Iranian Journal of Basic Medical Sciences
ijbms.mums.ac.ir

Liquiritin ameliorates atherosclerosis in ApoE$^{-/-}$ mice by modulating SIRT1 and lipid metabolism

Kang Ye [1], Jing Shi [1], Ping Gong [1], Zhongsheng Zhu [1]*

[1] Department of Internal Medicine-Cardiovascular, Guangming Traditional Chinese Medicine Hospital of Pudong New Area, Shanghai 201321, China

ARTICLE INFO

Article type:
Original

Article history:
Received: Oct 20, 2025
Accepted: Jul 20, 2026

Keywords:
Atherosclerosis
Dyslipidemia
Hepatic steatosis
Lipid metabolism
Liquiritin
SIRT1

ABSTRACT

Objective(s): Atherosclerosis (AS) and dyslipidemia are major contributors to cardiovascular disease; however, safe and effective therapeutic strategies remain limited. Liquiritin, a natural flavonoid, exhibits potential metabolic and cardiovascular benefits in human beings. This study investigated the effects of liquiritin on AS, lipid metabolism, and hepatic steatosis in high-fat diet (HFD)-induced ApoE-/- mice.

Materials and Methods: Mice were intraperitoneally administered liquiritin (20 mg/kg/day) for 12 weeks. Histological and biochemical analyses revealed that chronic liquiritin treatment did not induce hepatotoxicity, nephrotoxicity, or growth impairment, as evidenced by preserved liver and kidney architecture, unchanged serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and blood urea nitrogen (BUN) levels, and stable body weight.

Results: Liquiritin markedly attenuated aortic root plaque formation, lipid deposition, and fibrotic areas (P<0.001) in ApoE-/- mice. Furthermore, liquiritin significantly improved serum and hepatic lipid profiles by reducing triglyceride, total cholesterol, and LDL-C levels (P<0.001) and alleviating hepatic steatosis (P<0.001), as confirmed by histological analyses. Mechanistic studies demonstrated that liquiritin restored hepatic SIRT1 expression, down-regulated PPARγ and LXRα, and up-regulated ABCA1 (P<0.001), suggesting the modulation of key lipid metabolism pathways.

Conclusion: These findings demonstrate that liquiritin protects against AS and hepatic lipid accumulation by modulating SIRT1-mediated lipid metabolism (P<0.001), emphasizing its potential as a treatment option for metabolic and cardiovascular disorders.

▶ Please cite this article as:
Ye K, Shi J, Gong P, Zhu Z. Liquiritin ameliorates atherosclerosis in ApoE$^{-/-}$ mice by modulating SIRT1 and lipid metabolism. Iran J Basic Med Sci 2026; 29: 1406-1412. doi: https://dx.doi.org/10.22038/ijbms.2026.92111.19881

Introduction

Atherosclerosis, a chronic inflammatory condition of the arterial wall, involves the accumulation of lipids and fibrous material. It is the primary pathological basis for cardiovascular diseases (CVDs), which are the leading cause of global mortality (1). A critical initiating event in atherogenesis is the retention and modification of low-density lipoprotein cholesterol (LDL-C) and other lipoproteins, including apolipoprotein B, within the subendothelial space, which triggers a cascade of inflammatory and oxidative responses (2). Dyslipidemia, defined by increased levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), constitutes a major risk factor for the initiation and progression of atherosclerotic lesions (3).

The apolipoprotein E knockout (ApoE-/-) mouse model is a widely used and robust tool for studying atherosclerosis. These mice develop severe hypercholesterolemia and spontaneous atherosclerotic plaques on a chow diet, and the process is dramatically accelerated by a high-fat diet (HFD), closely mimicking human conditions (4). Research using this model has been instrumental in elucidating the intricate pathways linking lipid metabolism and vascular inflammation.

In recent years, sirtuin 1 (SIRT1), an NAD+-dependent class III histone deacetylase, has drawn considerable attention in the field of metabolic and cardiovascular health. SIRT1 is a pivotal regulator of energy metabolism, oxidative stress, and inflammation (5). SIRT1 activation promotes cholesterol efflux, reduces lipoprotein secretion, and inhibits inflammatory pathways in macrophages and endothelial cells, thereby exerting atheroprotective effects (6, 7). Consequently, identifying natural compounds that modulate SIRT1 activity is a promising therapeutic strategy for combating atherosclerosis.

Liquiritin is a major flavonoid isolated from the roots of Glycyrrhiza uralensis (licorice), a plant widely used in traditional Chinese medicine and known for anti-inflammatory, antioxidant, and hepatoprotective activities (8, 9). Preliminary evidence suggests that liquiritin may influence metabolic pathways; however, its potential effects on atherosclerosis and the underlying mechanisms, particularly its interaction with the SIRT1 pathway and systemic lipid metabolism, remain largely unexplored.

