubility rate in the ALS disease condition (Rakotoarisoa and Angelova, 2018).

The ability of curcumin to interact indirectly with a diverse array of transcription factors, including NF-κB, activator protein 1 (AP-1), β-catenin, and signal transducer and activator of transcription (STAT) proteins, and to act as a partial agonist of the PPAR-γ, a ligand-activated transcription factor involved in both neuroprotective and anti-inflammatory signaling pathways (Chen et al., 2015; Kunnumakkara et al., 2017). Curcumin has been demonstrated to help with a variety of diseases, including multiple sclerosis (MS) (Mohajeri et al., 2015). Curcumin-D-monoglucuronide (curcumin monoglucuronide, CMG) was developed as a prodrug form of curcumin due to its low bioavailability in the body. CMG is deemed to be safe for use and can be injected intravenously, revealing an anticancer impact on mice implanted with human colorectal cancer cells by achieving a 1,000-fold higher blood concentration of free-form curcumin than curcumin administered orally (Ozawa et al., 2017). In mouse xenograft models, CMG given intraperitoneally appears to have antitumor effects on oxaliplatin-resistant colon cancer with minimal toxicity (Ozawa-Umeta et al., 2020). After CMG delivery, the microbiota changes, which may be linked to immunopathology suppression in an autoimmune model for MS (Chearwae and Bright, 2008) and experimental autoimmune encephalomyelitis (EAE). The gut microbiota has been suggested to play a major role in the development and severity of MS. When compared to healthy controls, MS patients had an increased number of bacteria from the genera *Akkermansia, Blautia*, and *Pseudomonas*, as well as a lower number of bacteria from the genera *Prevotella* and *Parabacteroides* (Chen et al., 2016; Park et al., 2017; Tsunoda, 2017).

Antioxidant Properties

C. longa and its curcumin constituent have significant antioxidant activity, equivalent to both vitamin C and vitamin E, in both water- and fat-soluble extracts. Curcumin can help the body rid itself of hydroxyl radicals, singlet oxygen, superoxide radicals, nitrogen dioxide, and NO. Curcumin pretreatment was proven to reduce ischemia-induced mutations in the heart (Dikshit et al., 1995). The efficiency of curcumin on endothelial heme oxygenase-1 (inducible stress protein) employing bovine aortic endothelial cells was discovered in an *in vitro* investigation that resulted in increased cellular resistance to oxidative stress. Curcumin can also help *Caenorhabditis elegans* live longer by lowering intracellular ROS and lipofuscin levels during aging (Liao et al., 2011). Previous research into the potential of *C. longa* to sustain hippocampal cells of male Wistar rats from lead-induced damage and reduces lipid peroxidation caused by toxic heavy metals. Resveratrol and curcumin alleviate and synergistically repair oxidative stress to the tissues by enhancing antioxidant response through free radical scavenging (Al-Basher et al., 2020). In one of the earlier studies, the anti-inflammatory and antioxidant capability of curcumin was detected to be synergistically enhanced with quercetin, and a synergistic protective effect was also demonstrated in diazinon-induced rats (Abdel-Diam et al., 2019). The anti-inflammatory impact of berberine and curcumin may decrease oxidative stress, liver inflammation, and lipid metabolism (Feng et al., 2018), and the berberine

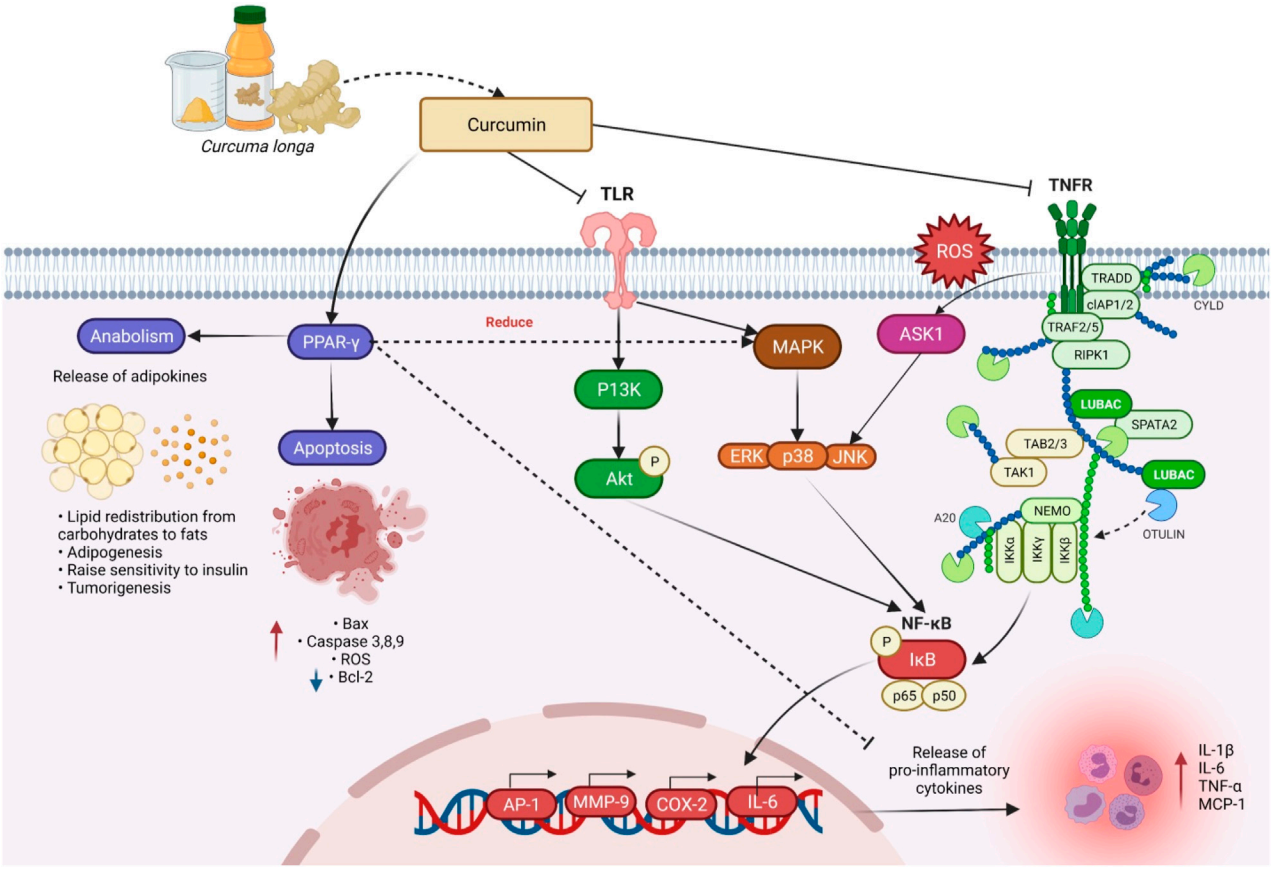


FIGURE 6 | Curcumin's mechanism of action in reducing inflammation, anabolism, and apoptosis. By inhibiting the pro-inflammatory transcription factor (NF-κB), and activation of PPAR-γ, curcumin aids in anabolism and apoptosis, suppression of pro-inflammatory cytokines, as well as the expression and release of TNF-α. Abbreviations: TLR, Toll-like receptors; TNFR, Tumor necrosis factor receptor; ROS, Reactive oxygen species; TRADD, Tumor necrosis factor receptor type 1-associated death domain protein; CYLD, CYLD lysine 63 deubiquitinase; cIAP1/2, Cellular inhibitor of apoptosis protein 1/2; TRAF 2/5, Tumor necrosis factor receptor-associated factor 2/5; RIPK1, Receptor-interacting serine/threonine-protein kinase 1; LUBAC, Linear ubiquitin chain assembly complex; SPATA2, Spermatogenesis-associated protein 2; NEMO, NF-κB essential modulator; TAB2/3, TGF-beta activated kinase 1 (MAP3K7) binding protein 2; TAK1, Transforming growth factor-β-activated kinase 1; IKKα, IKKβ, and IKKγ, Inhibitory kappa b kinase alpha, beta, and gamma; IkB, Inhibitor of nuclear factor kappa Bv; PPAR-γ, Peroxisome proliferator-activated receptor gamma; P13K, Phosphoinositide 3-kinases; Akt, Ak strain transforming; ERK, Extracellular-signal-regulated kinase; JNK, Jun N-terminal kinase; Bax, Bcl-2-associated X-protein; AP-1, Activated protein-1; MMP-9, Matrix metallopeptidase 9; COX-2, Cyclooxygenase 2; IL-6 and 1β, Interleukin 6 and 1 beta; TNF-α, Tumor necrosis factor alpha; MCP-1, Monocyte chemoattractant protein-1.

combination also reduced inflammatory and oxidative stress responses in the cortex and hippocampus of rats (Lin et al., 2020).

Anticancer Activity

Annapurna et al. (2011) evaluated the ability of *C. longa* prophylactically and therapeutically, i.e., pre-induction treatment and post-induction treatment *via* oral and topical application to modulate the N-methyl-N-nitrosourea-induced mammary cancer in rats for 24 weeks. Prophylactic topical application given at 200 mg/kg of *C. longa* has significantly reduced the mean tumor volume compared with therapeutic topical application. This was the first report to show the anticancer activity of *C. longa* with topical application in a breast cancer model. In an *in vivo* research involving the topical application of curcumin in CD-1 mice and dietary administration of 1% *C. longa*, 0.05% of its ethanol extract significantly reduced tumor incidence, tumor burden, and tumor volume in dimethyl benz[a]anthracene (DMBA)-initiated and 12,O-tetradecanoylphorbal-13-acetate (TPA)-promoted skin tumors (Huang et al., 1988). Kuttan and his colleague's work was the first to demonstrate curcumin's anti-cancer potential in both *in vitro* and *in vivo* experimental models (Kuttan et al., 1985). Curcumin activates DNA damage response, laying the foundation for the therapeutic use of these nutraceuticals in prostate cancer chemoprevention (Horie, 2012). The general anti-carcinogenic effect of curcumin involves mechanisms like induction of apoptosis and inhibition of cell-cycle progression in rat aortic smooth muscle cells (Chen and Huang, 1998). The antiproliferative effect is regulated partly through hindrance of protein tyrosine kinase activity and c-myc mRNA expression, while the apoptotic effect may partly be mediated *via* preventing the functioning of protein tyrosine kinase, protein kinase C, and expressions of c-myc mRNA and bcl-2 mRNA (Chen and Huang, 1998). Curcumin

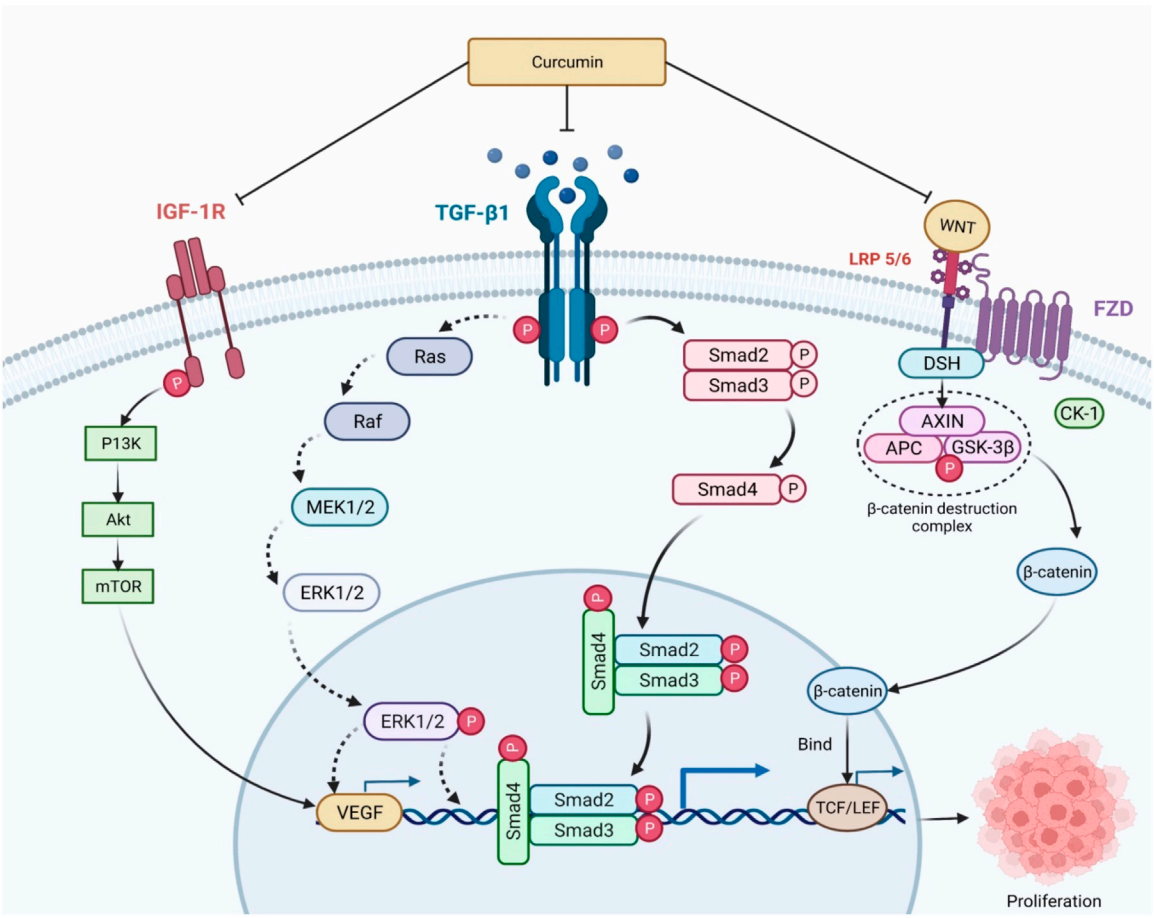


FIGURE 7 | Mechanism of curcumin in regulation of cancer proliferation. TGF-β1/smad3, IGF, PI3K/Akt, Wnt/β-catenin, and vascular endothelial growth factor (VEGF) are some of the signaling pathways and molecular targets that curcumin modulates to inhibit cancer. Blocking these receptors has the potential to reduce chronic inflammation and oxidative damage. DSH and AXIN are recruited once the WNT binds to LRP 5/6, producing the β-catenin destruction complex. β-catenin that has escaped into the nucleus promotes the transcription of genes including cyclin D1 and P13k, which promote cell proliferation and growth. IGF-1R, Insulin-like growth factor 1 receptor; TGF-β1, Transforming growth factor beta 1; WNT, Wingless/integrated; LRP 5/6, Low-density lipoprotein receptor-related protein 5/6; Smad, small mothers against decapentaplegic; Ras, Rat sarcoma virus; Raf, Rapidly Accelerated Fibrosarcoma; MEK 1/2, Mitogen-activated protein kinase 1/2; ERK ½, Extracellular-signal-regulated kinase ½; P13K, Phosphoinositide 3-kinases; Akt, Ak strain transforming; mTOR, mammalian target of rapamycin; TCF/LEF, T-cell factor/lymphoid enhancer factor; FZD, Frizzled; DSH, Dishevelled; AXIN, Axis Inhibitor; APC, Adenomatous polyposis coli; GSK-3β, Glycogen synthase kinase-3 beta; CK-1, casein kinase 1.

inhibits the transcription factor NF-κB (**Figure 6**) and various downstream gene products like c-myc, Bcl-2, COX-2, nitric oxide synthase (NOS), Cyclin D1, TNF-α, ILs, and matrix metallopeptidase 9 (MMP-9) and has anti-proliferative activities in a diversity of malignancies. Curcumin could be used to avoid colorectal cancer (CRC) in diabetics with type 2 diabetes by lowering leptin blood levels and increasing adiponectin levels. Poloxamer 407 can be employed as a polymer to expand the colorectal medicine liberation mechanism for curcuminoids in CRC treatment, according to the study of Chen et al. (2012).

A novel approach in adjuvant treatment for osteosarcoma is by combining a synthetic counterpart of the natural chemical pancratistatin with curcumin (Ma et al., 2011). One controlled study found that employing poly-lactic-co-glycolic acid to create and characterize nano-curcumin improves the water solubility as well as anticancer activity of the nanoparticulate emulsion (Nair et al., 2012). Curcumin also has an impact on numerous growth factor receptors and adhesion molecules that are implicated in tumor growth, angiogenesis, and metastasis (Wilken et al., 2011) and exerts antitumor action in cancer cells by the suppression of NF-κB and signal transducers and activators of the transcription 3 (STAT3) pathways (Jiménez-Flores et al., 2014).

A study on *in vitro* and *in vivo* models revealed that both *C. longa* and curcumin exhibited the ability to lessen the impacts of numerous known causative agents of mutation and cancer in different body tissues. Curcumin (50 μM) initiates destruction in the human kidney cells and causes the colorectal HT-29 cancerous cells to grow larger, which is most probably due to cell cycle arrest (Kössler et al., 2012). Curcumin also triggers programmed cell death in colon cancerous cells and inhibits micro-inflammation in the

gastrointestinal system linked to inflammatory bowel illnesses, according to laboratory research (Nita, 2003). Okanlawon et al. (2020) determine the influence of the inclusion of powdered *C. longa* on carcass yield and intestinal increase in rabbit production. Farombi et al. (2007) explored the combined effects of curcumin and kolaviron (a bioflavonoid extracted from Garcinia kola seeds) on DBP-induced testicular injury in rats. Curcumin treatment of mice infected with human prostate cancer cells resulted in a lowered microvessel density, cell proliferation, an improvement in apoptosis. Endothelial cells derived from bovine aorta exposed to curcumin (5–15 μM) under normoxic (oxygen tensions within 10–21%) or hypoxic (oxygen tensions within 1–5%) conditions were reported to increase heme oxygenase activity and resistance to oxidative stress. Consumption of alcohol sensitizes the pancreas to give an inflammatory response through NF-κB activation *via* protein kinase C epsilon. One pilot study concluded that an oral dosage of 500 mg of curcumin with 5 mg of piperine could restore lipid peroxidation in patients suffering from tropical pancreatitis (Durgaprasad et al., 2005).

EGFR-, miRNA-, autophagy-, and cancer stem cell-based treatments with curcumin could be proven as potential processes and targets for tackling lung cancer (Ye et al., 2012). Curcumin also seems to promote tumor progression, reducing the efficiency of docetaxel in lung cancer patients. Meanwhile, synchronized curcumin and docetaxel treatment causes minor toxicity in normal organs, as well as the bone marrow and liver (Yin et al., 2012). *In vivo* curcumin lessens the migratory and invasive capabilities of A549 cells and inhibited adiponectin expression thought to be mediated through the NF-κB/MMP pathways and has been proposed as an adjuvant in lung malignancy (Tsai et al., 2015).

Yu and his colleagues evaluated the role of curcumin in inhibiting the human hepatoma SMMC-7721 cells significantly by promoting apoptosis *via* modulation of Bax/bcl-2 (Yu et al., 2011). Apoptosis was associated with increases in p53 levels as well as its DNA-binding ability, along with protein expression of Bax. Phosphorylation of CDC27 (cell division cycle 27) is the main mechanism of anticancer efficacy of curcumin by obstructing cell growth and proliferation in an apoptotic pathway, leading to the death of the cells (Lee and Langhans, 2012). It has been discovered that circulating miR-21 is elevated in patients with hepatocellular carcinoma (HCC); it can be exploited as a diagnostic marker and therapeutic target for HCC, and is being linked to distant metastasis (Zhang et al., 2019). According to Li and his colleagues, in human hepatoma cell lines such as HepG2 and HCCLM3, suppression of miR-21 improved anticancer action of curcumin like cell growth suppression, apoptosis *via* upregulated target gene, and TIMP3 expression, and the mechanism may refer to TGF-β1/smad3 signaling pathway inhibition (Li J. et al., 2020). Curcumin inhibits cancer through modulating several signaling pathways and molecular targets, including TGF-β1/smad3, IGF, PI3K/Akt, Wnt/β-catenin, and vascular endothelial growth fact (VEGF) (**Figure 7**) (Mohebbati et al., 2017). Although several reasons for curcumin's antitumor potential have been hypothesized, the exact molecular mechanism of this activity against HCC is unknown.

Curcumin therapy of Burkitt's lymphoma cell lines in combination with ionizing radiation shows that it boosts lymphoma cells' susceptibility to ionizing radiation-induced apoptosis and improves cell cycle arrest at the G2/M phase. Curcumin and L-ASP show synergism in patients with blood and bone marrow malignancy (Jiang et al., 2015). Curcumin also hinders the cellular growth of uterine leiomyosarcoma and reduces the spread of castrate-resistant disease and human leiomyosarcoma cells *via* modulating the AKT-mammalian target of rapamycin pathway for inhibition (Wong et al., 2011). Curcumin or *C. longa* extract's potential in decreasing tumors induced chemically was investigated. It was documented that curcumin and crude extract of *C. longa* is useful in reducing papilloma development throughout carcinogenesis and progression. About 0.2% and 1.0% of dietary curcumin can reduce the number of papilloma by acting on 7,12-dimethyl benz[a] anthracene (DMBA) and 12,0-tetradecanoylphorbol-13-acetate (TPA) that promoted skin tumor, which was explored by Limtrakul et al. (2001) as ras-p21 and fos-p62 oncogene expression was decreased dose-dependently by curcumin. Mohanty et al. (2006) examined the potency of *C. longa* on apoptosis of myocardial cells in experimentally produced myocardial ischemic–reperfusion injury because it exhibited considerable anti-apoptotic effects that lead to the preservation of cardioprotective characteristics and heart function. Aqueous *C. longa* extract exhibited antimutagenic activity against mutagens and also inhibited the progression of forestomach tumors induced by benzo[a]pyrene against *Salmonella typhimurium* strains.

Insulin-like growth factor-binding protein-3 (IGFBP-3) is a high-affinity binding protein that alters the mitogenic functions of IGFs while also having anti-proliferative and proapoptotic characteristics. Apoptosis is induced by transfection of IGFBP-3 cDNA into breast cancer cell lines expressing either mutant (T47D) or wild-type p53 (MCF-7). IGFBP-3 results in a higher ratio of pro-apoptotic to anti-apoptotic Bcl-2 family members. Curcumin induced cell apoptosis in MCF-7 *via* a p53-dependent pathway and could offer therapeutic promise in patients with breast cancer (Choudhuri et al., 2002). In the mouse model, the combination of curcumin and cyclophosphamide negated the efficacy of cyclophosphamide and then hindered the reduction of tumor size. Curcumin causes DNA fragmentation and base degradation in the presence of copper and cytochrome p450 isoenzymes. Furthermore, Frank et al. (2003) demonstrated that curcumin bonded to copper did not suppress spontaneous hepatic tumor growth in a rat model of liver cancer. The enhanced toxicity and oxidative stress may be explained by the excess load of copper.

Curcumin can limit the absorption and effectiveness of irinotecan, a chemotherapy medication used in colon cancer. Curcumin in combination with paclitaxel (Taxol) effectively decreased breast cancer dissemination to the lung in a xenograft mouse model of human breast cancer, relative to either curcumin or paclitaxel alone (Frank et al., 2003). Curcumin reduces T cells significantly, but a low

dosage of curcumin boosts T cells retrieved from mice with the 3LL tumor. Consequently, increased $CD8^+$ T cells demonstrated improved IFN-γ secretion and proliferation, especially against 3LL tumor cells (Han et al., 2014). *C. longa* extracts (containing curcuminoids, volatile oil, and water-soluble polysaccharides) could be employed as an adjuvant supplement for cancer patients who have their immune systems impaired by chemotherapy and saw an improvement in the expansion of peripheral blood mononuclear cells and the composition of cytokines. *In vitro* and *in vivo* analyses have indicated that aromatic-turmerone exhibits anti-angiogenic abilities on human endothelial cells (Yue et al., 2015).

Dietary substances such as curcumin and docosahexaenoic acid (DHA) have varying antiproliferative abilities among multiple breast cell lines and indicated synergism in SK-BR-3 (human breast cancer cell line) cells, which might be due to DHA elevation of cellular curcumin absorption and, thus, an occurrence unique to the combined action of substances (Altenburg et al., 2011). The study shows the synergistic interaction between curcumin and garcinol in pancreatic cancer cells (BxPC-3 and Panc-1) in a dose-dependent manner (Parasramka and Gupta, 2012). *In vitro* screening is carried out on human multiple myeloma cell lines (U266) by Ghoneum and Gollapudi (2011) to explain the synergistic apoptotic potency of arabinoxylan rice bran (MGN-3/Biobran) and curcumin (*C. longa*) in the United States.

Anti-Allergic Activity

Curcumin inhibited the degranulation and release of histamine from rat peritoneal mast cells caused by compound 48/80. Calcium uptake measurements and cAMP tests in mast cells were used to investigate the mechanism of action. In an animal model, curcumin dramatically reduced the mast cell-mediated passive cutaneous anaphylactoid reaction. Curcumin enhanced intracellular cAMP levels and inhibited both nonspecific and selective mast cell-mediated allergy reactions (Choi et al., 2010). Curcumin significantly reduced IgE/Ag-induced PSA (passive systemic anaphylaxis), as measured by serum-dependent leukotriene C4, dependent prostaglandin D2, and histamine levels, indicating that it might be useful to produce drugs for allergic inflammatory illnesses (Li et al., 2014). Curcumin can suppress expression of CD80, CD86, and class II antigens by dendritic cells and blocks the release of inflammatory cytokines like IL1β, IL-6, and TNF-α from LPS-stimulated dendritic cells.

Antidermatophytic Activity

Rhizome juice is utilized as an antiparasitic in the therapeutics of numerous skin problems, and rhizome powder is added to cow's urine to relieve internal itching and dermatitis (Paranjpe and Pranjpe, 2001). Leaves hold great potential against human pathogenic fungi on account of their various *in vitro* and *in vivo* antifungal activities, for instance, strong fungicidal ability, long shelf life, high inoculum density tolerability, thermostability, a large number of antidermatophytic effects, and lack of any side effects. Curcumin has been proven to have antimutagenic, antioxidant, free radical scavenging, anti-inflammatory, and anti-carcinogenic abilities, allowing it to protect the skin from detrimental UV-induced impacts (Binic et al., 2013).

Pregnancy/Neonates

C. longa and curcumin caused a considerable increase in hepatic glutathione S-transferase (GST) and sulfhydryl (SH), Cytochrome b5, and cytochrome P450 levels, implying that *C. longa* and/or curcumin metabolites can be passed down through the milk supply. *C. longa* and curcumin are nontoxic and non-mutagenic during pregnancy in animals, although additional research in humans is needed (Soleimani et al., 2018).

Irritable Bowel Syndrome

IBS patients have far more inflammatory cells in the mucosa of the colon and ileum part of the intestine. Ng et al. (2018) looked into the possibility that curcumin can aid in IBS manifestation. Crohn's disease (CD) and ulcerative colitis (UC) are the two primary forms of inflammatory bowel disease (IBD), characterized by abdominal pain, bloating, altered bowel habits, and increased stool frequency. Holt et al. (2005) carried out a pilot study to see how curcumin therapy affected IBD patients who had earlier received standard UC or CD therapy. Curcumin with standard treatment exerts more beneficial effects than placebo plus conventional UC treatment in maintaining recovery, according to Hanai et al. (2006). Bundy et al. (2004) examined that abdominal pain or discomfort score was lowered significantly by 22% and 25% in the one- and two-tablet group volunteers, respectively, and revealed the role of *C. longa* on IBS pathology.

Dyspepsia and Gastric Ulcer

Six hundred milligrams of curcumin five times a day for 12 weeks to individuals with peptic ulcers could prevent ulcer development but also cause symptomatic erosions, dyspepsia, and gastritis in some patients. Abdominal pain along with other symptoms has greatly decreased with curcumin within 1–2 weeks. Kim et al. (2005) found that orally administered ethanolic *C. longa* extract decreased stomach acid, gastric juice secretion, and ulcer initiation in male rats by inhibiting H2 histamine receptors, which is similar to the effects of ranitidine. Similarly, the antiulcer action of *C. longa* ethanolic extract was seen as it lowers ulcer index in addition to stomach acidity significantly. *C. longa* extract also suppressed hypothermic-restraint stress depletion of stomach wall mucus and diminished the severity of necrotizing agent-induced lesions.

Antidepressant Properties

Rats suffering from chronic mild stress (CMS) lead to much less sucrose intake; increased IL-6, TNF-α, CRF, and cortisol levels; smaller medulla oblongata; and reduced splenic NK cellular activity. The condition created in CMS was cured with ethanolic extract, which even caused the medulla oblongata to be lower than normal. *C. longa* has antidepressant potential because it tends to hinder monoamine oxidase accumulation in the central nervous system (Yu et al., 2002). Curcumin has a wide range of characteristics that are important to depression pathogenesis. The ethanolic *C. longa* extract prevented the decrease in serotonin, noradrenalin, and dopamine concentrations while increasing serotonin turnover, cortisol levels, and serum corticotrophin-releasing factor levels (Xia

et al., 2007). The consequences of orally administered curcumin seem on behavior under chronic stress or depression condition in the rat model. Curcumin administration showed a similar impact to imipramine, a known antidepressant drug, and it has been indicated by various authors to be a feasible alternative source in depression condition (Mohammed et al., 2019; Qi et al., 2020).

Curcumin Prevents Drug Resistance

Curcumin possesses a powerful anti-drug resistance agent. It has a novel capacity to suppress adriamycin-induced elevation of P-glycoprotein and its mRNA, and this ability is linked to increased intracellular drug accumulation, thereby increasing ADM lethality (Xu et al., 2011). Curcumin blocks NF-κB activation, which results in chemosensitivity in drug-resistant cancer cells. Furthermore, curcumin and tamoxifen co-therapy has also been illustrated to expose tamoxifen-resistant breast cancer cells, suggesting that it could be a viable method for either minimizing tamoxifen resistance or re-sensitizing refractory illness to tamoxifen therapy (Mimeault and Batra, 2011).

Antimicrobial and Synergistic Property

The essential oil as well as extracts of *C. longa* can suppress a diverse range of bacteria, infectious fungus, and parasites.

Antibacterial Activity

Some of the researchers assessed the antimicrobial activity of curcumin against different bacterial strains such as *Salmonella paratyphi*, *Trichophytongypseum*, *Staphylococcus aureus*, *Streptococci mutans*, and *Mycobacterium tuberculosis* (Tajbakhsh et al., 2008; Maghsoudi et al., 2017). The extract showed antimicrobial activity towards *Trichophyton longifusus*, *Microsporum canis*, and *S. aureus* while toxicity was indicated against *Lemna minor*. The *C. longa*-treated rabbit group had a significantly greater mean value for wound contraction, and therefore, wounds revealed decreased inflammation and a rising tendency in collagen formation. The extract of *C. longa* in ethanol was active for *Shigella flexneri*, *Staphylococcus epidermis*, *Klebsiella pneumoniae, Lactobacillus*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Salmonella typhi* (Oghenejobo and Bethel, 2017). Drug combinations can cause powerful or reductive pharmacokinetic effects, which may enhance or diminish the clinical efficiency of one another *via* regulation of absorption, distribution, metabolism, and excretion (Chou, 2006). Another study revealed the synergistic combinatorial impact of copper metal ions with aqueous extracts of *C. longa* against *Paenibacillus popilliae*, a known food spoilage bacterium, and detected the phytoconstituents including alkaloid, flavonoid, anthocyanin, steroids, and coumarin in *C. longa* extracts (Jassal et al., 2015). *C. longa* aqueous extract and chitosan possess significant synergism and antibacterial potency at 512 μg/ml and 1,024 μg/ml against MDR pathogens such as methicillin-resistant *S. aureus*, carbapenem-resistant *Pseudomonas*, carbapenem-resistant Enterobacteriaceae and AmpC-producing Enterobacteriaceae, and antibiofilm producers (Etemadi et al., 2021). The aqueous extract, curcumin component, and oil fraction of *C. longa* revealed antibacterial activity and suppresses *H. pylori*, *Streptococcus, Staphylococcus,* and *Lactobacillus* species (Mahady et al., 2002).

Synergistic Interaction

Curcumin has been identified to have synergistic effects but not antagonistic effects when combined with antibiotics such as oxacillin, ampicillin, ciprofloxacin, gentamicin, amikacin, polymyxin B, and norfloxacin, and anti-inflammatory effects when combined with a certain cytotoxic agent, with chemotherapy (Lin et al., 2007), or with a polyphenol derivative-containing diet (Strimpakos and Sharma, 2008). Various researchers have employed different extracts prepared from medicinal plants to treat MDR bacteria (Mehta and Jandaik, 2016; Urmila Jandaik et al., 2016; Mehta et al., 2021; Mehta et al., 2022a), which has been recognized as a global concern. A mixture of *C. longa*, galanga powder, and essential oil of lemongrass slowed the deterioration of raw white hard clam muscle, which improved the seafood quality during preservation (Nguyen, 2020).

Antifungal Activity

Curcumin was shown to improve the activity of common azole and polyene anti-fungal (Sharma et al., 2010). Another study indicated that *C. longa* oil suppressed dermatophytes and pathogenic fungus when applied externally on guinea pigs infected with dermatophytes, molds, and yeast. The guinea pigs with dermatophytes and fungal infected lesions improved, and the lesions become invisible after 7 days of *C. longa* administration. The antifungal, antibacterial, phytotoxic, cytotoxic, and insecticidal activities of a *C. longa* ethanolic extract were explored by Khattak et al. (2005). Ether, chloroform, and ethanol extracts of *C. longa* along with its oil possess antifungal effect against *Aspergillus flavus*, *Aspergillus parasiticus, Fusarium moniliforme*, and *Penicillium digitatum*, as suggested by several authors (Wuthi-Udomlert et al., 2000; Jayaprakasha et al., 2001). *C. longa* methanolic extract exhibited antifungal action for *Cryptococcus neoformans* and *Candida albicans*, which indicates minimum inhibitory concentration (MIC) values of 128 and 256 g/ml, respectively. Curcumin has antifungal action over all Candida test strains, at MICs varying from 250 to 2,000 g/L, but is less efficacious than fluconazole, according to a recent analysis. It could be attributable to changes in membrane-associated ATPase activity, ergosterol production, or proteinase secretion (Neelofar et al., 2011).

Antiviral and Antiparasitic Activity

Curcumin has antiviral potential (von Rhein et al., 2016) even for HIV; inhibiting HIV-1 LTR promoter directed gene expression with no effect on cell viability (Ashraf, 2018). Curcumin had moderate effectiveness towards *Plasmodium falciparum* and *Leishmania* organisms. The ethanol extracts exhibit anti-*Entamoeba histolytica* activity while curcumin has anti-*P. falciparum* and anti-*Leishmania* effect *in vitro*. Curcumin seems to have its antiviral activity for Epstein–Barr virus and

TABLE 2 | Curcumin of *C. longa* with immunomodulating activity and their mechanisms of action.

Main constituent	Subjects	Study design	Immunomodulatory activities	Modulation in parameters/mediators affected	References
Curcumin	Healthy albino mice	*In vivo*	White blood cell production and weight lymphoid organs	Stimulates lymphoid organs and white blood cells	Afolayan et al. (2018)
	Dendritic cells	*In vitro*	Surface molecule expression	Suppresses expression of CD80, CD86, MHC class II, and IL-1	Kim et al. (2005)
	Dendritic cells	*In vitro*	Cytokine production	Decreases production of IL-6, IL-12, and TNF-α	Kim et al. (2005)
	Dendritic cells	*In vitro*	Phosphorylation of mitogen-induced protein kinases (MAPKs) and NF-κB p65 translocation	Inhibition of LPS-induced MAPK activation and nuclear translocation of NF-κB p65	Kim et al. (2005)
	Bronchoalveolar of Balb/c mice	*In vivo*	Allergic response	Decreases number of eosinophils	Ravikumar and Kavitha (2020)
	Bronchoalveolar of Balb/c mice	*In vivo*	Cytokine production	Decreases level of IL-4	Ravikumar and Kavitha (2020)
	PBMCs	*In vitro*	T-cell proliferation	Inhibit the proliferation of lymphocyte	Yadav et al. (2005)
	PBMCs	*In vitro*	Cytokine production	Inhibits the production of IL-2 and TNF-α	Yadav et al. (2005)
	PBMCs	*In vitro*	NF-κB	Inhibit lipopolysaccharide-induced NF-κB	Yadav et al. (2005)
	Erythroleukemic cell line K562	*In vitro*	Cytotoxicity	Increases NK cell cytotoxicity	Yadav et al. (2005)
	Lupus BALB/c mice	*In vivo*	Adaptive immune response	Decreases the percentage of Th1, Th2, and Th17	Kalim et al. (2017)
	Lupus BALB/c mice	*In vivo*	Antinuclear antibody (ANA) levels	Decreases level of ANA	Kalim et al. (2017)
	Monocytes and liver macrophages	*In vivo*	ROS production	Increased the production of ROS	Inzaugarat et al. (2017)
	Monocytes and $CD4^+$ cells	*In vivo*	TNF-α and IFN-γ production	Enhanced the production of TNF-α in monocytes and IFN-γ in $CD4^+$ cells	Inzaugarat et al. (2017)
	Fish	*In vivo*	Immune response	Increased the expression of antimicrobial peptides	Alambra et al. (2012)

HIV (Taher et al., 2003). An extract of *C. longa* in both aqueous and ethanol is used in aquaculture as a treatment for bacterial infections (Sahu et al., 2005). Curcumin exerts anti-parasitic action against African trypanosomes, has schistosomicidal activities against *Schistosoma mansoni* adult worms, and has anti-malarial in addition to nematocidal effects. Diets supplemented with *C. longa* reduced small intestine lesion scores and boosted weight gain in chicks infected with the cecal protozoan, *Eimeria maxima*. Curcumin fits well to the active site of the protease, according to *in silico* modeling studies (Vajragupta et al., 2005) and proved to be a powerful inhibitor of HIV integrase, as it can bind acidic residues in the integrase's catalytic site, limiting it from interacting with its substrates. Molecular docking analysis revealed that particularly the keto-enol and terminal o-hydroxyl group of curcumin are tightly linked to the integrase's binding region formed by residues such as Glu92, Thr93, Asp116, Ser119, Asp64, His67, Thr66, Asn120, and Lys159 (Vajragupta et al., 2005). Recent analysis has also shown the therapeutic potential of *C. longa* against coronavirus disease 2019 (COVID-19) (Emirik, 2020), and its ability to modulate cytokine storm in COVID-19 patients (Valizadeh et al., 2020; Mehta et al., 2022b) has produced formidable renewed interest in *C. longa*.

Antifertility

Traditional medicine has been recommended by the World Health Organization as a cost-effective substitute to manufactured antifertility medicines. Parkes mouse strain was given aqueous rhizome extract of *C. longa via* the oral route (600 mg/kg body weight/day for 8 and 12 weeks), which causes reversible spermatogenesis, decreased seminiferous tubules diameter, and loosening of germinal epithelium, thus indicating its potential in male fertility. Hembrom et al. (2015) also examined the influence of an aqueous *C. longa* rhizome extract in sperm count, spermatozoa motility, and seminal pH in Swiss Albino male mice leading to infertility. The combined action of curcumin and andrographolide significantly suppressed the number of implants and litter size in female Sprague–Dawley rats, changed the duration of phases involved in the estrus cycle, and lowered the number of ovarian follicles (Shinde et al., 2015). Petroleum ether in addition to aqueous extract of rhizome shows antifertility impact on rats *via* oral administration and results in complete inhibition of implantation. Curcumin also reduces human sperm motility, suggesting its usage as intravaginal contraceptive and its antispermatogenic activity.

Immunomodulatory

In the management and therapy of diseases caused by immune system malfunctioning, immune response modulation is necessary. The most commonly used immunosuppressant in transplant rejection is cyclosporin A, a microbial peptide (Elgert, 2009); however, cyclosporin A exhibited toxicities

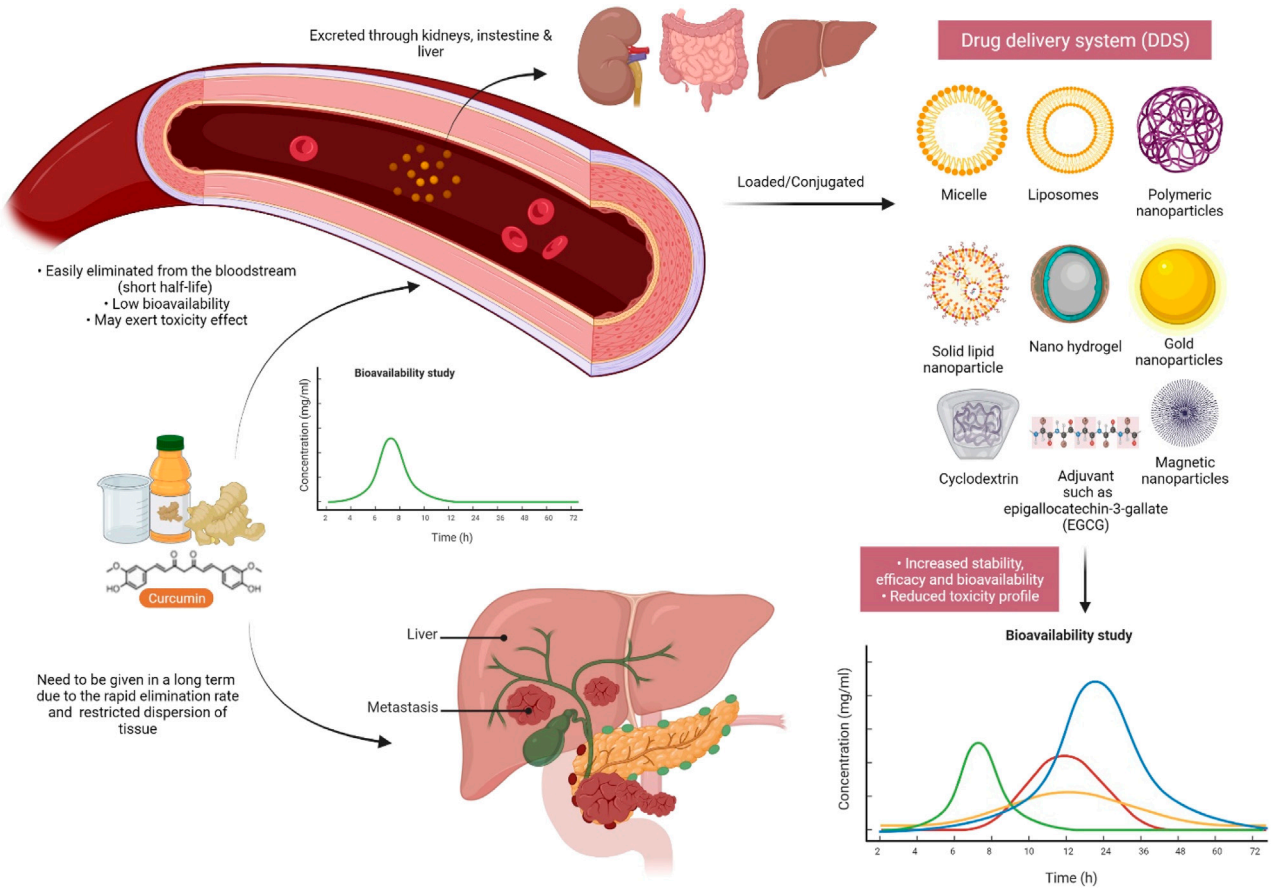


FIGURE 8 | Curcumin must be modified/improved prior to future research due to its extremely low bioavailability. Due to the quick elimination of curcumin in the systemic circulation as a result of enzyme metabolism, conjugating curcumin to adjuvants or other delivery systems may be beneficial in order to increase its half-life and bioavailability.

and adverse effects like nephrotoxic activity and gingival hypertrophy. Unfortunately, the majority of commercially available medications include adverse effects. The main important side effects of NSAIDs include injury to the stomach and intestinal mucosa. Corticosteroids, an immunosuppressive medicine, have several negative health risks, including decreased bone marrow and skin fragility. Natural products continue to be a valuable source of innovative and safe anti-inflammatory compounds (Elgert, 2009). Due to the existence of bioactive metabolites, many *Curcuma* species, including *C. longa, C. zanthorrhiza* Roxb., *C. amada* Roxb., *C. manga* Valeton & Zijp, *C. aeruginosa* Roxb., and *C. zedoaria* Rosc., have been shown to have a variety of immunomodulatory effects. Several recent analyses on the phytochemistry, biological, and pharmacological action of the *Curcuma* genus have been published (Rajkumari and Sanatombi, 2017; Sun et al., 2017; Mahadevi and Kavitha, 2020; USDA, 2021). Curcumin can inhibit the expansion of T cells triggered by plant lectin concanavalin A (Con A), according to a report on the role of the genus *Curcuma* and its bioactive metabolites to control the immunological response. Curcumin inhibits lymphoma B-cell proliferation by lowering the potency of c-MYC, BCL-XL, and NF-κB. Curcumin has also been demonstrated to suppress the production of ROS in macrophages. Curcumin also stimulates NK cell apoptosis by modulating the NF-κB pathway and inhibiting BCL-XL and Cyclin D. Curcumin inhibits IL-1 and IL-6 inflammatory cytokines such as from LPS-stimulated dendritic cells and suppresses the expressions of CD80, CD86, and MHC class II by dendritic cells. Curcumin also causes reduced LPS-induced MAPK activation and NF-κB p65 translocation in dendritic cells (Nair et al., 2017) along with impaired activation of Th1 responses. Curcumin significantly suppressed the formation of IL-6, IL-8, TNF-α, and MCP-1 from higher glucose-cultured monocytes, according to Jain et al. (2009). Curcumin decreased NOS activity and macrophages' ability to secrete nitric oxide (NO) at low doses. Curcumin has been revealed to be linked to the viral S1 protein, which is required for SARS-CoV-2 entry in an *in silico* approach; thus, it may inhibit cytokine storm in the severe stage of COVID-19 (Pawitan, 2020). **Table 2** shows the immunomodulating activity of curcumin with its mechanisms of action.

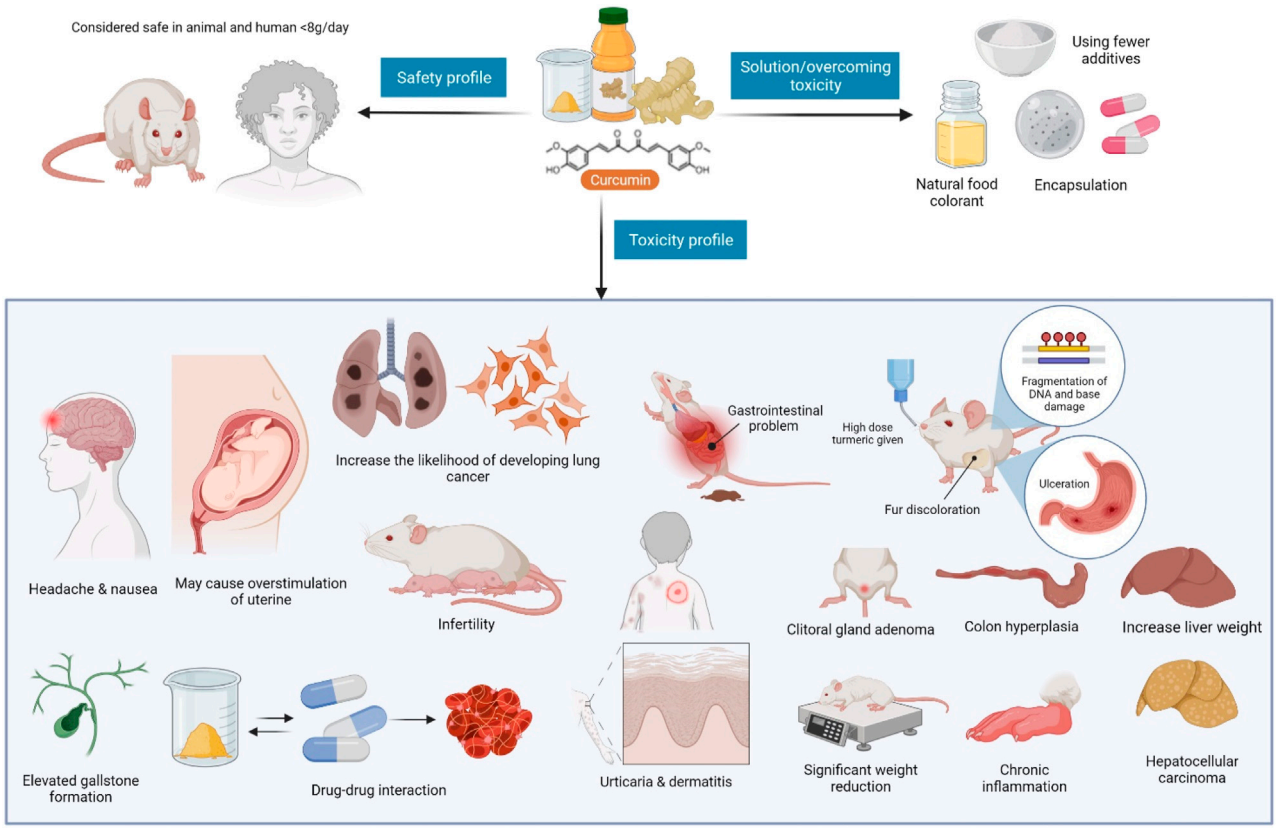


FIGURE 9 | Curcumin safety, toxicity profiles, and solution to overcome it. Curcumin safety, toxicity profiles, and a way to circumvent it. Despite its recognized safety, certain investigations have highlighted unprecedented side effects of curcumin, including gastrointestinal problems, chronic inflammations, and others. To address this issue, fewer additives should be utilized, and curcumin microencapsulation should be tailored.

TABLE 3 | Clinical uses of *C. longa* and its major compound curcumin (https://clinicaltrials.gov/).

S. No.	Clinical use	Condition	Intervention	Status
1.	Curcumin as nutraceutical in patients of depression	Major depressive disorder	Dietary Supplement: Curcumin Drug: Fluoxetine	Completed
2.	Premedication with curcumin on post-endodontic pain	Acute pulpitis	Drug: Curcumin Dietary Supplement: 400 mg starch	Completed
3.	Curcumin during the off treatment periods in patients with prostate cancer undergoing intermittent androgen deprivation therapy	Prostate cancer	Dietary Supplement: Curcumin and placebo	Completed
4.	Curcumin and function in older adults	Older adults, physical function, and cognitive function	Drug: Curcumin or microcrystalline cellulose	Completed
5.	Turmeric on new onset primary dysmenorrhea	Primary dysmenorrhea	Drug: Naproxen Dietary Supplement: Turmeric	Completed
6.	Turmeric and exercise-induced muscle damage and oxinflammation	Muscular injury	Dietary Supplement: Turmeric strength for joint and placebo	Completed
7.	Topical application of commercially available *Curcuma longa* gel on superoxide dismutase and malondialdehyde levels in saliva of chronic periodontitis patients	Chronic periodontitis	Drug: Topical application of Curenext gel Dietary Supplement: Placebo	Completed
8.	Turmeric on oxidative modulation in ESRD (end-stage renal disease) patients	End-stage renal failure	Drug: Turmeric Dietary Supplement: Placebo	Completed
9.	Turmeric in gingival massaging and adjunct to scaling and root planing in chronic periodontitis patient	Chronic periodontitis	Procedure: Tooth brushing with dentifrice, turmeric massaging, scaling, and root planing with turmeric massaging	Completed
10.	Turmeric and turmeric-containing tablets and sebum production	Skin inflammation	Dietary Supplement: Turmeric or turmeric-containing combination tablet or placebo tablets	Completed

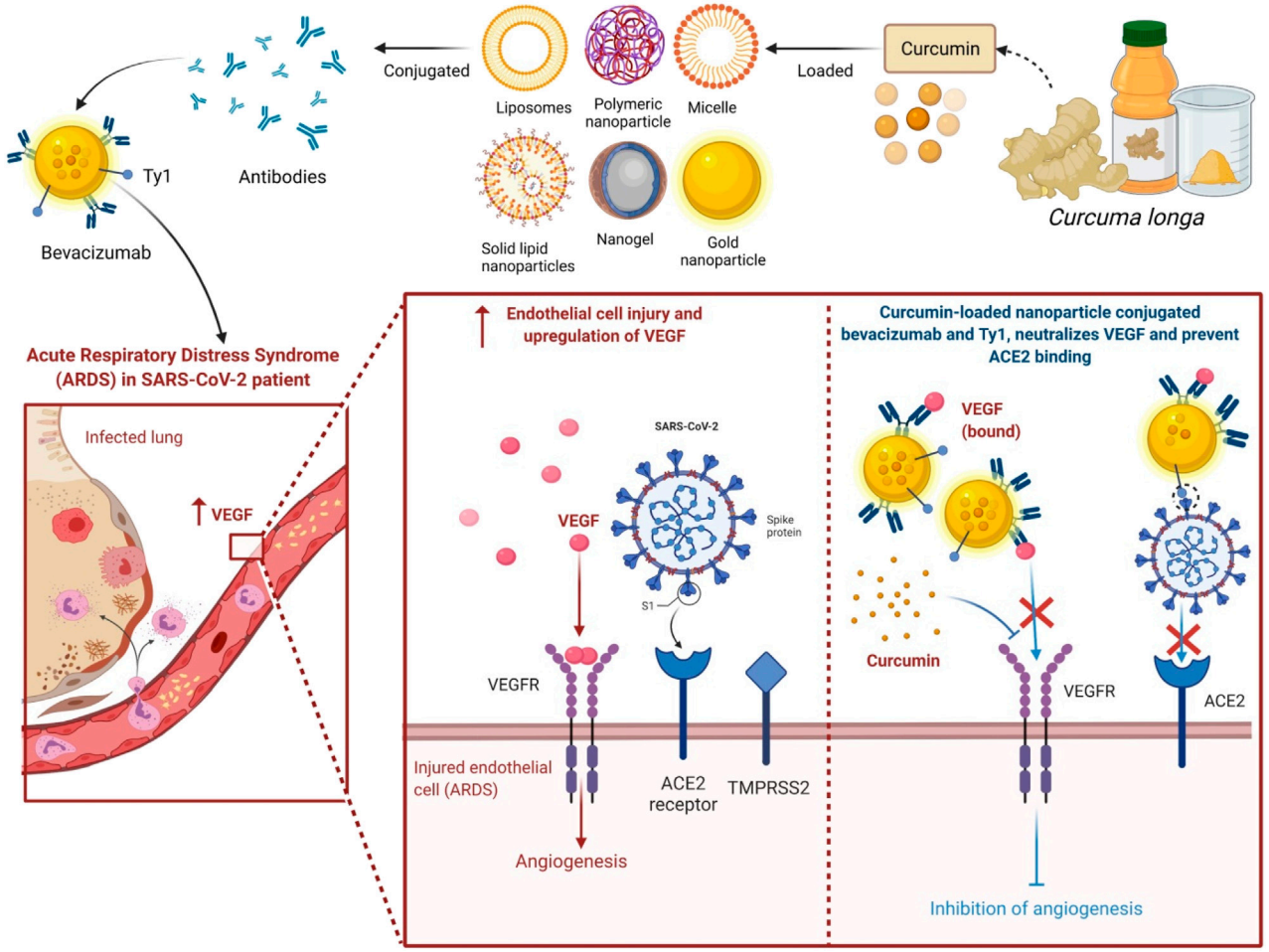


FIGURE 10 | Future perspective of curcumin-loaded gold nanoparticles functionalized with Ty1 and bevacizumab in a patient infected with SARS-CoV-2 (COVID-19).

NANO-FORMULATIONS AND GREEN SYNTHESIS OF *C. LONGA* AND ITS RELATED COMPOUNDS

In the last few years, the use of nanoformulation antibiotics has become increasingly popular in improving pharmacological therapeutic benefits. Nanomedicine is a relatively new field that is rapidly expanding in conjunction with nanotechnology and pharmaceutical disciplines (Caster et al., 2017). Nanopharmaceuticals, on the other hand, confront numerous hurdles, including improved characterization, toxicity issues, regulatory standards, high expenses, and healthcare warnings. Curcumin's practical applications are frequently limited by factors such as poor water solubility and physicochemical instability, poor bioactive absorption, rapid metabolization, low pharmacokinetics, bioavailability, penetration and targeting efficacy, sensitivity to metal ions, alkaline conditions, heat, and light, among others (Flora et al., 2013). These barriers are being overcome by encapsulating curcumin in nanoformulations (nano curcumin) (Yallapu et al., 2012). Integrating curcumin into nanocarriers is an effective and efficient way to improve curcumin's biological features, such as bioavailability and solubility, long-term circulation, and long-term retention in the body, as well as overcome curcumin's physiological challenges (Das et al., 2010; Li et al., 2013; Fonseca-Santos et al., 2016). It can also limit unintentional toxicity to neighboring normal cells/tissues by dispersing the intended tissues. Curcumin encapsulated in poly(lactic-co-glycolic acid) nanoparticles showed a ninefold increase in nano curcumin when compared to natural curcumin (Shaikh et al., 2009). Experiments demonstrate that nanoforms of curcumin are effective in the treatment of liver and heart problems (Shimatsu et al., 2012), cancer (Mohanty and Sahoo, 2010), and brain tumors (Mohanty and Sahoo, 2010; Lim et al., 2011). Moreover, curcumin nanoformulation exhibited threefold higher anti-HIV activity in comparison with its free form; obstructed the HIV-1-triggered expression of interleukin-1β (IL-1β), Topo II α, and cyclooxygenase 2 (COX-2); and blocked the synthesis of viral complementary DNA (cDNA) (Gandapu et al., 2011).

Moreover, metal-based green synthesis is gaining importance due to its chemical, optical, photochemical, and electronic properties (Mohanpuria et al., 2008). Among several metals, silver has gained huge attention for the green synthesis of NPs because of its numerous applications in various industries, particularly because of its nonlinear optical, biolabeling, and antibacterial capacity. Silver nanoparticles (AgNPs) are widely used in various fields like in drug delivery (Basu et al., 2018), nanomedicine (Carabineiro, 2017), agriculture, and cosmetics, and most importantly, they are used as an antimicrobial agent (Zhang et al., 2017). However, many scientists report that AgNPs also cause toxicity (McGillicuddy et al., 2017), but still, they play a major role as a disinfectant and as a antimicrobial agent. An emergence of nanotechnology that helps in the production of AgNPs has served as a new therapeutic modality. Because of their characteristic broad-spectrum antimicrobial ability, AgNPs have gained increasing attention in biomedical applications including wound management (Parveen et al., 2018; Ahsan and Farooq, 2019; Ravindran et al., 2019), but the hydrophobic nature of curcumin limits its biomedical applications. Hydrogels are in natural or synthetic forms including bacterial cellulose as one of the promising synthetic candidates used in wound dressings because of its ability to maintain a moist microclimate at the wound site, which has been proven to facilitate healing (Tao et al., 2019; Xue et al., 2019). The hydrophobicity of curcumin was overcome by its microencapsulation in cyclodextrins. A combination of AgNP with curcumin in the biosynthetic bacterial cellulose is used for the preparation of hydrogel dressings that exhibit antimicrobial activity against wound-infecting pathogenic microbes like *S. aureus, P. aeruginosa*, and *Candida auris* (Gupta et al., 2020). Among several different approaches to synthesizing AgNP, the use of extracts from natural plant sources has received wide research consideration because of the safe and eco-friendly procedure (Alsammarraie et al., 2018; Hemmati et al., 2019; Ravindran et al., 2019; Keshari et al., 2020). The antibacterial action of nano formed curcumin against a wide range of microorganisms, including fungi, bacteria, and viruses, has been carried out by researchers. Curcumin-modified AgNPs, for example, are utilized to inhibit respiratory syncytial virus (RSV) infection cells and reduce viral loads while having no deleterious side effects (Yang et al., 2016). Naseri et al. then investigated the antiviral effects of curcumin nanomicelles on the attachment and entrance of hepatitis C virus (HCV) infection, finding out that the viral load in HCV cells treated with curcumin nanomicelles was reduced (Naseri et al., 2017). Nanocurcumin was shown to exhibit improved antibacterial activity compared to curcumin because of its enhanced aqueous-phase solubility and simple dispersibility. An efficient antibacterial potential was found against *Bacillus subtilis*, *H. pylori*, *S. aureus,* and *P. aeruginosa* (Basniwal et al., 2011). An aqueous extract from *C. longa*-coated cotton fabrics, as well as formulated metallic AgNPs, also exhibited noticeable antimicrobial potency against *S. aureus*, *P. aeruginosa, S. pyogenes*, and *C. albicans*, and even AgNP-loaded cotton fabrics displayed potent wound healing activity in fibroblast (L929) cells (Maghimaa and Alharbi, 2020). Moreover, recently, it has been found that the combination of AgNP with the rhizome extract of *C. longa* showed antimicrobial effect towards plant affecting bacteria such as *Xanthomonas axonopodis* and *Erwinia amylovora*, which could be useful for nano-drug delivery applications (Gaurav, 2021). Curcumin's bioavailability was also discussed in **Figure 8**, as well as a method for dealing with it.

Side Effects, Contraindications, Precautions, and Safety Aspects of *C. longa*

Facts suggest that excessive turmeric consumption may trigger uterine contraction in pregnancy, and may hinder iron absorption (and so must be used with caution in iron-deficient individuals). Turmeric has been reported to reduce testosterone levels and sperm movement in men (when administered orally) and delay blood clotting (and so its use must be terminated at least 2 weeks before a scheduled surgery). According to some reports, turmeric should not be consumed if one has gallbladder and bleeding problems (Deshpande et al., 1998; Park et al., 2000). **Figure 9** depicts curcumin's safety and toxicity profiles, and a way to manage them (Cheng et al., 2001; Perkins et al., 2002; Rithaporn et al., 2003; Liddle et al., 2006; Sharma et al., 2007; Vareed et al., 2008; Dance-Barnes et al., 2009). In addition, **Table 3** summarizes the clinical uses of *C. longa* and its major component curcumin.

