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Total flavonoids of *Selaginella tamariscina* (Beauv.) Spring alleviate glucolipid metabolic disorders in rats and endothelial injury in TNF- α -induced cellsXiao-Lan Wang^{1,2}, Tao-Li Ning^{1,2}, Cai-Xia Liu^{1,2}, Pei-Pei Yuan^{1,2}, Wei-Sheng Feng^{1,2}✉, Xiao-Ke Zheng^{1,2}✉¹School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou 450046, Henan, China²The Engineering and Technology Research Center for Chinese Medicine Development of Henan Province, Zhengzhou 450046, Henan, China

ABSTRACT

Objective: To evaluate the effects of total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST) on glucolipid metabolic disorders (GLMD) in rats and TNF- α -stimulated human umbilical vein endothelial cells, as well as elucidate the underlying mechanisms.

Methods: A rat model of GLMD was established using a high-fat diet combined with vitamin D₃. Body weight and liver weight were recorded, and serum lipid profiles and biochemical parameters were determined. Hepatic histopathological changes were evaluated using hematoxylin and eosin (H&E) staining. The potential mechanisms underlying the protective effects of TFST against vascular inflammation were further explored using network pharmacology and molecular docking. In *in vitro* studies, following TNF- α stimulation and TFST treatment, the levels of inflammatory mediators in cell culture supernatants were measured by ELISA. The subcellular localization of NF- κ B p65 was detected by immunofluorescence microscopy to evaluate its nuclear translocation. In addition, Western blot analysis was performed to quantify the protein expression levels of NF- κ B p65, I κ B α , vascular cell adhesion molecule 1 (VCAM-1), and E-selectin.

Results: TFST significantly normalized body weight, liver weight and liver index ($P < 0.05$) and improved lipid parameters ($P < 0.05$), while reducing plasma viscosity ($P < 0.05$) in GLMD rats. It also reduced hepatic lipid accumulation and injury, and enhanced the activities of rate-limiting enzymes involved in hepatic lipid metabolism ($P < 0.05$). In the endothelial inflammatory cell model, TFST decreased the levels of inflammatory mediators and oxidative stress ($P < 0.05$). Furthermore, TFST downregulated the protein expression of VCAM-1, E-selectin, and NF- κ B p65, and restored I κ B α levels ($P < 0.05$), while also suppressing NF- κ B p65 nuclear

translocation.

Conclusions: TFST ameliorates GLMD and vascular endothelial inflammation *via* NF- κ B pathway inhibition.

KEYWORDS: *Selaginella tamariscina* (beauv.) Spring; Total flavonoids; Glucolipid metabolic disorders; Metabolic fatty liver disease; Vascular inflammation; NF- κ B pathway

1. Introduction

Glucolipid metabolic disorders (GLMD) pose a major threat

Summary

Question: Can total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST) ameliorate glucolipid metabolic disorders and vascular endothelial dysfunction?

Findings: TFST attenuated dyslipidemia and endothelial dysfunction *via* NF- κ B pathway inhibition.

Meaning: TFST ameliorates glycolipid metabolic disorders and vascular endothelial dysfunction. These effects are mediated, at least in part, through modulation of the NF- κ B pathway.

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to global cardiovascular health and are strongly associated with life-threatening cardiovascular complications[1]. Persistent dysregulation of glucolipid metabolism not only disrupts systemic lipid homeostasis but also causes abnormal hemorheology, hepatic lipid deposition, and impaired liver function, which can contribute to endothelial dysfunction and chronic vascular inflammation[2,3]. Statins are associated with different adverse reactions, among which fluvastatin, lovastatin, and pravastatin tend to cause rhabdomyolysis, whereas pitavastatin and atorvastatin result in liver dysfunction[4,5]. Traditional Chinese medicine (TCM), with its holistic and multi-target properties, can modulate multiple inflammatory signaling pathways, improve glucolipid metabolism, alleviate metaflammation, and protect multiple organs. It therefore represents a potential approach for managing GLMD[6]. Numerous studies have demonstrated that active compounds in TCM, such as berberine and curcumin, exert significant regulatory effects on insulin resistance and lipid accumulation[7,8]. By targeting key nodes in metabolic networks, these natural agents restore homeostasis while avoiding the adverse side effects typically associated with conventional synthetic drugs. Furthermore, the synergistic interactions among multiple herbal components enhance therapeutic efficacy, offering a comprehensive strategy for long-term management. This multifaceted mechanism underscores the value of integrating TCM into modern clinical practices for treating complex metabolic syndromes.

The total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST)—a classic TCM used for hemostasis, inflammation, and circulatory disorders—are composed primarily of amentoflavone, quercetin, kaempferol, azaleatin, and other flavonoid monomers[9,10]. According to earlier research, TFST possesses antioxidant, anti-inflammatory, and GLMD-regulating activities. It also reduces tissue and organ damage by controlling multiple signaling pathways[11,12]. However, previous studies have largely focused on amentoflavone alone as an individual flavonoid monomer[13], leaving the holistic effects of the total flavonoid extract (TFST) largely unexplored[14]. Although TFST has been reported to ameliorate hemorheological abnormalities in GLMD rats, its direct protective actions against fatty liver, endothelial inflammation, and vascular endothelial injury, and the underlying mechanisms remain poorly understood. Therefore, this study aimed to investigate the effect of TFST on GLMD and elucidate its underlying mechanisms.

