



Astragaloside IV Relieves Atherosclerosis and Hepatic Steatosis via MAPK/NF- κ B Signaling Pathway in LDLR^{-/-} Mice

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Astragaloside IV (AS-IV) is the main active compound of *Astragalus membranaceus*. In this study, we investigated whether AS-IV could attenuate atherosclerosis and hepatic steatosis in LDLR^{-/-} mice and its potential mechanisms. After 12 weeks of high fat diet, the LDLR^{-/-} mice were randomly divided into four groups. Then, the mice were administrated with 0.9% saline or AS-IV (10 mg/kg) or atorvastatin (1.3 mg/kg) for 12 weeks. Serum lipid profiles and inflammatory cytokines were detected by ELISA, hepatic TC and TG by colorimetric enzymatic kits, gene expression by RT-qPCR, plaque sizes by H&E staining, Oil Red O, liver pathology by H&E staining, collagen content by Masson, α -SMA, caspase-3 and NF- κ B p65 production by immunofluorescence staining. MAPK/NF- κ B pathway and inflammation related proteins were detected by Western Blot. The results showed that AS-IV decreased the levels of serum lipids, reduced plaque area and increased plaque stability in HFD-induced LDLR^{-/-} mice. AS-IV also decreased the levels of inflammatory cytokines in the serum, aortas and liver tissue, and NF- κ B p65 in aortic roots. The phosphorylation of JNK, ERK1/2, p38 and NF- κ B, and inflammatory proteins (iNOS, VCAM-1 and IL-6) was inhibited in AS-IV-treated group. In summary, AS-IV inhibited inflammation to attenuate atherosclerosis and hepatic steatosis via MAPK/NF- κ B signaling pathway in LDLR^{-/-} mice.

Keywords: Astragaloside IV, atherosclerosis, hepatic steatosis, inflammation, MAPK/NF- κ B signaling pathway

INTRODUCTION

Atherosclerosis (AS) is the pathological basis of a variety of cardiovascular diseases (CVDs) (Roth et al., 2017). Risk factors include hypertension, diabetes, smoking and adiposity (Herrington et al., 2016). As a lipid-driven inflammatory disease within the arterial wall, AS is always treated with lipid-lowering and anti-inflammatory therapies (Taleb, 2016). In recent decades, statins are used to treat AS, but with lots of adverse effects such as myopathy, rhabdomyolysis, and acute renal failure (Šimić and Reiner, 2015). It is necessary to find more effective methods to prevent and treat AS.

Dyslipidemia, inflammation, and oxidative stress closely link AS with nonalcoholic fatty liver disease (NAFLD). NAFLD has three subtypes: nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH), and related liver cirrhosis. The prevalence of NAFLD has been estimated to be 20–30% in the general population, and associated with several cardiovascular risk factors (Targher et al., 2008). A study has found that the prevalence of CVD is higher in patients with NAFLD (Targher et al., 2010a). NAFLD patients were found to have higher levels of subclinical

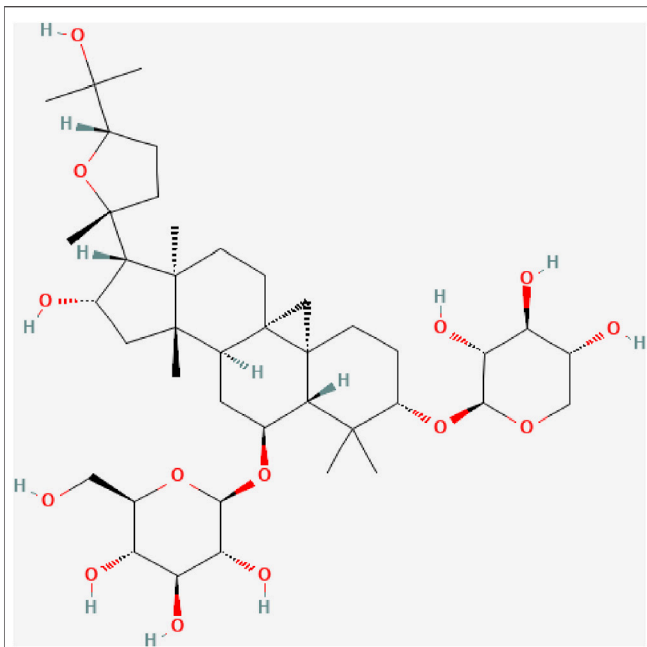


FIGURE 1 | The chemical structure of Astragaloside IV.

AS indexes, such as carotidintima-media thickness, and brachial artery flow-mediated dilatation (Villanova et al., 2005; Targher et al., 2006). However, whether NAFLD is the cause or consequence of CVD remains controversial. More researches are needed to explain the role of NAFLD in atherosclerotic cardiovascular events.

Astragaloside IV (AS-IV, $C_{41}H_{68}O_{14}$, **Figure 1**), a cycloartane-type triterpene glycoside chemical, is a major compound in the aqueous extract from *Astragalus membranaceus* (Zhang et al., 2020). Many studies have demonstrated that AS-IV can protect the cardiovascular system, lungs, kidneys and brain, an effect attributed to its anti-inflammatory, antioxidant, anti-apoptotic and immunoregulation properties (Yan et al., 2017; Meiqian et al., 2018; Zhang et al., 2019; Tan et al., 2020). It has been shown that AS-IV could counter inflammation through multiple signaling pathways, such as Toll like receptor four/nuclear factor- κ B (TLR4/NF- κ B) signaling pathway, mitogen-activated protein kinases (MAPK) signaling pathway, and Janus kinase two/signal transducer and activator of transcription 6 (JAK2/STAT6) signaling pathway (Leng et al., 2019; Yang and Wang, 2019; Hsieh et al., 2020).