Given the established roles of dyslipidemia and SIRT1 dysregulation in the pathogenesis of atherosclerosis, this study was designed to address the following research

***Corresponding author:** Zhongsheng Zhu. Department of Internal Medicine-Cardiovascular, Guangming Traditional Chinese Medicine Hospital of Pudong New Area, Shanghai 201321, China. Tel/ Fax: +86-18117209753, Email: drzzs66@163.com

question: Can liquiritin attenuate atherosclerosis and lipid metabolic disorders in high-fat diet-induced ApoE-/- mice by modulating hepatic SIRT1 and lipid metabolism-related signaling pathways? To address this question, the specific objectives of the study were as follows: (i) to evaluate the safety profile of long-term liquiritin administration in mice; (ii) to determine the effects of liquiritin on aortic atherosclerotic plaque formation, lipid accumulation, and fibrosis; (iii) to assess the impact of liquiritin on systemic and hepatic lipid metabolism as well as hepatic steatosis; and (iv) to investigate whether these effects are associated with modulation of hepatic SIRT1 and its downstream lipid metabolism–related proteins, including PPARγ, LXRα, and ABCA1. Although liquiritin has been reported to possess anti-inflammatory and hepatoprotective properties, its role in atherosclerosis and systemic lipid metabolism remains unclear. The novelty of the present study lies in demonstrating, for the first time, that liquiritin attenuates atherosclerotic plaque formation and vascular fibrosis in high-fat diet-induced ApoE-/- mice. Moreover, this study provides new mechanistic insights by identifying hepatic SIRT1 restoration and the coordinated regulation of lipid metabolism-related proteins as potential pathways through which liquiritin improves dyslipidemia and hepatic steatosis. By linking vascular protection with hepatic lipid metabolic regulation, these findings reveal a previously unrecognized SIRT1-dependent mechanism underlying the anti-atherosclerotic effects of liquiritin.

Materials and Methods

Experimental animals

Male C57BL/6 mice aged 6-8 weeks and weighing 22–24 g, along with male apolipoprotein E-deficient (ApoE -/-) mice, were obtained from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. (Jiangsu, China). C57BL/6 mice (n=8) served as the control group and were fed a maintenance diet containing 4% fat (Beijing HFK Bioscience Co., Ltd.). ApoE-/- mice were fed a Western diet for 12 weeks to establish an atherosclerosis model. After 12 weeks, ApoE-/- mice were randomly divided into two groups (n=8 each): the atherosclerosis model group and the liquiritin treatment group. Mice in the Control and AS groups received intraperitoneal saline injections for 12 consecutive weeks, whereas those in the Liquiritin group were administered liquiritin at 20 mg/kg/day. Fasting peripheral blood samples and aortas were collected at the end of the treatment period for analysis. All animals were housed under standard laboratory conditions with free access to food and water ad libitum. The experimental procedures were approved by the Ethics Committee of the Guangming Traditional Chinese Medicine Hospital of the Pudong New Area (approval number: 2025-KY-002) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Liver and renal function assessment

In this study, sixteen (16) C57BL/6 mice were randomly assigned to two groups and received either saline or liquiritin at 20 mg/kg/day via intraperitoneal injection for 12 weeks. Blood samples were collected after treatment, and serum was obtained for subsequent biochemical analysis. Serum alanine aminotransferase (ALT; C009-2-1, Nanjing Jiancheng), aspartate aminotransferase (AST; C010-2-1, Nanjing Jiancheng), creatinine (S0291S, Beyotime), and blood urea nitrogen (BUN; S0574S, Beyotime) concentrations were quantified using a microplate reader according to the manufacturers' protocols. Furthermore, liver and kidney tissues were harvested, fixed in 4% paraformaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histopathological examination (10).

Body weight and lipid levels measurement

The body weights of each mouse were measured at 4, 8, and 12 weeks after liquiritin administration. Lipid changes in the serum and liver tissue of mice were analyzed by measuring the concentrations of TG (S0219S, Beyotime), TC (S0211S, Beyotime), and LDL-C (A113-1-1, Nanjing Jiancheng) using the Lipid Biochemistry Kits.

Aortic root and liver histological analysis

The aortic root and liver tissues were collected and fixed in 4% paraformaldehyde for 24 h. The samples were then dried and embedded in paraffin. Thin slices (5 μm) were prepared using a specific cutting tool. The tissues were stained with hematoxylin and eosin (H&E; C0105S, Beyotime, Shanghai, China) to examine their structure. Hematoxylin was used as a counterstain after Oil Red O (C0157S; Beyotime, Shanghai, China) was applied to frozen liver and aortic root slices to assess lipid accumulation. Collagen deposition and fibrotic changes in the aortic root were examined using Masson's trichrome staining (D026-1-1, Nanjing, Jiancheng). The stained sections were examined using a light microscope (Olympus, Tokyo, Japan), and selected images were captured for further analysis. Lipid accumulation and collagen areas were measured using ImageJ software (NIH, Bethesda, MD, USA)(11).

Measurement of hepatic free fatty acids (FFA) and malondialdehyde (MDA)

Based on previous reports (12, 13), we examined the levels of malondialdehyde (MDA) and hepatic free fatty acids (FFA). The liver tissues were removed, sliced into small pieces, and weighed precisely. After homogenizing the samples in an ice-cold buffer, they were centrifuged for 10 min at 4 ˚C at 1500 ×g. The supernatants were collected for further biochemical analyses. MDA, a marker of lipid peroxidation, was measured using a commercial kit (A003-1-2, Nanjing Jiancheng) according to the manufacturer's instructions, and hepatic free fatty acid (FFA) levels were assessed using the Amplex Red Free Fatty Acid Assay Kit (S0215S, Beyotime). The BCA Protein Assay Kit (P0012, Beyotime) was used to measure protein concentrations. FFA and MDA levels were normalized to the total protein content to ensure comparability across samples.