CONCLUSION

C. longa with its various pharmacological features has been characterized as a universal panacea among herbal remedies, as per the literature survey. This plant is regarded as a potent medicinal plant with a wide range of potent pharmacological properties due to the presence of numerous chemical components including starch, essential elements, proteins, vitamins, volatile oils, curcumin, and curcuminoids. Curcumin has a long history of use as a culinary spice and food color, as well as a component of Ayurvedic and Chinese medicine. Curcumin has a variety of beneficial effects on humans, according to science. Curcumin is still utilized as a cooking ingredient today, but modern technology has made it possible to use it in a range of food- and health-related applications. Curcumin's efficacy, safety, and pharmacokinetics have all been examined extensively in clinical studies over the last 50 years (Gupta et al., 2013; Subramani et al., 2018). The development of innovative nanomedicine formulations to increase curcumin targeting, pharmacokinetics, efficacy, and cellular uptake has been prompted by a significant therapeutic limitation (Salehi et al., 2020a; Salehi et al., 2020b). Cancer, CVD, arthritis, atherosclerosis, diabetes, gastric illness, IBD, psoriasis, acquired immunodeficiency syndrome, and other inflammatory disorders are all examples of pleiotropic activities. Several studies in this review discovered the anti-inflammatory effects of *C. longa* and curcumin, including decreased white blood cell, neutrophil, and eosinophil numbers, as well as protective effects on serum levels of inflammatory mediators like phospholipase A2 and total protein in various inflammatory disorders. Curcumin has anticancer properties by interfering with many cellular systems and inhibiting/inducing the production of multiple cytokines, enzymes, or IκKβ, TNF-α, STAT3, COX-2, PKD1, NF-κB, epidermal growth factor, and MAPK, among others. Under oxidative stress conditions, *C. longa* extracts and curcumin decreased MDA and NO levels while increasing thiol, SOD, and catalase levels. Curcumin also influenced the lifespan of organisms

by regulating important signaling pathways such as the mTOR, PKA, and FOXO signaling pathways. In conditions where the immune system was disturbed, treatment with *C. longa* and curcumin enhanced IgE, IL-4, TGF-β, IL-17, IFN-γ, and the Th1/Th2 ratio. The pharmacological effects of *C. longa* extracts and curcumin on respiratory, allergy, and immunologic problems suggest that *C. longa* and curcumin may have a possible therapeutic effect on these illnesses. *C. longa* extracts and curcumin delay the onset of diabetes, improve β-cell functioning, prevent β-cell death, and reduce insulin resistance in animal models. Curcumin's use has been limited due to its low water solubility, which can result in poor chemical stability, oral bioavailability, and cellular uptake. Other strategies that have been aggressively studied include delivering medications at a controlled rate, slow delivery, and targeted delivery. Curcumin nanoformulations have been produced to improve the solubility and bioavailability of the compound. Curcumin's medicinal applications and clinical efficacy could be expanded if biotechnology and nanotechnology were used to address the current limitations.

This review adds to the growing body of evidence supporting the use of turmeric as a preventative and therapeutic strategy. We believe that more progress in the development of strategies incorporating natural products can be exploited to be used against COVID-19 in the upcoming years. Hence, this paper also suggests the use of gold nanoparticles in combination with neutralizing antibody Ty1, which may assist selectively in the receptor binding domain of the SARS-CoV-2 spike, directly hindering angiotensin-converting enzyme 2 interaction. The inclusion of curcumin and bevacizumab further enhanced the efficacy of the proposed strategy as both may target and potently neutralize VEGF, thereby decreasing and slowing tumor growth (**Figure 10**).

FUTURE DIRECTIONS

C. longa entailed extensive research and development work to fully exploit its medicinal value, and efforts should be made to investigate the possibilities of practical clinical applications as well as the details of hidden and untold areas in order to maximize its utility for the benefit of humanity. It is suggested to inhibit cytokine storm in the severe stage of COVID-19. So, we can further work on it to get out of this pandemic situation of COVID-19 mutations. For the rational usage of turmeric and curcumin in the therapies of human diseases especially COVID-19, an accurate knowledge of effective dose, safety, and mode of action is necessary.

AUTHOR CONTRIBUTIONS

Writing—original draft: JM, AC, SF, MS, and NF; Conceptualization: JM and AC; Resources: SF, JM, AC, MS, NR, MB, VS, KC, LT, RN, YW, KS, PL, DM, VK, AA, and NF; Data curation: SF, JM, AC, MS, NR, MB, VS, KC, LT, RN, YW, KS, PL, DM, VK, AA, and NF; Writing—review and editing: SF, JM, AC, MS, NR, MB, VS, KC, LT, RN, YW, KS, PL, DM, VK, AA, and NF. All authors have read and agreed to the published version of the manuscript.

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Channa Striatus Protects Against PTZ-Induced Seizures in LPS Pre-conditioned Zebrafish Model

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Epilepsy is a neurological disorder characterized by recurrent unprovoked seizures. Mounting evidence suggests the link between epileptogenesis and neuroinflammation. We hypothesize that eliminating neuroinflammation can alleviate seizure severity and prolong seizure onset. *Channa striatus* (CS) is a snakehead murrel commonly consumed by locals in Malaysia, believed to promote wound healing and mitigate inflammation. This study aims to unravel the anticonvulsive potential of CS extract on neuroinflammation-induced seizures using an adult zebrafish model. Neuroinflammation was induced *via* cerebroventricular microinjection of lipopolysaccharides from *E. coli* and later challenged with a second-hit pentylenetetrazol at a subconvulsive dose of 80 mg/kg. Zebrafish behaviour and swimming pattern analysis, as well as gene expression analysis, were done to study the pharmacological property of CS. CS extract pre-treatment in all doses significantly reduced seizure score, prolonged seizure onset time and slightly improved the locomotor swimming pattern of the zebrafish. CS extract pre-treatment at all doses significantly reduced the expression of NF_KB gene in the brain, and CS extract at 25 mg/L significantly reduced the IL-1 gene expression suggesting anti-neuroinflammatory properties. However, there were no significant changes in the TNFα. Besides, CS extract at 50 mg/L also elevated the expression of the CREB gene, which exerts neuroprotective effects on the neurons and the NPY gene, which plays a role in modulating the inhibition of the excitatory neurotransmission. To sum up, CS extract demonstrated some anticonvulsive and anti-inflammatory activity on neuroinflammation-induced seizures. Still, more studies need to be done to elucidate the mechanism of action of CS extract.

Keywords: neuroinflammation, epilepsy, *Channa* striatus, seizures, anti-inflammatory, pentylenetetrazol

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INTRODUCTION

Epilepsy is a dynamic brain disorder that affects approximately 50 million people worldwide (WHO, 2018). The main hallmark of epilepsy is the spontaneous, unprovoked and synchronic hyperactivity of the neurons caused by sudden and excessive electrical discharges (Yaksi et al., 2021). The root cause of epilepsy is idiopathic, but there is compelling evidence suggesting the link between epileptogenesis and a wide array of factors stemming from neuroinflammation (Lee and Shaikh, 2019). Over the past decade, the connection between inflammation and epilepsy has been extensively studied using *in vitro* and *in vivo* experimental models, as inflammation was said to be detrimental to

cell survival, but at the same time, neuroinflammation was also believed to be neuroprotective (Vezzani et al., 2011).

The gold standard of treatment for epilepsy is anti-seizure drugs (ASDs). However, studies have shown that about one-third of individuals with epilepsy still experience seizures resistant to medication (Laxer et al., 2014). This is because they only provide symptomatic relief by blocking seizures rather than treating the underlying pathology of seizures (Pitkänen and Sutula, 2002). Hence, there is an urgent need to develop new therapies to alleviate the burden of drug-resistant epilepsy. In neuroinflammation-mediated epilepsy, it is believed that the inflammation promotes the release of cytokines and prostaglandins, which can lead to neuronal hyperexcitability and generation of seizures (Vezzani et al., 2011). Therefore, targeting and ameliorating neuroinflammation can eliminate the root cause of seizures and, at the same time, possibly treat neuroinflammation-mediated epilepsy.

In Malaysia, *Channa striatus* (CS), more commonly known as "haruan" fish by the locals, is an indigenous, predatory freshwater snakehead fish traditionally consumed for its pharmacological benefits. Numerous scientific studies have deciphered the therapeutic benefits of CS, such as its wound healing and analgesic ability, as well as boosting energy for the sick. In the lab, extracts of the fish can be made from the whole fish, roe, mucus and skin of the fish, but the most common preparation method is from the fish fillet, to mimic the traditional preparation method of making fish soup using the fillet (Mat Jais et al., 1997). Amino acids and fatty acids, found in high concentrations in the fish extract, might have contributed to its pharmacological properties. Important amino acids of the fish include glycine, alanine, lysine, aspartic acid and proline. In contrast, its major fatty acids are palmitic acid, oleic acid, stearic acid, linoleic acid and arachidonic acid (Zakaria et al., 2007). These amino acids are the essential component of collagen found in human skin. For example, glycine and other amino acids combine to form a polypeptide associated and responsible for skin growth and wound healing (Chyun and Griminger, 1984; Zakaria et al., 2007). The fatty acid, arachidonic acid, is a precursor to prostaglandins which may induce platelet aggregation and adhesion to endothelial tissue to initiate blood clotting (Zakaria et al., 2007). Although the prostaglandins derived from the breakdown of arachidonic acid are said to be mediators of nociception and inflammation, numerous studies are showing the antinociceptive, anti-inflammatory and wound healing effect of Haruan extract (HE) (Mat Jais et al., 1997; Baie and Sheikh, 2000; Baie and Sheikh, 2000; Somchit et al., 2004; Zakaria et al., 2004; Zakaria et al., 2005).

In this study, adult zebrafish (*Danio rerio*) was used as an animal model. Zebrafish have 70% genes homologous to humans, with approximately 85% genes related to recognized epilepsy genes (Hortopan et al., 2010; Howe et al., 2013). To unravel the anticonvulsive potential of CS extract under inflammatory conditions, we established a neuroinflammation model using adult zebrafish, challenged with a second-hit pentylenetetrazol (PTZ) at a subconvulsive dose.

MATERIALS AND METHODS

Chemicals and Equipment

Professor Abdull Manan Mat Jais from Abmanan Biomedical Sdn. Bhd. (Malaysia), provided CS extract which was extracted following a protocol described previously (Mat Jais et al., 1997). Pentylenetetrazole (PTZ) was used as a proconvulsant and purchased from Sigma-Aldrich (St. Louis, MO, United States) and lipopolysaccharide (LPS) derived from *Escherichia coli* 026: B6 was obtained from Sigma Aldrich (St. Louis, United States). Levetiracetam (Lev; Keppra®) was used as a positive control and manufactured by UCB Pharma (Brussels, Belgium). The zebrafish behavior was recorded using Sony Digital Handycam video camera (HDR-PJ340E) and later analyzed using SMART V3.0.05 tracking software (Panlab, Harvard Apparatus, Massachusetts, United States). For the cerebroventricular microinjection, stereoscopic microscope Nikon, SMZ 1500 (Nikon, Tokyo, Japan), pneumatic microjector, IM-11-2 (Narishinge, Tokyo, Japan) and borosilicate capillaries G-1 (Narishinge, Tokyo, Japan) were used.

Animals

Heterogeneous wild-type adult zebrafish (*Danio rerio*) of approximately 8 months old were purchased from *Danio* Assay (UPM, Malaysia). The zebrafish were housed at the animal facility unit of Monash University Malaysia under standardized husbandry conditions. Fish tanks (36 cm × 26 cm x 22 cm) were used to hold the zebrafish. The water temperature was maintained between 26 and 28°C, and pH 6.8 and 7.1 with a 14:10 light to dark cycle. They were fed three times a day with TetraMin® Tropical Flakes with occasional supplementation of live brine shrimps (artemia). Constant aeration was provided to the tanks with water circulation and filtration system. All animal experimentations were approved by the Monash University Malaysia Animal Ethics Committee (Project ID: 18499). Before any invasive procedures, the zebrafish were anaesthetized with 0.6 mg/L of benzocaine and precautions were taken to minimise suffering.

Neuroinflammation Induction With Cerebroventricular Microinjection (CVMI)

Adapted from Kizil et al., 2013 study, cerebroventricular microinjection was performed on the adult zebrafish to induce neuroinflammation. Lipopolysaccharide (LPS) derived from *Escherichia coli* 026: B6 (Sigma Aldrich, St. Louis, United States) was used as an inflammatory agent for the induction. Firstly, a small opening was incised on the zebrafish skull using a 30G needle on the cranial bone close to the midline located above the optic tectum. The slit exposes the zebrafish's cerebroventricular fluid (CVF), allowing LPS solution to be microinjected using thin glass capillaries without damaging the brain. This injection can disperse the LPS solution throughout the brain, targeting the ventricular

and periventricular cells, inducing generalized neuroinflammation. For this study, 100 nL of 2 mg/ml LPS were injected into zebrafish in groups 6-10, and distilled water of the same volume was injected into zebrafish in group 5.

Anti-Convulsant Study

Drug Treatment and Groups

The zebrafish were divided into ten groups, consisting of 8 zebrafish per group. Pentylenetetrazol (PTZ, Sigma Aldrich, United States) was used as a chemiconvulsant to induce seizures. PTZ was dissolved in distilled water to a concentration of 80 mg/kg and injected via the intraperitoneal route. This study used Levetiracetam (Lev, Keppra®) from UCB Pharma (Braine-l'Alleud, Belgium) as a positive control. It was administered by dissolving in system water (5 g/L) and poured into a tank where the zebrafish were placed. CS extract treatment was also given by dissolving into system water and poured into the zebrafish tank. The zebrafish were exposed to CS extract for 2 h.

Group 1: ControlGroup 2: LPS onlyGroup 3: Lev onlyGroup 4: CS (100 mg/ml) onlyGroup 5: Sham + PTZ (80 mg/kg)Group 6: LPS + PTZ (80 mg/kg)Group 7: LPS + Lev + PTZ (80 mg/kg) Group 8: LPS + CS 25 mg/ml + PTZ (80 mg/kg)Group 9: LPS + CS 50 mg/ml + PTZ (80 mg/kg)Group 10: LPS + CS 100 mg/ml + PTZ (80 mg/kg).

The anti-convulsant study was adapted from the protocol established in our lab (Chung et al., 2020). Group 1 acted as the control group and did not receive any treatments. Groups 2, 3 and 4 acted as the per se group and received only one treatment: LPS, lev, and CS extract, respectively. Group 5 was the sham control, receiving saline during the CVMI. Group 6 and 7 were negative and positive controls, respectively. Groups 8, 9 and 10 were treatment groups receiving three different doses of CS extract representing low, medium and high doses. LPS administration via CVMI was given 24 h before treatments to allow the development of neuroinflammation. Lev and CS extract treatment lasted for 2 h before the PTZ administration.

In this study, PTZ was administered at 80 mg/kg ip., a subconvulsive dose. Usually, a subvconvulsive dose is given to induce chronic seizures, but in our study, the subconvulsive dose allowed us to investigate if the neuroinflammation aggravated the susceptibility to seizures. After administering seizures, the zebrafish movements were recorded using a Sony Digital Handycam video camera (HDR-PJ340E) for behavioural analysis. Then, the zebrafish brains were harvested for gene expression analysis.

Seizure Behavioural Analysis

The following scoring system was used to evaluate the zebrafish seizure scores, which indicated the severity of seizures:

Score 1—Short swim mainly at the bottom of the tankScore 2—Elevated swimming activity and increased frequency of opercular movements Score 3—Burst swimming patterns with left and right and erratic movements Score 4—Circular swimming movements.

The highest seizure score displayed and the onset time for seizure score 4 (if any) were noted when viewing the recorded videos. Then, the videos were analyzed using tracking software SMART V3.0.05 (Pan Lab, Harvard Apparatus) to evaluate the swimming patterns of the zebrafish and the total distance travelled.

Gene Expression Analysis

Brain Harvesting

The extracted individual fish brains were immediately transferred to a 200 µl of ice-cold TRIzol® (Invitrogen, United States) for gene expression analysis and stored at -80 °C until further investigation.

RNA Isolation and Synthesis of the First-Strand cDNA

According to the protocol supplied by the kit's manufacturer (Qiagen, United States), the mRNA was isolated and was identical to the protocol used by Kundap et al. (2017). Briefly, the zebrafish brain was first homogenized in TRIzol® before the isolation of mRNA. The mRNA obtained was quantified with Nanodrop Spectrophotometer was then converted to cDNA as per the instructions given in the Omniscript Reverse-transcription Kit (Qiagen, United States).

StepOne® Real-Time PCR

The gene expression levels were determined via real-time quantitative RT-PCR (Applied Biosystems, United States). The QuantiNova SYBR® Green PCR Kit and the appropriate Qiagen primer set were used for each gene; using a similar protocol as Choo et al. (2018). The genes that were studied are nuclear factor kappa B (NF-κB), tumour necrosis factor-alpha (TNFα), interleukin (IL-1), high mobility group box 1 (HMBG1), neuropeptide Y (NPY), and cAMP Response Element-Binding Protein (CREB). Eukaryotic translation elongation factor 1 alpha 1b (eef1a1b) was used as the housekeeping gene. The following are their respective primers used for the PCR:

NF-κB: Dr_nfkb1_2_SG QuantiTect Primer Assay (Cat no. QT02498762).
TNF-α: Dr_tnf_1_SG QuantiTect Primer Assay (Cat no. QT02097655).
IL-1: Dr_il1rapl1a_1_SG QuantiTect Primer Assay (Cat no. QT02131850).
HMGB1: Dr_hmgb1b_2_SG QuantiTect Primer Assay (Cat no: QT02088555).
NPY: Dr_npy_1_SG QuantiTect Primer Assay (Cat no. QT02205763).
CREB_1: Dr_crebbpa_1_SG QuantiTect Primer Assay (Cat no. QT02197503).
eef1a1b: Dr_eef1a1b_2_SG QuantiTect Primer Assay (Cat no. QT02042684).

The PCR began with first incubation at 95°C for 2 min before thermal cycling. According to the manufacturer's protocol, the thermal cycling settings for the PCR were 40 cycles of 95°C for 5 s and 60°C for 15 s. We calculated the relative expression level (fold change) of the genes of interest by normalizing the threshold cycle (Ct) values obtained from the genes of interest against the Ct

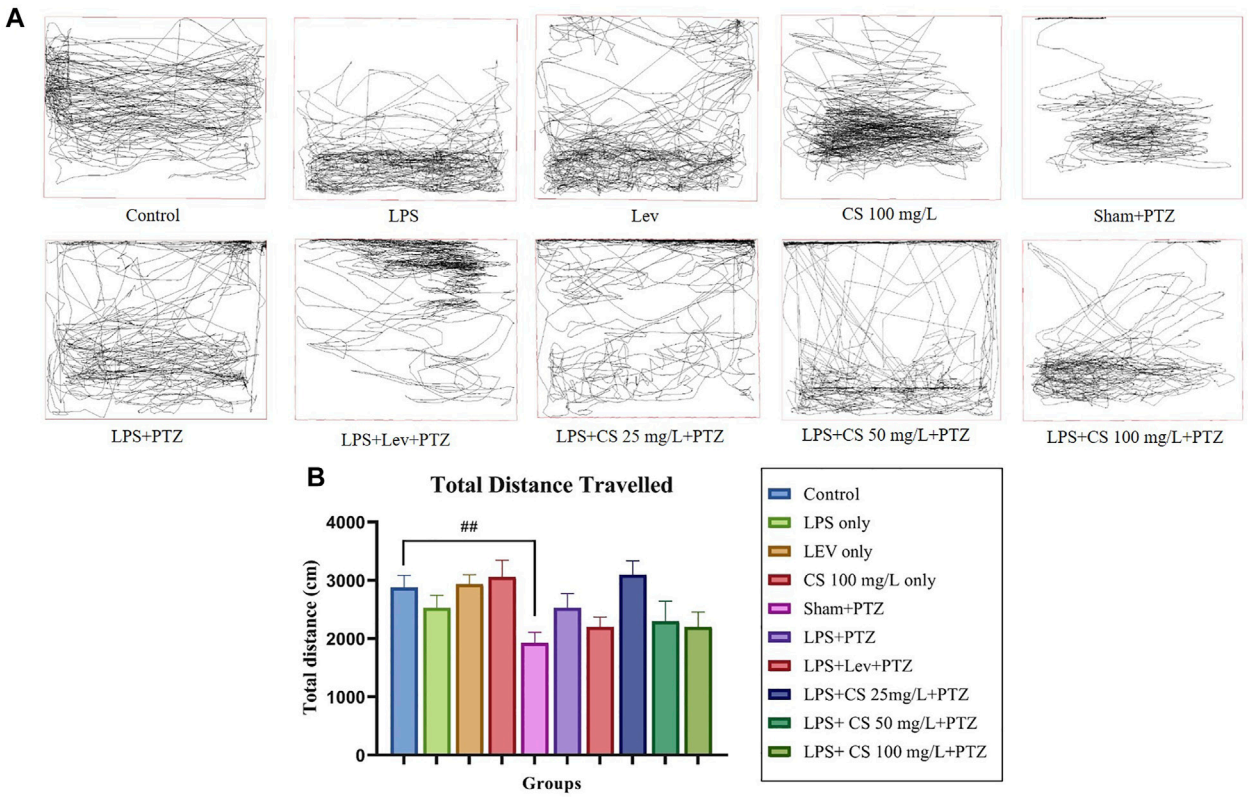

FIGURE 1 | (A) Representative swimming pattern of zebrafish in each group. **(B)** Mean total distance travelled (cm) by zebrafish in each group. The *p*-values $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$ were regarded as statistically significant for the treatment groups compared to the negative control (LPS + PTZ). The *p*-values $^{\#\#\#\#}p < 0.0001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ was regarded as statistically significant for the *per se* groups compared to the control.

value of the eef1a1b housekeeping gene. The formula for the calculation is:

$$\textit{Relative expression level} = 2^{\left(Ct\,eef1a1b - \hat{Ct}\,\textit{Gene of interest}\right)}$$

RESULTS

Anti-Convulsant Study

Zebrafish swimming pattern was analyzed by SMART software, and one swimming pattern representative was selected for each group to be displayed in **Figure 1**. Zebrafish in the control group showed no preference toward any part of the tank. This observation can similarly be seen in zebrafish in lev, CS 100 mg/L and sham + PTZ groups. Conversely, zebrafish in the LPS group showed a preference for the lower part of the tank. Zebrafish from the negative control (LPS + PTZ) group showed a more erratic swimming pattern with more dwelling activity on the top of the tank. Lev pre-treatment reduced swimming activity, but top dwelling was still observed. Zebrafish in the CS extract pre-treated groups showed less erratic swimming behaviour, but top dwelling was still observed in groups treated with 25 mg/L and 50 mg/L. Zebrafish pre-treated with 100 mg/L did not show top dwelling activity. The mean total distance travelled was analyzed and compared. There was a significant reduction in the mean total distance travelled in the sham + PTZ group ($p < 0.01$). The changes in mean total distance travelled were insignificant in other groups.

The swimming behaviour of the zebrafish was observed for 10 min post recovery from anaesthesia as the acute seizures induced via PTZ usually last less than that. Therefore, zebrafish with no seizures were reported to have an onset time of 600 s. Our study observed no significant changes in the seizure onset time between the control and groups receiving only LPS, lev or CS extract treatments as shown in **Figure 2**. There is a significant dip in seizure onset time for the negative control (LPS + PTZ) group compared to the control ($p < 0.0001$). Levetiracetam treatment post-CVMI was able to delay the seizure onset time significantly ($p < 0.001$), and this is also observed in zebrafish receiving CS extract treatment of 50 mg/ml ($p < 0.05$) and 100 mg/ml ($p < 0.01$). As for mean seizure score, CVMI of LPS and PTZ administration induced seizure score 4 significantly ($p < 0.0001$) even at a subconvulsive dose of 80 mg/kg. Levetiracetam treatment reduced the mean seizure score significantly ($p < 0.001$). This pattern is also observed in CS extract treatment at all doses, with a significance of $p < 0.05$ at the dose of 100 mg/ml.

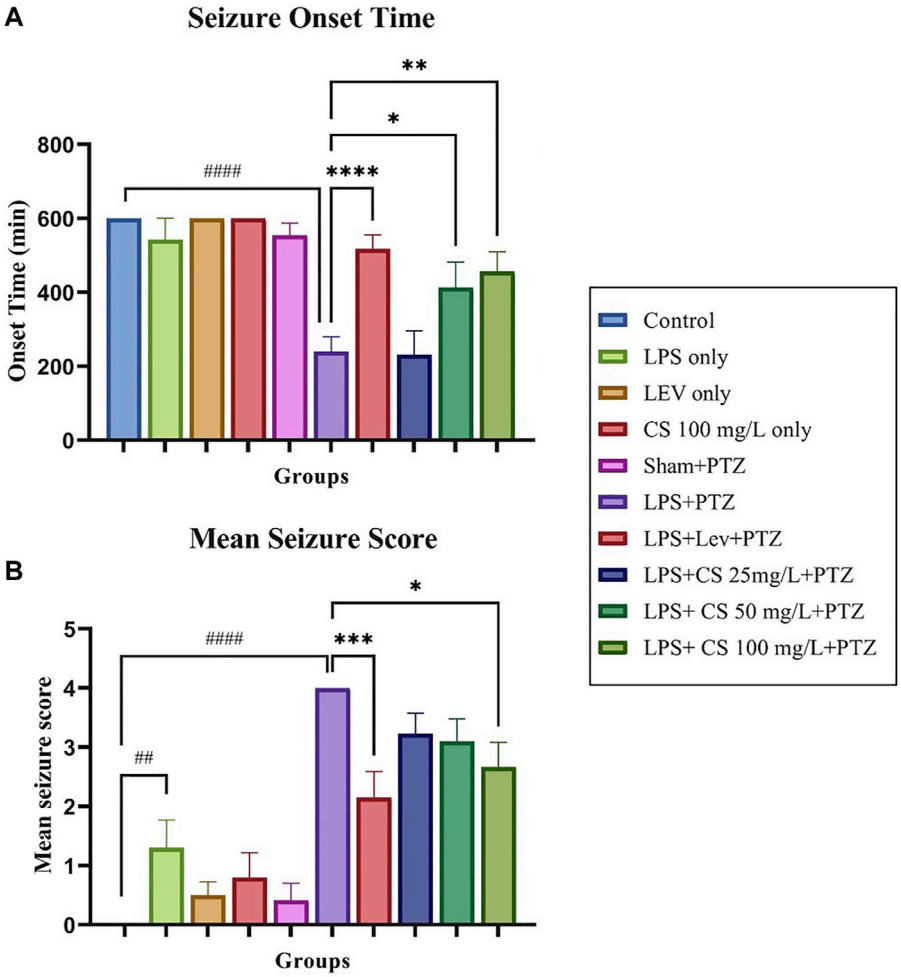

FIGURE 2 | (A) Seizure score 4 onset time for each group. **(B)** Mean seizure scores for each group. The *p*-values $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$ were regarded as statistically significant for the treatment groups compared to the negative control (LPS + PTZ). The *p*-values $^{\#\#\#\#}p < 0.0001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ was regarded as statistically significant for the *per se* groups compared to the control.

Gene Expression Analysis

A Gene expression study was performed to evaluate the changes in the expression levels of several inflammatory genes and genes implicated in epilepsy. As per **Figure 3A**, the expression of CREB was upregulated significantly in zebrafish in the negative control (LPS + PTZ) compared to the control group ($p < 0.05$). Interestingly, the zebrafish in the LPS only group had a significantly higher expression of CREB ($p < 0.005$). In contrast, other *per se* groups did not show any significant changes compared to the control group. The treatment of lev and CS extract further increased the expression of CREB, and this is significant at the CS extract dose of 50 mg/ml ($p < 0.05$) compared to the negative control (LPS + PTZ). CS extract treatment at 100 mg/ml reduces the CREB expression to a level lower than the negative control (LPS + PTZ), but this is not significant.

As per **Figure 3B**, the expression of HMGB1 was significantly upregulated in zebrafish receiving CS extract treatment at 100 mg/ml ($p < 0.0001$) compared to the control group. For the negative control (LPS + PTZ), we found a significant downregulation of the expression of HMGB1 compared to the control group ($p < 0.01$). Lev and CS extract at doses of 25 mg/ml treatments slightly increased the expression of HMGB1, but these increments were not significant compared to the negative control (LPS + PTZ).

As illustrated in **Figure 3C**, the expression of the NPY gene in the negative control (LPS + PTZ) group was significantly higher than in the control group ($p < 0.0001$). A similar pattern was observed in the LPS only and CS treatment at 100 mg/ml *per se*, compared to the control group. Lev treatment slightly reduced the expression of the NPY gene, while CS treatment at 50 mg/L significantly increased the NPY expression ($p < 0.0001$) with no significant changes at other concentrations.

The expression of inflammatory genes TNFα, IL-1 and NF_KB, were demonstrated in **Figures 4A–C**, respectively. TNFα gene expression was significantly reduced in the Sham + PTZ group, compared to control ($p < 0.01$). In the negative control group, the

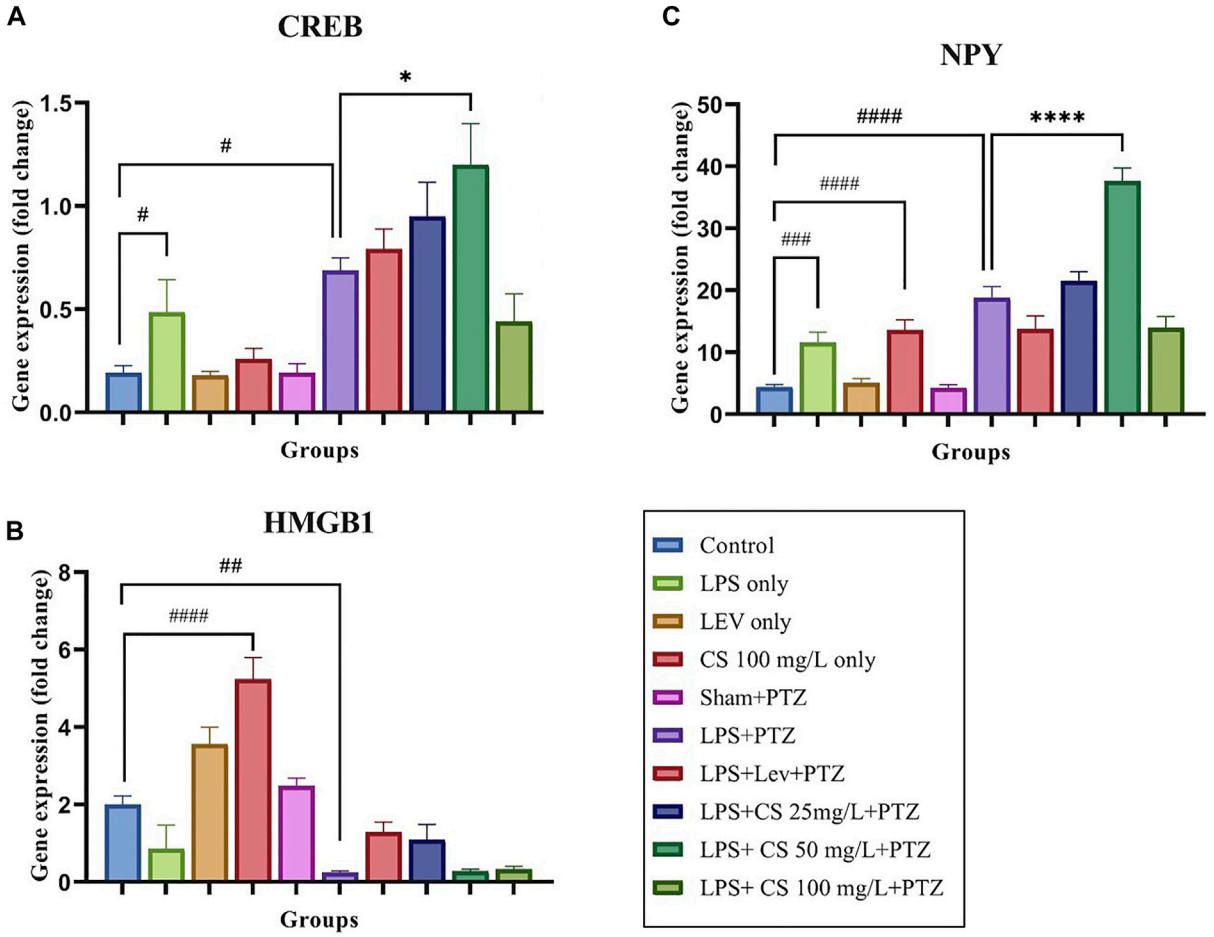

FIGURE 3 | Gene expression levels for the following genes: **(A)** CREB, **(B)** NPY, and **(C)** HMGB1. The *p*-values $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$ were regarded as statistically significant for the treatment groups compared to the negative control (LPS + PTZ). The *p*-values $^{\#\#\#\#}p < 0.0001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ was regarded as statistically significant for the *per se* groups compared to the control.

TNFα expression was increased slightly and not significant compared to the control group. Lev and CS extract at a low dose of 25 mg/L and 100 mg/L reduced the TNFα expression but were not significant.

IL-1 expression was upregulated in the lev *per se* group, compared to the control ($p < 0.01$). There were no significant changes in the other *per se* groups. The expression of IL-1 was unexpectedly decreased in the negative control group (LPS + PTZ) compared to the control group, but this was not significant. There were no significant changes in the treatment groups except groups pre-treated with 50 mg/L of CS extract ($p < 0.05$).

For the expression of NF_KB, we observed no significant changes in the *per se* groups compared to the control. The expression of NF_KB was significantly elevated in the negative control (LPS + PTZ) group compared to the control ($p < 0.01$). Lev and CS extract treatments significantly lowered the expression of NF_KB at a significance of $p < 0.01$ for CS extract 50 mg/L, $p < 0.001$ for lev and CS extract 100 mg/ml and $p < 0.0001$ for CS extract 25 mg/L.

DISCUSSION

This work aims to determine the anti-seizure potential of CS extract on seizures induced by PTZ aggravated by neuroinflammation. In our previous study (unpublished), we had established that 652.9 mg/L is the LD_{50} for CS extract in adult zebrafish, from which we selected 25 mg/L, 50 mg/L and 100 mg/L as the low, medium, and high dose for treatment in this study.

Firstly, neuroinflammation with the second hit PTZ model of adult zebrafish was established. We adapted the use of LPS, which is an inflammatory agent, and the CVMI procedure, to induce neuroinflammation to adult zebrafish. LPS can trigger the inflammation cascade of zebrafish embryos and inflict neuroinflammation in adult zebrafish via systemic injections (Ko et al., 2017; Fasolo et al., 2021). Considering PTZ will be injected intraperitoneally, we decided not to induce neuroinflammation systematically with LPS ip. But via CVMI instead, to prevent double injections to the same injection site and for targeted neuroinflammation induction (Kizil et al., 2013).

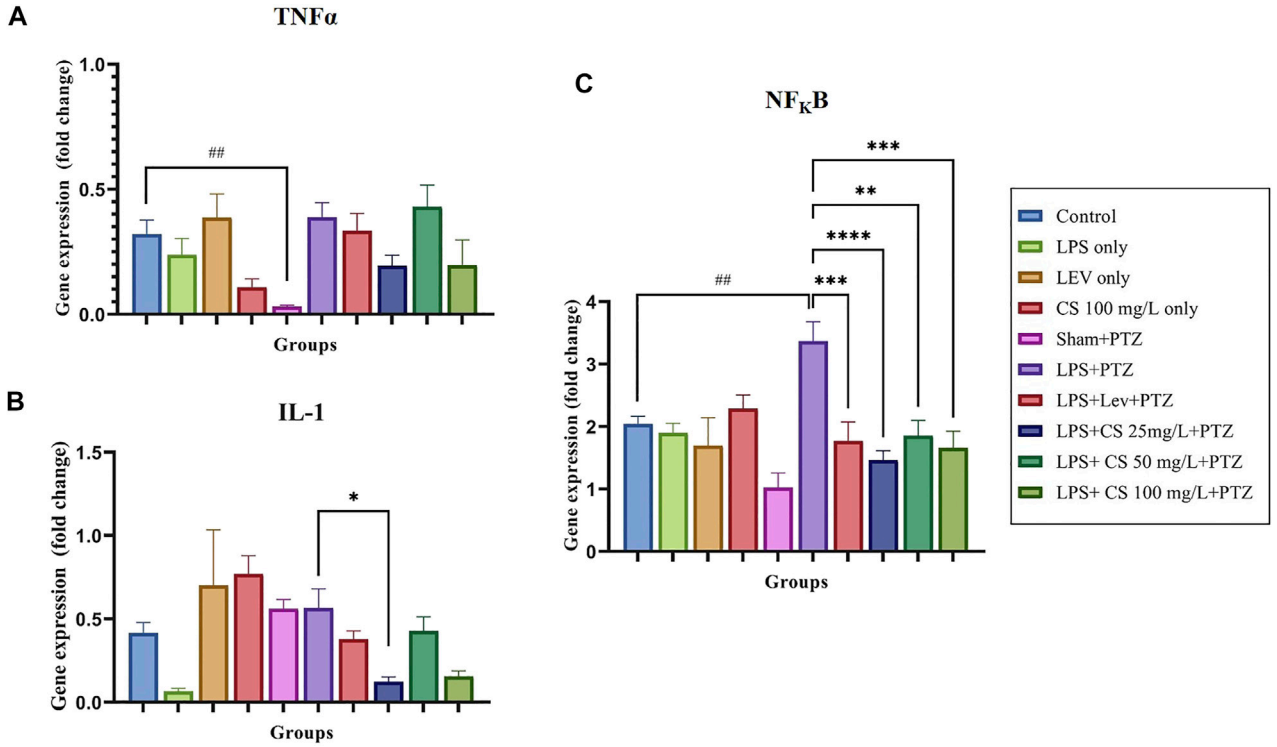

FIGURE 4 | Gene expression levels for the following inflammatory genes: **(A)** TNFα, **(B)** NF$_K$B, and **(C)** IL1. The *p*-values $^{****}p < 0.0001$, $^{***}p < 0.001$, $^{**}p < 0.01$, and $^{*}p < 0.05$ were regarded as statistically significant for the treatment groups compared to the negative control (LPS + PTZ). The *p*-values $^{\#\#\#\#}p < 0.0001$, $^{\#\#\#}p < 0.001$, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ was regarded as statistically significant for the *per se* groups compared to the control.

After induced neuroinflammation, we challenge the zebrafish with a subconvulsive dose of PTZ (80 mg/kg). Based on the findings from the behavioural study, LPS CVMI alone does not produce clonic-like seizures (score 4) but relatively mild symptoms of seizures (Score 1 and 2). However, after a challenge with a second hit PTZ, clonic-like seizures were significantly induced. Therefore, this result suggested that the model for neuroinflammation-induced seizures is established. This is a novel model as no previously published evidence suggested such a model.

Swimming pattern analysis showed that zebrafish in the LPS *per se* group showed an increase in bottom-dwelling time. This is a classical characteristic of anxiety in zebrafish (Choo et al., 2019). The swimming pattern of zebrafish in the negative control (LPS + PTZ) group showed more erratic movements, and this can be explained by the inhibition of GABA by the action of PTZ, which promotes neuronal excitability leading to uncontrolled motor movements such as kindling or twitching (Chung et al., 2020). Lev pre-treatment seemed to alleviate the anxiety behaviour, which was also seen in groups pre-treated with CS extract at all doses, although the effects were mild. Coupled with the significant reduction of seizure scores and increase in seizure onset time, CS extract could exhibit anticonvulsant activity which were comparable to levetiracetam, an antiepileptic drug with anti-inflammatory property.

Based on the results of gene expression analysis, we observed a significant increase in the expression of CREB in the negative control group (LPS + PTZ) compared to the control group ($p < 0.05$). This was expected as CREB is believed to be involved in the epileptogenesis process (Guo et al., 2014). There was also a significant rise in CREB expression in the LPS *per se* group compared to the control ($p < 0.05$). This could be due to the neuroinflammation induced by LPS, which triggered the rise in CREB expression as it is a neuroprotective transcription factor (Feldmann et al., 2019). Lev and CS pre-treatment further increased the expression of the CREB gene, and this increment is significant at CS extract at a dose of 50 mg/L ($p < 0.05$). This finding establishes the neuroprotective potential of CS extract, which can similarly be seen in antiepileptic drugs such as cenobamate (Wiciński et al., 2021). NPY plays a role in modulating the different processes in the brain and is expressed in multiple areas of the brain. One of its major roles is regulating the inhibition of excitatory synaptic transmission, particularly glutaminergic synaptic transmission (Tekgul et al., 2020; Cattaneo et al., 2021). In the present study, NPY expression was found to be significantly increased in the negative control group (LPS + PTZ) compared to the control ($p < 0.0001$) and lev treatment was able to reduce the expression of NPY insignificantly. This finding was similar to that of a study by Tekgul et al. (2020). Interestingly, CS extract treatments further increased the expression of NPY at 25 mg/L (insignificant) and

50 mg/L (significant with $p < 0.0001$). Worsened seizure conditions usually accompany this observation, but adequate seizure control was observed with these groups. This could suggest that CS extract exerts its anticonvulsant activity via the enhanced activity of NPY and inhibitory processes.

HMGB1 is an endogenous protein that acts as a 'danger signal' in response to stress or damage to the neurons (Lee and Shaikh, 2019). Usually elevated in epileptic conditions, HMGB1 activates IL-1 and toll-like receptor 4 (TLR4) pathways and triggers the generation of seizures (Paudel et al., 2018). However, in our study, we found a significant reduction in HMGB1 expression in the negative control (LPS + PTZ) group compared to the control ($p < 0.01$). This is not uncalled for, as there were reports of a reduction of HMGB1 after kainic acid-induced seizures. A study by Luo et al. (2014) observed a drop in HMGB1 levels in the brain and a surge of serum HMGB1 levels. It was reported that HMGB1 was sequestered from neurons into the blood after acute cell death, explaining our results (Luo et al., 2014). The involvement of HMGB1 in the IL-1 pathway is noteworthy. The drop in brain levels of HMGB1 can explain the lack of a significant change in IL-1 of zebrafish in the negative control (LPS + PTZ) group compared to the control. Similar observations were also previously reported in a study by Choo et al. (2018). CS extract pre-treatment can reduce the expression of the inflammatory gene IL-1, and this is significant at 25 mg/L compared to the negative control (LPS + PTZ) group ($p < 0.05$). This can be due to the potent anti-inflammatory property of CS extract that was previously reported (Zakaria et al., 2008; Shafri and Abdul Manan, 2012).

The gene expression analysis also demonstrated an insignificant change in the inflammatory gene, TNFα, expression in the negative control group (LPS + PTZ) compared to the control. This was unexpected because acute seizures are most likely to increase the expression of TNFα. This may be contributed by the fact that the induction of neuroinflammation and second hit PTZ was insufficient to cause an increase in TNFα expression levels. On the other hand, we observed a significant increase in NF_KB expression level in the negative control (LPS + PTZ) group compared to the control ($p < 0.01$). Lev and CS extract pre-treatment significantly reduced the NF_KB expression level at significance of $p < 0.001$ for lev and CS extract 100 mg/L, $p < 0.0001$ for CS extract 25 mg/L, and $p < 0.01$ for CS extract 50 mg/L. Similar observations have been reported by Teocchi et al. (2013). This is most likely contributed by the anti-inflammatory and potential anticonvulsant properties of CS extract.

The present study demonstrated a novel model of neuroinflammation with second-hit PTZ in adult zebrafish. This model could be helpful in future studies involving neuroinflammation-induced epilepsy. While CS extract was shown to exhibit some anti-inflammatory and anti-convulsant properties, more studies need to confirm these findings. A protein expression study would be useful in studying the different proteins modulated by CS extract, revealing the pathways in which CS extract is involved.

CONCLUSION

Overall, it may be said that CS extract can have an anti-neuroinflammatory and anticonvulsive effect as it was protective against seizures induced by the second-hit PTZ model. CS extract treatment displayed neuroprotective and anti-neuroinflammatory properties by modulating gene expression levels.

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 animal study was reviewed and approved by Monash University Malaysia Animal Ethics Committee.

AUTHOR CONTRIBUTIONS

VL was responsible for performing all the experiments and writing the manuscript. VL, AN, and SN worked together to establish the techniques for model development. VL and MS conceptualized and designed the experiment. All authors approved the final manuscript for submission.

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Methyl 6-O-cinnamoyl-α-D-glucopyranoside Ameliorates Acute Liver Injury by Inhibiting Oxidative Stress Through the Activation of Nrf2 Signaling Pathway

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Excessive stimulation of hepatotoxins and drugs often lead to acute liver injury, while treatment strategies for acute liver injury have been limited. Methyl 6-O-cinnamoyl-α-D-glucopyranoside (MCGP) is a structure modified compound from cinnamic acid, a key chemical found in plants with significant antioxidant, anti-inflammatory, and antidiabetic effects. In this study, we investigated the effects and underlying mechanisms of MCGP on acetaminophen (APAP)- or carbon tetrachloride (CCl_4)-induced acute liver injury. As a result, MCGP inhibited cell death and apoptosis induced by APAP or CCl_4, and suppressed the reactive oxygen species (ROS) generation stimulated by H_2O_2 in liver AML12 cells. *In vivo*, MCGP alleviated APAP/CCl_4-induced hepatic necrosis and resumed abnormal aminotransferase activities and liver antioxidase activities. In addition, MCGP depressed APAP- or CCl_4-induced oxidative stress through the suppression of CYP2E1 and activation of nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. MCGP also enhanced the number of PCNA-positive hepatocytes, increased hepatic PCNA and Bcl-XL, and decreased BAX expression in APAP-/CCl_4-intoxicated mice. Furthermore, MCGP activated the GSDMD-N/cleaved caspase 1 pathway. In summary, MCGP might act as a potential therapeutic drug against drug-induced and chemical-induced acute liver injuries, and its underlying mechanisms might engage on the pressing of oxidative stress, refraining of hepatocyte apoptosis, and facilitating of liver regeneration.

Keywords: methyl 6-O-cinnamoyl-α-D-glucopyranoside, acute liver injury, oxidative stress, liver regeneration, hepatocyte apoptosis

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Abbreviations: MCGP, methyl 6-O-cinnamoyl-α-D-glucopyranoside; APAP, acetaminophen; CCl_4, carbon tetrachloride; PCNA, proliferating cell nuclear antigen; CYP2E1, cytochrome P450 family 2 subfamily E member 1; ROS, reactive oxygen species; NAPQI, N-acetyl-p-benzoquinone imine; GSH, glutathione; CCl_3−, trichloromethyl; Nrf2, nuclear factor erythroid 2-related factor 2; NQO1, NAD(P)H quinine oxidoreductase 1; SOD, superoxide dismutase; HO-1, heme oxygenase 1; GST, glutathione S-transferase; HNF4A, hepatocyte nuclear factor 4 alpha; CSF1, colony stimulating factor 1 receptor; MYB, MYB proto-oncogene, transcription factor; GSTs, glutathione S-transferases; Gclc, glutamate-cysteine ligase catalytic subunit.

INTRODUCTION

The liver is necessary as a major site for metabolism, providing energy, and protein synthesis. In addition, the liver is a mediator of systemic and local innate immunity (Gu and Manautou 2012). Excessive stimulation of hepatotoxins and drugs often leads to acute liver injury, which may result in life-threatening clinical problems (Bernal and Wendon 2013). Acetaminophen (APAP) and carbon tetrachloride (CCl_4) are two well-used hepatotoxins that can induce acute liver injury (Mossanen and Tacke 2015; Scholten et al., 2015). Acute liver injury induced by APAP overdose is reported to be the main cause for drug-induced acute liver injury (DILI) in many Western countries (Chun et al., 2009). APAP is a widely used antipyretic and analgesic drug, which can induce serious DILI when taken as overdose (Zheng et al., 2017). In addition, CCl_4 induces oxidative damage, inflammation, fatty degeneration, and fibrosis in the liver. CCl_4, an often-used solvent in organic chemistry, can also induce acute liver injury (Scholten et al., 2015). Nowadays, the acute liver injury models induced by these two compounds have been widely used to screen hepatoprotective agents.

Cytochrome P450 CYP2E1 is critical in oxidative stress, reactive oxygen species (ROS) generation, and hepatotoxic injury (Chowdhury et al., 2006; Zhai et al., 2008). APAP is metabolized mainly in the liver and transformed into a reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) by the liver CYP2E1 (Yan et al., 2018). In APAP overdose–induced liver injury, the overactivation of CYP2E1 depletes cellular glutathione (GSH), leading to oxidative stress and resultant liver damage (Lancaster et al., 2015). Similarly, CYP2E1 also catalyzes the generation of a free radical trichloromethyl (CCl_3^-) from CCl_4 dehalogenation, thus inducing hepatotoxicity (Weber et al., 2003). CCl_3^- reacts with oxygen to form trichloromethylperoxy (CCl_3OO^-) and stimulates oxidative stress resulting in calcium homeostasis and leading to apoptosis and necrosis (Weber et al., 2003).

Oxidative stress is also closely relevant with the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway in CCl_4- or APAP-induced acute liver injury (Liu et al., 2013; Lu et al., 2016; Mitazaki et al., 2018). Nrf2 is a member of the Cap-n-collar basic leucine zipper family that regulates the expression of antioxidant genes, including superoxide dismutase (SOD), heme oxygenase 1 (HO-1), NAD(P)H quinine oxidoreductase 1 (NQO1), and glutathione S-transferase (GST) (Liu et al., 2013). The activation of the Nrf2 pathway protects liver injury from hepatotoxins such as APAP or CCl_4 (Liu et al., 2013). Nrf2-deficient mice are highly susceptible to APAP hepatotoxicity (Enomoto et al., 2001). Thus, the Nrf2 signaling may be a promising target on suppression of oxidative stress for attenuating APAP- or CCl_4-induced acute liver injury.

Natural products and their derivatives played a highly significant role in new drug discovery and development. In this concern, our group established compound libraries of natural products and their derivatives with nearly 6,000 compounds. Compounds with anticancer, antioxidative, hepatoprotective, anti-inflammatory, and antibacterial effects are focused in our screening work (Qiao et al., 2016; Xiang et al., 2016; Xu et al., 2021). Among these, we found an effective compound methyl 6-O-cinnamoyl-α-D-glucopyranoside (MCGP) with a pronounced hepatoprotective effect. MCGP is a glycosylation product of cinnamic acid, an organic acid occurring naturally in plants that have low toxicity and a broad spectrum of biological activities (Zhang et al., 2007). Cinnamic acid and its derivatives have been found to present potent anti-inflammatory and anticancer activities (Sova 2012; Ruwizhi and Aderibigbe 2020). In addition, MCGP also exhibited predominant hepatoprotective and antioxidative effects.

In this study, we determined the hepatoprotective effect of MCGP on APAP- or CCl_4- induced liver injury through *in vivo* and *in vitro* experiments. Moreover, we uncovered the underlying mechanisms for the hepatoprotective effect of MCGP. Mechanistically, MCGP exerts a therapeutic effect through the pressing of oxidative stress, refraining of hepatocyte apoptosis, and facilitating of liver regeneration.

MATERIALS AND METHODS

Materials

The general procedure for synthesis of MCGP (98% or higher purity) and its structure was characterized by one-dimensional nuclear magnetic resonance (NMR) spectrometer (Supplementary Materials). MCGP was dissolved in dimethyl sulfoxide (DMSO, Biosharp, China) to prepare a 40 mM stock solution and stored at −20°C before use for cell administration. MCGP was resuspended in 5% CMC-Na solution for the intragastric administration.

Cell Culture, Administration, and Detection

Human liver cancer cell line 7,721 and HepG2, human hepatic stellate cell line LX-2, human monocytic leukemia cell line THP-1, and murine normal liver cell AML12 were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). Cells were cultured as monolayers in DMEM-high glucose (7,721 and HepG2)/DMEM (LX-2)/RIPM-1640 (THP-1) and D/F12 (AML12) medium (Hyclone, United States) supplemented with 10% fetal bovine serum (Procell, China) and 1% antibiotics of penicillin/streptomycin (100 units/mL) (Invitrogen, United States). Cells were grown in an atmosphere of 5% CO_2 in air at 37 °C.

For the detection of the cytotoxicity effect, 25, 50, 100, 200, 400, 800, and 1,000 μM of MCGP were applied to 7,721, LX-2, and THP-1 cells. 25, 50, 100, 200, and 400 μM of MCGP were applied to HepG2 and AML12 cells for 48h. DMSO (0.1%) was used as control. Then, cell counting kit 8 (CCK8, Biosharp, China) was added to each well for 1–4 h, and absorbance was detected at 450 nm using a microplate reader (Thermo Fisher Scientific, United States).

For the cell viability assay, cells were incubated with 50, 100, and 200 μM of MCGP for 18 h followed by 30 mM of CCl_4 for 6 h. Then, cell counting kit 8 was added to each well for 1–4 h, and absorbance was detected at 450 nm. For the cell apoptosis assay, cells were incubated with 50, 100, and 200 mM of MCGP for 18 h

followed by 30 mM of CCl_4 or 25 mM of APAP for 6 h. Then, the cells were determined using an Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (KeyGEN, Jiangsu, China) according to the manufacturer's protocol. For the ROS generation assay, the cells were incubated with 50, 100, and 200 mM of MCGP administration for 18 h, after which 400 μM of H_2O_2 was introduced to generate ROS for 6 h. Then, the cells were fixed with 4% cold paraformaldehyde for 15 min. Thereafter, ROS (Sigma, United States) were stained and scanned using a digital microscopy scanner Pannoramic MIDI (3DHISTECH, Hungary).

$ABTS^{\bullet+}$ Radical Inhibition Assay

To evaluate the antiradical activity of MCGP, 25–1,000 μM of MCGP was placed in 96-well plates (Corning, NY, United States), and 130 μl of 2,20-AzinoBis-(3-ethylbenzoThiazoline-6-Sulfonic acid) ($ABTS^{\bullet+}$) (Macklin, Shanghai, China) radicals was added. The $ABTS^{\bullet+}$ reagent was produced by reacting the ABTS solution (10 mM) with 30% H_2O_2 in advance. After incubation for 16 h at room temperature, 10 ml of this solution was diluted in 80 ml of sodium acetate hydrochloric acid buffer (Macklin, Shanghai, China). Ascorbic acid (25–1,000 μM) (Macklin, Shanghai, China) was used as a standard antioxidant. After 30 min of incubation in the dark at room temperature, the plates were read at 650 nm using a microplate reader (Thermo Fisher Scientific, United States).

RNA-Seq and Data Analysis

Total RNAs were extracted with TRIzol reagent (Invitrogen, Thermo Fisher Scientific, United States) from HepG2 cells treated with 40 μM of MCGP or DMSO (control) for 24 h. Three biological replicates for the control group and the sample group were sequenced by Beijing Genomics Institute (BGI) using the BGISEQ-500 platform for each group. The differentially expressed genes (DEGs) were screened with a q value (adjusted p value) $\leq$ 0.01 and $|\text{fold change}| \geq 2$. Bioinformatics Workflow including data filtering, mapping transcript prediction, differential gene expression analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis, and GO function analysis were performed by the platform established at BGI.

Animal Administration

Specific-pathogen–free (SPF) male C57BL/6 and Balb/c mice (20–22 g) were purchased from Beijing HFK Bio-Technology Co. Ltd. (Beijing, China) and housed in a SPF environment at the experimental animal center of Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). After adapting feed for 1 week, mice were subjected to the following administration.

For the CCl_4-induced acute liver injury, male C57BL/6 were randomly divided into 5 groups. 1) Control group (n = 10). 2) Model group (n = 10). 3) MCGP-M group (n = 10, 100 mg/kg). 4) MCGP-H group (n = 10, 200 mg/kg). 5) Positive group. (n = 10, 50 mg/kg of silymarin). Mice were first orally given MCGP once a day for 10 days, and then followed with a single intraperitoneal injection of 1% CCl_4 (in olive oil, 5 ml/kg) 1 h after the last MCGP administration. Mice were killed 24 h after CCl_4 injection, and plasma and liver tissue were collected.

For the acetaminophen (APAP)-induced acute liver injury, male Balb/c mice were randomly divided into 6 groups. 1) Control group (n = 10). 2) Model group (n = 10). 3) MCGP-L group (n = 10, 50 mg/kg). 4) MCGP-M group (n = 10, 100 mg/kg). 5) MCGP-H group (n = 10, 200 mg/kg). 6) Positive group (n = 10, 50 mg/kg of silymarin). Mice were first orally given MCGP once a day for 10 days, and then followed with intragastric administration of APAP (300 mg/kg). Mice were killed 6 h after APAP administration, and plasma and liver tissue were collected.

All mice were cared for and killed according to guidelines provided by the Institutional Animal Care and Use Committee of Tongji Medical College.

Histology and Immunohistochemistry Analysis

The livers were fixed in 10% formaldehyde and embedded in paraffin. The liver tissue lesions were stained with hematoxylin and eosin (H&E) for histopathology. Immunohistochemistry was performed using CYP2E1 (Proteintech), BCL-xL (Abcam), PCNA, and Bax antibodies (Cell Signaling Technology). Aperio Image Scope software was applied to analyze the quantification of PCNA positive cells.

Biochemical Assay

Whole-blood samples were allowed to coagulate for 15 min at room temperature and then centrifuged at 4,000 g at 4°C for 10 min to separate the serum. The liver homogenate was centrifuged at 12000 g, 4°C for 10 min. Serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), liver homogenate of glutathion (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA) were detected with commercial test kits according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Quantitative Real Time PCR Tests

Total RNA was reverse-transcribed into cDNA using a transcription kit (ABP, United States). Quantitative RT-PCR (qRT-PCR) was performed using SYBR Green qPCR Mix (Vazyme Biotech Co., Ltd., China) with 0.2 μM forward and reverse primers in a final volume of 10 μl, and detected by ABI QuantStudio 5 (Thermo Fisher Scientific, United States). The resulting cDNA was amplified by incubating at 95°C for 5 min, 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 30 s. Values were exhibited relative to β-actin. The corresponding primer sequences are listed in **Supplementary Table S1**.

Western Blot Analysis

Total proteins from livers were lysed in radioimmunoprecipitation assay (RIPA, Beyotime, China) buffer, and 30 μg total proteins were used for each blot. The samples were separated by SDS-PAGE and transferred onto a

nitrocellulose filter (NC, Millipore, United States) membrane by electroblotting. The membranes were blocked for 1 h in blocking buffer (Beyotime, China) and then incubated overnight with 1: 1,000 dilutions of anti-Nrf2, anti-NQO1, anti-HO-1, anti-Keap1, anti-BCL-xL, anti-GSDMD, anti-caspase 1, anti-PCNA, anti-NLRP3, and anti-ASC (Cell Signaling Technology, United States) and 1:2,000 dilutions of anti-Bax, anti-IL1β, and anti-IL18 (Abcam, United States). After incubation with the secondary antibodies anti-mouse IgG (H + L) (DyLight™ 800, Cell Signaling Technology, United States) at 1:50,000 dilutions, membranes were imaged using a LiCor Odyssey scanner (LI-COR, United States). The protein expressions were normalized using β-actin as the reference (Cell Signaling Technology, United States) in the same sample.

Terminal-Deoxynucleotidyl Transferase Mediated Nick End Labeling Assay

The liver tissues were fixed for at least 24 h in 4% paraformaldehyde, dehydrated in graded ethanol, and embedded in paraffin. The paraffin sections (5 μm) were incubated in a TUNEL reaction mixture from a kit according to the manufacturer's instructions. The slides were scanned using a digital microscopy scanner Pannoramic MIDI (3DHISTECH, Hungary).

Enzyme Linked Immunosorbent Assay

The liver homogenate was centrifuged at 12000 g, 4°C for 10 min. The liver homogenate was collected for IL1β and IL18 ELISA assay (Boster, Wuhan, China) according to the manufacturer's instruction.

Statistical Analysis

The experimental results are expressed as means ± SEM of at least triplicate measurements. The differences were evaluated by Student's t-test with GraphPad Prism software (GraphPad Prism version 5.01 for Windows, Sand Diego, CA). The differences with $^{*}P$ and $\#p < 0.05$ were considered statistically significant.

RESULTS

Methyl 6-O-Cinnamoyl-α-D-Glucopyranoside Protected AML12 Cells From Acetaminophen or Carbon Tetrachloride–Induced Hepatotoxicity

MCGP was synthesized according to the methods in **Figure 1A**. We first detected the cytotoxicity effect of MCGP on different cell lines. As a result, MCGP exhibited little cytotoxicity on 7,721 and LX-2 at dosages of 800 and 1,000 μM (**Figures 1B,D**). Interestingly, MCGP slightly promoted the cell growth at dosages of 25–400 μM at all the detected cell lines (**Figures 1B–F**). Accordingly, we presumed that MCGP might also promote cell growth in other conditions.

APAP and CCl_4 are two well-used hepatotoxins that can induce hepatocyte apoptosis and liver injury *in vitro* and *in vivo*. Therefore, we identified the cell viability of AML12 cells co-treated by MCGP and APAP or CCl_4. As expected, MCGP promoted the cell viability of AML12 cells treated by APAP or CCl_4 (**Figures 2A,B**). Moreover, MCGP also inhibited cell apoptosis of AML12 cells injured by APAP or CCl_4 (**Figures 2C,D**). In addition, we also found that MCGP could suppress the ROS generation induced by H_2O_2 (**Figure 2E**). This result prompted that MCGP might suppress oxidative stress by scavenging free radicals. However, MCGP exerted little effect on $ABTS^{\bullet+}$ radical inhibition (**Figure 2F**). Considering this, we supposed that MCGP could not directly scavenge free radicals, while it could scavenge through intracellular processes.

RNA-Seq Reveals the Differentially Expressed Genes of Methyl 6-O-Cinnamoyl-α-D-Glucopyranoside–Administrated HepG2 Cells

In order to comprehensively understand the effect of MCGP on liver cells, RNA-seq was conducted to compare the gene expression between 40 μM MCGP-treated HepG2 cells and DMSO control. As a result, 100 differentially expressed genes were screened between MCGP and control, including 58 down-expressed and 42 up-expressed genes regulated by MCGP (**Figure 3A**). The KEGG pathway analysis of these genes indicated an enrichment in metabolic pathways and PI3K-Akt signaling pathway (**Figures 3B,C**). The gene ontology (GO)-function analysis showed an enrichment in components of membranes (**Figures 3D,E**).

Oxidative stress, hepatocyte apoptosis, and liver regeneration are common processes engaged in APAP- or CCl_4-induced hepatotoxicity (Scholten et al., 2015; Ramachandran and Jaeschke 2019). Hence, we screened the gene expression of related genes in RNA-seq results. As shown in **Figure 3F**, the Nrf2/HO-1/NQO1 signaling pathway components were almost upregulated by MCGP. In addition, MCGP also increased the liver regeneration–related genes PCNA, Ki67, BCL-2, and IL18, while it decreased hepatocyte nuclear factor 4 alpha (HNF4A) (**Figure 3G**). Taking together, we supposed that MCGP might protect the liver cell from APAP- or CCl_4-induced hepatotoxicity through the activation of the Nrf2 signaling pathway. However, the effect and mechanism of MCGP *in vivo* still remains unknown.