In this study, we investigated whether AS-IV can attenuate HFD-induced AS and hepatic steatosis by repressing MAPK/NF- κ B pathway in the aortas and liver tissue of LDLR^{-/-} mice.

METHODS

Materials and Reagents

Astragaloside IV was extracted from PP (purity: 99.63%, available at: <http://www.cdmust.com>) by Mansite Biological Company (Chengdu, China). The high-fat diet (HFD, 78.85% normal

diet+21% lard +0.15% cholesterol) was bought from SYSE Biotechnology Co., Ltd. (Changzhou, China); atorvastatin calcium tablets from Pharmacy of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine; enzyme immunosorbent assay (ELISA) kits for mouse IL-1 β , IL-6 and TNF- α from Shanghai XiTang Biological Technology Co., Ltd. (Shanghai, China); oil red O staining kit, Masson staining kit, total cholesterol assay kit and triglyceride assay kit from Jiancheng Bioengineering Institute of Nanjing (Nanjing, China); primary antibodies against ERK, p-ERK, P38 MAPK, p-P38 MAPK, JNK, p-JNK, NF- κ B, p-NF- κ B, IL-6, iNOS, VCAM-1, α -SMA, caspase 3 and GAPDH from Cell Signaling Technology Inc. (Beverly, Massachusetts, United states). ECL Star kit and Hematoxylin, Eosin (H&E) Staining Kit, 4', 6-diamindino-2-phenylindole (DAPI), Triton X-100, radio immunoprecipitation assay (RIPA), and BCA protein assay kit were from Beyotime Biotechnology (Shanghai, China); primers for IL-1 β , IL-6 and TNF- α from Sangon Biotech Co., Ltd. (Shanghai, China); primeScript RT Reagent Kit for RT-PCR amplification and TB Green Premix EX Taq from TAKARA Biomedical Technology Co., Ltd. (Beijing, China); RNA Purification Kit from EZBioscience (Shanghai, China).

Animals and Treatment

Six-week-old LDLR^{-/-} male mice (18–22 g) were obtained from the GemPharmatech Co., Ltd. (Nanjing, Jiangsu, <http://www.gempharmatech.com>) (SCXK 2018-0008). All mice were housed in the Animal Center of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. After 1 week of environmental adaptation, all mice were randomly assigned to four groups: control group (CON), model group (MOD), Astragaloside IV group (AS-IV, 10 mg/kg), and Atorvastatin group (ATO, 1.3 mg/kg). The mice in CON group were fed with normal diet for 24 weeks, and those in other groups with HFD. The dose of AS-IV is based on the references (Min Li et al., 2017; Liu et al., 2018; Yang et al., 2020). The dose of ATO is calculated according to the human and mouse dose conversion, and refers to the reference (Xu et al., 2017). After the mice were fed for 12 weeks, the LDLR^{-/-} mice in AS-IV group were intraperitoneally injected with AS-IV, and ATO group received oral atorvastatin for 12 weeks. CON and MOD groups were injected with the same volume of 0.9% saline. After a 12-h fasting, all the mice were sacrificed via carbon dioxide asphyxiation. The animal study was approved by the Ethics Committee of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (No. 2019-N002, shown in **Supplementary Material**).

Serum Biochemical Analysis and Determination of Hepatic Lipids

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), and triglyceride (TG) levels in the blood serum were analyzed in the Clinical Laboratory Department of Longhua Hospital Affiliated to Shanghai University of Traditional Chinese Medicine. The hepatic levels of TC and TG were measured using colorimetric enzymatic kits.

TABLE 1 | List of primers for real-time qPCR analysis.

Gene		Oligonucleotide sequence
β-Actin	Forward	5'- GAGACCTTCAACACCCAGC - 3'
	Reverse	5'- ATGTCACGCACGATTTCCC - 3'
IL-1β	Forward	5'- GCTTCAGGCAGGCAGTATCA - 3'
	Reverse	5'- TGCAGTTGCTAATGGGAACG - 3'
IL-6	Forward	5'-AAAGCAGCAAAGAGGCACTG- 3'
	Reverse	5'-TACCTCAAACCTCCAAAGACCAG- 3'
TNF-α	Forward	5'- CCCTCCAGAAAAGACACCATG- 3'
	Reverse	5'- CACCCCGAAGTTCAGTAGACAG- 3'

Determination of Cytokine

The concentrations of IL-1β, IL-6 and TNF-α in the serum were detected by ELISA kits according to the manufacturer's instructions. The final concentrations were calculated in accordance with corresponding standard curves.

Histopathological Analysis

The aortic roots and liver tissue were fixed in 4% paraformaldehyde for 48 h. The aortic roots were embedded in the optimal cutting temperature (OCT) compound and cut into circular sections (10 μm) with a microtome. The sections of aortic root were stained with H&E, Oil Red O and Masson solution. The lipid-rich plaque areas were observed under the microscope and analyzed by ImageJ software. Liver tissues that had been fixed in 4% paraformaldehyde were dehydrated in ethanol and finally embedded in paraffin. Paraffin sections were then sliced 10 μm thick and stained with H&E.