Diet plays an important role in maintaining cholesterol homeostasis, while a high-fat diet (HFD) promotes excessive lipid deposition in adipose tissue and contributes to the development of GLMD, obesity, and associated liver injury[15]. Although a combination of vitamin D₃ and HFD is widely used to induce atherosclerosis in animals, we did not observe obvious vascular lipid deposition in our model. Instead, the model consistently caused GLMD and metabolic fatty liver

injury. The network pharmacology analysis in this study indicated that metabolic dysfunction-associated fatty liver disease is closely linked to vascular inflammation and the NF- κ B pathway in atherosclerosis. Accordingly, we performed subsequent *in vitro* studies.

In this study, a GLMD rat model was established by HFD and vitamin D₃ co-administration, in which serum lipid profiles, hemorheological parameters, and hepatic function markers were systematically evaluated. Network pharmacology and molecular docking identified TNF and NF- κ B signaling as pivotal targets. To further elucidate the underlying mechanisms, we employed a model of TNF- α -stimulated human umbilical vein endothelial cells (HUVECs) to assess the endothelial-protective effects of TFST and to determine the involvement of the NF- κ B pathway. Collectively, these findings provide experimental evidence supporting TFST as a promising candidate for the prevention and treatment of GLMD and its related cardiovascular complications.

2. Methods

2.1. Materials and reagents

TFST was prepared and identified in our laboratory. The original herb material, *Selaginella tamariscina* (P. Beauv.) Spring was morphologically authenticated by Dr. Chen SQ. Reference sample (No. HZY201203) has been deposited at Laboratory of Chinese materia medica chemistry, School of Pharmacy, Henan University of Chinese Medicine, Henan, China. The total flavonoid content of TFST was determined to be no less than 55%. The custom-formulated HFD was also prepared in our laboratory and consisted of basal diet (78.8%), cholesterol (1%), lard (10%), egg yolk powder (10%), and sodium cholate (0.2%). Vitamin D₃ was acquired from General Pharmaceutical Co., Ltd. (catalog number: H31021404, Shanghai, China).

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine transaminase (ALT), aspartate transaminase (AST), superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) kits were purchased from Nanjing Jiancheng Bioengineering Institute. Enzyme-linked immunosorbent assay (ELISA) kits for interleukin-6 (IL-6), interleukin-8 (IL-8), and endothelin-1 (ET-1) were acquired from R&D Systems (catalog numbers: D6050, D8000C, DET100, respectively; USA).

Primary antibodies targeting NF- κ B p65, I κ B α , vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and Lamin B1 were acquired from Santa Cruz Biotechnology (catalog numbers: sc-8008, sc-1643, sc-13160, sc-13704, sc-377000, Dallas, TX, USA). A rabbit monoclonal anti- β -actin antibody and HRP-conjugated goat anti-

rabbit IgG secondary antibody were sourced from Beijing ComWin Biotech (catalog numbers: CW0264M, CW0103S, Beijing, China). Recombinant human TNF- α was obtained from PeproTech (Rocky Hill, NJ, USA). All other reagents and solvents were of analytical grade and commercially available.

2.2. Animals and grouping

Sprague-Dawley (SD) rats (SPF, male, 180–220 g) were obtained from Henan Experimental Animal Center (Zhengzhou, China). All rats were housed under controlled conditions [temperature (22 ± 2) °C, humidity (55 ± 5)%, 12 h light/dark cycle] with *ad libitum* access to standard chow and water. Rats were subsequently randomly divided to 4 groups ($n = 6$): Normal control (NC) group fed a standard diet, GLMD model group fed the HFD and given a single intraperitoneal injection of vitamin D₃ (600 000 IU/kg) on day 1, TFST low-dose group (TFST-L) subjected to the GLMD model and treated with TFST (65 mg/kg) by gavage, TFST high-dose group (TFST-H) subjected to the GLMD model and treated with TFST (130 mg/kg). The treatment lasted for 12 weeks, and the TFST dosages were selected based on a previous study^[13]. Body weight and liver weight were recorded at the experimental endpoint. At the end of the experiment, all rats were fasted for 12 h, then anesthetized, and blood and tissue samples were collected for histopathological and biochemical analyses.

2.3. Measurement of body and liver weights

At the end of the experiment, the livers were excised immediately after euthanasia, rinsed with ice-cold saline, blotted dry, and weighed. The liver index was calculated according to the following formula: liver index = liver wet weight (g) / body weight (g) $\times$ 100%.

2.4. Biochemical analysis

Blood samples were centrifuged at 3 000 rpm for 15 min at 4 °C to obtain serum. Serum levels of TC, TG, HDL-C, LDL-C, calcium, ALT, and AST were then measured using commercial kits according to the manufacturer's protocols.

2.5. Hemorheological assay

Whole blood was collected into heparinized tubes for the measurement of hemorheological parameters. Whole blood viscosity (WBV) was measured at low (10/s), middle (50/s), and high (200/s) shear rates, and plasma viscosity (PV) was determined at 37 °C using a cone-plate viscometer (LBYN6, Pricell, Beijing, China).

2.6. Hepatic histopathological examination

Liver tissue samples were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 5 μ m thickness. Sections were then deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E). Histopathological changes, including lipid droplet accumulation, hepatocellular ballooning, and inflammatory cell infiltration, were observed using a light microscope (BX53, Olympus, Japan) at $\times$ 400 magnification.

2.7. Determination of hepatic lipid metabolism-related enzymes

Liver tissue was homogenized in ice-cold saline. The homogenates were centrifuged at 4 000 rpm for 15 min at 4 °C, and the clear supernatants were collected. The enzymatic activities of acyl-CoA synthetase (ACS) and carnitine palmitoyltransferase-1 (CPT-1) in the supernatants were determined using commercial colorimetric assay kits (catalog numbers: CK-E30058R, CK-E90744R, Cwbio, Beijing, China), following the manufacturer's instructions.