Methyl 6-O-Cinnamoyl-α-D-Glucopyranoside Treatment Alleviated Liver Injury and Oxidative Stress in Acetaminophen-/Carbon Tetrachloride-Intoxicated Mice

To further verify the hepatoprotective effect of MCGP *in vivo*, we used APAP- or CCl_4-induced liver injury mice models. As shown in **Figure 4A**, the liver histological evaluation in mice 6 h after APAP administration showed that pretreatment of MCGP alleviated APAP-induced intrahepatic hemorrhage and nuclear pyknosis in mice. Similarly, pretreatment of MCGP also

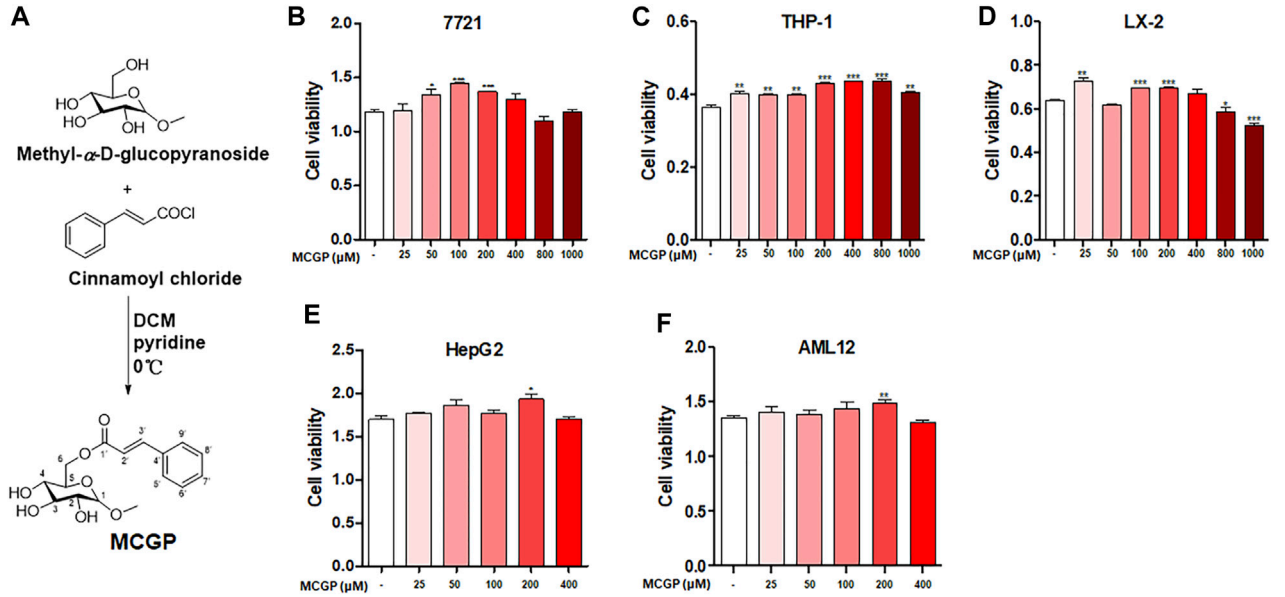

FIGURE 1 | (A) Synthetic route and structure of MCGP. **(B-D)** Cytotoxicity of MCGP on 7,721 **(B)**, THP-1 **(C)**, and LX-2 **(D)** cells at 25–1,000 μM. **(E,F)** Cytotoxicity of MCGP on HepG2 **(E)** and AML12 **(F)** cells at 25–400 μM. Data were presented as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs control.

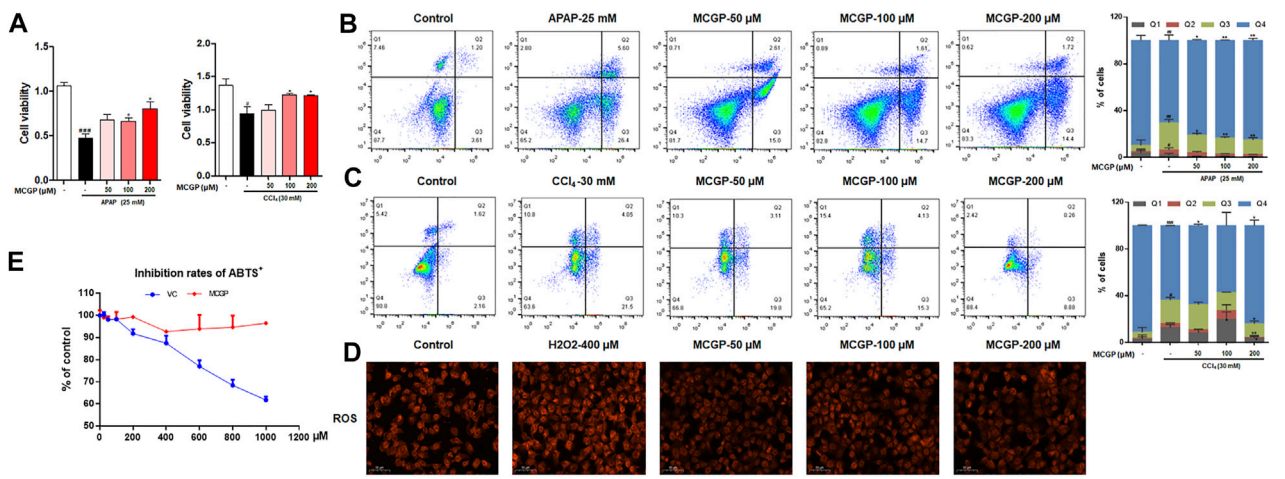

FIGURE 2 | MCGP protected AML12 cells from APAP- or CCl_4-induced hepatotoxicity. **(A)** The cell viability of AML12 cells co-treated with MCGP at 50–200 μM and 25 mM of APAP or 30 mM of CCl_4. **(B,C)** Cell apoptosis of AML12 cells co-treated with MCGP at 50–200 μM and 25 mM of APAP **(B)** or 30 mM of CCl_4 **(C)**. **(D)** The ROS generation of MCGP at 50–200 μM on AML12 cells stimulated by 400 μM of H_2O_2. **(E)** The inhibition rates of $ABTS^{\bullet+}$ of MCGP and ascorbic acid at 25–1,000 μM. Data were presented as the mean ± SEM. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs control, *$p < 0.05$, **$p < 0.01$ vs APAP or CCl_4.

improved liver damage in mice 24 h after CCl_4 induction (**Figure 5A**). MCGP also inhibited the APAP-/CCl_4-induced mice weight loss, and decreased liver weight and liver index (**Figures 4B,C** and **Figure 5B**). The liver necrotic areas were increased in CCl_4-administrated mice, while MCGP reduced the increased necrotic areas (**Figure 5C**). MCGP also significantly inhibited the increase of the serum ALT/AST level in APAP-/CCl_4-induced mice (**Figure 4D** and **Figure 5D**).

GSH, SOD, and MDA are critical markers indicating liver damage during APAP-CCl_4-injured liver. GSH and SOD can protect cells from oxidative damage. Previous studies have revealed significant decrease in liver antioxidase activity GSH and SOD in APAP-/CCl_4-induced acute liver injury (Xie et al., 2016). In addition, the lipid peroxidation product MDA was detected to reflect the liver oxidative damage (Ingawale et al., 2014). As a result, the activity of GSH and SOD were notably decreased in APAP-/CCl_4-treated mice, accompanied by a significant increase in the MDA level. The pretreatment of MCGP improved GSH and SOD levels and inhibited the MDA level in both APAP- or CCl_4-treated mice (**Figures 4E,F**

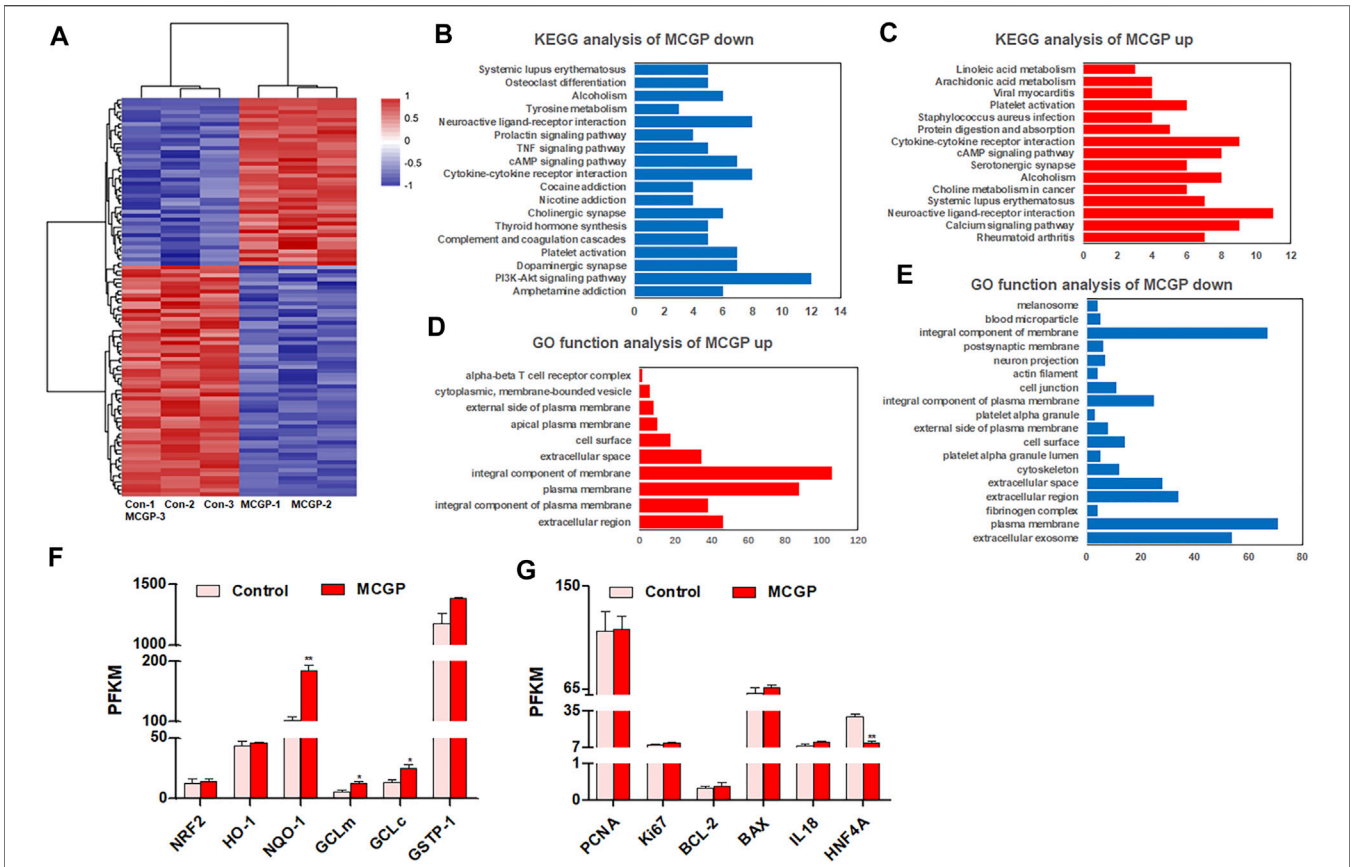

FIGURE 3 | RNA-seq of control or MCGP-treated HepG2 cells. **(A)** The expression heat map of differentially expressed genes in MCGP-treated HepG2 cells. **(B)** KEGG pathway enrichment of MCGP upregulated genes. **(C)** KEGG pathway enrichment of MCGP downregulated genes. **(D)** GO function analysis of MCGP upregulated genes. **(E)** GO function analysis of MCGP downregulated genes. **(F,G)** The PKFM value of NFR2 signaling pathway components **(F)** and liver regeneration related genes of MCGP administration in HepG2 cells. $^{*}p < 0.05$, $^{**}p < 0.01$ vs control.

and **Figure 5E**). Silymarin, a well-used hepatoprotective herbal drug, was used as the positive control.

Methyl 6-O-Cinnamoyl-α-D-Glucopyranoside Treatment Inhibited CYP2E1 and Activated the Nrf2/HO-1 Signaling Pathway in Acetaminophen-/Carbon Tetrachloride-Intoxicated Mice

A small amount of hepatotoxins (such as APAP and CCl_4) are metabolized by cytochrome P450 enzymes, mainly *via* CYP2E1 isoform. APAP is metabolized by CYP2E1 to NAPQI, which exhausts GSH and leads to mitochondrial damages and necrotic cell death (Lancaster et al., 2015). Similarly, CCl_4 is metabolized by CYP2E1 to highly reactive free radical metabolites that initiate membrane lipid peroxidation (Fahmy et al., 2016). The overactivation of CYP2E1 induced by APAP or CCl_4 leads to oxidative stress and resultant liver damage. In our result, MCGP significantly depressed the protein increase of liver CYP2E1 on APAP-/CCl_4-induced mice (**Figures 6A,E**). Glutamate–cysteine ligase catalytic subunit (Gclc) and glutamate–cysteine ligase modifier subunit (Gclm) are two subunits of glutamate–cysteine ligase, the first rate-limiting enzyme of glutathione synthesis (Jin et al., 2019). The glutathione S-transferase (GST) family includes glutathione S-transferase pi 1 (Gstp1) and glutathione S-transferase pi 2 (Gstp2), which play an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione (Mari et al., 2009). CCl_4 administration inhibits the transcription level of Gclc, Gclm, Gstp1, and Gstp2, while MCGP rescued their decrease (**Figure 6B**).

Oxidative stress is closely relevant with APAP-/CCl_4-induced acute liver injury (Lu et al., 2016; Wang S. et al., 2017). Nrf2 is a transcription factor that plays an important role against oxidative stress by mediating the expression of many endogenous antioxidants such as GSTs, GCLs, and quinine oxidoreductase 1 (NQO1) (Bataille and Manautou 2012). MCGP significantly activated the gene and protein expression of Nrf2, NQO1, and HO-1 in APAP-/CCl_4-induced mice liver (**Figures 6C–F**). In addition, MCGP suppresses the expression of kelch-like ECH associated protein 1 (Keap1) (**Figures 6D–F**), thus dissociating Nrf2 from Keap1 and leading to the nuclear translocation of Nrf2.

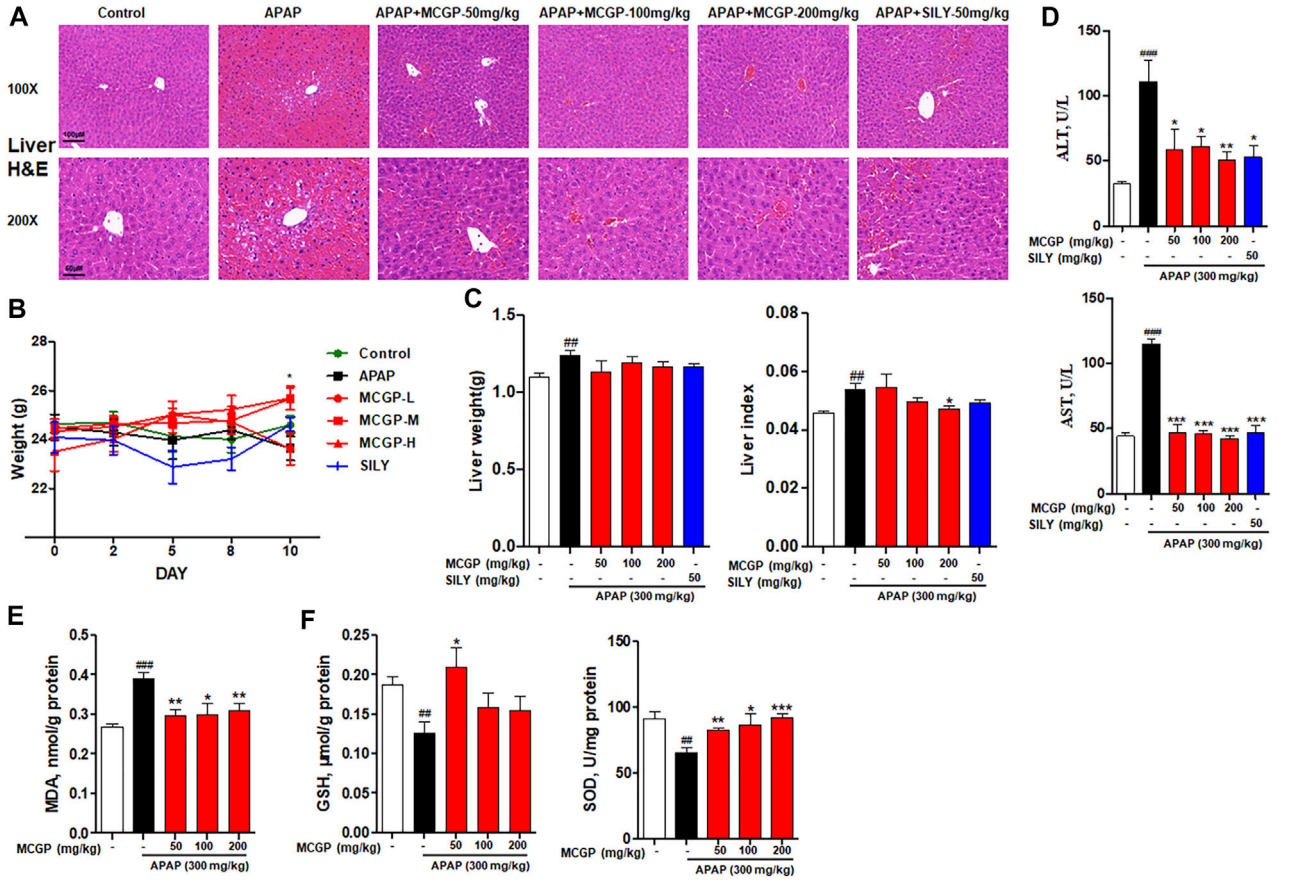

FIGURE 4 | MCGP treatment alleviated APAP-intoxicated liver injury. **(A)** Liver histological observation. Mice were pretreated with vehicle or various dosages of MCGP (50, 100 or 200 mg/kg) or positive control silymarin once a day for 10 days, and then followed with intragastric administration of APAP (300 mg/kg). Typical images are chosen from each experimental group: Control, APAP, MCGP-L + APAP, MCGP-M + APAP, MCGP-H + APAP, SILY (silymarin + APAP). **(B)** Mice weight of each group. **(C)** Liver weight and liver index of each group. **(D)** Serum ALT and AST activity. **(E,F)** Liver levels of GSH, MDA, and SOD in each group. Data were presented as the mean ± SEM. ##p < 0.01, ###p < 0.001 vs control, *p < 0.05, **p < 0.01, ***p < 0.001 vs APAP.

These results are also consistent with the RNA-seq result that MCGP upregulated the transcription of Nrf2 signaling molecules (**Figure 3F**).

Methyl 6-O-Cinnamoyl-α-D-Glucopyranoside Treatment Promoted Liver Regeneration and Protected Against Hepatocyte Apoptosis in Acetaminophen-/Carbon Tetrachloride-Intoxicated Mice

Nrf2 has been confirmed with the function on promotion of liver regeneration and inhibition of hepatocyte apoptosis (Chan et al., 2021; Ghanim and Qinna 2022). Therefore, we explored the effect of MCGP on liver regeneration and apoptosis. As shown in **Figure 7A**, both APAP and CCl_4 increased the number of PCNA-staining hepatocytes in livers from APAP-/CCl_4-intoxicated mice. Similarly, MCGP markedly increased the number of PCNA-staining hepatocytes in livers from APAP-/CCl_4-intoxicated mice (**Figures 7A,B**). In addition, MCGP promoted the protein expression of PCNA in APAP-/CCl_4-intoxicated mice livers (**Figure 7G**). Hepatocyte nuclear factor 4 alpha (HNF4A), colony stimulating factor 1 receptor (CSF1), MYB proto-oncogene, transcription factor (MYB), and cAMP responsive element binding protein 5 (CREB5) are downregulated genes revealed by RNA-seq after MCGP administration, while research studies showed that the four genes were related to liver regeneration, anti-apoptosis, and cell proliferation (Lancaster et al., 2015; Wang Y. et al., 2017; Wu et al., 2018; Zhou et al., 2020). As expected, we found the increase of these genes in MCGP-treated livers from CCl_4-intoxicated mice, which is incompatible with the RNA-seq result (**Figure 7C**).

CCl_4 exposure significantly increased hepatocyte apoptosis, while MCGP remarkably decreased the TUNEL-positive hepatocytes in livers from CCl_4-intoxicated mice (**Figure 7D**). In addition, the apoptosis-related gene and protein expressions of Bax and Bcl-xL in the liver tissue were also detected by immunohistochemistry, qRT-PCR, and Western blotting. As a result, CCl_4 had a slight effect on the expression of BCL2-like 1 (Bcl-XL) (**Figures 7E,G**), and significantly increased the transcription level of BCL2 apoptosis regulator (BCL-2)

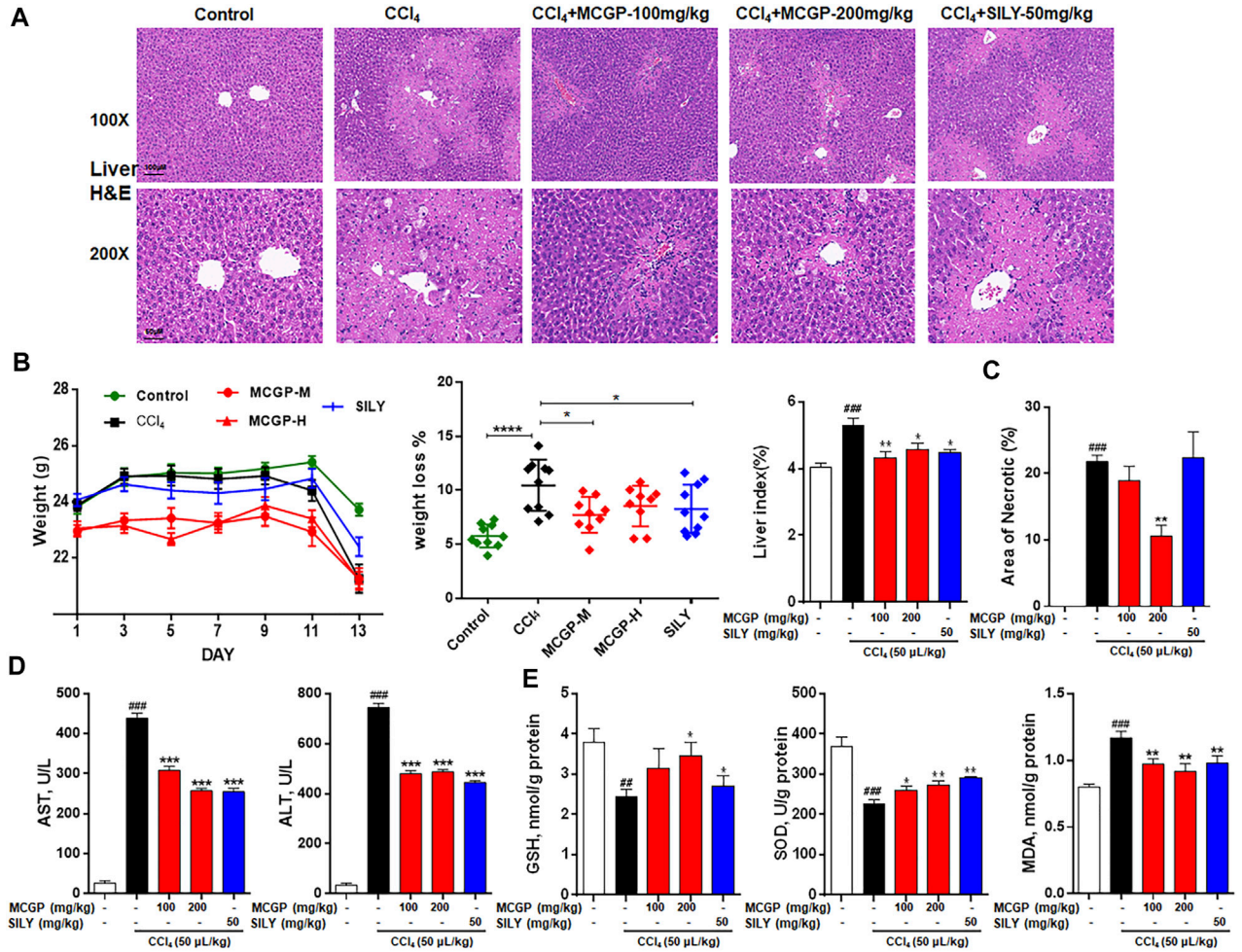

FIGURE 5 | MCGP treatment alleviated CCl_4-intoxicated liver injury. **(A)** Liver histological observation. Mice were pretreated with vehicle or various dosages of MCGP (100 or 200 mg/kg) or positive control silymarin (50 mg/kg) once a day for 10 days, and then followed with a single intraperitoneal injection of 1% CCl_4 (in olive oil, 5 ml/kg) 1 h after the last MCGP administration. Typical images are chosen from each experimental group: Control, CCl_4, MCGP-M + CCl_4, MCGP-H + CCl_4, SILY (silymarin + CCl_4) (n = 10). **(B)** Mice weight, weight loss, and liver index of each group. **(C)** Counted necrotic areas of each group (n = 3). **(D)** Serum ALT and AST activity. **(E)** Liver levels of GSH, MDA and SOD in each group. Data were presented as the mean ± SEM. ##$p < 0.01$, ###$p < 0.001$ vs control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs CCl_4.

(**Figure 7F**). MCGP notably promoted the expression of Bcl-XL and BCL-2 in APAP-/CCl_4-induced mice livers (**Figures 7E–G**). In addition, MCGP also decreased the gene and protein level of BCL2 associated X, apoptosis regulator (BAX) (**Figures 7E–G**).

Methyl 6-O-Cinnamoyl-α-D-Glucopyranoside Treatment Promoted IL18 Secretion and Inhibited Inflammation in Acetaminophen-/Carbon Tetrachloride-Intoxicated Mice

Previous studies revealed that IL18 accelerated liver regeneration after partial hepatectomy or APAP-induced liver injury (Zhang et al., 2014; Shi et al., 2020). IL-1β and IL-18 are two main pro-inflammatory cytokines produced during the recruitment of caspase 1 to cleaved GSDMD into its active form GSDMD-N and insert in cell membranes (de Vasconcelos et al., 2016; Shojaie et al., 2020). Here, we found that MCGP activated the transcription of caspase 1, IL1β, and IL18 and elevated the liver content of IL1β and IL18, inducing the protein expression of GSDMD-N, cleaved caspase 1, IL1β, and IL18 (**Figures 8A–C**).

APAP- or CCl_4-induced liver injury is characterized by sterile inflammation, functioning as damage-associated molecular patterns (DAMPs), which can transcriptionally activate inflammatory cytokines (TNFα, IL1β, IL6, and IL10) and chemokines (MCP-1, MIP-2, and IL8) (Dambach et al., 2002; James et al., 2005). As a result, CCl_4 markedly elevated the serum level of pro-inflammatory cytokines IL6 and TNFα (**Figure 8D**). In contrast, MCGP decreased the level of IL6 and TNFα (**Figure 8A**). In addition, MCGP inhibited the elevated transcription level of inflammation factors IL6, TNFα, MCP-1, and IL12 by CCl_4, while it increased the expression of anti-inflammatory factor IL10 (**Figure 8E**).

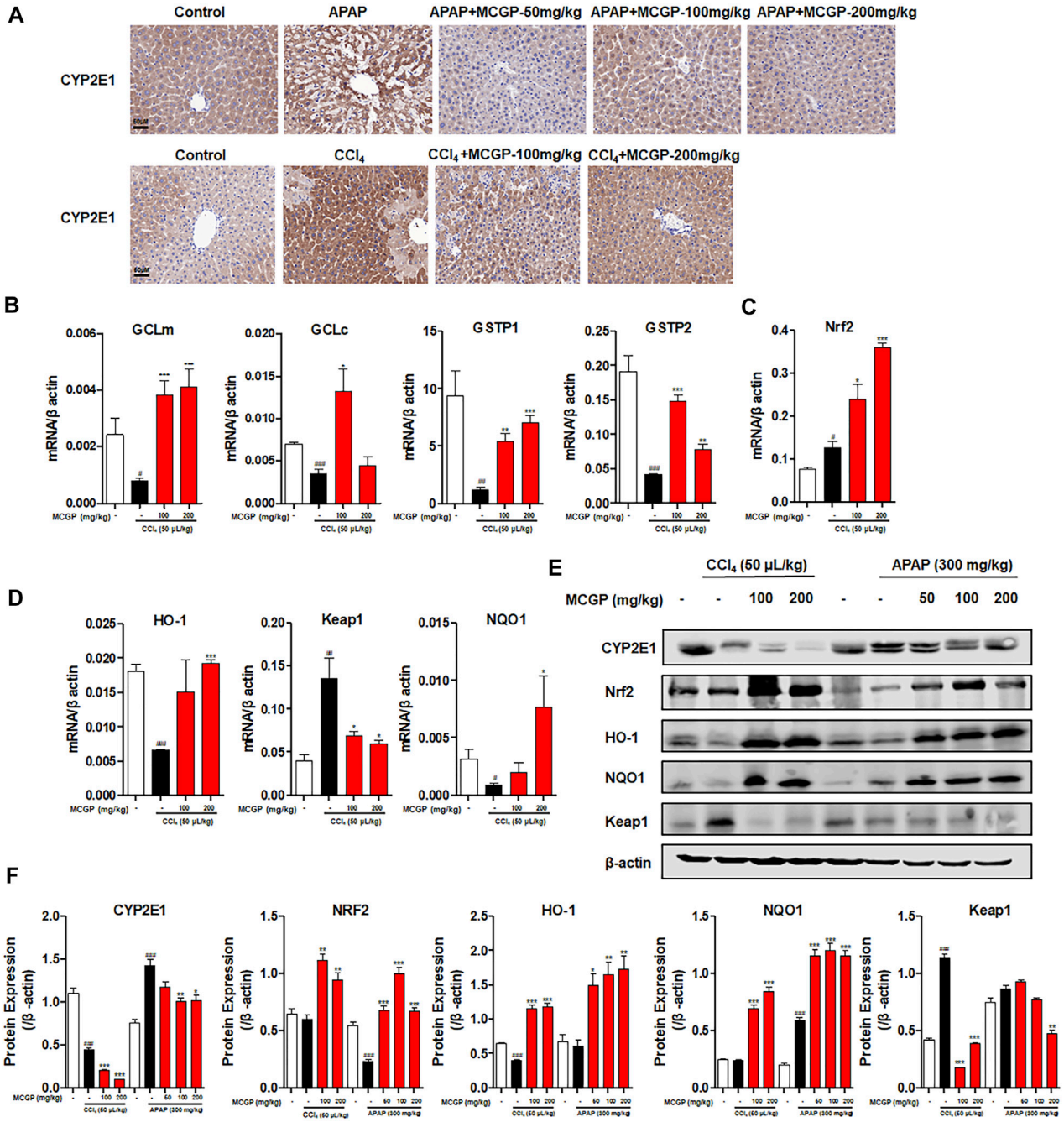

FIGURE 6 | MCGP inhibited CYP2E1 and activated the NRF2/HO-1 signaling pathway in APAP-/CCl_4-intoxicated mice. **(A)** Hepatic CYP2E1 immunohistochemical staining of each group in APAP- or CCl_4-intoxicated mice. **(B-D)** Transcription level of GClm, GCLc, GSTP1, NRF2, HO-1, Keap1, and NQO1 of each group in CCl_4-intoxicated mice. **(E,F)** The protein expression of CYP2E1, Nrf2, HO-1, NQO1, and Keap1 in liver tissues from the APAP or CCl_4 groups **(E)**. The β-actin was used as an invariant control for equal loading, followed by the integrity optical density analyses of each blot **(F)**. Data were presented as the mean ± SEM (n = 3). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs model.

DISCUSSION

In this study, we first identified the hepatoprotective effect of MCGP on two different types of acute liver injury animal models. Our results indicated that MCGP pretreatment efficiently alleviated intrahepatic hemorrhage, nuclear pyknosis, liver necrosis, and transaminase levels in APAP-/CCl_4-intoxicated mice. Further exploration revealed that the effects of MCGP were relevant to inhibition of oxidative stress, hepatocyte apoptosis, and promotion of liver regeneration.

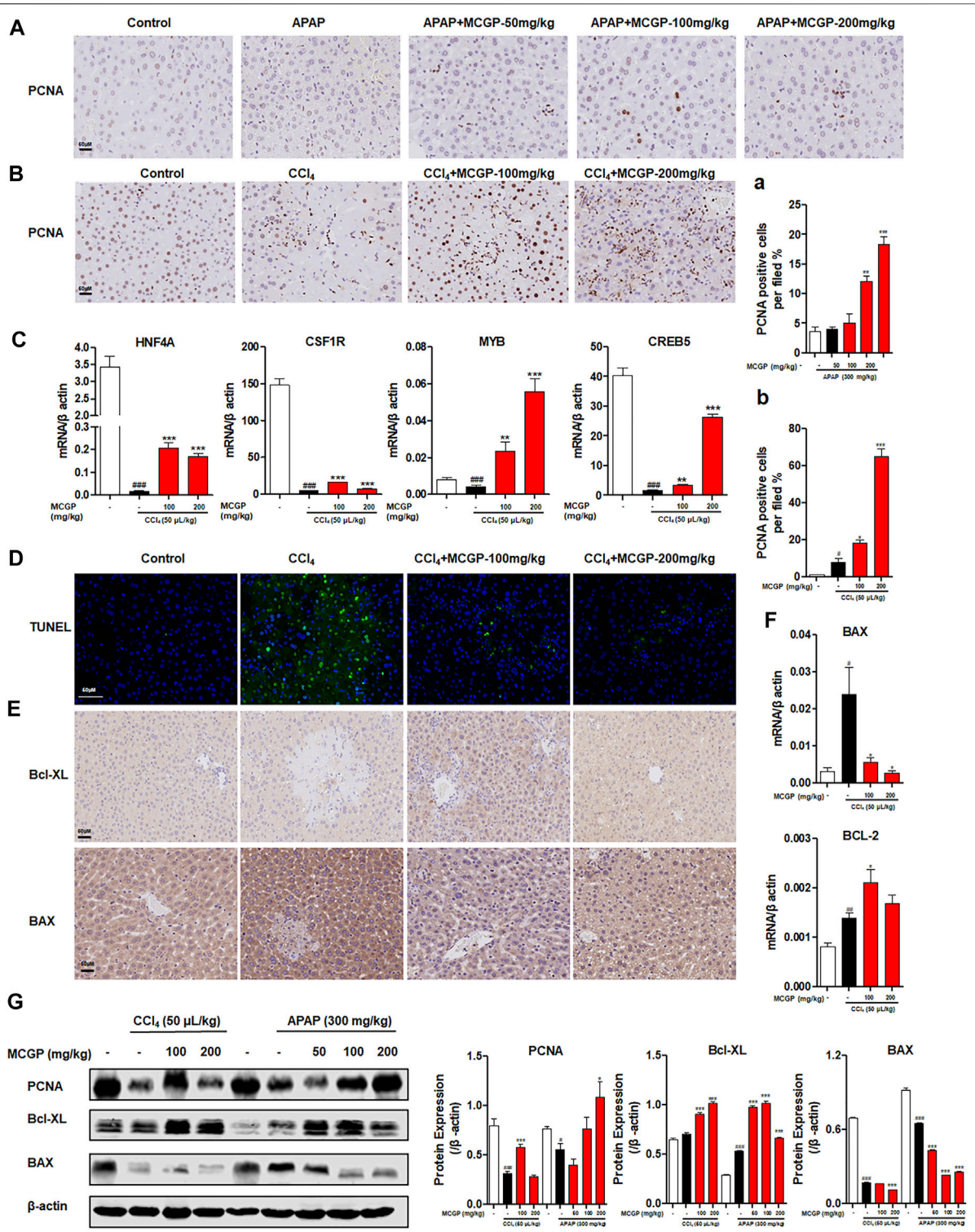

FIGURE 7 | MCGP promoted liver regeneration and protected against hepatocyte apoptosis in APAP-/CCl_4-intoxicated mice. **(A)** Hepatic PCNA immunohistochemical staining of each group in APAP-intoxicated mice. **(A)** The number of PCNA-positive hepatocytes per field. **(B)** Hepatic PCNA immunohistochemical staining of CCl_4-intoxicated mice. **(B)** The number of PCNA-positive hepatocytes per field, scale bar: 50 μM. **(C)** Transcription level of HNF4A, CSF1R, MYB, and CREB5 of each group in CCl_4-intoxicated mice. **(D)** TUNEL-staining of each group in CCl_4-intoxicated mice, 200x. **(E)** Hepatic Bcl-XL and BAX immunohistochemical staining of each group in CCl_4-intoxicated mice. **(F)** Transcription level of BAX and BCL-2 of each group in CCl_4-intoxicated mice. **(G)** The protein expression of PCNA, Bcl-XL, and BAX in liver tissue from the APAP or CCl_4 groups. The β-actin was used as an invariant control for equal loading, followed by the integrity optical density analyses of each blot. Data were presented as the mean ± SEM (n = 3). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs model.

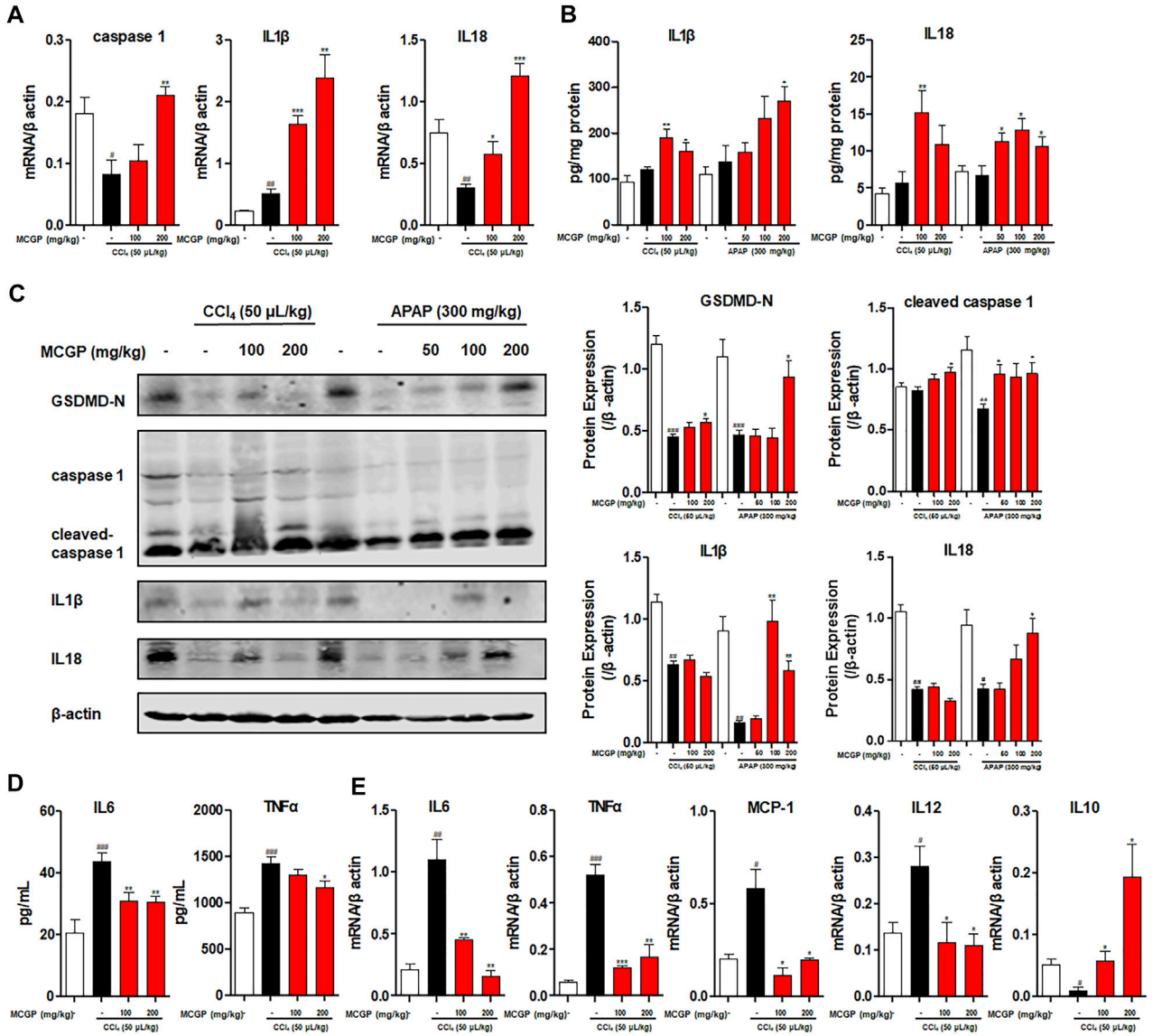

FIGURE 8 | MCGP promoted IL18 secretion and inhibited inflammation in APAP-/CCl_4-intoxicated mice livers. **(A)** Transcription level of caspase 1, IL1β, and IL18 of each group in CCl_4-intoxicated mice. **(B)** The liver content of IL1β and IL18 of each group in APAP- or CCl_4-intoxicated mice. **(C)** The protein expression of GSDMD-N, caspase 1, cleaved-caspase 1, IL1β, and IL18 in liver tissue from the APAP or CCl_4 groups. The β-actin was used as an invariant control for equal loading, followed by the integrity optical density analyses of each blot. **(D)** The serum level of IL6 and TNFα of each group in CCl_4-intoxicated mice. **(E)** Transcription level of IL6, TNFα, MCP-1, IL12, and IL10 of each group in CCl_4-intoxicated mice. Data were presented as the mean ± SEM (n = 3). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs control, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs model.

APAP- or CCl_4-induced acute liver injuries are widely used to explore novel liver-protective agents. CYP2E1 catalyzes APAP to its toxic intermediate NAPQI, and excess NAPQI causes significant depletion of GSH (Lancaster et al., 2015). GSH can protect cells from oxidative damage by scavenging free radicals and other oxygen species through nonenzymatic and enzymatic processes (Lancaster et al., 2015). Overloaded NAPQI covalently conjugated to sulfhydryl groups in cellular proteins triggers mitochondrial oxidative stress and dysfunction, which ultimately initiates hepatocellular apoptosis and necrosis (Lancaster et al., 2015). CCl_4 is also metabolized into radicals CCl_3• and $OOCCl_3$• by CYP2E1.[32] Abundant radicals induce lipid peroxidation and ROS generation, ultimately causing hepatocyte necrosis and liver dysfunction (Fahmy et al., 2016). In this study, we demonstrated that MCGP can increase the liver level of SOD and GSH, inhibit the expression of CYP2E1, and promote the transcription level of glutathione synthesis rate-limiting enzymes (GCLs) and glutathione transferases (GSTPs), thus protecting liver from oxidative damage. In addition, MCGP also decreased the ROS content in H_2O_2-stimulated murine normal liver cell AML12. However, MCGP could not directly inhibit $ABTS^{•+}$ radical by

in vitro oxidation–reduction reactions, which suggested that MCGP might suppress oxidative stress through cellular progress. Nrf2 signaling pathway is a classical way for antioxidants. Activation of Nrf2 transcribes antioxidant enzymes, including microsomal epoxide hydrolase, HO-1, NQO-1, and glutamate GCL, acting as a cell defense system to detoxify NAPQI (Zhao et al., 2021). Further results exhibited that MCGP promoted the protein expression of Nrf2, HO-1, and NQO1 and inhibited the expression of Keap1. Taking together, we presume that MCGP can ameliorate APAP/CCl_4-induced oxidative stress through the inhibition of CYP2E1 and the activation of the Nrf2 signaling pathway. These results were consistent with the improvement of liver antioxidant enzymes SOD, GSH, and MDA.

Hepatocyte apoptosis is a representative attribution of acute liver injury induced by oxidative stress; APAP or CCl_4 is reported to induce notable elevation of hepatocyte apoptosis (Lu et al., 2016; Yan et al., 2016). In this study, we exhibited that APAP or CCl_4 obviously increased the number of TUNEL-positive cells, while MCGP significantly decreased apoptotic hepatocytes. Consistent with the decreased TUNEL-positive cells, apoptosis-related protein Bax was down-expressed while Bcl-XL was up-expressed on MCGP pretreated APAP-/CCl_4-intoxicated mice livers. Apart from this, MCGP also ameliorated the cell viability of APAP- or CCl_4-intoxicated AML12 cells and decreased the number of apoptotic cells. All these results suggested that MCGP significantly ameliorated APAP- or CCl_4-induced liver damage by restraining hepatocyte apoptosis induced by oxidative stress.

On the other hand, the restoration of liver normal function after acute liver injury can be acquired through promoting liver regeneration, which is proved to be efficient in APAP- or CCl_4-induced liver injury (Yan et al., 2018; Humpton et al., 2021). In addition, activation of Nrf2 enhances functional liver regeneration (Chan et al., 2021; Ghanim and Qinna 2022). Hence, we supposed that MCGP could also promote liver regeneration through the activation of the Nrf2 signaling pathway. PCNA and HNF4A were reported to be critical on regulating liver regeneration (Sherr 1994; Lee et al., 2020). CSF1R and its ligand colony stimulating factor 1 (CSF1) regulate the proliferation, differentiation, and function of macrophages, including Kupffer cells (Santamaria-Barria et al., 2019). In damaged tissues, CSF1R and CSF1 assist macrophages to suppress immune response and promote vascular regeneration to accelerate repairing; inhibition of CSF1R delays liver regeneration (Santamaria-Barria et al., 2019). MYB, CREB5, and BCL-2 are molecules in the PI3K/AKT signaling pathway, which is relevant to cell survival and proliferation (Lin et al., 2020). Our results showed that MCGP pretreatment can markedly promote the PCNA-positive cells and increase the expression of PCNA, HNF4A, CSF1R, MYB, and CREB5 in livers. These results indicate that MCGP can, on the one hand, inhibit APAP-/CCl_4-induced hepatocyte apoptosis by regulating the liver expression of apoptosis-related proteins, and on the other hand, promote liver repairing and regeneration by motivating the expression of cell survival and proliferation protein.

Reports revealed that necrosis of hepatocytes trigger the releasing of inflammatory mediators such as cellular contents, which can function as DAMPs (Ramachandran and Jaeschke 2019). DAMPs can activate Kupffer cells to generate large amounts of cytokines, thus recruiting circulating neutrophils and monocytes into the liver, resulting in the initiation of inflammatory-related injury (Ramachandran and Jaeschke 2019). The major function of NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome is to recognize a wide variety of danger signals including DAMPs, and thus lead to the activation of caspase-1, which further conducts the production of mature IL-1β and IL-18 (Yang et al., 2019). A previous study also covered the crucial role of Nrf2 on the protection effect of baicalin on APAP-induced liver injury (Shi et al., 2018). The follow-up report from the group reported that baicalin promoted the interaction between Nrf2 and Nlrp3, ASC, and caspase 1 in APAP-intoxicated mice, thus leading to the assembly of NLRP3 inflammasome and the subsequent IL-18 product, thereby promoting hepatocyte proliferation after APAP-induced acute liver injury (Shi et al., 2020). In our study, MCGP administration promoted the activation of cleaved caspase 1, GSDMD-N, IL1β, and IL18.

IL-1β and IL-18 are two typical pro-inflammatory cytokines produced during NLRP3 inflammasome activation. However, the promotion of IL1β and IL18 by MCGP did not enhance liver inflammation but reduced liver inflammation in CCl_4-intoxicated mice. In addition, administration of high pharmacological doses of IL1β directly do not aggravate APAP-induced liver injury (Williams et al., 2010). Moreover, previous studies revealed that IL18 accelerated liver regeneration after partial hepatectomy or APAP-induced liver injury (Zhang et al., 2014; Shi et al., 2020). In this study, MCGP can significantly increase the liver IL1β and IL18 protein expression. In addition, MCGP enhanced the amount of hepatic IL1β and IL-18 in APAP-/CCl_4-intoxicated mice. Taking together, we suspect that MCGP ameliorated acute liver injury partially through the promotion of IL18 content to accelerated liver regeneration.

In conclusion, this study, for the first time, identified the protective effect of MCGP in APAP-/CCl_4-intoxied acute liver injury. Mechanically, MCGP may exert its hepatoprotective effect through the alleviation of oxidative stress, the suppression of hepatocyte apoptosis, and the promotion of liver regeneration. Furthermore, these results also suggest that MCGP may be protective against other types of acute liver injury, such as those induced by lipopolysaccharide or concanavalin A, which is what we are identifying at present.

DATA AVAILABILITY STATEMENT

The raw sequences of this study have been deposited in the Sequence Read Archive (accession number: SRR18347304 and SRR18347305).

ETHICS STATEMENT

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Tongji Medical College.

AUTHOR CONTRIBUTIONS

YZ: conceptualization, funding acquisition. QX: methodology, investigation, formal analysis, and writing—original draft preparation. YD: methodology, investigation, and formal analysis. JM: investigation. ZL: conceptualization. XC: formal analysis. TC: investigation. YW: resources. SY, LG, JZ and LM: validation, investigation. WS: writing—review and editing. QZ: project administration. HR: project administration.

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Caloric Vestibular Stimulation Induced Enhancement of Behavior and Neurotrophic Factors in Chronic Mild Stress Induced Rats

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Background: Caloric Vestibular Stimulation (CVS) is a non-invasive technique for stimulating the vestibular system. The vestibular system maintains equilibrium and acts as a moderator of mood, emotional control, and stress levels. Stress is a disruption of psychological, behavioral, and physiological homeostasis that affects people of all ages in today's world. Thus, modest therapeutic procedures like vestibular stimulation can be practiced to effectively reduce stress. Hence, the purpose of the study was to determine the effect of vestibular stimulation on improving behavioral alterations and neurotrophic factors in rats exposed to **Chronic Mild Stress (CMS)**.

Methodology: The study employed 24 healthy male Sprague Dawley rats divided into four groups (n = 6). CMS was induced for 28 days with a variety of stimuli. Bilateral CVS with hot water (temperature ≈40°C) was started on Day 14 of CMS and continued for 15 days. On days 1, 15, and 28, locomotor activity (LA), wire grip strength (WGS), fall off time (FT), and immobilization time (IT) were measured, and the data were analyzed statistically. Additionally, neurotrophic factors such as Brain Derived Neurotrophic Factor (BDNF) and Glial cell line-Derived Neurotrophic Factor (GDNF) were observed in rats' hippocampus.

Results: On days 15 and 28, the CMS-induced group showed a significant reduction in LA, WGS, FT and IT in comparison to the control group. On day 28, the CVS-induced group demonstrated a significant increase in WGS, FT and IT when compared to the CMS group. Immunohistochemical analysis revealed that animals subjected to CMS had decreased BDNF and GDNF expression compared to the control group, indicating neuronal dysfunction in the hippocampus in response to stress. However, therapy with CVS increased BDNF and GDNF expression, thereby regenerating damaged hippocampus nerve terminals.

Conclusion: The findings of the current study revealed that CVS is a safe and simple neuroprotective treatment against stress and a promising non-invasive technique for overcoming the motor symptoms associated with it. The findings may pave the way for future research and therapeutic applications of CVS for stress management.

Keywords: caloric vestibular stimulation, chronic mild stress, brain derived neurotrophic factors, glial cell line-derived neurotrophic factors, behavior

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INTRODUCTION

The last century, stress has become an inevitable aspect of our lives, and its incidence rate has soared, particularly among the younger generations. One-third of the global population has reported feeling stressed, and the number is increasing constantly[1]. Stress is depicted as a change in psychological, behavioral, and physiological equilibrium (Bekris et al., 2005) which causes the "wear and tear" of the body when it responds to pressure or a potentially dangerous circumstance (Almojali et al., 2017). The stimulation of the hypothalamic-pituitary-adrenal (HPA) axis is one of the essential systems reacting to stress to guarantee an optimal response to stress. Chronic stress, which is linked to hippocampal alterations, may be linked to the inception of psychotic ailments (Kajantie and Phillips, 2006). The Chronic Mild Stress (CMS) model of depression (Willner et al., 1992; Willner, 1997) is generally regarded as a paradigmatic example and has been shown to generate an anhedonic-like state in rats, which mirrors some stress symptoms in humans (Bekris et al., 2005) and may thus be applied to gain a better understanding of human psychopathology (Willner, 2017).

The vestibular system, which regulates posture and equilibrium, is inextricably linked to the whole physiology of the body (Goldberg et al., 2012). Vestibular information is transmitted to the hippocampus through brain regions that receive vestibulo-thalamocortical projections, such as the parietal cortex. According to electrophysiological observations, the brainstem vestibular nucleus complex and the hippocampus were polysynaptically coupled, and hippocampal cells responds to vestibular stimulation. Damage to the vestibular system has a long-term influence on the hippocampus's electrophysiological and neurochemical function (Smith et al., 2005). Vitte *et al* used caloric vestibular stimulation (CVS) to explore the changes in blood oxygenation in the hippocampus with magnetic resonance imaging, and he established direct evidence for vestibular-hippocampal communication in humans. They concluded that CVS engaged the hippocampus mostly ipsilaterally, as well as Brodmann's area 39–42, the posterior insular cortex, Brodmann's area 7 in the superior parietal lobe, and the retrosplenial cortex and subiculum (Vitte et al., 1996).

The vestibular system also helps with a wide range of tasks, from reflexes to cognition and coordination. As a result, vestibular sense is sometimes known as "The Sixth Sense" (Baizer et al., 2013). The controlled stimulation of the vestibular system has consistently been applied for neurological diagnosis (Miller and Ngo, 2007) and has proven to be beneficial in the treatment of dementia (Kranthi et al., 2021), regulation of brain ageing neurotransmitters (Nishiike et al., 1997), and the alleviation of depression and anxiety (Kranthi et al., 2021). However, studies relating vestibular stimulation to behavior and neurotrophic factors in stress are sparse. Therefore, the current study was intended to assess the effect of vestibular stimulation on behavior and neurotrophic factors in CMS-induced rats.

[1]https://www.singlecare.com/blog/news/stress-statistics/

MATERIALS AND METHODS

Reagents

The materials used in this study were primary antibody: Anti-BDNF (ab108319) and anti- GDNF (ab18956) which were purchased from Abcam, United States. Secondary antibody: Rabbit specific HRP_DAB (horseradish peroxidase_ 3,3′ Diaminobenzidine) IHC Detection Kit - Micro-polymer was bought from Abcam, United States. Antigen retrieval buffer was obtained from Abcam, United States. Bovine Serum Albumin (BSA), Tris- Ethylenediaminetetraacetic acid (EDTA) buffer and xylene were purchased from Sigma, United States. Tris-Buffered Saline, 0.05% Tween 20 (TBST), Phosphate Buffered Saline (PBS), Phosphate Buffered Saline plus Tween 20 (PBS-T), Diamidine-2′-phenylindole dihydrochloride (DAPI), 10% paraformaldehyde, paraffin, hydrogen peroxide was purchased from Eman Biodiscoveries Sdn Bhd, Penang, Malaysia.

Experimental Animals

The study employed healthy, adult, male Sprague Dawley (SD) rats weighing 180 ± 20 g. The rats were housed and maintained in large, spacious polyacrylic cages with a 12-h light/12-h dark cycle maintained at ambient room temperature. Water and standard rat pellet food were provided *ad libitum* to the animals. The study was approved by the University Human and Animal Ethics Committee (AUAEC/FOM/2020/03) and was conducted in accordance with the requirements of the Animal Research Review Panel guidelines. All behavioral experiments were conducted between 9.00 am and 12.00 p.m.

Chronic Mild Stress (CMS) Administration

The CMS model employed in this study is a modified version of the CMS approach used in the earlier studies (Willner, 2017). Various stressors with varying durations were applied continuously for 28 days. Throughout these 28 days, the control group animals were not exposed to CMS. Each day, the following stressors were introduced one by one, and the cycle was repeated.

Restraint Stress: For an hour, rats were placed in a restraining device constructed of flexible nylon, limiting movement but permitting free respiration and air circulation.

Overcrowding: Overnight placement of two home-cages of rats from the same experimental group in a single cage.

Wet bedding: Rats were wetted by placing them in a cage with wet husk (5 cm high) for 5 h.

Forced swimming: Each rat was placed in a 1-L cylindrical plastic Baxter container full of 850 ml tap water (24–26°C) and forced to swim for 5 min.

Tail pinch in restrainer: For 20 min, the rat was placed in the previously described restraining device and a clothespin was fastened 2 cm from the base of the tail.

Cold water swim test: The rat was placed in a cylindrical tank (60 cm height × 30 cm diameter) filled with water to a depth of 30 cm at 8°C for 5 min.

Inversion of the day and night cycle: Cages were housed in a separate dwelling, and the inversion of the day and night cycle was retained.

Caloric Vestibular Stimulation (CVS)

The animal was placed in a rat restrainer and the middle ear cavity of each ear was irrigated with a syringe filled with 2 ml of warm water at 42 ± 2°C. Constant vestibular stimulation was achieved by maintaining the flow rate at 0.1 ml/s. To generate convection currents in the semicircular canals, the rats were positioned such that the horizontal canal was tilted approximately 30° degrees with regard to the horizontal plane (Nishiike et al., 1997; Nishiike et al., 2000).

Experimental Design

The experimental rats were randomly divided into four different groups (each group consisting of six animals).

Group 1 – Control.

Group 2 – CMS.

Group 3 – CVS.

Group 4 – CMS + CVS.

The group I animals were normal animals and free from CVS or CMS. The animals in group II were induced with CMS once daily for 28 days. The animals in group III were given CVS treatment, once daily for 15 days and the animals in group IV were induced with CMS once daily for 28 days and were given CVS treatment from day 15 onwards i.e., the rats were stressed for 28 days (verified by behavioral changes), and on the day 15, CVS was delivered for 15 days concurrently with stress induction. At the end of the study, the animals were sacrificed, and the brain sample was collected for immunohistochemical analysis.

Behavioral Assessment

On pre-study day, day 14 and day 28, locomotor activity, muscular coordination, grip strength, and immobilization time were evaluated.

Locomotor activity

The actophotometer (rat activity cage) was used to record the locomotor activity of the rats. It is comprised of an acrylic cage and eight infrared light beams along both the *x* and *y* axis. At room temperature, each rat's activity was recorded for 10 min (Parasuraman et al., 2020).

Rotarod test

The motor coordination of the rat was evaluated using rotarod instrument. The instrument is composed of a rotating horizontal metal rod. All rats were trained for 5 days before the experiment could start. On the first day of training, the rotation speed was set to 17 rpm and gradually increased to 20 rpm on the last day. Three trials were conducted on the rat, with a 5-min break in between. The time interval between the rat's retention on the rotarod and its fall was recorded. Each rat was subjected to the same technique. As a reading, the mean of three trials was considered (Kishore Kumar et al., 2017; Parasuraman et al., 2020).

Hanging wire Grip strength test

The metallic wire string was linked horizontally to two vertically arranged rods. The rat was placed in the center of the string, with its forelimb clinging to the string while its body and tail were suspended in the air over 30 cm from the ground. The distance between the rope and the ground was kept as low as possible to prevent injuring the rodent during its fall. The time required for the rat to fall was recorded, i.e., "fall off time" (Parasuraman et al., 2020).

Forced swimming test

Forced swimming test was used to measure the immobilization time. The tank was filled with water in a temperature range of 24–30°C to ensure that the rodents' tails and feet did not contact the bottom. After that, the rat was placed in the tank. The time interval between the rodent ceasing to swim and beginning to sink was recorded. As soon as the rodent began to sink, it was promptly removed. All rodents were subjected to the same treatment. The water tank was subsequently cleansed whenever excrement and urine accumulated, as this could result in bacterial contamination (Palanimuthu et al., 2016).

Brain Tissue Pre-processing

After 28 days of intervention, the rats were euthanized by cervical dislocation, and the hippocampus was dissected from fresh rat brain and transferred to tissue cassettes immersed in 10% neutral buffered formalin (Sigma) for overnight fixing. Following fixation, the cassettes were transported to a Thermo tissue processor for 16 h according to manufacturer's protocol, which included fixation, dehydration, clearing, and wax infiltration. Tissues were imbedded in wax (Thermo) and chilled to room temperature before being trimmed into 4 µm sections using a Leica microtome. The sections were fished out using Poly-Lysin coated slides (Thermo) for Immunohistochemistry (IHC) staining. All prepared slides were dehydrated at room temperature (Badroon et al., 2020; Al-Suede et al., 2021).

Determination of the Expression of Brain Derived Neurotrophic Factor (BDNF) and Glial Cell Line-Derived Neurotrophic Factor (GDNF) in Hippocampus by Using IHC Technique

Brain tissues were harvested and preserved in a 10% paraformaldehyde solution. The paraffin-embedded tissue block was sliced into 4 µm sections. The tissue was deparaffinized and rehydrated prior to IHC staining using a graded series of ethanol. The sections were incubated in a microwave with antigen retrieval buffer and then rinsed with

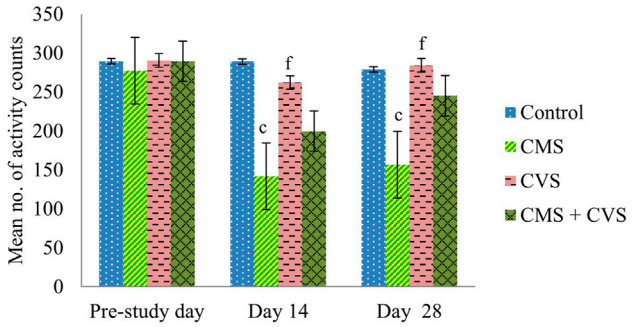

FIGURE 1 | Effect of CVS on locomotor activity. Values are expressed as mean ± SEM ($n = 6$). $^{c}p < 0.001$ compared with that of control group; $^{f}p < 0.001$ compared with that of CMS group. (One-way ANOVA followed by Tukey's *post hoc* test).

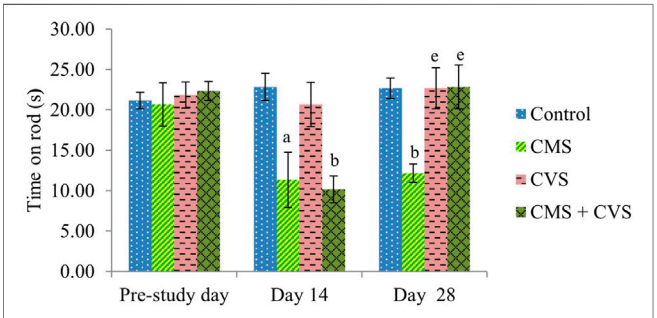

FIGURE 2 | Effect of CVS on fall off time. Values are expressed as mean ± SEM ($n = 6$). $^{a}p < 0.05$, $^{b}p < 0.01$ compared with that of control group; $^{e}p < 0.01$ compared with that of CMS group. (One-way ANOVA followed by Tukey's *post hoc* test).

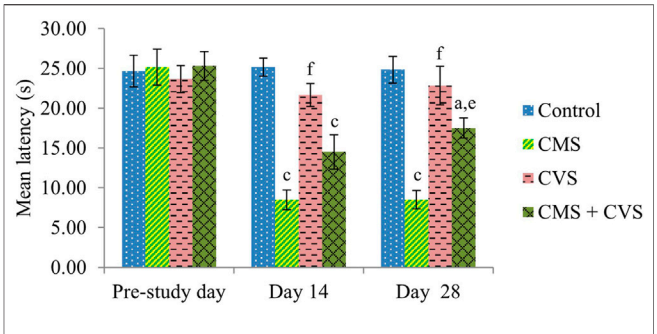

FIGURE 3 | Effect of CVS on wire grip. Values are expressed as mean ± SEM ($n = 6$). $^{a}p < 0.05$, $^{c}p < 0.001$ compared with that of control group; $^{e}p < 0.01$, $^{f}p < 0.001$ compared with that of CMS group. (One-way ANOVA followed by Tukey's *post hoc* test).