Western Blotting

The thoracic aorta or liver tissue were lysed in RIPA lysis solution for 30 min. The supernatant was collected after a 10-min centrifugation. The total protein was quantified using BCA kit, separated on 10% SDS-PAGE and transferred onto the PVDF membranes. The PVDF membranes were immersed in 5% BSA in Tris-buffered saline with Tween-20 (TBST) for 1 h. The blocked PVDF membranes were incubated with primary antibodies (ERK, p-ERK, P38 MAPK, p-P38 MAPK, JNK, p-JNK, NF-κB, p-NF-κB, IL-6, iNOS, VCAM-1 and GAPDH) diluted with 5% BSA in TBST at 4°C overnight. Having been washed with TBST, the membranes were immersed in secondary antibodies for 1 h. The contents of proteins were imaged using ECL reagent and photographed using the ChemiScope 6,000. Finally, the density of protein bands was analyzed with ImageJ software.

Real-Time Quantitative PCR (RT-qPCR) Assay

Total RNA was extracted for the mice aorta tissue and liver tissue using RNA Purification Kit, and reverse-transcribed into cDNA using PrimeScript RT Reagent Kit following the standard protocol. The Real-Time Quantitative PCR (RT-qPCR) assay was conducted using TB Green PremixEX Taq with the Applied Biosystems 7,500 Real-Time PCR System. The amplification parameters were set at 95°C for 1 min, followed by 40 cycles of 95°C for 5 s and 58°C for 15 s, 72°C for 30 s, and 95°C for 15 s. The relative expression of mRNA was normalized to that of β-actin. All primer sequences used are listed in **Table 1**.

Immunofluorescence Study

Frozen slices of aortic sinus were used for immunofluorescence study. Having been fixed with 4% paraformaldehyde for 15 min and permeabilized by 0.1% Triton X-100 for 10 min, the cells were blocked with PBS containing 5% BSA for 1 h. Then, the frozen slices were incubated with anti-α-SMA or caspase 3 or anti-NF-κB p65 at 4°C overnight. On the following day, the cells were washed with PBS and incubated with goat anti-rabbit IgG H&L for 1 h. Finally, the cells were treated with DAPI. All images were captured with a fluorescence microscope.

Statistical Analysis

All experimental results were shown as the mean ± standard deviation (SD). The statistical analyses were accomplished with GraphPad Prism 7.0. software and SPSS 21.0. Significant differences between groups were defined by the one-way ANOVA. $p < 0.05$ was considered statistically significant.

RESULTS

Effect of Astragaloside IV on Serum Lipid Profiles and Atherosclerotic Lesions in the Aortic Sinus of Mice

To explore the effect of AS-IV on blood lipids in AS mice, the serum levels of TC and TG were detected. As shown in **Figures 2A,B**, TC and TG serum levels were lower in CON group. The TC and TG serum levels were significantly increased in MOD group ($p < 0.01$); while after 12 weeks' intervention with AS-IV or ATO, the indexes were lower than those in MOD group ($p < 0.01$). The above results showed that AS-IV could correct lipid metabolism in AS mice.

Lipid plaques in the aorta were observed by H&E and Oil Red O staining. As shown in **Figures 2C–F**, the lipid accumulation in the aortic roots was more significant in MOD group, but not detected in CON group ($p < 0.01$). In contrast, the atherosclerotic lesion reduced in AS-IV-treated and ATO-treated groups ($p < 0.01$), compared to MOD group. The above results suggested that AS-IV could reduce AS plaques in HFD-induced-LDLR^{-/-} mice.

The characteristics of a vulnerable plaque include a high necrotic core size and a low fibrous cap area. Then the collagen content and the expression levels of α-SMA and caspase 3 were detected by immunofluorescence to explore the effect of AS-IV on the stability of atherosclerotic plaques. **Figures 2G–L** showed that the atherosclerotic lesions of the MOD group had a lower collagen content, a lower expression level of α-SMA and a higher expression level of caspase 3 than CON group ($p < 0.01$). AS-IV or ATO increased collagen content and α-SMA expression, and decreased caspase 3 expression ($p < 0.05$). The results suggested that the stability of atherosclerotic plaque could be enhanced by AS-IV, reducing the risk of plaque rupture.

Effect of Astragaloside IV on Transaminase, Liver Lipogenesis and Hepatic Steatosis in Mice

Long-term HFD leads to lipid deposition in the liver, leading to liver inflammation. To investigate the effect of AS-IV, we also