Western blotting

Liver tissue proteins were extracted and analyzed by western blotting as previously described (14). Tissues were homogenized in ice-cold RIPA lysis buffer (Beyotime, China) containing protease and phosphatase inhibitors, then centrifuged at 12,000 ×g for 15 min at 4 ˚C. Protein concentration in the supernatant was determined by BCA assay (Beyotime, China). Proteins (50 μg per lane) were resolved by SDS-PAGE, transferred to PVDF membranes

(Millipore, USA), and blocked with 5% non-fat milk in TBST. Membranes were probed overnight at 4 ˚C with primary antibodies against SIRT1 (Santa Cruz, sc-74465, 1:1000), PPARγ (Santa Cruz, sc-7273, 1:500), LXRα (Solarbio, K003251P, 1:500), ABCA1 (Abcam, ab18180, 1:400), and GAPDH (Abcam, ab8245, 1:2000). After incubation with horseradish peroxidase-conjugated secondary antibodies (Beyotime, 1:5000), bands were detected using ECL reagents (Beyotime) on a ChemiDoc imaging system (Bio-Rad). Densitometric analysis was performed using ImageJ software (NIH, USA), and protein expression was normalized to GAPDH.

Statistical analysis

All data were expressed as the mean and standard deviation (SD). Group differences were analyzed using analysis of variance (ANOVA), followed by the least significant difference (LSD) *post hoc* test for pairwise comparisons when ANOVA indicated significance. All analyses were two-tailed and performed using SPSS (version 20.0; SPSS Inc.). A *P*-value < 0.05 was considered statistically significant.

Results

Liquiritin does not induce hepatotoxicity, nephrotoxicity, or growth impairment in mice

The chemical structure of liquiritin, with a molecular weight of 418.39 and a molecular formula of $C_{21}H_{22}O_9$, is shown in Figure 1A. To assess potential toxic effects, mice were administered saline or liquiritin (20 mg/kg/day) intraperitoneally for 12 weeks. Histopathological evaluation of liver and kidney tissues was performed to assess the potential toxic effects of liquiritin. Hematoxylin and eosin (H&E) staining revealed normal hepatic architecture in the liquiritin-treated group, characterized by well-organized hepatocyte cords and intact central veins, comparable to that of the control group (Figure 1B). No pathological alterations were observed in renal tissues, with preservation of glomerular and tubular structures and no signs of inflammation or degeneration. Serum biochemical analyses further supported the absence of hepatic and renal toxicity in the rats. No statistically significant differences were observed in serum alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels between the control and liquiritin-treated groups (P>0.05, Figure 1C). Similarly, renal function parameters, including serum creatinine and blood urea nitrogen (BUN) levels, remained unchanged after liquiritin administration compared with the control group (P>0.05; Figure 1D). Body weight was monitored throughout the experimental period to evaluate systemic effects. Compared to the control group, animals in the AS group showed a significant increase in body weight at weeks 4 (P<0.05), 8 (P<0.01), and 12 (P<0.01). In contrast, the body weights of the liquiritin-treated group did not differ significantly from those of the control group at any time point (Figure 1E-G). Collectively, these results indicate that liquiritin administration does not induce detectable hepatic or renal toxicity and is well tolerated *in vivo*, supporting its safety for subsequent pharmacological studies.

Liquiritin attenuates aortic root plaque formation, lipid deposition, and fibrosis in ApoE-/- mice

To evaluate the effect of Liquiritin on atherosclerosis, aortic roots from control C57BL/6 mice, ApoE-/- mice fed a Western diet (AS model), and ApoE-/- mice treated with Liquiritin (20 mg/kg/day, IP, 12 weeks) were analyzed by HE, Oil Red O, and Masson trichrome staining. Representative images (200× magnification) show extensive plaque formation, lipid accumulation, and fibrotic deposition in AS model mice compared with controls, whereas liquiritin treatment markedly reduced these pathological changes

