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journal homepage: <https://www.sciopen.com/journal/2097-0765>Hypolipidemic activity and mechanism of active components of *Dracocephalum moldavica* L. teaSiqi Li^{a,b,1}, Qinyu Li^{a,b,1}, Na Zhang^{b,*}, Minhui Li^{a,b,c,*}^a Inner Mongolia Autonomous Region Hospital of Traditional Chinese Medicine, Hohhot 010020, China^b Department of Pharmacy, Baotou Medical College, Baotou 014040, China^c Inner Mongolia Traditional Chinese and Mongolian Medical Research Institute, Hohhot 010010, China

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ABSTRACT

Hyperlipidemia is associated with many diseases and considered the main contributing factor in the development of metabolic syndrome. The traditional Mongolian medicine *Dracocephalum moldavica* L. is commonly consumed as a tea (non-*Camellia* tea) for the prevention and treatment of coronary heart disease, hypertension, atherosclerosis, and other diseases. Here, we attempted to investigate the hypolipidemic activity and mechanisms underlying the active components of *D. moldavica*. First, the mechanism underlying the lipid accumulation-reducing effects of the *D. moldavica* ethanol extract was determined using *in vitro* experiments, and 4 polar fractions were screened for activity. Then, the effect of the ethyl acetate fraction of *D. moldavica* (EAD) on lowering blood lipid was confirmed *in vivo*. The combination of network pharmacological and serum pharmacokinetics analyses showed that apigenin, luteolin, and naringenin of EAD existed in prototype form in the drug-containing serum of rats. We also determined the content of the 3 active ingredients in EAD. Finally, *in vitro* experiments showed that apigenin, luteolin, and naringenin improved oleic acid-induced lipid accumulation in HepG2 cells by regulating the PPAR signaling pathway (peroxisome proliferator-activated receptor alpha (PPARα), carnitine palmityl transferase I (CPT1), and fatty acid binding protein-3 (FABP3)). Our findings provide mechanistic insights and application prospects for the prevention and treatment of hyperlipidemia using *D. moldavica*.

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1. Introduction

Hyperlipidemia is considered a lipid metabolism disorder rather than a disease; it is characterized by abnormal lipid levels^[1-2]. With societal and economic development, living standards have improved, work pressure has increased, convenience foods have become more popular, and poor lifestyle and eating habits have emerged; thus, the prevalence of dyslipidemia is rising annually^[3] and observed worldwide^[4]. In China, the overall prevalence of dyslipidemia in

adults is 40.4%^[5]. Currently, the drugs used for the clinical treatment of hyperlipidemia include statins and fibrates, and they may cause liver injury and further aggravate impaired liver function^[6-7]. Therefore, identifying effective and non-toxic drugs from traditional ethnic medicines that can lower blood lipids and performing relevant pharmacological efficacy research is of vital importance.

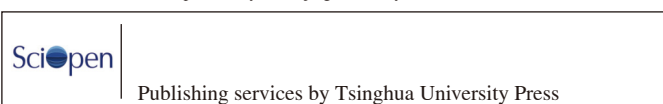
Dracocephalum moldavica L. tea, as a non-*Camellia* tea^[8], can lower blood sugar and lipid levels and is often consumed in northern regions of China, such as Shanxi and Shaanxi, for the prevention and treatment of diseases^[9]. In recent years, with the increasing demand for herbal medicines in the global market, non-*Camellia* tea has attracted increasing attention^[10]. *D. moldavica* is an annual herb belonging to the Lamiaceae family (Fig. 1), and its common name in Mongolia is beriyenggü. The earliest record of Xiangqinglan (*D. moldavica*) use was in *Ren Yao Bai Jing Jian* published in the 18th century,

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where it was listed as a Mongolian medicine with a sweet property and the ability to remove heat from the stomach and liver. Modern pharmacological studies have shown that it is commonly used for the prevention and treatment of coronary heart disease, hypertension, atherosclerosis, and other diseases^[11–13], and drinking it as a tea is a simple and convenient means of consumption. However, the mechanism underlying blood lipid regulation by *D. moldavica* is unclear.

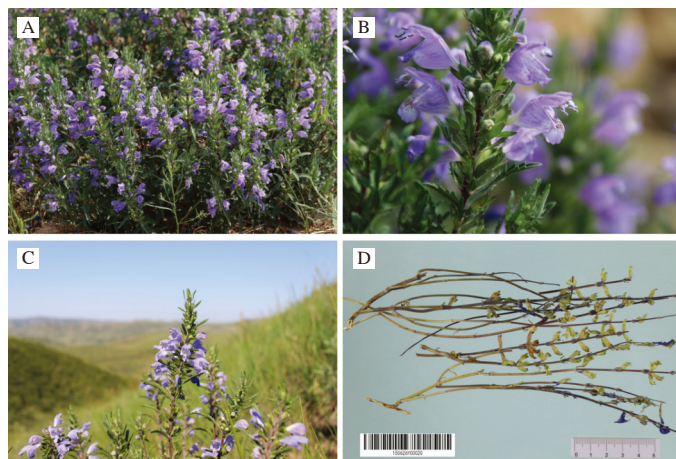


Fig. 1 *D. moldavica*: morphology of fresh whole plants (A–C) and whole plant specimen (D).

Network pharmacology is an emerging scientific discipline based on theories from traditional pharmacology, chemoinformatics, bioinformatics, network biology, and network analysis. The pharmacological mechanisms of drugs have been revealed at the system level and the entire biological network level^[14]. Elucidating the active components and mechanisms of action of traditional Chinese and Mongolian medicines is a significant challenge in drug research. Therefore, network-based global predictions of drug ingredients–drug targets–disease genes have been promoted to identify disease-associated genes and predict the target profiles and pharmacological actions of herbal compounds^[15]. Serum pharmacochimistry analysis of traditional Chinese medicine (TCM) is an effective method of rapidly screening effective substances, and it has become universally accepted and widely applied over the past 2 decades. Compared with previous approaches, the interdisciplinary integration of serum pharmacochimistry and network pharmacology or omics can better explain drug changes *in vivo* and *in vitro*, interactions between drugs, and interactions between medicines and the body^[16].

Owing to the complex interaction mechanism between the active components and target signaling pathways, the specific molecular mechanism underlying the ability of active components of *D. moldavica* to inhibit dyslipidemia remains to be elucidated. The combination of serum pharmacochimistry and network pharmacology is suitable for solving this problem. Therefore, in this study, after determining the effectiveness of the ethanol extract of *D. moldavica*, we screened the activity of four polar fractions of *D. moldavica* *in vitro* and validated the active parts that present a curative effect *in vivo*. The chemical composition of the active part was then identified using liquid chromatography–tandem mass spectrometry (LC/MS–MS). Network pharmacology was used to predict the potentially effective components, targets, and signaling pathways of the parts that are active in the treatment of dyslipidemia. Furthermore,

the active components of *D. moldavica* were determined by serum pharmacochimistry, and the content of active components was determined by high-performance liquid chromatography (HPLC). The regulatory effects of the active components of *D. moldavica* on core targets and the mechanism underlying the reduction in blood lipids were further verified through *in vitro* experiments. Our findings will provide a reference for studies focused on pharmacodynamic substances of *D. moldavica* in regulating blood lipid levels.

2. Material and methods

2.1 *D. moldavica* preparation and reagents

D. moldavica was collected from Xilinguole League, Inner Mongolia Autonomous Region, China, in July 2020 and identified by Prof. Chunhong Zhang (Baotou Medical College (China)). A voucher specimen was deposited at the Herbarium (Specimen No. 150204190830007LY) at Baotou Medical College for future reference. After drying the whole *D. moldavica* specimen, it was beaten into a powder (1.91 kg) and successively extracted with 70% ethanol to obtain approximately 184.89 g of extract. The extract was dissolved in water and extracted successively with petroleum ether, ethyl acetate, and *n*-butanol to produce 5.88, 14.42, and 35.50 g dry sections, respectively. The aqueous phase contained 71.71 g of the residue.

Analytical-grade ethanol, petroleum ether, ethyl acetate, and *n*-butanol were purchased from Tianjin Fengchuan Chemical Reagent Technology Co., Ltd. (Tianjin, China). Luteolin, apigenin, naringenin, luteoloside, naringin, apin (98% purity by HPLC), bovine serum albumin V, *cis*-9-octadecenoic acid, Dulbecco's modified Eagle medium (DMEM), trypsin, cell counting kit-8 (CCK-8), Oil red O staining kit, TRIzol, and phosphate-buffered saline (PBS) were purchased from Solarbio (Beijing, China). Fetal bovine serum (FBS), penicillin, and streptomycin were purchased from HyClone (Logan, UT). PrimeScript RT reagent kits and TB Green Premix Ex Taq II were purchased from TaKaRa Biotechnology Co. (Shiga, Japan). Antibodies against peroxisome proliferator-activated receptor- α (PPAR α) and carnitine palmitoyltransferase 1 (CPT-1) were purchased from Absin Bioscience (Shanghai, China). GAPDH was purchased from Affinity Biosciences (Cincinnati, OH, USA). Horseradish peroxidase (HRP)-conjugated affinity-pure goat anti-rabbit IgG (H + L) was purchased from Beyotime (Shanghai, China).

2.2 Cell lines and cell culture

The human hepatocellular carcinoma cell line HepG2 was acquired from the cell bank of the BeNa Culture Collection (Beijing, China) and cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin. The cells were cultured and incubated at 37 °C and 5% CO₂ conditions for all experiments.

2.3 Cytotoxicity assay

Toxicity was assessed using the CCK-8 assay. Briefly, HepG2 cells were seeded at a density of 8×10^3 cells/well in 96-well plates (Corning Inc., Corning, NY, USA). After overnight incubation, the cells were divided into 2 groups: the control and drug groups (different doses of corresponding drugs were given). After establishing the

groups, they were incubated for 24 h. Then, 10 μ L of the CCK-8 solution was added and incubated for another 2 h. The absorbance of the samples was measured at 450 nm using a Multimode Microplate Reader (Enspire, PerkinElmer, MA, USA).

2.4 Oil red O staining

HepG2 cells were seeded in 6-well plates at a density of 5×10^5 cells/well and incubated for 24 h. Then, the cells were divided into the following treatment groups: (1) control group (CK); (2) model group (sodium oleate, M); (3) positive control group (50 μ g/mL simvastatin, Y); and (4) drug treatment groups. Three parallel wells were prepared for each group. After 24 h of treatment with different concentrations of the drugs and sodium oleate, the supernatant of the original medium was collected and discarded. The cells were washed twice with PBS, fixed with 4% paraformaldehyde solution for 15 min, and washed with PBS after discarding. Next, 60% isopropanol was added for 10 s and then discarded. Oil red O staining solution was added, and the cells were stained for 15 min. The staining solution was then discarded, and the cells were rinsed three times with 60% isopropyl alcohol and PBS buffer. The size and quantity of accumulated lipids were observed using an inverted microscope (Motic, Xiamen City, China).

2.5 Measurement of intracellular serum total cholesterol (TC) and triglyceride (TG) contents

The cells were lysed using ultrasound in an ice water bath, and the resulting cell lysates were used to assess the TC and TG content using a commercial kit (Jiancheng, Nanjing, China) according to the manufacturer's instructions.