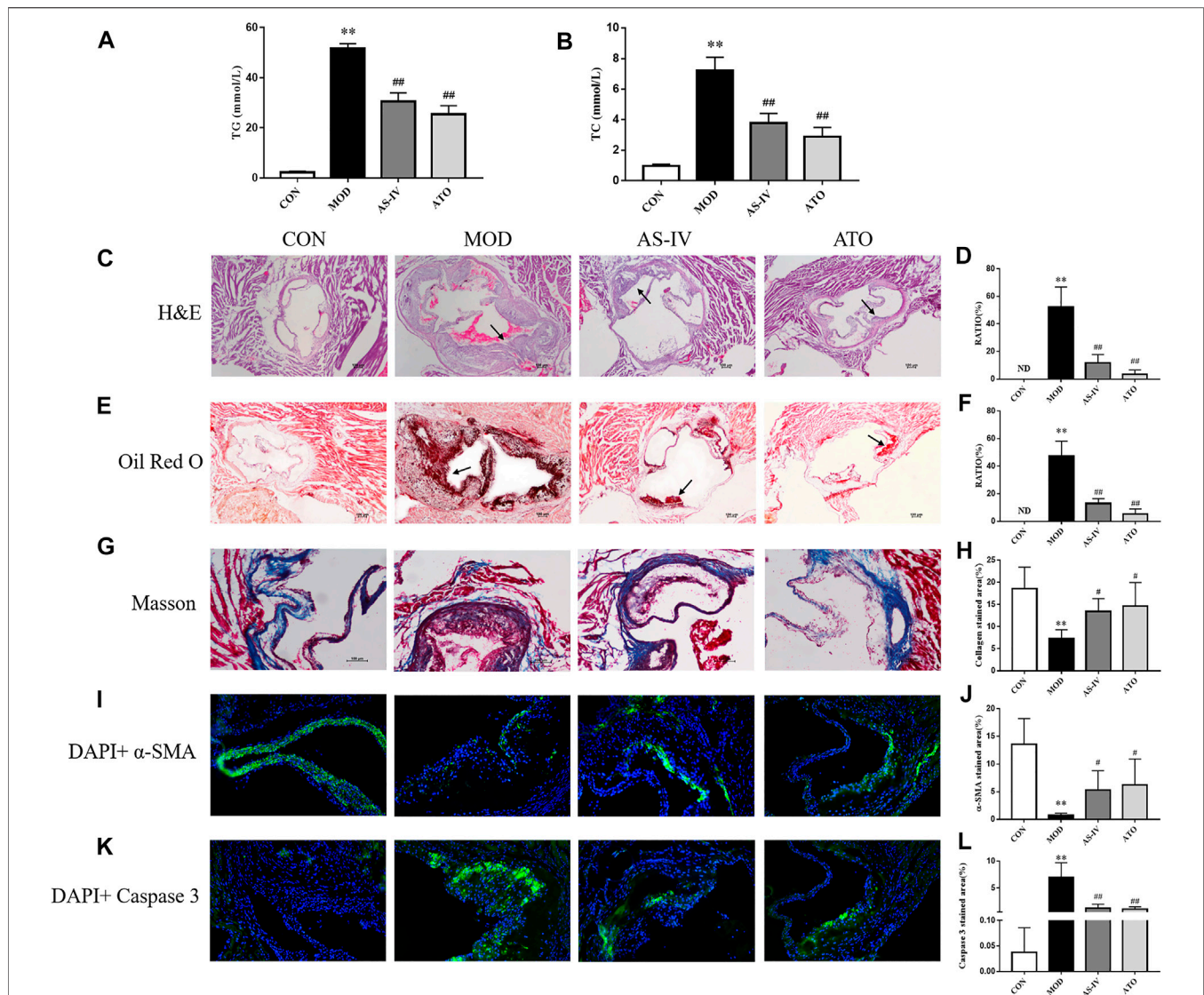


FIGURE 2 | Serum lipid profiles and atherosclerosis of aortic sinus in the $LDLR^{-/-}$ mice. (A–B) TC and TG levels of serum were detected, $n = 8$. H&E (C) and Oil Red O staining (E) were used to detect the progress of lipid-rich plaques, magnification $\times 40$, $n = 6$. (B,D) The areas of lesion were calculated respectively by ImageJ analysis software. Masson staining (G) was used to detect the collagen content followed by the quantification (H), magnification $\times 100$, $n = 6$. (I–L) Representative photomicrographs of aortic root sections stained with α -SMA (I), and caspase 3 (K) in atherosclerotic plaque followed by the quantification (J,L), magnification $\times 200$, $n = 6$. CON means control group, MOD means model group, AS-IV means Astragaloside IV group, ATO means Atorvastatin group. TC, total cholesterol; TG, triglyceride; ND, not detected; RATIO = plaque area/aortic sinus lumen area $\times 100\%$. Data are expressed as mean \pm SD. ** $p < 0.01$ versus CON group; # $p < 0.05$, ## $p < 0.01$ versus MOD group.