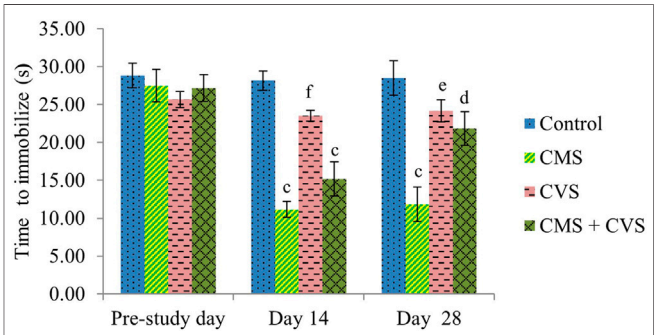

FIGURE 4 | Effect of CVS on immobilization time. Values are expressed as mean ± SEM (n = 6). $^{c}p < 0.001$ compared with that of control group; $^{d}p < 0.05$, $^{e}p < 0.01$, $^{f}p < 0.001$ compared with that of CMS group. (One-way ANOVA followed by Tukey's *post hoc* test).

TBST (1×), followed by a 10-min incubation with hydrogen peroxide block. Following that, the section was blocked with 5% BSA for 10 min and incubated overnight at 2°C with primary antibodies (anti-BDNF or anti-GDNF), followed by washing with PBS. For 10 min at room temperature, sections were treated with secondary antibody (Rabbit specific HRP DAB). The negative control sections received the identical treatment as the positive control sections, but without the addition of primary antibodies. Sections were washed three times in PBS-T followed by mounting with DAPI (Fluorescent mounting medium). Finally, sections were examined under a microscope with ×100 objective. The percentage of BDNF and GDNF protein expression was measured using imageJ software (Badroon et al., 2020; Al-Suede et al., 2021).

Statistical Analysis

The mean and standard error of the mean (SEM) were used to express descriptive data. Statistical significance was fixed at p value less than 0.05 for all behavioral tests. The data for behavioral studies were analyzed using one-way ANOVA followed by Tukey's *post hoc* test.

RESULTS

Effect of CVS on the Behavior of Rats

At the end of the study, when compared to control rats, CMS-induced rats had significantly lowered locomotor activity (**Figure 1**), motor coordination (**Figure 2**), muscle grip strength (**Figure 3**), and immobilization time (**Figure 4**) whereas the CMS-induced rats which were administered CVS showed a significant increase in locomotor activity (**Figure 1**), motor coordination (**Figure 2**), muscle grip strength (**Figure 3**), and immobilization time (**Figure 4**) when compared to the CMS group. The rats which were administered CVS alone showed no significant differences in locomotor activity (**Figure 1**), muscle grip strength (**Figure 3**), or immobilization time (**Figure 4**) when compared to control rats.

Effect of CVS on BDNF Levels in CMS-Induced Rats

The immunohistochemical staining for BDNF protein is clearly apparent in the sections of the control group, demonstrating that BDNF is expressed normally and constitutively in the

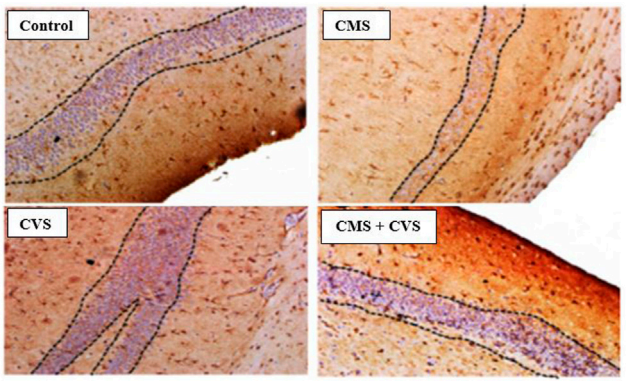

FIGURE 5 | Immunohistochemical staining for BDNF in rat hippocampus section. Shown are representative photomicrographs of different groups of rats. BDNF staining is prominently visible in the sections as dotted lines.

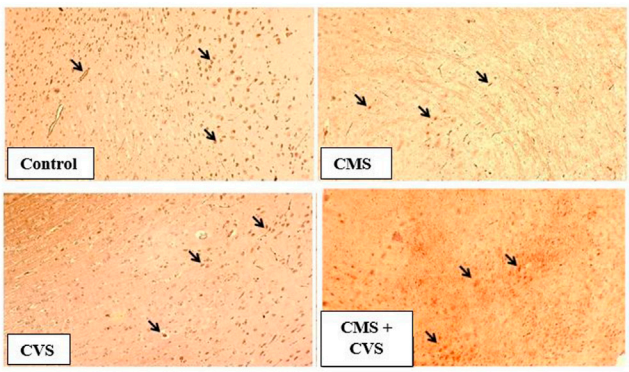

FIGURE 7 | Immunohistochemical staining for GDNF in rat hippocampus section. Shown are representative photomicrographs of different groups of rats. GDNF staining is prominently visible in the sections marked with arrows.

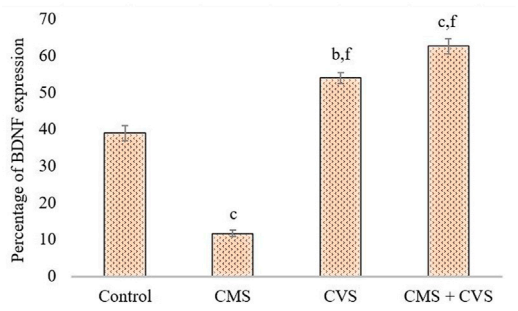

FIGURE 6 | Quantitative immunohistochemical analysis of BDNF. Values are expressed as mean ± SEM (n = 6). $^{b}p < 0.01$, $^{c}p < 0.001$ compared with that of control group; $^{f}p < 0.001$ compared with that of stress group. (One-way ANOVA followed by Tukey's *post hoc* test).

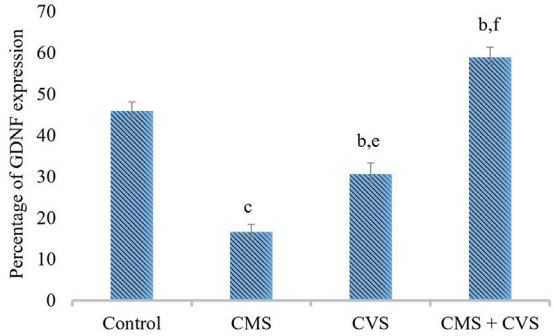

FIGURE 8 | Quantitative immunohistochemical analysis of GDNF. Values are expressed as mean ± SEM (n = 6). $^{b}p < 0.01$, $^{c}p < 0.001$ compared with that of control group; $^{e}p < 0.01$, $^{f}p < 0.001$ compared with that of stress group. (One-way ANOVA followed by Tukey's *post hoc* test).

hippocampus of the control animals (**Figure 5**). CMS-exposed rats, on the other hand, had lower levels of BDNF expression than the control group. Among the treated groups, CMS+CVS animals had the highest level of BDNF protein expression, followed by the group which was given CVS which is evident with the quantitative analysis (**Figure 6**).

Effect of CVS on GDNF Levels in CMS-Induced Rats

The results showed that rats exposed to CMS had lower levels of GDNF protein expression in the hippocampus when compared to the control and treatment groups (**Figure 7**). When compared to the untreated group, the treated groups that received CVS and CMS+CVS showed a strongly significant increase in GDNF protein expression. GDNF expression was substantially higher in the CMS + CVS group than in the CMS group. Among the treated groups, CMS+CVS group had the highest level of GDNF expression, followed by the group administered with CVS only indicating that CVS enhances GDNF expression in the brain tissue as proven by quantitative analysis (**Figure 8**).

DISCUSSION

The current study was established to assess the protective role of CVS in CMS-induced rats in terms of behavioral changes and modifications of neurotrophic factors (BDNF and GDNF). In this study, CVS prevented the CMS-induced changes in locomotor behaviour, muscle grip strength and immobilization time. The results indicate that CVS can be helpful to modulate CMS-induced behavioral changes and improve the psychomotor function.

Stress affects the hippocampus, amygdala, and prefrontal cortex of the brain (Bremner, 2006), by stimulating the HPA axis, which is mediated through the hippocampus (Zhu et al., 2014). The principal stress-response mechanism is the HPA axis, which regulates various neurological functions at both the central and peripheral levels of the brain. Any type of stress has an impact on brain functions and can lead to a variety of neurodegenerative disorders (Esch et al., 2002; McEwen, 2004). The CMS model of stress is a prototypical example in which rats are subjected progressively to a series of severe stressors. In humans it can be associated to suffering from depression due to the normal unpredictable pressures of human existence (Willner, 2017).

Hence, CMS model was employed to analyze the antidepressant-like effects of CVS in this study. As a result of chronic exposure to unpredictable micro-stressors, a cascade of behavioral changes ensue, all of which are linked to the clinical core symptom of stress. The symptoms of CMS reflect an enhanced physiological stress response. Previous experiments revealed that the glucocorticoid receptor antagonist mifepristone, the corticosterone synthesis inhibitor metyrapone, or adrenalectomy restrict the development of a depressive phenotype after CMS exposure. This demonstrates the vital relevance of HPA system in the impacts of CMS. The critical factor is that negative feedback systems operating through forebrain structures keep HPA activity in check, with the primary feedback occurring at the level of the hippocampus (Welberg and Seckl, 2001), and hence the levels of neurotrophic factors were assessed in the hippocampus which is an area of continued neurogenesis throughout the life of the brain. The hippocampus is a primary stress track owing to its role in regulating HPA axis function as well as its susceptibility to stress (Zaidi and Banu, 2004; Ansari et al., 2012). CMS exposure causes a preliminary activation of microglia, a marker of neuropathology that is triggered by increasing glucocorticoid exposure. The long-term exposure of the hippocampus to glucocorticoids disrupts cell metabolism by slowing glucose absorption and renders neurons more susceptible to metabolic stimuli (Sapolsky, 1987). Stress decreases the inhibitory input to the HPA axis, leading to overactivation of the HPA axis, which boosts the corticosterone level. The vestibular system has extensive connections with many cognitive parts of the brain, including the hippocampus, basal ganglia, parieto-frontal cortices, and cerebellum, which are referred collectively as vestibular cortices (Brandt, 2003). CVS can directly suppress the Sympatho-Adrenal-Medullary (SAM) and HPA axis by boosting GABA release, and higher GABA inhibits the HPA axis. It also indirectly suppresses the HPA axis by boosting GABA release and stimulating hippocampal formation (Vitte et al., 1996; Cuthbert et al., 2000; Herman et al., 2004; Cullinan et al., 2008; Mody and Maguire, 2012), resulting in a spike in BDNF and GDNF levels. Bilateral CVS has been shown to improve different aspects of brain function in neurodegenerative pathologies (Devi and Mukkadan, 2017). However, only few research studies have been conducted to investigate the tangible impacts of bilateral CVS on CMS-induced rats. In the current work we have employed CVS to ameliorate behavior and neurotransmitters such as BDNF and GDNF levels in CMS-induced rats and it must be presumably owing to symmetrical stimulation of the cerebral hemispheres (Bense et al., 2003). Previous studies established the benefit of CVS in enhancing auditory and visual response speed under stress (Rajagopalan et al., 2017).

The behavioral analysis of the current research indicated that rats exposed to CMS, displayed a considerable decline in the locomotor count by actophotometer, muscle grip strength by hanging wire grip strength test, and immobilization time by forced swimming test. These behavioral aberrations in the CMS rats were recognized as a behavior of stress. The rats that were treated with CVS reversed the behavioral alterations which is obvious from the significant restoration of the locomotor count, muscle grip strength, and immobilization time, providing considerable protection for the neurons. These were ascribed as favorable effects of CVS in the inhibition of stress axis. These findings are in agreement with prior studies supporting the decrease in the depression, anxiety, and stress ratings followed by CVS (Kumar et al., 2016; Rajan et al., 2016).

The forced swimming test is performed in this study to assess immobilization. Immobility in a forced swimming test represents behavioral despair caused by the realization that escape is unattainable. The forced swimming test has also been used to assess active coping strategies, with immobility suggesting a passive coping response (Lam et al., 2018; Molendijk and de Kloet, 2019). CMS animals showed lower immobilization in the current study, indicating that the animals were less capable of coping with inescapable stressors, whereas CVS prevented CMS-induced changes in coping with inescapable stressors.

In the rotarod experiment, CMS animals showed a decrease in fall off time when compared to control animals, however the CMS animals co-administered with CVS prevented the CMS-induced alterations. The rotarod test is employed to evaluate the motor coordination of rodents and to detect cerebellar dysfunction (Shiotsuki et al., 2010). The decrease in locomotion in CMS group implies that the animal lost motor coordination due to stress which was prevented by CVS.

BDNF is generated from BDNF pro-isoform, which is then cleaved proteolytically (N-terminal domain is deleted) within the neuron or after it is released, forming its final protein form (Ahmed et al., 2015). This mature neurotrophin binds to protein-kinase neurotrophin receptors - tropomyosine-related kinase (Trk) receptors. The immunohistochemistry screening of hippocampal slices exhibiting BDNF and GDNF demonstrated substantial deterioration following CMS induction. Stress, diet, metabolism, and behavior modulates the expression of BDNF in the central and peripheral neural systems (Fuchikami et al., 2009). Stress results in morphological changes (McEwen, 2001), dendritic atrophy in hippocampal pyramidal neurons, particularly in the CA3, CA4 area, and an impairment of neurogenesis in the dentate gyrus (McEwen, 1999; Fuchs et al., 2001; Gill and Grace, 2013), as well as motor cortex thinning (Khan et al., 2018). Our present study also proves that stress causes pathological and morphological changes in the hippocampus, which is consistent with prior studies that show that several brain related issues, such as stress, seizure, ischemia, and hypoglycemia, alter BDNF expression in the central nervous system (Yan et al., 1997; Tapia-Arancibia et al., 2004). Changes in its expression may have a role in several disorders, including depression, Alzheimer's disease, Parkinson's disease, and epilepsy (Tapia-Arancibia et al., 2004). The treatment group (CMS+CVS) animals had higher levels of BDNF and GDNF, followed by the CVS group of rats. The surge in BDNF levels implies vestibular neurogenesis and modification of potassium-chloride cotransporter (KCC2) and GABA receptor expression in the vestibular nuclei. By boosting neurogenesis and modifying the expression of KCC2 and GABA receptors in the vestibular nuclei, BDNF signaling enhances vestibular compensation. The neurotrophic effects of GDNF against neuronal

dysfunction are well documented. This GDNF neuroprotective action generated a powerful upregulation of BDNF, suggesting that together may crucial against neurons atrophy and degeneration (Revilla et al., 2014). There is a definite connection between the vestibular system and the brain. The vestibular system is intricately linked to the cerebral cortex, and vestibular system lesions trigger cortical and hippocampal atrophy (Brandt et al., 2005). Previous research has shown that bilateral loss of vestibular function is attributed to decrease hippocampal volume, cell number, proliferation, dendritic length, and morphology, all of which contribute to memory loss, anxiety, and autonomic dysfunction (Smith et al., 2010; Smith and Darlington, 2013; Balabhadrapatruni et al., 2016). Controlled CVS accelerates dendritic arborization in hippocampal pyramidal cells and boosts cell proliferation in the dentate gyrus and perhaps neurogenesis (Cuthbert et al., 2000; Khan et al., 2018). Vestibular stimulation impacts the physiology of the cortex due to its extensive interactions with brain regions. The current research presents data on stress-induced hippocampal morphological alterations, as well as the impact of CVS on stress-induced changes.

CONCLUSION

The findings of the current study indicated that Chronic Mild Stress for 28 days induces behavioral and immunohistochemical modifications which is a significant indicator of neurodegeneration. The results further indicate that Caloric Vestibular Stimulation has ameliorating impact on Chronic Mild Stress with its therapeutic potential and can serve as a neuroprotectant in the treatment of stress-related disorders (Jinu and Archana, 2018), (Sailesh et al., 2014).

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by AIMST University Animal Ethics Committee (AUAEC), AIMST University, Kedah, Malaysia.

AUTHOR CONTRIBUTIONS

Conceptualization, AR, SG, and SP, Methodology, AR, SG, and SP, Validation, AR, SG, and SP, Investigation, SG and SP, Data curation, SG and SP, Writing, Original Draft preparation, SG and AR, Writing, Review and editing, AR and SP, Visualization, AR and SG, Supervision, AR and SP. This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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REVIEW
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Brain-Derived Neurotrophic Factor-Mediated Neuroprotection in Glaucoma: A Review of Current State of the Art

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Retinal ganglion cells (RGCs) are neurons of the visual system that are responsible for transmitting signals from the retina to the brain *via* the optic nerve. Glaucoma is an optic neuropathy characterized by apoptotic loss of RGCs and degeneration of optic nerve fibers. Risk factors such as elevated intraocular pressure and vascular dysregulation trigger the injury that culminates in RGC apoptosis. In the event of injury, the survival of RGCs is facilitated by neurotrophic factors (NTFs), the most widely studied of which is brain-derived neurotrophic factor (BDNF). Its production is regulated locally in the retina, but transport of BDNF retrogradely from the brain to retina is also crucial. Not only that the interruption of this retrograde transport has been detected in the early stages of glaucoma, but significantly low levels of BDNF have also been detected in the sera and ocular fluids of glaucoma patients, supporting the notion that neurotrophic deprivation is a likely mechanism of glaucomatous optic neuropathy. Moreover, exogenous NTF including BDNF administration was shown reduce neuronal loss in animal models of various neurodegenerative diseases, indicating the possibility that exogenous BDNF may be a treatment option in glaucoma. Current literature provides an extensive insight not only into the sources, transport, and target sites of BDNF but also the intracellular signaling pathways, other pathways that influence BDNF signaling and a wide range of its functions. In this review, the authors discuss the neuroprotective role of BDNF in promoting the survival of RGCs and its possible application as a therapeutic tool to meet the challenges in glaucoma management. We also highlight the possibility of using BDNF as a biomarker in neurodegenerative disease such as glaucoma. Further we discuss the challenges and future strategies to explore the utility of BDNF in the management of glaucoma.

Keywords: brain-derived neurotrophic factor, glaucoma, neurodegeneration, neuroprotection, retina, retinal ganglion cell

INTRODUCTION

Retinal ganglion cells (RGCs) are essential to processing perceived images, and their loss can lead to irreversible blindness, such as that seen in glaucoma (Gupta et al., 2016). Optic neuropathies, such as glaucoma, the second leading cause of blindness globally, are associated with the loss of RGCs and gradual degeneration of the optic nerve head (ONH); hence producing a characteristic pattern of visual field loss (Weinreb et al., 2018; Smith et al., 2020).

Glaucoma is a group of ocular disorders with multiple clinical phenotypes, but regardless of the subtypes, increased intraocular pressure (IOP) remains a widely recognized risk factor for the development and progression of glaucoma. Hence, currently lowering IOP to a target level is the only treatment option for glaucoma (Weinreb et al., 2014). Its etiology is unclear and what constitutes a "major contributor" to the disease development remains ambiguous. Numerous studies have been conducted to understand the pathophysiology of glaucoma and to identify the cellular and molecular targets for therapeutic intervention. A comprehensive review by Agarwal et al. (2009) stated that IOP elevation and vascular dysregulation remain the primary pathophysiologic factors, while the excitotoxic and the oxidative damage of the neurons are the secondary factor contributing to glaucomatous RGC loss (Agarwal et al., 2009).

Numerous investigators have documented the potential cytotoxic stimuli that contribute to the RGC death in glaucoma, including neurotrophin deprivation, glutamate excitotoxicity, mitochondrial dysfunction, glial activation, inflammation, endoplasmic reticulum (ER) stress, ischemia, and oxidative stress (Almasieh et al., 2012; Almasieh and Levin, 2017). From a therapeutic standpoint, each of these mechanisms could be a potentially attractive strategy for the intervention to achieve neuroprotection. Thus, neuroprotectants that can block the downstream cascades evoked by various cytotoxic stimuli have extensively been studied in an attempt to eradicate or slow down the optic neurodegeneration. The favourable effects of any of the currently investigated neuroprotective candidates, although observed in animal glaucoma models have not been replicated in clinical trials (Claes et al., 2019) and, in fact, the proposed benefits of some of these potential agents have now been challenged (Gribkoff and Kaczmarek, 2017). Besides, the consensus on the neuroprotective properties of potential therapeutic intervention, mode of its delivery also remains a challenge (Cvenkel and Kolko, 2020). Of note, poor drug delivery has always been one of the main concerns in the treatment of glaucoma, hence there is ever growing need for novel drug delivery systems. This includes the applications of nanotechnology-based formulations such as nano-fibre (Omer and Zelkó, 2021; Rohde et al., 2022), hydrogels (Lynch et al., 2020; Balla et al., 2022), contact lenses (Fan et al., 2020; Dang et al., 2022), and implants (Adrianto et al., 2021; Boia et al., 2022). Discussion on this area is beyond the scope of this paper. Comprehensive reviews by Akhter et al. and others (Akhter et al., 2022; Peng et al., 2022).

The RGC loss in glaucoma is accomplished through apoptosis irrespective of the initiating pathological stimuli (Munemasa and Kitaoka, 2013). Although the precise factors that contribute to glaucoma are still being debated, the neurotrophin deprivation theory, having arisen from the observed failing of the axonal transport, currently presents as one of dominant contributors. Neurotrophins are used in neuroprotective therapies because of their effective role in maintaining and improving the survival of neuronal cells (Jeanneteau et al., 2020). The deprivation of essential neurotrophins leads to induction of the apoptosis. Studies have shown that the neurotrophin-dependent mechanisms of cell death inhibition include the regulation of Bcl-2 and Bad proteins (Miller and Kaplan, 2001). The repetitive neuronal activity increases the secretion and action of neurotrophins at the synapses and modulates the synaptic transmission and connectivity (Schinder and Poo, 2000). Brain-derived neurotrophic factor (BDNF), a potent trophic factor, is predominantly expressed in the central nervous system (CNS) and is crucial for synaptic and structural plasticity. Its enhanced expression offers protection after injury (Feng et al., 2017).

BDNF exerts neuroprotective effects directly *via* the Tropomyosin receptor kinase B (TrkB) expressed in RGCs (Vecino et al., 2002; Osborne et al., 2018) and/or indirectly *via* the TrkB expressed in glia (Dekeyster et al., 2015a). In addition to the RGCs, the amacrine cells in the retina also produce BDNF, which can be transported retrogradely, from the brain to retina *via* axons (Cohen-Cory et al., 1996; Vecino et al., 2002; Grishanin et al., 2008; Harada et al., 2015). There is evidence that both the local synthesis and retrograde transport of BDNF, get reduced subsequent to excitotoxic insult causing changes in the synaptic dynamics, which in turn leads to retinal neurodegeneration (Quigley et al., 2000). In this review, the authors discuss the role of BDNF deficiency in the glaucomatous RGC loss. Many published studies describe the link between the lack of BDNF support to the RGCs as a trigger for their apoptosis (Ko et al., 2001; Vecino et al., 2002; Johnson et al., 2009; Shoeb Ahmad et al., 2013). Therefore, it is likely that aberrant BDNF expression and the underlying signaling pathways in the visual system paly a key role in the pathophysiology of glaucoma. Indeed, previous studies revealed that BDNF preserves the RGCs after the optic nerve axotomy in chronically hypertensive rats (Peinado-Ramón et al., 1996; Ko et al., 2001; Dahlmann-Noor et al., 2010). Similarly, there is a consensus on the association of central and local alterations in the BDNF-TrkB signaling pathway with the retinal or the optic nerve damage, indicating the role of BDNF in preserving the inner retinal elements. (Pease et al., 2000; Gupta et al., 2014; Dekeyster et al., 2015a). However, the different roles of the BDNF/TrkB signaling pathway in RGC versus other retinal neurons and glia cells have yet to be elucidated.

In glaucomatous eyes, BDNF expression was observed to be significantly lower in aqueous humor, lacrimal fluid, and serum relative to the healthy subjects (Almasieh et al., 2012), suggesting a possible correlation between low BDNF levels and the early stages of glaucoma (Ghaffariyeh et al., 2011). Considering involvement of multiple interacting mechanisms, blocking a specific pathway at the point of onset may not be adequate to stop pathological progression (Almasieh et al., 2012). Therefore, a focus on altering pathological cascade close to the merging

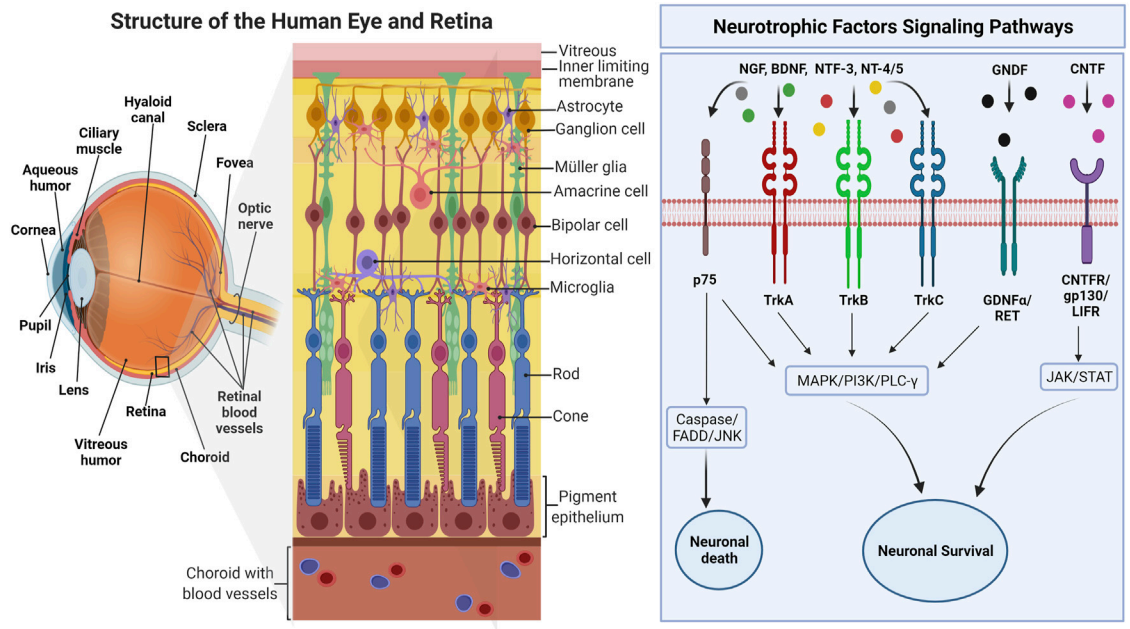

FIGURE 1 | The overview of human eye anatomy with focus on the retinal structure. The right side of the figure depicts the interactions of various NTFs with major intracellular signaling pathways activated through corresponding receptors. Adapted from "Structure of The Retina" by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.

endpoints may prove to be more meaningful to stop the RGC death.

In this review, the authors discuss the role of BDNF as a potential biomarker for the early detection of glaucoma. We propose BDNF-based neuro-repair as a novel strategy to complement neuroprotection achieved by the current treatments, focusing primarily on cell death and conferring continuous neurotrophic support after the initial injury. It is noteworthy that, despite significant advances, no neuroprotective agent that protects the RGCs from damage has shown benefits in clinical trials. Therefore, investigations into molecular and cellular events leading to the RGC death in glaucoma are warranted. The authors highlight the exogenous application of BDNF in the experimental model of glaucoma and its limitations when translating research findings into clinical application. Indeed, future studies conducted to better understand the critical role of BDNF and its signaling in healthy versus glaucomatous retinas will provide new insights that may prove to be essential as neuroprotective strategies to preserve RGCs. This review was carried out using the key words, brain-derived neurotrophic factors; retinal ganglion cells; neurodegeneration; neuroprotection; retina; glaucoma on PubMed, SCOPUS, and Web of Science databases. English language papers published from the year 1951, 1982 to 2022 are included in this review.

NEUROTROPHIC FACTORS

Nerve growth factor (NGF) was the first growth factor identified in 1950s for its trophic (survival- and growth-promoting) effects on sensory and sympathetic neurons (Levi - Montalcini and Hamburger, 1951). Later, in 1982, BDNF was discovered as the second member of the "neurotrophic" family of growth factors through isolation and purification from the pig brain. It was shown to promote survival of a subpopulation of neurons in dorsal root ganglion (Barde et al., 1982). Since the NGF and BDNF discovery, other members of the neurotrophin family have been described, such as neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), ciliary neurotrophic factor (CNTF), and glial cell line-derived neurotrophic factor (GDNF) (**Figure 1**), each with a distinct profile of trophic effects on the subpopulations of neurons in the nervous system (Ip and Yancopoulos, 1996; Blum and Konnerth, 2005; Ibáñez and Andressoo, 2017). These molecules share several similarities, including their homologies in sequence, structure, and processing. They are synthesized as proneurotrophins, the immature precursors, and are converted to mature proteins after the proteolytic cleavage (Reichardt, 2006). These molecules bind to Tropomyosin receptor kinase (Trk) receptors and p75 neurotrophin receptor (p75NTR), and their affinity towards each of these receptors depends on their maturity (Lu et al., 2005). Mature Neurotrophic Factors (NTF) have a high affinity towards Trk receptors, which leads to cell survival and growth, while proneurotrophins have a high affinity towards p75NTR, which mainly elicits cell apoptosis. Each type of NTF binds selectively to specific Trk receptors: NGF binds specifically to TrkA; NT4 and BDNF activate TrkB; NT3 binds to TrkC, and all NF can bind to p75NTR (Reichardt, 2006). All the NTF-receptors bindings are not necessarily high affinity bindings. For example, the binding of BDNF to TrkB is of low affinity, but it can be

changed when interacting with the Trk receptor and p75NTR (Chao, 2003). Upon activation, each receptor regulates several signaling pathways that are essential for neuronal development and function. Trk receptors regulate three major signaling pathways that mediate differentiation and survival, namely, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and phospholipase Cγ (PLC-γ).

p75NTR with the intracellular death domain, similar to that in tumor necrosis factor receptors (TNFR), regulates survival and inflammation through nuclear factor kappa B (NF-κB), neuronal apoptosis through Jun-N terminal kinase (JNK), and reduced growth cone motility through RoA/ROCK signaling pathway (Huang and Reichardt, 2003). However, these two types of receptors could interact with each other or another type of receptor and transduce different binding affinities and signaling pathways that further contribute to additional functions of NTF and its receptors (Chao, 2003). Apart from forming complexes that produce high-affinity binding sites for NTF, activation of the Trk signaling pathway, such as NF-KB, by p75NTR has shown a synergistic contribution to neuronal survival (Huang and Reichardt, 2003). Although proapoptotic signaling of p75NTR is suppressed by Trk signaling, primarily through PI3K, this interaction is not always fully efficient, given that the crosstalk of p75NTR, and Trk signaling could also induce apoptosis in the presence of ceramide and regulate the number of mature cells (González-Hoyuela et al., 2001). The specificity of Trk receptors to each NTF is regulated by the presence or absence of an insert, a brief sequence of amino acids in the juxtamembrane region (Huang and Reichardt, 2003). For instance, TrkB without the insert can be activated by BDNF only, whereas the presence of the insert on TrkB makes it activable by NT3 and NT4 as well (Yacoubian and Lo, 2000). Hence, the receptors, either full length (TrkB-FL) or truncated (TrkB-Tc), would regulate distinct features of the NTF signaling. Compared to other NTFs, BDNF is highly expressed in the adult brain, mainly in the hippocampus, and is tightly regulated by neural activity. Apart from neuronal survival, BNDF is widely accepted to play a critical role in synaptic plasticity and memory (Sasi et al., 2017).

BDNF AND ITS RECEPTORS

The broad range of functions served by BDNF owe to the complexities of neurotrophin production, secretion, and receptor signaling in the nervous system. Once secreted, BDNF can be activated in two forms: prodomain of BDNF (cleaved precursor protein; pro-BDNF) and mature BDNF (mBDNF), which exert their functions primarily through p75NTR and sortilin signaling and TrkB and p75NTR/TrkB, respectively (Kutsarova et al., 2021). Although mBDNF has a high affinity of binding to TrkB, it binds to p75NTR when the expression level is aberrant, hence stimulating signaling cascades in the manner opposite to the TrkB receptor. Because of the opposing affinities, the intra-/extracellular cleavage of BDNF becomes another critical factor in regulating the downstream signaling effects of BDNF (Lee et al., 2001).

The Trk receptors dimerize in response to a ligand binding and autophosphorylate. There are several isoforms of TrkB, and the most abundantly expressed are the full-length (TrkB-FL) and the truncated (TrkB-Tc) forms. TrkB-Tc lacks an intracellular kinase domain (Eide et al., 1996), hence it functions as a dominant-negative receptor, forming heterodimers with full-length receptors and blocking neurotrophin signaling. In astrocytes and Schwann cells, the truncated form has been suggested to regulate the pool of neurotrophins and keep them from degrading or signaling until they are released into the extracellular space (Alderson et al., 2000). The pro-death receptor, p75NTR, comprises a cytosolic death domain that is highly expressed during development (Dechant and Barde, 2002). It acts canonically by mediating both pro-death and pro-survival signals, which depend entirely on associations with cytoplasmic proteins (Dechant and Barde, 2002). In contrast, several intracellular tyrosine residues of TrkB-FL can be phosphorylated (Huang and Reichardt, 2003). Because of this, the three signaling cascades (i.e., MAPK, PI3K, and PLC-γ pathways) promote and govern the activity-dependent and tissue-specific expression of BDNF (Chen et al., 2003). The promoters translocate to the nucleus, where transcription of mRNAs responsible for producing the heterogeneous population of BDNF occurs (Minichiello, 2009). The BDNF mRNA splice variant has been described in multiple species (Dekeyster et al., 2015a). Of importance, environmental experiences such as stress-induced epigenetic modifications can influence the BDNF gene activity and epigenetic marking of the BDNF gene (Roth and Sweatt, 2011).

Since these receptor-mediated actions are thought to act contradictory, the dynamics may help in balancing the growth and death of neurons. Of note, preferentially, pro-BDNF signaling through presynaptic p75NTR is essential for axonal retraction in growing neuromuscular synapses and results in antigrowth signaling (Lee et al., 2001). The modulator, pro-BDNF, would selectively promote N-methyl-D-aspartate (NMDA) activity, along with glutamate, through p75NTR. Unlike pro-BDNF, mBNDF *via* presynaptic TrkB leads to axonal stabilization and results in pro-growth signaling (Je et al., 2013). Mature BDNF-TrkB signaling mediates long-term potentiation (LTP) through pre- and post-synaptic mechanisms, such as by influencing local protein synthesis, spine remodeling, or gene transcription (Park and Poo, 2012). Since BDNF is predominantly secreted as pro-BDNF, proteins that cleave the prodomain may influence which receptors are triggered by the BDNF release, giving yet another regulatory mechanism for BDNF signaling (Chen et al., 2005). BDNF-TrkB signaling can act as both mediator and modulator for plasticity-inducing neuronal activity. Moreover, BDNF with neurotransmitter signaling released within a critical time window can act as the instructor for immediate synaptic plasticity. This is why BDNF has been of interest as a stimulant for protective and restorative treatments in both neurological and psychiatric disorders. What is also known about BDNF is that compared to other NTFs, the former is the superior factor for the RGC survival under

glaucomatous conditions (Almasieh and Levin, 2017). This has been proven by the exogenous application of BDNF in the developing retinotectal system where the RGC axons showed arborization and growth, contrariwise to the depleted endogenous BDNF, which hampered presynaptic trafficking and axonal branch stabilization (Kutsarova et al., 2021).

BDNF IN RETINA

Emerging evidence on the importance of BDNF as a neurotrophin in addition to NGF, was discovered by Barde et al. (1982). In this seminal study, BDNF was shown to exert trophic effects in the survival of a subpopulation of dorsal root ganglion neurons and the fiber growth in cultured embryonic chicks (Barde et al., 1982). The same effect was later identified in the adult human brain, where a sustained expression of BDNF was associated with increased number of receptors specific to dendrite growth indicating stimulation of neurogenesis and perhaps appearance of new neurons (Tyler et al., 2002). Importantly, BDNF is required for development and survival of dopaminergic, GABAergic, serotonergic, and cholinergic neurons. Indeed, an in-depth interpretation of the effects of BDNF on the development and survival of retinal neurons may provide more significant insights into role of BDNF/TrkB pathway in the pathogenesis and, ultimately, loss of RGCs.

Unlike other retinal neurons, the axons from RGCs project to various areas of brain *via* the optic nerve (Crair and Mason, 2016). Much of the information regarding projection of optic nerve fibres in brain has been gathered from studies involving animals like rodents, chicks, and tadpoles (*Xenopus*). However, the significant difference between these experimental species and humans is the distribution of RGC axon projections in brain. In higher mammals, such as macaque, the most RGC projections synapse in the lateral geniculate nucleus (LGN), with fewer axons extending to the superior colliculus (SC) (Perry et al., 1984). However, in other experimental species such as mice, 85%–95% of the RGCs project to the SC (Ito and Feldheim, 2018; Reinhard et al., 2019). The development of retinal axons and their projections undergoes changes over a broad time frame to regulate the structural morphology and connectivity. Most prominently 50% of RGCs undergo apoptosis during pre- and early postnatal period (Guerin et al., 2002). BDNF exhibits a spatiotemporal expression at this stage, which may play an essential role in maintaining the growth of the neuroretina as well as other structures of eye such as cornea, lens, trabecular meshwork, and ciliary body (Bennett et al., 1999).

As Frost et al. (2001) reported, while the BDNF protein expression in the hamster SC declines significantly to attain adult level by postnatal day 15, the same in retina increases significantly to attain adult level at the same time point. In SC, the BDNF protein level increases only during RGC branching but plateaus by the time arborization nears completion and then declines once the adult level is reached on the postnatal day 18 (Frost et al., 2001). It is clear that the relationship between the enhanced BDNF level and the neuronal activity of developing RGCs impacts their survival into adulthood. Likewise, when the RGC axons arborize in the SC, mature BDNF levels rise (Frost et al., 2001). Multiple events in the anatomical and functional maturation of the hamster retinal projection system are temporarily linked with developmental changes in retinal and SC BDNF protein concentrations. Moreover, BDNF expression is activity-dependent during the period of RGC death and synapse formation (Cohen-Cory and Lom, 2004). Both BDNF and TrkB mRNA and protein are expressed in the retina and SC at this time and are exceptionally high in RGC target sites (Perez and Caminos, 1995; Cohen-Cory et al., 1996; Frost et al., 2001; Cohen-Cory and Lom, 2004).

BDNF can be locally produced by RGCs and astrocytes in the retina and is transported to target areas *via* paracrine and autocrine actions (Cohen-Cory et al., 1996). However, it is still debatable whether BDNF/TrkB support of RGC survival throughout the development is due to retrograde or anterograde transport or the retinal BDNF sources persist into adulthood. What is undisputable is that BDNF promotes neuronal survival, axonal guidance and regulates the excitatory and inhibitory synaptic transmission in the visual system (Tyler et al., 2002). Indeed, BDNF was the major player of activity-dependent branching within the SC and remodeling of the RGC axons arborization (Marler et al., 2014). Owing to its highly regulated expression due to transcription, translation, and post-translational modifications (Ruiz et al., 2014), BDNF is believed to modulate critical protein synthesis in activity-dependent synaptic plasticity (Pollock et al., 2001). This complex regulation demonstrates the vitality and diversity of BDNF in supporting existing neurons after cellular insults in multiple neurodegenerative diseases (Pollock et al., 2001). For instance, reduced expression of BDNF and polymorphism -are closely associated with Alzheimer's disease (AD) progression (Kunugi et al., 2001; Beeri and Sonnen, 2016). A meta-analysis reported that serum BDNF decrease in individuals affected by Parkinson Disease (PD), supporting the association of reduced BDNF level and PD (Jiang et al., 2019). The same was documented in the person with relapse-remitting multiple sclerosis, where BDNF concentration was significantly lower than in healthy individuals (Wens et al., 2016).

NTF IN GLAUCOMA

It is now well established from various experimental glaucoma studies that NTFs effectively promote the survival of neurons and prevent apoptotic ganglion cell death (Mallone et al., 2020). This was supported by the finding that the therapies that significantly preserved the RGCs in the rat model of glaucoma were associated with elevated BDNF expression as compared to the untreated controls (Martin et al., 2003). Further cementing the significance of BDNF as a neuroprotective agent, it has been observed that exogenously applied BDNF inhibits the RGC loss and optic nerve damage in various acute and chronic glaucoma models (Gao et al., 2002; Mohd Lazaldin et al., 2020; Fudalej et al., 2021). Similar evidence of reduced RGC loss was also observed in response to the topical application of NGF (Colafrancesco et al., 2011; Lambiase et al., 2011). Topically administered

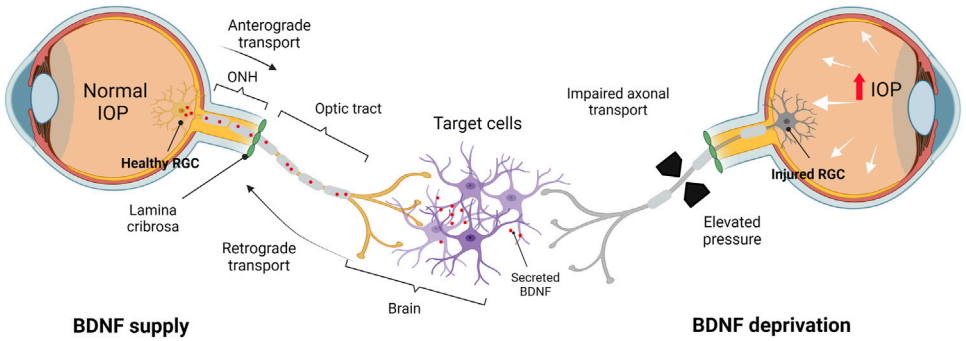

FIGURE 2 | BDNF deprivation theory in the adult human visual system. BDNF supply: in healthy conditions, BDNF is synthesized and secreted by cells in the brain and transported retrogradely *via* the optic nerve toward the somas (RGCs). BDNF deprivation: axonal transport is perturbed due to the elevated IOP and RGCs are deprived of target-derived (brain) BDNF, leading to the stressed RGCs undergoing apoptosis. IOP, intraocular pressure; ONH, optic nerve head. Created by Biorender.com (https://biorender.com/).

NGF rescued RGCs from degeneration and enhanced the visual function of individuals with advanced glaucoma (Lambiase et al., 2011). Interestingly, in a case-control study, serum BDNF and NGF levels were low in patients with early and moderate glaucoma, indicating that the NTFs have a potential to serve as diagnostic biomarkers for glaucoma (Oddone et al., 2017). The overexpressed CNTF has also been shown to exert a strong protective effect on RGCs in an experimental rat model (Pease et al., 2009). In an ocular hypertension-induced rat model of glaucoma, the administration of CNTF resulted in substantial reduction of the RGC loss, suggesting that CNTF promotes the survival of RGCs (Ji et al., 2004). CNTF has also been shown to promote regeneration in various retinal degeneration models (Li et al., 2010; Wen et al., 2012). Despite the evidence supporting the neuroprotective effects, the use of NTFs is challenging in clinical settings due to difficulties in their passage *via* anatomical barriers, such as, the blood-brain barrier, the blood-retinal barrier and the blood-aqueous barrier. Moreover, the challenges posed by their short half-lives and wide-ranging effects requires target-specific formulations (Mallone et al., 2020).

BDNF DEPRIVATION AND ITS LINK TO GLAUCOMA

One of the hypotheses proposes that the hindered defense mechanism of RGCs stems from the compromised neurotrophin transport to the cell bodies (Munemasa and Kitaoka, 2013; Guo et al., 2020). Since neurotrophins, particularly BDNF, are transported to the retina primarily in a retrograde manner, the transport blockade prevents BNDF synthesized locally, in soma and dendrites of neurons, to bind to the Trk receptors at the axon terminals (Dekeyster et al., 2015a). The lack of trophic support to RGCs may trigger apoptotic signalling and resulting in RGC loss (Kimura et al., 2016). In theory, BDNF deprivation in RGCs exerts stress, which triggers the cellular apoptotic pathways via JNK-mediated signaling, resulting in activation of proapoptotic BCL-2 family of proteins and leading to mitochondrial dysfunction. As a response to disease or injury, RGCs are known to upregulate the BDNF gene expression to circumvent apoptosis signaling and support the survival of the remaining RGC population. The same trend can be seen in axonal growth rate; however, it occurs only within the axonal terminals (de Rezende Corrêa et al., 2015). Apart from the RGCs, the inner retinal cells and photoreceptors are responsive to BDNF, implying that the neurotrophins are locally synthesized in the inner nuclear layers (Perez and Caminos, 1995). TrkB is highly expressed in RGCs, amacrine, and Müller cells, suggesting that the cellular target of the trophic action of BDNF is in the inner retinal elements (Zhang et al., 2005; Weber et al., 2010).

Considering the role of neurotrophins in RGC survival, dampening the endogenous stimuli, especially during episodes of insult, leads to substantial RGC damage (**Figure 2**). Deficient BDNF-TrkB signaling has been shown to be associated with RGC loss in various studies (Pease et al., 2000; Quigley et al., 2000; Iwabe et al., 2007; Osborne et al., 2018). Quigley et al. (2000) demonstrated that acute IOP elevation substantially suppresses the retrograde BDNF delivery to the ONH from the SC in adult rats, contributing to neuronal loss due to BDNF deficits. This has been attributed to the aberrant distribution of the axoplasmic transport of the trophic factors from target neurons in the SC and dLGN (Pease et al., 2000; Tanaka et al., 2009). Similarly, Pease et al. (2000) reported that the obstructed retrograde transport of BDNF gives rise to abnormal TrkB axonal distribution, focal accumulation of TrkB and BDNF, increased levels of TrkB in GCL, and increased TrkB in glia (Pease et al., 2000).

Multiple *in vivo* studies have suggested that the deficits of BDNF expression mark the RGC damage in glaucoma, and its interrupted axonal transport has been implicated in the progressive development of optic neuropathy in experimental models of glaucoma (Gupta et al., 2014; Feng et al., 2016; Osborne et al., 2018; Chitranshi et al., 2019; Wójcik-Gryciuk et al., 2020; Conti et al., 2021; Lazzara et al., 2021). The BDNF axonal transport in injured RGCs analysed *via* live-cell imaging was shown to exhibit reduced activity before the death of RGCs (Takihara et al., 2011). This finding is consistent with that in glaucomatous human eyes (Ghaffariyeh et al., 2009; Ghaffariyeh

et al., 2011; Gupta et al., 2014; Shpak et al., 2018; Igarashi et al., 2021) as BDNF deficits were detected in serum (Ghaffariyeh et al., 2011; Shpak et al., 2018), aqueous humour (Shpak et al., 2018; Igarashi et al., 2021), and lacrimal fluid (tears) (Ghaffariyeh et al., 2009; Shpak et al., 2018) of patients with early glaucomatous changes. Although it stimulates the expression of BDNF and its receptors, excitotoxicity induced by NMDA may also alter the retrograde transport of BDNF in the optic nerve and deprive it of the neurotrophins (Lambuk et al., 2017). It is also noteworthy that upon acute insult, the expression of BDNF-TrkB in the mouse retina is enhanced above the normal levels with extended axon survival (Feng et al., 2017). It is also hypothesized that the neuronal compartments, including the soma, axon terminal, and dendrites, appear to start the orchestration of BDNF-TrkB signaling differently (Chowdary et al., 2012). Manipulating the RGC target regions in which the signal is initiated may be a way of preventing the RGC death and delaying the progression of glaucoma or.

While the interruption in the retrograde transport is present at the early phase of the damage, the BDNF protein is synthesized rapidly in RGCs as an endogenous neuroprotective response, corroborating the idea of locally produced vs. retrogradely transported BDNF (Vecino et al., 2002). In addition, BDNF and TrkB is abundantly expressed in RGCs after axotomy, indicating that the endogenous protective response may contribute to the short-term survival of the neurons (Hirsch et al., 2000). Similar to BDNF, TrkB may be transported and stored at the axon. The intense and consistent TrkB expression was detected in the nerve fiber layer (NFL) post optic nerve lesion in the adult rat retina (Cui et al., 2002). In short, TrkB receptors could be synthesized in the soma and transported anterogradely or isolated at the nerve terminals and retrogradely transported to the soma of RGCs. However, they are not likely to promote long-term survival of the cells due to the reduced expression of BDNF expression subsequent to initial upregulation in RGCs. The limited presence of BDNF could also be attributed to the metabolic changes in injured neurons (Hu et al., 2010).

It is also argued that interrupted retrograde BDNF delivery can not be considered as the only cause of RGC death in glaucoma. This argument is supported by the observation that the adult porcine RGCs *in vitro* continued to survive and maintain their regular interaction with neighbouring neurons despite the lack of exogenous BDNF and dissociation from target tissues and presynaptic inputs (García et al., 2003). BDNF is also anterogradely transported to the CNS, where it serves as a survival factor for postsynaptic neurons in the SC and dLGN (Caleo et al., 2000).

Axonal Transport

It is suggested that RGC axonal transport alterations are a critical pathological component concomitant with the early increase in the IOP. Anterograde axonal transport delivers proteins, lipids, and mitochondria to the distal synapse (Fahy et al., 2016). Since neuronal proteins and molecules are predominantly synthesized in the cell body, the long axon hinders soma-derived proteins from reaching their presynaptic destinations at the axonal terminals (Chowdary et al., 2012). However, anterograde transport is the vital means of transferring newly generated synaptic proteins, ion channels, lipids, and mitochondria to their axonal destinations (Chowdary et al., 2012). Conversely, retrograde axonal transport from the axon to the soma is involved in the transport of waste substances, for instance, degraded molecules and organelles for clearance (Guo et al., 2020). This axonal transport also serves as a channel for the intracellular transport of distal chemical and biological trophic signals back to the cell body. Alongside downregulated RGC-specific genes and metabolic changes, functional and mechanical impairment of the retrograde axonal transport can be an early indicator of glaucomatous damage (Vidal-Sanz et al., 2012). The failure could result from the distortion of the elements, including defects in the cytoskeletal filaments and motor protein, which is the key to the axonal traffic machinery (Perlson and Holzbaur, 2007). It may impact the delivery of factors essential for the cell survival and the retinal function (Lu et al., 2014; Fahy et al., 2016). Indeed, this idea corroborates the neurotrophin deprivation theory as one of the mechanisms of RGC loss (Fahy et al., 2016).

The anterograde axonal transport from RGCs may occur for both the endogenous and exogenously administered BDNF (Caleo et al., 2000; Caleo et al., 2003; Butowt and von Bartheld, 2005). A fraction of BDNF transported *via* the anterograde path is newly produced by RGCs or the neighbouring retinal cells (Butowt and von Bartheld, 2001). Several attempts have been made to show that the role of BDNF secreted and delivered from RGCs in an anterograde direction along axons is to promote survival factors for post-synaptic neurons after retinal injury (Caleo et al., 2003; Dengler-Crish et al., 2014). During the development of rodents, deficits of retinal BDNF-TrkB signaling retracted the RGC axons from the dLGN and affected the inner retinal neuronal circuit development, implying that the retrograde transport blockade in the retina does not affect the retinogeniculate connectivity (Menna et al., 2003; Grishanin et al., 2008). RGCs also continuously deliver BDNF to the SC in adulthood (Avwenagha et al., 2006). In adult rats RGCs express BDNF-TrkB post-axotomy, supporting the idea that the survival of damaged RGCs may depend on the sufficient BDNF levels (Vecino et al., 2002).

The overproduction of BDNF in RGCs ensures fine-tuning of proper target innervation in the visual cortex of the brain, including the dLGN, SC, the suprachiasmatic nucleus, and the pretectum (Leinonen and Tanila, 2018). Inadequate trajectory to these target areas could be due to the insufficient endogenous retinal BDNF or perturbed axonal transport of the neurotrophin (Johnson et al., 2009). BDNF is also synthesized in the SC, the primary target of optic projections, and can be delivered to the retina retrogradely *via* RGC axons (Spalding et al., 2004). These pieces of information highlight that BDNF is excessively produced after the onset of target innervation and at the early stages of RGC development. However, adult RGCs are supported by BDNF, which is primarily produced locally (Beros et al., 2021). The enhanced retrograde transport of BDNF is triggered when the local trophic support is progressively interrupted. In theory, although local intra-retinal BDNF supplies may be delivered anterogradely for long-term survival, RGCs are eventually

considered to be dependent on the competition for limited amounts of retrograde BDNF support (Beros et al., 2021). Further studies are needed to explore this support of RGC survival by BDNF during the development and adulthood when experiencing injuries or stress.

The obstruction of BDNF delivery and accumulation of TrkB at the ONH plays a vital role in the pathogenesis of glaucoma (Pease et al., 2000). In animal models with elevated IOP, the retrograde transport of BDNF-TrkB was blocked at the ONH contributing to BDNF deficits eventually leading to gliosis and neuronal loss in the retina (Quigley et al., 2000; Gupta et al., 2014). Similar observations were made by Dekeyster et al. (2015a) in the mouse model of optic nerve crush due to blockage of the retrograde delivery of BDNF. Temporary upregulation of retinal BDNF-TrkB after injury suggests that it acts as a natural protection mechanism to overcome neurotrophin transport deficits (Gao et al., 1997; Johnson et al., 2009; Dekeyster et al., 2015a).

Interestingly, in mice, the absence of BDNF did not affect the number of RGCs in the mature retina (Chitranshi et al., 2019; Beros et al., 2021). Noteworthy, that for the target-dependent survival during the early retinal development, BDNF-TrkB signaling is not required, whereas in adult animals, it intensifies to reduce RGC degeneration in the presence of pathogenic stimuli. However, exogenous administration of BDNF to the optic tectum of developing *Xenopus* and chick improved the RGC dendritic arbor complexity (Lom and Cohen-Cory, 1999; Cohen-Cory and Lom, 2004). In fact, during the RGC development BDNF can be said to affect the RGC and optic tectum architecture differently depending on its source and transport. Despite the elegant series of studies, the interplay between retrograde and anterograde BDNF axonal transport in the human retina remains unclear. Most studies observed that BDNF differentially modulates the survival of RGCs in rodents, and there remains a need to investigate the same effects in humans.

BDNF AS A NEUROPROTECTIVE AGENT

Neuroprotection is an ideal therapeutic approach in glaucoma to keep RGCs alive (Almasieh and Levin, 2017). The goal of neuroprotection in glaucoma is to preserve the optic nerve independent of the IOP reduction and thus prevent or delay the RGC apoptosis and axonal degeneration (Tsai, 2020; Shalaby et al., 2021). Hence, any protective intervention that directly aims at promoting the health and survival of RGCs has the potential as an antiglaucoma agent. From this standpoint, BDNF seems an attractive option for further investigations.

As highlighted earlier, BDNF increases the number of receptor sites in neurons that lead to dendrite and axonal growth and stimulate neurogenesis (Miranda et al., 2019). It is required for both the development and survival of dopaminergic, GABAergic, serotonergic, and cholinergic neurons (Park and Poo, 2012). The cellular basis for learning and memory rests with the synapses within the hippocampus. The activation of the BDNF associated TrkB intracellular pathway was shown to improve cognition, which correlated with an increase in the synaptic density (Castello et al., 2014). Accordingly, the upregulation of both BDNF and TrkB was detected in the brain areas with neuronal plasticity. Because of this relationship, BDNF is considered a molecular mediator for regulating the synaptic plasticity, playing a pivotal role in memory formation and consolidation (Zeng et al., 2012). Disruption of the pathways that transport and produce BDNF can cause clinical symptoms of deteriorating memory and cognitive dysfunction (Leal et al., 2017). Clinical studies have shown a causal relationship between lower levels of BDNF and cognitive decline observed with aging, schizophrenia, and Rett syndrome (Zuccato et al., 2011; Autry and Monteggia, 2012; Soares et al., 2016).

In animal models of glaucoma, disrupted BDNF axonal transport was observed, and BDNF injection into the superior colliculus (SC) of neonatal hamsters resulted in a 13–15-fold reduction in RGC pyknosis, showing that the BDNF plays a significant role in promoting the RGC survival (Ma et al., 1998). In addition, clinical and experimental studies have shown that the BDNF/TrkB complex is downregulated in the inner retina and the optic nerve head of glaucomatous eyes (Pease et al., 2000; Gupta et al., 2014). TrkB also gets gradually downregulated in response to the neuronal damage, suggesting it may be less responsive to the BDNF levels (Shen et al., 1999). Compared to the control group, BDNF levels in the blood of primary open-angle glaucoma patients and in the tears of normal-tension glaucoma patients were significantly lower (Ghaffariyeh et al., 2011; Oddone et al., 2017). A study by Rudzinski et al. (2004) showed that changes in the retinal expression of neurotrophic factors significantly correlate with the RGC death. The same study observed a transient upregulation of both retinal NGF and TrkA receptors after 7 days of ocular hypertension (Rudzinski et al., 2004). The sustained upregulation of retinal BDNF after 28 days of ocular hypertension was also recorded. However, the expression of TrkB receptors as well as NT-3 levels remained unchanged; although, there was an early and sustained increase of TrkC receptors in Müller cells, but not in RGCs (Rudzinski et al., 2004). Thus, the asymmetric upregulation of neurotrophin and its receptor patterns may suggest that the dysregulated activity of neurotrophic factors plays a role in the RGC apoptosis.

Other studies also provide convincing evidence about the neuroprotective effects of BDNF in retina. Ma et al. (1998) showed a reduction in the RGC death after BDNF was injected into the SC (Ma et al., 1998). Studies by Pease et al. (2000) using an experimental model of glaucoma in monkeys and rats with acute IOP elevation showed that the BDNF transport in the optic nerve head was dysregulated (Pease et al., 2000). However, once BDNF was injected *via* an intravitreal injection, it reduced the RGC degeneration (Mansour-Robaey et al., 1994). Ko et al. (2001) also found that the injection of BDNF to rats with elevated IOP increased the survival rate of RGCs as compared to the untreated animals (Ko et al., 2001). Exogenous, topical, or intravitreal BDNF was found to be potent in activating the pro-survival signaling pathways in RGCs following induction of ocular hypertension in experimental animals (Ma et al., 1998; Ji et al., 2004). In

another study, recombinant human BDNF eye drops caused recovery of the pattern electroretinogram (P-ERG) and the visual cortex evoked potential (VEP) damage (Domenici et al., 2014) in the presence of chronic intraocular hypertension. As measured by the Brn3 immunopositive cell density in the RGC layer using retinal immunocytochemistry, this was linked to an increase in the RGC survival (Domenici et al., 2014). In addition, three consecutive intraocular injections of BDNF at 1.0 g/L in moderately chronic hypertensive rat eyes resulted in a 2-week increase in the RGC survival with no cumulative effect (Ko et al., 2001). High-dose BDNF, when injected intravitreally in animal models of the optic nerve injury, induced a rapid and considerable downregulation of TrkB expression, reducing the BDNF efficiency (Chen and Weber, 2004). On the other hand, it was shown that cyclic AMP (cAMP) induced neuronal sensitivity and axonal regeneration in BDNF-treated culture. Enhanced survival was associated with the increased availability of TrkB (Park et al., 2004). This allowed more TrkB to bind to BDNF on the surface of RGCs, enhancing the cell survival. By combining the treatment with forskolin, a TrkB agonist, the cAMP level gets elevated and the responsiveness of RGCs to BDNF is enhanced (Hu et al., 2010). This may imply that injured RGCs are less active and more sensitive to BDNF, which may affect their overall activity more than that of their healthy counterparts. Furthermore, studies have shown that increased BDNF/TrkB expression might harm neuronal homeostasis by increasing the glutamate excitotoxicity (Maki et al., 2015). TrkB activation has been shown to accelerate the glutamate-induced mortality in rat neuroblastoma cells (Maki et al., 2015), and higher BDNF levels have been reported in muscles of amyotrophic lateral sclerosis (ALS) patients.

Use of high-dose subcutaneous or intrathecal rhBDNF in patients with ALS did not provide neuroprotective effect (Ochs et al., 2000). As a result, several efforts have been made to target TrkB specifically with low molecular weight substances. A flavonoid-based TrkB agonist, 7,8-dihydroxyflavone (7,8 DHF) has been tested for this purpose. In an animal model of Parkinson's Disease and in an *in vitro* model of excitotoxic and oxidative stress-induced RGC apoptosis, this compound proved effective in activating TrkB downstream signaling and exerting neuroprotective effects (Jang et al., 2010; Gupta et al., 2013). Distinct TrkB agonist antibodies have enhanced the RGC survival *in vitro* and *in vivo* in acute and chronic glaucoma models (Bai et al., 2010; Hu et al., 2010). A cell-permeable phosphine–borane compound promoted the RGC protection in a rat model of optic nerve injury by stimulating the ERK1/2 pathway to directly activate the survival signaling downstream of TrkB (Almasieh et al., 2011). However, further research is needed to weigh the benefits and the drawbacks of activating BDNF/TrkB signaling pathway in the management of neurodegenerative diseases.

CURRENT STATUS

BDNF plays a role in a myriad of pathophysiologic pathways (TGF-β, MAP kinase, Rho kinase, JNK, PI-3/Akt, PTEN, Bcl-2, Caspase, Calcium-Calpain) and could serve as a promising candidate for devising therapies to enhance RGC survival in glaucoma (Chitranshi et al., 2018). Various studies targeting the BDNF-TrkB signaling pathways, primarily through topical or intravitreal applications, have shown BDNF to impede the RGC loss effectively in animal models. Indeed, NTFs have been a subject of interest for neuroprotection in the past two decades due to their pivotal roles in maintaining and enhancing neuronal survival (Poo, 2001; Mandolesi et al., 2005). Although, in the glaucomatous retina, BDNF and its receptor showed no distinct differences in the expression levels as compared to the normal retina, the treatment with topical drugs, such as prostaglandin analogs, caused an increase in the expression of BDNF and TrkB (Harper et al., 2020). The studies, however, have also demonstrated that the effect of the BDNF treatment on the RGC survival is short-lived, whereas the repeated or over-exposure decreased the responsiveness or even desensitized TrkB activation by BDNF (Klöcker et al., 1998; Dekeyster et al., 2015b).

Although, some agents have shown promising results in preclinical studies, most are not ready for application in human trials. Some of these agents such as alpha 2-agonist brimonidine (BMD) (Lafuente et al., 2002), NMDA receptor antagonist (memantine) (Weinreb et al., 2018), ciliary neurotrophic factor (CNTF) (Kauper et al., 2012), rhNGF (Gala et al., 2021) or nicotinamide (vitamin B3) (Hui et al., 2020) have advanced to comprehensive randomized controlled trials (Osborne et al., 2018; Lee et al., 2012). However, the results of some of these trials are not favourable (Kolko, 2015). For example, BMD, commonly prescribed in clinics to reduce the IOP, was found to improve the BDNF production and preserve RGCs when administered systematically to mouse and rat models of IOP-independent glaucoma (Lee et al., 2012; Lee et al., 2010; Metoki et al., 2005). The neuroprotective effect of BMD was also shown in ocular hypertensive animals and those with optic nerve injury in terms of prevention of visual defects (Kitaoka et al., 2015; Semba et al., 2014). Clinically, monotherapy with 0.2% BMD tartrate causes significantly greater reduction in the progression of visual field defects compared to 0.5% timolol maleate eye drops in a 30-months Low-Pressure Glaucoma Study group trial (ClinicalTrials.gov identifier NCT00317577) (Krupin et al., 2011). However, the report raised concerns because the progression rate in the individuals treated with timolol was worse than that in the untreated group as observed in other trials, such as the Collaborative Normal Tension Glaucoma Study, suggesting that timolol enhances the disease progression rather than BMD reducing it, or it could be a combination of the two (Group, 1998). Besides, topical administration of BMD is associated with greater side effects, including hyperemia, discomfort, and hypersensitivity, as compared to other topical anti-glaucoma medications. A selectively higher dropout rate could have skewed the results in the BMD arm as compared to the timolol arm (Storgaard et al., 2021). Unfortunately, this observation is not limited to BMD only. None of the completed double-blind clinical trials for NTF administrations have met predefined endpoints for clinical

efficacy (Shen et al., 2021). The overall progress of clinical trials demonstrated findings that could raise the uncertainty caused by ocular drug delivery challenges, thus highlighting the need to develop more relevant and appropriate clinical endpoints (Rusciano et al., 2017).