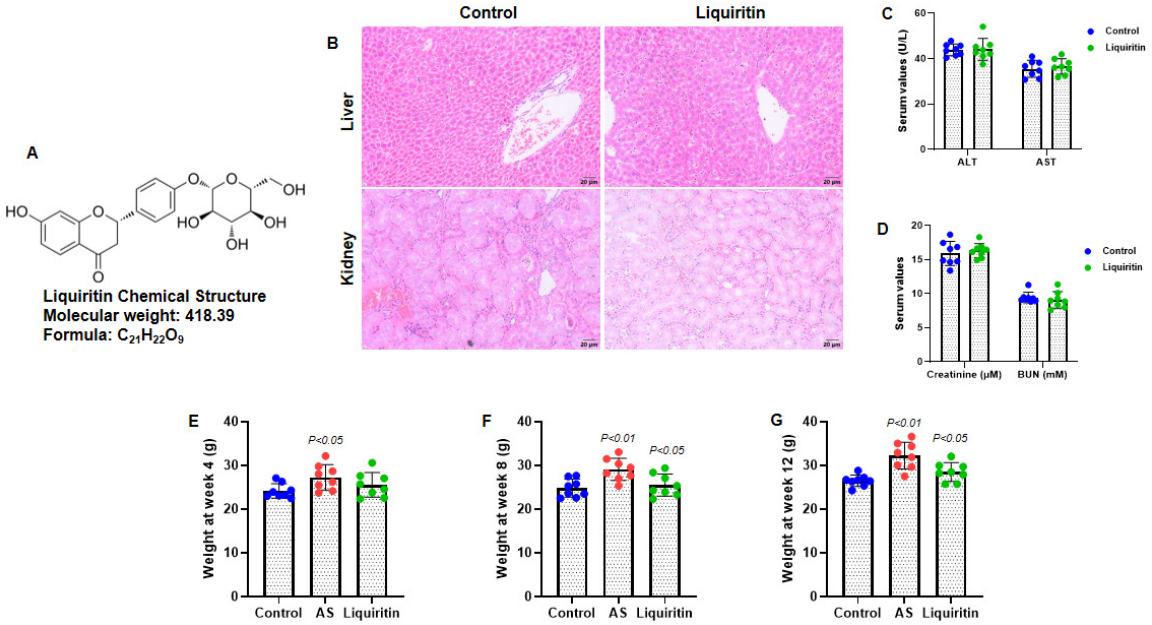

Figure 1. Liquiritin shows no toxicities to the liver and kidney in mice
(A) Chemical structure, molecular weight, and chemical formula of Liquiritin. (B) Mice were intraperitoneally injected with saline or Liquiritin (20 mg/kg/day) for a continuous 12 weeks, and the hepatic and renal tissues were stained with hematoxylin and eosin (HE)(magnification 200×). (C, D) Serum concentrations of ALT, AST, creatinine, and BUN were measured to evaluate the hepatic and renal function of mice. ApoE-/- mice were fed with a western diet and intraperitoneally injected with saline or Liquiritin (20 mg/kg/day) for a continuous 12 weeks; the control mice were fed with a maintenance diet and intraperitoneally injected with the same volume of saline. (E, F, G) Body weight of mice was measured at weeks 4, 8, and 12. Data are expressed as mean±SD (n=8 per group) and analyzed using ANOVA followed by the Bonferroni test
ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, BUN: Blood urea nitrogen (BUN), ANOVA: Analysis of variance (ANOVA)

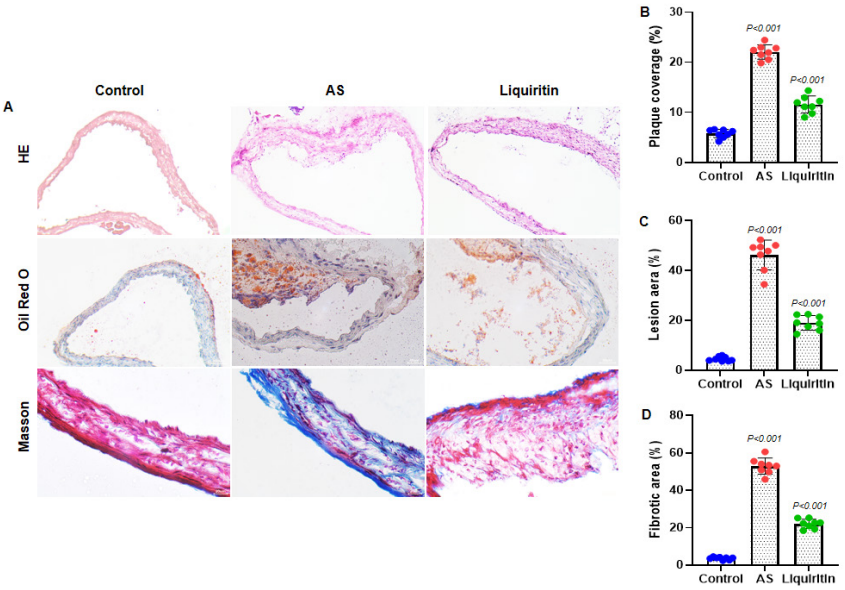

Figure 2. Effects of liquiritin on the aortic root plaque, atherosclerotic lesion, and fibrosis
The control group is C57BL/6 mice given a maintenance diet; the AS model group is ApoE-/- mice given a western diet; and the Liquiritin group is ApoE-/- mice given a western diet and intraperitoneally injected with Liquiritin (20 mg/kg/day) for 12 weeks. (A) Representative images of HE, Oil Red O, and Masson trichrome staining of aortic roots (200×). (B) Quantification of the percentage of plaque coverage by HE staining. (C) Quantification of the percentage of lesion area by Oil Red O staining. (B) Quantification of the percentage of the fibrotic area. Data are expressed as mean±SD (n=8 per group) and analyzed using ANOVA followed by the Bonferroni test
HE: Hematoxylin and eosin, ANOVA: Analysis of variance

(Figure 2A). Quantitative analysis revealed that liquiritin significantly decreased the percentage of plaque coverage (P<0.001, Figure 2B), lesion area (P<0.001, Figure 2C), and fibrotic area (P<0.001, Figure 2D) compared with the AS model group. These findings indicate that liquiritin effectively mitigates aortic root atherosclerosis, lipid deposition, and fibrosis in ApoE-/- mice.