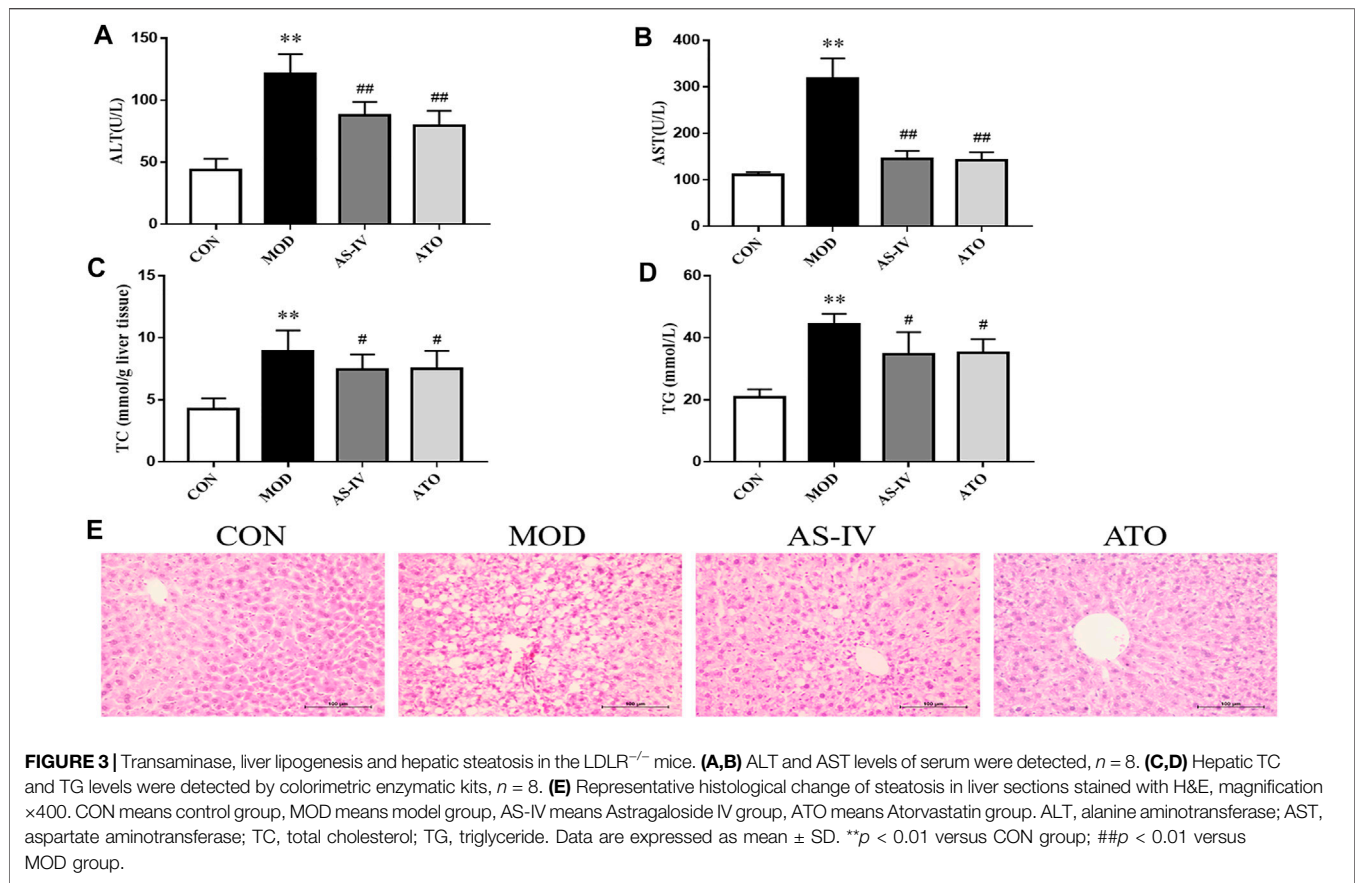
tested serum ATL and AST levels in each group. As shown in Figures 3A,B, the serum AST and ALT levels in the MOD group were significantly higher than those in the CON group ($p < 0.01$). AS-IV and ATO treatments showed a significantly decreased level of serum AST and ALT ($p < 0.01$). This suggested that AS-IV significantly reduced the release of transaminase.

To investigate the effect of AS-IV on HFD-induced hepatic steatosis, we observed H&E staining of liver tissue, and examined the hepatic triglyceride and cholesterol accumulation (Figures 3C–E). After 12 weeks of HFD, the MOD group mice showed significantly liver fat deposition. 12-week administration of AS-IV and ATO reduced the liver lipid deposition through

microscope observation. Hepatic TC and TG levels in the MOD group were significantly higher than those in the CON group ($p < 0.01$). AS-IV and ATO significantly reduced the hepatic TC and TG content ($p < 0.01$), indicating that hepatic steatosis was attenuated by AS-IV.

Effect of Astragaloside IV on Inflammation Cytokines in Mice

In order to explore the effect of AS-IV on the inflammation, immunofluorescence staining of NF- κ B p65 was performed and the expression levels of inflammation cytokines were detected. As



shown in **Figures 4A, B**, NF- κ B p65 content was significantly increased in the atherosclerotic lesions of the MOD group ($p < 0.01$). AS-IV and ATO inhibited the increase of NF- κ B p65 ($p < 0.05$).

Then, the serum levels of inflammatory cytokines IL-1 β , IL-6, and TNF- α were detected by ELISA kits (**Figures 4C–E**). The levels of IL-1 β , IL-6 and TNF- α in the MOD group increased compared with those in the CON group ($p < 0.01$). While 12 weeks' intervention with AS-IV or ATO significantly decreased the levels of IL-1 β , IL-6 and TNF- α expression ($p < 0.01$). The mRNA levels of those inflammatory cytokines in mice aortas and liver tissue were detected by RT-qPCR (**Figures 4F–K**). Similarly, AS-IV or ATO decreased the mRNA levels of IL-1 β , IL-6 and TNF- α expression in mouse aortas and liver tissue ($p < 0.01$). The expression levels of inflammatory proteins iNOS, VCAM-1, and IL-6 were detected by Western blotting (**Figures 4L,M**). AS-IV also down-regulated the expression of iNOS, VCAM-1, and IL-6 ($p < 0.01$). The above results suggested that AS-IV could reduce the inflammatory response through inhibiting the generation of inflammatory factors.