2.8. Network pharmacology and molecular docking

The active components of TFST, such as amentoflavone, quercetin, kaempferol, and azaleatin, were extracted from two databases, TCMSP and PubChem. Potential targets related to GLMD and inflammation were obtained from OMIM, GeneCards, and DisGeNET databases. The compound-target-disease network was established and displayed in Cytoscape 3.9.1. To find out the important signaling pathways, pathway enrichment analysis was done with the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database using Metascape. Using AutoDock Vina 1.2.3, molecular docking was carried out to confirm the binding affinity between key flavonoids and TNF protein. Binding activity was suggested for binding energy ≤ -7.0 kcal/mol.

2.9. Cell culture and treatment

HUVECs were generously provided by Professor Li Qiang from the First Affiliated Hospital of Henan University of Chinese Medicine, Zhengzhou, China. Cells were cultured in RPMI-1640 medium supplemented with fetal bovine serum (FBS, 10%) and penicillin-streptomycin (1%) at 37 °C in a humidified incubator containing 5% CO₂. HUVECs were separated into 6 groups: Control group (untreated), model group (stimulated with 10 ng/mL TNF- α for 24 h), and TFST treatment groups (pretreated with 5, 10, 15, and 20 μ g/mL TFST for 24 h, followed by co-incubation with 10 ng/mL TNF- α for 24 h).

2.10. Measurement of inflammatory and oxidative stress markers

After treatment, culture media were collected and centrifuged ($300 \times g$, 10 min, 4°C). The levels of IL-6, IL-8, and ET-1 were measured using commercial ELISA kits, and NO, SOD and MDA were determined using biochemical assay kits, following the manufacturer's instructions.

2.11. Immunofluorescence staining

HUVECs were fixed with paraformaldehyde (4%), permeabilized with Triton X-100 (0.1%), and blocked with bovine serum albumin (5%). Subsequently, cells were incubated with anti-NF- κB p65 antibody overnight at 4°C . Following thorough washes with phosphate-buffered saline, cells were incubated with fluorescently labeled secondary antibody (1:500) for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI and finally observed using a laser scanning confocal microscope (FV1000, Olympus, Japan).

2.12. Western blot analysis

Total, nuclear, and cytoplasmic protein fractions were isolated using commercially available kits according to the manufacturers' protocols. Protein concentrations were determined *via* the BCA assay. Equal amounts of protein were separated by SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with 5% (*w/v*) non-fat milk, which was prepared in TBST, for 1.5 h at room temperature. and subsequently probed with primary antibodies against NF- κB p65 (1:1 000), I $\kappa\text{B}\alpha$ (1:1 000), VCAM-1 (1:1 000), E-selectin (1:1 000), Lamin B1 (1:1 000), and β -actin (1:3 000) overnight at 4°C . After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibodies (1:2 000) for 1 h at room temperature in the dark. Protein bands were visualized using an enhanced chemiluminescence (ECL) kit and captured using a BOX gel imaging system (Syngene, Cambridge, UK). Densitometric analysis was performed using ImageJ software. Protein expression levels in nuclear fractions were normalized to Lamin B1, while those in cytoplasmic fractions were normalized to β -actin.

2.13. Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). IBM SPSS Statistics 18.0 was applied for statistical analysis. Differences among multiple groups were compared using one-way analysis of variance (ANOVA). $P < 0.05$ was defined as statistically significant.

2.14. Ethical approval

All animal experiments were approved by the Laboratory Animal Ethics Committee of Henan University of Chinese Medicine (approval number: DWLLCXY202511003, dated November 10, 2025). Animal operations and treatments complied with the requirements of the Guide for the Welfare and Ethics Review of Laboratory Animals (GB/T 35892-2018), the Guiding Opinions on the Humane Treatment of Laboratory Animals, and the constitution of the Laboratory Animal Ethics Committee of Henan University of Chinese Medicine.

3. Results

3.1. Effect of TFST on body and liver weights

As shown in Figure 1, GLMD rats exhibited a significant reduction in body weight and a marked increase in liver weight ($P < 0.05$). In contrast, body weight was significantly increased by TFST (65 mg/kg) treatment ($P < 0.05$) (Figure 1A), and liver weight was notably lowered at 65 and 130 mg/kg (Figure 1B) ($P < 0.05$).

3.2. Effect of TFST on lipid parameters and hemorheological indices

The GLMD group showed significantly higher TC (Figure 2A), TG (Figure 2B), LDL-C (Figure 2D), and Ca (Figure 2E), as well as lower HDL-C (Figure 2C) ($P < 0.05$). TFST treatment restored the levels of lipid parameters ($P < 0.05$). Compared with the normal control group, the GLMD group exhibited significantly elevated WBV across all shear rates (Figure 2F-H) and PV ($P < 0.05$) (Figure 2I). TFST treatment significantly restored these levels ($P < 0.05$).

3.3. Effect of TFST on liver injury indices

Histological evaluations indicated that TFST alleviated hepatic lipid accumulation and tissue injury in GLMD rats. The GLMD rats showed extensive lipid accumulation in the liver, whereas TFST treatment reduced lipid deposition. H&E staining revealed marked hepatocellular damage in the GLMD group, characterized by cytoplasmic rarefaction and lipid droplet accumulation. These morphological abnormalities were notably improved following TFST treatment. The higher dose of TFST (130 mg/kg) largely preserved normal hepatocyte architecture and reduced intracellular lipid vacuolization (Figure 3A). Liver injury markers (ALT and AST) were significantly increased in GLMD rats ($P < 0.05$). However, TFST treatment significantly lowered these markers