Liquiritin attenuates dyslipidemia in HFD-induced ApoE-/- mice

In HFD-fed ApoE-/- mice, serum and hepatic lipid profiles were evaluated to assess the effects of liquiritin on lipid metabolism. HFD-fed ApoE-/- mice showed markedly higher levels of serum TG, TC, and LDL-C than control C57BL/6 mice (P<0.001, Figures 3A-C and Table 1). Administration of liquiritin significantly reduced serum TG, TC, and LDL-C levels (P<0.001), indicating improved systemic lipid metabolism. Similarly, hepatic lipid analysis revealed that HFD feeding led to accumulation of TG, TC, and LDL-C in the liver (P<0.001, Figures 3D-F and Table 1). In contrast, liquiritin treatment significantly decreased these lipid levels (P<0.001), suggesting a hepatoprotective effect against lipid deposition. Collectively, these results demonstrate that liquiritin effectively ameliorates hyperlipidemia and hepatic lipid accumulation in HFD-fed

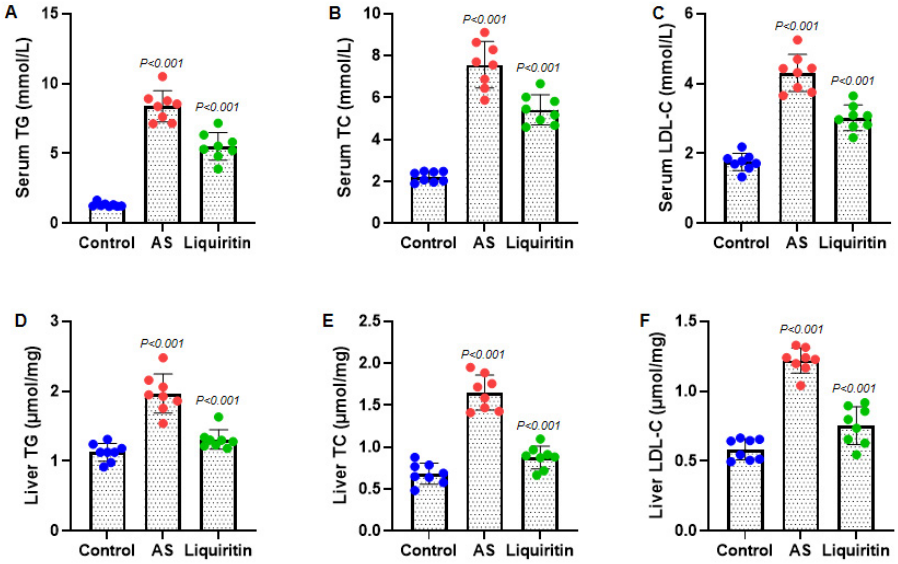

Figure 3. Liquiritin reduces the lipid levels in HFD-induced ApoE-/- mice
Concentrations of (A) TG, (B) TC, and (C) LDL-C were measured in the serum of mice. Concentrations of (D) TG, (E) TC, and (F) LDL-C were measured in the liver of mice. Data are expressed as mean±SD (n=8 per group) and analyzed using ANOVA followed by the Bonferroni test
TG: Triglycerides, TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol , ANOVA: Analysis of variance

Table 1. Effects of liquiritin on serum and hepatic lipid profiles in high-fat diet (HFD) induced ApoE-/- mice

Parameter	Control	AS	Liquiritin
Serum TG (mmol/l)	1.32±0.17	8.38±1.26***	5.51±1.04###
Serum TC (mmol/l)	2.22±0.21	7.56±1.12***	5.41±0.78###
Serum LDL-C (mmol/l)	1.75±0.21	4.30±0.52***	3.02±0.4###
Hepatic TG (μmol/mg)	1.12±0.15	1.97±0.22***	1.31±0.16###
Hepatic TC (μmol/mg)	0.68±0.14	1.65±0.22***	0.88±0.15###
Hepatic LDL-C (μmol/mg)	0.58±0.07	1.22±0.10***	0.76±0.15###

Data are expressed as mean±SD (n=8). ***P<0.001 vs. Control; ###P< 0.001 vs. AS group
TG; Triglyceride, TC; Total cholesterol, LDL-C; Low-density lipoprotein cholesterol

ApoE-/- mice.

Liquiritin alleviates hepatic steatosis in HFD-induced ApoE-/- mice

Histological analyses assessed the effects of liquiritin on liver morphology and fat accumulation. Hematoxylin and eosin (H&E) staining showed that livers from HFD-fed ApoE-/- mice exhibited pronounced hepatocellular swelling and vacuolation, indicative of steatosis, compared with those from control C57BL/6 mice (Figure 4A, top panel). Oil Red O staining further confirmed extensive lipid droplet accumulation in the livers of HFD-fed mice (Figure 4A, bottom panel). Treatment with liquiritin markedly ameliorated these histopathological changes, reducing hepatocellular vacuolation and lipid deposition. Quantitative analyses corroborated these findings: hepatic TG, TC, and LDL-C levels were elevated in HFD-fed mice, whereas liquiritin administration significantly decreased these lipid levels (P<0.001, Figures 4B-D). These findings indicate that liquiritin effectively mitigates HFD-induced hepatic steatosis and fat accumulation in ApoE-/- mice.