The rationale for the use of NTFs as therapeutic agents for glaucoma is their ability to promote RGC survival, regenerate axons, and increase the neuronal function and interconnectivity in such a way that their protection is not limited to preserving the remaining viable RGCs under the glaucomatous condition, but also to foster regeneration of the already lost nerve cells. For example, CNTF delivered by an encapsulated cell technology implant known as the NT-501 device is currently undergoing Phase II clinical trials against the geographic atrophy (age-related macular degeneration) and has been shown to slow down the progression of the vision loss (Zhang et al., 2011). Through this technology, the engineered retinal pigment epithelial cells with encapsulated CNTF were intravitreally implanted into the eyes to give a selective and sustained delivery of CNTF to the RGCs. More trials are conducted using the CNTF implants in POAG patients, although the outcomes are not yet published (Tian et al., 2015). Importantly, unlike CNTF, the human trials failed to show the benefit of the BDNF treatment for repairing the retinal damage. This could be because of the inability of the neurotrophic agents to cross blood ocular barriers. Hence, alternative methods of delivery to bypass the intrinsic biological barriers, should be sought.

It is reasonable to predict that BDNF will require a non-invasive delivery method in humans. Undoubtedly, the unfavourable pharmacokinetic properties (e.g., short half-life and low blood-brain/ocular barrier permeability) are the major hurdles to using BDNF-based therapies in clinical investigations (Houlton et al., 2019). Delivering BDNF to target site remains challenging due to its instability. Poor protein stability is detrimental to the therapeutic efficacy and may elicit potential immunogenic effects associated with the exposure of non-native peptide epitopes, which may act as the adjuvant for BDNF. Ultimately, the biological effects of BDNF will depend on the ability of the drug delivery system to provide a sustained and adequate drug release. With the right approach, it is possible to ameliorate functional axonal regeneration over a short period (Madduri and Gander, 2012). Furthermore, enormous challenges could arise for its human use due to a variety of genetic backgrounds, lifestyles, patterns of physical activity, and age of the patients with variable pathologies and additional medications, which may all affect the overall efficacy. Conclusively, successful NTF delivery requires dosage customization taking into account each of these factors and use of a delivery system optimal for clinical use.

ADJUVANT THERAPY TO BOOST BDNF SIGNALING

Currently, there is an ongoing effort to achieve the selective activation of BDNF-TrkB *via* the administration of exogenous BDNF or its conjugation with other molecules with a high affinity to TrkB in order to achieve target-specific delivery (Ruiz et al., 2014). One of the approach is the exogenous administration of BDNF with nanoparticles as carriers (Schmidt et al., 2018). In cats with optic nerve damage, combined intravitreal injection of other molecules together with BDNF prolonged the RGC survival (Weber et al., 2010; Weber and Harman, 2013). It is likely that targeting BDNF-TrkB pathways *via* specific upstream or downstream molecules, such as inhibition of the Shp2 phosphatase and GSK-3β activity (Kimura et al., 2016) may prove to be beneficial. To achieve greater therapeutic efficacies, it may also be necessary to combine different compounds that target multiple mechanisms of RGC loss (Konstas et al., 2020). For instance, pharmacological approaches to reduce inflammation and oxidative stress in combination with gene therapy are currently being developed (Katsu-Jiménez et al., 2016; Yin et al., 2020). Although the use of combination therapies has been recommended, there are still no reports on their clinical efficacy.

ENDOGENOUS BDNF MODULATION THROUGH STEM CELL THERAPY

Stem cell therapy is another approach with the potential to modulate BDNF signaling either by enhancing its production *via* activating multiple neuroprotective pathways or by acting as a nanocarrier. Stem cell-derived RGCs are an ideal treatment option to replace diseased or dead RGCs; however, the complexity of the retinal architecture makes the idea of the cell replacement difficult for functional repair. Alternatively, transplantation of stem cells, such as mesenchymal stem cells (MSCs), also holds a great prospect due to their capacity to secrete exosomes that can serve as extracellular vesicles encapsulating BDNF (Harrell et al., 2019). Interestingly, MSC-derived Exos (MSC-Exos) can survive in the vitreous humor for at least 4 weeks after the intravitreal injection and, because of their nanoscale dimensions, may rapidly reach RGCs to supply them with neurotrophins (Mathew et al., 2019). Accordingly, the intravitreal transplantation of MSCs that were engineered to produce and secrete BDNF at a constant and optimized level were found to preserve the functional and structural integrity of retina in a rat model of chronic ocular hypertension (Harper et al., 2011). Harrell et al. have extensively reviewed the therapeutic potential of the transplantation of MSCs-derived NTFs in glaucoma. The authors suggested that MSCs induced the production of neurotrophins and vasoactive and immunomodulatory factors, which triggered the expansion and regeneration of RGCs in animal models of glaucoma (Harrell et al., 2019). These findings suggest that the emerging role of stem-cell-based therapies as vectors for the delivery of BDNF may be beneficial for the glaucoma treatment. Exciting discoveries are underway by utilizing stem cell therapies, such as the engineered BDNF-producing cells that can be encapsulated

(Deng et al., 2016; Pollock et al., 2016) or directly grafted with various moieties (Harper et al., 2011). BDNF gene delivery through recombinant adeno-associated viruses also seems to elicit a sustained increase in the BDNF concentration in the retina and support the survival of RGCs (Osborne et al., 2018). This method seems to hold a great potential for ensuing the BDNF delivery and release to the SC, which the RGCs target. Although viral vector-induced BDNF overexpression in the SC may improve the retrograde transport, it did not improve the BDNF levels in the retina nor did it protect RGCs in glaucomatous animals (Wójcik-Gryciuk et al., 2020).

BDNF AS A DIAGNOSTIC BIOMARKER FOR GLAUCOMA

The diagnostic criteria for glaucoma have been extensively debated and specific guidelines are now followed. Currently, diagnosis largely relies on the detection of abnormal changes in the optic disc and the visual field using various tools such as fundoscopy, optical coherence tomography (OCT) and the standard automated perimetry (SAP). However, it is suggested that most of the currently used methods can detect the disease only when 30%–50% of the RGCs have been irreparably lost (Quigley et al., 1992). Nonetheless, early detection of glaucomatous damage is ideal for preventing the progressive loss of RGCs (Aquino and Aquino, 2020). Hence, it is important to look for biomarkers that can predict the onset and/or progression of disease and can be objectively measured and evaluated as an indicator of biological processes in both normal and pathological conditions (Fiedorowicz et al., 2021). For example, an investigation into the relationship between the systemic levels of BDNF and the risk for the development and/or the rate of glaucoma progression may prove beneficial in predicting its possible utility as a biomarker (Oddone et al., 2017). It would also be interesting to explore the relationship of BDNF levels with treatment outcomes and prognosis. This proposition is based on the observations that BDNF levels are considerably lower in the sera, aqueous humors, and lacrimal fluids of patients with early stages of POAG (Shpak et al., 2018). A similar correlation of BDNF levels has been observed in patients with Alzheimer disease (Karege et al., 2005; Beeri and Sonnen, 2016; Eyileten et al., 2021). Since, BDNF is also generated by some non-neural cells, it remains debatable if the source of systemically detected BDNF is in fact the neuronal tissue. Studies have, however, shown that systemic BDNF levels corresponds to BDNF levels in the brain (Mojtabavi et al., 2020). The blood BDNF concentrations across species have been extensively reviewed by Klein et al. (2011). The authors suggest that the blood and plasma BDNF levels very closely reflect BDNF levels in brain tissues. These findings not only give insight into the pathophysiology of the disease but also indicate possible use of systemic BDNF levels as a biomarker for monitoring the onset and progression of neurodegenerative diseases such as glaucoma and Alzheimer disease.

CHALLENGES AND FUTURE DIRECTION

To date, the bonafide intervention for glaucoma, whether it is a pharmaceutical or a surgical procedure, aims to slow down the progression of optic neuropathy and reduce visual field defects by lowering the IOP just enough to maintain good visual functions. Several published clinical trials have explicitly proven that the reduction of IOP could reduce the progression of the visual loss in both early and late stages of the disease. Yet, as it was reported in many cases, patients with excellent IOP readings have had worsening vision despite extensive therapy (Kim and Caprioli, 2018). Even with substantial improvements in therapeutic precision and knowledge on the disease progression, a subset of individuals with glaucoma is prone to aggressive progression, possibly owing to non-IOP-associated factors contributing to the RGC loss (Forchheimer et al., 2011). Besides that, there have been no substantial evidence of non-IOP lowering medications that could alter the glaucoma progression, and none of them could provide neuroprotection to recover the retinal and neural function in clinical trials (Lusthaus and Goldberg, 2019). An analytical review by Storgaard et al. suggests that despite several glaucoma related preclinical and clinical trials in the last 30 years, a successful translations to actual clinical use has not been achieved (Storgaard et al., 2021). The barriers to translate from preclinical into clinical practice may include heterogeneous nature of disease that is difficult to mimic fully in animal models leading to the variability in outcome measures, differences in ocular bioavailability, and the optimal timing of intervention. As opposed to therapeutic intervention after the diagnosis in human, similar interventions in animal studies are employed either before or during induction of disease process. Not to forget, human studies must account for disease variability induced by comorbidities and polypharmacy, which is generally not a component in preclinical studies. Furthermore, development of a formulation to circumvent anatomical and physiological barriers to drug permeation and allow a suitable route of administration, preferably topical, remains challenging.

CONCLUSION

RGC degeneration underlies a number of ocular disorders associated with terminal blindness, including glaucoma. Although various pathophysiological mechanisms have been described, deprivation of NTFs is a well-known factor contributing to RGC loss in glaucoma. Among the NTFs, BDNF has widely been investigated for its role in maintaining the integrity of retinal neuronal structure and function. It has a variety of roles extending from embryonic to adult life. The neuroprotective effects of BDNF have been observed by various researchers in preclinical studies; however, it application in clinical setting as monotherapy or adjuvant therapy remains to be explored. This review has highlighted various sources of BDNF, its transport mechanisms within neuronal cells and wide array of its functions. Considering the crucial role of BDNF in physiological functions, manipulations of its cellular pathways to specifically targeting the pathophysiological derangement would be the key to its

successful therapeutic application. Additionally, its possible use as a biomarker requires further investigations. Additional medications that can operate concomitantly with the current IOP-lowering medications are desperately needed. However, various forms of glaucoma may require different additional therapies, necessitating an individualized therapeutic approach that considers the patients' overall health and disease predispositions. Another critical matter is the need to create thoroughly reliable and precise screening procedures, which would enable an early detection of the neuronal injury in glaucoma. The development of clinical tools that are sensitive to retinal structure and changes in function on the scale of months instead of years will profoundly impact clinical trials by shortening their duration and fast-tracking the therapeutic development. This review suggests that BDNF is an exciting target as a biomarker. However, this vast subject still needs further investigation. Therefore, it is important that physicians remain updated on the most recent discoveries, particularly with respect to those highlighting therapeutic benefits of neuroprotective agents.

AUTHOR CONTRIBUTIONS

LL, MAML, and SA, performed literature search and drafted the manuscript. II, RA, VU, and RM supervised and revised the manuscript. All authors contributed to the manuscript and approved the submitted version.

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REVIEW
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Herb and Spices in Colorectal Cancer Prevention and Treatment: A Narrative Review

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Colorectal cancer (CRC) is the second most deadly cancer worldwide. CRC management is challenging due to late detection, high recurrence rate, and multi-drug resistance. Herbs and spices used in cooking, practised for generations, have been shown to contain CRC protective effect or even be useful as an anti-CRC adjuvant therapy when used in high doses. Herbs and spices contain many bioactive compounds and possess many beneficial health effects. The chemopreventive properties of these herbs and spices are mainly mediated by the BCL-2, K-ras, and MMP pathways, caspase activation, the extrinsic apoptotic pathway, and the regulation of ER-stress-induced apoptosis. As a safer natural alternative, these herbs and spices could be good candidates for chemopreventive or chemotherapeutic agents for CRC management because of their antiproliferative action on colorectal carcinoma cells and inhibitory activity on angiogenesis. Therefore, in this narrative review, six different spices and herbs: ginger (*Zingiber officinale* Roscoe), turmeric (*Curcuma longa* L.), garlic (*Allium sativum* L.), fenugreek (*Trigonella foenum-graecum* L.), sesame (*Sesamum indicum* L.), and flaxseed (*Linum usitatissimum* L.) used in daily cuisine were selected for this study and analyzed for their chemoprotective or chemotherapeutic roles in CRC management with underlying molecular mechanisms of actions. Initially, this study comprehensively discussed the molecular basis of CRC development, followed by culinary and traditional uses, current scientific research, and publications of selected herbs and spices on cancers. Lead compounds have been discussed comprehensively for each herb and spice, including anti-CRC phytoconstituents, antioxidant activities, anti-inflammatory properties, and finally, anti-CRC effects with treatment mechanisms. Future possible works have been suggested where applicable.

Keywords: biomolecules, colon cancer, drug resistance, functional foods, management, nutraceuticals, prevalence

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1 INTRODUCTION

1.1 Colorectal Cancer

Cancer is a leading cause of death that significantly affects the life expectancy of every nation. Colorectal cancer (CRC) is the third most commonly diagnosed and second most deadly cancer globally (WHO, 2021) that accounting for approximately 9.39% of death of all recorded cancers in 2020 (Ferlay et al., 2020; Hossain et al., 2022). CRC incidence is expected to double by 2035 worldwide due to the fast acceleration of diagnosed cases in the elderly. Less developed countries are expected to rise in diagnosed cases of CRC (Papamichael et al., 2015; Hossain et al., 2022).

The term CRC is specific to the large intestine and rectum, where it develops from the abnormal growth of glandular epithelial cells. This development occurs when epithelial cells acquire a succession of genetic or epigenetic alterations that provide them with a selective advantage of hyper-proliferation (Testa et al., 2018). These out of control growing cells produce a benign adenoma, which then progresses to carcinoma and metastasis by three major pathways: microsatellite instability (MSI), chromosomal instability (CIN), and CpG island methylator phenotype (CIMP) (Vogelstein et al., 1988; Nguyen and Duong, 2018; Malki et al., 2020). Like any other tumour or cancer, CRC is classified into stages: Stage 0 (carcinoma *in situ*) to stage IV. Standard treatment options for the stages 0 –II CRC are surgery, whereas stage III requires surgery and adjuvant chemotherapy, and stage IV and recurrence CRC involve surgery, chemotherapy, and targeted therapy (PDQ, 2021).

1.2 Colorectal Cancer Treatment Opportunity and Challenges

Regular screening can prevent CRC. As a polyp takes 10–15 years to be cancerous, detecting and removing polyps at an early stage is critical. However, only 40% of CRC are found at early stages, and sometimes CRC recurs after treatment (ACS, 2020). For CRC treatment strategy, Food and Drug Administration (FDA) approved at least 30 different drugs (**Supplementary Table S1**), and either singly or in combination with other drugs (**Supplementary Table S2**) are used for CRC treatment. These chemotherapeutic drugs are exposed to the cancer cells and simultaneously damage healthy cells. Consequently, these drugs manifest several adverse effects, including fatigue, headache, muscle pain, stomach pain, diarrhoea and vomiting, sore throat, blood abnormalities, constipation, neuronal damage, skin changes, memory problems, loss of appetite, and hair loss (ACS, 2020).

Even though the overall survival of individuals with advanced CRC has increased in recent decades due to new chemotherapy regimens (**Supplementary Tables S1, S2**); however, in nearly all patients with CRC, current systemic chemotherapies developed resistance (Cho and Hu, 2020), limiting the therapeutic efficacy of anti-cancer medicines and ultimately leading to chemotherapy failure. Chemotherapeutic drug resistance is a major issue in CRC treatment in the current clinical practice. Apart from this limitation, the access to diagnosis and treatment of CRC for survival is less accessible in developing countries, particularly for rural people, where about 44% of the world's people currently live (World Bank, 2021). Therefore, nearly half the world's population lacks the means to diagnose and treat.

1.3 Natural Remedies for Colorectal Cancer

According to World Health Organization (WHO), 75–80% of the world's population solely rely on traditional medical systems for their first line of treatment due to concerns about the safety and efficacy of synthetic drugs (Hossain et al., 2014; Urbi and Zainuddin, 2015; Hossain and Urbi, 2016; Hossain M. S. et al., 2021). On the contrary, for being comparatively safe, natural products have gained tremendous importance as sources of polypharmacological drugs for infectious diseases, cancers, and neurological disorders (Hossain S. et al., 2021; Farooq et al., 2021). Moreover, indications of the importance of plants for diverse ailments in religious scripts attracted more researchers focusing on evaluating the scientific validity of traditional claims (Hossain et al., 2014; Hossain, 2016; Hossain et al., 2016). So, finding safer alternatives to systematic chemotherapeutic drugs from natural sources are an important and worthy study.

Herbs and spices have been commonly used as condiments to enrich aroma, taste, and colour for thousands of years. Even though they are consumed in small amounts, these herbs and spices contain many bioactive compounds and beneficial health effects. The role of spices and herbs in the inhibition of CRC cells growth has been reported in many recent studies (Zheng et al., 2016; Jaksevicius et al., 2017; Aasim et al., 2018; DeLuca et al., 2018; Khor et al., 2018; Wani and Kumar, 2018; Aiello et al., 2019; Rajasekaran, 2019; Buhrmann et al., 2020; Dandawate et al., 2020; Ganesan et al., 2020; Hallajzadeh et al., 2020; Hu et al., 2020; Malmir et al., 2020; Martínez-Aledo et al., 2020;; Pricci et al., 2020; Badsha et al., 2021; Kammath et al., 2021; Karthika et al., 2021). There is increasing evidence of preventing CRC by consuming fruits and vegetables, while red meat enhances the risk factors (Hallajzadeh et al., 2020). Similarly, dietary fibre was contradictory until recent findings showed that high dietary fibre could prevent cancers, including CRC (DeLuca et al., 2018; Masrul and Nindrea, 2019; O'Keefe, 2019). For example, consumption of one to three tablespoons of ground flaxseeds (FS) per day (8–24 g/day) has been suggested as part of a healthy eating pattern that works as a chemotherapeutic agent against CRC development (DeLuca et al., 2018).

Since many disease conditions, including CRC, commonly treated with culinary herbs and spices in traditional medical systems, are considered self-limiting, their purported benefits need critical evaluation intended for CRC management. This would be a worthy study for the community, particularly clinical practitioners and CRC patients. Therefore, in this study, six commonly used herbs and spices: ginger (*Zingiber officinale* Roscoe), turmeric (*Curcuma longa* L.), garlic (*Allium sativum* L.), fenugreek (*Trigonella foenum-graecum* L.), sesame (*Sesamum indicum* L.), and flaxseed (*Linum usitatissimum* L.) were chosen to evaluate their chemoprotective chemotherapeutic roles in CRC management critically and explored the possibility of developing these agents as anti-CRC pharmaceuticals. Scientific evaluation of these plants against cancers, particularly CRC, is increased over

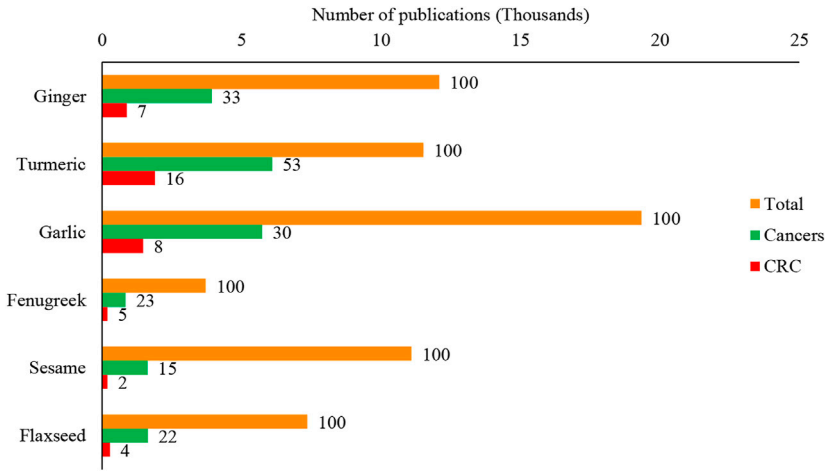

FIGURE 1 | Scientific evaluation of culinary spices and herbs for the different research, including cancers and colorectal cancer. The value top on the bar represents the per cent (%) value of the total publications of each plant. The data were retrieved from the Scopus database on 15 August 2021 using the search keywords: Ginger OR *Zingiber officinale* Roscoe, Turmeric OR *Curcuma longa* L., Garlic OR *Allium sativum* L., Fenugreek OR *Trigonella foenum-graecum* L., Sesame OR *Sesamum indicum* L., Flaxseed OR *Linum usitatissimum* L., Cancer*, "Colorectal cancer" OR "Colon cancer". CRC: Colorectal cancer.

the last few years, and turmeric is the most extensively used spice evaluated for CRC (**Figure 1**).

Initially, this study comprehensively discussed the molecular basis of CRC development, followed by culinary and traditional uses, current scientific research, and publications of selected herbs and spices on cancers and their role in CRC management with underlying molecular mechanisms of action. Lead compounds have been discussed comprehensively for each herb and spice, including anti-CRC phytoconstituents, antioxidant activities, anti-inflammatory properties, and finally, anti-CRC effects with treatment mechanisms. Future possible works have been suggested where applicable.

2 MOLECULAR BASIS OF COLORECTAL CANCER

Cancer is a genetic illness caused by oncogene activation, tumour suppressor gene dysfunction, or environmental mutagenesis (Imran et al., 2017). The effective control of cancer solely lies in a better understanding of its pathophysiology, and significant progress has been achieved in comprehending the molecular basis of cancer. Genetic and epigenetic changes play a role in the onset of neoplastic transformation of the healthy epithelium into malignant phases (Gorga, 1998). Progression of CRC is mainly involved with the silencing of tumour suppressor genes and activation of an oncogene (Malki et al., 2020).

As mentioned earlier, both genetic and epigenetic changes in the key genes are responsible for CRC development. This alteration is involved with three major pathways: CIN, MSI, and CIMP pathways (Pino and Chung, 2010). The CIN route is responsible for most CRC cases. This pathway is characterized by widespread abnormalities in chromosomal number (aneuploidy) and loss of heterozygosity. It can be caused by errors in chromosome segregation, telomere stability, or the DNA damage response, though the genes involved are currently unknown (Pino and Chung, 2010). Fearon and Vogelstein described the first multistep genetic model of colorectal tumorigenesis in 1990 (Fearon and Vogelstein, 1990). Later in 2010, Pino and Chung (Pino and Chung, 2010) critically evaluated the CIN pathway of CRC and discussed the role of each gene involved with CRC progression.

According to the model proposed, the formation of aberrant crypt foci (ACF) is the initial step of CRC progression. Inactivating the *adenomatous polyposis coli* (*APC*) tumour suppression gene through the mutations can activate the Wnt signalling pathway at this stage. Subsequently, activating mutations in the proto-oncogene *KRAS*, mutations in the tumour suppressor gene *TP53*, as well as loss of heterozygosity at chromosome 18q are required for progression to larger adenomas and early carcinomas. In a tiny fraction of colorectal tumours, mutational activation of the *PIK3CA* gene occurs late in the adenoma-carcinoma sequence. Consistent with the evolution of adenomas that are not malignant, CIN is detected in benign adenomas and increases in tandem with tumour progression (**Figure 2**).

Nuclear Factor-kappa B (NF-κB) is a ubiquitous transcription factor regulating gene expression of inflammatory and immunological cytokines, cytokine receptors, and adhesion molecules in various cell signalling pathways. NF-κB activation also affects the control of apoptotic pathways, cell proliferation, differentiation, migration, angiogenesis, and tumour cell resistance to chemo/radiotherapy (Soleimani et al., 2020). NF-κB binds to an inhibitor, I-kappa B (IκB), present in the cytoplasm of most the quiescent cells and inactivates NF-κB by covering the nuclear localization sequence, blocking DNA binding and nuclear uptake of NF-κB (Baeuerle and Henkel, 1994). However, extracellular stimuli such as oncogenic molecules, and chemo/radiotherapy, cell surface receptors, including tumour necrosis factor receptors, interact with their

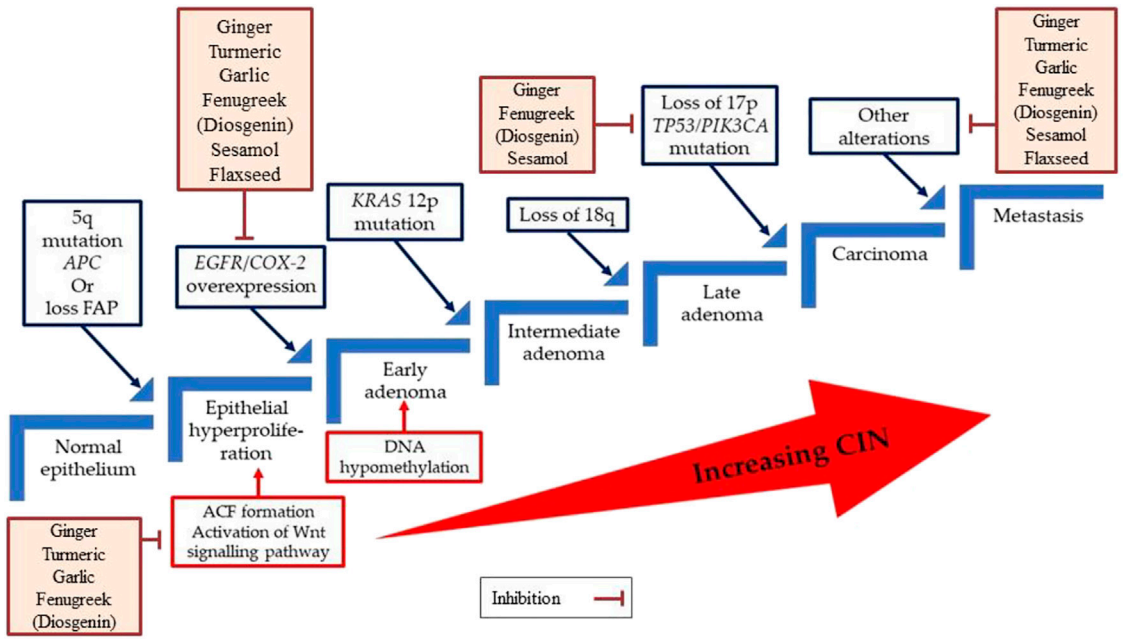

FIGURE 2 | A multistep genetic model of colorectal carcinogenesis sequence. ACF, Aberrant crypt foci; APC, Adenomatous polyposis coli, CIN, Chromosomal instability, COX-2, Cyclooxygenase-2; EGFR, Epidermal growth factor receptor; FAP, Familial adenomatous polyposis.

specific ligands to cause an upregulation of the IκB kinase complex, which triggers down-stream genes expression that potentially promotes inflammation and cancer initiation/progression (Soleimani et al., 2020). In addition, active NF-κB in tumours with wild type *Kirsten Rat Sarcoma Virus* (*KRAS*) and *KRAS* mutations increased the activity of NF-κB signalling in patients with *KRAS* mutations, and patients exhibited a lower survival and weaker response to first-line treatment compared to other cases (Lin et al., 2012a; Lin et al., 2012b). Therefore, the NF-κB signalling pathway plays a vital role in accelerating cell proliferation, cell survival, and inhibition of apoptosis.

The well-established molecular basis of cancer helps determine confirmatory biomarkers that can improve clinical outcomes in patients with CRC and increase the survival of patients with metastatic cancer. Chemopreventive or chemotherapeutic agents target those biomarkers for the best outcomes to control CRC. In other words, identified biomarkers, such as *KRAS and TP53* genes, can be targeted to prevent or control CRC as inhibition of the *KRAS* gene or activation of the TP53 gene modulate the normal function of cells; thus, cancerous cells cannot sustain growth. Additionally, inhibition of the NF-κB signalling cascade limits cell proliferation; therefore, targeting this cascade may lead to preventive measures, and novel treatment approaches against CRC.

In our current review, we have annexed six culinary herbs that have strong vigour to inhibit the adenomas cell, carcinomas cell, and even several cancerous cell lines such as colorectal cancer, breast cancer, and prostate cancer (Matsuura et al., 2006). Ginger and its components may operate as chemopreventive agents by lowering COX-2 expression, according to *in vitro* and animal studies (Kim et al., 2005; Citronberg et al., 2013)(Kim et al., 2005; Citronberg et al., 2013). Gingerol works by activating key cell-signalling regulators and pathways such as Bax/Bcl2, p38/MAPK, Nrf2, p65/NF-B, TNF-, ERK1/2, SAPK/JNK, ROS/NF-B/COX-2, caspases-3, -9, and p53 (Wee et al., 2015; de Lima et al., 2018). Turmeric extract suppresses metastasis by regulating several targets, including molecules involved in the Wnt and Src pathways, EMT, and EGFR-related pathways. It also restricts FAK/Src, STAT3, Erk, and Akt pathways suppressing cell proliferation, motility, and migration (Li et al., 2018; Li et al., 2021). Garlic and its constituents suppress tumour biomarker aberrant crypt foci (ACF), NF-κB, anti-apoptotic genes (Bcl-2, cIAP1/2, and XIAP), and inflammatory genes (iNOS and COX-2), and EGFR, whereas it induces apoptotic gene expression (Ban et al., 2007; Ngo et al., 2007; Saud et al., 2016; Mondal et al., 2022). A few studies found a significant reduction of ACF with 1% fenugreek or 0.1% or 0.05% diosgenin, which is also implicated in the suppression of COX-2 as well as the stimulation of nuclear factor-B, p53, and p21 expression (Moalic et al., 2001; Raju et al., 2004). Sesamol (100 μM) inhibits the expression of COX-2 and cytosolic prostaglandin E2 synthase mRNA in polyp sections and targets the p53, MAPK, JNK, PI3K/AKT, TNFα, NF-κB, PPARγ, caspase-3, Nrf2, eNOS, and LOX signalling pathways (Shimizu et al., 2014; Majdalawieh and Mansour, 2019). Flaxseed meal elevates the mitochondrial apoptosis genes such as p53 and cyclin-dependent kinase inhibitor 1A (p21) as well as cell cycle arrest genes (Hernández-Salazar et al., 2013). Furthermore, the COX-1 and COX-2 protein level in the colonic tissue is considerably reduced (Bommareddy et al., 2006).

3 Correlation of Oxidative Stress, Inflammation, and Carcinogenesis

Reactive oxygen species (ROS) are produced after exposure to different physical agents, including ultraviolet rays and heat, as

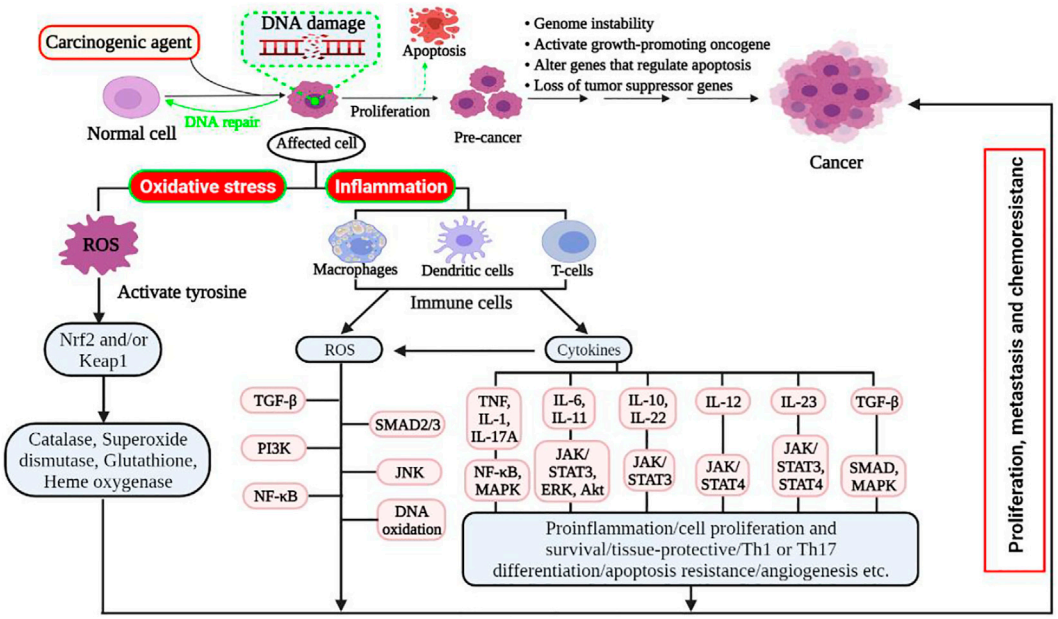

FIGURE 3 | Schematic mechanism of oxidative stress and inflammation-induced cancer development. Damaged cells or tissues produce ROS resulting in oxidative stress and/or inflammation. Oxidative stress: regenerated ROS can be detoxified with the presence of balanced detoxifying agents, such as antioxidants. However, excessive ROS induces apoptotic signalling pathways and promotes carcinogenesis in cells with faulty signalling by deregulating biomolecules. Hence, it targets *Nrf2* and its regulator *Keap1* and downregulates antioxidant enzymes that result in high intracellular ROS levels, which induce cell proliferation, metastasis, and chemoresistance by rescuing *Nrf2* transcription. Inflammation: various diseases and stress conditions causes inflammatory cell infiltration that induces ROS and different cytokines. The elevated ROS activates latent TGF-complex, which binds to its receptor and activates signalling pathways such SMAD2/3, PI3K, and JNK. It also activated tyrosine kinase that allowed NF-κB (active form) to enter the nucleus, further activating target genes for chemokines, cytokines, adhesion molecules, and receptors to cause cell proliferation, growth, and differentiation. Akt, protein kinase B; Erk, extracellular signal-regulated kinase; IL, interleukin; JNK, c-Jun N-terminal kinase; Keap1, kelch-like ECH (enoyl-CoA hydratase)-associated protein 1; MAPK, mitogen-activated protein kinase; Nrf2, nuclear-related factor 2; NF-κB, nuclear factor-kappa B; Pi3K, Pi3 kinase; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; SUZ12, suppressor of zeste 12; TGF, transforming growth factor; TNF, tumour necrosis factor.

well as after chemotherapy and radiotherapy in cancer treatment. Over the past few decades, researchers have realized that ROS plays a significant role in the aetiology of various diseases, including cancer, cardiovascular disease, and inflammation injury. Excessive production of ROS in cellular life needs to be regulated tightly. Because ROS have the potential to initiate the degenerative process in cells; however, living organisms have several antioxidant systems that scavenge the adverse effects of ROS on cells (Karker et al., 2016). These drastic impacts on cells include oxidative stress, damaging biomolecules, including DNA, lipid oxidation, protein degradation, and cellular apoptosis (**Figure 3**) (Valko et al., 2007).

ROS are by-products of regular cellular metabolism that play crucial roles in activating signalling pathways in cellular life (Perillo et al., 2020). When cells or tissues are exposed to prolonged environmental stress, ROS are created over an extended period, resulting in irreversible damage to cell structure and function, as well as the induction of somatic mutations and neoplastic transformation (Khandrika et al., 2009). Indeed, oxidative stress is associated with cancer onset and development, either by increasing DNA mutations or generating DNA damage, genome instability, and cell proliferation (**Figure 3**) (Fang et al., 2009; Visconti and Grieco, 2009). Additionally, proteins and lipids are also key oxidative targets, and altering these molecules increases the risk of mutagenesis (Schraufstätter et al., 1988). The adverse effects of ROS can be tightly controlled through a sophisticated enzymatic antioxidant system [e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, and catalase] (Soraya and Mozafar, 2018).

Chronic inflammation is caused by various biological, pharmacological, and physical factors, and it has been linked to an elevated risk of numerous types of cancer in humans, including CRC. Epidemiological and experimental evidence suggests that oncological illnesses like cancer have been linked to this inflammation (Reuter et al., 2010). This inflammation is now regarded as a "secret killer" for diseases such as cancer. For example, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis are associated with an increased risk of colon adenocarcinoma, and chronic pancreatitis is related to an increased rate of pancreatic cancer (Reuter et al., 2010).

An elevated ROS is produced in the inflammatory cells due to increased oxygen absorption in the damaged area. As a result of the increased ROS, the latent TGF-complex is activated, which binds to its receptor and activates different signalling pathways, such as SMAD2/3, PI3K, MAPK/AP-1, and JNK. (Coussens and Werb, 2002). In addition, inflammatory cells also generate soluble mediators, such as cytokines and chemokines, which persuade

TABLE 1 | Traditional uses of herb and spice along with their scientific name, family, culinary uses, part used, and lead compounds.

Common name	Scientific name	Family	Type	Culinary use	Part used	Lead compound(s)	Traditional uses	References
Ginger	*Zingiber officinale* Roscoe	Zingiberaceae	Spice	Use for pungent flavour and taste in foods and beverages	Rhizomes, leaves	Gingerols, paradols, shogaols, quercetin	Common cold, digestive disorders, rheumatism, neuralgia, colic and motion sickness, migraines, hypertension, abdominal distension, dropsy, cancer, and diabetes	Mascolo et al. (1989), Surh et al. (1998)
Turmeric	*Curcuma longa* L.	Zingiberaceae	Spice	Used for a specific flavour and yellow colour	Rhizomes	Curcumin, calebin A	Rheumatoid arthritis, chronic anterior uveitis, conjunctivitis, skin cancer, smallpox, chickenpox, wound healing, urinary tract infections, liver ailments, digestive disorders; to reduce flatus, jaundice, menstrual difficulties, colic, abdominal pain and distension, and dyspeptic conditions	Dixit et al. (1988), Bundy et al. (2004), Prasad and Aggarwal, (2011)
Garlic	*Allium sativum* L.	Amaryllidaceae	Spice	Used for pungent flavour as a seasoning or condiment.	Bulb, leaves, flower	Diallyl sulfide, diallyl disulfide, diallyl trisulfide, diallyl tetra-sulfide, S-allyl mercaptocysteine, allicin, selenomethionine and se-methyl-L-selenocysteine	Typhus, dysentery, cholera, influenza maintain and increase their strength, abnormal growths, circulatory ailments, general malaise and infestations with insects and parasites, alleviation of joint disease and seizures	Rivlin, (2001), Ayaz and Alpsoy, (2007), Petrovska and Cekovska, (2010)
Fenugreek	*Trigonella foenum-graecum* L.	Fabaceae	Spice	Used as leafy vegetables and seasonings	Seed, leaves	Diosgenin	Menstrual pains, sedating tummy, boost physique, to treat weakness of body, gout, breast milk stimulant, tonic, digestive and respiratory problems, and ease childbirth	Yoshikawa et al. (1997), Bahmani et al. (2016), Wani and Kumar, (2018)
Sesame	*Sesamum indicum* L.	Pedaliaceae	Herb		Seed	Sesamol	Benefits the liver, kidney, spleen, and stomach, lubricates the intestines, nourishes all the internal viscera, blackens the hair, kills intestinal worms such as Ascaris, tapeworm	Anilakumar et al. (2010), Pathak et al. (2014)
Flaxseeds	*Linum usitatissimum* L.	Linaceae	Herb	Used as a featured ingredient in cereals, pasta, whole-grain bread and crackers, energy bars, meatless meal products, and snack foods	Seed, leaves	Linolenic acid, lignans, p-coumaric and ferulic acid	Dyspnoea, asthma, dysphonia, bad cough, bronchitis, constipation, pulmonary tuberculosis, hemoptysis, splenomegaly, and stomach ulcer	Goyal et al. (2014)

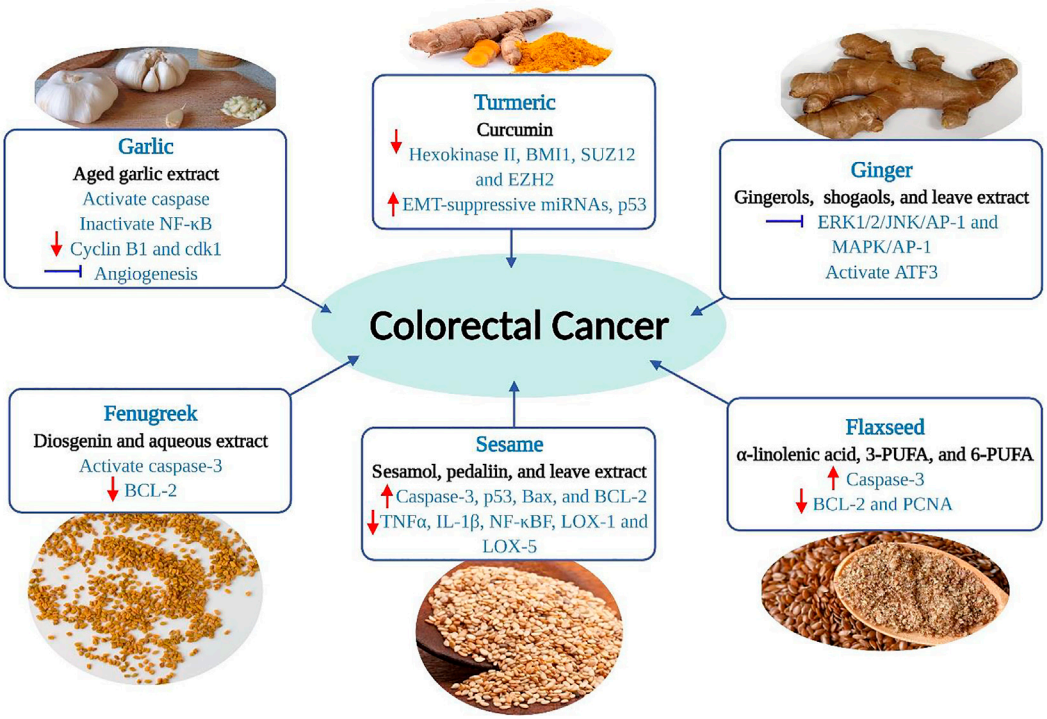

FIGURE 4 | Chemopreventive effects of selected herbs and spices against colorectal cancer by targeting diverse mechanisms of action. The red arrow (up) denotes stimulation or up-regulation, the red arrow (down) denotes down-regulation, and a bar perpendicular to the end of the line (blue) denotes inhibition. AP-1, activator protein 1; ATF3, activating transcription factor 3; Bax, Bcl-2 associated X protein; BCL-2, B-cell lymphoma-2; cdk1, cyclin-dependent kinase 1; BMI1, B cell-specific Moloney murine leukemia virus integration site 1; COX-2, cyclooxygenase 2; EMT, epithelial-to-mesenchymal transition; Erk, extracellular signal-regulated kinase; EZH2, enhancer of zeste homolog 2; JNK, c-Jun N-terminal kinase; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; p53, tumor protein p53; PCNA, proliferating cell nuclear antigen; PUFA, polyunsaturated fatty acids; SUZ12, suppressor of zeste 12; TNF, tumor necrosis factor.

changes in transcription factors and can trigger different signal transduction cascades, including NF-κB, STAT3, and activator protein-1 (AP-1), Nrf2 (**Figure 3**). The aberrant expression of inflammatory cytokines like TNF, different interleukins (*i.e.*, IL-1, IL-6, IL-10, IL-11, IL-12, IL-22, IL-23), and chemokine IL-8 have also been reported to play a pivotal role in oxidative stress-induced inflammation (Kanda et al., 2017). Therefore, this sustained inflammatory/oxidative stress leads to damage to neighbouring healthy epithelial and stromal cells, and prolonged time may lead to carcinogenesis because of genome instability, activate of the growth-promoting oncogene, alteration of genes that regulate apoptosis, and loss of tumour suppressor genes (Federico et al., 2007; Liu et al., 2021).

4 THERAPEUTIC POTENTIAL OF CULINARY HERB AND SPICE FOR COLORECTAL CANCER MANAGEMENT

Food is consumed either raw or cooked to provide energy and nutritional support for an organism. In addition to enhancing taste, aroma, and colour, herbs and spices also provide nutritional value. Apart from their culinary uses, ginger, turmeric, garlic, fenugreek, sesame, and flaxseeds are traditionally used for different ailments, including cancers (**Table 1**).

These plants are widely investigated for the scientific validity of health benefits or traditional uses, particularly their anti-cancerous role. The extracts or compounds that possess antioxidants showed potential anti-cancerous effects. Antioxidants are substances that, when present in low concentrations compared to the substrate, prevent or delay the oxidation of the substrate. On the other hand, the substrate would otherwise be oxidized by the pro-oxidants. Different parts, plant extracts, and isolated compounds of the selected herbs and spices have potential antioxidant properties that show anti-inflammatory and anti-cancer effects. An overview of their chemopreventive or chemotherapeutic role in CRC management by targeting diverse mechanisms of action is shown in **Figure 4**.

4.1 Ginger

The botanical name of ginger is *Zingiber officinale* Roscoe, which belongs to the family Zingiberaceae. It is a herbaceous perennial flowering plant that originated in Southeast Asia. It is one of the most consumed dietary condiments globally and is now produced worldwide, including in Bangladesh, India, China, Nigeria, Nepal, Indonesia, and Japan (Surh, 1999). The rhizome, the horizontal stem from which the roots grow, is the central portion of ginger that is widely used and consumed in numerous forms, such as fresh, dried, pickled, preserved, crystallized, candied, powdered or ground (Bode and Dong, 2011).

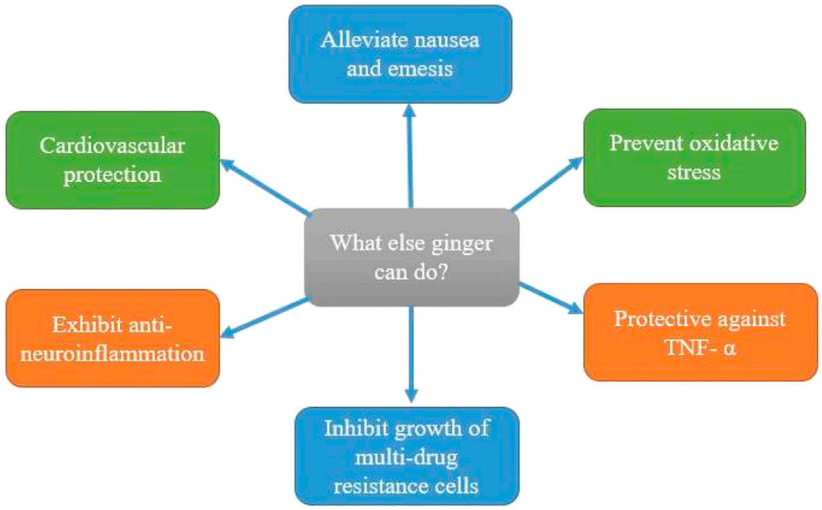

FIGURE 5 | Health benefits and anticancer properties of ginger.

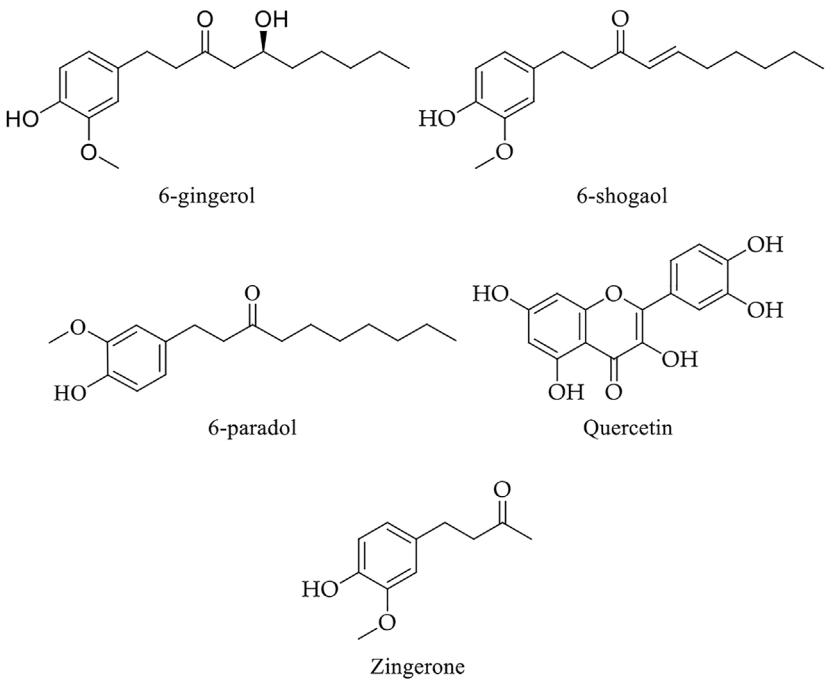

FIGURE 6 | Chemical structure of main non-volatile bioactive compounds of ginger.

Ginger is an excellent source of antioxidants used to treat ailments from colds to cancer (Bode and Dong, 2011). The popularity of ginger for scientific research has surged in recent years. As of 15 August 2021 (Scopus database), approximately 12,092 papers with a focus on the beneficial effects of ginger have been published between 1853 and 2021. The papers focused on only cancer is 33%, while 7% focused mainly on CRC (**Figure 1**). This plant has many other health benefits related to cancers (**Figure 5**).

4.1.1 Lead Compounds of Ginger

Ginger has at least 115 compounds (volatile and non-volatile) in fresh and dried rhizomes identified in different extracts. Among these, gingerols, parasols, shogaols, and quercetin are the most common constituents and exert various powerful therapeutic and preventive effects (Bode and Dong, 2011). Examples of volatile components are hydrocarbons, zingiberene, sequiphellandrene, α-curcumin, and other sesquiterpenes, while non-volatile

TABLE 2 | An overview of cytotoxic effects of culinary herbs and spices on colorectal cancer.

Name	Extract/ compound	Cell line	Cellular effect	Mechanism	References
Ginger	Gingerols, shogaols, and leave extract	HCT116, SW480, LoVo	Inhibit cell proliferation and induce apoptosis in CRC, but not in normal colorectal cells Inhibit the growth of cells and induce apoptosis Prevent PMA-induced proliferation in CRC	Inhibit ERK1/2/JNK/AP-1 pathway Activate ATF3 promoter and increase ATF3 expression Inhibit MAPK/AP-1 signalling	Fu et al. (2014), Park et al. (2014), Radhakrishnan et al. (2014)
Turmeric	Curcumin extract	HT29 HCT 116 Colon 26-M01	Inhibit production of mucosal concentrations of pro-carcinogenic eicosanoids 5-HETE and PGE-2 PGE-2 could reverse induced apoptosis Inhibit the growth of hCAC Curcumin+5-FU enhance cellular apoptosis and inhibit proliferation in 5-FU resistant cells Decrease cell motility and migration	G0/G1 phase arrest, down-regulation of cell cycle progression Down-regulate expression of hexokinase II Induced dissociation of hexokinase II from the mitochondria led to mitochondrial-mediated apoptosis Upregulate EMT-suppressive miRNAs in 5-FU resistant cells Down-regulate BMI1, SUZ12, and EZH2 transcripts Upregulating p53 molecule expression Multiple signalling pathways such as AKT, Erk, and STAT3 inhibit colony formation in murine colorectal cancer cells	Carroll et al. (2011), Manikandan et al. (2012), Shehzad et al. (2014), Guo et al. (2015), Toden et al. (2015), Wang et al. (2015), Rajitha et al. (2016), Li et al. (2018)
Garlic	AGE Aged garlic extract	DLD-1, Colo 205, HT29, SW480, SW620	Decrease ACF Showed a lower number of adenoma and adenocarcinoma lesions Suppressed the proliferative activity in adenoma and adenocarcinoma lesions but showed no effect on normal colon mucosa Regulate ER-stress induce apoptosis (80% apoptosis) Inhibits angiogenesis and proliferation	Caspase activation Inactivation of NF-κB Delayed cell cycle progression by downregulating cyclin B1 and cdk1 expression *via* inactivation of NF-κB Prevent tumour formation by inhibiting angiogenesis through the suppression of endothelial cell motility, proliferation, and tube formation Increase cellular adhesion to collagen and fibronectin, and inhibit angiogenesis in the colorectal cancer cell.	Matsuura et al. (2006), Jikihara et al. (2015), Tung et al., 2015)
Fenugreek	Diosgenin and aqueous extract	HT29	Induce apoptosis Inhibit the production of AOM and induce ACF Reduce LPO and increase GPx, GST, SOD	Suppress BCL-2 and activate caspase-3 protein expression	Raju et al. (2004), Sushma and Devasena, (2010)
Sesame	Sesamol, pedaliin, and leave extract	HT29, HCT116	Induce apoptosis Induce G0/G1 and S-phase cell cycle arrest	Suppress TNFα and IL-1β expression, NF-κB signalling, and LOX-1 and 5-LOX activity Modulate caspase-3, p53, Bax, and BCL-2 expression	Gupta et al. (2009), Chu et al. (2010a), Chu et al. (2010b), Wu et al. (2015), Kim et al. (2021)
Flaxseed	α-linolenic acid, 3-PUFA, and 6-PUFA Extract (Oil)	CaCo-2, SW480, Colo 201 LoVo RKO	Inhibit cell proliferation and induce apoptosis Induce S-phase cell cycle arrest, elevate cyclin A protein levels, and increase the proportion of apoptotic cells Mitochondrial disfunction and trend to apoptosis	Upregulate Caspase-3 Down-regulate BCL-2 and PCNA protein Augmenting ROS production, accumulating intracellular ca^{2+} decreasing mitochondrial membrane potential and production of ATP	Danbara et al. (2005), Bommareddy et al. (2010), Chamberland and Moon, (2015), Zhang et al. (2015)

5-HETE, 5-hydroxyeicosatetraenoic acid; AGE, Aged garlic extract, cdk1, Cyclin-dependent kinase 1; PGE-2, Prostaglandin E-2; hCAC, Human colon adenocarcinoma cell lines; EMT, Epithelial-mesenchymal transition; AOM, azoxymethane; ACF, aberrant crypt foci; LPO, Plasma lipid peroxides; GPx, Glutathione peroxidase, GST, Glutathione S-transferase; SOD, Superoxide dismutase; NF-κB, Nuclear Factor-kappa B; PCNA, proliferation cell nuclear antigen; PUFA, Polyunsaturated fatty acid.

pungent phenolic compounds are used 6-gingerol, 6-shogaol, 6-paradol, quercetin, and quercetin zingerone (**Figure 6**) (Vedashree et al., 2020). The non-volatile compounds exert chemopreventive and therapeutic efficacy (**Figure 4** and **Table 2**) (Fu et al., 2014; Park et al., 2014; Radhakrishnan et al., 2014).

4.1.2 Antioxidant Activity of Ginger

Ginger extracts, powder, and constituents have shown potential antioxidant activity *in vitro* and *in vivo* models (Kikuzaki and Nakatani, 1993; Stoilova et al., 2007; Wang et al., 2018; Tanweer et al., 2020; Naliato et al., 2021). These studies showed that solvent has significant effects on the effectiveness of antioxidant

properties. Aqueous ethanolic solution (0.02%) showed high antioxidant activity (Kikuzaki and Nakatani, 1993). The solution was prepared from different extracts of ginger like dichloromethane, methanol, and α-tocopherol. The dichloromethane extract exhibited higher activity than α-tocopherol and methanol extract. The ginger extract inhibited the hydroxyl radicals 79.6% at 37°C and 74.8% at 80°C, which showed higher antioxidant activity than quercetin and chelated Fe^{3+} in the solution (Stoilova et al., 2007). Gingerol related compounds substituted with an alkyl group bearing 10-, 12- or 14-carbon chain length might contribute to both radical scavenging effect and inhibitory effect of autoxidation of oils; however, there was no significant difference in the activity among the compounds with different alkyl chain length (Masuda et al., 2004). These results suggested that the antioxidant action may be attributed to radical scavenging and substrate affinity. Ginger bioactive compounds can also stimulate a plethora of enzymes, such as glutathione reductase, glutathione S–transferase, and glutathione peroxidase, that help mitigate free radicals that induce oxidative stress startlingly suppress colon carcinogenesis (Manju and Nalini, 2005). Consumption of ginger extracts may reduce or delay the progression of diseases that oxidative stress occurs due to a lack of antioxidant supplementation (Tohma et al., 2017) because ginger cakes or bread showed high antioxidants activity by scavenging peroxyl radicals (Martinez-Villaluenga et al., 2009; Balestra et al., 2011; Ademosun et al., 2021). Therefore, it can assume that ginger-based bakeries or beverages would be effective functional dietary products in managing and preventing cancers.

4.1.3 Anti-Inflammatory Effects of Ginger

Phytocompounds isolated from ginger, such as gingerol and shogaol, can suppress the synthesis of pro-inflammatory cytokines such as IL-1, IL-8, and TNF- α. (Tjendraputra et al., 2001). An NF-κB signalling pathway is linked with chronic inflammatory diseases like cancer, allergy, myocardial infarction, asthma, arthritis, multiple sclerosis, and atherosclerosis. Habib et al. (2008) demonstrated that the ginger extract has the magnificent potential to lessen the expression of the NF-κB signalling pathway. Cyclooxygenases (*i.e.*, COX-1, COX-2) enhance prostaglandin-mediated inflammation. Gingerol impedes COX-2 expression induced by lipopolysaccharides (LPS) (Lantz et al., 2007). A double-blind, placebo-controlled, randomized experiment reported that daily consumption of raw and heat-treated ginger (2 g) for 11 consecutive days resulted in moderate-to-large reductions in muscle pain compared to the placebo (Black et al., 2010). In LPS induced inflammation, one of the lead compounds of ginger, [6]-shogaol reduced the levels of nitric oxide synthases (iNOS), COX-2, and phospho-NF-kB, suppressed histone deacetylase-1 (HDAC-1) expression, and increased histone H3 acetylation expression. [6]-Shogaol can inhibit HDAC-1 expression, comparable to that of commonly used HDAC inhibitors Trichostatin A and MS275 (Shim et al., 2011). This result indicates that a ginger supplement rich with [6]-shogaol could significantly attenuate various inflammatory responses.

4.1.4 Anti-Colorectal Cancer Effects and Mechanisms of Actions of Ginger

Ginger leaves extract induced apoptosis in human colorectal cancer cells, HCT116, SW480 (human colon adenocarcinoma cells), and LoVo by activating transcription factor 3 (ATF3). ATF3 is responsible for the induction of apoptosis in CRC cells by regulating the ERK1/2 pathway, where ginger leaves (50, 100, and 200 μg/ml for 24 and 48 h) interact with the cAMP-responsive element-binding (CREB) site and activate ATF3 (Tang et al., 2002; Hsu et al., 2005; Park et al., 2014). Another investigation reported that ginger extract inhibited CRC cell growth (HCT-116) by down-regulating the K-ras and MMP-2 marker gene expressions. K-ras is crucial in colorectal metastasis by regulating VGEF, protease expression, apoptosis, adhesion, and motility (Lavrado et al., 2015).

4.2 Turmeric

Turmeric (*Curcuma longa* L.) is also derived from the Zingiberaceae family. In addition to improving taste, turmeric was one of the spices used to preserve food. Yellow turmeric rhizomes give an aromatic flavor and slightly bitter taste. (Dhakal et al., 2019). Turmeric rhizomes are an excellent antioxidant source, and it has free-radical scavenging properties (Restrepo-Osorio et al., 2020). Its extracts (1–2%) are considered a natural preservative and free from microbial contamination at least for 90 days of storage (Gul and Bakht, 2015). Turmeric is generally given at a dose of 5–500 mg/kg for nutritional purposes depending on the food categories like dairy products, beverages, cereals, mustard, food concentrates, pickles, sausages, confectionery, and ice cream, meat, fish, eggs, and other confectionaries. It is also mixed with other compounds, including annatto, seasonal sauces, mayonnaise, and butter (Sharifi-Rad et al., 2020). Turmeric is rich in polyphenols and universally known as the "wonder drug of life." The lead compound, curcumin, is responsible for a wide range of pharmacological activities, including antioxidant, anti-cancer, anti-arthritic, anti-microbial, anti-diabetic, anti-inflammatory activities, and avails in the treatment of many ailments, including tendinitis, liver cirrhosis, Alzheimer's disease, heart attack, hypoglycemia, gastrointestinal problems, worms, swelling, cancer, skin and ocular perceiver infections (Gera et al., 2017). Currently, the major focus has been given by scientists on cancer. As of 15 August 2021 (Scopus database), the effect of turmeric/curcumin on cancer; between 1881 and 2021, was the topic of 11,519 papers, with 53% related to cancer and 16% associated exclusively on CRC (**Figure 1**). The wide application of curcumin in diverse fields had caused its global market to expand exponentially. The pharmaceutical industry, particularly those focused on anti-cancer medication formulations, is the most significant application category, accounting for more than half of the global market, followed by the food and cosmetic industries (Sharifi-Rad et al., 2020).

4.2.1 Lead Compounds of Turmeric

Turmeric powder contains various bioactive components. Dry turmeric contains 69.43% carbohydrates, 6.3% proteins, 5.1% oils,

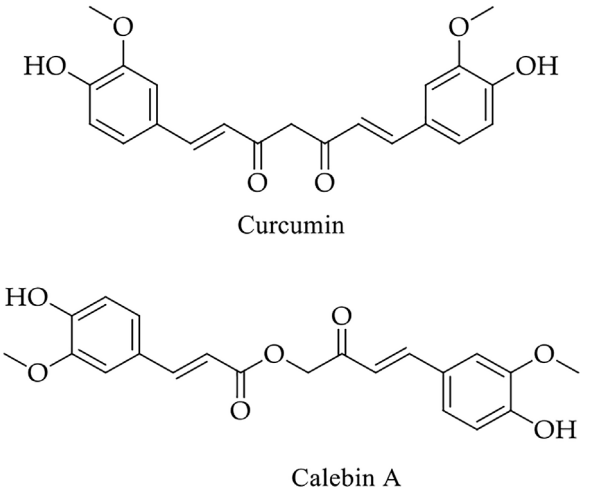

FIGURE 7 | Chemical structure of main bioactive compounds of turmeric.

3.5% minerals, and other elements (15.67%). Curcumin and calebin A are lead compounds (**Figure 7**) with a magnificent biological role in CRC patient management (**Figure 4** and **Table 2**).