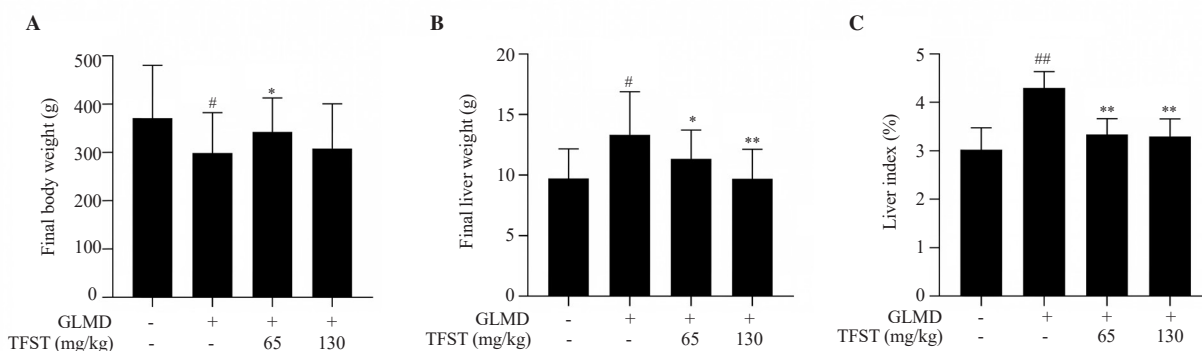


Figure 1. Effect of total flavonoids of *Selaginella tamariscina* (Beauv.) Spring (TFST) on (A) body and (B) liver weights as well as (C) liver index in rats with glucolipid metabolic disorders (GLMD). Data are shown as mean±SD ($n=6$). # $P<0.05$, ## $P<0.001$ compared with the normal control group. * $P<0.05$, ** $P<0.001$ compared with the GLMD group.

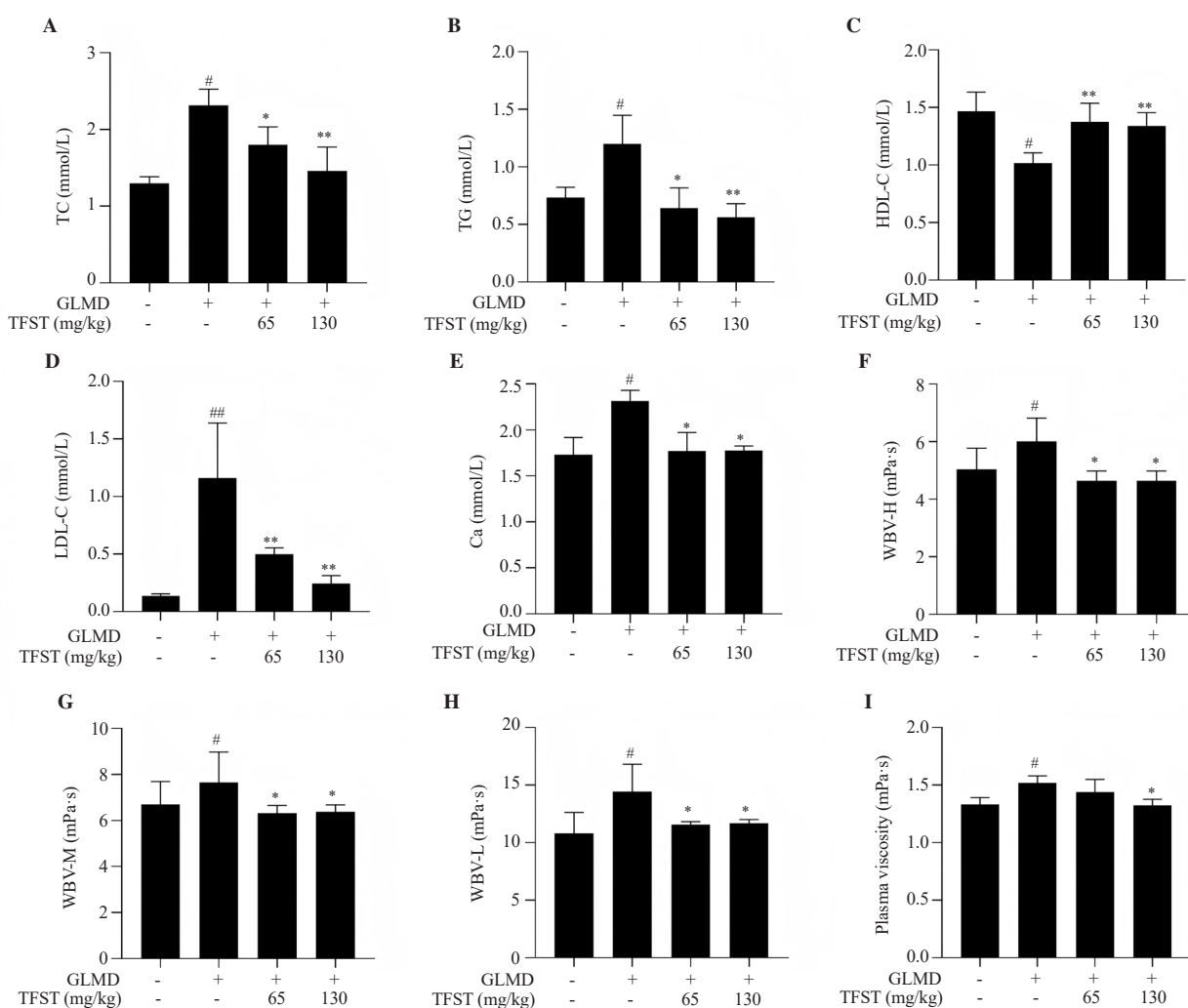


Figure 2. Effect of TFST on serum biochemical parameters and hemorheological indices in rats. (A) Total cholesterol (TC), (B) triglycerides (TG), (C) high-density lipoprotein cholesterol (HDL-C), (D) low-density lipoprotein cholesterol (LDL-C), (E) serum calcium (Ca), (F-H) whole blood viscosity at high (WBV-H; 200/s), middle (WBV-M; 50/s), and low (WBV-L; 10/s) shear rates, respectively, (I) plasma viscosity. Data are shown as mean±SD ($n=6$). # $P<0.05$, ## $P<0.001$ compared with the normal control group. * $P<0.05$, ** $P<0.001$ compared with the GLMD group.