2.6 Animals and treatments

Forty-eight male Sprague–Dawley (SD) rats were purchased from SPF Biotechnology Co., Ltd. (Beijing, China) (License No. SCXK[Jing]2019-0010). The experiments were approved by the Ethical Review Committee of Baotou Medical College (approval number: Baotou Medical Lun Audit Animal 2021 No. 017; approval date: April 19, 2021). During the experiment, the rats were housed in cages containing three rats and kept under the following conditions: 12/12 h dark/light conditions, room temperature of 22–26 $^{\circ}$ C, relative humidity of 40%–70%, and *ad libitum* access to water. After 1 week of adaptive feeding, the rats were used in the following experiment.

Thirty-six SD rats were used for the pharmacodynamic studies. SD rats were randomly divided into normal (CK, $n = 6$) and modeling groups and fed a basal or high-fat diet (HFD) for 4 weeks, respectively. After 4 weeks of feeding, the modeling groups were randomly divided into 5 groups ($n = 6$ in each group, with continuous gavage once daily for 4 weeks): untreated model (M), positive control (Y, HFD + 0.21 mg/200 g atorvastatin per day), low-dose (VL, HFD + 7.32 mg/200 g ethyl acetate fraction of *D. moldavica* (EAD) per day), medium-dose (VM, HFD + 14.65 mg/200 g EAD per day), and high-dose (VH, HFD + 21.97 mg/200 g EAD per day) groups. Body weight was recorded once a week. Four weeks later, after administering the final dose of the drug, the rats were fasted and allowed free access to water for 24 h; they were

then subjected to an abdominal injection of pentobarbital sodium anesthesia.

Twelve SD rats were used for the serum pharmacochemical studies. SD rats were divided into an administration group ($n = 6$) and a blank group ($n = 6$) for the blood composition analysis. The administration group was administered 10 times the clinical dose of EAD (73.2 mg/200 g per day), and the blank group was administered the same volume of distilled water solution once daily for 3 days. At 1.5 h after the last intragastric administration, blood was collected from the abdominal aorta after anesthesia.

The dosage of EAD was calculated by converting the body surface area between humans and rats based on the extraction rate.

2.7 Serum biochemical assays

Blood was sampled from the abdominal aorta of rats. Plasma was separated by centrifugation at $644 \times g$ for 20 min, and the supernatant was collected. The levels of TC, TG, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using an automatic biochemical analyzer (Poch-80i; Xisen Mekang Biotechnology Co., Ltd., Shanghai, China).

2.8 Histological examination

The liver tissues were immediately removed, weighed, preserved in 4% paraformaldehyde, dehydrated in graded ethanol, and immersed in paraffin wax. Approximately 5 μ m sections were stained with hematoxylin-eosin (H&E) and then examined under a light microscope (Leica DM2000, Leica, Germany).

2.9 LC-MS/MS analysis of EAD

LC-MS/MS analyses of the *in vitro* screening and *in vivo* experimental verification results were performed to identify the chemical constituents of EAD. Chromatographic analyses of the extracted compounds were performed using a SCIEX X500 QTOF system (SCIEX, Framingham, MA, USA). An ACQUITY UPLC BEH C_{18} chromatography column (100 mm $\times$ 2.1 mm, 1.7 μ m) from Phenomenex (Torrance, CA, USA) was used for the chromatographic separations. The mobile phase consisted of water containing 0.1% formic acid (solvent A) and acetonitrile (solvent B), and gradient elution was performed for 0–40 min at 5%–95% B. The flow rate was set to 0.3 mL/min, the injection volume was 5 μ L, and the column temperature was 40 $^{\circ}$ C.

Mass spectrometry (MS) data were captured using a SCIEX X-500R QTOF instrument equipped with an electrospray ionization interface in positive ion mode. The following parameters were selected: temperature, 500 $^{\circ}$ C; spray voltage, 5 500 V; curtain gas, 30 psi; and collision gas, 7 psi. The time-of-flight (TOF) start mass was generated across a range of 100–1 500 Da in the TOF MS and 50–1 500 in the TOF MS/MS. The step-normalized collision energy settings were 20, 40, and 60 V.

2.10 Network pharmacology analysis

Mol2 files of the EAD components were retrieved from the PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) and Swiss Target

Prediction (<http://www.swisstargetprediction.ch>) databases. The target proteins and genetic code were extracted using the search function in the UniProt database (<http://www.uniprot.org/>), and the source and genetic information of the obtained drug targets were verified. Dyslipidemia-related targets were searched for in disease target databases, including OMIM (<https://omim.org/>), Disgenet (<https://www.disgenet.org/>), and Genecards (<https://www.genecards.org/>). The retrieved targets were normalized to the UniProt database. Duplicate targets in the search results were removed, and targets related to dyslipidemia were identified to establish a disease target dataset.

The relevant targets of the components of EAD and the targets of hyperlipidemia were intersected by Jvenn, and the common intersected targets may represent key targets for EAD to ameliorate dyslipidemia. The screened key targets were uploaded to the STRING platform (<https://cn.string-db.org/>) and select multiple protein modes, the protein species was set to *Homo sapiens*, and a protein-protein interaction (PPI) network was established. To obtain the PPI interaction network, the screening condition was set to the lowest interaction threshold at medium confidence (≥ 0.4), while the rest of the parameters were set to the default. The above components and predicted targets were imported into Cytoscape 3.7.1 for drawing and analysis, and a “components-target” network was constructed, with the edge used to represent the relationship between the components and the targets, and the plug-in used to filter the core compounds according to the degree of node connection (Degree).

The obtained key targets were subjected to Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using Metascape (<https://metascape.org/gp/index.html#/main/step1>), and the species was limited to *H. sapiens*. The results were visualized using an online bioinformatics tool (<https://www.bioinformatics.com.cn/>). GO enrichment analyses were performed on the intersection targets using the Metascape database, and the results corresponded to 3 categories: biological processes (BP), molecular functions (MF), and cell components (CC).

2.11 Molecular docking

Ten targets screened from the PPI network were selected as receptors, and ten compounds screened from the “components-targets” network were selected as ligands. The CAS number of small drug molecules was then searched in the PubChem database, and the 3D structure was downloaded. Small drug molecules were minimized using Chem3D and saved in the mol2 format. The 3D structure (PDB format) of the target protein was downloaded from the PDB database. Pymol software was used to dehydrate the proteolytic enzyme and remove the original ligand of the active center, and AutoDock software was used to hydrogenate the target protein and convert the format to pdbqt format. The rotation bond of the small drug molecule was set and saved in pdbqt format. The corresponding box parameters were set, and Vina was run for docking simulations. The conformation with the lowest energy was set as the optimal conformation, and Pymol was used to process the results.

2.12 Serum pharmacochemistry analysis

Four times the volume of acetonitrile was added to rat plasma samples, vortexing was performed for 3 min, and centrifugation was performed at 10 000 r/min for 10 min. The supernatant was then collected and dried under N_2 , and the residue was dissolved in 100 μ L methanol and centrifuged at 10 000 r/min for 5 min. The supernatant liquid was then collected for analysis using a high-performance liquid chromatography-triple quadrupole-mass spectrometer (HPLC-QQQ/MS) with a TSQ Endura (Thermo Fisher Scientific, Inc., Waltham, MA, USA)^[17-19]. The analysis was performed in positive and negative electrospray ionization modes. The spray voltage was set to 5 500 eV in the positive mode and $-4\ 500$ eV in the negative mode. The gas settings for gas 1, CAD gas, and curtain gas were 20, 7, and 25 psi, respectively. The selective reaction-monitoring (SRM) scanning mode was used to scan the ion pairs. The declustering potential was 50 V in the positive mode and -80 V in the negative mode. A 5 μ L extraction sample was injected into the column (XTERRA MS C₁₈ column, 2.1 mm $\times$ 100 mm, 3.5 mm) and eluted at 0.3 mL/min with a gradient elution of water with 0.1% (V/V) formic acid (A) and acetonitrile (B) (0–8 min, 8%–40% B; 8–40 min, 40%–92% B).

Based on the chromatograms of Chinese herbal samples, drug-containing serum samples, and reference substances, the precise relative molecular mass and molecular fragment peaks were used for searching, and the relative chromatographic retention time and mass spectrometry secondary fragment information were used for identification. The MS secondary fragment information and compound optimization results reported in the literature were compared to identify the drug prototype components in the serum, and the results were further determined by the reference substance.

2.13 Determination of active compounds content

An appropriate amount of EAD was weighed, and a 10 mg/mL solution was prepared with methanol (sample solution). Then, we accurately weighed 0.66 mg of naringenin, 0.41 mg of luteolin, and 0.82 mg of apigenin and added 10 mL of methanol to prepare a mixed control solution (reference solution). The reference and sample solutions were filtered through a 0.45 μ m filter. The mobile phase components (40:60 ratio of methanol:water containing 0.2% phosphoric acid) were filtered through a 0.2 μ m filter before use and pumped from the solvent reservoir at a flow rate of 1.0 mL/min. The column temperature was 30 $^{\circ}$ C, and the measured wavelength was 320 nm^[20].

We then accurately weighed 0.43 mg of luteoloside, 0.56 mg of naringin, and 0.49 mg apiin and added 10 mL of methanol to prepare a mixed control solution (reference solution). The sample solution was prepared as described above. The mobile phase components (20:80 ratio of acetonitrile:water containing 0.1% phosphoric acid) were filtered through a 0.2 μ m filter before use and pumped from the solvent reservoir at a flow rate of 1.0 mL/min. The column temperature was 28 $^{\circ}$ C, and the measured wavelength was 340 nm^[21].

The above analysis was performed using an HPLC system (Thermo Scientific Dionex UltiMate 3000; ZORBAX Eclipse Plus C₁₈, 4.6 mm $\times$ 250 mm, 5 μ m).

2.14 Total RNA extraction and real-time quantitative polymerase chain reaction (qRT-PCR)

Total RNA was extracted from HepG2 cells using the TRIzol reagent (Solarbio, Beijing, China) and reverse transcribed using the PrimeScript RT reagent kit with gDNA Eraser (Perfect Real Time) (TaKaRa Biotechnology, Shiga, Japan). The expression of *PPARα*, *CPT1*, and fatty acid-binding protein 3 (*FABP3*) mRNA in HepG2 cells was detected. Aliquots of the obtained cDNA samples were then amplified using PCR with the following schedule: 40 cycles at 95 °C for 30 s, primer annealing at 95 °C for 5 s, and extension at 60 °C for 34 s. The experiment was repeated 3 times, and the relative expression of the target gene was calculated by the $2^{-\Delta\Delta C_t}$ method. The specifically designed primers are listed in Table 1.

2.15 Western blotting assays

Proteins in HepG2 cells from each group were extracted with high-efficiency RIPA buffer, and protein concentrations were quantified using BCA kits. Equal amounts of protein samples (60 µg) were separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked in 5% non-fat milk for 1 h before incubating with the respective primary antibodies: anti-PPARα (abs124681, 1:1 000), anti-CPT1 (abs116565, 1:1 000), and anti-GAPDH (cat. #AF7021, 1:2 000) at 4 °C overnight. The membranes were then washed three times for 10 min each with TBST. After washing three times, the membranes were incubated with the corresponding secondary antibody, HRP-conjugated affinity-pure goat anti-rabbit IgG (H + L, A0208, 1:2 000). Western blot signal strength was measured using Western Bright ECL spray (Advansta, San Jose, CA, USA) and quantitatively assessed using Syngene GeneTools software (Cambridge, UK).