4.2.2 Antioxidant Activity of Turmeric

Turmeric can mitigate the rise of free radical formation in the living cells responsible for damaging the biomolecules, such as lipid, protein, and DNA (Goud et al., 1993). A study reported by Tilak et al. (2004) showed that turmeric, as used in cooking and in-home remedies, and its major compounds have significant antioxidant abilities at different levels of action. This study prepared six different types of standardized aqueous and ethanol extracts using the processed powder or raw turmeric rhizome as per the cooking mood. The ethanol contains more phenolic and flavonoid content than aqueous extracts. In all antioxidant tests using raw and processed turmeric, ethanol extracts were performed over the aqueous extracts. Boiled ethanol extract (10 min) had the strongest activity in two chemical assays - ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picryl hydroxyl (DPPH) radical scavenging test. However, in the ferryl myoglobin assay, ethanol extracts (raw turmeric stirring in ethanol for 1 h) exhibited the highest total antioxidant activity (TAA). Boiling the aqueous extracts increased their potency compared to the aqueous extracts. Turmeric boiling extracts were more efficient at scavenging 2,2-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radicals than aqueous turmeric extracts. There is no doubt numerous studies reported on the antioxidant activity of turmeric extracts and their different isolated compounds. At the same time, the *in vitro* antioxidant activities of turmeric extracts have been supported by the *in vivo* studies to support its pharmacological applications (Sreekanth et al., 2003; Dall'Acqua et al., 2016; Mohammed et al., 2020). A proprietory formulation containing extract of turmeric obtained by supercritical carbon dioxide gas extraction and post-supercritical hydroethanolic extraction is known as Smoke Shield. Administration of Smoke Shield to mice increased antioxidant enzymes in blood, liver, and kidney (Sreekanth et al., 2003). Smoke Shield increased glutathione-S-transferase activities in the liver and kidney. Additionally, it increases superoxide dismutase and glutathione, whereas it decreases glutathione peroxidase in smokers' blood. Smoke Shield contains significant antioxidant action, inhibits phase I enzymes, and increases detoxification enzymes, making it a chemoprotective herbal preparation (Sreekanth et al., 2003). The oral administration of turmeric extract to healthy rats decreased urinary levels of allantoin, m-tyrosine, 8-hydroxy-2′-deoxyguanosine, and nitrotyrosine. This finding supports the *in vivo* antioxidant effect of turmeric (Dall'Acqua et al., 2016). Another recent *in vivo* antioxidant study of methanol extract of turmeric showed a significant decrease in SOD, catalase (CAT) and GPx levels in both liver and kidney of Alloxan-induced diabetic rats (Mohammed et al., 2020). These findings suggested that turmeric supplements could potentially neutralize the ROS level in cells even if used in cooking, either stirring on oil for 10 min or 30 min in aqueous; however, further investigation on the complex role of antioxidant curcumin effects is required before making a precise conclusion.

The main bioactive compound of turmeric, curcumin (42 μM) integrated with rat liver mitochondria, reduces ascorbate-Fe^{2+} driven lipid peroxidation significantly. During incubation (0–60 min), the per cent inhibition was almost 100% without any lag period of inhibition (Tilak et al., 2004). Another phytoconstituent of turmeric called turmeric was an effective antioxidant/DNA protectant/antimutagen. It has three methionine residues that are responsible for its antioxidant properties. Turmeric at a 183 nM is highly protective (80%) to membranes and DNA against oxidative injury. Additionally, it is noncytotoxic up to milligrams doses in human lymphocytes (Srinivas et al., 1992). Curcumin and turmeric controlled oxidative stress by reducing the level of thiobarbituric acid-reactive substances (TBARS) and protein carbonyls and repealing altered antioxidant enzyme activities in rat models (Suryanarayana et al., 2007). Many other studies also reported that turmeric extracts and their constituents have potential free radical scavenging activity, and ethanol extracts are more efficient than aqueous extracts (Cousins et al., 2007; Singh et al., 2010; Lim et al., 2011; Tanvir et al., 2017; Arumai Selvan et al., 2018). The formulation of turmeric extracts using modern technology like spray-dried microparticles (Martins et al., 2013), silver nanoparticles (Arumai Selvan et al., 2018), and puffing (Choi et al., 2019) enhance the antioxidant activity of turmeric.

4.2.3 Anti-Inflammatory Effect of Turmeric

Traditionally, as an anti-inflammatory antidote in ayurvedic medicine, Turmeric powder is implicated as an anti-inflammatory antidote. In a rat model, the production of these enzymes was elevated by curcumin treatment (Tvrdá et al., 2016). In *the in-vitro* experiment, the macrophage-mediated inflammation due to ROS production has reduced by giving 10 μM curcumin (Amano et al., 2015). The increasing detrimental bacterial population in the colon so prompts to produce of carcinogenic chemicals, toxins that are responsible

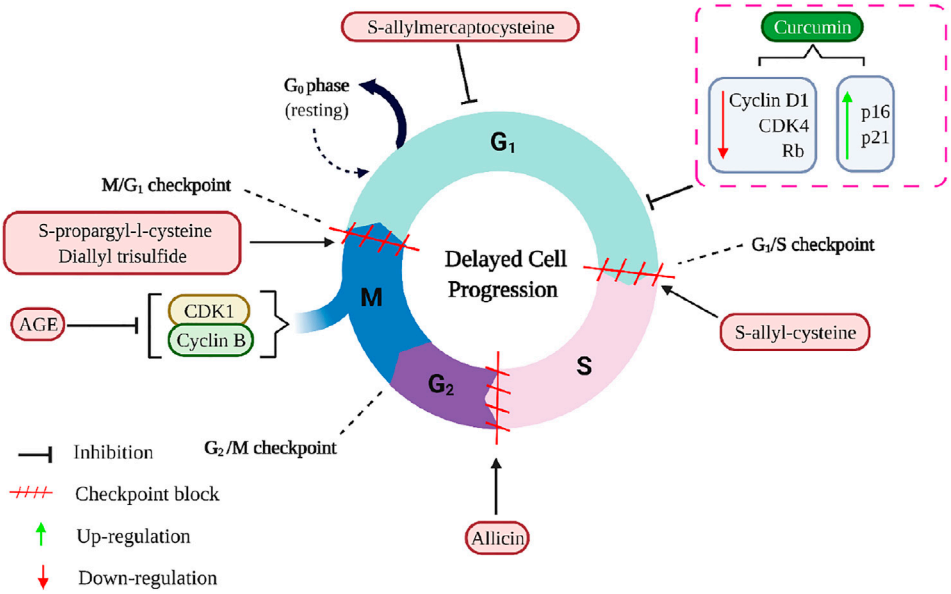

FIGURE 8 | The mechanisms of curcumin and aged garlic extracts, including its active compounds, inhibit the cell cycle in cancer cells. AGE down-regulates Cyclin B1 and CDK1 and arrest the cell cycle in the G2/M- checkpoint. S-propargyl-L-cysteine and diallyl trisulfide arrest the cell cycle in the G2/M- checkpoint, whereas S-allyl-cysteine induced cell cycle arrest in the G1/S- checkpoint, and allicin induced cell cycle arrest during the S phase. Additionally, S-allylmercaptocysteine shortens the duration of the S phase and lengthens the duration of the G0/G1 phase. The dotted rectangle indicates the curcumin (lead compound of turmeric) functions. AGE, Aged garlic extract; CDK1, Cyclin-dependent kinase 1; CDK4, Cyclin-dependent kinase 4; Rb, Rb Protein.

for the development of colon cancer; for instance, *Bacteroides fragilis* produces *Bacteroides fragilis* toxins (BFTs) that subsequently activate the STAT3 signalling pathway and stimulate IL-17 cytokines production that subsequently promotes NF- κB and Wnt signalling pathway activation leading to abnormal cell division (Chung et al., 2018). Caleb in A is a potent anti-inflammatory component of turmeric responsible for inhibiting cancer formation through this NF-κB signalling pathway (Buhrmann et al., 2020). Another investigation has demonstrated that the inflammation mediated by NF-κB activation is suppressed by averting IκBα kinase and AKT signalling pathway because of using curcumin (Aggarwal et al., 2006). The administration of curcumin is also thought to have reduced the expression of inflammatory cytokines, such as C-reactive protein, cyclooxygenase-2 (COX-2), TNF-, CXCR-4, MIP-1, IL-1, IL-6, and IL-8 (Gonzales and Orlando, 2008; Wang and Dubois, 2010; Kim et al., 2012; Afzali et al., 2021).

4.2.4 Anti-Colorectal Cancer Effects and Mechanisms of Actions of Turmeric

Calebin A suppressed the expression of Nuclear Factor-kappa B (NF-κB), which promotes the anti-apoptotic B cell lymphoma extra-large (BCL-xL), B-cell lymphoma (BCL-2), surviving, proliferation (Cyclin D1), invasion (MMP-9), metastasis (CXCR4) biomarkers, as well as down-regulated apoptosis (Caspase-3) gene biomarkers, ultimately leading to apoptosis in human colorectal adenocarcinoma (HCT116) cells (Buhrmann et al., 2020). Rajitha et al. (Rajitha et al. (2016) demonstrated that the administration of 25 μM curcumin, a potential NF-κB inhibitor, significantly suppressed NF-κB activation *via* inhibiting the transcription factor E2F-1 and thymidylate synthase as compared to untreated cell lines. This treatment resulted in cell cycle arrest at the G0/G1 phase with a concomitant decrease in the number of cells in the S, and tumour growth was significantly reduced in the CRC cell lines HCT116 and HT-29. The western blotting analysis further revealed that curcumin significantly decreased the levels of cyclin D1, CDK4, and pRb and increased p16 and p21 in both cell lines compared to controls (**Figure 8**) (Rajitha et al., 2016). It has also been shown to emerge with anti-inflammatory and anti-tumour properties by the induction of apoptosis and modulating different signalling pathways, such as mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinase (ERK), p38, Jun N-terminal kinase (JNK) in gastric cancer, and neurofibroma (Li et al., 2008; Lee et al., 2019). Curcumin and its analogues have been an effective chemotherapeutic agent and chemosensitizer by regulating specific microRNAs, signalling pathways, and epithelial-mesenchymal transition (Cai et al., 2018).

4.3 Garlic

Another commonly consumed spice is garlic (*Allium sativum* L.), a member of the family Liliaceae (Shang et al., 2019). Garlic can be consumed raw or cooked and in powder or oil form. Garlic as traditional medicine has been documented in ancient writings of Egypt, Greece, China, and India as early as 3,000 years ago (Rivlin, 2001; Omar and Al-Wabel, 2010). Garlic has been shown to reduce the incidence of heart disease and cancer in epidemiologic and preclinical investigations, and it has also been claimed to be an anti-cancer dietary component (Bayan et al., 2014). There are around 33 sulfur compounds, including alliin,

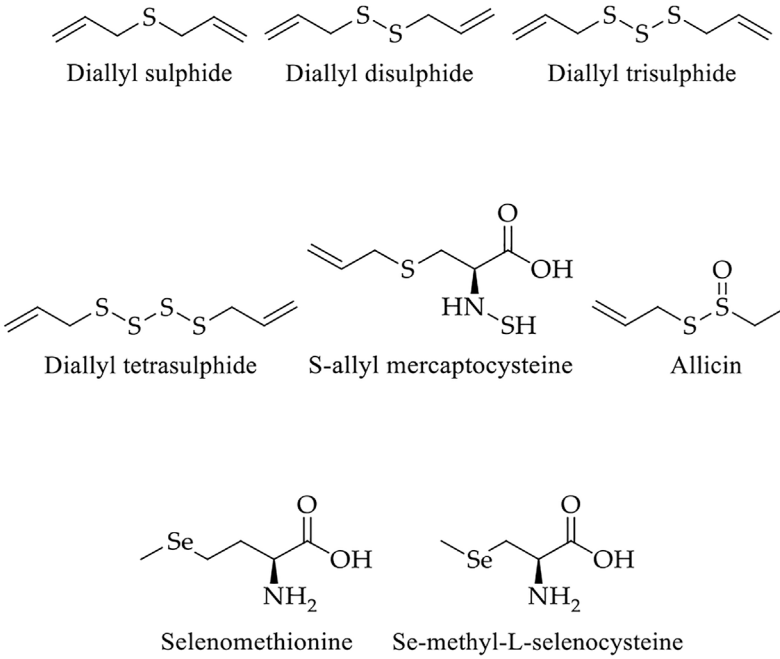

FIGURE 9 | Chemical structure of main bioactive compounds of garlic.

allicin, ajoene, allyl propyl disulfide, diallyl trisulfide, S-allyl cysteine, vinyldithiines, S-allyl mercapto cysteine, and others, several enzymes (i.e., allinase, peroxidases, myrosinase), 17 amino acids like arginine and others, and minerals, such as selenium, germanium, tellurium and other trace minerals (Newall et al., 1996; Martins et al., 2016; Abe et al., 2020). As of 15 August 2021 (Scopus database), 19,339 papers related to the merits of garlic consumption have been documented between 1854 and 2021. One-third of these published records were related to its benefits in cancer modulation, while 8 % pivoted on its benefits for CRC prevention and management (**Figure 1**). There is compelling evidence that garlic and related sulfur components can reduce cancer risk and affect the biological behaviour of tumours. A high intake of garlic is associated with decreased risks for stomach and CRC (Omar and Al-Wabel, 2010).

4.3.1 Lead Compounds of Garlic

Garlic's pungent flavour renders it a daily seasoning or condiment in Asian cuisine. Prominent medicinal values and uses of garlic have been seen since ancient times. Garlic has more than 200 chemicals. The leading bioactive molecules among these compounds are diallyl sulfide, diallyl disulfide, diallyl trisulfide, diallyl tetrasulfide, S-allyl mercaptocysteine, allicin, selenomethionine, and se-methyl-L-selenocysteine (**Figure 9**) (Czepukojc et al., 2014). The potential chemotherapeutic activities of the aforementioned compounds in colorectal cell lines are listed in **Figure 4** and **Table 2**.

4.3.2 Anti-Colorectal Cancer Effects and Mechanisms of Actions of Garlic

Aged garlic extract (AGE) and its bioactive compounds are good chemopreventive agents for CRC because of their antiproliferative action on colorectal carcinoma cells and inhibitory activity on angiogenesis. AGE suppressed the proliferation of different CRC cell lines, namely DLD1, COLO205, HT29, SW480, and SW620 (Matsuura et al., 2006; Jikihara et al., 2015; Tung et al., 2015). It had diverse impacts on the invasive activities of these cell lines, and AGE showed a significant reduction in invasive activity on SW480 and SW620 cells; however, it did not affect the invasive activity of HT29 cells (Matsuura et al., 2006). There appears to be a relationship between the effect of AGE and the type of cancer cells being treated. AGE improved the endothelial cells' adherence to collagen and fibronectin, whereas its bioactive compounds reduced cell motility and invasion. In addition, AGE had a strong inhibitory effect on the proliferation and tube formation of endothelial cells (Matsuura et al., 2006). SW620 is a metastasized SW480, and both cell lines have been documented to have increased p53 levels, while HT-29 cells consist of mutated p53 (Eng et al., 2021). Thus, it is plausible that the bioactive compounds interact with the molecules such as p53 that ensure cell cycle checkpoints are conducted rigorously.

AGE decreased the number of ACF but did not affect gross tumour pathology in the DLD1 human CRC cell line. AGE inhibited the proliferation of adenoma and adenocarcinoma lesions but did not affect normal colon mucosa. It delayed cell cycle progression by inhibiting cyclin B1 and Cyclin-dependent kinase 1 (cdk1) expression but did not trigger apoptosis in DLD1 (Jikihara et al., 2015). Bioactive compounds of garlic, particularly selenomethionine and se-methyl-L-selenocysteine, decreased ACF and induced apoptosis by about 80% by activating caspase 3. In addition, AGE delayed cell cycle progression by inactivation of NF-κB signalling and downregulation of cyclin B1

and cdk1 expression during the G2/M-phase (**Figure 8**) (Jikihara et al., 2015; Tung et al., 2015). Se-methyl-L-selenocysteine increased Fas and FasL expression, followed by the caspase-3, caspase-8, DNA fragmentation factor, and poly(ADP-ribose) polymerase cleavage. Se-methyl-L-selenocysteine also increased Bax protein levels while decreasing Bid and BCL-2 protein levels. However, this compound caused apoptosis *via* endoplasmic reticulum stress rather than reactive oxygen species stress. The cleavage of caspase-12 and caspase-9 increases growth arrest and protein levels of GADD 153 and 45. In COLO 205 cells, Se-methyl-L-selenocysteine reduced ERK1/2 and PI3K/AKT protein levels while increasing p38 and JNK protein levels (Tung et al., 2015).

These results suggested that AGE or garlic's bioactive compounds, mainly selenomethionine and se-methyl-L-selenocysteine, could prevent tumour formation by inhibiting angiogenesis by suppressing endothelial cells motility, proliferation, and tube formation. Therefore, they could be good chemopreventive agents for CRC because of their antiproliferative action on colorectal carcinoma cells and inhibitory activity on angiogenesis.

4.4 Fenugreek

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family Leguminosae. It has long been used as a spice to improve the sensory quality of cuisines across the world, including in Bangladesh, India, and Pakistan (Wani and Kumar, 2018; Singh et al., 2020). Its seeds and green leaves, commonly used as leafy vegetables and seasonings, are now widely cultivated for medicinal purposes. They also enhance flavour, colour, and texture in foods (Tewari et al., 2020). Although modern medicine has made incredible advances, the usage of herbal plants for treating or preventing diseases is still widely used due to their diverse nutraceutical capabilities and safety. Among many spice crop plants that are nutritious, functional, and therapeutic, fenugreek is popular with all these characteristics. Recently, it has gained tremendous scientific attention for further evaluation and validation of nutraceutical and health benefits, especially lifestyle-related diseases and cancer. Our systematic investigation in the Scopus database revealed that scientists published around 3,713 papers between 1931 and 2021. Almost a quarter of the articles focused on cancers, and 5% of papers precisely focused only on CRC (**Figure 1**). The health benefits of fenugreek that lead to anti-cancer effects are summarised in **Figure 10**.

4.4.1 Lead Compounds of Fenugreek

Since antiquity, fenugreek has been a member of the Fabaceae family and has been extensively utilized as Ayurveda in traditional and alternative medicine systems (Aasim et al., 2018; Rajasekaran, 2019). It is rich in several phytochemicals, of which diosgenin (a saponin) (**Figure 11**) has anticarcinogenic properties (**Table 2**) (Raju et al., 2004).

4.4.2 Anti-Colorectal Cancer Effects and Mechanisms of Actions of Fenugreek

Multiple functional and molecular targets are involved in the anti-cancer effects of fenugreek or its bioactive compounds, such as apoptosis in tumour cell lines, especially in human CRC (**Table 2**) (Raju et al., 2004). In 1,2-Dimethylhydrazine-treated mice, a diet rich in fenugreek seed powder reduced colon tumour incidence and lipid peroxidation LPO while simultaneously increasing GPx, glutathione S-transferase (GST), SOD, and catalase activity in the liver (Sushma and Devasena, 2010). Another study demonstrated that diosgenin inhibited the production of azoxymethane (AOM)-induced aberrant crypt foci, a preneoplastic colonic lesion in F344 rats. This compound induced apoptosis in HT-29 human colon cancer cells by suppressing BCL-2 and activating caspase-3 protein expression, implying its potential as a colon cancer preventive agent (Raju et al., 2004).

4.5 Sesame

Sesame (*Sesamum indicum*) from the Pedaliaceae family is one of the earliest domesticated oilseed crops known to humankind with its multifarious uses. It is mainly consumed in various cuisines and preferably used with bread, biscuits, crackers, and so forth and as a seasoning in food worldwide (Namiki, 2007). Sesame has an essential role in human nutrition due to its rich chemical compositions like oil (44–58%), protein (18–25%), carbohydrates (~13.5%), minerals and vitamins (Elleuch et al., 2007; Hassan, 2012; Lim, 2012). Sesame seeds have multiple potential bioactive compounds that are beneficial components in food and are accountable for disease-preventing properties. These chemical compounds include phenolics, carotenoids, phytosterols, and polyunsaturated fatty acids, often utilized as antioxidants and for other purposes (Pathak et al., 2017). Recent studies demonstrated that the leaves and shoots of sesame plants are used as vegetables, and the leaves contain valuable nutrients such as amino acids responsible for various traditional uses, including pain relief, catarrh, eye pain, bruises, and erupted skin lesions. In Japan, young sesame leaves (30–70 cm tall, 40–60 days after planting) are dried and sold as a health food supplement (Fuji et al., 2018).

The seeds contain lignans such as sesamin and sesaminol and are highly valued as traditional health and nutraceutical food. Young sesame leaves contain three iridoids (lamalbid, sesamoside and shanzhiside methyl ester) and seven polyphenols (cistanoside F, chlorogenic acid, pedalitin-6-O-laminaribioside, pedaliin, isoacteoside, pedalitin and martynoside), and acteoside. These compounds show potential radical scavenging effects in assays like the DPPH, ABTS, and superoxide anion radicals test (Matsufuji et al., 2011; Fuji et al., 2018). However, sesamin, a major lignan in sesame oil did not show antioxidant *in vitro* activity (Nakai et al., 2003). Interestingly, on the other hand, this compound showed protective effects against oxidative damage in rat liver. The same metabolites were found as glucuronic acid and/or sulfuric acid conjugates in substantial amounts in rat bile after oral administration of sesamin (Nakai et al., 2003). Therefore, sesamin is considered as a prodrug. On giving 10 mg/kg or 100 mg/kg of sesamin (S10, S100) to 32 male ddY mice 2 h before swimming exercise using a new forced-swimming apparatus, their plasma lipid peroxide level was significantly suppressed, while the level in the control group increased significantly after exercise ($p < 0.01$). S100 showed

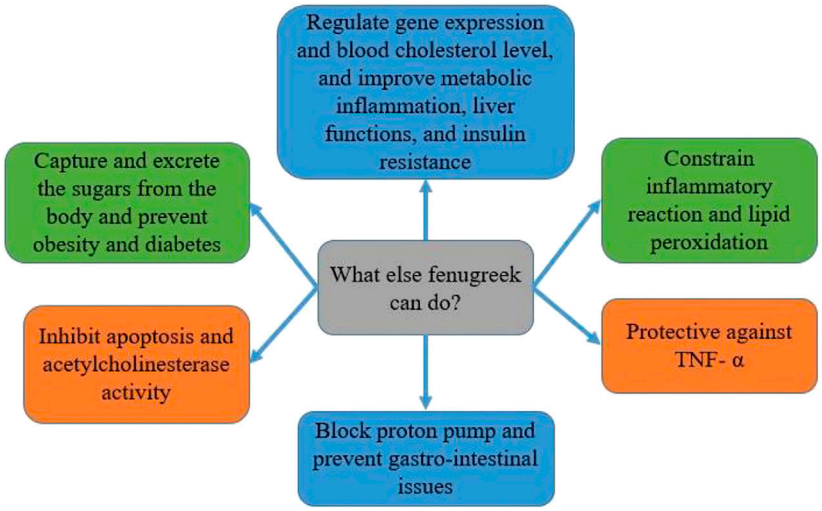

FIGURE 10 | Health benefits and anticancer properties of fenugreek.

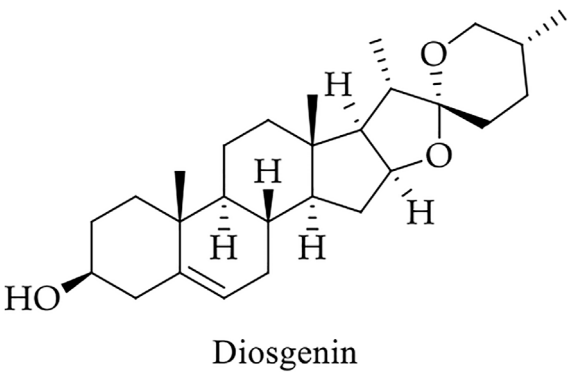

FIGURE 11 | Chemical structure of the chemopreventive bioactive compound of fenugreek.

significantly higher total GPx activity and GST activity in the liver compared to control ($p < 0.05$) (Ikeda et al., 2003). This finding suggested that sesamin may enhance liver LPO degradation, resulting in strong protective effects against exercise-induced plasma lipid peroxidation. Sesamin *in vivo* metabolites with the catechol group is the most efficient antioxidants (Papadopoulos et al., 2016). The highly antioxidative action of sesame oil has been clarified, and it has been determined that recently discovered lignans mediate with tocopherols. A novel synergistic effect of sesame lignans with tocopherols has been found, and it is believed to be responsible for the antiaging effect of sesame. Sesame lignans inhibit metabolic decomposition of tocopherols, which results in the antiaging effect of sesame being attributed to strong vitamin E activity (Namiki, 2007). A systematic search in the Scopus database showed that around 11,089 articles were published between 1898 and 2021. Among these publications, about 15% emphasized cancers, and 2% precisely concentrated on CRC alone (**Figure 1**).

4.5.1 Lead Compounds of Sesame

Sesame from the Pedaliaceae family generally refers to sesame seeds. It is one of the oldest condiments and a commercially significant oilseed crop (Queen of oilseed crops) with 40–60% oil and medicinal value due to the presence of a broad spectrum of bioactive molecules, including sesamin, sesamol, sesamolin, and sesaminol (**Figure 12** and **Table 2**) (Zohary and Hopf, 2000; Aasim et al., 2018).

4.5.2 Anti-Colorectal Cancer Effects and Mechanisms of Actions of Sesame

Sesamol is one of the prominent biomolecules of sesame seeds that confers chemopreventive properties and analgesic effects (**Table 2**). It targets p53, MAPK, JNK, PI3K/AKT, TNF, NF-B, PPAR, caspase-3, Nrf2, eNOS, and LOX signalling pathways, suggesting that sesamol possesses potent anti-cancer properties. It has a wide range of biological functions, including inhibition of lipid peroxidation and enhancement of radical scavenging, upregulation of antioxidant enzymes, suppression of TNFα and IL-1β expression, inhibition of NF-κB signalling, suppression of LOX-1 and 5-LOX activity, induction of apoptosis, arresting cell growth at different phases of the cell cycle and modulation of caspase-3, p53, BAX, and BCL-2 expression (Gupta et al., 2009; Chu et al., 2010a; Chu et al., 2010b; Wu et al., 2015; Kim et al., 2021). In HCT116 cells, sesame leaf extract (250 g/ml and 500 g/ml) induced apoptosis and cell cycle arrest during the G2/M phase. This extract increased the G2/M cell population to 2.3–6.6-fold of the control, with a concurrent drop in G0/G1 and S phase cell populations, demonstrating that sesame leaf extract has a G2/M arresting function (Kim et al., 2021).

4.6 Flaxseed

Flaxseed (*Linum usitatissimum* L.) from the Linaceae family is one of the world's oldest cultivated herbaceous crops. It is still

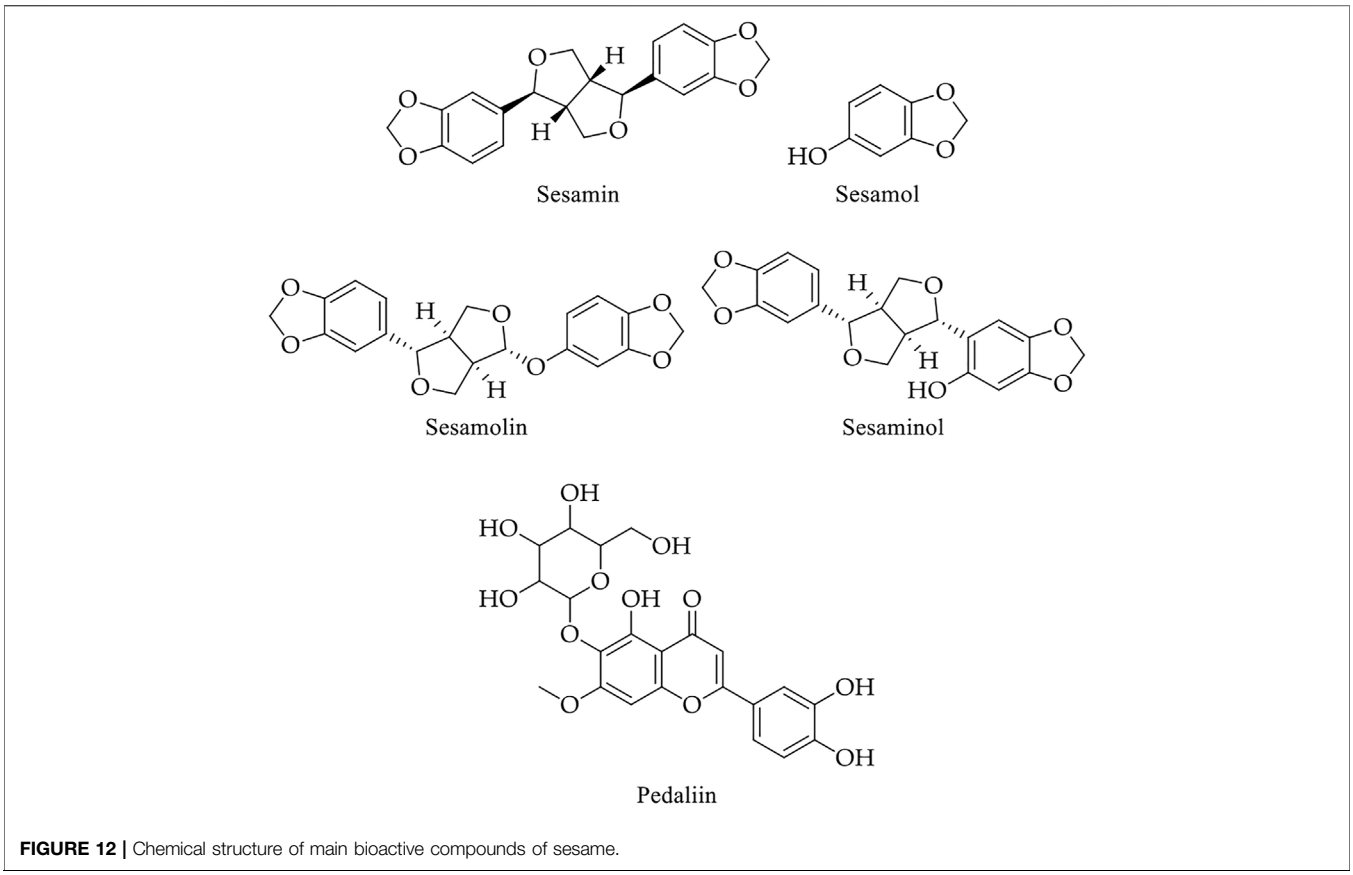

FIGURE 12 | Chemical structure of main bioactive compounds of sesame.

widely grown for its oil, fibre, and nutritional value. Flaxseed oil is high in omega-3 fatty acid linolenic acid (55%), an attribute that boosts its role as a functional food. Additionally, flaxseeds are used in animal feed to boost reproductive health (Oomah, 2001; Turner et al., 2014). The flaxseed products include whole seed (ground), flaxseed oil (partially defatted), fully defatted (solvent extraction), mucilage extract, flaxseed hull, oleosomes, and alcohol extract. Each of these products has particular health benefits. Reports typically neglect the presence of many bioactive chemicals in flaxseed fractions or attribute the impact to a single component. However, whole flaxseed is widely accepted as a healthy food with anti-cancer activity (Shim et al., 2014). In female rat mammary glands, flaxseed flour reduces epithelial cell proliferation and nuclear abnormalities that indicate the reduction of mammary tumour growth in the later stages of carcinogenesis (Serraino and Thompson, 1991; Thompson et al., 1996). Recently the growth of cancer research using flaxseeds has increased significantly. Our team's systematic literature search (Scopus database) revealed that about 7,357 articles were published between 1844 and 2021. About 22% concentrated on cancers among these publications, and 4% was specifically spotlighted on CRC (**Figure 1**).

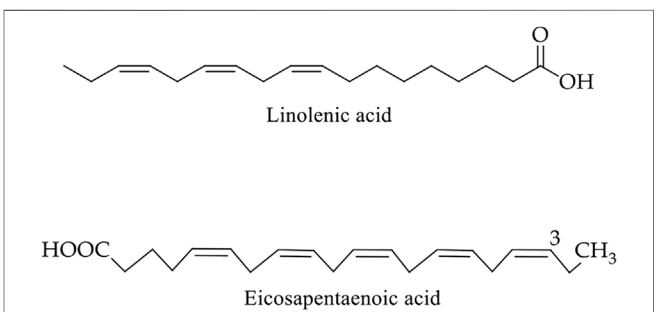

FIGURE 13 | Chemical structure of main bioactive compounds of flaxseed. α-Linolenic acid (18:3n-3) and eicosapentaenoic acid (20:5n-3) belong to the omega-3 fatty acids group.

4.6.1 Lead Compounds of Flaxseed

Flaxseed is one of the world's oldest crops, grown since the dawn of civilization. Flaxseed treated various ailments in India, Sri Lanka, Greece, Rome, Egypt, and many other countries and enriched the Ayurveda and traditional Chinese medicine system (Goyal et al., 2014). Due to its high fibre level, omega-3 fatty acids, flavonoids, and phytoestrogens, flaxseed usage in reducing human CRC risk are gaining attention (Calviello et al., 2007; Lattimer and Haub, 2010; Kajla et al., 2015). Moreover, flaxseed is one of the most significant plant sources of linolenic acid, an omega-3 polyunsaturated fatty acid (PUFA) (49–60%), which is linked to a lower risk of colonic neoplasms (**Figure 13** and **Table 2**) (Goyal et al., 2014; DeLuca et al., 2018).

4.6.2 Anti-Colorectal Cancer Effects and Mechanisms of Actions of Flaxseed

In vitro studies and animal models suggest that diets high in 3-PUFA may protect against malignancies, such as colon cancer, whereas treatment with 6-PUFA may inhibit cancer cells proliferation. For example, mice fed diets enriched in α-linolenic acid, which enhanced plasma levels of α-linolenic acid (ALA) and its metabolites eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), showed a reduction in the growth of transplanted colon cancer cells (Danbara et al., 2005; Bommareddy et al., 2010; Chamberland and Moon, 2015). In case-2 human colon adenocarcinoma cells, ALA has been demonstrated to inhibit cell proliferation and induce apoptosis (Bommareddy et al., 2010). SW480 cells treated with enterolignans (enterodiol (ED) alone or in combination with enterolactone (EL)) showed a dose-dependent reduction in cell number, induction of S-phase cell cycle arrest, elevated cyclin A protein levels, an increased proportion of apoptotic cells (0–40 mol/L). Similar results were observed in a study using Colo 201 cells treated with EL, where apoptosis was modulated. Cell proliferation was decreased by the up-regulation of an apoptosis-inducing protein (a cleaved form of Caspase-3) and the down-regulation of both an apoptosis-inhibiting protein (BCL-2) and proliferation cell nuclear antigen (PCNA) protein (Danbara et al., 2005). Regulation of transcription in apoptotic genes (BCL-2, CCND1, and c-Myb) and cell cycle regulation were reported using Young Adult Mouse Colonocytes treated with low levels of EL (1 M) and ED (5 M) (DeLuca et al., 2018).

5 THE WAY FORWARD

Significant ground-breaking knowledge of molecular mechanisms behind CRC development revealed that dietary factors might be associated with CRC development at an increasing rate. However, the evidence to date is regrettably inadequate due to its highly complex mechanisms. Another significant association of intestinal microbiota with CRC has been predicted. Again, intestinal microbiota balance depends on the dietary habits and alterations of balanced intestinal microbiota involved with CRC development and progression (Leeming et al., 2019; Wong and Yu, 2019). However, modulation of the gut microbiota is a promising strategy to enhance treatment efficacy and reduce the adverse effects of CRC therapies (Wong and Yu, 2019). Many challenging issues, including aetiology, diagnosis, treatment, and management, need to be addressed to properly manage CRC and identify the key concerns for a long-term solution.

For appropriate management of CRC, several steps are required to follow for this global issue. Besides early identification and screening of high-risk communities and individuals, taking preventive measurements through consuming high dietary foods and maintaining a normal lifestyle is essential. This study revealed that consuming culinary herbs and spices might help prevent and cure CRC. They showed potential growth inhibition of human colorectal cancer cells by regulating relevant molecular signalling pathways. The bioactive compounds from these herbs and spices can be isolated and purified through several techniques, including convention extraction or green techniques like supercritical or subcritical fluid extraction. Some sophisticated analytical tools can be applied to purify and identify pure compounds, such as the High-Pressure Liquid Chromatography (HPLC) technique, Liquid chromatography-mass spectrometry (LC-MS) analysis, LC-MS-mass spectrometry (LC-MS-MS), and Gas chromatography-mass spectrometry (GC-MS) (Hossain et al., 2014; Hossain S. et al., 2021). To develop stable drugs, isolated compounds can be formulated in different drug forms like tablets, suspension or emulsion (Hua, 2019). They also might be incorporated with nanoparticles and encapsulated in the biodegradable polymer for target-specific drug delivery (Begines et al., 2020).

Some additional measurements are highly recommended as follows: 1) public cancer registration for tracking CRC incidence and survival, 2) government should provide quality medical care for timely diagnosis and treatment, 3) ensure better-personalized therapy and easy access to clinical trials for CRC patients, and 4) increased awareness of CRC as well as about other comorbidities to improving cancer care and research for proper management of this global issue.

6 CONCLUSION

Literature has provided evidence that herbs and spices have potential roles in preventing and reducing CRC severity. All the six common herbs and spices, namely ginger, turmeric, garlic, fenugreek, sesame, and flaxseed, are useful in preventing CRC. Apart from chemotherapeutic uses, these culinary herbs and spices-derived substances could have a salubrious indication for CRC prevention and management. Their mechanisms of action are mainly mediated through BCL-2, K-ras, and MMP pathways, caspase activation, the extrinsic apoptotic pathway, and the regulation of ER-stress-induced apoptosis. Therefore, these herbs and spices are good candidates for chemopreventive agents for CRC due to their antiproliferative action on colorectal carcinoma cells and inhibitory activity on angiogenesis.

AUTHOR CONTRIBUTIONS

Data curation, MH, MK, MI, MK, and MHA-R; writing manuscript—original draft, MH, MK, MI, MK, and MHA-R; writing manuscript—review and editing, MH, KG, DO, HC, YW, SM, KK, LM and YL; Conceptualization, visualization and project administration, MH; Supervision, MH and LM All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2022.865801/full#supplementary-material

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Biological activities of meroterpenoids isolated from different sources

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Meroterpenoids are natural products synthesized by unicellular organisms such as bacteria and multicellular organisms such as fungi, plants, and animals, including those of marine origin. Structurally, these compounds exhibit a wide diversity depending upon the origin and the biosynthetic pathway they emerge from. This diversity in structural features imparts a wide spectrum of biological activity to meroterpenoids. Based on the biosynthetic pathway of origin, these compounds are either polyketide-terpenoids or non-polyketide terpenoids. The recent surge of interest in meroterpenoids has led to a systematic screening of these compounds for many biological actions. Different meroterpenoids have been recorded for a broad range of operations, such as anti-cholinesterase, COX-2 inhibitory, anti-leishmanial, anti-diabetic, anti-oxidative, anti-inflammatory, anti-neoplastic, anti-bacterial, antimalarial, anti-viral, anti-obesity, and insecticidal activity. Meroterpenoids also possess inhibitory activity against the expression of nitric oxide, TNF- α, and other inflammatory mediators. These compounds also show renal protective, cardioprotective, and neuroprotective activities. The present review includes literature from 1999 to date and discusses 590 biologically active meroterpenoids, of which 231 are from fungal sources, 212 are from various species of plants, and 147 are from marine sources such as algae and sponges.

KEYWORDS

cytotoxicity, anti-inflammatory, anti-proliferative, anti-microbial, anti-fungal, anti-viral, anti-oxidant, meroterpenoids

Introduction

The name "meroterpenoid" was conceived by Cornforth for a group of secondary metabolites, which are partially derived from the terpenoid biosynthetic pathway (Matsuda and Abe, 2016). Meroterpenoids have wide structural diversity consisting of a prenyl unit connected to a phenolic derivative from basic compounds to the more complex meroterpenoids consisting of functionalized carbon chains (Geris and Simpson, 2009a). The diversity is observed not only in the non-terpenoid component of the structure but also in the chain length of the terpenoid and the mode in which the terpenoid portion of the molecule undergoes cyclization. These compounds are derived from various natural sources, such as animals, fungi, marine organisms, and plants (Matsuda and Abe, 2016). However, fungi and aquatic organisms are the richest sources of meroterpenoids (El-Demerdash et al., 2020a). Higher plants from genera such as *Psidium*, *Eucalyptus*, *Arnebia*, and *Eugenia* show the presence of biologically active meroterpenoids.

The classification of meroterpenoids was based on the biosynthetic pathway of origin of these compounds: the initial classification focused on the chemical composition of the polyketide-terpenoid and non-polyketide-terpenoid components (Geris and Simpson, 2009b). Some researchers relied on the same terpene component, whereas a few others realized that the immense diversity and complexity of the structures of the non-terpenoid component should help define the meroterpenoids chemically. Broadly, the meroterpenoids of fungal origin fall under three major categories: those possessing triketide-terpenoid scaffold, those with tetraketide-terpenoid scaffold, and those containing indole-3-glycerolphosphate moiety. This rigid classification fits in a wide variety of aromatic and non-aromatic polar molecules, possessing groups such as the carboxylic acid, hydroxy group, and lactone/ester moieties in the non-terpenoid component. Subtle changes in the stereochemistry of the attached substituents bring these groups in close spatial vicinity, which aids the formation of unique groups such as epoxide, imparting such isomers' modified biological potency. Non-polyketide terpenoids are derived from the shikimic acid pathway and include quinine derivatives, dehydroquinic acid, protocatechuic acid derivatives, or subunits attached to terpenoid moiety with one C-C bond. On the contrary, polyketides are a large family of natural compounds synthesized by fungi, plants, or bacteria by condensing carboxylic acid compounds. The polyketide moiety is predominant in meroterpenoids derived from fungi (Birch, 1967). Meroterpenoids with the 5/6/6/6 or the 6/6/6/6 tetracyclic rings seemed to be formed through the mevalonate pathway. Jiang et al. reported a comprehensive analysis of the chemical scaffolds seen in meroterpenoids and a distribution of the meroterpenoids discovered in the last decade within these classes (Jiang et al., 2021). Similarly, the focus on the chemical diversity of meroterpenoids from fungi of marine origin by El-Demerdash et al. proves useful in comprehending the structural features of the meroterpenoids (El-Demerdash et al., 2020a).

Meroterpenoid compounds have been studied in the recent decade for a wide spectrum of biological activity. These compounds possess many activities such as anti-cholinesterase, alpha-glucosidase, COX-2 inhibitory, anti-bacterial, anti-viral, anti-leishmanial, anti-obesity, anti-diabetic, anti-oxidative, anti-neoplastic, insecticidal, and cardioprotective. This diverse but promising spectrum of biological activities has also surged a simultaneous interest in the study of total synthesis of meroterpenoids; to name a few, berkeleyone A, from a fungal origin, merochlorins A and B, from marine origin, lingzhiol, from various species of mushrooms, and tomentosenol A and (±)-guajadial B from a plant origin have been explored for total synthesis (Liu et al.; Gao et al., 2012; Teufel et al., 2014; Gautam, 2016; Yu et al., 2016; Elkin et al., 2017). Semisynthetic analogs from isocupressic acid (strongylophorines), (+)-bicyclogermacrene ((+)-ledene, (+)-viridiflorol, (-)-patrol, (+)-spathulenol, and psiguadials A, C, and D) and many others have also been structurally explored (Tran and Cramer, 2014; Yu et al., 2016). Even several workers have scrutinized the structure-activity relationships of meroterpenoids to improve the observed biological activity. Limited review articles are published on meroterpenoids. The first review of meroterpenoid obtained from fungi was published by Shiomi et al. (1999). Later. Geris and Simpson (2009a) published one more review of meroterpenoids obtained from fungi, and the review was mainly focused on the phytochemistry aspects of meroterpenoids. Then, Matsuda and Abe (2016) published a review of the biosynthesis of meroterpenoids from fungi. Recently, two reviews have been published on the chemistry and biology of meroterpenoids derived only from fungi (El-Demerdash et al., 2020b; Jiang et al., 2021). However, a comprehensive review of meroterpenoids derived from different sources such as plants, fungi, and marine sources is unavailable. Thus, the present review mainly focuses on meroterpenoids from these sources with respect to chemistry, biological activity, and the synthesis approach of biologically active meroterpenoids.

Methods

The data have been collected from various sources such as PubMed, ScienceDirect, Scopus, ProQuest, EBSCO, and google scholar. Research and review articles from the year 1999 onward were thoroughly reviewed. Meroterpenoids, fungi, algae, and plants in combination with meroterpenoids have been used as keywords to collect the data.

Strategies for total or partial synthesis of meroterpenoids

The natural biosynthesis of meroterpenoids involves the pathways of terpenoids and polyketide synthesis, which makes the overall process intriguing. Considering the complex stereochemistry existing within the meroterpenoids makes synthesizing pure enantiomers synthetically a challenging and humongous task. Several researchers have reported the total synthesis of meroterpenoids or precursor molecules leading to the synthesis of meroterpenoids. Strongylophorines; gujadial; psidial A; (+) yahazunol; guadials B and C; guapsidial A and psiguajadial D; drimane meroterpenoids; naphthoquinone-based meroterpenoids; ganocins B and C; (+) ledene; (+)-viridiflorol; (-)-palustrol; (+)-spathulenol; psiguadials A, C, and D; (±) berkeleyone A; and biscognienyne B have been attempted (Laube et al., 2002; Lawrence et al., 2010; Tran and Cramer, 2014; Liu Y. et al., 2016; Yu et al., 2016; Elkin et al., 2017; Miles et al., 2017; Dethe et al., 2018; Wang et al., 2020). Petrovčič et al. have critically reviewed the synthesis protocols adopted by various studies that have attempted the total synthesis of meroterpenoids since 2015. Cycloadditions, Suzuki reaction, Diels Alder reaction using dienophiles such as caryophyllene and α-humulene, and groups leading to innovative polyene cyclization termination have been thoroughly exploited for the total synthetic procedures. Similarly, chemoenzymatic methods have been exploited for oxidation reactions in several methods (Petrovčič et al., 2021).

Biological activities of meroterpenoids

Cytotoxic activity of meroterpenoids

Cytotoxicity studies of meroterpenoids isolated from the fungus

Meroterpenoids of different types isolated from various fungal species such as *Phoma* sp., *Pseudocosmospora* sp., *Ascochyta viciae* Lib., *Neosetophoma*, *Ganoderma cochlear* (Blume & T. Nees) Bres., *Stachybotrys chartarum* (Ehrenb.), *Antrodia cinnamomea* (Chang & Chou), *Streptomyces* sp., *Neosartorya spinosa* (Raper & Fennell) Kozak., *Emericella nidulans*, *Gliomastix* sp., *Xylaria humosa*, *Penicillium* sp., *Eurotium chevalieri*, *Guignardia mangiferae* A.J. Roy, *Peyronellaea coffeae-arabicae* FT238, *Aspergillus terreus* Thom, *Aspergillus insuetus* (Bainier) Thom & Church, *Stachybotrys bisbyi* G.L. Barron, and *Pestalotiopsis fici* have been reported for their moderate-to-potent cytotoxic effect in various cancer cell lines.

Nakamura et al. reported the cytotoxic effect of two isolated meroterpenoids, namely, rel-(6′S, 10′R)-decarboxy-Δ^9-tetrahydrocannabinolic acid B and rel-(6′S, 10′R)-Δ^9-tetrahydrocannabinolic acid B, against promyelocytic leukemia (HL60) with IC_{50} of 1.6 and 24.1 μM, respectively (Nakamura et al., 2019). Qin et al. isolated dimeric meroterpenoid compounds from *Ganoderma cochlear* (Blume & T. Nees) Bres. fruiting bodies, namely, (+) and (-)-gancochlearols A and B, and cochlearoids N–P. The study demonstrated that (+) and (-)-gancochlearols A and B were cytotoxic against erythroleukemic and hepatocarcinoma cells and also inhibited COX-2 expression (Qin et al., 2018b). Cochlearoids N and P showed a potent cytotoxic effect against erythroleukemia-type cells (Qin F.-Y. et al., 2019). Two more meroterpenoids, gancochlearol D and ganomycin F, have been reported for their cytotoxic effect against lung cancer cells of various types, with ganomycin F being more potent than gancochlearol D (Cheng et al., 2018). Spirocochlealactones A–C also have a potential cytotoxic effect against A549, Huh-7, and K562 cancer cell lines (Qin F.-Y. et al., 2018). Zhang et al. isolated two tropolonic meroterpenoids, phomanolides D and F, which exhibited a cytotoxic effect against glioma, breast cancer, and cervical cancer cells (Zhang et al., 2019c). Ascochlorin isolated from *Ascochyta viciae* also showed a potent cytotoxic effect on breast cancer cells (Quan et al., 2019). Eupenifeldin and dehydroxyeupenifeldin isolated from *Neosetophoma* reported a cytotoxic effect against a board cancer cell lines (i.e., ovarian, breast, lung cancer, and mesothelioma cells) (El-Elimat et al., 2019). Jagels et al. isolated moderately cytotoxic meroterpenoids, stachybotrychromenes A and B, from *Stachybotrys chartarum* (Ehrenb.) (Jagels et al., 2018). Antroquinonol A biosynthesized by the fungus *Antrodia cinnamomea* (Chang & Chou) has been reported as a potent tumor growth inhibitor against lung and prostate cancer with GI_{50} values of 13.5 ± 0.2 and 5.7 ± 0.2 μM. Furthermore, antroquinonol V reported growth inhibitory activity with GI_{50} values of 8.2 ± 0.8 μM against lung cells (Chen M. C. et al., 2017). Quinadoline A, 1-hydroxychevalone C, 1,11-dihydroxychevalone C, and 1-acetoxychevalone C, isolated from the fungus *Neosartorya spinosa* (Raper & Fennell) Kozak., displayed cytotoxicity against lung and breast cancer cells (Rajachan et al., 2016). Emeriphenolicins E, which is an isoindolone containing meroterpenoid isolated from *Emericella nidulans*, has been reported with a potent cytotoxic effect in hepatic cancer cells (Zhou et al., 2016). Purpurogemutantin, macrophorin A, 4′-oxomacrophorin, 2,3-hydrodeacetoxyyanuthone A, 22-deacetylyanuthone A, and anicequol isolated from fungus *Gliomastix* sp. exhibited potent-to-moderate cytotoxic effect in various cell lines (He W. J. et al., 2017). Arisugacin B and arisugacin F isolated from the fungus *Penicillium* sp. exhibited weak cytotoxicity with IC_{50} values in the range of 24–60 μM against cervical cancer and leukemia cells (Sun et al.,

TABLE 1 Sources and biological activity of fungus meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Pseudocosmospora sp. Bm-1-1	Rel-(6′S, 10′R)-Δ^9 -tetrahydrocannabinolic acid B; rel-(6′S, 10′R)-decarboxy-Δ^9-tetrahydro cannabinolic acid B	Cytotoxicity	Nakamura et al. (2019)
Ganoderma cochlear (Blume & T. Nees) Bres.	(±) Gancochlearols A and B	Cytotoxicity; COX-2 inhibitory	Qin et al. (2018b)
Ganoderma cochlear (Blume & T. Nees) Bres.	(±) Cochlearoids N–P	Cytotoxicity, anti-bacterial, BRD4 inhibitors	Qin et al. (2019a)
Ganoderma cochlear (Blume & T. Nees) Bres.	Gancochlearols D and C; ganomycin F	Cytotoxicity, N-acetyltransferase	Cheng et al. (2018)
Ganoderma cochlear (Blume & T. Nees) Bres.	(+)- and (−)-Spirocochlealactones A–C; ganodilactone	Cytotoxicity, COX2 inhibitors	Qin et al. (2018a)
Phoma species	Phomanolides D (2); phomanolide F (4)	Cytotoxicity	Zhang et al. (2019c)
Ascochyta viciae	Ascochlorin; 5, 6, 7a, 7b	Cytotoxicity	Quan et al. (2019)
Neosetophoma species	Eupenifeldin; dehydroxyeupenifeldin	Cytotoxicity	El-Elimat et al. (2019)
Stachybotrys chartarum (Ehrenb.) DSMZ 12880 (chemotype S)	Stachybotrychromens A and B	Cytotoxicity	Jagels et al. (2018)
Antrodia cinnamomea	Antroquinonols A, V, W	Cytotoxicity	Chen et al. (2017b)
Neosartorya spinosa	1-hydroxychevalone C; 1-acetoxychevalone C; 1,11-dihydroxychevalone C; Quinadoline A	Cytotoxicity	Rajachan et al. (2016)
Emericella nidulans HDN12-249	Emeriphenolicins E	Cytotoxicity	Zhou et al. (2016)
Gliomastix sp. ZSDS1-F7	Purpurogemutantin, macrophorin A, 4′-oxomacrophorin, 2,3-hydro-deacetoxyyanuthone A, 22-deacetylyanuthone A anicequol	Cytotoxicity; anti-tubercular activity	He et al. (2017a)
Penicillium sp. SXH-65	Arisugacins B and F	Cytotoxicity	Sun et al. (2014)
Xylaria humosa	Chevalones B and C	Cytotoxicity	Sodngam et al. (2014)
Penicillium sp. Sh18	Isopenicin A	Cytotoxicity	Tang et al. (2019)
Eurotium chevalieri	Chevalones B, C, and D	Cytotoxicity	Kanokmedhakul et al. (2011)
Ignardia mangiferae A348	Guignardones Q and S	Cytotoxicity	Sun et al. (2015)
Aspergillus terreus Thom OUCMDZ-2739	Rubrolide S; 5-[(3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-yl)-methyl]-3-hydroxy-4(4-hydroxyphenyl)-2(5H)-furanone; terretonin C	Cytotoxicity	Sun et al. (2018)
Periconia sp. F-31	Periconones B and E	Cytotoxicity, anti-HIV	Liu et al. (2017a)
Aspergillus insuetus (Bainier) Thom & Church (OY-207)	Insuetolides A and C, (E)-6-(40-hydroxy-20-butenoyl)-strobilactone A; strobilactone A, (E,E)-6-(60,70-dihydroxy-20,40-octadienoyl)-strobilactone A	Cytotoxicity, anti-fungal	Cohen et al. (2011)
Pestalotiopsis fici	Pestalofones J	Cytotoxicity	Wang et al. (2016a)
Phoma sp.	Phomanolide A, eupenifeldin	Anti-proliferative	Zhang et al. (2015)
Peyronellaea coffeae-arabicae FT238	11-Dehydroxy epoxyphomalin A	Anti-proliferative	Li et al. (2016b)
Ganoderma cochlear (Blume & T. Nees) Bres.	(±)-Cochlearins A–I	Anti-proliferative, anti-oxidant	Peng et al. (2018b)
Aspergillus terreus	Terreustoxin C, terretonin	Anti-proliferative	Feng et al. (2019)
Ganoderma cochlear (Blume & T. Nees) Bres.	(±)-Cochlactones A and B	Anti-inflammation	Peng et al. (2018a)
Stachybotrys chartarum (Ehrenb.) 952	Stachybonoids A and F, stachybotrysin C, Stachybotrylactone	Anti-inflammation, anti-viral	Zhang et al. (2017)
Aspergillus terreus Thom	Austinoid, 1,2-dehydroterredehydroaustin	Anti-inflammation	Liu et al. (2018b)
Aspergillus terreus Thom	Yaminterritrem B	Anti-inflammation	Liaw et al. (2015)
Talaromyces amestolkine YX1	Amestolkolide B	Anti-inflammation	Chen et al. (2018)
Alternaria sp. JJY-32	Tricycloalternarenes A, B, and C; bicycloalternarenes A, B, C, D, and F; monocycloalternarenes A, B, Cm and D	Anti-inflammation	Zhang et al. (2013)
Penicillium purpurogenum MHz 111	Purpurogenolides B, C, and D; berkeleyacetal C	Anti-inflammation	Sun et al. (2016)
Penicillium brasilianum WZXY-m122-9	Brasilianoids A–E	Anti-inflammation, dermatological diseases	Zhang et al. (2018a)
Guignardia mangiferae A.J. Roy	Mangiterpene C; 2′,3′-seco-manginoid C	Anti-inflammation	Chen et al. (2019)

(Continued on following page)

TABLE 1 (*Continued*) Sources and biological activity of fungus meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Ganoderma theaecolum	Ganotheaecoloid J	COX-2 inhibitory	Luo et al. (2018b)
Ganoderma theaecolum	(±)-Ganotheaecolumols C, D, I, and K; iso-ganotheaecolumol I	COX-2 inhibitory	Luo et al. (2018a)
H. caput-medusae	Caputmedusins A, B, and C	α-Glucosidase inhibitors	Chen et al. (2017a)
Aspergillus terreus Thom 3.05358	Amauromine B, austalides N	α-Glucosidase inhibitors	Shan et al. (2015)
Myrothecium sp. OUCMDZ-2784	Myrothecisins A–D, myrothelactone A, myrothelactone C, tubakialactone B, acremonone G	α-Glucosidase inhibitors	Xu et al. (2018)
Ganoderma leucocontextum	Ganoleucins A and C; ganomycins I, B, and C; fornicins C and B	α-Glucosidase inhibitors, HMG-CoA inhibitors	Wang et al. (2017)
Ganoderma sinense	Applanatumol I	Anti-oxidant	Gao et al. (2018)
Ganoderma capensa	Ganocapensins A and B; ganomycins E, F, I, and C; fornicins E and B	Anti-oxidant	Peng et al. (2016b)
Perenniporia medulla-panis	Perennipins A–C, (+)-fornicin A	Anti-oxidant	Kim et al. (2019)
Phyllosticta sp. J13-2–12Y	(*S,Z*)-Phenguignardic acid methyl ester	Anti-microbial	Yang et al. (2017)
Penicillium sp. T2-8	Preaustinoid D, dihydroxyneogrifolic acid; preaustinoid A1, austin, (S)-18,19-dihydroxyneogrifolin	Antimicrobial, anti-bacterial	Duan et al. (2016)
Cytospora spieces	Cytosporolides A–C	Antimicrobial	Li et al. (2010)
Aspergillus sp. TJ23	Spiroaspertrione A, andiconin B	Anti-microbial	He et al. (2017c)
Ganoderma orbiforme	Ganoboninone G, ganomycin I	Anti-bacterial	Li et al. (2018d)
Emericella sp. TJ29	Emervaridone A	Anti-bacterial	He et al. (2017b)
Penicillium sp. SCS-KFD09	Chrodrimanins K and N, verruculides B2, 3-hydroxypentcecilide A	Anti-bacterial, anti-viral	Kong et al. (2017)
Penicillium citrinum	Penicimarins G and H, dehydroaustin, 11β-acetoxyisoaustinone, austinol	Anti-bacterial	Huang et al. (2016)
Dysidea sp.	Dysidphenols A and C, smenospongimine, smenospongine, smenospongorine, smenospongiarine, smenospongidine	Anti-bacterial	Zhang et al. (2016)
Aspergillus terreus	Terreusterpenes A, B, and D	BACE1 inhibitory, AchE inhibitors	Qi et al. (2018b)
Aspergillus terreus	Asperterpenes E, F, and J	BACE1 inhibitory	Qi et al. (2018a)
Aspergillus terreus	Asperterpenes A and B	BACE1 inhibitory	Qi et al. (2016)
Aspergillus terreus Thom	Spiroterreusnoids A–F	BACE1 inhibitory, AchE inhibitory	Qi et al. (2019)
Ganoderma applanatum	Applanatumols A and (+) B	Renal fibrosis	Luo et al. (2016)
Aspergillus sp. 16-5c	Isoaustinol, dehydroaustin, dehydroaustinol	AchE inhibitors	Long et al. (2017)
Ganoderma cochlear (Blume & T. Nees) Bres.	Ganocin D	AchE inhibitors	Peng et al. (2014)
Ganoderma species	(+)-Zizhines G, (−)-zizhines G, (−)-ganosinensols A, (+) zizhines P, (−) zizhines P, (+)-zizhines Q, (−) zizhines Q	AchE inhibitors	Luo et al. (2019a)
Ganoderma capense	Ganocapenoids C, ganocalidin E, cochlearin I, patchiene A	AchE inhibitors	Liao et al. (2019)
Penicillium spices	Arisugacins D, M, O, P, and Q	AchE inhibitors	Dai et al. (2019)
Verticillium albo-atrum	Acetoxydehydroaustin A, austin	Activation of sodium channel	Wu et al. (2018)
Aspergillus aureolatus HDN14-107	Austalides U and I, merochlorin D, austalide P acid	Anti-viral	Peng et al. (2016a)
Penicillium funiculosum GWT2-24	Chrodrimanins A, E, and F	Anti-viral	Zhou et al. (2015)
Talaromyces sp. CX11	Talaromyolide D (4)	Anti-viral	Cao et al. (2019)
Ganoderma lingzhi	Lingzhilactone B	Renal protective activity	Yan et al. (2015b)
Ganoderma lingzhi	Spirolingzhines A, B, C, and D; lingzhines B, D, E, and F; 4-(2,5-dihydroxyphenyl)-4-oxobutanoic acid	Neural stem cell (NSC) proliferation	Yan et al. (2015a)
Penicilium purpurogenum	Dhilirolide L	Insecticidal	Centko et al. (2014)
Penicillium lividum KMM 4663 and *Penicillium thomii* KMM 4645	Austalide H acid, austalide H acid butyl ester, 13-O-deacetylaustalide I, 13-deacetoxyaustalide I	Inhibition of AP-1	Zhuravleva et al. (2014)
Endophytic Penicillium brasilianum found in the *Melia azedarach* root bark	Brasiliamide A	Antimicrobial	Fill et al. (2009)
Ganoderma lucidum	Dayaolingzhiols D–E	AchE inhibitors	Luo et al. (2019b)

(Continued on following page)

TABLE 1 (*Continued*) Sources and biological activity of fungus meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Ganoderma austral	Ganomycin C, (−)-ganoresinain A, ganotheaecoloid G	Neuroprotective activity	Zhang et al. (2019b)
Ganoderma applanatum	Spiroapplanatumines G and H	Inhibitors of JAK3	Luo et al. (2017)
Ganoderma petchii	Petchiethers A and B	Renal protective activity	Li et al. (2016a)
Ganoderma petchii	Petchienes B and (-) D	Increase intracellular free calcium	Gao et al. (2015)
Ganoderma cochlear (Blume & T. Nees) Bres.	Cochlearoids F –I, cochlearoid K	Renal protective activity	Wang et al. (2016b)
Ganoderma cochlear (Blume & T. Nees) Bres.	Cochlearols S, U, X, and Y	Renal protective activity	Wang et al. (2019b)
Ganoderma cochlear (Blume & T. Nees) Bres.	Cochlearol K, cochlearin E	Renal protective activity	Wang et al. (2019a)
Ganoderma cochlear (Blume & T. Nees) Bres.	(+)- and (−)-cochlearols A and B	Renal protective activity	Dou et al. (2014)
Ganoderma lucidum	Chizhine F, fornicin B, ganomycin I	Renal protective activity	Luo et al. (2015)
Ganoderma lucidum	Lingzhifuran A, lingzhilactone D	Anti-fibrotic activity	Ding et al. (2016b)
Mangrove endophytic fungus Diaporthe sp. SCSIO 41011	Chrodrimanins A, B, E, H, G, and F	Insecticidal	Luo et al. (2019c)
Boletinus asiaticus	Asiaticusinol C, asiachromenic acid, asiaticusin A	BACE1 inhibitory	Yatsu et al. (2019)
Phyllosticta capitalensis	Guignardianone C	Phytotoxic activity (plant toxicity)	Ma et al. (2019)

2014). Sodngama et al. isolated chevalones B and C and reported their cytotoxicity activity against the human lung cancer cell line, NCI-H187, with IC_{50} values of 21.4 and 17.7 μg/ml (Sodngam et al., 2014). An unprecedented terpenoid-polyketide meroterpenoid (isopenicin A) isolated from the culture of *Penicillium* sp. sh18 exhibited stronger growth inhibitory effects on colon cancer cells. Isopenicin A selectively suppresses the Wnt signaling pathway-induced ST-Luc transcription with an IC_{50} value of 9.80 μM. Moreover, elevated ST-Luc activity was significantly decreased by isopenicin A in both SW620 and HCT116 cells (Tang et al., 2019). Kanokmedhakul et al. reported the potent cytotoxic meroterpenoid (chevalone B) with IC_{50} values of 3.9 and 2.9 μg/ml against lung and epidermal carcinoma cells. Chevalones C and D also showed cytotoxic effects with IC_{50} values of 8.7 and 7.8 μg/ml against the BC1 cell line (Kanokmedhakul et al., 2011). Guignardones Q and S isolated from the fungal strain *Guignardia mangiferae* A.J. Roy were reported for their cytotoxic effects against breast cancer cells. However, these compounds showed a weak inhibitory effect on tumor growth (Sun et al., 2015). Terretonin C and rubrolide S, 5-[(3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-yl)-methyl]-3-hydroxy-4(4-hydroxyphenyl)-2(5H)-furanone isolated from *Aspergillus terreus* Thom demonstrated potent cytotoxic effects against breast cancer and leukemia cells (Sun et al., 2018). Meroterpenoid periconones E isolated from the fungus *Periconia* reported a cytotoxic effect against breast cancer cells with an IC_{50} value of 4.2 μmol/L (Liu J. M. et al., 2017). Meroterpenoid insuetolides C, (E)-6-(40- hydroxy-20-butenoyl)-strobilactone A, and (E,E)-6-(60,70-dihydroxy-20,40-octadienoyl)-strobilactone A isolated from the ethyl acetate extract of the fungus *Aspergillus insuetus* (Bainier) Thom and Church (1929) inhibited the MOLT-4 cell line proliferation at 50 μg/ml by 51%, 55%, and 72%, respectively (Cohen et al., 2011). Wang et al. also isolated meroterpenoid pestalofones J and reported a weak cytotoxic activity from the fungus *Pestalotiopsos fici* (Wang B. et al., 2016). Recently, two more meroterpenoids (phomeroids A and B) isolated from the fungus *Phomopsis tersa* FS441 reported their cytotoxic effect in various cell lines (SF-268, HepG-2, A549, and MCF-7) (Chen et al., 2020). Andrastin-type meroterpenoids, namely, penimeroterpenoid A, recently isolated from *Penicillium* species, showed a moderate cytotoxic effect against A549, HCT116, and SW480 cell lines (Ren et al., 2021). Tropolactones A, B, and C isolated from the fungus *Aspergillus* reported a cytotoxic potential against human colon carcinoma (HCT-116) with IC_{50} values of 13.2, 10.9, and 13.9 μg/ml (Table 1 and Figure 1).