(Figure 3B and 3C). Moreover, a marked decrease in ACS and CPT-1 was observed in GLMD rats ($P < 0.05$), while both doses of TFST treatment significantly ameliorated these changes ($P < 0.05$) (Figure 3D and 3E).

3.4. Network pharmacology analysis, target prediction and molecular docking

To elucidate the possible mechanism of TFST in GLMD, a compound-target-disease network was constructed (Figure 4A). Based on topological analysis of the network, the core hub targets were identified according to degree centrality, with TNF, NF- κ B1, AKT1, and PIK3R1 exhibiting the highest connectivity.

KEGG pathway enrichment analysis (Figure 4B) revealed that potential targets were mainly concentrated in inflammation- and immune-related pathways, such as the TNF signaling pathway, NF- κ B signaling pathway, and the lipid and atherosclerosis pathway. These findings indicate that TFST may exert its anti-inflammatory effects *via* the regulation of these pathways.

Molecular docking was performed to evaluate the binding affinity of the candidate compounds to TNF (Figure 4C-F). Amentoflavone showed the lowest binding energy (-10.1 kcal/mol), indicating the strongest binding affinity, compared with quercetin (-8.3 kcal/mol), kaempferol (-8.1 kcal/mol), and azaleatin (-7.3 kcal/mol). Notably,

amentoflavone formed multiple hydrogen bonds with crucial residues of TNF, suggesting a potential direct interaction.

3.5. Effect of TFST on TNF- α -induced HUVECs

In comparison with the control group, the release of IL-6, IL-8, NO, MDA, and ET-1 ($P < 0.05$) was significantly enhanced in TNF- α -induced HUVECs, along with reduced SOD activity ($P < 0.001$). TFST treatment at all doses lowered these elevated mediators ($P < 0.05$), while markedly elevating SOD activity at 10, 15, and 20 μ g/mL ($P < 0.05$) (Figure 5A-D, 5L and 5M).

Immunofluorescence staining revealed that TNF- α stimulation promoted the nuclear translocation of NF- κ B p65 (Figure 5E), which was quantitatively confirmed by Western blot analysis (Figure 5F-H). Compared with the normal control group, TNF- α significantly increased the nuclear protein expression of NF- κ B p65 ($P < 0.001$) and concomitantly decreased the cytoplasmic expression of I κ B α ($P < 0.001$). These TNF- α -induced alterations were reversed by TFST pretreatment ($P < 0.001$).

TNF- α stimulation also significantly upregulated the expression of VCAM-1 and E-selectin compared with the control group ($P < 0.001$), which was significantly attenuated by TFST pretreatment ($P < 0.05$) (Figure 5I-K).

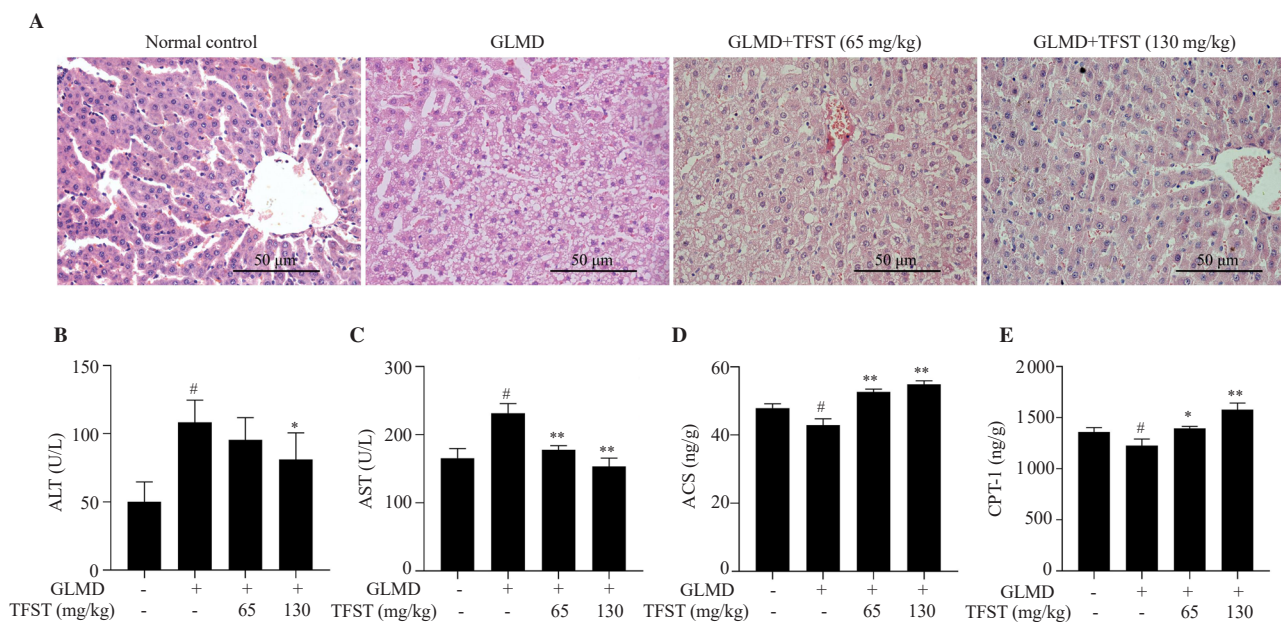


Figure 3. Effect of TFST on liver injury in GLMD rats. (A) Representative histopathological images of liver sections stained with hematoxylin and eosin (scale bar = 50 μ m, 400 $\times$ magnification). (B) Alanine transaminase (ALT), (C) aspartate transaminase (AST), (D) acyl-CoA synthetase (ACS), and (E) carnitine palmitoyltransferase-1 (CPT-1). Data are shown as mean $\pm$ SD ($n=6$). # $P < 0.05$ compared with the normal control group. * $P < 0.05$, ** $P < 0.001$ compared with the GLMD group.