2.16 Statistical analysis

The data in this study are expressed as the means ± standard deviations. Statistical comparisons were performed using the *t*-test or one-way analysis of variance (ANOVA) using GraphPad Prism 9 software. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Establishment of lipid accumulation model in HepG2 cells induced by oleic acid

We established a lipid accumulation model in HepG2 cells. HepG2 cells were stimulated with 0.2, 0.4, 0.5, and 0.8 mmol/L oleic acid. The HepG2 cells treated with 0.2 and 0.4 mmol/L sodium

oleate for 24 h had clear cell boundaries, although only a few of them were dyed reddish brown (Figs. 2A, B). After stimulation with 0.8 mmol/L sodium oleate for 24 h, most cells died (Fig. 2D). After treatment with 0.5 mmol/L sodium oleate for 24 h, the cell spacing gradually increased, and red lipid droplets densely surrounded the cells, indicating that the lipid accumulation model of HepG2 cells was successfully established (Fig. 2C).

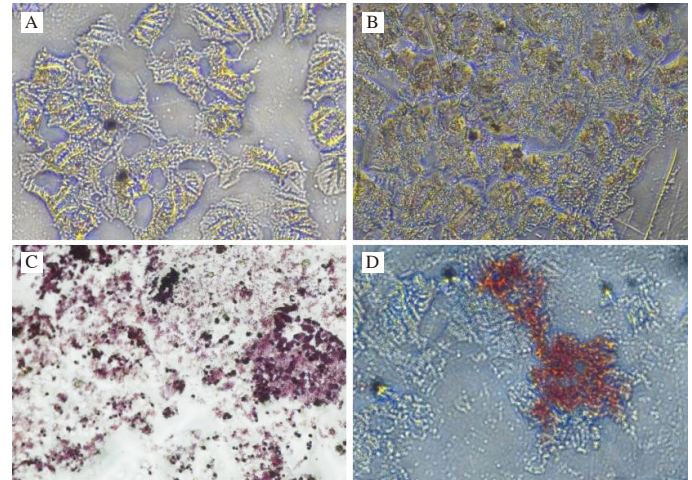


Fig. 2 Effects of different concentrations of sodium oleate on lipid accumulation in HepG2 cells (magnification: 100×). (A) 0.2 mmol/L sodium oleate; (B) 0.4 mmol/L sodium oleate; (C) 0.5 mmol/L sodium oleate; (D) 0.8 mmol/L sodium oleate.

3.2 Validation of the efficacy of *D. moldavica* ethanol extract on HepG2 cells

The toxicity of the *D. moldavica* ethanol extract to HepG2 cells was determined using the CCK-8 method. The results showed that the ethanol extract of *D. moldavica* was non-toxic at ≤ 80 µg/mL, as shown in Fig. 3A. Furthermore, the inhibitory concentration at 5% (IC_5) and IC_{10} were selected in this range for subsequent experiments.

The lipid accumulation-alleviating effect of the ethanol extract was evaluated by changes in the TC and TG contents. Compared with the 0.5 mmol/L sodium oleate group, the ethanol extract produced significant differences in the TC ($IC_5 = 45$ µg/mL, $IC_{10} = 119$ µg/mL) and TG contents ($IC_{10} = 119$ µg/mL) (Fig. 3B). These results indicated that lipid accumulation induced by oleic acid in HepG2 cells was alleviated by the ethanol extract of *D. moldavica*.

3.3 Screening of activity of different polar fractions of *D. moldavica*

3.3.1 Toxic effects of the different polar fractions of *D. moldavica* on HepG2 cells

The toxicity of the 4 polar fractions to HepG2 cells was measured at concentrations of 25–400 µg/mL (Fig. 4). HepG2 cell

Table 1
Primer sequence.

Gene	Forward (5'–3')	Reverse (5'–3')
<i>PPARα</i>	TCGCGAGGATAGTTCTGGAAGC	ACCACAGGATAAGTCACCGAGGAG
<i>CPT1</i>	GAGCGACTGGTGGGAGGAGTAC	TGCTGCCTGAATGTGAGTTGGAAG
<i>FABP3</i>	ACACAGCACCTTCAAGAACACAGAG	GGTGAACAAGTTTCCTCCATCCAG
<i>β-Actin</i>	TGCTGTCCCTGTATGCCTCT	TTTGATGTCACGCACGATTT

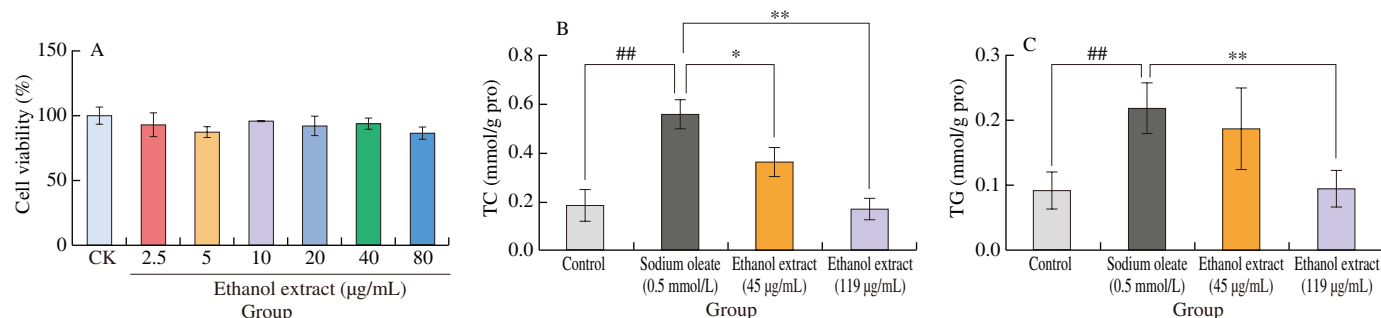


Fig. 3 *D. moldavica* ethanol extract alleviates oleic acid-induced lipid accumulation in HepG2 cells. (A) Effect on cell viability; (B, C) Changes of TC and TG contents. $^{##}P < 0.01$ compared with that of the control group; $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with that of the 0.5 mmol/L sodium oleate group.

proliferation did not significantly differ from that of the control group when treated with the ethyl acetate and petroleum ether fractions at concentrations of 25 and 50 μg/mL ($P > 0.05$), *n*-butanol fraction at concentrations ≤ 200 μg/mL ($P > 0.05$), and water fraction at concentrations ≤ 400 μg/mL ($P > 0.05$).

3.3.2 Oil red O staining results of oleic acid-stimulated HepG2 cells treated with different polar fractions of *D. moldavica*

We determined the concentrations for subsequent experiments based on the IC_5 and IC_{10} values of the different polar fractions. The ethyl acetate, *n*-butanol, water, and petroleum ether fractions of *D. moldavica* inhibited the formation of lipid droplets in HepG2 cells within the experimental concentration range, and the number of lipid droplets was significantly reduced compared to that in the model group (Fig. 5). The EAD significantly inhibited the formation of lipid droplets in HepG2 cells and led to decreased cell spacing, and the cell morphology was similar to that of the control group.

3.3.3 Changes in the TC and TG contents in oleic acid-stimulated HepG2 cells treated with different polar fractions of *D. moldavica*

Compared to the control group, the TC content in the model group was significantly increased after sodium oleate induction. Compared with the model group, the *n*-butanol fraction ($IC_5 = 144$ μg/mL, $IC_{10} = 265$ μg/mL), EAD ($IC_{10} = 51$ μg/mL; $IC_5 = 1$ μg/mL), and water fraction ($IC_5 = 53$ μg/mL) produced significant differences ($P < 0.05$). The results showed that the *n*-butanol fraction, water fraction, and EAD could reduce the TC content and inhibit lipid accumulation in HepG2 cells to some extent. Compared with that in the control group, the TG content in the model group was significantly increased after sodium oleate induction. Compared with the model group, EAD ($IC_{10} = 51$ μg/mL, $P < 0.05$) led to significant differences. The results showed that EAD reduced the TG content and inhibited lipid accumulation in HepG2 cells to some extent. However, the positive drug simvastatin showed an abnormal increase in TC content, and the specific reasons need to be further determined (Fig. 6).

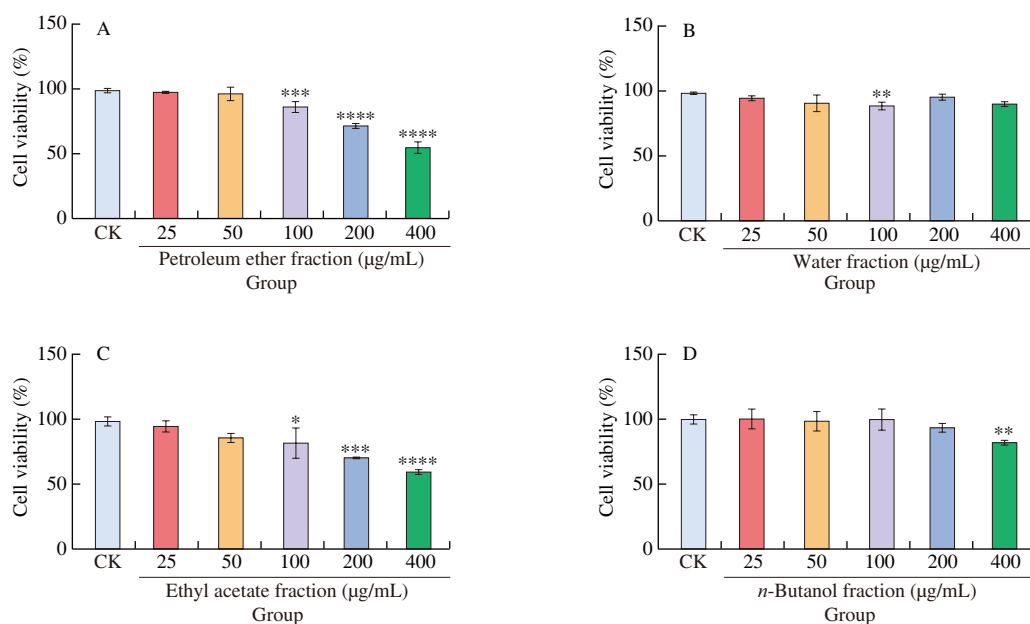


Fig. 4 Effects of different polar fractions of *D. moldavica* on the proliferation of HepG2 cells. (A) Petroleum ether fraction; (B) water fraction; (C) ethyl acetate fraction; (D) *n*-butanol fraction. $^{****}P < 0.0001$, $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ compared with that of the control group.