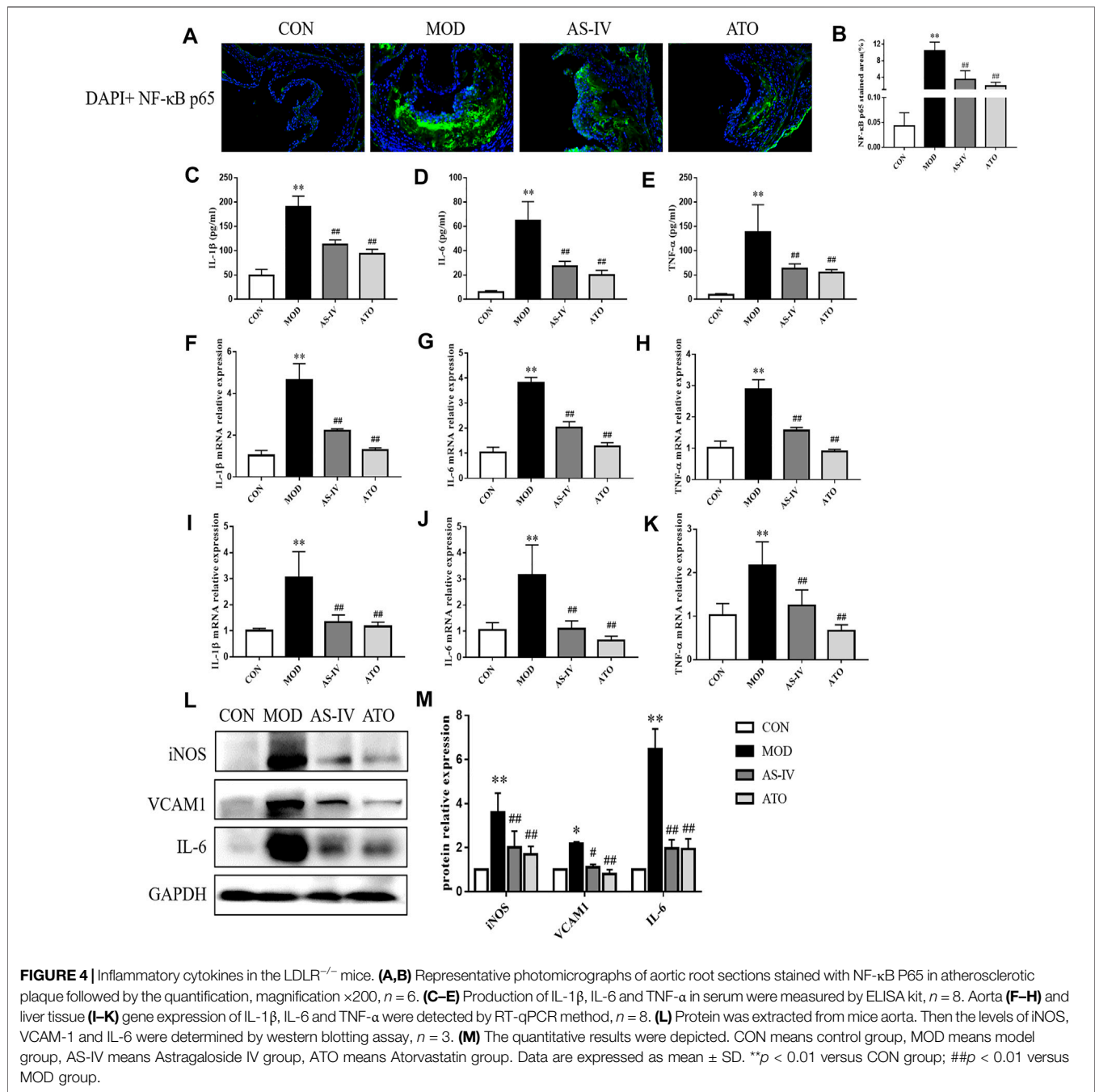
Effect of Astragaloside IV on MAPK Signal Pathway in Mice

Long-term fat deposition could lead to inflammation. Inflammation plays an important role in the development of

AS and NAFLD. MAPK and NF- κ B are critical factors in inflammatory responses. To determine whether the beneficial effect of AS-IV on the aortic roots and liver was associated with MAPK/NF- κ B signaling pathway, we examined the phosphorylation of JNK, ERK1/2, p38 and NF- κ B p65 in the aortas and liver tissue (**Figures 5A–D**). Compared to the CON group, the Western blotting showed that the protein expression of MAPK/NF- κ B in the MOD group was markedly upregulated and restored after AS-IV administration ($p < 0.01$). These results suggested AS-IV could mitigate the activation of MAPK/NF- κ B in atherosclerotic mice.