4. Discussion

Insulin resistance, oxidative stress, meta-inflammation, and intestinal flora dysbiosis are the drivers of GLMD, which clinically manifests as fatty liver and atherosclerosis[16]. Due to the intricate interplay among these pathological processes, reliance on a single-disease approach is unsatisfactory, and an integrated therapeutic

strategy is imperative[17]. Natural flavonoids exhibit a variety of remarkable properties, including potent antioxidant and anti-inflammatory activities, as well as comprehensive regulation of glucolipid metabolism[18]. Baicalin attenuated GLMD and aortic vasoconstriction by modulating intracellular Ca^{2+} homeostasis in GLMD rats. Furthermore, it alleviated vascular inflammation and oxidative stress in high glucose-induced endothelial cells[19,20].

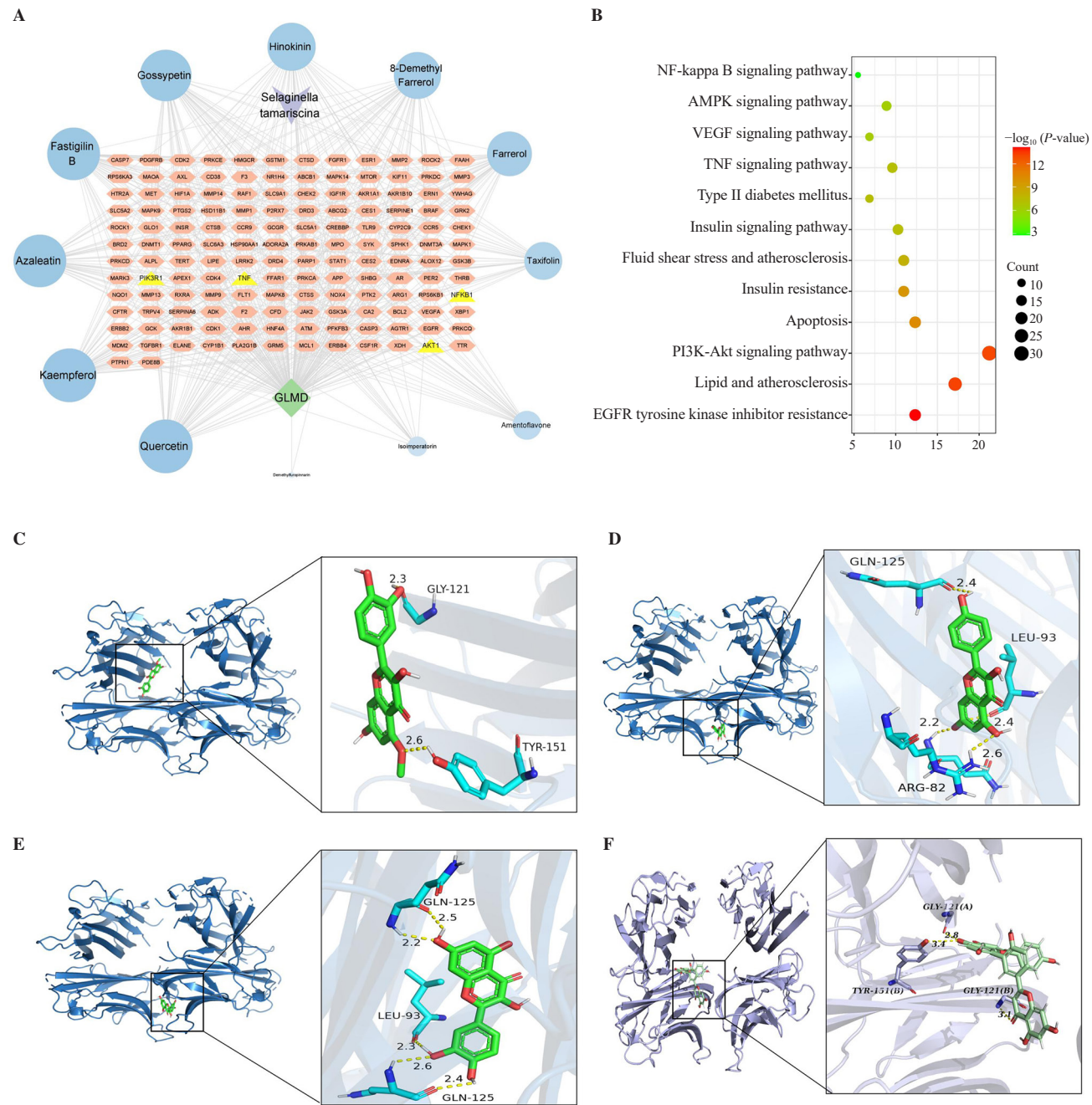


Figure 4. Target prediction and molecular docking analysis of bioactive flavonoids of *Selaginella tamariscina*. (A) Compound-target-disease interaction network showing the relationships between bioactive flavonoids, potential targets, and GLMD. (B) KEGG pathway enrichment analysis of the core targets. Bubble size corresponds to the number of enriched genes, and color indicates the significance of enrichment (P -value). (C-F) Molecular docking models of TNF with azaleatin (−7.3 kcal/mol), kaempferol (−8.1 kcal/mol), quercetin (−8.3 kcal/mol), and amentoflavone (−10.1 kcal/mol), respectively. The detailed binding interactions (including hydrogen bonds) are shown in the magnified views and 2D interaction diagrams.