Liquiritin modulates SIRT1 and lipid metabolism-related proteins in the liver of HFD-induced ApoE-/- mice

The expression of SIRT1 and key lipid metabolism-related proteins was assessed in the livers of HFD-induced ApoE-/- mice to determine how liquiritin affects hepatic lipid metabolism. Western blot analysis showed that SIRT1 protein levels were markedly decreased in the AS model group compared with the control group, whereas liquiritin treatment markedly restored SIRT1 expression (P<0.001, Figure 5A-B). Similarly, PPARγ and LXRα expression, which were up-regulated in the AS model group, were significantly attenuated by liquiritin administration (P<0.001, Figure 5C-D). Moreover, ABCA1, a critical regulator of cholesterol efflux, was decreased in AS model mice and substantially increased following liquiritin treatment (P<0.001, Figure 5E). All protein levels were normalized to GAPDH, which served as an internal control. These findings indicate that modulation of hepatic SIRT1 and lipid metabolism-associated proteins by liquiritin may underlie its lipid-

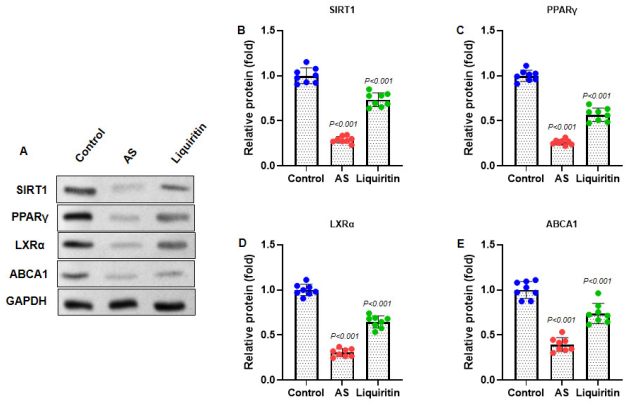

Figure 5. Liquiritin modulates SIRT1 and lipid metabolism-related proteins in the liver of HFD-induced ApoE-/- mice
(A) Representative gel blots of SIRT1 and lipid metabolism-related protein expression by Western blot. These gel blots were quantified for (B) SIRT1, (C) PPARγ, (D) LXRα, and (E) ABCA1. All proteins were normalized to GAPDH as an internal control. Data are expressed as mean±SD (n=8 in each group), and analyzed using ANOVA followed by the Bonferroni test
SIRT1: Sirtuin 1, HFD: High-fat diet, PPARγ: Peroxisome Proliferator-Activated Receptor Gamma, LXRα: Liver X Receptor alpha, ABCA1: ATP-binding cassette transporter A1, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, ANOVA: Analysis of variance

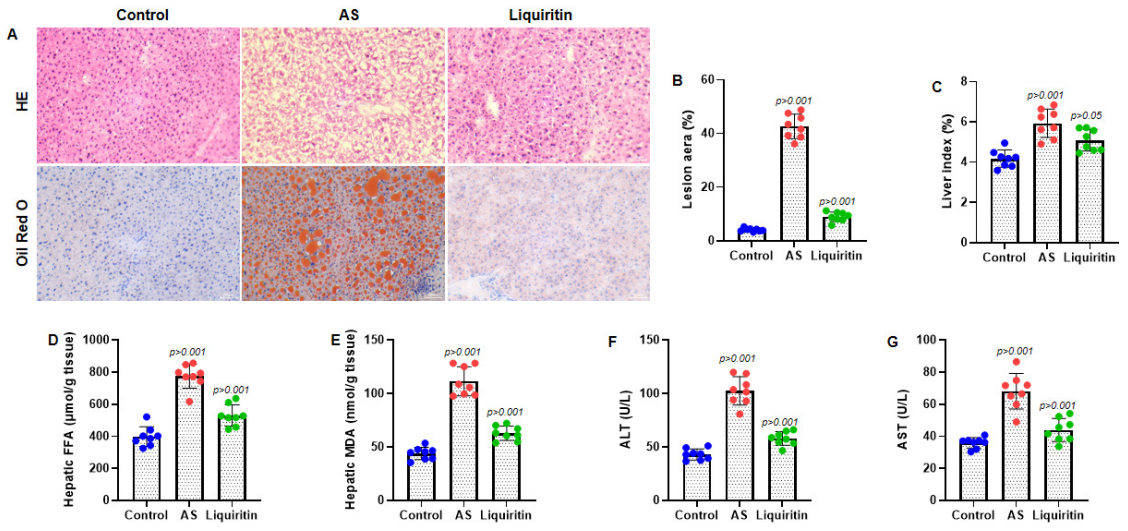

Figure 4. Liquiritin alleviates hepatic steatosis in HFD-induced ApoE-/- mice
(A) Representative image of the liver section stained with HE and Oil Red O (200×). (B) Quantification of the percentage of positive area of liver stained with Oil Red O. (C) The liver index was calculated by the formula: wet weight of liver (g)/body weight (g) ×100%. (D) The content of hepatic free fatty acids (FFA). (E) The content of hepatic MDA. (F, G) The activities of serum ALT and AST levels. Data are expressed as mean±SD (n=8 per group) and analyzed using ANOVA followed by the Bonferroni test
HFD: High-fat diet (HFD), MDA: Malondialdehyde (MDA), ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ANOVA: Analysis of variance

lowering effects in atherosclerotic mice.

Discussion

In this study, we demonstrated that liquiritin exerts potent anti-atherosclerotic and lipid-lowering effects in HFD-induced ApoE-/- mice without causing hepatotoxicity, nephrotoxicity, or growth impairment. Chronic administration of liquiritin (20 mg/kg/day, IP, for 12 weeks) did not alter the histological architecture of the liver and kidney tissues or affect serum levels of ALT, AST, creatinine, or BUN, indicating a favorable safety profile for prolonged treatment. These findings are consistent with previous reports highlighting the low *in vivo* toxicity of liquiritin (15-17). Importantly, no substantial changes in body weight were observed, suggesting that liquiritin does not adversely affect growth or overall metabolism under the experimental conditions used in this study.