DISCUSSION

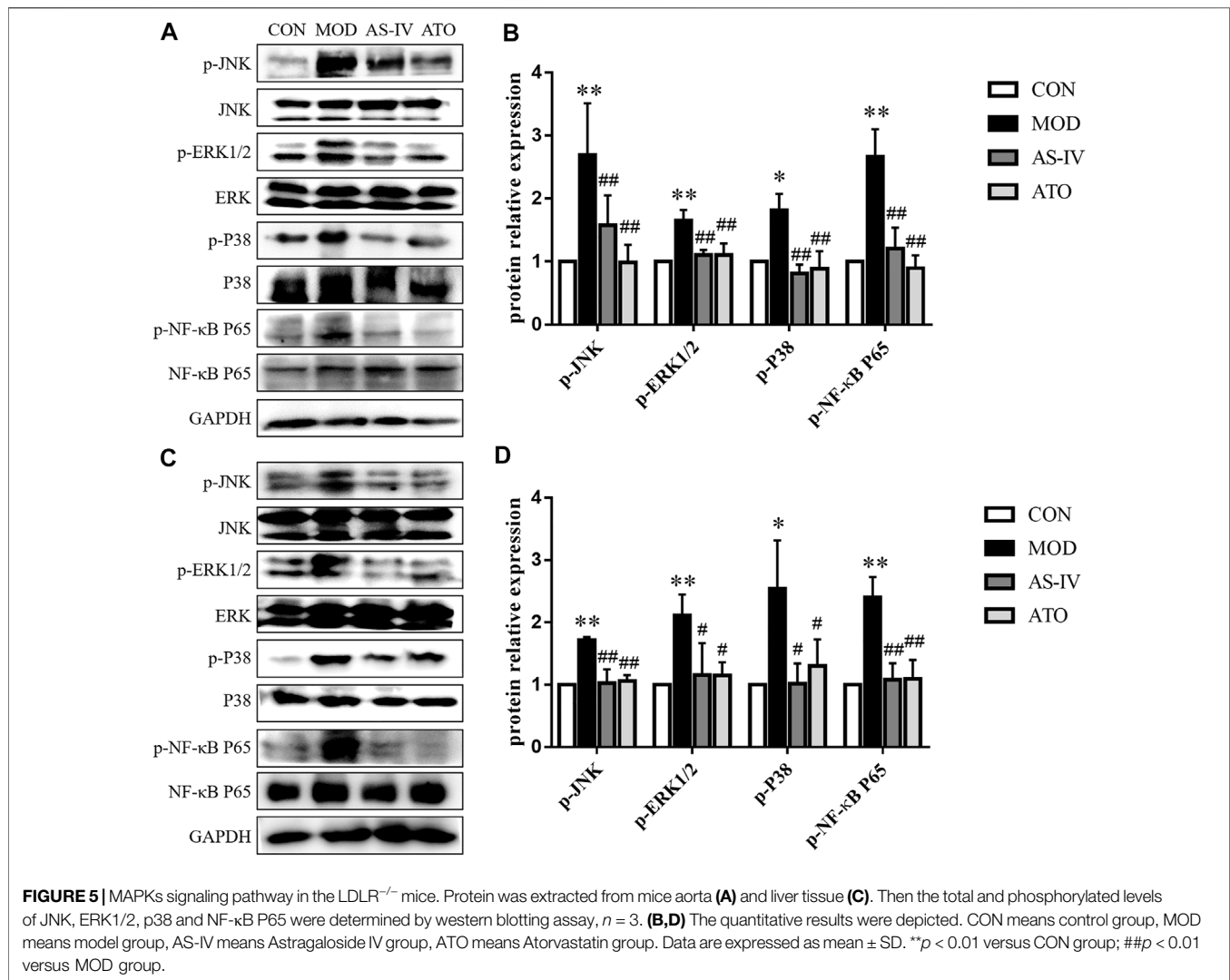
The development of AS requires lipid accumulation and inflammatory response (Moore et al., 2013). AS-IV is a type of polysaccharide extracted from *Astragalus membranaceus*, which is a traditional Chinese herbal medicine for CVD (Zhang et al., 2011; Ma et al., 2013). In this study, we investigated whether AS-IV could alleviate AS and hepatic steatosis, and uncovered the underlying mechanism. The results showed that AS-IV inhibited the development of AS and hepatic steatosis, which may be attributed to its beneficial effects on lipid metabolism and inflammation. Mechanistically, we found that AS-IV fights against inflammation by inactivating MAPK/NF- κ B signaling



pathway. Although our results show that atorvastatin has better lipid metabolism and anti-inflammatory effects. And studies have confirmed that statins affect atherosclerosis through several other effects, such as plaque stabilization, reducing inflammation, reversing endothelial dysfunction, and reducing thrombosis (Girotra et al., 2012; Okuyama et al., 2015; Niedzielski et al., 2020). Myopathy, rhabdomyolysis, acute renal failure and liver enzyme abnormalities were the most frequently reported adverse events of statins (Šimić and Reiner, 2015; Newman et al., 2019). Therefore, we need to find more effective traditional Chinese medicine for the treatment of atherosclerosis. AS-IV has no

obvious toxicity or adverse reactions (Yu et al., 2007). It has been documented that oral administration of AS-IV did not affect liver and renal function (Gui et al., 2012). The numerous experiments provide substantial evidence for AS-IV prevented the progression of cardiovascular diseases (Qin et al., 2015; Xiong-Zhi Li et al., 2017; Nanding Wang et al., 2020). Besides, recent studies have shown the potential therapeutic benefits that AS-IV confers in the treatment of kidney injury and liver cirrhosis (Xu et al., 2014; Xin et al., 2015; Wei et al., 2019).

Increased plasma cholesterol and triglyceride levels are risk factors for AS (Beverly and Budoff, 2020). Chylomicrons, VLDL



and LDL carry these lipids into the arterial wall where the lipids accumulate and deposit to initiate early-stage AS (FERENCE et al., 2018). LDL is prone to undergoing various atherogenic modifications, the most common of which is oxidation (Quinn et al., 1987). Modified LDL serves as the primary source of cholesterol accumulation and chronic inflammation in atherosclerotic plaques (Tabas et al., 2015). Oxidized LDL (ox-LDL) can be taken up by macrophages, thereby turning them into foam cells presented as fat streaks in the early stage of AS (Maguire et al., 2019). Therefore, lipid-lowering is the cornerstone in the treatment of AS (Mourikis et al., 2020). The results of our study showed that AS-IV decreased serum lipid levels and plaque accumulation in the aortic roots. With the development of plaques, their thinning fibrous caps and enlarging necrotic cores make plaques brittle, resulting in severe cardiovascular events (Insull, 2009). Activation of caspase 3 regulates inflammation and cell death. Previous studies have identified cleaved caspase 3 in atherosclerotic plaques, where it colocalizes with dead macrophages and lipid-rich plaque components (Hutter et al., 2004; Kong et al., 2019). It has also

been shown in an animal study that caspase-3 inhibitors protect cholesterol-loaded hepatocytes (Gan et al., 2014). We found that AS-IV increased collagen and α -SMA content, and reduced caspase 3 content in the plaque. These results suggested that AS-IV improved AS through correcting lipid metabolism and enhancing plaque stability.