Cytotoxicity studies of meroterpenoids isolated from marine source

Meroterpenoids isolated from marine sources such as *Dactylospongia*, the marine strain of actinomycetes,

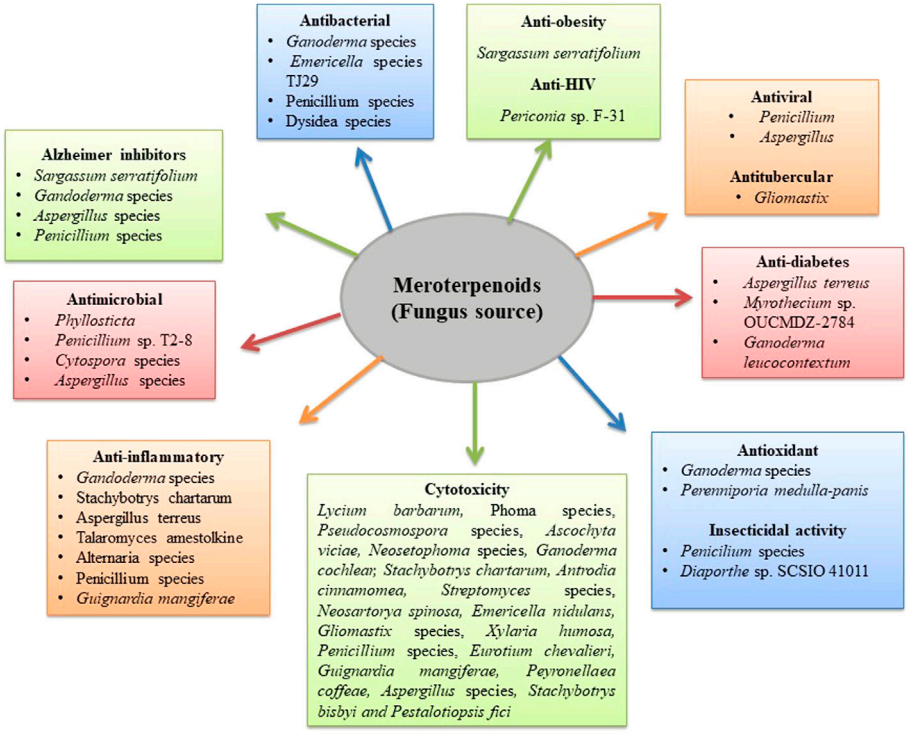

FIGURE 1
Biological activity of fungus meroterpenoids.

Lobophytum crissum von Marenzeller, Dysidea, and *streptomyces* have also been reported for their potential cytotoxic effects. Sesquiterpene and drimane meroterpenoids isolated from *Dactylospongia elegans* (Thiele, 1899) and other species of *Dactylospongia* have been reported as potential cytotoxic agents in various cancer cell lines. Reports show that 19-O-methylpelorol demonstrated a potential cytotoxic effect with an IC_{50} value of 9.2 μM in lung cancer cell lines (PC-9) (Li J. et al., 2018). Yu et al. evaluated the cytotoxic potential of 19-methoxydictyoceratin-A, smenospongiarine, smenospongorine, smenospongimine, and dictyoceratin-C meroterpenoids isolated from *Dactylospongia elegans* (Thiele, 1899) against prostate, pancreatic, and liver cancer cells. They reported that 19-methoxydictyoceratin-A exhibited a moderate activity, whereas smenospongiarine, smenospongorine, smenospongimine, and dictyoceratin-C demonstrated a potent effect with IC_{50} values in the range of 2–37.85 μM in all cancer cell types (Yu et al., 2019). Ebada et al. isolated drimane meroterpenoid metabolites, 5-epi-ilimaquinone, 5-epi-smenospongine, isospongiaquinone, isosmenospongine, and nakijiquinones A and G, from marine sponge *Dactylospongia elegans* (Thiele, 1899), which were assessed for *in vitro* cytotoxicity in mouse lymphoma cells. Results displayed that among the isolated compounds, 5-epi-smenospongidine and isospongiaquinone were the most active with similar IC_{50} values of 1.34 μM in addition to 5-epi-ilimaquinone, isosmenospongine, and nakijiquinones A and G, which showed potent activity (Ebada et al., 2017). A marine strain of actinomycetes has also been reported to contain meroterpenoids with a potent cytotoxic effect. Marinocyanins A and B demonstrated a potent cytotoxic effect against colon cancer cells (Asolkar et al., 2017). Additionally, napyradiomycins 1 to 4 isolated from actinomycete also confirmed a cytotoxic effect *via* cell apoptosis in colon adenocarcinoma cells with an IC_{50} value of around 1 and 2 μM (Farnaes et al., 2014). Cheng et al. also reported the cytotoxic potential of napyradiomycins A and B4 isolated from *Streptomyces* strain with an IC_{50} value between 1 and 5 μg/ml against colon cancer cells (Cheng et al., 2013). The soft coral *Lobophytum crissum* von Marenzeller has also been reported for the presence of potential cytotoxic meroterpenoid, namely, pseuboydone C, cyclo-(Phe-Phe), speradine C, 24,25-dehydro-10,11-dihydro-20-hydroxyaflavinin, and aflavinine, with the IC_{50} mean values of 0.7, 0.8, 0.9, 0.5, and 0.4 μM, respectively, against insect cell line SF9 (Lan et al., 2016). Kim et al. isolated six new drimane sesquiterpene hydroquinone meroterpenoids along with arenarol from *Dysidea* sp. Sponge. The cytotoxic investigations on K562 and A549 cell lines showed that aureol B; melemeleones C and D; cycloaurenones A, B, and C; and arenarol showed cytotoxic activity comparable to doxorubicin

TABLE 2 Sources and biological activity of marine meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Dactylospongia sp.	Dactylospongins A, B, and D, Ent-melemeleone B, dysidaminone N, 19-O-methylpelorol	Cytotoxicity, Anti-inflammation	Li et al. (2018c)
Dactylospongia elegans	19-Methoxy-dictyoceratin-A, smenospongiarine, smenospongorine, smenospongimine, dictyoceratin-C	Cytotoxicity	Yu et al. (2019)
Dactylospongia elegans	5-Epi-ilimaquinone, 5-epi-smenospongidine, isospongiaquinone, isosmenospongine, nakijiquinones A and G	Cytotoxicity	Ebada et al. (2017)
Dysidea species	Aureol B; melemeleones C and D, cycloaurenones A, B, and C; Arenarol	Cytotoxicity	Kim et al. (2015)
Dysidea avara	Dysideanones A and B	Cytotoxicity	Haque et al. (2018)
Smenospongia aurea (08FL-20-B), *Smenospongia cerebriformis* (08FL-20)	(+)-5-Epi-ethylsmenoquinone	Cytotoxicity	Hwang et al. (2015)
Haliclona (Soestella) mucosa	Panicein A hydroquinone, paniceins B2, B3, and C	Cytotoxicity	Fiorini et al. (2015)
Dysidea villosa	Dysivillosins A–D	Anti-inflammation	Jiao et al. (2017)
Dysidea septosa	Septosones A and C	Anti-inflammation	Gui et al. (2019)
Okinawan marine sponge (SS-1202)	Nakijiquinone S, nakijinol C	Anti-microbial	Suzuki et al. (2014)
Spongia species	Langcoquinpne C, smenospongorine	Anti-bacterial	Nguyen et al. (2017)
Spongia spieces	Langcoquinones A and B, dictyoceratin A, ilimaquinone, smenospongine, smenospongidine, nakijiquinone L	Anti-bacterial	Li et al. (2018b)
Callyspongia spices	Isoakaterpin	Anti-leishmanial	Gray et al. (2007)
Dysidea species	Avinosol, avarone, avarol, avinosone	Anti-invasion activity	Diaz-Marrero et al. (2006)
Acanthodendrilla species	(+)-Makassaric acid, (+)-subersic acid	Inhibitors of protein kinase MK2	Williams et al. (2004)
Actinomycete strains CNS-284 and CNY-960	Marinocyanins A and B	Cytotoxicity	Asolkar et al. (2017)
Actinomycete species	Napyradiomycins 1–4	Cytotoxicity	Farnaes et al. (2014)
Streptomyces strains	Napyradiomycins A and B4	Cytotoxicity	Cheng et al. (2013)
MAR 4 *Streptomyces* Strains	Napyradiomycins A and B3	Anti-microbial	Cheng et al. (2013)
Streptomyces sp.	Merochlorins E and F	Anti-bacterial	Ryu et al. (2019)
Streptomyces sp. strain CNQ-525	A80915A, A80915B	Anti-bacterial	Haste et al. (2011)
Kappaphycus alvarezii (Doty) Doty ex Silva (family Solieriaceae)	2-Ethyl-6-(4-methoxy-2-((2-oxotetrahydro-2Hpyran-4-yl) methyl) butoxy)-6-oxohexyl 5-ethyloct-4-enoate (C29)	Anti-inflammation Antioxidant	Makkar and Chakraborty, (2018)
Stypopodium flabelliforme	Sargaol, epitaondiol, stypodiol, isoepitaondiol	Gastroprotective	Areche et al. (2015)
Aspergillus sp. ZL0-1b14	Aspertetranones A–D	Anti-inflammation	Wang et al. (2015b)
Penicillium sp. YPGA11	Conidiogenone C	Anti-oxidant	Cheng et al. (2019)
Aspergillus terreus Thom EN-539	Aperterpenes N, terretonin G	Anti-microbial	Li et al. (2019b)
Aspergillus terreus	(22E,24R)-Stigmasta-5,7,22-trien-3-b-ol, stigmast-4-ene-3-one, aspernolides F	Anti-microbial, anti-leishmanial	Ibrahim et al. (2015)
Aspergillus versicolor	Asperversins G	AchE inhibitors	Li et al. (2018b)
Penicillium sp. SK5GW1L	3-Epiarigsugacin E, arisugacin B, territrem C, terreulactone C	AchE inhibitors	Ding et al. (2016a)
Penicillium sp. SF-5497	Preaustinoid A6, berkeleyone C	PTP1B inhibitors	Park et al. (2019)
Aspergillus insuetus	Terretonins E and F, aurantiamine	Mammalian mitochondrial respiratory chain Inhibitors	López-Gresa et al. (2009)
Corbiculid bivalve clam and *Villorita cyprinoides*	Dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl) furan-2(3H)-one; tetrahydro-3-methoxy-5-((E)-8,12-dimethyloct-8 enyl)-pyran-2-one; (12E)-(3,4,6,7,8,8a-hexahydro-1H-isochromen-3-yl)-methyl-hept-12-enoate; (10E)-butyl-9-(6-ethyl-3,4,6,7,8,8a-hexahydro-1H-isochromen-3-yl)-pent-10-enoate	Anti-inflammation; COX2 inhibition; Anti-oxidant	Joy and Chakraborty (2018)
Ascidian *Aplidium scabellum*, 322	2-Geranyl-6-methoxy-1,4- hydroquinone-4-sulfate, scabellone B, 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzo-pyran- 6-ol, 2-geranyl-6-methoxy-1,4-hydro-quinone	Anti-inflammatory, anti-plasmoid activity	Chan et al. (2011)

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TABLE 2 (*Continued*) Sources and biological activity of marine meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Antarctic Ascidian, *Aplidium* species	Rossinones A and B	Anti-oxidant	Appleton et al. (2009)
Botryllus tuberatus	Tuberatolides A and B, 2′-*epi*-tuberatolide B, yezoquinolide (*R*)-sargachromenol, (*S*)-sargachromenol	Human farnesoid X receptor (Hfxr), activated chenodeoxycholic acid (CDCA)	Choi et al. (2011)
Dysidea species	(+)-Yahazunone, (+)-chromazonarol	Anti-fungal	Zhang et al. (2018b)
Cystoseira baccata	(3R)- and (3S)-tetraprenyltoluquinol; (3R)- and (3S)-tetraprenyltoluquinone	Anti-leishmanial	Bruno de Sousa et al. (2017)
Lobophytum crissum, 200	Pseuboydone C; cyclo-(Phe-Phe), speradine C; aflavinine; 24,25-dehydro-10,11-dihydro-20-hydro-xyaflavinin	Cytotoxicity	Lan et al. (2016)

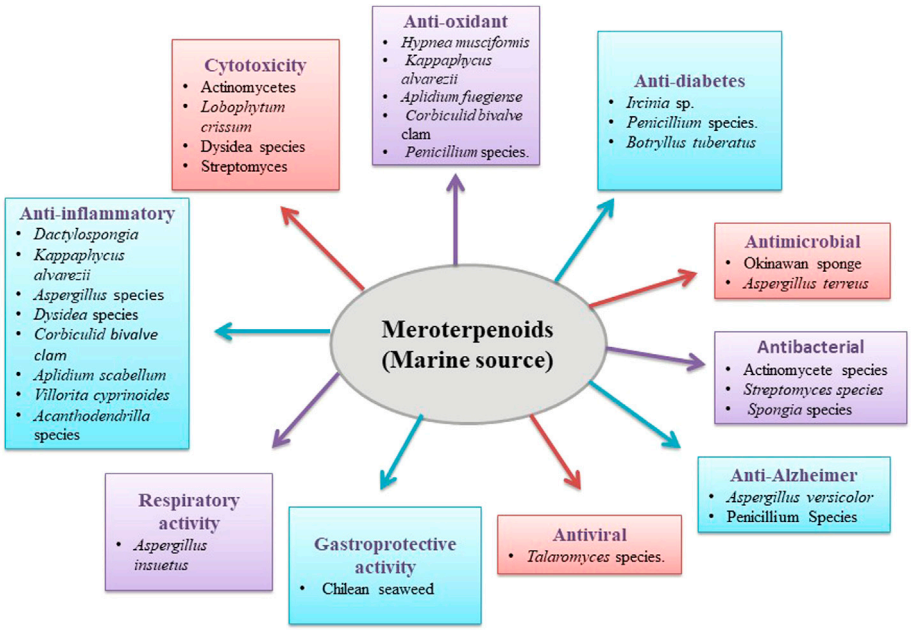

FIGURE 2
Biological activity of marine meroterpenoids.

and showed an IC_{50} value below 10 μM. It was reported that aureol B and arenarol were the most potent meroterpenoids with a potent cytotoxic effect (Kim et al., 2015). Dysideanones A and B, two meroterpenoids isolated from *Dysidea avara* (Schmidt, 1862), also showed moderate cytotoxic activity against colon cancer cells (Haque et al., 2018). (+)-5-Epi-ethylsmenoquinone isolated from *Smenospongia* was reported as cytotoxic meroterpenoid against two different colon cancer cell lines with IC_{50} values of 3.24 and 2.95 μM (Hwang et al., 2015). Fiorini et al. reported that paniceins B2, B3, and C and particularly panicein A hydroquinone, which is a natural meroterpenoid formed by the mucosa of the Mediterranean sponge *Haliclona (Soestella)*, could inhibit the function of the patched model doxorubicin efflux built from AcrB structure, and *in vitro* melanoma cells cytotoxicity was enhanced by the doxorubicin. Four meroterpenoids, panicein B2, B3, and C and panicein A hydroquinone were tested for cytotoxicity. These meroterpenoids exhibited moderate cytotoxicity above the micromolar range with panicein A hydroquinone inhibiting CCRF-CEM leukemia cells most selectively with a cytostatic effect (TGI) of 25 μM (Fiorini et al., 2015) (Table 2 and Figure 2).

Cytotoxicity studies of meroterpenoids isolated from plants

Herbal plants are also one of the major sources of different types of meroterpenoids with cytotoxic activity. Plants from approximately 12–13 different genera, such as *Lycium*

barbarum L., *Psidium*, *Eucalyptus*, *Arnebia*, *Baeckea*, *Pogostemon*, *Eugenia*, *Euphorbia*, *Rhododendron*, *Belamcanda*, *Myrtus*, *Rhodomyrtus*, *Calocedrus*, and *Callistemon*, have been reported to date to possess cytotoxic meroterpenoids in their different parts.

The tetracyclic meroterpenoid, namely, bipolahydroquinones C, cochlioquinones I-M, and cochlioquinones D, isolated from the fungus *Lycium barbarum* L. demonstrated a cytotoxic effect against breast cancer (MDA-MB-231) cell line and squamous cell carcinoma (NCI-H226). The results suggested that meroterpenoids from this species showed a cytotoxic effect in both cell lines. Bipolahydroquinones C and cochlioquinone D showed significant effects with IC_{50} values of 5.5 and 6.9 μM against squamous cell carcinoma cells, respectively. Cochlioquinones I-M were reported to have an IC_{50} value of more than 10 μM against squamous cell carcinoma cells. Similarly, significant inhibition was shown against breast cancer cells by cochlioquinone K (IC_{50} 9.5 μM), bipolahydroquinone C (IC_{50} 6.7 μM), cochlioquinone I (IC_{50} 8.5 μM), cochlioquinone L (IC_{50} 7.5 μM), and cochlioquinone M (IC_{50} 5.6 μM) (Long et al., 2019). Two species of *Psidium* were reported to have cytotoxic meroterpenoids in their leaves. Four sesquiterpene-based meroterpenoid (i.e., psiguadials A, B, C, and D) and monoterpene-based meroterpenoid (guadials C) isolated from *Psidium guajava* L. demonstrated a cytotoxic effect against two hepatic cancer cell line. Psiguadials A, B, C, and D confirmed a potent effect with IC_{50} values below 1 μM against HepG2. However, guadial C and psiguadials A and B showed moderate cytotoxic effects against HepG2/ADM cells (Shao et al., 2010, 2012; Jian et al., 2015). Guajadial, a dialdehyde meroterpenoid, demonstrated a potent cytotoxic effect with an IC_{50} value less than that of the standard drug cisplatin against A549 and H1650 cell lines (Wang et al., 2018a). Other meroterpenoids, namely, guajavadials A–C isolated from *Psidium guajava* L. showed moderate activity against five human cell lines (HL-60, A-549, SMMC-7721, MCF-7s, and SW480), with guajavadial C being the most effective with an IC_{50} value of 3.54 μM toward SMMC-7721 cell lines (Qin et al., 2016). Additionally, meroterpenoids, such as 4,5-diepipsidial A and guajadial B, were also isolated from *Psidium guajava* L. with a weak cytotoxic potential (Qin et al., 2017c). Littordials B, C, and E, formyl phloroglucinol-β-caryophyllene meroterpenoids isolated from *Psidium littorale* Raddi, were active against the MDA-MB-321 cell line, whereas littordials C and E were reported as active compounds against the murine model for human melanoma cells and human lung cancer cells, respectively (Xu et al., 2019). Qin et al. isolated cytotoxic formyl phloroglucinol-terpene meroterpenoid eucalypglobulusal F from *Eucalyptus globulus* Labill. fruits, which demonstrated a potent action with an IC_{50} value of 3.3 μM against T lymphoblastoid cells (Qin et al., 2018e). Three more formyl phloroglucinol meroterpenoids (eucalteretials C, euglobal IX, and euglobal Ib) isolated from the twigs and leaves of *Eucalyptus tereticorni* Sm. by Liu et al. exhibited cytotoxic potential in different cancer cells. Eucalteretial C and euglobal IX were significantly toxic with IC_{50} values of 4.8 and 9.5 μM against HCT116 cells, whereas euglobal Ib was active against DU145 cells with an IC_{50} value of 7.8 μM (Liu H. et al., 2018). *Eucalyptus robusta* Sm. leaves also showed the presence of formyl phloroglucinol meroterpenoid eucalrobusone C with a cytotoxic effect against liver, breast, and bone cancer cells (Shang et al., 2016a). In a similar study, eucalrobusone C demonstrated a cytotoxic effect against liver cancer cells through p38 MAPK pathway-induced apoptosis (Jian et al., 2017). From the roots of *Arnebia euchrome* (Royle) Johnston, thirteen meroterpenoids have been isolated with cytotoxic potential. Arnebinone B and 6S,11Z-2-methoxy-arnebinone B demonstrated a cytotoxic effect against different liver cancer cells. 6S,11Z-2-Methoxy-arnebinone B exhibited the most potent activity against SMMC-7721, HepG2, QGY-7703, and HepG2/ADM human liver cancer cell lines, whereas arnebinone B exhibited moderate growth inhibitory effects against HepG2/ADM (Wang et al., 2018b). Furthermore, arnebinols A and C, 8-O-dimethyl-11-deoxyalkannin, arnebinone B, clavilactone A, and shikonofurans A, B, and C isolated from the roots of the same species confirmed potent cytotoxic effect against osteosarcoma. However, deoxyalkannin, arnebinone, and shikonofuran A demonstrated strong inhibition against human liver cancer cells (Wang L. et al., 2015). Xu-Jie Qin isolated polymethylated phloroglucinol meroterpenoids (baeckfrutones (-)-B, F, and K) from the leaves and twigs of *Baeckea frutescens* Linnaeus, which exhibited a remarkable activity with IC_{50} values of 1.33, 15.61, and 12.89 μM against human prostate, lung, and colon cancer cells, respectively (Qin et al., 2018f). Nguyen et al. isolated pyrone-sesquiterpenoid meroterpenoids pogostemins A, B, and C from the aerial parts of *Pogostemon auricularius* (L.) Hassk., reporting cytotoxicity against the lung cancer cells, keratin forming tumor cell line, liver, gastric cancer, and colorectal adenocarcinoma cells. The study concluded that pogostemins A showed a potent cytotoxic effect, and pogostemins B and C exhibited a moderate effect against the tested cell lines (Nguyen et al., 2018). Eugenials C, D, and E isolated from the fruit extract of *Eugenia umbelliflora* O. Berg showed cytotoxic potential against myelogenous leukemia and murine melanoma cell (Farias et al., 2018). Rubiginosins A, D, and G and anthopogochromene B, isolated from the flowers of *Rhododendron rubiginosum* Franch. var. *rubiginosum* showed a moderate cytotoxic effect against hepatic and leukemia cells (Yang et al., 2018). Similarly, four meroterpenoids (belamcanoxide A, iridobelamal A, isoiridogermanal, and iridal) isolated from rhizomes of *Belamcanda chinensis* (L.) DC. showed a moderate cytotoxic effect against liver and stomach cancer cells (Ni et al., 2017). Liu et al. isolated meroterpenoids rhodomentones A and B from the

TABLE 3 Sources and biological activity of plant meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Lycium Barbarum	Bipolahydroquinone C, cochlioquinone I, cochlioquinone J, cochlioquinone K, cochlioquinone L, cochlioquinone M, cochlioquinone D	Cytotoxicity	Long et al. (2019)
Psidium guajava L.	Psiguadials A and B, guajadial	Cytotoxicity, anti-proliferative	Shao et al. (2010)
Psidium guajava L.	Guadial C	Cytotoxicity	Jian et al. (2015)
Psidium guajava L.	Guajadial	Cytotoxicity	Wang et al. (2018a)
Psidium guajava L.	Guajavadials A–C	Cytotoxicity	Qin et al. (2016)
Psidium guajava L.	4,5-Diepipsidial A, guajadial B	Cytotoxicity, anti-tumor	Qin et al. (2017c)
Psidium littorale	Littordials B, C, and E	Cytotoxicity	Xu et al. (2019)
Eucalyptus globulus	Eucalypglobulusal F	Cytotoxicity	(Qin et al., 2018e)
Eucalyptus tereticorni	Eucalteretial C, euglobals IX and Ib	Cytotoxicity	Liu et al. (2018a)
Eucalyptus robusta	Eucalrobusone C	Cytotoxicity	Shang et al. (2016a)
Arnebia euchroma	Arnebinone B, 6S,11Z-2-methoxy-arnebinone B	Cytotoxicity	Wang et al. (2018b)
Arnebia euchroma	Arnebinols A and C, 8-odimethyl-11-deoxyalkannin, arnebinone B, clavilactone A, shikonofurans A, B, and C	Cytotoxicity	Wang et al. (2015a)
Baeckea frutescent	Baeckfrutones (-)-B, F, G, (+) I, J, and K	Cytotoxicity, anti-inflammation	Qin et al. (2018f)
Pogostemon auricularius	Pogostemins A–C	Cytotoxicity	Nguyen et al. (2018)
Eugenia umbelliflora fruits	Eugenials C, D, and E	Cytotoxicity	Farias et al. (2018)
Rhododendron rubiginosum Franch.	Rubiginosins A, D, and G, anthopogochromene B	Cytotoxicity	Yang et al. (2018)
Rhododendron dauricum L.	Daurichromenic acid (DCA)	Anti-HIV	Saeki et al. (2018)
Belamcanda chinensis	Belamcanoxide A, iridobelamal A, isoiridogermanal, iridal	Cytotoxicity	Ni et al. (2017)
Rhodomyrtus tomentosa	Rhodomentones A and B	Cytotoxicity	Liu et al. (2016a)
Calocedrus macrolepis var. *Formosana*	Ferrugimenthenol	Cytotoxicity	Hsieh et al. (2011)
Callistemon salignus	Isomyrtucommulone B, callisalignones A, 2,6-dihydroxy-4-methoxy-3-methylisopropiophenone, 2,6-dihydroxy-4-methoxyisovalerophenone, myrtucommulone	Cytotoxicity; anti-microbial	Qin et al. (2017a)
Callistemon salignus	Callisalignenes G, H, and I	Cytotoxicity	Qin et al. (2017b)
Euphorbia fischeriana	Fischernolides B and D	Cytotoxicity	Zhang et al. (2019c)
Baeckea frutescens	Baefrutones A–D	Anti-inflammation	Hou et al. (2018)
Baeckea frutescens	Baeckfrutones (+) N, baeckfrutones S	Anti-inflammation	Zhi et al. (2018)
Baeckea frutescens	Baeckfrutones F, G, (+) I, and J	Anti-inflammation	(Qin et al., 2018f)
Clinopodium chinense (Benth.) O. Kuntze	Clinoposides G and H	Anti-inflammation, Aanti-oxidant	Zhu et al. (2018)
Baeckea frutescens	Frutescones O	Anti-inflammation	Hou et al. (2017)
Hypericum yojiroanum	Yojironin A	Anti-microbial	Mamemura et al. (2011)
Dryopteris championii	Aspidin BB, desaspidin BB, Ddesaspidin PB	Anti-bacterial	Chen et al. (2016)
Eugenia umbelliflora O. Berg	Eugenials C and D	Anti-bacterial	Li et al. (2018b)
Eucalyptus robusta	Eucalrobusones T, U, and (+) X	Anti-fungal	Shang et al. (2019)
Eucalyptus robusta	Eucalrobusones J and O	Anti-fungal	Shang et al. (2016b)
Psoralea glandulosa	Bakuchiol, 3-hydroxy-bakuchiol	Anti-fungal	Madrid et al. (2012)
Eucalyptus robusta	Eucalyptus dimer A, eucalyprobusone A	AchE inhibitors	Qin et al. (2018d)
Rhodomyrtus tomentosa	Rhodomyrtusials A and B, tomentodiones Q	AchE inhibitors	Qin et al. (2019b)
Magnolia officinalis var. *biloba*	Magterpenoids A and C	PTP1B inhibitors	Li et al. (2018a)
Rhododendron capitatum	(−)- and (+)-Rhodonoid B	PTP1B inhibitors	Liao et al. (2015)
Rhododendron nyingchiense	Nyingchinoids (+)A, (+)B, (-)C, (-)D and (+/-)H, grifolin	PTP1B inhibitors	Huang et al. (2018)
Magnolia officinalis var. *biloba*	Magmenthanes E and H	PTP1B inhibitors	Li et al. (2019a)

(Continued on following page)

TABLE 3 (*Continued*) Sources and biological activity of plant meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Hypericum japonicum	Japonicols E and H	Anti-KSHS activities	Hu et al. (2018)
Rhododendron capitatum	(+)-Rhodonoid C	Anti-viral	Liao et al. (2017)
Hypericum japonicum	Hyperjaponols B and D	Anti-viral	Hu et al. (2016)
Cordia oncocalyx	rel-1,4,8α-Trihydroxy-5-furanyl-2-methoxy-8aβ-methyl-6,7,8, 8a,9,10-hexahydro-10-anthracenone; 6- formyl-2-methoxy-9-methyl-1,4-phenanthrendione, rel-10β,11β- epoxy-11β-ethoxy-8α-hydroxy-2-methoxy-8aβ-methyl- 5α,6,7,8,8a,9,10aβ-octahydro-1,4-anthracendione	Neuroinhibitory	Matos et al. (2017)
Melaleuca Leucadendron L.	Melaleucadines A and B	Neuroprotective activity	Xie et al. (2019)
Clinopodium chinense	Clinoposides B, D, and F	Cardioprotective activity	Zhu et al. (2016)
Okara fermented with *Talaromyces* sp. strain YO-2.	Chondrimanins D–F	Insecticidal	Hayashi et al. (2012)
Psoralea corylifolia L.	Bakuchiols, acetylbakuchiol, O-methyl, and O-ethyl bakuchiols	Hypoxia-inducible factor-1 (HIF-1) inhibitory	Wu et al. (2008)
P. corylifolia	(S)-Bakuchiol	Hypoxia-inducible factor-1 (HIF-1) inhibitory	Wu et al. (2007)
Eucalyptus robusta	Eucarobustol E (EE)	Anti-biofilm activity	Liu et al. (2017b)
Psidium guajava L.	Psiguajadials A–L, guajavadials A and C, psiguadials A and D, guapsidial A, psidial A, guajadial, guajadials C–F, guadial A	Phosphodiesterase-4 inhibitors	Tang et al. (2017)

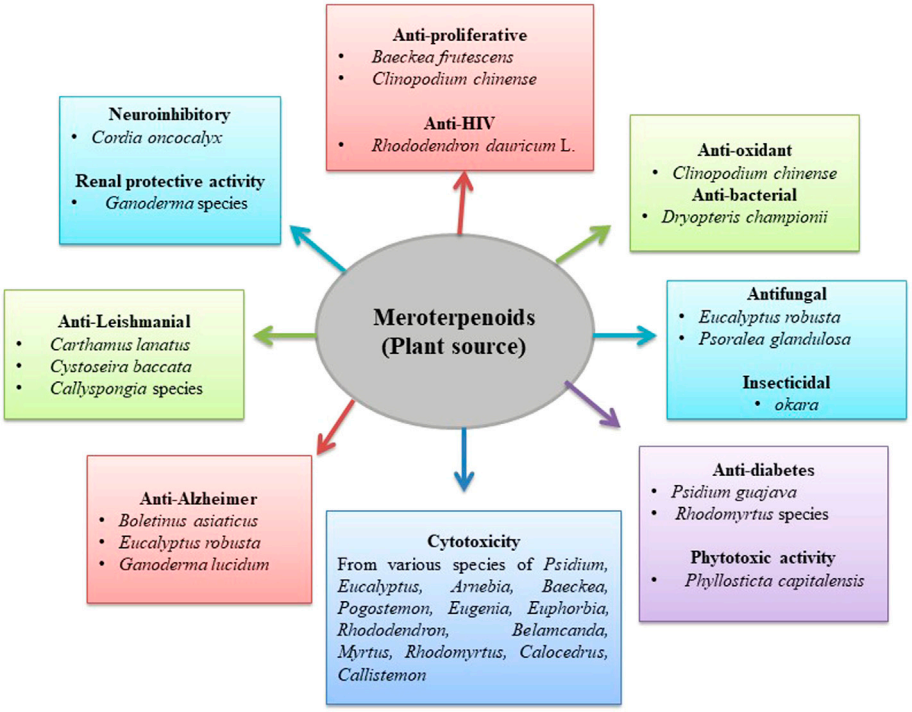

FIGURE 3
Biological activity of plant meroterpenoids.

Rhodomyrtus tomentosa (Aiton) Hassk. leaves, showing a moderate cytotoxic effect (Liu H. X. et al., 2016). Saleh et al. isolated the xanthomonic acid from the mango pathogenic organism *Xanthomonas citri* (Hasse, 1915), which has been reported to show a cytotoxic effect *via* the induction of autophagy. Furthermore, it showed potential effect against embryonic kidney, cervical, and breast cancer cell lines, with higher selectivity toward estrogen-independent breast cancer cells (MDA-MB-231) compared to the estrogen-dependent type (MCF-7) (Saleh et al., 2016). Hsieh et al. isolated secoabietane-type diterpenoid meroterpenoid ferrugimenthenol from the bark of *Calocedrus macrolepis* Kurz var. *formosana*. Results of the study indicated that ferrugimenthenol displayed potent activity against human oral epidermoid carcinoma cells (Hsieh et al., 2011). Qin et al. isolated myrtucommulone D, isomyrtucommulone B, and callisalignenes G–I from the *Callistemon salignus* leaves and twigs. Myrtucommulone D, isomyrtucommulone B, callisalignene G, and H were reported to have potent inhibitory activity. However, callisalignenes I showed a cytotoxic effect against human colon cancer cells. Additionally, callisalignenes G and I displayed cytotoxicity against lung cancer cells, which was more potent than the standard drug VP-16 (Qin et al., 2017a; 2017b). Zhang et al. isolated fischernolides B and D from *Euphorbia fischeriana* Steud. with cytotoxic activity against hepatic, colon, lung, breast, and cervical cancer cell lines. It has been reported that fischernolide B demonstrates a cytotoxic effect by the induction of apoptosis through caspase activation (Zhang et al., 2019a) (Table 3 and Figure 3).

Cytotoxicity studies of meroterpenoids isolated from algae

Meroterpenoids of different types isolated from various algal species such as *Sargassum* and *Cystoseira* were tested against various cancer cell lines and reported cytotoxic activity.

Meroterpenoids isolated from two genera of brown algae have been reported for their cytotoxic effects in various cancer cell lines. Lee et al. isolated sargachromanols J, Q, and R, from *Sargassum* algae, which reported potential cytotoxic effects against human gastric, colon, and fibrosarcoma cancer cell lines with IC_{50} values of 6.5 μg/ml (sargachromanol J), 3.4 μg/ml (sargachromanol Q), and 13.9 μg/ml (sargachromanol R), respectively (Lee et al., 2014). They also isolated sargachromanols E, D, and P meroterpenoids from *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh, 1820. All compounds were tested for their cytotoxic potency against human gastric, colon, fibrosarcoma, and breast cancer cell lines. The results indicated that sargachromanols E, D, and P displayed potent cytotoxicity in AGS cell lines (IC_{50} values of 0.7, 6.1, and 0.7 μg/ml), HT-29 (IC_{50} values of 0.5, 1.0, and 3.3 μg/ml), and HT-1080 cell lines (IC_{50} values of 5.7, 0.8, and 1.8 μg/ml), respectively (Lee et al., 2013). Six new tetraprenyltoluquinol derivatives, two triprenyltoluquinol derivatives, and two new tetraprenyltoluquinone derivatives, 2-[(2′E,6′Z,10′E, 14′Z)-5′-Oxo-15′-hydroxymethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methylhydroquinone, 2-[(2′E,6′E,10′E, 14′Z)-5′-Oxo-15′-hydroxymethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methylhydroquinone, 5-oxoisocystofuranoquinol 2-[(2′E,6′E,10′E, 14′Z)-5′-hydroxy-15′-hydroxymethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methylhydroquinone and 5-oxocystofuranoquinol, were isolated from the brown algae *Cystoseira crinite* Duby, 1830, with moderate cytotoxic activity toward gastric, hepatic, and breast cancer cells (Fisch et al., 2003) (Table 4 and Figure 4).

Anti-proliferative activity of meroterpenoids

Anti-proliferative activity of meroterpenoids isolated from the fungus

Meroterpenoids isolated from various fungus species, such as *Phoma, Peyronellaea coffeae-arabicae FT238*, and *Aspergillus terreus* Thom, have been studied for their anti-proliferative activity against various cancer cells. Reports reveal that phomanolide A and eupenifeldin isolated from the fermentation cultures of solid substrate fungus *Phoma* sp. eupenifeldin effectively inhibited the proliferation of neuroblastoma, glioblastoma, and neuroglioma cells. Similarly, phomanolide A reported an inhibitory effect with an IC_{50} value of 81.1 μM against the neuroblastoma cells. In addition, phomanolide A demonstrated an anti-proliferative effect with an IC_{50} value of 14.3 μM only on cervical cancer cells (HeLa), comparable to that of cisplatin (Zhang et al., 2015). Li et al. isolated meroterpenoid 11-dehydroxy epoxyphomalin A from fungus *Peyronellaea coffeae-arabicae* FT238, showing inhibitory activity against OVCAR3 (mt-p53R248) with an IC_{50} value of 0.5 μM. Furthermore, Stat3 strongly at 5 μM (Li C. S. et al., 2016) (±)-cochlearin D isolated from *Ganoderma cochlear* (Blume & T. Nees) Bres. demonstrated anti-proliferative activity when tested on HSC-T6 cells through inhibition of TGF-β1-induced HSCs proliferation. However, the non-toxic, effective concentration of (±)-cochlearin D has a weak inhibitory effect on TGF-β1 and thus demonstrates a weak anti-proliferative effect (Peng X. et al., 2018). Feng et al. isolated highly oxygenated meroterpenoids from *Aspergillus terreus* Thom (the Antarctic fungus), namely, terreustoxin C and terretonin. The isolated compounds were tested for concanavalin A- (Con A-) induced T-cell proliferation for *in vitro* immunomodulation. It was found that compounds significantly inhibited murine Con A-induced T-cell proliferation at the concentration of 10 μM (Feng et al., 2019). Novel sesquiterpenoid diphenylmethane

TABLE 4 Sources and biological activity of algae meroterpenoids.

Source of meroterpenoid	Name of meroterpenoids	Biological activity	References
Sargassum	Sargachromanols J, Q, and Ra	Cytotoxicity	Lee et al. (2014)
Sargassum siliquastrum	Sargachromanols E, D, and P	Cytotoxicity	Lee et al. (2013)
Cystoseira crinita Duby	2-[(2′E,6′E,10′E,14′Z)-5′-Oxo-15′-hydroxymethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methylhyd- roquinone	Cytotoxicity, anti-oxidant	Fisch et al. (2003)
	2-[(2′E,6′Z,10′E,14′Z)-5′-Oxo-15′-hydroxymethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methylhyd- roquinone		
	2-[(2′E,6′E,10′E)-5′-Oxo-13′-hydroxy-3′,7′,11′,15′-tetra-methylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methyl hydroquinone		
	2-[(2′E,6′Z,10′E)-5′-Oxo-13′-hydroxy-3′,7′,11′,15′-tetra-methylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methyl hydroquinone		
	2-[(2′E,6′E,10′E)-5′-Oxo-3′,7′,11′,15′-tetramethyl hexadeca-2,6,10′,14′-tetraenyl]-6-methyl hydroquinone		
	2-[(2′E,6′Z,10′E)-5′-Oxo-3′,7′,11′,15′-tetramethyl hexadeca-2′,6′,10′,14′-tetraenyl]-6-methyl hydroquinone,		
	2-[(2′E,6′E)-5′-Oxo-3′,7′,11′-trimethyldodeca-2′,6′10′-trie-nyl]-6-methyl hydroquinone		
	2-[(2′E,6′Z)-5′-Oxo-3′,7′,11′-trimethyldodeca-2′,6′,10′-trie- nyl]-6-methyl hydroquinone		
	5-Oxo-cystofuranoquinol		
	5-Oxo-isocysto furanoquinol		
	2-[(2′E,6′E,10′E)-5′,13′-dioxo-3′,7′,11′,15′-tetrameth- ylhexadeca-2′,6′,10′,14′-tetraenyl]-6-methyl hydroquinone		
	2[(2′E,6′E,10′E, 14′Z)-5′-Hydroxy-15′-hydroxym- ethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6- methyl hydro quinone		
Cystoseira usneoids	11-Hydroxy-11-O-methylamentadione (AMT-E)	Anti-inflammation	Zbakh et al. (2016)
Cystoseira usneoides	Cystodione A and B, Amentadione-1′-methyl ether, 6-cis-Amentadione-1′-methyl ether, Usneoidone Z, 11-Hydroxyamentadione-1′-methyl ether	Anti-inflammation, anti-oxidant	De Los Reyes et al. (2013)
Sargassum siliquastrum	Sargachromanols S and T	Anti-oxidant	Kang and Kim (2017)
Sargassum siliquastrum	Sargachromanols A–P	Anti-oxidant	Jang et al. (2005)
Cystoseira tamariscifolia	Cystophloroketals A–D	Anti-microbial	El Hattab et al. (2015)
Sargassum siliquastrum and *C. albicans*	Sargachromanols D, F, H, L, M, and P	Anti-bacterial inhibitors of Na+/K + ATPase, isocitrate lyase (ICL) inhibitors	Chung et al. (2011)
Sargassum serratifolium	Sargahydroquinoic acid, sargachromanol, sargaquinoic acid	BACE1 inhibitory, AchE inhibitory	Seong et al. (2017)

meroterpenoids (psiguadials A and B) along with a pair of known epimer guajadial isolated from the leaves of *Psidium guajava* L. also showed moderate inhibitory activity against hepatocellular carcinoma cells (Shao et al., 2010) (Table 1 and Figure 1).

Anti-inflammatory activity of meroterpenoids

Anti-inflammatory activity of meroterpenoid isolated from the fungus

Meroterpenoids isolated from different natural sources have been extensively studied as anti-inflammatory agents. In order to study the anti-inflammatory effect of meroterpenoids, these compounds were tested on RAW 264.7-induced lipopolysaccharide (LPS) macrophage cells. These cells exhibited increased production of NO, TNF-alpha and other inflammatory parameters. If meroterpenoids could decrease the production of these parameters, it meant that they have the potential to be used for anti-inflammatory effects.

Polycyclic-meroterpenoid (±)-cochlactones A and B and their isomers isolated from *Ganoderma cochlear* (Blume & T. Nees) Bres. reported a stronger inhibitory effect on NO production (Peng X.-R. et al., 2018). Polyketide-terpenoid hybrid meroterpenoids, stachybonoids C and F and stachybrotrylactone, isolated from the fungus *Stachybotrys chartarum* (Ehrenb.), displayed moderate inhibitory activity

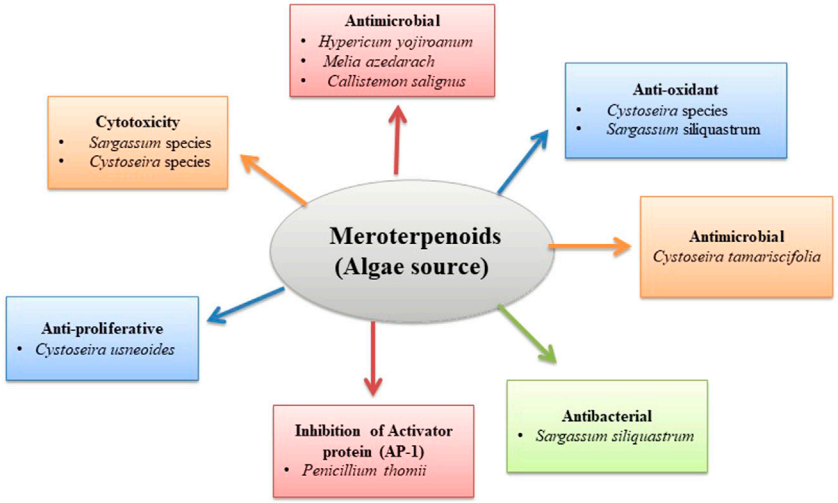

FIGURE 4
Biological activity of algae meroterpenoids.

on NO production (Zhang et al., 2017). Meroterpenoids austinoid and 1,2-dehydroterredehydroaustin isolated by Liu et al. from the *Aspergillus terreus* Thom mangrove endophytic fungus showed weak inhibitory action toward the NO production (Liu Z. et al., 2018). Yaminterritrems B, isolated by Liaw et al. from *Aspergillus terreus* Thom with the EC_{50} value at 18.3 μM, demonstrated a reduction in the expression of COX-2-induced LPS at the protein and RNA levels (Liaw et al., 2015). Meroterpenoid amestolkolide B isolated from mangrove endophytic fungus *Talaromyces amestolkiae* Yilmaz, Houbraken, Frisvad & Samson 2012 displayed potent inhibitory activity by inhibiting RAW264.7 cells activated lipopolysaccharide NO production (Chen et al., 2018). The NF-κB inhibitory activity of tricycloalternarene A; bicycloalternarenes A, B, C, D, and F; tricycloalternarenes B and C; monocycloalternarenes A, B, C, and D; and hydrogenated cyclopenta[b]chromans isolated from the *Alternaria* sp. JJY-32 sponge-associated fungus was tested, and all compounds showed activity in RAW264.7 cells with IC_{50} values between 39 and 85 μM (Zhang et al., 2013). Jing Sun et al. isolated purpurogenolides B–D and berkeleyacetal C from *Penicillium purpurogenum* Stoll. (1923) MHz 111. These exhibited inhibition activity with IC_{50} values of 30.0, 15.5, and 0.8 μM against NO production (Sun et al., 2016). A study on fungus *Penicillium brasilianum* Bat. by Zhang et al. led to the isolation of 3,5-dimethylorsellinic acid- (DMOA-) based meroterpenoids, brasilianoids A, B, and C. Brasilianoids A exhibited stimulation of filaggrin and caspase-14 expression in a dose-dependent manner in HaCaT cells, whereas brasilianoids B and C caused moderate inhibition of RAW 264.7 macrophages LPS-induced NO production (Zhang J. et al., 2018). Mangiterpenes C and 2′,3′-secomanginoid C isolated from *Guignardia mangiferae* A.J. Roy markedly decreased NO production-induced LPS with observed IC_{50} values of 5.97 and 6.82 μM, respectively (Chen et al., 2019) (Table 1 and Figure 1).

Anti-inflammatory activity of meroterpenoids isolated from marine sources

Meroterpenoids isolated from multiple marine sources, such as *Dactylospongia, Kappaphycus alvarezii* (Doty) Doty ex Silva, *Aspergillus*, *Dysidea villosa* (Lendenfeld, 1886), *Dysidea septosa* (Lamarck, 1814), *Corbiculid*, and *Aplidium scabellum* (Michaelsen, 1924), have reported significant anti-inflammatory activity. The report shows that sesquiterpene hydroquinone meroterpenoid dactylospongins A, B, and D, ent-melemeleone B, dysidaminone N, and 19-O-methylpelorol were isolated from the *Dactylospongia* sp. by Jing li et al. These compounds exhibited inhibitory activity with IC_{50} values ranging from 5.1 to 9.2 μM on PEG2, IL-6, IL-1β, and IL-8, respectively (Li J. et al., 2018). From *Kappaphycus alvarezii* (Doty) Doty ex Silva, red seaweed ethyl acetate fraction isolated 2-ethyl-6-(4-methoxy-2-((2-oxotetrahydro-2H-pyran-4-yl)methyl)butoxy)-6-oxohexyl-5-ethyloct-4-enoate (C29) reported *in vitro* potential inhibitory activity with IC_{50} 1.04 μg/ml toward 5-lipoxidase pro-inflammatory mediators (Makkar and Chakraborty, 2018). Wang et al. isolated triketide-sesquiterpenoid meroterpene

aspertetranones A–D from the *Aspergillus* sp. ZL0-1b14 marine algal-associated fungus. Aspertetranones A and D suppressed the IL-1β and IL-6 production in a dose-dependent manner, whereas aspertetranones B and C, at 33.3 μM concentration, exhibited weak anti-inflammatory effects. Similarly, aspertetranones A–D exhibited weak TNF-α and NO production (less than 35% inhibition) inhibitory effects (Wang Y. et al., 2015). Terpene-polyketide-pyridine hybrid meroterpenoids dysivillosins A–D, isolated from *Dysidea villosa* (Lendenfeld, 1886) by Jiao et al., reported potent inhibitory effect with IC_{50} values of 8.2, 10.2, 19.9, and 16.2 μM in the release of degranulation marker β-hexosaminidase in a dose-dependent manner. The development of LTB4 and IL-4 in antigen-stimulated RBL-2H3 mast cells at 6 and 12 μM, dose-dependently, may be downregulated by all the four meroterpenoids (Jiao et al., 2017). Septosones A and C were isolated from the *Dysidea septosa* (Lamarck, 1814) marine sponge by Gui et al. The study showed that septosone A could inhibit NF-κB activation-induced TNF-α with an IC_{50} value of 6.8 μM in human HEK-293T cells, whereas septosone C with an IC_{50} value of 27.2 μM reported weak inhibitory activity (Gui et al., 2019). Dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl)furan-2(3H)-one compound reported potential inhibitory activity against pro-enzymes 5-LOX and COX-2 (IC_{50} 0.84 and 0.76 μg/ml), which were obtained from *Corbiculid* bivalve clam (Joy and Chakraborty, 2018). Chan et al. isolated 2-geranyl-6-methoxy-1,4-hydroquinone-4-sulfate, scabellone B, 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-6-ol, and 2-geranyl-6-methoxy-1,4-hydroquinone meroterpenoids from an extract of *Aplidium scabellum* (Michaelsen, 1924) and reported inhibitory activity with IC_{50} values of 21, 125, 92, and 0.2 μM; *in vitro* human neutrophils stimulated PMA by superoxide production (Chan et al., 2011) (Table 2 and Figure 2).

Anti-inflammatory activity of meroterpenoids isolated from plants

Meroterpenoids studied from various parts of plants such as *Baeckea frutescens* Linnaeus and *Clinopodium chinense* (Benth.) have been reported as exerting anti-inflammatory activity *via* regulating the signaling NF-κB pathway and also increasing anti-oxidant enzyme activity, Nrf2 levels, and mitochondrial membrane potential.

A study on rare triketone-phloroglucinol-monoterpene baefrutones A–D isolated by Hou Ji Qin et al. from the *Baeckea frutescens* Linnaeus aerial parts with IC_{50} values 9.15–18.04 μM range reported moderate inhibitory activity as comparable to the positive control L-MMMA (Hou et al., 2018). Similarly, methanol extract of leaves and twigs isolated meroterpenoids, baeckfrutones (+) N and S, showed potential inhibitory effects with IC_{50} values of 36.21 ± 1.18 and 20.86 ± 0.60 μM on RAW 264.7 macrophages stimulated LPS NO production (Zhi et al., 2018). At concentrations less than 50 μM, baeckfrutone compounds F, G, (+) I, and J reported significant inhibitory activity with rates of 74.64, 75.37, 55.13, and 75.01%, respectively, compared to positive control L-MMMA (54.07%) (Qin et al., 2018f). Kuntze et al. from *Clinopodium chinense* (Benth.) aerial parts isolated clinoposides G and H flavonoid-triterpene saponin meroterpenoids significantly reported apoptosis and cell injury inhibition, improved mitochondrial membrane potential, increased anti-oxidant enzymes activity, and reduced the cytokines inflammatory levels. In addition, the compounds also increased the Nrf2 level and decreased the p65 levels in the cell nucleus (Zhu et al., 2018). Hou et al. isolated new monoterpene or sesqui-based meroterpenoid frutescones O from the *Baeckea frutescens* Linnaeus aerial parts. This compound showed potent inhibitory activity that could decrease the pro-inflammatory markers TNF-α and IL-6 and influence p65 suppression of nuclear translocation via the NF-κB signaling pathway (Hou et al., 2017) (Table 3 and Figure 3).

Anti-inflammatory activity of meroterpenoids isolated from algae

Zbakh et al. examined the 11-hydroxy-11-O-methylamentadione (AMT-E) algae meroterpene inhibitory effects in a colitis induced-dextran sodium sulfate (DSS) murine model. The administration of 10 and 20 mg/kg doses of AMT-E significantly decreases 60% and 67% cytokines levels and also decreases IL-10 concentration (Zbakh et al., 2016). Reyes et al. isolated meroterpenoids, usneoidone Z, and 11-hydroxyamentadione-1′-methyl ether from algae *Cystoseira usneoides* (Linnaeus) M. Roberts, 1968, and reported inhibitory activity of TNF-α production by 73% and 64% in LPS-stimulated THP-1 cells (De Los Reyes et al., 2013) (Table 4 and Figure 4).

COX-2 inhibitory activity of meroterpenoids

COX-2 inhibitory activity of meroterpenoids from fungus

Meroterpenoids isolated from fungus *Ganoderma* species have been majorly reported as anti-COX-2 agents to date. Luo et al. isolated meroterpenoid ganotheaecoloid J from *Ganoderma* species and reported its potent COX-2 inhibitory activity (Luo et al., 2018b). From fruiting bodies of *Ganoderma cochlear* (Blume & T. Nees) Bres., (±)-gancochlearols A and B were

isolated and reported to have potent COX-2 inhibitory activity (Qin et al., 2018c). Similarly, (±)-spirocochlealactones A–C, new spiro meroterpenoid podimeric enantiomers, and ganodilactone, with IC_{50} values of 1.29–3.63 μM showed potent COX-2 inhibitory activity against lung, immortalized myelogenous leukemia, and hepatic cell lines (Qin F.-Y. et al., 2018). From *Ganoderma* mushrooms, Luo et al. isolated meroterpenoids, ganotheaecolumols A–K, and iso-ganotheaecolumol I, which were tested against COX-2 and JAK3 kinase for their inhibitory activity. It was reported that (±)-ganotheaecolumols C and D, iso-ganotheaecolumol I, and ganotheaecolumols I and K showed inhibitory activity with IC_{50} values of 1.05, 1.38, 2.61, 3.47, and 4.84 μM (Luo et al., 2018a) (Table 1 and Figure 1).

COX-2 inhibitory activity of meroterpenoids from marine sources

From *Villorita cyprinoides* (Gray et a, 2007), two irregular pyranoids and isochromenyl meroterpenoids dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl) furan-2(3H)-one and tetrahydro-3-methoxy-5-((E)-8,12-dimethyloct-8-enyl)-pyran-2-one and two hexahydro-isochromenyl-meroterpenoids were identified by Joy et al. The result showed that isolated compounds tetrahydro-3-methoxy-5-((E)-8,12-dimethyloct-8-enyl)-pyran-2-one, (10E)-butyl-9-(6-ethyl-3,4,6,7,8,8a-hexahydro-1H-isochromen-3-yl)-pent-10-enoate, dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl)furan-2(3H)-one and (12E)-(3,4,6,7,8,8a-hexahydro-1H-isochromen-3-yl)-methyl-hept-12-enoate exhibited COX2 inhibitory activity with $IC_{50} > 1.10$ (Joy and Chakraborty, 2018) (Table 2 and Figure 2).

Anti-HIV activity of meroterpenoids

Anti-HIV activity of meroterpenoids from the fungus

The anti-HIV activity reported by Liu et al. from the *Periconia* sp. F-31 endophytic fungus isolated new polyketide-terpenoid hybrid molecule periconones B with an IC_{50} value of 18.0 μmol/L compared with positive control efavirenz (Liu J. M. et al., 2017) (Table 1 and Figure 1).

Anti-HIV activity of meroterpenoids from plants

Tetsuro et al. isolated meroterpenoid daurichromenic acid (DCA) from *Rhododendron dauricum* L. (Ericaceae), which consists of orsellinic acid (OSA) and sesquiterpene moiety. Daurichromenic acid (DCA) was found to be an anti-HIV meroterpenoid produced *via* oxidative cyclization of the farnesyl group of the grifolic acid (Saeki et al., 2018) (Table 3 and Figure 3).

Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity of meroterpenoids from the fungus

Meroterpenoids, studied from different fungal species such as *H. caput-medusae* (Bull.) Pers., *Aspergillus terreus* Thom, *Myrothecium* sp. OUCMDZ-2784, and *Ganoderma leucocontextum*, have been reported to show moderate-to-potent α-glucosidase inhibitory activity.

A detailed investigation by Chen et al. led to the isolation of meroterpene dimers containing isoindolinone and caputmedusins A–C from the *H. caput-medusae* (Bull.) Pers. fermentation broth. When evaluated for their α-glucosidase inhibitory function, all isolates displayed moderate inhibition with IC_{50} values of 39.2, 36.2, and 40.8 μM, respectively (Chen L. et al., 2017). In a study by Shan et al., diketopiperazine alkaloidal meroterpenoids, amauromine B and austalide N, were isolated from the *Aspergillus terreus* Thom fungus culture broth. These compounds showed potent inhibitory effects compared with positive control acarbose (Shan et al., 2015). Xu et al. from the *Myrothecium* sp. OUCMDZ-2784 isolated myrothecisins A–D, myrothelactone A, myrothelactone C, tubakialactone B, acremonone G. recombinant expressed in *Saccharomyces cerevisiae* Meyen ex E.C. Hansen. All the compounds demonstrated strong inhibitory action against the recombinant human-sourced recombinant α-glucosidase expressed in *Saccharomyces cerevisiae* Meyen ex E.C. Hansen. compared with that of positive control acarbose (Xu et al., 2018). Triterpenes meroterpenoids; ganoleucins A and C; ganomycins I, B, and C; fornicins C and B were isolated by Wang et al. from *Ganoderma leucocontextum* fruiting bodies. These noncompetitively inhibited alpha-glucosidase isolated from yeast and rat small intestine mucosa (Wang et al., 2017) (Table 1 and Figure 1).

Anti-oxidant activity of meroterpenoids

Anti-oxidant activity of meroterpenoids from the fungus

Meroterpenoids from fungal species, such as *Ganoderma sinense*, *Ganoderma capensa* (Lloyd), *Ganoderma cochlear* (Blume & T. Nees) Bres., and *Perenniporia medulla-panis* (Jacq.) Donk (1967) have been studied for anti-oxidant activity using ABTS and DPPH radical scavenging assay. Gao et al. isolated meroterpenoids applanatumol I, from a 95% ethanolic extract of *Ganoderma sinense* fruiting bodies. The outcome revealed that (+)-applanatumol I treatment effectively shielded LO2 cells from cell loss and apoptosis

caused by H_2O_2. Increased levels of Nrf2, phosphorylation Akt, upregulation of anti-oxidant enzymes, and heme oxygenase 1 (HO-1) were detected in (+)-applanatumols I treated cells; it indicates that the anti-oxidative effects of (+)-applanatumols I by PI3K/Akt-mediated activation of the Nrf2/HO-1 pathway could defend LO2 cells against oxidative harm (Gao et al., 2018). From *Ganoderma capensa* (Lloyd), Peng et al. isolated aromatic meroterpenoids, ganocapensins A and B, ganomycin E, ganomycin F, fornicin E, ganomycin I, fornicin B, and ganomycin C, and reported strong inhibitory activity with IC_{50} values of 6.00 ± 0.11–8.20 ± 0.30 μg/ml compared with positive control Trolox (Peng X. et al., 2016). Additionally, Peng et al. also isolated (±)-cochlearins A–E and G, and three new analogs from *Ganoderma cochlear* (Blume & T. Nees) Bres. cochlearins F, H–I, compared with positive control Trolox. All of the meroterpenoids exhibited inhibitory activity with IC_{50} values in the range of 3.1 ± 0.1–5.3 ± 0.1 μM (Peng X. et al., 2018). From *Perenniporia medulla-panis* (Jacq.) Donk (1967) culture broth, which is a wood-rotting fungus in the Polyporaceae family, Kim et al. isolated xylopyranosyl meroterpenoid. Compound (+) fornicin A with an IC_{50} value of 106.0 μM significant demonstrated DPPH radical scavenging activity, compared with BHA and Trolox as positive controls. On the contrary, perennipins A–C and (+)-fornicin A with IC_{50} values 12.8–190.3 μM range showed anti-oxidant activity against radical scavenging ABTS activity. However, compound (+) fornicin A showed much higher ABTS radical scavenging activity than other compounds (Kim et al., 2019) (Table 1 and Figure 3).