A key pathological feature of atherosclerosis is the formation of atherosclerotic plaques, accompanied by lipid deposition and vascular wall fibrosis. Our histological analyses revealed that liquiritin significantly reduced plaque formation, lipid accumulation, and fibrotic deposition in the aortic roots of ApoE-/- mice fed a Western diet. Quantitative reductions in plaque coverage, lesion area, and fibrotic area support the efficacy of liquiritin in mitigating structural and vascular damage. These results align with prior studies reporting the cardiovascular-protective effects of flavonoids through anti-inflammatory and anti-fibrotic mechanisms (18, 19).

Dyslipidemia is a central contributor to atherosclerosis and hepatic steatosis. Consistent with this, HFD-fed ApoE-/- mice exhibited elevated serum and hepatic levels of TG, TC, and LDL-C. Liquiritin treatment markedly improved both systemic and hepatic lipid profiles, indicating its potent lipid-lowering effects. Histopathological analysis of the liver further confirmed the protective effect of liquiritin against HFD-induced hepatic steatosis, as evidenced by reduced hepatocellular vacuolation and lipid accumulation. These findings suggest that liquiritin targets vascular pathology and alleviates hepatic lipid deposition, highlighting its dual role in cardiovascular and metabolic protection.

Mechanistically, liquiritin modulates key regulators of hepatic lipid metabolism. SIRT1, an NAD+-dependent deacetylase that regulates lipid and glucose homeostasis, was down-regulated in HFD-induced ApoE-/- mice but was restored with liquiritin treatment. Concurrently, liquiritin reduced expression of PPARγ and LXRα, transcription factors that promote lipogenesis and cholesterol accumulation, and up-regulated ABCA1, a critical mediator of cholesterol efflux. These findings suggest that liquiritin enhances lipid homeostasis by coordinating the regulation of SIRT1 and lipid metabolism-related proteins, thereby reducing hepatic lipid accumulation and systemic dyslipidemia. These results align with earlier research showing that SIRT1 activation suppresses PPARγ- and LXRα-mediated lipogenesis while promoting reverse cholesterol transport via ABCA1 (20, 21).

Study limitations and future directions

Despite these encouraging findings, the present study has several shortcomings. First, while we demonstrated the protective effects of liquiritin on atherosclerosis, dyslipidemia, and hepatic steatosis in HFD-induced ApoE-/- mice, the study was limited to a single dose and route of administration (20 mg/kg/day, intraperitoneally). Dose-response relationships and alternative administration routes, such as oral delivery, remain to be explored to better reflect potential clinical applications. Second, although our data indicate that liquiritin modulates hepatic SIRT1 and key lipid metabolism-related proteins, the precise molecular mechanisms underlying these effects, including potential involvement of upstream signaling pathways and epigenetic regulation, remain incompletely elucidated. Third, this study focused primarily on the liver and aortic root tissues; the systemic effects of liquiritin on other metabolic organs, such as adipose tissue, skeletal muscle, or gut microbiota, were not assessed.

These drawbacks should be addressed in future studies by evaluating multiple doses and routes of liquiritin administration, investigating its long-term safety and efficacy, and delineating the detailed molecular mechanisms underlying SIRT1-mediated lipid regulation. Additionally, expanding the investigation to other relevant tissues and assessing the impact of liquiritin in combination with existing lipid-lowering or anti-atherosclerotic therapies could provide further insights into its translational possibilities. Finally, clinical studies are essential to confirm the effectiveness and safety of liquiritin in human populations at risk of atherosclerosis and metabolic disorders.

Conclusion

Collectively, our study provides compelling evidence that liquiritin is a safe and effective agent for mitigating atherosclerosis and associated lipid abnormalities in HFD-fed ApoE-/- mice (Figure 6). The observed benefits are likely mediated by modulation of hepatic SIRT1 and downstream lipid metabolism pathways, which could offer a viable treatment option for the management and prevention of atherosclerosis and associated metabolic diseases in humans.

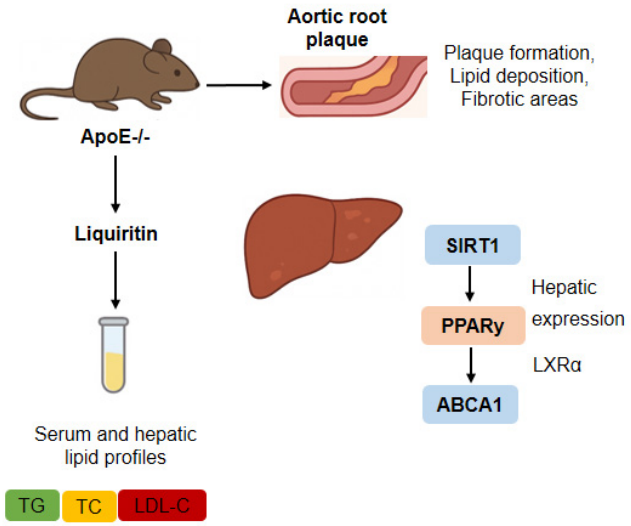

Figure 6. Schematic illustration of how liquiritin attenuates aortic plaque formation and improves lipid metabolism in ApoE-/- mice via the sirtuin 1 (SIRT1) pathway

Acknowledgment

Not applicable.