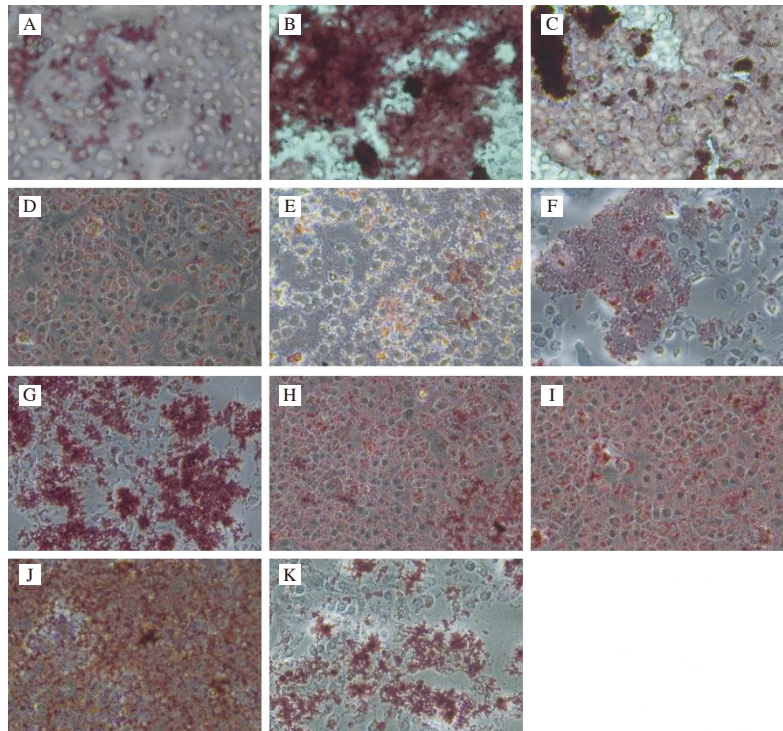


Fig. 5 Effects of different polar fractions of *D. moldavica* on lipid accumulation in HepG2 cells (magnification: 100 $\times$). (A) Control group; (B) 0.5 mmol/L sodium oleate; (C) 50 μ g/mL simvastatin; (D) ethyl acetate fraction (IC_{10} = 51 μ g/mL); (E) ethyl acetate fraction (IC_5 = 1 μ g/mL); (F) *n*-butanol fraction (IC_{10} = 265 μ g/mL); (G) *n*-butanol fraction (IC_5 = 144 μ g/mL); (H) water fraction (IC_{10} = 318 μ g/mL); (I) water fraction (IC_5 = 53 μ g/mL); (J) petroleum ether fraction (IC_{10} = 80 μ g/mL); (K) petroleum ether fraction (IC_5 = 38 μ g/mL).

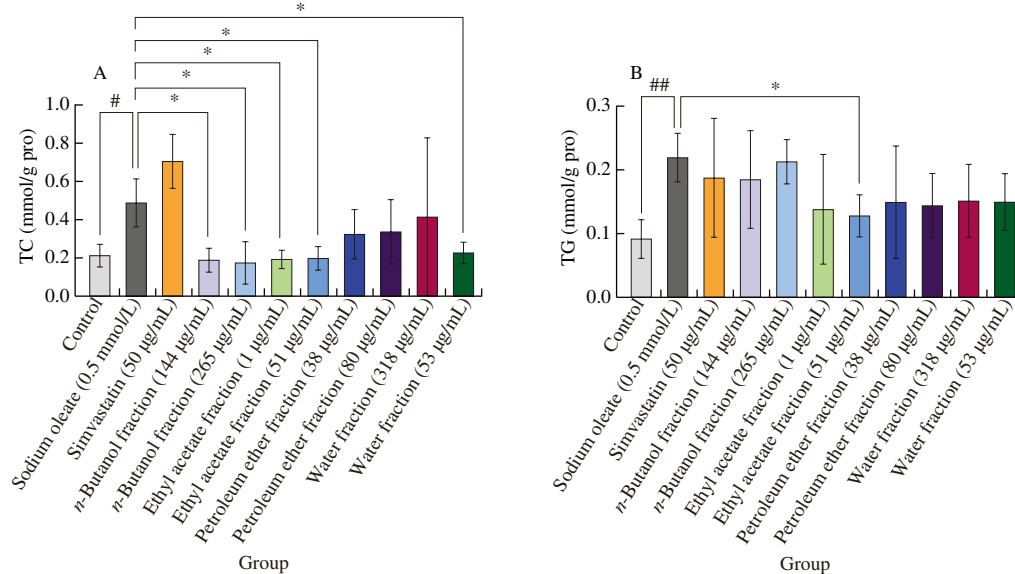


Fig. 6 Effects of different polar fractions of *D. moldavica* on biochemical indices. $^{##}P < 0.01$, $^*P < 0.05$ compared with that of the control group; $^*P < 0.05$ compared with that of the 0.5 mmol/L sodium oleate group.

Based on the above Oil red O staining results and changes in TC and TG contents, it can be concluded that the EAD has the most vigorous activity and can be used for subsequent experimental studies.

3.4 EAD alleviates pathological indexes in rats fed a HFD

3.4.1 Effect of EAD on the serum lipid profiles of hyperlipidemic rats

The effects of EAD on HFD-induced serum lipid profiles are

shown in Fig. 7A. Compared with that in the CK group, the content of TG and LDL-C in the M group significantly increased, while the content of HDL-C significantly decreased, indicating successful model establishment. After administering atorvastatin or EAD, the levels of TG and LDL-C decreased, while those of HDL-C increased ($P < 0.05$). However, in this experiment, the TC content did not change in each group, and the specific reason needs to be determined through further research.

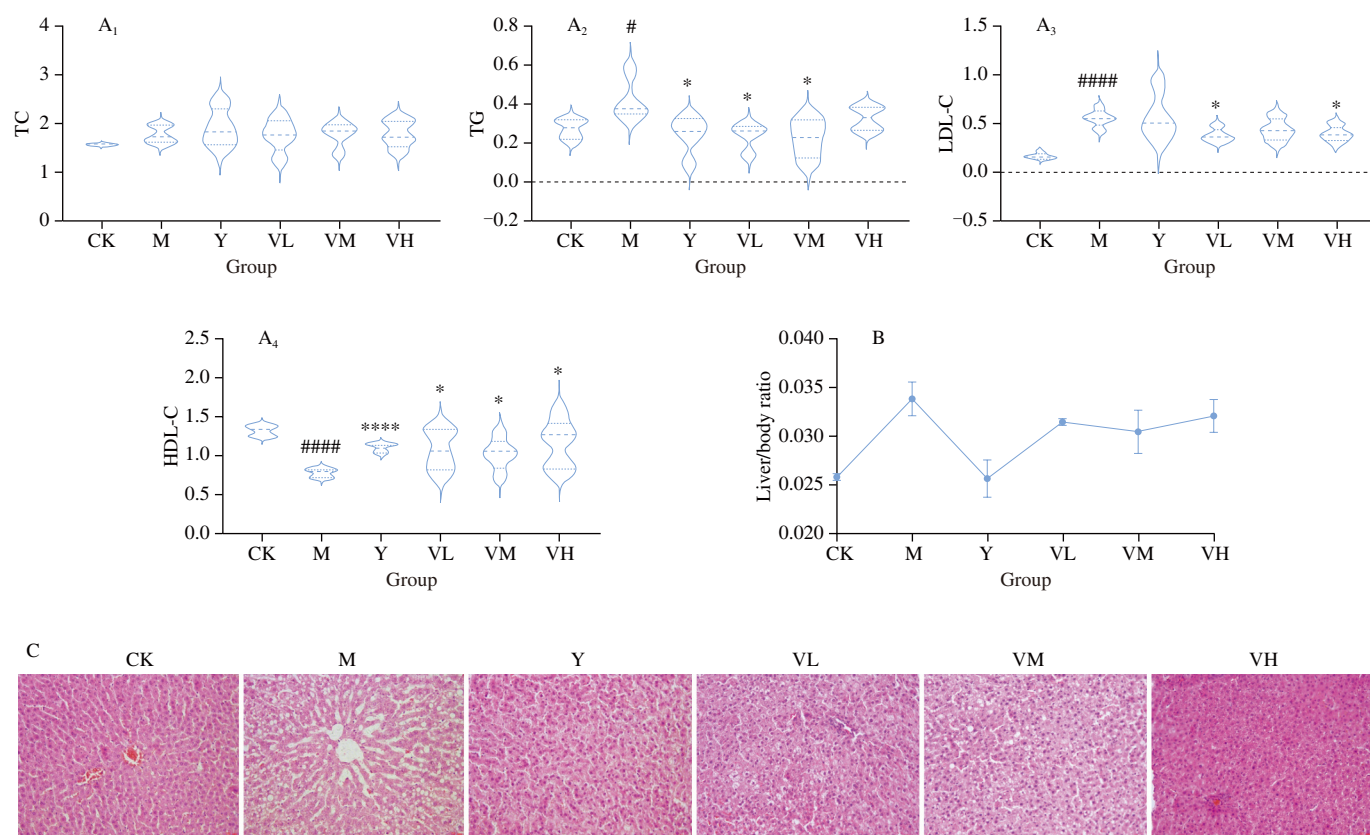


Fig. 7 Effect of EAD in rats fed a HFD. (A) Four items of blood lipids; (B) liver-to-body ratio; (C) liver histopathology, magnification: 100×. #### $P < 0.0001$ and * $P < 0.05$ compared with that of the CK group. * $P < 0.05$ compared with that of the M group.

3.4.2 Effect of EAD on the body and liver weights of hyperlipidemic rats

As shown in Fig. 7B, with the establishment of the model, the liver-to-body ratio significantly increased. After drug administration, the liver-to-body ratio significantly decreased, indicating that atorvastatin and EAD can relieve the accumulation of lipids in the liver of hyperlipidemic animals.

3.4.3 Effect of EAD on liver histopathology

The representative images of H&E-stained liver pathological changes are shown in Fig. 7C. Compared with those in the CK group, the cells in the M group were disordered and showed a large number of gaps, indicating that HFD intake led to liver steatosis. However, compared to the M group, the atorvastatin and EAD groups showed a significant reduction in vacuoles and an improvement in lipid accumulation, indicating a reduction in liver lipid degeneration.

3.5 Chemical composition analysis of EAD

SCIEX OS software was used to process the sample data through the TCM MS/MS Library 2.1 and secondary fragmentation. Finally, a total of 39 chemical constituents were identified, and their secondary fragments were found. The total ion chromatogram (TIC) of EAD in the positive ion mode is shown in Fig. 8. Detailed information on these compounds is presented in Table 2. The relevant secondary fragment information is shown in the Supplementary Material.

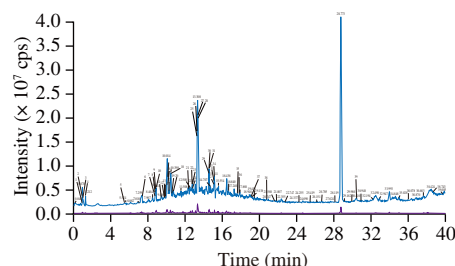


Fig. 8 TIC results of the EAD in positive mode.

3.6 Network pharmacology analysis

3.6.1 Intersection analysis and PPI network of EAD component targets and dyslipidemia targets

Through Jvenn, the relevant targets of the EAD components and dyslipidemia were intersected, and a total of 163 intersected targets were obtained. (Fig. 9A).

We imported the 163 targets into an online STRING database to construct a PPI network. Subsequently, the PPI network was imported into Cytoscape 3.7.2 software, and the network topology attributes were analyzed using the Tools function. According to the “node connectivity (degree)”, ≥ 40 were selected as the key targets (Fig. 9B). The top 10 core targets were serine/threonine protein kinase 1 (AKT1), caspase-3 (CASP3), prostaglandin endoperoxidase synthase 2 (PTGS2), tumor necrosis factor (TNF), estrogen receptor 1 (ESR1), tumor protein p53 (TP53), peroxisome proliferator-activated receptor

Table 2

Identification of chemical constituents in EAD.