Anti-oxidant activity of meroterpenoids from marine sources

Meroterpenoids studied from different marine species such as *Hypnea musciformis* (Wulfen), *Kappaphycus alvarezii* (Doty), *Aplidium fuegiense* (Cunningham, 1871), *Corbiculid bivalve* clam, and *Penicillium* sp. YPGA11 has been reported for anti-oxidant activity using radical scavenging ABTS and DPPH assay. Chakraborty et al. studied *Hypnea musciformis* (Wulfen) red seaweed as a potential anti-oxidant. The ethyl acetate fraction of the seaweed yielded three aryls substituted meroterpenoids, namely, 2-(tetrahydro-5-(4-hydroxyphenyl)-4-pentylfuran-3-yl)-ethyl-4-hydroxy benzoate, 2-2-[(4-hydroxybenzoyl)-oxy]-ethyl-4-methoxy-4-2-[(4-methylpentyl) oxy]-3,4-dihydro-2H-6-pyranylbutanoic acid and 3-((5-Butyl-3-methyl-5,6-dihydro-2H-pyran-2-yl)-methyl)-4-methoxy-4-oxobutyl benzoate. Compound 2-(tetrahydro-5-(4-hydroxyphenyl)-4-pentylfuran-3-yl)-ethyl-4-hydroxy benzoate exhibited DPPH radical inhibiting and Fe^{2+} ion chelating activity with IC_{50} 25.05 and 350.7 μM, respectively, followed by 3-((5-butyl-3-methyl-5,6-dihydro-2H-pyran-2-yl)-methyl)-4-methoxy-4-oxobutyl benzoate with IC_{50} 231.2 and 667.9 μM, and 2-2-[(4-hydroxybenzoyl)-oxy]-ethyl-4-methoxy-4-2-[(4-methylpentyl)oxy]-3,4-dihydro-2H-6-pyranylbutanoic acid with IC_{50} 322.4 and 5,115.3 μM (Chakraborty et al., 2016). Makkar et al. isolated and purified meroterpenoid 2-ethyl-6-(4-methoxy-2-((2-oxotetrahydro-2Hpyran-4-yl) methyl) butoxy)-6-oxohexyl-5-ethyloct-4-enoate (C29) from the *Kappaphycus alvarezii* (Doty), (family Solieriaceae) red seaweed methanol: ethyl acetate fraction. The highly oxygenated meroterpenoid C29 showed potential anti-oxidant activity (IC_{50} < 0.35 μg/ml) (Makkar and Chakraborty, 2018). The biologically active derivatives of meroterpene, rossinones A and B, were isolated from the antarctic ascidian *Aplidium* fuegiense array. The inhibitory function of the compounds was tested by Appleton et al. with active human peripheral blood neutrophils. When either N-formyl methionylleucyl phenylalanine (fMLP) (IC_{50} 1.9 and 2.5 μM) or phorbol myristate acetate (PMA) (IC_{50} 0.8 and 0.7 μM) were used to cause the respiratory blast, rossinones A and B were found to inhibit the production of superoxide (Appleton et al., 2009). Joy et al. reported two irregular pyranoids and isochromenyl meroterpenoids from the *Corbiculid bivalve* clam, tetrahydro-3-methoxy-5-((E)-8,12-dimethyloct-8-enyl)-pyran-2-one, and dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl) furan-2(3H)-one while studying bioactivity-guided ethyl acetate: methanol extract of black clam purification. Compound dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl) furan-2(3H)-one exhibited significantly greater DPPH radical scavenging ability with IC_{50} value < 0.65 μg/ml. Moreover, tetrahydro-3-methoxy-5-((E)-8,12-dimethyloct-8-enyl)-pyran-2-one and dihydro-5-(8-(9,12-dihydro-8-methyl-11-propyl-2H-pyran-8-yl)-ethyl)furan-2(3H)-one was reported for ferrous ion (Fe^{2+}) chelating ability with IC_{50} value ~0.84 μg/ml (Joy and Chakraborty, 2018). Cheng et al. isolated meroterpenoid from the *Penicillium* sp. YPGA11 deep-sea fungus. The isolated compounds were tested in LPS-activated RAW 264.7 macrophages for an inhibitory effect against NO production, whereas quercetin was selected as a positive control. The result showed that compound conidiogenone C exhibited inhibitory effects with an IC_{50} value of 7.58 μM (Cheng et al., 2019) (Table 2 and Figure 2).

Anti-oxidant activity of meroterpenoids from algae

Meroterpenoids studied from diverse algae species, such as *Cystoseira usneoides* (Linnaeus) M. Roberts, *Cystoseira crinite* Duby, 1830, and *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh, have been reported to show strong radical scavenging activity.

Reyes et al. studied the *Cystoseira usneoides* (Linnaeus) M. Roberts and isolated tetraprenyltoluquinol meroterpenoids, cystodiones A and B, 6-cis-amentadione-1′-Me ether, and amentadione-1′-Me ether. These compounds showed excellent radical scavenging activity (De Los Reyes et al., 2013). Six new derivatives of tetraprenyltoluquinol, two new derivatives of triprenyltoluquinol, and two new derivatives of tetraprenyltoluquinone were isolated along with four known derivatives of tetraprenyltoluquinol from the brown algae *Cystoseira crinita* Duby. All the isolated compounds were tested for anti-oxidant activity. In the DPPH assay, the hydroquinones-based meroterpenoids showed a strong radical scavenging effect in comparison to alpha-tocopherol. These compounds showed inhibitory activity between 13% and 41% in PCL assay (Fisch et al., 2003). *Sargassum serratifolium* (C. Agardh) contains isoprenoid quinones and chromanol meroterpenoids with anti-oxidant activity. DPPH scavenging activity studies revealed that ethyl acetate extract (IC_{50} 34.6 ± 0.47 μg/ml) displayed the strongest activity and ABTS radical scavenging activity followed by methanol extract (IC_{50} 43.2 ± 0.24 μg/ml) (Lim et al., 2019). Kang et al. isolated sargachromanols S and T, two new meroterpenoids, from *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh, with EC_{50} values of 57.1 and 31.1 μM exhibiting mild scavenging activity against the DPPH radical (28.1 μM) and against ABTS radical (15.8 μM) (Kang and Kim, 2017). Similarly, sargachromanols A–P were isolated from the brown alga *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh, sixteen new meroterpenoids of the chromene class in a study by Jang et al. It was reported that chromene class of compounds show anti-oxidant activity; these meroterpenoids were also tested for anti-oxidant activity using DPPH assay. It was found that sargachromanols A–P possessed significant radical scavenging activity with values ranging from concentration 87–91% of 100 μg/ml (Jang et al., 2005) (Table 4 and Figure 4).

N-acetyltransferase inhibiting activity of meroterpenoids

From the aqueous ethanolic extract of *Ganoderma cochlear* (Blume & T. Nees) Bres. fruiting bodies, Cheng et al. isolated (+)- and (-)-gancochlearol C and ganomycin F, the compounds were tested for N-acetyltransferase inhibition. The findings indicate that (+)-gancochlearol C with an IC_{50} value of 5.29 μM could inhibit N-acetyltransferase (Cheng et al., 2018).

Anti-microbial activity of meroterpenoids

Anti-microbial activity of meroterpenoids from the fungus

Meroterpenoids studied from different fungal species such as *Phyllosticta*, *Penicillium* sp. T2-8, *Cytospora*, and *Aspergillus* have reported moderate-to-potent anti-bacterial activity.

Yang et al. isolated phyllomeroterpenoids A–C and six biosynthetically related compounds (S, Z)-guignardianone C, (S, Z)-botryosphaerin B, (4S, 6R, 9S, 10R, 14R) −17-hydroxylated guignardone A, (S, Z)-phenguignardic acid methyl ester (4S, 6R, 9, 10, 12S, 14R)−12-hydroxylated guignardone A, and (4S, 6R, 9S, 10R, 14R)-guignardone B from fungus *Phyllosticta* sp. Only compound (S, Z)-phenguignardic acid methyl ester with MIC values of 4 μg/ml showed significant anti-microbial activity against *S. aureus* 209P and *C. albicans* FIM709 (Yang et al., 2017). Duan et al. isolated meroterpenoids preaustinoid D and dihydroxyneogrifolic acid, a neogrifolin derivative, Austin, and (S)-18,19-dihydroxyneogrifolin from *Gastrodia elata* Blume, associated with *Penicillium* sp. T2-8 endophytic fungus. The study showed that preaustinoid D and dihydroxyneogrifolic acid with MIC of 128 μg/ml exhibited moderate inhibitory activity against *C. albicans*. Similarly, dihydroxyneogrifolic acid exhibited inhibitory activity against *Bacillus subtilis* (MICs of 8 μg/ml) and *S. Aureus* (MICs of 32 μg/ml), respectively. In addition, Austin and (S)-18,19-dihydroxyneogrifolin with MICs of 4 μg/ml showed activities pointed out against *S. aureus* (Duan et al., 2016). Yun Li isolated from the fungus Cytospora sp. meroterpenoids cytosporolides A–C, three caryophyllene-derived meroterpenoids with a special peroxylactone skeleton. The outcome shows the behavior displayed by all compounds against *S. aureus* and *S. pneumoniae* Gram-positive bacteria, and cytosporolides C was the most potent compound, with IC_{50} values of 1.98 μg/ml and 1.16 μg/ml (Li et al., 2010). Yan He et al. isolated spiro meroterpenoids, spiroaspertrione A, and andiconin B from *Aspergillus* sp. endophytic fungus. Both compounds demonstrated inhibition activity against MRSA with MIC values of 4 and 16 μg/ml, respectively (He et al., 2017c). Meroternoidal alkaloid oxalicine C isolated from endophytic fungus *penicillium chrysogenum* has also been reported to have moderate anti-bacterial activity against *Ralstonia solanacearum* (Xu et al., 2020) (Table 1 and Figure 1).

Anti-microbial activity of meroterpenoids from marine sources

Meroterpenoids studied from diverse species of Okinawan marine sponge and *Aspergillus terreus* Thom (1918) have reported anti-microbial activity for various strains such as *E. coli*, *M. luteus*, *B. subtilis*, *S. aureus*, *C. albicans*, *A. niger*, and *C. neoformans*.

New meroterpenoid compounds, namely, nakijinol C and nakijiquinone S, have been isolated from marine sponge Okinawan of Spongiidae family by Suzuki et al. Anti-microbial assay of nakijiquinone S and nakijinol C revealed against several bacteria and fungi

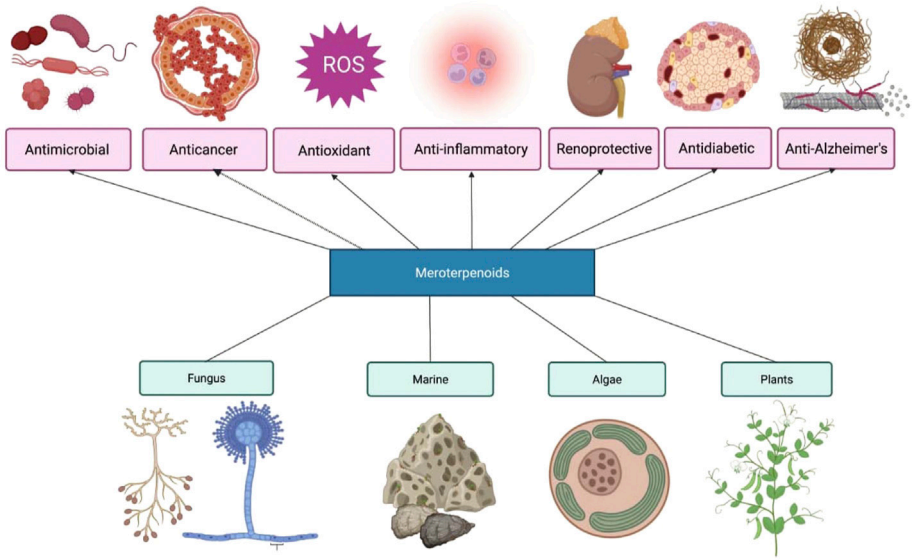

FIGURE 5
Biological activities of meroterpenoids obtained from various sources.

(*E. coli*, *A. Niger*, *B. subtilis*, *M. luteus*, *T. mentagrophytes*, *S. aureus*, and *C. neoformans*) showed anti-microbial activity (Suzuki et al., 2014). Lei Li et al. identified and isolated aperterpene N and O meroterpenoids, along with terretonins A and G, structurally two known related derivatives, from the marine fungus *Aspergillus terreus* Thom (1918), EN-539. Aperterpene N with an IC_{50} value of 18.0 μM displayed neuraminidase (NA) inhibitory activity. Furthermore, terretonin G demonstrated activity against *M. luteus* (MIC value 32 μg/ml) and *S. Aureus* (8 μg/ml), compared with that of positive control chloramphenicol (Li H. L. et al., 2019). Similarly, Ibrahim et al. isolated (22E, 24R)-stigmasta-5,7,22-trien-3-b-ol and aspernolides F from *Aspergillus terreus* Thom (1918), reporting good activity against *C. neoformans* and *S. aureus*. The compound exhibited a potent action against MRSA, and *C. neoformans* showed 0.96 μg/ml and 4.38 μg/ml IC_{50} values. In addition, aspernolides F showed activity against *C. neoformans* (IC_{50} 5.19 μg/ml) and mild activity against MRSA (IC_{50} 6.39 μg/ml) (Ibrahim et al., 2015). Cheng et al. isolated napyradiomycins A and B3 from *Streptomyces* strains of the MAR4 group. The result showed that these compounds exhibit the most active analogs against MRSA (16 and 2 μg/ml, respectively) (Cheng et al., 2013) (Table 2 and Figure 2).

Anti-microbial activity of meroterpenoids from plants

Meroterpenoids isolated from various plants, such as *Hypericum yojiroanum* M. Tatewaki & K. Ito, *Melia azedarach* (Linnaeus) and *Callistemon salignus* Craven, were studied for anti-microbial activity on various strains. Reports showed that yojironin A isolated from the entire *Hypericum yojiroanum* M. Tatewaki & K. Ito, vine, action exhibited activity against *A. niger* (IC_{50} 8 μg/ml), *C. albicans* (IC_{50} 2 μg/ml), *C. neoformans* (IC_{50} 4 μg/ml), *Trichophyton mentagrophytes* (IC_{50} 2 μg/ml), *S. aureus* (MIC 8 μg/ml), and *B. subtilis* (MIC 4 μg/ml) (Mamemura et al., 2011). From *Penicillium brasilianum* Bat. found in the root and bark of *Melia azedarach* (Linnaeus), Fill et al. obtained bisphenylpropanoid N-acetylamides, brasiliamide A showed only a weak bacteriostatic effect against *B. subtilis* (MIC of 250 μg/ml) (Fill et al., 2009). Acylphloroglucinol derivatives, callisalignones A–C, and known meroterpenoids, myrtucommulone D and isomyrtucommulone B, were isolated from *Callistemon salignus* in a study by Qin et al. The results reported that isomyrtucommulone B exhibited significant activity against *E. coli* (MIC value of 0.122 μg/ml), and myrtucommulone D exhibited potent activity against *S. aureus* and other drug-resistant *S. aureus* strains.

Compounds of callisalignone A, 2,6-dihydroxy-4-methoxy-3-methylisopropiophenone, and 2,6-dihydroxy-4-methoxyisovalerophenone displayed moderate activity against *A. fumigatus* (MIC value of 15.625 μg/ml) (Qin et al., 2017a) (Table 3 and Figure 3).

Anti-microbial activity of meroterpenoids from algae

Phloroglucinol-meroterpenoid cystophloroketals A–D were extracted from alga *Cystoseira tamariscifolia* (Hudson) in a study conducted by Hattab et al. The study showed that cystophloroketals A, B, and D could inhibit the growth of marine bacteria and fungi with MICs values of 1 μg/ml, and cystophloroketals C had the highest inhibitory activity (El Hattab et al., 2015) (Table 4 and Figure 5).

Anti-bacterial activity of meroterpenoids

Anti-bacterial activity of meroterpenoids from the fungus

Meroterpenoids isolated from different fungal species such as *Ganoderma orbiforme* (Fr.) Ryvarden (2000), *Ganoderma cochlear (Blume & T. Nees) Bres.*, *Emericella* species TJ29, *Penicillium*, and *Dysidea* have shown moderate-to-potent anti-bacterial activity against various strains such as *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. epidermidis*.

From the cultivated fruiting bodies of *Ganoderma orbiforme* (Fr.) Ryvarden (2000), basidiomycete, norlanostane-type triterpenoids ganoboninone G, and ganomycin I were isolated by Li et al. This research revealed that these compounds exhibited poor action toward *M. tuberculosis* H37Ra (MIC value of 50 μg/ml) and also ganomycin I reported activity against *E. faecium* (MIC 25 μg/ml) Gram-positive bacteria, *B. cereus* (MIC 25 μg/ml), and *S. aureus* (MIC 12.5 μg/ml) (Li W. et al., 2018). In another study, Qin et al. isolated phenolic meroterpenoids (±) cochlearoids O and P from *Ganoderma cochlear* (Blume & T. Nees) Bres. These compounds exhibited strong inhibitory activity with IC_{50} values ranging 5.43–17.99 μM against *S. aureus* (Qin F.-Y. et al., 2019). Terpene-polyketide hybrid meroterpenoid, namely, emervaridone A, was isolated from *Emericella* species TJ29. The compounds showed activity against five drug-resistant microbial pathogens [MRSA, *P. aeruginosa*, *Enterococcus faecalis*, *K.* pneumoniae, and β-lactamase-producing *E. coli* (ESBL-producing *E. coli*)]. Emervaridone A also displayed anti-bacterial activity against ESBL-producing *E. coli* and *P. aeruginosa*, in which emervaridone A had MIC values of 2 and 8 μg/ml (He et al., 2017b). Drimane-type sesquiterpene meroterpenoid verruculides B2 isolated from *Penicillium* sp. displayed weak inhibitory with an MIC of 32 μg/ml activity against *S. aureus* (Kong et al., 2017). In another similar study, a fungus *Penicillium citrinum* (Thom, C. 1980), meroterpenoids penicimarins G–H, dehydroaustin, 11β-acetoxyisoaustinone, and austinol exhibited selective anti-bacterial activity. Penicimarin H and austinol showed activity against *S. epidermidis* and *S. aureus* with the same MIC values of 10 μM. Moreover, penicimarins G and H showed a large action spectrum against pathogenic bacteria *S. epidermidis*, *E. coli*, *B. Cereus*, *S. aureus*, *E. coli*, *B. cereus*, and *Vibrio alginolyticus* (Huang et al., 2016). Duan et al. isolated meroterpenoids preaustinoid A1 and (S)-18,19-dihydroxyneogrifolin from *Penicillium* sp. T2-8. The result showed preaustinoid A1 exhibited inhibitory activity against *B. subtilis* (MIC value 4 μg/ml) and (S)-18,19-dihydroxyneogrifolin exhibited potent inhibitory activity against *E. Coli* (MIC value 8 μg/ml) (Duan et al., 2016). Meroterpenoids, dysidphenols A and C, smenospongimine, smenospongine, smenospongorine, smenospongiarine, and smenospongidine isolated from *Dysidea* sp. showed anti-bacterial activity against *E. coli* (25,922), *B. subtilis* (6,633), and *S. aureus* (25,923) strains. Dysidphenols A and C exhibited weak activity against the three strains. However, smenospongimine, smenospongine, smenospongorine, smenospongiarine, and smenospongidine showed potent inhibitory activity in all three strains (Zhang et al., 2016) (Table 1 and Figure 1).

Anti-bacterial activity of meroterpenoids from marine source

Meroterpenoids studied from different marine species such as *Actinomycete*, *Streptomyces*, and *Spongia* have reported anti-bacterial activity against Gram-positive strains. The report showed that merochlorins E and F, isolated by Ryu et al. from *Streptomyces*, exhibited strong inhibitory activity against *B. subtilis*, *S. aureus*, and *Kocuria rhizophila* (MIC values from 1 to 2 μg/ml) (Ryu et al., 2019). Nguyen investigated Vietnamese marine sponge *Spongia* species and isolated sesquiterpene hydroxyquinone langcoquinone C and smenospongorine, which had significant activity against *S. aureus* and *B. subtilis* with MIC ranging from 6.25 to 25 μM (Nguyen et al., 2017). Sesquiterpene aminoquinones langcoquinones A–B, dictyoceratin A, ilimaquinone, smenospongine, smenospongidine, and nakijiquinone L from the marine sponge *Spongia* species exhibited significant inhibitory activity against *S. aureus* and *B. subtilis* with MICs in a

range of 6.25–12.5 μM (Li H. et al., 2018). Haste et al. isolated two naphthoquinone meroterpenoids (A80915A and A80915B) produced by actinomycete, marine-derived, *Streptomyces* sp. CNQ-525 strain. These compounds demonstrated strong and fast bactericidal action against modern strains of MRSA (Haste et al., 2011) (Table 2 and Figure 2)

Anti-bacterial activity of meroterpenoids from plants

Three phloroglucinols meroterpenoids, aspidin BB, desaspidin BB, and desaspidin PB, isolated from *Dryopteris championii* (Benth.), were tested against the *S. aureus*, *E. coli*, *B. subtilis*, and *Dickeya zeae* (MIC values between 4 and 16 μg/ml) (Chen et al., 2016). Two meroterpenoids, eugenials C and D, isolated from the *Eugenia Umbelliflora* (O.Berg) fruits, reported strong activity against *B. Subtilis*, *S. aureus*, and MRSA (Li H. et al., 2018) (Table 3 and Figure 3).

Anti-bacterial activity of meroterpenoids from algae

Meroterpenoid sargachromanol L of the chromene class was isolated from *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh brown algae. The result showed that sargachromanols L exhibited weak anti-bacterial activity (Chung et al., 2011) (Table 4 and Figure 4).

Antitubercular activity of meroterpenoids

Quinone and hydroquinone-based meroterpenoids, deacetoxyyanuthone A, macrophorin A, and 4′-oxomacrophorin, were isolated by Jun He et al. from fungus *Gliomastix* sp. ZSDS1-F7. The result showed that these compounds showed important inhibitory action against *M. tuberculi* with IC_{50} values of 22.1, 2.44, and 17.5 μM, respectively (He W. J. et al., 2017).

Anti-fungal activity of meroterpenoids

Anti-fungal activity of meroterpenoids from fungus

Zhang et al. synthesized and explored the anti-fungal activity of meroterpenoid (+)-chromazonarol and (+)-yahazunone. The findings revealed that these compounds showed beneficial activity with EC_{50} values of 24.1 and 28.7 μM against *Sclerotinia scleotiorum* (Zhang S. et al., 2018). Endophytic fungus *Phyllosticta* sp. WGHL2 also showed four new meroterpenoids, namely, guignardones U–X, along with known meroterpenoids. However, none of the four newly isolated compounds showed anti-fungal activity (Yan et al., 2021) (Table1 and Figure 1).

Anti-fungal activity of meroterpenoids from marine sources

Cohen et al. isolated meroterpenoid insuetolides A, strobilactone A, and (E, E)-6-(60,70-dihydroxy-20,40-octadienoyl)-strobilactone A from ethyl acetate extract of the culture medium of the marine-derived fungus *Aspergillus insuetus* (Bainier) Thom & Church (1929). The MIC values of these compounds against the fungus *Neurospora crassa* were 140, 242, and 162 μM, respectively (Cohen et al., 2011). Merosesquiterpene 24-methylsulfinyllancoquinone B isolated from marine sponge *Spongia pertusa* has been reported for its moderate anti-fungal activity against human pathogens, namely, *Candida albicans* and *Trichophyton* species (Tang et al., 2022) (Table 2 and Figure 2).

Anti-fungal activity of meroterpenoids from plants

Meroterpenoids studied from various species of plants, such as *Eucalyptus robusta* Smith and *Psoralea glandulosa* L., have been reported to date to possess anti-fungal activity in their different parts.

From the leaves of *Eucalyptus robusta* Smith, formyl phloroglucinol (FPM) meroterpenoids, namely, eucalrobusones T, U, and X, were isolated by Shang et al. The results showed that eucalrobusones T and U exhibited significant activity MIC_{50} values less than 10 μg/ml against *C. glabrata*. Eucalrobusone X showed the strongest activity with an MIC_{50} value of 10.78 μg/ml against *C. albicans*. It was also found that FPMs are more effective against *C. glabrata* than *C. albicans* (Shang et al., 2019). A similar study was conducted on FPMs, namely, eucalrobusones J and O, isolated from the leaves of *Eucalyptus robusta* Smith by Shang et al. The result showed that compounds eucalrobusones J and O exhibited significant inhibitory activity against *C. glabrata* and eucalrobusone O also showed moderate activity against *C. albicans* (Shang et al., 2016b). Similarly, from extracts of *Psoralea glandulosa* L., Madrid et al. isolated meroterpenoids, namely, bakuchiol and 3-hydroxybakuchiol. Both compounds demonstrated potent activity with the MIC_{80} ranging from 4 to 416 and 0.125–16 μg/ml, respectively, against the strains of *C. albicans* ATCC7978 and *Candida parapsilosis* ATCC22019 (Madrid et al., 2012) (Table 4 and Figure 4).

Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) inhibitory activity of meroterpenoids

BACE1 inhibitory activity of meroterpenoids

Meroterpenoids studied from two fungal species, namely, *Aspergillus terreus* Thom (1918) and *S. serratifolium* (C. Agardh), have been reported to show moderate-to-potent BACE1 inhibitory activity.

Qi et al. investigated various DMOA meroterpenoids from the fungus *Aspergillus terreus* Thom (1918) for BACE1 inhibitory activity. Terreusterpenes A and B inhibited BACE 1 with IC_{50} values of 5.98 and 11.42 μM. Terreusterpene D exhibited promising inhibitory activity (IC_{50} values of 1.91 μM); asperterpenes E, F, and J exhibited significant inhibitory activity (IC_{50} values of 3.3, 5.9, and 31.7 μM); and asperterpenes A and B demonstrated moderate activity (IC_{50} values of 78 and 59 μM) (Qi et al., 2016; 2018b; 2018a). Seong et al. isolated sargahydroquinoic acid, sargaquinoic acid, and sargachromenol meroterpenoids from *S. serratifolium* (C. Agardh) and tested them for anti-Alzheimer's disease (AD) activity. The study demonstrated that all three compounds exhibited potent inhibitory activity compared with quercetin (Seong et al., 2017). A study on spiroterreusnoids A–F spiro-dioxolane meroterpenoids isolated by Changxing et al. from *A. terreus* with IC_{50} values 5.86–27.16 μM range showed potential BACE1 inhibitory effects (Qi et al., 2019). Yatsu et al. isolated 4-hydroxybenzoic acid-based meroterpenoids from fruiting bodies of *B. asiaticus*. Asiaticusinol C, asiachromenic acid, and asiaticusin A showed BACE1 inhibitory activity with IC_{50} values between 2 and 14 μM (Yatsu et al., 2019) (Table 1 and Figure 1).

Renal protective effect of meroterpenoids

Luo et al. isolated applanatumols A and (+)-B from *Ganoderma applanatum* (Pers.) Pat. 1887. The biological activity of these compounds toward renal fibrosis was evaluated in rat proximal tubular epithelial cells. The results show that applanatumols A and (+)-B could inhibit extracellular matrix (ECM) components (fibronectin and collagen I) (Luo et al., 2016).

Acetylcholinesterase inhibitory activity of meroterpenoids

Acetylcholinesterase inhibitory activity of meroterpenoids from the fungus

Various species of *Ganoderma*, *Aspergillus*, and *Penicillium* fungus have yielded meroterpenoids that have shown potent AchE inhibiting activity.

Qi et al. investigated DMOA-based meroterpenoid, terreusterpene D, obtained from *A. terreus*. The compounds with an IC_{50} value of 8.86 μM exhibited promising AchE inhibitory activity, which could also serve for Alzheimer's disease treatment (Qi et al., 2018b). From *Aspergillus* 16-5c, Long et al. isolated polyketide-terpenoid meroterpenoids, namely, isoaustinol, dehydroaustin, and dehydroaustinol, and reported potent AchE inhibiting activity (Long et al., 2017). Polycyclic-meroterpenoid enantiomers ganocin D isolated by Peng et al. from the *Ganoderma cochlear* (Blume & T. Nees) Bres. fruiting bodies showed weak inhibition with an inhibition of 32% (50 μM) (Peng et al., 2014). Luo et al. isolated (+)-zizhines G, (−)-zizhines G, (−)-ganosinensols A, (+) zizhines P, (−) zizhines P, (+)-zizhines Q, and (−) zizhines Q from *Ganoderma* species. All the compounds exhibited inhibitory activity with inhibition rates of 88.77%, 87.68%, 82.18%, 89.24%, 87.73%, 83.43%, and 83.71%, respectively, at the concentration of 50 μM using tacrine as a positive control (Luo et al., 2019a). Aromatic meroterpenoid ganocapenoid C, ganocalidin E, cochlearin I, and patchiene A were isolated from *Ganoderma capense* (Lloyd). These compounds showed inhibition with the IC_{50} values of 28.6 ± 1.9, 8.7 ± 1.6, 8.2 ± 0.2, and 26.0 ± 2.9 μM, respectively (Liao et al., 2019). Dai et al. isolated meroterpenoid arisugacins D, M, O, P, and Q from *Penicillium* species in a phenotype-based zebrafish assay. The compound arisugacin D has been reported as a selective inhibitor with an IC_{50} value of 3.5 μM. Compounds arisugacin M, O, P, and Q induced paralysis in zebrafish embryos, with arisugacin O demonstrating potent and selective inhibitory activity (Dai et al., 2019). A study on spiroterreusnoids A–F spiro-dioxolane meroterpenoids extracted by Changxing et al. from fungus *Aspergillus terreus* Thom (1918) showed moderate AchE inhibitory effects, with IC_{50} values ranging from 22.18 to 32.51 μM (Qi et al., 2019) (Table 1 and Figure 1).

Acetylcholinesterase inhibitory activity of meroterpenoids from marine sources

Huaqiang Li et al. obtained asperversins G from the fungus *Aspergillus versicolor* (Vuill), which exhibited an inhibitory effect (IC_{50} of 13.6 μM) (Li H. et al., 2018). Ding et al. isolated α-pyrone meroterpenoids 3-epiarigsugacin E, territrem C, arisugacin B, and terreulactone C from the fungus *Penicillium* sp. SK5GW1L. The result showed that compound 3-epiarigsugacin E exhibited weak inhibitory activity compared to arisugacin B, territrem C, and terreulactone C (IC_{50} values of 3.03, 0.23, and 0.028 μM) (Ding B. et al., 2016) (Table 2 and Figure 2).

Acetylcholinesterase inhibitory activity of meroterpenoids from plants

Qin et al. isolated dimeric phellandrene-derived meroterpenoids *Eucalyptus* dimer A, (±) eucalyprobusone A, from fruits of *Eucalyptus robusta* Smith, and triketone sesquiterpene type meroterpenoid rhodomyrtusials A, rhodomyrtusials B, and tomentodione Q from *Rhodomyrtus tomentosa*. *Eucalyptus* dimer A, (±) eucalyprobusone A, rhodomyrtusials A, rhodomyrtusials B, and tomentodione Q with IC_{50} values of 17.71, 13.61, 8.8, 6.0, and 6.6 μM exhibited inhibitory activity, respectively (Qin X.-J. et al., 2018; Qin et al., 2019 X.). Luo et al. isolated meroterpenoids dayaolingzhiols D and E from *Ganoderma lucidum* (Karst). These reported strong inhibitory activity with IC_{50} values of 8.52 and 7.37 μM, respectively (Luo et al., 2019b) (Table 3 and Figure 3).

Acetylcholinesterase inhibitory activity of meroterpenoids from algae sources

Seong et al. isolated sargahydroquinoic acid, sargachromanol, and sargaquinoic acid meroterpenoids for anti-Alzheimer's disease (AD) activity from *S. serratifolium* (C. Agardh). The result showed that all three compounds exhibited moderate inhibitory activity compared with berberine (Seong et al., 2017) (Table 4 and Figure 4).

Protein tyrosine phosphatase (PTP1B) inhibitory activity of meroterpenoids

PTP1B activity of meroterpenoids from marine

Preaustinoid-related meroterpenoids, preaustinoid A6, and berkeleyone C were isolated and identified from *Penicillium* species on the chemical investigation by Park et al. The compounds inhibited PTP1B activity with IC_{50} values of 17.6 and 58.4 μM. It was also found that compound preaustinoid A6 lowered the apparent value of V_{max} and increased the K_i value of 17 μM, indicating that it inhibited PTP1B in a non-competitive manner (Park et al., 2019) (Table 2 and Figure 2).

PTP1B activity of meroterpenoids from plants

Meroterpenoids from species *Magnolia* and *Rhododendron* have been extensively studied for PTP1B inhibiting activity. Li et al. isolated polycyclic meroterpenoid magterpenoids A and C from ethanolic extract bark of *Magnolia officinalis* (Rehder & Wilson) var. biloba. The result displayed PTP1B with IC_{50} values of 1.44 and 0.81 μM, respectively (Li C. et al., 2018). Meroterpenoids enantiomeric pairs, (−) and (+)-rhodonoid B, were extracted from partly racemic mixtures that existed naturally in *Rhododendron capitatum* (Maxim.). The result demonstrated inhibition (IC_{50} values of 43.56 and 30.38 μM) compared to positive control oleanolic acid (Liao et al., 2015). From *Rhododendron nyingchiense* (R.C. Fang & S.H. Huang), Huang et al. isolated meroterpenoids, (+) nyingchinoids A and B, (−) nyingchinoids C and D, (±)-nyingchinoids H, and grifolin. The study showed that the compounds with IC_{50} values between 5.7 ± 0.5 and 61.0 ± 4.8 μM exhibited weak inhibitory effects (Huang et al., 2018). Li et al. isolated compounds of magmenthanes E and H from *Magnolia officinalis* (Rehder & Wilson) var. Biloba bark. The compounds displayed significant inhibition against PTP1B (IC_{50} values of 4.38 and 3.88 μM) (Li C. et al., 2019) (Table 3 and Figure 3).

Bromodomain-containing protein 4 (BRD4) inhibitory activity of meroterpenoids

Bromodomain-containing protein 4 is a transcriptional and epigenetic protein in humans encoded by the *BRD4* gene. *BRD4* plays a critical role in cancer growth and embryogenesis and is responsible for the development of many diseases. BRD4 inhibited by molecules can be developed as anti-viral, anti-inflammatory, anti-proliferative, and anticancer drugs (Qin F.-Y. et al., 2019).

The fruiting bodies of *Ganoderma cochlear* (Blume & T. Nees) Bres. have isolated (±) cochlearoids N–P, three pairs of meroterpenoids. The outcome revealed that (±) cochlearoid N showed a *BRD4* inhibitory effect against K562 cells with IC_{50} values of 7.68 and 6.68 μM (Qin F.-Y. et al., 2019) (Table 1 and Figure 1).

Anti-Kaposi's sarcoma-associated herpes virus activities of meroterpenoids

Kaposi's sarcoma-associated herpes virus (KSHV) is a double-stranded DNA-based carcinogenic pathogen. KSHV is involved in Kaposi's sarcoma diseases, AIDS, Castleman's disease, and primary lymphoma drugs such as ganciclovir, cidofovir, or nelfinavir, and the target is generally used to inhibit KSHV replication. However, this drug cannot restrain the virus effectively. Therefore, natural products such as meroterpenoids were investigated as KSHV inhibitors (Hu et al., 2018).

Hu et al. investigated acylphloroglucinol-based meroterpenoid japonicols E and H from *H. japonicum* (Thunb.). The result exhibited strong inhibition toward the lytic replication in Vero cells (IC_{50} values of 8.30 and 4.90 μM) (Hu et al., 2018).

Immunosuppressive activity of meroterpenoids

By effective genome mining, arthripenoid C was isolated from two fungi targeting genetically proximal genes from polyketide and terpenoid biosynthesis. These compounds inhibit concanavalin- (ConA-) induced T-cell proliferation (IC_{50} values of 8.8 μM). In addition, both TNF-α and IFN-γ were substantially secreted from activated T cells in response to stimulation with ConA, which was markedly attenuated with IC_{50} 4.2 and 12.1 μM treatment with arthripenoid C (Zhang X. et al., 2018).

Effect of meroterpenoids in obesity and non-alcoholic fatty liver disease

Kwon et al. investigated the effect of meroterpenoids from ethyl acetate fraction of *Sargassum serratifolium* (C. Agardh) (ESS) on obesity and related stenosis on the administration of a high-fat diet to C57BL/6J mice. EES supplementation restored the phosphorylation levels of AMP-activated protein kinase (AMPK) and reduced lipogenic proteins. Thus, ESS exerted the anti-obesity and lipid-lowering effects by activating AMPK-related fatty acid oxidation signaling in the adipocyte's cells. The study concluded that EES has the ability to prevent diet-induced obesity and related metabolic disorders by inhibiting lipogenesis and adipogenesis in 3T3-L1 preadipocytes and activating energy expenditure (Kwon et al., 2018a; 2018b).

Effect of meroterpenoids in sodium channel activation, inactivation, and window currents

Electrophysiological influences on the gating kinetics of voltage-gated sodium channels in central neurons were tested for acetoxydehydroaustin A and austin, isolated from *Verticillium albo-atrum* (Reinke & Berthold, 1879) fungus. They also improved the recovery time from rapid sodium channel inactivation. These findings found that both compounds affected the activation, inactivation, and window currents of the sodium channel (Wu et al., 2018).

Anti-viral activity of meroterpenoids

Anti-viral activity of meroterpenoids from the fungus

The anti-viral activity of *Penicillium* and *Aspergillus* isolated meroterpenoids has been reported. Austalide U, merochlorin D, austalide I, and austalide P acid meroterpenoids were isolated from *Aspergillus aureolatus* (Muntañola-Cvetkovic & Bata, 1964) HDN14-107 sponge-derived fungus culture by Peng et al. The CPE inhibition assay assessed the anti-influenza A virus (H1N1) activities of these compounds. The results showed that compounds with IC_{50} values of 90, 99, 131, and 145 μM exhibited inhibitory effects (Peng J. et al., 2016). Drimane-type sesquiterpene meroterpenoids chrodrimanins K and N and 3-hydroxypentacecilide A isolated from *Penicillium* sp. SCS-KFD09 displayed anti-H1N1 activity (IC_{50} values of 74, 58, and 34 μM) (Kong et al., 2017). Chrodrimanins A, E, and F isolated from *Penicillium funiculosum* (Thom, 1910) GWT2-24 showed inhibition against influenza A virus (H1N1) (IC_{50} values of 21, 55, and 57 μM) compared to that of the positive control ribavirin (Zhou et al., 2015) (Table 1 and Figure 1).

Anti-viral activity of meroterpenoids from marine sources

Polycyclic meroterpenoid talaromyolide D, obtained from the marine fungus *Talaromyces* sp. CX11, exhibited an inhibitory activity with a CC_{50} value of 3.35 μM against the pseudorabies virus (PRV) (Cao et al., 2019) (Table 2 and Figure 2).

Anti-viral activity of meroterpenoids from plants

Liao et al. performed a chemical investigation on the *Rhododendron capitatum* (Maxim.) aerial parts and isolated enantiomeric meroterpenoid and (+)-rhodonoid C. The anti-viral activity was evaluated against the HSV-1 *in vitro* study using the cytopathic effect (CPE) assay with acyclovir as the positive control. The compound showed inhibitory activity against HSV(IC_{50} value of 80.6 ± 4.7 μM) (Liao et al., 2017). The hybrid polyketide-terpenoid stachybonoid A isolated from fungus *Stachybotrys chartarum* (Ehrenb.) 952 reported inhibitory activity against the dengue virus replication (Liu Z. et al., 2018). Linzhen hu et al. isolated filicinic acid-based meroterpenoid hyperjaponols B and D from *Hypericum japonicum* (Thunb.). The compounds were assessed for activity against the anti-Epstein–Barr virus. The compounds with EC_{50} values of 0.57 and 0.49 μM showed an inhibitory effect on the Epstein–Barr virus (Hu et al., 2016) (Table 3 and Figure 3).

Neuroinhibitory activity of meroterpenoids

Matos et al. investigated hydroquinones and benzoquinone-based meroterpenoid compounds from

Cordia oncocalyx (F. Allum.). They isolated a new compound rel-1,4,8α-trihydroxy-5-furanyl-2-methoxy-8aβ-methyl-6,7,8,8a,9,10-hexahydro-10-anthracenone, reported to possess the neuroinhibitory activity, and none of the pharmacological antagonists was reversed. Additionally, compounds rel-1,4,8α-trihydroxy-5-furanyl-2-methoxy- 8aβ-methyl-6,7,8, 8a, 9,10-hexahydro-10-anthracenone and 6-formyl-2-methoxy-9-methyl-1,4-phenanthrendione were able to inhibit the 69% and 63% contractions, respectively (Matos et al., 2017).

Neuroprotective activity of meroterpenoids

From *Ganoderma austral*, meroterpenoids ganomycin C, (−)-ganoresinain A, ganotheaecoloid G were isolated by Zhang et al. The compounds were tested in glutamate-induced SH-SHY cells for neuroprotective activity. The result showed that these compounds prevent glutamate-mediated cellular toxicity of neural cells (Zhang J. J. et al., 2019). Benzylic phloroglucinol-terpene hybrid type meroterpenoid, namely, melaleucadines A and B, were isolated by Kie et al. from branches and leaves of *Melaleuca Leucadendron* (L.) L. These compounds possessed neuroprotective activity on Cort-induced PCI-2 cell injuries with cell viability of 53.72% and 58.38%, respectively, at 50 μM (Xie et al., 2019).

JAK3 inhibitory activity of meroterpenoids

Spiroapplanatumines G and H spiro meroterpenoids were isolated from *Ganoderma applanatum* (Pers.) Pat. 1887, fungus. The results showed that these compounds with IC_{50} values of 7.0 ± 3.2 and 34.8 ± 21.1 μM display inhibitory properties on JAK3 kinase (Luo et al., 2017).

Anti-plasmoid activity of meroterpenoids

Cadelis et al. studied thiaplidiaquinones A and B and their effect against the NF54 strain of chloroquinone-sensitive *P. falciparum*. The prenyl and farnesyl analogs exhibited moderate activity against *P. falciparum* (Welch, 1897) (IC_{50} 0.29 mM), with the farnesyl series exhibiting greater selectivity (Cadelis et al., 2017).

Chan et al. conducted a bioassay of the New Zealand ascidian *Aplidium scabellum* (Michaelsen, 1924) that yielded pseudodimeric meroterpenoid, namely, scabellone B. The compound exhibited selectivity toward *Plasmodium falciparum* (Welch, 1897) (IC_{50} 4.8 μM) (Chan et al., 2011).

HMG-CoA reductase inhibitory activity of meroterpenoids

Triterpene meroterpenoids ganomycins I, B, and C were isolated by Wang et al. from fruiting bodies of *Ganoderma leucocontextum* (T. H. Li, W. Q. Deng, Dong M. Wang & H. P. Hu, 2015). These compounds exhibited stronger inhibition compared to the positive control atorvastatin against HMG-CoA reductase (Wang et al., 2017).

Renal protective activity of meroterpenoids

Petchiethers A and B, isolated from *Ganoderma petchii* (Lloyd) Steyaert, 1972, were tested for the inhibition of overproduction of fibronectin. The results show that both compounds could inhibit the development of fibronectin in a dose-dependent manner and achieve maximal effects at 20 μM concentrations (Li C. G. et al., 2016). Phenolic meroterpenoids, namely, cochlearoids (F–I, K), cochlearol (K, S, U, X, and Y), and cochlearin E, isolated from *Ganoderma cochlear* (Blume & T. Nees) Bres. demonstrated an inhibitory effect against TGF-β1-induced HKC-8 cells and TGF-β1-induced NRK-49F cells, respectively. Cochlearoids (F–I, K) showed a potential inhibitory effect on fibronectin overproduction in TGF-β1-induced HKC-8 cells. Similarly, cochlearols (K, S, U, X, and Y) and cochlearin E inhibited fibronectin overproduction in TGF-β1-induced NRK-49F cells (Wang X. L. et al., 2016; 2019b; 2019a). Racemic polycyclic meroterpenoid (+)- and (−)-cochlearols A and B isolated from *Ganoderma cochlear* (Blume & T. Nees) Bres. reported inhibitory activity of collagen I, fibronectin, and α-SMA in a dose-dependent manner in TGF-β1- induced rat renal proximal tubular cells. Also, (−)-cochlearol B showed strong inhibitory activity against p-Smads in TGF-β1-induced rat renal proximal tubular cells (Dou et al., 2014). Luo et al. isolated chizhine F, fornicin B, and ganomycin I from *Ganoderma lucidum* (Curtis) P. Karst., which inhibited the MCP-1 expression in high-glucose-induced mesangial cells in a dose-dependent manner (Luo et al., 2015). Lactone fused meroterpenoid lingzhilactone B isolated from *Ganoderma lingzhi* (Sheng H. Wu, Y. Cao & Y.C. Dai, 2012) reported an inhibitory effect in adriamycin-induced nephropathy mice. The *in vitro* and *in vivo* results suggested that lingzhilactone B inhibits various activities such as ROS generation, increased expression of Nrf2, mRNA expression of collagen IV, and fibronectin in rat tubular epithelial cells. It also could reduce urinary albumin levels, inhibit the phosphorylation of Smad3, and protect against renal injuries by inhibiting inflammation and increasing the activity of anti-oxidants (Yan et al., 2015b).

Anti-fibrotic activity of meroterpenoids

Ding et al. isolated lingzhifuran A and lingzhilactone D, phenolic meroterpenoids, from the fruiting bodies of *Ganoderma lucidum* (Curtis.) P Karst. The compounds exhibited Smad3 phosphorylation inhibition (Ding W. Y. et al., 2016).

Cardioprotective activity of meroterpenoids

Zhu et al. isolated flavonoid-triterpene saponin meroterpenoids, namely, clinoposides B, D, and F, which showed cell viability of 87.2 ± 7.7%, 82.7 ± 8.3%, and 90.8 ± 6.5% at 25.0 μg/ml using quercetin and ginsenoside Rb 1 as a positive control. All three compounds showed better protective effects as evidenced by increased levels of SOD, CAT, and GSH-Px and reduced MDA, LDH, caspase-3, and caspase-9 levels (Zhu et al., 2016).

Anti-leishmanial activity of meroterpenoids

Two stigmasterol derivatives, (22E, 24R)-stigmasta-5,7,22-trien-3-β-ol, stigmast-4-en-3-one, isolated from the roots of *Carthamus lanatus* L. (Asteraceae) showed good exhibition toward *L. donovani* (IC_{50} values of 4.61 and 6.31 μg/ml) (Ibrahim et al., 2015) (3R)- and (3S)-tetraprenyltoluquinol and (3R)-tetraprenyltoluquinone and (3S)-tetraprenyltoluquinone, isolated from *Cystoseira baccata* (S. G. Gmelin) P. C. Silva, 1952, could inhibit the growth of the *L. infantum* (Nicolle, 1908) promastigotes (IC_{50} 44.9 and 94.4 μM). Compound (3R)- and (3S)-tetraprenyltoluquinol decreased the intracellular infection index (IC_{50} = 25.0 ± 4.1 μM). Disulfated meroterpenoids, isoakaterpin, from extracts of *Callyspongia* sp. exhibited inhibition of *Leishmania* spp. adenosine phosphoribosyl transferase (IC_{50} of 1.05 μM) (Gray et al., 2007) (Table 3 and Figure 3).

Gastroprotective activity of meroterpenoids

Meroterpenoids sargaol, epitaondiol, stypodiol, and isoepitaondiol were isolated from the *Stypopodium flabelliforme* Weber-van Bosse, 1913, Chilean Seawood by Areche et al. The gastroprotective activity was evaluated using a gastric ulcer ethanol/HCL-induced mice model. Among meroterpenoids obtained, sargaol and epitaondiol with ED_{50} values of 35 and 40 mg/kg reported gastroprotective activity, respectively. Oral administration of stypodiol and isoepitaondiol at 40 mg/kg blocked 69% and 78% of the appearance of gastric mucosal lesions in mice, respectively (Areche et al., 2015). (Table 2 and Figure 2).

Neural stem cell proliferation activity of meroterpenoids

Yan et al. isolated spirolingzhines A–D, lingzhines (B, D–F), and 4-(2,5-dihydroxyphenyl)-4-oxobutanoic acid meroterpenoids from the fruiting bodies of the *Ganoderma lingzhi* (Sheng H. Wu, Y. Cao & Y.C. Dai), 2012, fungus. In order to determine whether the isolated compounds affect the CNS, their ability to regulate adult NSCs from P7 mouse dentate gyrus was evaluated. The results showed that these compounds promoted NSC proliferation (-)-spirolingzhine A, which was found to exhibit the highest NSC proliferation activity comparable to the positive control forskolin (Yan et al., 2015a).

Inhibition of AP-1 activity of meroterpenoids

In a study by Zhuravlena et al., isolated meroterpenoids, austalide H acid butyl ester, 13-O-deacetylaustalide I, austalide H acid, and 13-deacetoxyaustalide I, were isolated from *Penicillium lividum* Thom, C. KMM 4663 and *Penicillium thomii* Maire, R.C.J.E. 1917, KMM 4645. The outcome reported that the transcriptional activity of AP-1 oncogenic nuclear factor of JB6 Cl41 cells was inhibited at noncytotoxic concentrations after 12 h of treatment by these compounds. At 6.25 μM concentration, these compounds exhibited inhibitory activity, whereas the reduction of cell viability up to 100 μM was not observed (Zhuravleva et al., 2014).

Insecticidal activity of meroterpenoids

Meroterpenoid dhilirolide L isolated from the fungus *Penicillium purpurogenum* Stoll (1923) by Centko et al. showed inhibitory activity and exhibited sublethal developmental disruption at low concentrations in the *Trichoplusia ni* (Hübner, 1800–1803) cabbage looper (Centko et al., 2014). Chrodrimanin-type (A, B, E, H, G, and F) meroterpenoids from the solid cultures of a mangrove endophytic fungus *Diaporthe* sp. SCSIO 41011 showed inhibitory insecticidal activity of GABA-gated chloride channels as potent and selective blockers of insects (Luo X. W. et al., 2019). Chondrimanins D–F were isolated by Hayashi et al. from okara, which is the solid residue of soybean, fermented with the YO-2 strain of *Talaromyces* sp., showing inhibitory activity against silkworms with LD_{50} values of 20, 10, and 50 μg/g of diet (Hayashi et al., 2012). Bai et al. isolated meroterpenoids, namely, penicianstinoids A and B, furanoaustinol, austinol, 1,2-dihydro-7-hydroxydehydroaustin, 7-hydroxydehydroaustin, and dehydroaustinol from bioactive metabolites of *Penicillium* sp. The researchers reported inhibitory with EC_{50} values of 9.4, 9.9, 19.1, 19.5, 20.5, 20.6, and 38.2 μg/ml against *C. elegans* (Bai et al., 2019).

Selective inhibitors of the p-Smad3 activity of meroterpenoids

(+)-Lingzhiol and (-)-lingzhiol, a pair of rotary door-shaped meroterpenoid enantiomers, were isolated from *Ganoderma lucidum* Karst (1881) by Yan et al. to study the effect against diabetic nephropathy (+)lingzhiol and (-)-lingzhiol, demonstrating inhibition of TGF-β1-induced p-Smad3 in renal proximal tubular cells of rat and initiating the production of Nrf2/Keap1 in mesangial cells (Yan et al., 2013).

Inhibitors of Na+/K + ATPase activity of meroterpenoids

Sargachromanols D, F, H, and L are the meroterpenoids of the chromene class isolated from the *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh, 1820, brown algae. The study result indicated that compounds exhibited inhibitory activity toward Na+/K + ATPase from the porcine cerebral cortex in a study by Chung et al. (2011).

Isocitrate lyase inhibitory activity of meroterpenoids

Chung et al. isolated chromene class meroterpenoids, namely, sargachromanols L, M, and P, from the brown alga *Sargassum siliquastrum* (Mertens ex Turner) C. Agardh, reporting that compounds exhibited moderate ICL inhibitory activity (Chung et al., 2011).

Chenodeoxycholic acid-activated human farnesoid X receptor activity of meroterpenoids

Choi et al. isolated meroterpenoids tuberatolides A and B, 2′-*epi*-tuberatolide B, yezoquinolide, (*R*)-sargachromenol, and (*S*)-sargachromenol from the Korean marine tunicate *Botryllus tuberatus* Ritter & Forsyth, 1917. In a cotransfection cell-based assay, these compounds without significant cytotoxicity showed potent inhibition of hFXR transactivation. Also, tuberatolide A at low concentrations antagonized chenodeoxycholic acid- (CDCA-) dependent activation of hFXR without any cytotoxicity in both bioassay systems (Choi et al., 2011).

Mammalian mitochondrial respiratory chain inhibitory activity of meroterpenoids

Two meroterpenoids, terretonins E and F, along with the known compound aurantiamine, was isolated as fermentation products of the marine fungus *Aspergillus insuetus* (Bainier) Thom & Church (1929), associated with the sponge *Petrosia ficiformis* (Poiret, 1979). Meroterpenoids, terretonins E and F, showed potential inhibition of the integrated chain (NADH oxidase activity; also, aurantiamine was five times less potent than terretonin F (López-Gresa et al., 2009).

Hypoxia-inducible factor-1 inhibitory activity of meroterpenoids

Meroterpenoids, bisbakuchiols A–C, 12,13-dihydro-12,13-dihydroxybakuchiol, 12,13-dihydro-12,13-epoxybakuchiol and O-methyl, and O-ethyl bakuchiols, were isolated from the seeds of *Psoralea corylifolia* L. (Fabaceae) in a study by Wu et al. The result displayed that all compounds exhibited an HIF-1 inhibitory effect (Wu et al., 2008). In a similar study, a bioassay-guided phytochemical investigation by Wu et al. of the methanol extract of *P. corylifolia* using a HIF-1-mediated reporter gene assay in human gastric cancer cells led to the isolation of dimeric meroterpenoid (S)-bakuchiol inhibited hypoxic activation of HIF-1 with an IC_{50} value of 6.1 μM (Wu et al., 2007).

Larvicidal activity of meroterpenoids

Geris et al. conducted a study to determine the potential of larvicidal activity of meroterpenoids, dehydroaustin, acetoxydehydroaustin, and austin from *Penicillium* sp. against third-instar larvae of *A. aegypti*. The results showed that when the meroterpenoids at a concentration of 500 ppm each were exposed to third-instar larvae of *A. aegypti*, meroterpenoids dehydroaustin and acetoxydehydroaustin exhibited *in vitro* larvicidal activity of 100% and 70%, respectively, after 24 h of exposure and austin displayed a very low larval mortality compared with positive control temephos (Geris et al., 2008).

Anti-invasion activity of meroterpenoids

Meroterpenoids, namely, avinosol, avarone, avarol, and avinosone, were isolated from *Dysidea* sp. marine sponge collected in Papua New Guinea in a study by Marrero et al. The meroterpenoids were tested in the anti-invasion assay against MDA-MB-231 breast cancer cell lines and LS174T colon carcinoma cells. It was found that avinosol had an IC_{50} of ~50 μg/ml in the anti-invasion assay against both cell lines. Avarone, avarol, and avinosone were only active in the assay at a concentration of 100 μg/ml (Diaz-Marrero et al., 2006).

Protein kinase MK2 inhibitory activity of meroterpenoids

Williams et al. isolated (+)-makassaric acid and (+)-subersic acid, new meroterpenoid inhibitors of the protein kinase MK2m from the marine sponge *Acanthodendrilla* sp. The study concluded that (+)-makassaric acid and (+)-subersic acid inhibited MK2 with IC_{50} of 20 and 9.6 μM, respectively (Williams et al., 2004).

Antibiofilm activity of meroterpenoids

From the leaves of *E. robusta*, eucarobustol E (EE) meroterpenoid was isolated. The results showed strong inhibitory activity against *C. albicans* biofilms with 16 μg/ml concentration. The study concluded that EE blocked yeast-to-hypha transition and thus reduced cellular surface hydrophobicity cells of biofilm (Liu R. H. et al., 2017).

Phosphodiesterase-4 inhibitory activity of meroterpenoids

The isolation of *Psidium* meroterpenoids psiguajadials A–K was triggered by bioassay-guided fractionation of the ethanolic extract of *Psidium guajava L.* leaves, guajavadial C, psiguadial D, psiguadial A, guapsidial A, psidial A, guajadial, psiguajadial L, guajadials C–F, guajavadial A, and guadial A. The isolated compounds exhibited moderate inhibitory activity with IC_{50} values in the range of 1.34–7.26 μM compared with positive control rolipram (Tang et al., 2017).

Increase in intracellular free calcium activity of meroterpenoids

From the *Ganoderma petchii* (Lloyd) Steyaert (1972) fruiting bodies, Gao et al. isolated petchienes B and (-) D. Outcomes demonstrated that isolated compounds could significantly elevate the concentration of intracellular Ca^{2+} at 10 μM in HEK-293 cells (Gao et al., 2015).

Effect of meroterpenoids in dermatological diseases

3,5-Dimethylorsellinic acid- (DMOA-) related meroterpenoids, namely, brasilianoids A–E were isolated, from the fungus *Penicillium brasilianum* Bat. WZXY-m122-9 ethyl acetate extract. Compound brasilianoid A significantly increased the expression of caspase-14 and filaggrin in HaCaT cells in a dose-dependent manner., The cytotoxicity of brasilianoid A against HaCaT cells was measured by the MTT assay to test the skin protective activity against UVB irradiation. After exposure to UVB 30 mJ/cm^2, cell viability was decreased to 70% compared to the normal group. Brasilianoid A (20 μM) treated the damaged cells, increasing cell viability to 77% compared with positive control epigallocatechin gallate. NO production in LPS-induced RAW 264.7 macrophages was moderately inhibited by meroterpenoids, namely, brasilianoids B and C. In addition, brasilianoids C–E (10 μM) also resulted in the inhibition of DNA expression of the HBV virus in HepG2.2.15 cells with the inhibition rates of 25%, 15%, and 10%, respectively, the same as that of lamivudine (positive control) (Zhang J. et al., 2018).

Phytotoxic activity (plant toxicity) of meroterpenoids

Ma et al. isolated guignardianone C from the fermentation extract of *Phyllosticta capitalensis* Henn., (1908). The phytotoxic effects of guignardianone C on *Lactuca sativa* L. and *Lolium perenne* L. were evaluated. Guignardianone C displayed inhibition activity on the shoot growth of *L. sativa* and *L. perenne* and the root growth of *L. perenne* (Ma et al., 2019).

Growth inhibition activity of meroterpenoids against newly hatched larvae of *Helicoverpa armigera* (Hübner, 1808)

Bai et al. isolated bioactive metabolites from mangrove-derived fungal *Penicillium* sp. (penicianstinoids A and B; peniciisocoumarins A, B, E, F, and H; austinol; 1,2-dihydro-7-hydroxydehydroaustin; and austin). These were reported to have growth inhibitory activity with IC_{50} values between 50 and200 μg/ml, respectively (Bai et al., 2019).

Summary

Meroterpenoids are a group of partially derived secondary metabolites from terpenoid biosynthetic pathways. They exhibit huge structural diversity, from basic compounds containing a prenyl unit to more complex meroterpenoids formed with functionalized carbon chains. Meroterpenoids and their derivatives are isolated from natural resources, such as seeds, animals, fungi, and marine organisms. They have

been rigorously subjected to pharmacological screening and possess a broad spectrum of pharmacological activities. More than 190 meroterpenoids reported here were isolated from different species of fungi, such as *Penicillium*, *Aspergillus*, *Ganoderma*, and *Sargassum*, and have shown anticancer, anti-proliferative, anti-viral, anti-microbial, anti-inflammatory, anti-Alzheimer's, and anti-obesity activities. Similarly, algal-based meroterpenoids isolated from algae species such as *Cystoseira*, *Sargassum*, and *Hypericum* have shown anti-oxidant, anti-microbial, anti-proliferative, and cytotoxic activity. Species of *Ganoderma*, *Eucalyptus*, *Cordial*, *Rhododendron*, and *Psidium* are primary sources of plant-based meroterpenoids active against HIV, leishmaniasis, diabetes, fungal, and bacterial infections and Alzheimer's and cancer progression. More than 80 meroterpenoids were isolated from marine sources, such as seaweeds, clam, sponges such as *Dactylospongia*, Okinawan, Chilean, actinomycetes, and *Penicillium*. Species have reported pharmaco-biological activities such as anti-inflammatory, cytotoxicity, gastroprotective, anti-viral, antidiabetes, and anti-microbial. Meroterpenoids have also shown activity against alpha-glucosidase, Kaposi-sarcoma associated herpes virus, N-acetyltransferase, BACE1, acetylcholinesterase (AchE), PTP1B, and bromodomain-containing protein 4. They have also demonstrated renoprotective, cardioprotective, and neuroprotective activities. The plethora of research conducted on meroterpenoids from various sources suggests the potential of meroterpenoids being used against the spectrum of diseases and disorders. This review explicitly discusses the nomenclature and isolation of meroterpenoids from different sources and their reported biological activities. The promising range of biological activities and structural complexities exhibited by meroterpenoids make them valuable targets for in-depth study as novel drug candidates.

Author contributions

Conceptualization: MO, RR, KS, and NF; resources: NF, RR, KS, MO, YK, VS, MS, and SF; data curation: NF, RR, KS, MO, YK, VS, MS, and SF; writing—original draft preparation: NF, RR, KS, MO, YK, VS, MS, and SF; writing—review and editing: NF, RR, KS, MO, YK, VS, MS, and SF. All authors have read and agreed to the published version of the manuscript.

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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Glossary

MAPK Mitogen-activated protein kinase

LPS Lipopolysaccharide

NO Nitric oxide

COX-2 Cyclooxygenase

IL-6 Interleukin-6

IL-10 Interleukin-10

IL-1β Interleukin-1 β

IL-8 Interleukin-8

PEG2 Prostaglandin G2

TNF-α Tumor necrosis factor-α

LTB4 Leukotriene B4

NF-κB Nuclear factor kappa light chain enhancer of activated B cells

5-LOX 5-Lipoxygenase

Nrf2 Nuclear factor erythroid 2-related factor 2

HO-1 Heme oxygenase 1

PI3K Phosphoinositide 3-kinase

Akt Protein kinase B

ABTS- 2,2 Azinobis[3-ethylbenzothiazoline-6-sulfonate]

DPPH- 2,2 Diphenyl-1-picrylhydrazyl

TBARS Thiobarbituric acid reactive substances

TEAC Trolox equivalent anti-oxidant capacity

PCL Photo chemiluminescence

MIC Minimum inhibitory concentration

IC50 Half maximal inhibitory concentration

MRSA Methicillin-resistant *Staphylococcus aureus*

FPM Formyl phloroglucinol meroterpenoid

BACE1 Beta-site amyloid precursor protein cleaving enzyme 1

TGF-β1 Transforming growth factor beta 1

AchE Acetylcholinesterase

PTP1B Protein tyrosine phosphatase

ESS Sargassum serratifolium

H1N Anti-influenza A virus

MCP-1 Monocyte chemoattractant protein-1

SOD Superoxide dismutase

CAT Catalase

GSH-Px Glutathione peroxidase

MDA Malondialdehyde

LDH Lactate dehydrogenase

NSC Neural stem cell

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