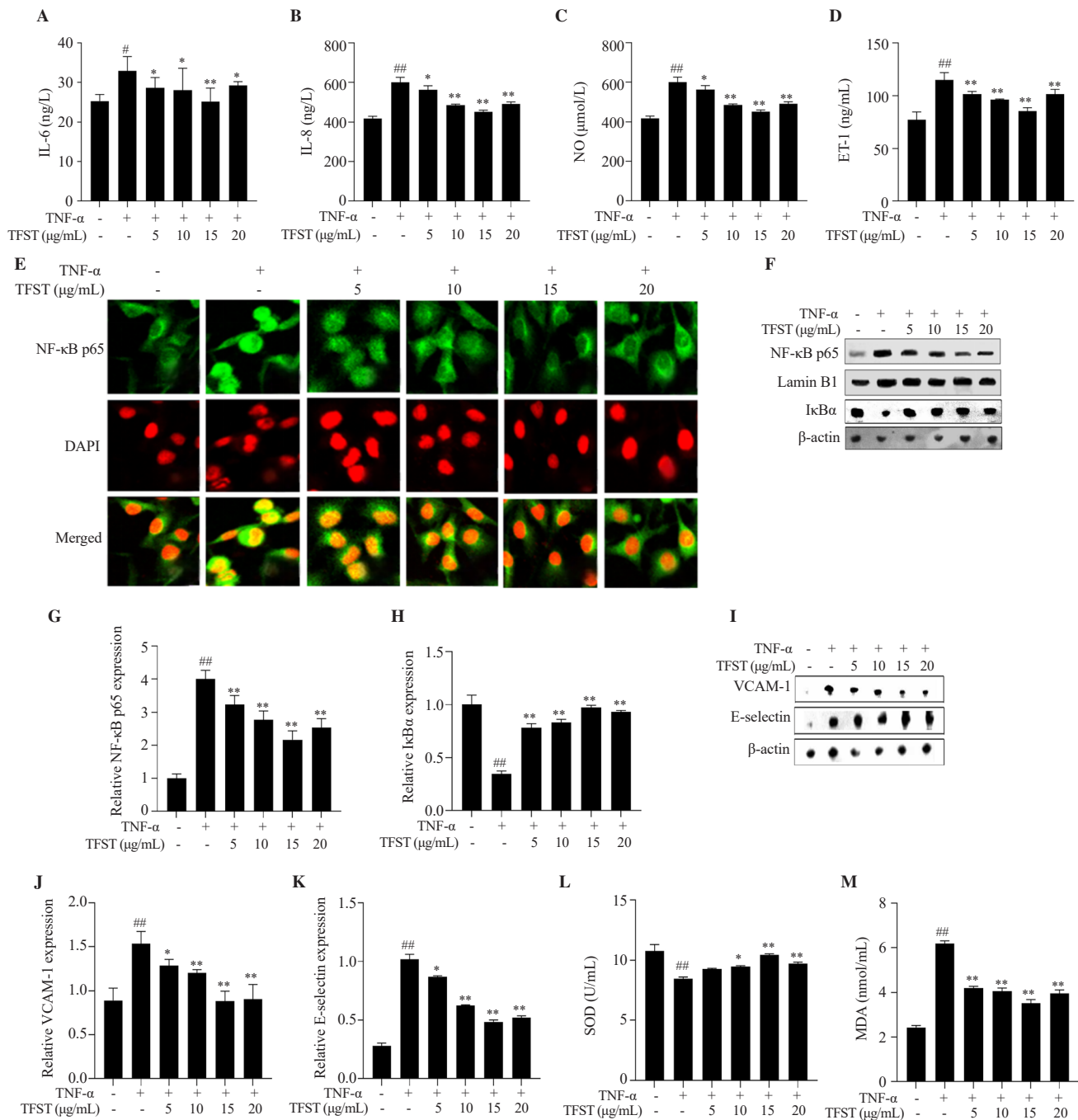


Figure 5. TFST inhibits TNF- α -induced inflammatory injury in human umbilical vein endothelial cells. (A-D) Levels of pro-inflammatory mediators IL-6, IL-8, endothelin-1 (ET-1), and nitric oxide (NO) in the culture supernatants after TNF- α stimulation and TFST treatment (5, 10, 15, and 20 μ g/mL). (E) Immunofluorescence staining showing the nuclear translocation of NF- κ B p65 (green) in TNF- α -stimulated cells. Nuclei were counterstained with DAPI. (F-H) Western blot analysis of nuclear NF- κ B p65 and cytoplasmic I κ B α , with quantitative densitometry. Lamin B1 and β -actin were used as nuclear and cytoplasmic internal controls, respectively. (I-K) Western blot analysis of VCAM-1 and E-selectin expression and quantitative results; β -actin served as the loading control. (L-M) SOD activity and MDA content in cell lysates. Data are shown as mean $\pm$ SD ($n=3$). [#] $P<0.05$, ^{##} $P<0.001$ compared with the control group; ^{*} $P<0.05$, ^{**} $P<0.001$ compared with the TNF- α model group.

Catechin normalized blood pressure and endothelial function in diabetic rats, while its metabolites reduced monocyte adhesion to IL-1 β -stimulated endothelial cells[21,22].

Multiple studies have underscored the therapeutic value of TCM in managing GLMD[23]. Among the various candidates, *Selaginella*

tamariscina (Beauv.) Spring—a perennial herbaceous fern of the Selaginellaceae family—has been extensively investigated for its phytochemistry and GLMD-related pharmacological activities[24–26]. However, its potential vascular protective effects remain largely unexplored.