No.	Component name	Molecular formula	Retention time (min)	Precursor mass	Adduct/charge
1	Betaine	C ₃ H ₁₁ NO ₂	0.835	118.1	[M + H] ⁺
2	Adenine	C ₅ H ₅ N ₅	0.866	136.1	[M + H] ⁺
3	Niacinamide	C ₆ H ₆ N ₂ O	0.881	123.1	[M + H] ⁺
4	Adenosine	C ₁₀ H ₁₃ N ₅ O ₄	1.208	268.1	[M + H] ⁺
5	Scopoletin	C ₁₀ H ₈ O ₄	5.196	193.0	[M + H] ⁺
6	Methyl salicylate	C ₉ H ₈ O ₃	7.275	153.1	[M + H] ⁺
7	Delphinidin-3- <i>O</i> - β -glucopyranoside	C ₂₁ H ₂₁ O ₁₂	8.684	465.1	[M] ⁺
8	Tricetin	C ₁₅ H ₁₀ O ₇	8.729	303.1	[M + H] ⁺
9	Scutellarin	C ₂₁ H ₁₈ O ₁₂	8.800	463.1	[M + H] ⁺
10	Luteoloside	C ₂₁ H ₂₀ O ₁₁	8.815	449.1	[M + H] ⁺
11	Butane-1,2,3-tricarboxylic acid 1-(4-hydroxybutyl) 2,3-dibutyl ester	C ₁₉ H ₃₄ O ₇	9.411	375.2	[M + H] ⁺
12	Rhoifolin	C ₂₇ H ₃₀ O ₁₄	9.893	579.2	[M + H] ⁺
13	Luteolin-7-glucuronide	C ₂₁ H ₁₈ O ₁₂	9.924	463.1	[M+H] ⁺
14	Apigenin-7- <i>O</i> -glucoside	C ₂₁ H ₂₀ O ₁₀	9.996	433.1	[M + H] ⁺
15	Daidzin	C ₂₁ H ₂₀ O ₉	10.081	417.1	[M + H] ⁺
16	Baicalin	C ₂₁ H ₁₈ O ₁₁	10.110	447.1	[M + H] ⁺
17	1-Mesityl-2-methylpropan-1-ol	C ₁₃ H ₂₀ O	10.197	193.2	[M + H] ⁺
18	Peonidin-3- <i>O</i> - β - <i>D</i> -glucopyranoside	C ₂₂ H ₂₃ O ₁₁	10.389	463.1	[M] ⁺
19	5-Hydroxy-2-(2-hydroxyphenyl)-6-methoxy-4-oxo-4 <i>H</i> -1-benzopyran-7-yl β - <i>D</i> -glucopyranosiduronic acid	C ₂₂ H ₂₀ O ₁₂	10.664	477.1	[M + H] ⁺
20	Apiin	C ₂₆ H ₂₈ O ₁₄	10.737	565.2	[M + H] ⁺
21	Cirsimaritin	C ₂₃ H ₂₄ O ₁₁	12.213	477.1	[M + H] ⁺
22	Fortunellin	C ₂₈ H ₃₂ O ₁₄	12.722	593.2	[M + H] ⁺
23	Luteolin	C ₁₅ H ₁₀ O ₆	12.737	287.1	[M + H] ⁺
24	Quercetin	C ₁₅ H ₁₀ O ₇	12.794	303.0	[M + H] ⁺
25	Diosmetin	C ₁₆ H ₁₂ O ₆	13.295	301.1	[M + H] ⁺
26	Glycetein	C ₁₆ H ₁₂ O ₅	13.295	285.1	[M + H] ⁺
27	Acacetin	C ₁₆ H ₁₂ O ₅	13.310	285.1	[M + H] ⁺
28	5,7-Dihydroxy-6-methoxy-2-phenylchromen-4-one	C ₁₆ H ₁₂ O ₅	13.310	285.1	[M + H] ⁺
29	Naringenin	C ₁₅ H ₁₂ O ₅	14.528	273.1	[M + H] ⁺
30	Genistein	C ₁₅ H ₁₀ O ₅	14.543	271.1	[M + H] ⁺
31	Apigenin	C ₁₅ H ₁₀ O ₅	14.543	271.1	[M + H] ⁺
32	Tricin	C ₁₇ H ₁₄ O ₇	15.001	331.1	[M + H] ⁺
33	Scutellarein 4'-methyl ether	C ₁₆ H ₁₂ O ₆	15.076	301.1	[M + H] ⁺
34	3- <i>O</i> -Methylfunicone	C ₂₀ H ₂₀ O ₈	15.106	389.1	[M + H] ⁺
35	Cirsimaritin	C ₁₇ H ₁₄ O ₆	17.478	315.1	[M + H] ⁺
36	Naringin	C ₂₇ H ₃₂ O ₁₄	17.823	581.2	[M + H] ⁺
37	Genkwanin	C ₁₆ H ₁₂ O ₅	19.135	285.1	[M + H] ⁺
38	5-Hydroxy-2',4',7,8-tetramethoxyflavone	C ₁₉ H ₁₈ O ₇	20.780	359.1	[M + H] ⁺
39	Corosolic acid	C ₃₀ H ₄₈ O ₄	30.426	473.3	[M + H] ⁺

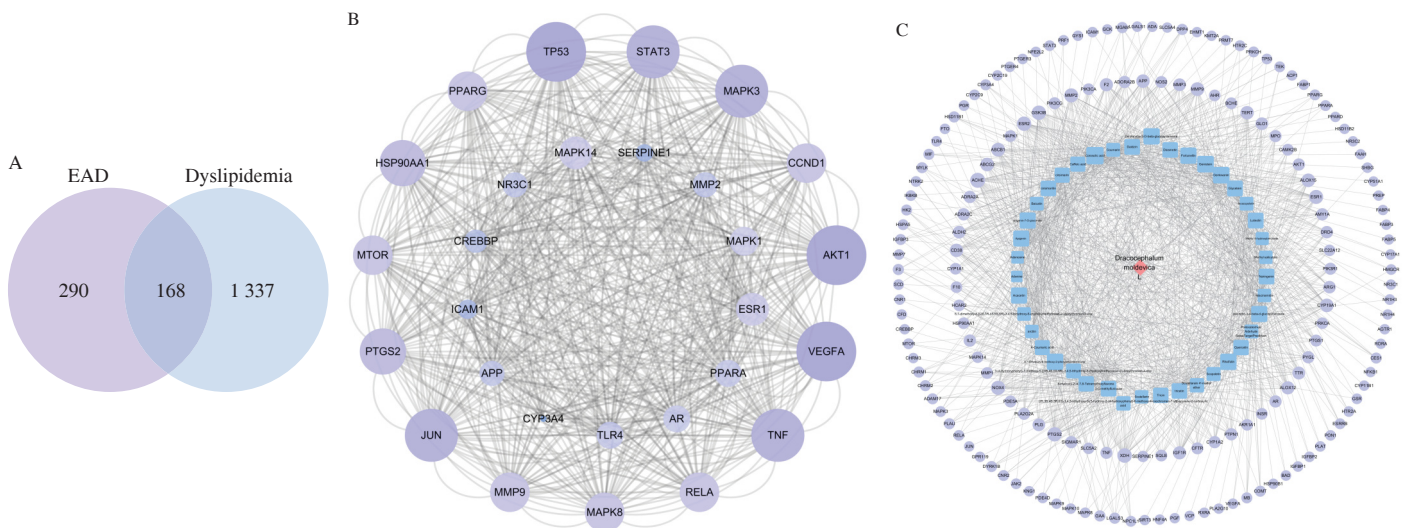


Fig. 9 Network pharmacology analysis of the EAD treatment for dyslipidemia. (A) Intersection analysis of the EAD component targets and dyslipidemia targets. (B) PPI networks of the core targets (where darker colors and larger circles represent greater correlations and may indicate potential key targets for EAD to treat dyslipidemia). (C) EAD “components-targets” network diagram.

gamma (PPARG), JUN, matrix metalloprotein 9 (MMP9), and mitogen-activated protein kinase 3 (MAPK3). These may be the core targets for the treatment of dyslipidemia with EAD.

3.6.2 EAD component-target network diagram

Based on these results, Cytoscape was used to draw and analyze the relationship network between the components and targets (C-T network), and key nodes were used to determine the components and targets that played an essential role. Furthermore, 209 nodes and 1 282 edges were obtained through Cytoscape network construction (Fig. 9C). The higher the node degree, the more important the compound or target was in the network. Sorted by degree, the top 10 components in the network were genkwanin, 5,7-dihydroxy-6-methoxy-2-phenylchromen-4-one, corosolic acid, acacetin, genistein, apigenin, luteolin, naringenin, apigenin-7-*O*-glucoside, and delphinidin-3-*O*- β -glucopyranoside.

3.6.3 Enrichment analysis of potential therapeutic targets

In total, 163 potential therapeutic targets were imported into the Metascape database and a histogram of the GO enrichment analysis was constructed (Fig. 10A). The BP category was mainly enriched in response to hormones, cellular response to organic cyclic compound, regulation of defense response, response to decreased oxygen levels, and circulatory system process; the MF category was mainly enriched in nuclear receptor activity, protein kinase activity, serine hydrolase activity, heme binding, and phosphatase binding; and the CC category was mainly enriched in the cytoplasmic vesicle lumen, apical part of cell, membrane raft, endoplasmic reticulum lumen, and receptor complex.

The KEGG pathway enrichment analysis was performed on the 163 genes through the Metascape database, and the results were screened according to the number of enriched genes, with the 20 enriched pathways selected as a bubble chart (Fig. 10B). Rich factor

refers to the ratio of the number of differentially expressed genes to the number of total genes located in this pathway. The size of the bubbles indicates the number of genes, and the depth of the bubble color indicates the level of significance. Genes were mainly enriched in linoleic acid metabolism, bladder cancer, PPAR signaling pathway, steroid hormone biosynthesis, cholinergic synapse, complement and coagulation cascades, and other pathways.

3.6.4 Molecular docking of EAD components and core targets

The molecular docking technology used to evaluate the degree of binding between a target and its corresponding component is generally based on the binding energy. Ten targets screened from the PPI network were selected as receptors, and 10 compounds screened from the “C-T network” were selected as ligands. The binding energies obtained by docking were plotted (Fig. 11A). Binding energy ≤ -5.0 kcal/mol indicated good binding activity; therefore, it was used as the basis for screening. The results showed that the docking binding energies between the top 10 compounds and targets predicted by network pharmacology were good, especially for the binding of apigenin-7-*O*-glucoside and delphinidin-3-*O*- β -glucopyranoside with AKT1 and PTGS2, respectively. The results with lower binding energies were further visualized, and the docking results are shown in Figs. 11B-E.