The hepatic functions, mainly determining the cholesterol metabolism and lipid profile in the serum, are critical for AS development (Purnak et al., 2011). The results showed that AS-IV reduced the levels of hepatic TC and TG, and liver fat deposition. A study has demonstrated that elevated ALT levels, as a marker for NAFLD, are associated with increased CVD-related mortality in Koreans (Yun et al., 2009). AS-IV also decreased the levels of ALT and AST. These results indicate that AS-IV reduces hepatic steatosis, a beneficial effect in treating AS.

Lipid metabolism is also associated with inflammation. Accumulated evidence demonstrates that ox-LDL produces pathophysiological effects, including the release of proinflammatory cytokines, overexpression of cell adhesion molecules, and impairment of endothelium-dependent

vasorelaxation (Di et al., 2017). Endothelial cells dysfunction, due to the increased ox-LDL, can activate the inducible isoform of nitric oxide synthase (iNOS) (Stancu et al., 2012; Gliozzi et al., 2019). In addition, ox-LDL can activate TLR4/NF- κ B signaling pathway via lipoprotein receptor-1 (Bai et al., 2017; Balzan and Lubrano, 2018), thus increasing the levels of iNOS and vascular cell adhesion molecule 1 (VCAM-1) (Zhong et al., 2018; Gliozzi et al., 2019). These molecules enhance inflammation and exacerbate AS. Therefore, anti-inflammatory strategies are essential for the treatment of AS (Pedro-Botet et al., 2020). Our results showed that AS-IV restored the serum levels of IL-1 β , IL-6 and TNF- α , and the mRNA expression levels of these cytokines in mice aortas. AS-IV also repressed the increase of inflammatory proteins (iNOS, VCAM-1 and IL-6) induced by HFD. These results indicate that AS-IV attenuates AS through inhibiting the release of inflammatory factors.

There is no doubt that inflammatory responses become more severe in the stage of NASH. Some studies have shown that patients with NASH are more likely to develop AS compared with those with simple steatosis (Targher et al., 2010b; Söderberg et al., 2010). Studies have shown that hepatic inflammation occurs earlier than the formation of early aortic lesions, and partial inflammatory factors that lead to AS are generated in the liver (Purnak et al., 2011). Excessive accumulation of lipids results in hepatic inflammation, activating interferon γ , IL-1, TNF- α and other inflammatory signal pathways to promote AS (Kleemann et al., 2007). Therefore, alleviating hepatic inflammation has potential benefits in AS treatment. In the AS-IV group, the mice showed decreased mRNA expression levels of IL-1 β , IL-6 and TNF- α . These results suggest that AS-IV ameliorates hepatic steatosis through suppressing the expression of inflammatory factors.

Both MAPK and NF- κ B are key signaling pathways responsible for inflammation in the development of AS and NAFLD (Hopkins, 2013; Hernández-Aquino and Muriel, 2018). Stimulated by endotoxin or lipopeptide, TLR4 triggers the myeloid differentiation factor 88-dependent pathway, rapidly activates the MAPK/NF- κ B signaling pathway, and upregulates the transcription of inflammatory factors, all stoking up hepatic inflammation to aggravate AS (Wang et al., 2014; Wu et al., 2018). Recent research found that inhibiting the activation of MAPK signaling pathway could inhibit AS (Yiru Wang et al., 2020; Jiang et al., 2020). And suppressing MAPK/NF- κ B signaling pathway could mitigate NASH (Li et al., 2020). Existing studies showed that AS-IV inhibits inflammation and oxidation by inhibiting NF- κ B, MAPK signaling pathways (Zhang and Frei, 2015; Hsieh et al., 2020). And the potential effects of AS-IV on cardiac disease have been described by regulating the MAPK signaling pathway (Sun et al., 2021). AS-IV administration could downregulate the phosphorylation of JNK, ERK1/2, p38 and NF- κ B p65 in the aortas and liver tissue. These suggest that AS-IV exerts anti-AS and anti-inflammation effects by suppressing HFD-induced activation of MAPK/NF- κ B signaling pathway.

In conclusion, AS-IV can attenuate AS development and hepatic steatosis via improving the lipid metabolism and inhibiting inflammation through suppressing MAPK/NF- κ B signaling pathway. Therefore, AS-IV may act as a promising anti-atherosclerotic drug. Further *in vitro* studies should be conducted to clarify the beneficial effects of AS-IV on AS and hepatic steatosis. Activation of MAPK may affect apolipoprotein B stability and/or degradation, providing a potential target for AS-IV against atherosclerosis and hepatic steatosis.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by the Laboratory Animal Management and Use (Animal Welfare) Committee, Longhua Hospital affiliated to Shanghai University of Traditional Chinese Medicine.

AUTHOR CONTRIBUTIONS

PL conceived and designed the experiments. YZ and MD contributed equality. YZ and MD performed the experiments. MD and JW analyzed the results. YZ was a major contributor in writing the manuscript. PL reviewed and edited the final manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphar.2022.828161/full#supplementary-material>

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