Our findings indicate that TFST exerts multifaceted benefits in vitamin D₃ and HFD-challenged rats, encompassing the amelioration of GLMD, the correction of hemorheological disturbances, and the mitigation of hepatic steatosis and injury, alongside potent cytoprotection against TNF- α -induced endothelial damage. Notably, no obvious histopathological alterations were observed in the arterial vessels across all groups, despite the model exhibiting profound GLMD and metabolic fatty liver disease—both of which were markedly alleviated by TFST. Interestingly, the model group displayed a significant decrease in body weight, contrasting with the typical weight gain seen in conventional diet-induced obesity models. This weight loss was effectively reversed by TFST treatment. Furthermore, concurrent administration of a high dose of vitamin D₃ with certain medications has been shown to not only induce GLMD but also potentiate weight loss and hepatic damage[27,28].

Hemorheological impairment has been implicated in both the pathogenesis of cardiovascular diseases and arteriosclerosis[29], and is also regarded as a key objective indicator of blood stasis in TCM practice[30–32]. To establish the GLMD model, we employed HFD combined with vitamin D₃ in rats, which successfully produced overt dyslipidemia and hemorheological dysfunction. Following TFST treatment, a remarkable reduction in serum levels of TC, TG, LDL-C, and calcium was observed, accompanied by a significant increase in HDL-C. Furthermore, TFST robustly reduced WBV across all shear rates as well as PV. These results highlight the therapeutic potential of TFST in ameliorating both lipid disorders and hemorheological abnormalities, thereby relieving microcirculatory impairment in GLMD.

The liver, on the other hand, is the main organ for lipid synthesis, decomposition, and transport. Hepatic steatosis, hepatocyte swelling, and impaired liver function—all resulting from excessive lipid deposition in hepatocytes—are the hallmarks of GLMD, as evidenced by elevated serum ALT and AST[33–35]. Our results indicated that TFST significantly reduced the elevated liver weight, alleviated vacuolar degeneration and lipid droplet accumulation in liver tissue, and normalized ALT and AST levels in GLMD rats. TFST also increased the activities of ACS and CPT-1, two key rate-limiting enzymes involved in hepatic fatty acid oxidation and metabolism. These findings suggest that TFST protects the liver by promoting fatty acid metabolism and limiting lipid accumulation, thereby maintaining systemic lipid homeostasis. Importantly, hepatic lipid metabolism disorder leads to vascular endothelial inflammation through multiple interconnected pathways—including dyslipidemia, systemic inflammation, oxidative stress, and hepatic extracellular vesicle-mediated signaling—all of which converge to impair endothelial function and trigger a pro-inflammatory cascade[36–38]. In recent years, TCM and natural products have demonstrated promising efficacy in the holistic regulation of fatty liver disease, inflammation, and atherosclerosis[39,40].

Further, network pharmacology and molecular docking

demonstrated that the key components of TFST, such as amentoflavone, quercetin, kaempferol, and azaleatin, could directly bind to TNF with high affinity. Among these, amentoflavone, as the most abundant constituent, yielded the most favorable docking score, suggesting that it might be the key effective monomer. KEGG pathway enrichment analysis identified the TNF signaling pathway, NF- κ B signaling pathway, and the lipid and atherosclerosis pathway as the most significantly enriched targets. Collectively, our cellular and animal experimental results corroborate these computational predictions, indicating that TFST exerts synergistic therapeutic effects through a multitargeted mechanism involving multiple constituents, signaling pathways, and biological processes.

Vascular endothelial dysfunction is regarded as one of the initial events in atherosclerosis, and TNF- α is an important pro-inflammatory cytokine implicated in endothelial injury[41]. Overactivated TNF- α signaling can initiate the NF- κ B cascade, accelerating I κ B α breakdown and driving NF- κ B p65 nuclear translocation, and upregulating downstream inflammatory mediators and adhesion molecules[42,43]. The present study showed that TNF- α -stimulated HUVECs resulted in elevated IL-6, IL-8, NO, and ET-1, enhanced oxidative stress (decreased SOD and increased MDA), and upregulated VCAM-1 and E-selectin expression, which were reversed by TFST treatment. Immunofluorescence and Western blot results further confirmed that TFST suppressed NF- κ B p65 nuclear translocation, restored I κ B α expression, and downregulated the expression of adhesion molecules. Collectively, these findings indicate that TFST exerts a protective effect against endothelial inflammatory injury through blockade of the NF- κ B signaling pathway.

In conclusion, TFST exerted anti-GLMD, hepatoprotective, and vascular endothelial-protective effects. Its mechanism of action is associated with the enhancement of lipid metabolism and hemorheology, and the attenuation of TNF- α /NF- κ B-mediated inflammatory responses and oxidative stress. Nevertheless, several limitations of the present study should be acknowledged. Although TFST exhibits protective effects against GLMD, liver injury, and endothelial inflammation, we did not systematically explore the characteristic signaling pathways and key targets that mediate the crosstalk among these pathological processes. Moreover, the mechanistic pathways of endothelial cell protection were only partially investigated, lacking *in vivo* animal validation as well as complementary experiments using pathway-specific inhibitors or gene-silencing strategies. Future investigations will focus on evaluating the anti-atherosclerotic effects of TFST in the context of GLMD, and determining its safe and effective dosage range.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

XLW designed the experimental study and drafted and proofread the manuscript. TLN and CXL performed the experimental study. PPY performed data analysis and verification. XKZ and WSF were responsible for the conception and design of the study, data interpretation, manuscript drafting and revision, and final approval of the version to be published. All authors read and approved the final manuscript.

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