3.7 Identification of the prototype components of EAD in rat serum

The components of EAD are complex, and multiple components work synergistically. After oral administration, the components absorbed into the blood may exert their effects. Next, we analyzed the changes of components in rat blood after oral administration of EAD. Through the analysis of the precursor ion and product ion of the compounds, among the top compounds in network pharmacology,

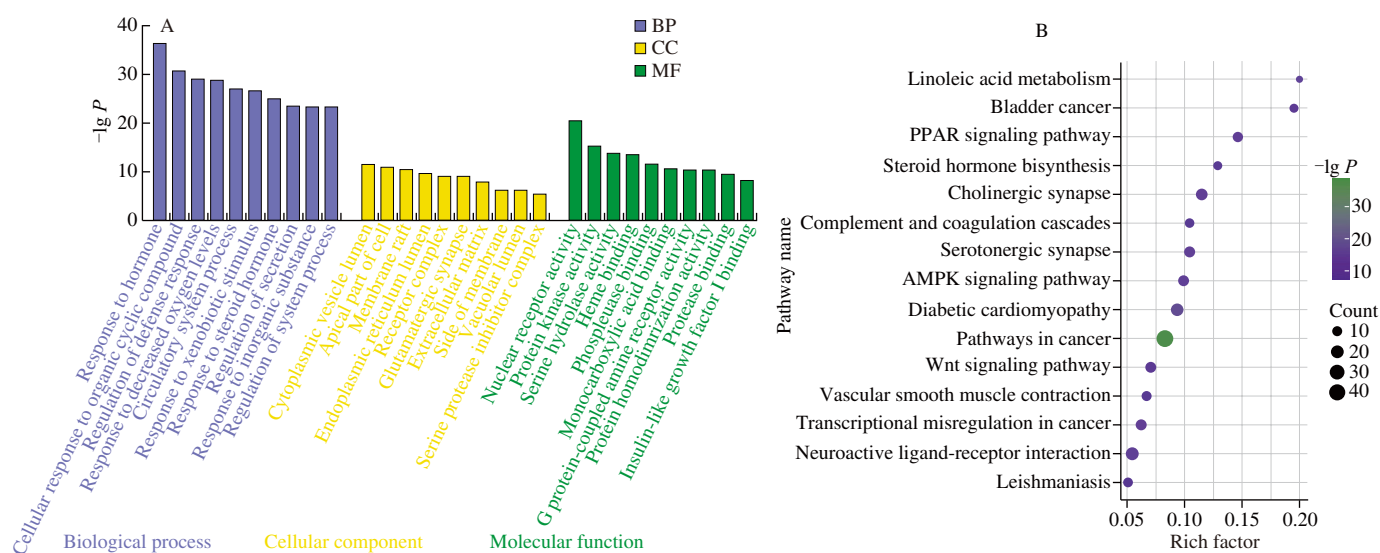


Fig. 10 Gene enrichment analysis of potential genes involved in the anti-dyslipidemia effects of EAD. (A) Histogram of GO secondary classification (the left ordinate is the number of genes, and the scales of up and down genes are given separately. The columns of each ontology are sorted in descending order of the number of genes). (B) KEGG enrichment analysis (the larger the rich factor, the higher the degree of enrichment; the larger the bubbles, the greater the number of genes enriched in the pathway. The depth of the bubble color indicates the significance level, with a bluer color indicating the higher significance of enrichment in the pathway).

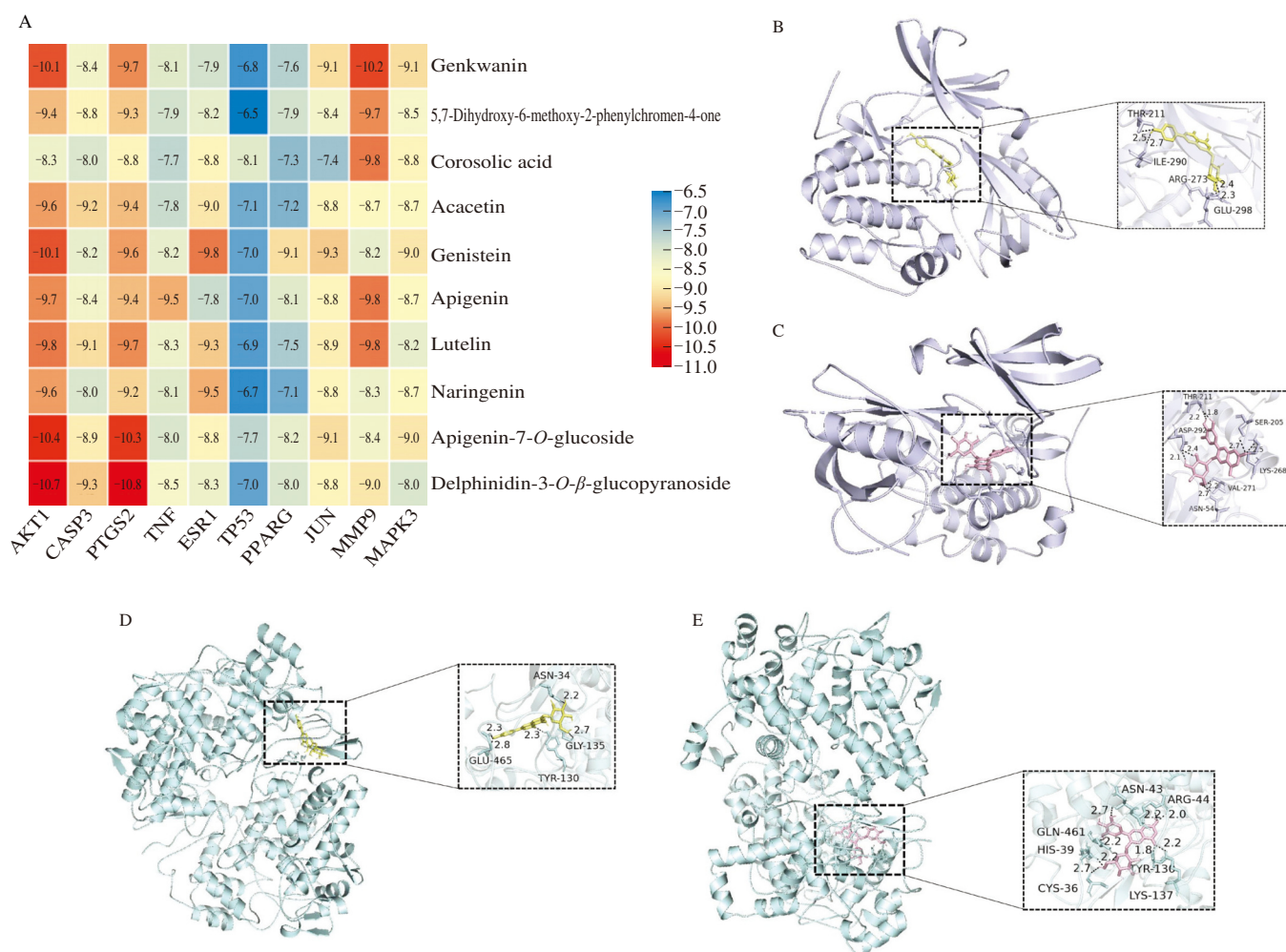


Fig. 11 Docking energy of key components in EAD and key target molecules regulating dyslipidemia. (A) Visualization results of molecular docking between key components in EAD and key targets for regulating dyslipidemia. (B) Molecular docking diagram of AKT1 and apigenin-7-O-glucoside, with one hydrogen bond at each of amino acids THR211, ILE290, ARG273, and GLU298; (C) molecular docking diagram of AKT1 and delphinidin-3-O- β -glucopyranoside, with one hydrogen bond at each of amino acids THR211, SER205, ASP292, LYS261, VAL271, and ASN54; (D) molecular docking map of PTGS2 and apigenin-7-O-glucoside, with one hydrogen bond each at amino acids ASN34, GLY135, TYR130, and GLU465; (E) molecular docking map of PTGS2 and delphinidin-3-O- β -glucopyranoside, with one hydrogen bond each at amino acids ASN43, ARG44, TYR130, LYS137, CYS36, HIS39, and GLN461.

Table 3
MS secondary fragment information.

Compound	Polarity	Precursor (<i>m/z</i>)	Product (<i>m/z</i>)	Collision energy (V)	Intensity	Source fragmentation	Radio frequency lens (V)
Apigenin	Positive	271.015	153.054	30.48	602 514.18	38.776	171
	Positive	271.015	119.137	32.21	253 558.42	38.776	171
	Positive	271.015	145.054	35.03	93 518.24	38.776	171
Luteolin	Positive	287.050	153.054	31.71	423 778.74	38.776	170
	Positive	287.050	135.125	32.13	138 122.92	38.776	170
	Positive	287.050	241.042	31.20	46 678.03	38.776	170
Naringenin	Negative	271.038	119.036	55.00	24.41	0	126

luteolin, apigenin, and naringenin were found in rat serum. Relevant data are shown in Table 3 and Figs. S1–S4.

3.8 Content analysis of active compounds

EAD 0.104 3 g was accurately weighed, among which the contents of naringenin, luteolin, apigenin, and luteoloside were 1.776 4, 3.391 9, 9.024 4, and 11.540 4 mg/g, respectively. Naringin and apiin were trace and not detected. HPLC chromatograms of the reference and sample solutions are shown in Figs. S5–S6.

3.9 Toxicity screening of prototype components of EAD (apigenin, luteolin, and naringenin) in HepG2 cells

We selected three compounds as prototypes in the EAD-containing serum for the following study. The cytotoxic effects of apigenin, luteolin, and naringenin on HepG2 cells were evaluated *via* CCK-8 assay. Compared with the CK group, apigenin had no cytotoxicity at ≤ 5 μ g/mL, luteolin had no cytotoxicity at ≤ 12.5 μ g/mL, and naringenin had no cytotoxicity at ≤ 50 μ g/mL (Fig. 12). For further studies, apigenin at concentrations of 1.25, 2.5, and 5 μ g/mL,

luteolin at concentrations of 3.125, 6.25, and 12.5 $\mu\text{g/mL}$, and naringenin at concentrations of 12.5, 25, and 50 $\mu\text{g/mL}$ were selected.

3.10 Oil red O staining of prototype components of EAD (apigenin, luteolin, and naringenin) in HepG2 cells

Based on the aforementioned constructed HepG2 lipid accumulation model, the concentrations identified in the toxicity screening were selected for the experiments. Only a few lipid droplets were observed in the CK group (Fig. 13A). In contrast, a diffuse distribution of lipid droplets was observed in the M group (Fig. 13B).

After treatment with simvastatin, apigenin, luteolin, and naringenin, the red lipid droplets decreased, indicating that lipid accumulation decreased (Figs. 13C-L).

3.11 Prototype components of EAD (apigenin, luteolin, and naringenin) play a role in reducing lipid accumulation through the PPAR signaling pathway

Based on the results of the KEGG enrichment analysis, the PPAR signaling pathway may be a vital signaling pathway for the hypolipidemic effects of the active components of EAD.