Availability of Data and Materials

The data from this study is available upon a reasonable request from Dr Zhongsheng Zhu, at drzzs66@163.com.

Ethical Approval

This study protocol was reviewed and approved by the Ethics Committee of Guangming Traditional Chinese Medicine Hospital of Pudong New Area (approval number 2025-KY-002). The authors adhered to all standard protocols in accordance with the 1964 Declaration of Helsinki. All methods used in this study were in accordance with the ARRIVE guidelines.

Authors' Contributions

K Y handled conceptualization, methodology, investigation, formal analysis, and data curation. J S and P G contributed to conceptualization, methodology, investigation, and formal analysis. Z Z was responsible for conceptualization, methodology, and supervision. All authors participated in writing this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

References

1. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, *et al.* Atherosclerosis. Nat Rev Dis Primers 2019; 5: 56.
2. Tabas I, García-Cardeña G, Owens GK. Recent insights into the cellular biology of atherosclerosis. J Cell Biol 2015; 209: 13-22.
3. Ference BA, Ginsberg HN, Graham I, Ray KK, Packard CJ, Bruckert E, *et al.* Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J 2017; 38: 2459-2472.
4. Meir KS, Leitersdorf E. Atherosclerosis in the apolipoprotein-E-deficient mouse: A decade of progress. Arterioscler Thromb Vasc Biol 2004; 24: 1006-1014.
5. Kitada M, Koya D. SIRT1 in type 2 diabetes: Mechanisms and therapeutic potential. Diabetes Metab J 2013; 37: 315-325.
6. Yu W, Fu YC, Chen CJ, Wang X, Wang W. SIRT1: A novel target to prevent atherosclerosis. J Cell Biochem 2009; 108: 10-13.
7. Winnik S, Auwerx J, Sinclair DA, Matter CM. Protective effects of sirtuins in cardiovascular diseases: From bench to bedside. Eur Heart J 2015; 36: 3404-3412.
8. Huang X, Wang Y, Ren K. Protective effects of liquiritin on the brain of rats with Alzheimer's disease. West Indian Med J 2015; 64: 468-472.
9. Yang R, Yuan BC, Ma YS, Zhou S, Liu Y. The anti-inflammatory activity of licorice, a widely used Chinese herb. Pharm Biol 2017; 55: 5-18.
10. Gargiulo S, Barone V, Bonente D, Tamborrino T, Inzalaco G, Gherardini L, *et al.* Integrated ultrasound characterization of the diet-induced obesity (DIO) model in young adult c57bl/6j mice: Assessment of cardiovascular, renal and hepatic changes. J Imaging 2024; 10: 217.
11. Bayol SA, Simbi BH, Fowkes RC, Stickland NC. A maternal "junk food" diet in pregnancy and lactation promotes nonalcoholic Fatty liver disease in rat offspring. Endocrinology 2010; 151: 1451-1461.
12. Liu J, Han L, Zhu L, Yu Y. Free fatty acids, not triglycerides, are associated with non-alcoholic liver injury progression in high fat diet induced obese rats. Lipids Health Dis 2016; 15: 27.
13. Arruda VM, Azevedo GT, Granato MJMG, Matos ACP, Araújo TG, Guerra JFDC. Oxidative stress and annexin A2 differential expression in free fatty acids-induced non-alcoholic fatty liver disease in HepG2 cells. Int J Mol Sci 2024; 25: 9591.
14. Kurien BT, Scofield RH. Western blotting: An introduction. Methods Mol Biol 2015; 1312: 17-30.
15. Wang Q, Xian M, Li C, Mi L, Ji J, Wu Z, Shi X, Han X. Protective effect of liquiritin against cisplatin-induced liver injury in mice through reduction of inflammation, oxidative stress, apoptosis and inhibition of p38 MAPK/p53 pathway based on network pharmacology and in experimental validation. J Ethnopharmacol 2025; 354:120536.
16. Jia SY, Yin WQ, Xu WM, Li J, Yan W, Lin JY. Liquiritin ameliorates painful diabetic neuropathy in SD rats by inhibiting NLRP3-MMP-9-mediated reversal of aquaporin-4 polarity in the glymphatic system. Front Pharmacol 2024; 15: 1436146.
17. Zhao Z, Wang W, Guo H, Zhou D. Antidepressant-like effect of liquiritin from Glycyrrhiza uralensis in chronic variable stress induced depression model rats. Behav Brain Res 2008; 194: 108-113.
18. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY. Antiartherosclerotic effects of plant flavonoids. Biomed Res Int 2014; 2014: 480258.
19. Chen Z, Zhang SL. The role of flavonoids in the prevention and management of cardiovascular complications: A narrative review. Ann Palliat Med 2021; 10: 8254-8263.
20. Hou X, Xu S, Maitland-Toolan KA, Sato K, Jiang B, Ido Y, *et al.* SIRT1 regulates hepatocyte lipid metabolism through activating AMP-activated protein kinase. J Biol Chem 2008; 283: 20015-20026.
21. Li Y, Wong K, Giles A, Jiang J, Lee JW, Adams AC, *et al.* Hepatic SIRT1 attenuates hepatic steatosis and controls energy balance in mice by inducing fibroblast growth factor 21. Gastroenterology 2014; 146: 539-549.