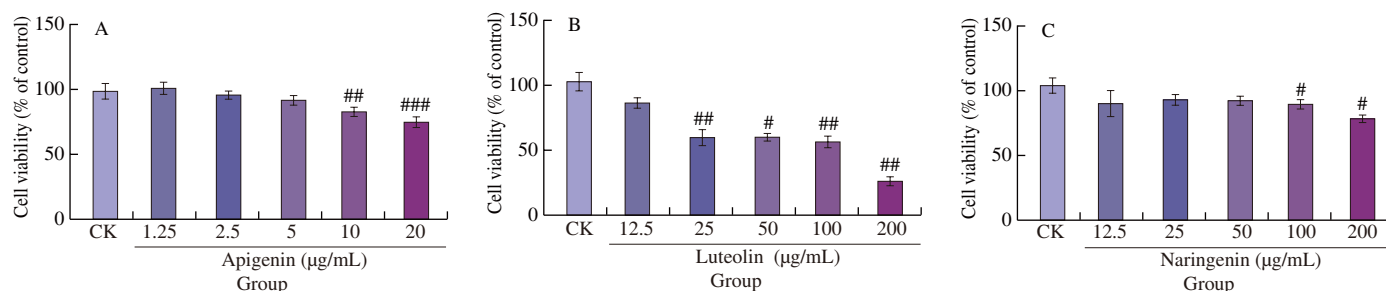


Fig. 12 Viability of HepG2 cells treated with different concentrations of apigenin (A), luteolin (B), and naringenin (C). ### $P < 0.001$, ## $P < 0.01$, * $P < 0.05$ compared with that of the CK group.

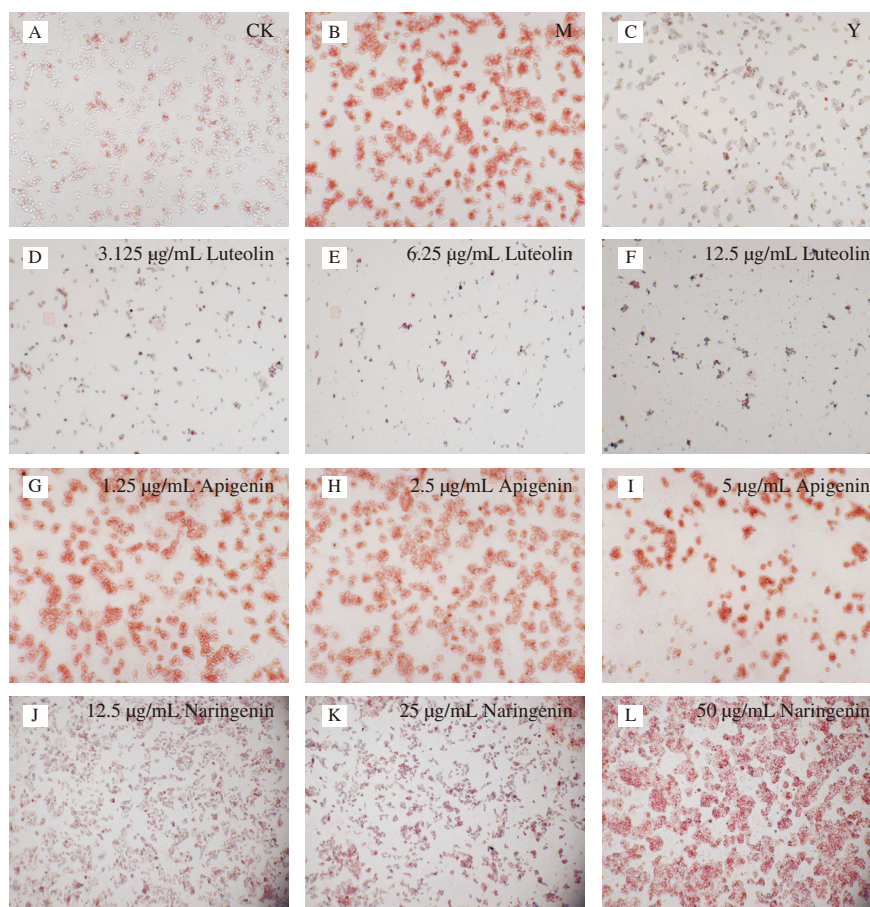


Fig. 13 Results of apigenin, luteolin, and naringenin Oil red O staining (magnification: 100 $\times$). (A) CK; (B) M; (C) Y; (D) 3.125 $\mu\text{g/mL}$ luteolin; (E) 6.25 $\mu\text{g/mL}$ luteolin; (F) 12.5 $\mu\text{g/mL}$ luteolin; (G) 1.25 $\mu\text{g/mL}$ apigenin; (H) 2.5 $\mu\text{g/mL}$ apigenin; (I) 5 $\mu\text{g/mL}$ apigenin; (J) 12.5 $\mu\text{g/mL}$ naringenin; (K) 25 $\mu\text{g/mL}$ naringenin; (L) 50 $\mu\text{g/mL}$ naringenin. The darker the red color, the more serious the lipid accumulation.

3.11.1 Apigenin, luteolin, and naringenin affect mRNA levels of the PPAR signaling pathway in HepG2 cells

We selected *PPARα*, *CPT1*, and *FABP3* in the PPAR signaling pathway to validate the potential mechanism by qRT-PCR analysis. After the HepG2 cells were stimulated with oleic acid, the *PPARα* expression in the M group increased and the expression of *CPT1* and *FABP3* decreased. Apigenin, luteolin, and naringenin administration improved oleic acid-stimulated *PPARα*, *CPT1*, and *FABP3* mRNA expression to varying degrees (Fig. 14). Thus, we tentatively suggest that these 3 components could alleviate lipid accumulation in HepG2 cells by regulating the PPAR signaling pathway.

3.11.2 Apigenin, luteolin, and naringenin affect protein expression of the PPAR signaling pathway in HepG2 cells

CPT1 is the downstream target of *PPARα*. We performed a Western blot analysis of these 2 key targets in the PPAR signaling

pathway. Interestingly, the western blotting results were not completely consistent with the qRT-PCR results (Fig. 15). *PPARα* expression decreased after oleic acid stimulation and increased after apigenin, luteolin, and naringenin treatment. *CPT1* expression was consistent with that of *PPARα*. Furthermore, apigenin and naringenin showed stronger therapeutic effects than luteolin.

Therefore, the prototype components of EAD identified in this study, namely, apigenin, luteolin, and naringenin, can regulate mRNA and protein expression in the PPAR signaling pathway. The results also suggest that *PPARα*, *CPT1*, and *FABP3* might be promising targets for treating dyslipidemia.

4. Discussion

In TCM, an improper diet is generally believed to harm the spleen and stomach, cause excessive paste fat turbidification, dampen heat and phlegm, and affect Qi functions and blood stasis, thus leading to dysfunction of the viscera and hyperlipidemia. Traditional Mongolian

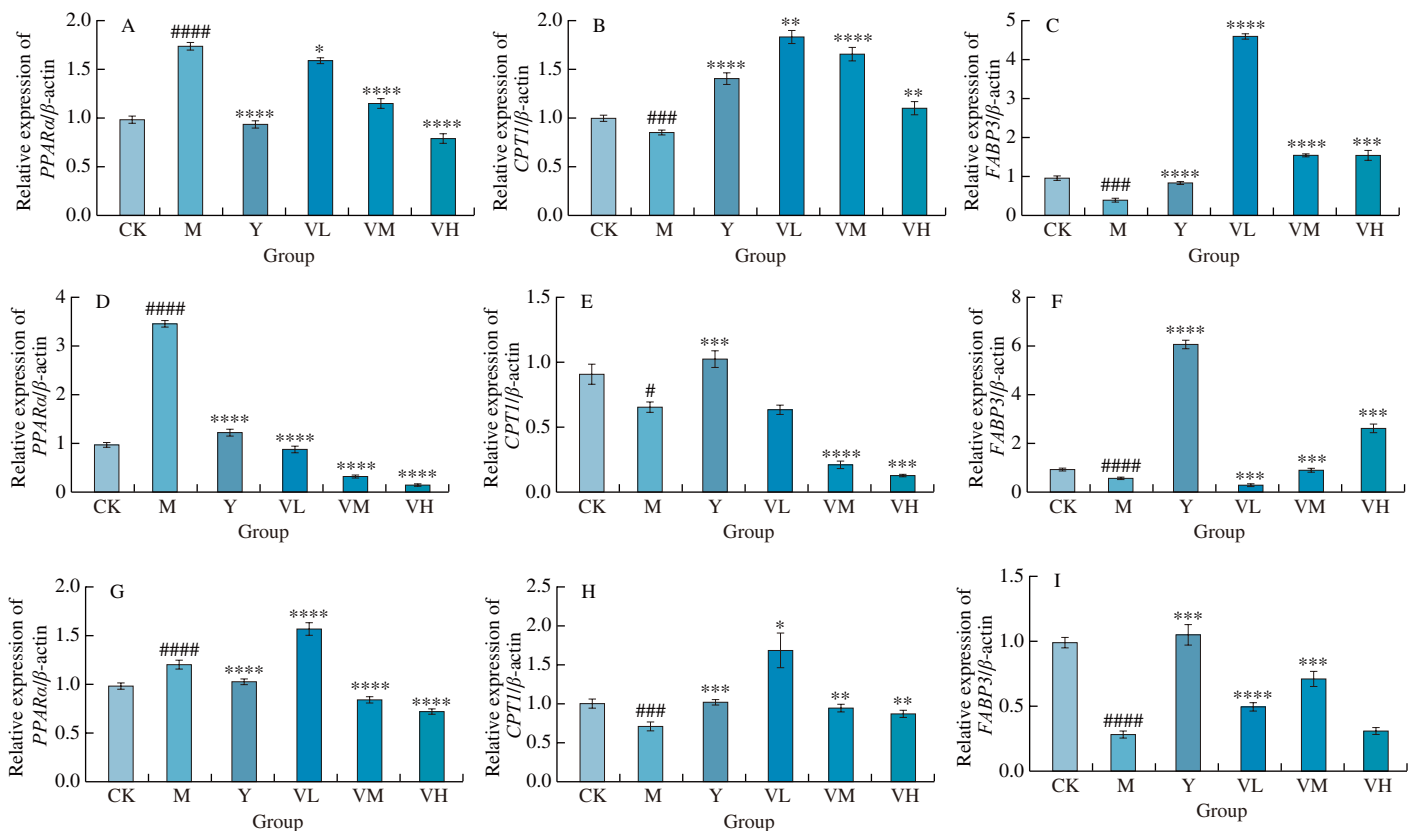


Fig. 14 Relative expression of *PPARα*, *CPT1*, and *FABP3* mRNA in HepG2 cells treated with apigenin (A-C), luteolin (D-F), and naringenin (G-I). #### $P < 0.0001$, ### $P < 0.001$, # $P < 0.05$ compared with that of the CK group; **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with that of the M group.

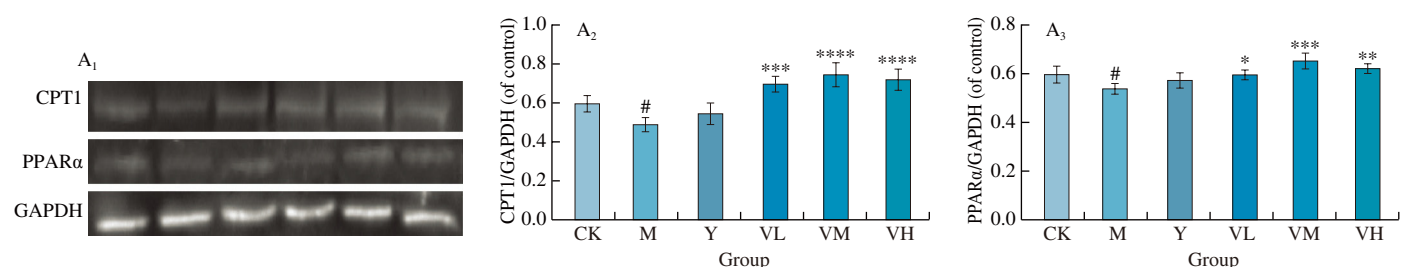


Fig. 15 Relative expression of *PPARα* and *CPT1* protein in HepG2 cells treated with naringenin (A), apigenin (B), and luteolin (C). #### $P < 0.0001$, ### $P < 0.001$, # $P < 0.05$ compared with that of the CK group; **** $P < 0.0001$, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ compared with that of the M group.

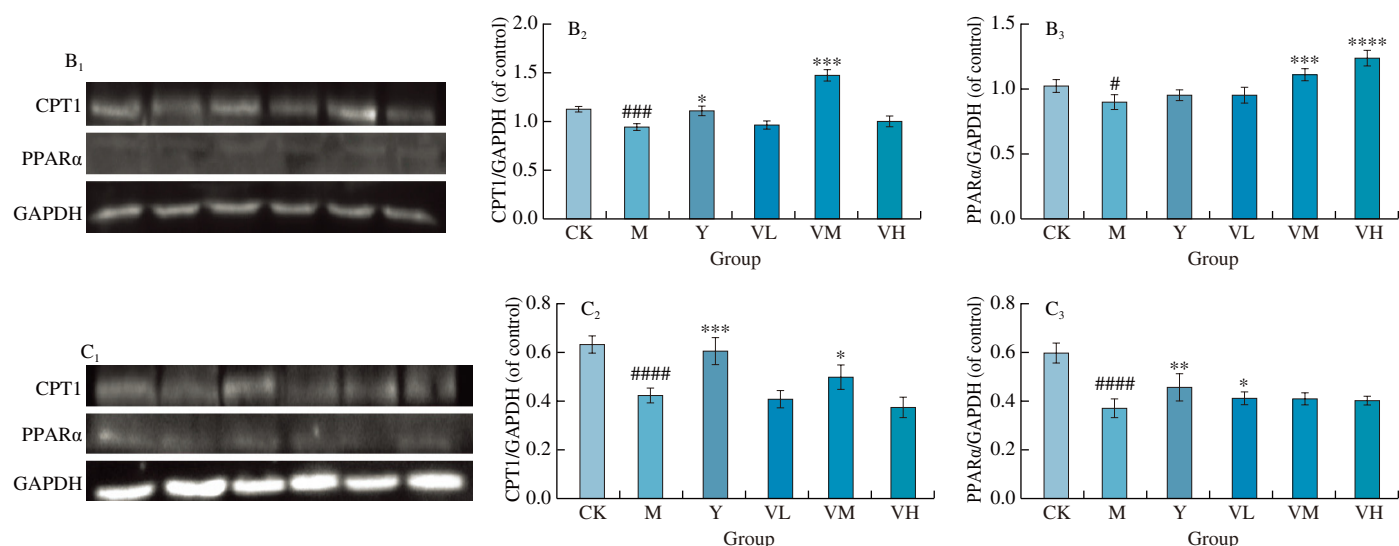


Fig. 15 (Continued)

medicine indicates that the human body experiences living and drinking disorders for a long time. Excessive oil intake leads to an imbalance in Sangen-Heyi, Xila, and Badagan. Excessive Badagan and mucus intake and excessive blood heat cause hyperlipidemia. Modern medicine considers dyslipidemia a metabolic disorder characterized by the increased intake of exogenous lipids or other substances, increased synthesis of endogenous lipids, and abnormal transport and catabolism of lipids^[22]. The human hepatocellular liver carcinoma cell line HepG2 is widely used to evaluate the lipid-lowering activity of TCM. Long-chain fatty acids can induce the formation of obvious lipid droplets and the accumulation of cellular fat in HepG2 cells. Oleic acid has the highest free fatty acid content^[23-24]. Therefore, oleic acid is commonly used in models that simulate a high-free fatty acid environment and induce intracellular lipid accumulation in HepG2 hepatocytes.

Chinese and Mongolian medicinal drugs have a long history of use, and an increasing number of TCM-based drugs are being widely used in clinical settings. However, there may be potential risks associated with using herbal products, including liver toxicity^[25]. Therefore, an inevitable trend for the modernization of Chinese and Mongolian medicine is to identify the active compounds in medicinal plants. The “holistic exploration and systematic regulation” of network pharmacology echoes the research on the “multi-component and multi-target” approach of TCM, and this method shows unique advantages in searching for the targets and pharmacodynamic material bases of TCM and elaborating on the mechanisms of action and drug-target compatibilities. The application of serum pharmacokinetics in TCM is mainly based on the rapid screening of pharmacodynamic substances; however, another method is needed to explore the mechanism of action^[26]. *D. moldavica*, as a plant with medicine and food homology, has significant potential based on its traditional use in reducing blood lipids. *D. moldavica* is commonly brewed with water into a tea, which can play a role in reducing blood lipids. *D. moldavica* as tea is a folk usage in some areas, has not been widely used. Screening the polar parts and determining the active ingredients further clarify the material basis of *D. moldavica* in treating diseases, and such research can provide a solid theoretical and experimental basis for the development of *D. moldavica* tea.

Previous studies have shown that different extracts of *D. moldavica* have therapeutic effects on hyperlipidemia; however, the material basis and related mechanisms have not been determined^[27]. Therefore, we combined serum pharmacokinetics and network pharmacology to identify the serum components, predict the targets, and analyze the pathways of *D. moldavica*. Finally, to determine the effectiveness and mechanisms of the active components in *D. moldavica*, a lipid accumulation model of HepG2 cells was constructed *in vitro*, the content of active components in EAD was determined, and their roles in EAD were validated from different perspectives.

Hyperlipidemia can be caused by HFDs. Disordered lipid metabolism is a sign of hyperlipidemia, and it can be detected based on the four components of blood lipids, namely, TC, TG, LDL-C, and HDL-C^[28]. In this study, we demonstrated that EAD can improve blood lipid abnormalities caused by HFDs. The liver-to-body ratio also gradually decreased and normalized, and HE staining revealed that EAD alleviates HFD-induced liver damage in rats.

Network pharmacology offers a way of thinking about drug discovery that simultaneously embraces efforts to improve clinical efficacy and understand side effects and toxicity^[29]. The active components of EAD that alleviated dyslipidemia were preliminarily screened using the network pharmacology method. The chemical components used for the network pharmacology analysis in this study were identified based on the LC-MS analysis of EAD, without considering oral absorption and drug-like properties. The components detected in the blood after oral administration are generally considered the components that take effect. Therefore, we tested the components that entered the blood and further screened the active ingredients. Among the top-ranked compounds in network pharmacology analysis, only naringenin, apigenin, and luteolin were detected in rat serum. How other components in EAD play a role still needs to be further studied. Considering that the content of these active ingredients would help improve the quality control and promotion of the application of EAD, we detected the content of apigenin, naringenin, and luteolin. Studies have shown that luteoloside, naringin, and apigenin are easily metabolized by intestinal flora into luteolin and absorbed into the blood after oral administration^[30-32]; therefore, we also determined

the content of luteoloside, naringin, and apigenin in EAD. Our results show that the content of active ingredients screened by network pharmacology and serum pharmacchemistry is also considerable, which can provide a basis for the subsequent research on the material basis of *D. moldavica*.

Mongolian medicine for disease treatment is complex; therefore, the mechanism of action of monomer compounds must be monitored while studying the overall therapeutic effect of Mongolian medicine^[33]. Modern basic research has shown that naringin regulates lipid metabolism disorders by affecting glucose uptake and cholesterol metabolism^[34]. Luteolin has significant hypolipidemic and anti-hepatic steatosis effects, and the hypolipidemic mechanism is complex and involves various BP, such as cholesterol, fatty acid metabolism, and glycolysis pathways^[35]. Studies have suggested that apigenin may improve lipid metabolism by activating the AMPK-SREBP pathway in HepG2 cells, thereby preventing hyperlipidemia and reducing lipid accumulation^[36-37]. Therefore, we reasonably believe that EAD can alleviate dyslipidemia mainly through the action of these three components. Long-chain fatty acids can cause lipid accumulation in HepG2 cells, and the cells store excess lipids in the form of TGs and cholesteryl esters. Oil red O is a strong lipid solvent and lipid dye that easily combines with TG to form small lipid droplets. Therefore, Oil red O staining solution adsorbs dyes from the solvent to stain lipid droplets, and positive staining results for fat are orange to red^[38]. In this study, the effects observed after Oil red O staining of HepG2 cells were consistent with the above predictions and showed that luteolin, naringin, and apigenin alleviate oleic acid-induced lipid accumulation.

PPARs are nuclear hormone receptors activated by fatty acids and their derivatives. PPAR has three subtypes (alpha, beta/delta, and gamma) that exhibit different expression patterns in vertebrates. Each subtype is encoded by a separate gene that binds fatty acids and eicosanoids. PPAR α can inhibit the expression of hepatic steatosis-related substances by enhancing fatty acid oxidation and plays an important role in regulating lipid metabolism. CPT1 is a key enzyme affecting the metabolism of fatty acids in mitochondria^[39-40]. FABP3 is the most widely distributed of these proteins, and its main functions are to combine with and promote the transport of long-chain fatty acids in cells from the plasma membrane to mitochondria, participate in β -oxidation after entering mitochondria, and finally generate ATP to provide energy for cells^[41]. Oleic acid is one of the most commonly used long-chain fatty acids, and PPAR α can increase its uptake and oxidative decomposition^[42-43]. Therefore, in terms of protein expression, luteolin, naringin, and apigenin suppress the lipid accumulation induced by oleic acid by activating the expression of PPAR α . As a key downstream target gene of PPAR, the expression of *CPT1* is regulated by PPAR. When fatty acids enter the cell, CPT1 is esterified into long-chain lipoyl coenzyme A, and 75% of the CPT-1 is synthesized into hexyl carnitine, which enters the mitochondria to participate in the oxidation process of fatty acid β ^[44]. Stimulating the activity of CPT-1 can promote the normal progression of the fatty acid oxidation pathway and improve lipid metabolism to a certain extent. However, at the mRNA level, the expression of *PPAR α* increases after oleic acid stimulation. mRNA and proteins are responsible for two aspects of gene expression: transcription and translation, respectively. mRNA is regulated by various factors during translation; therefore, inconsistent expression provides insights for our subsequent research. These results confirm the network pharmacology predictions showing

that the PPAR α signaling pathway is critical in alleviating oleic acid-induced lipid accumulation.

5. Conclusion

Our findings indicate that luteolin, naringin, and apigenin represent active components in EAD and play important roles in lowering lipid levels. In oleic acid-induced lipid accumulation in HepG2 cells, these compounds play a role in alleviating lipid accumulation by regulating the PPAR signaling pathway, which is an important signaling pathway in lipid metabolism, and regulating the two key factors PPAR α and CPT1. Non-*Camellia* tea is an important part of Chinese tea culture. The above findings present new mechanistic insights and application prospects for the prevention and treatment of hypolipidemia using *D. moldavica* tea and provide a precise theoretical and research basis for the further development and utilization of *D. moldavica*.

Conflicts of interest

Minhui Li is an editorial board member for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. The authors declare that they have no competing interests.

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