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Prophylactic and therapeutic effects of EsV3 on atherosclerotic lesions in ApoE$^{-/-}$ mice

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Abstract

Background Atherosclerosis (AS) is a major contributor to vascular disorders and represents a significant risk to human health. Currently, first-line pharmacotherapies are associated with substantial side effects, and the development of atherosclerosis is closely linked to dietary factors. This study evaluated the effects of a dietary supplement, EsV3, on AS in apolipoprotein E (ApoE) $^{-/-}$ model mice.

Methods The study utilized a high-fat diet-induced ApoE$^{-/-}$ hyperlipidemic mouse model. EsV3 was administered in prophylactic (P-EsV3) and therapeutic regimens for 16 and 12 weeks, respectively, with distinct high- and low-dose groups (0.36 and 1.8 g/kg/day). Serum lipid levels were measured and monitored for body weight and food consumption alterations in murine models. Aortic oil red O staining was conducted to assess plaque formation and calculate the plaque-to-vessel area ratio. Liver tissue changes were examined via HE staining. Moreover, serum oxidative stress markers (MDA, GSH, SOD) were measured to evaluate oxidative damage and lipid metabolism.

Results Both atorvastatin and P-EsV3 treatments significantly lowered TC, TG, and LDL-C levels, with P-EsV3-H enhancing HDL-C levels ($P < 0.05$). Prophylactic EsV3 administration was more effective than therapeutic administration in regulating TG and LDL-C levels and had comparable effects to atorvastatin on TC and HDL-C. All treatment groups exhibited reduced body weight compared to the model group, with no significant differences in food intake. Additionally, EsV3 administration significantly reduced the aortic plaque area and liver lipid droplets compared to the model group, while mitigating oxidative stress, as evidenced by decreased MDA levels and increased SOD and GSH levels, with outcomes comparable to those observed with atorvastatin.

Conclusions In ApoE$^{-/-}$ hyperlipidemic mice, EsV3 improved lipid profiles and reduced aortic plaque formation. EsV3's effects, attributed partly to its antioxidant properties, were comparable to atorvastatin, suggesting its potential as a preventive and therapeutic agent for hyperlipidemia and atherosclerosis.

Keywords Atherosclerosis, Hyperlipidemia, EsV3, Dietary supplement, Oxidative damage

Introduction

Atherosclerosis (AS) is the most common cause of vascular diseases and is a severe threat to human health [1]. AS often results in severe outcomes such as coronary heart disease, stroke, and peripheral artery disease, which in turn increase the occurrence of cardiovascular events. Cardiovascular disease is a prominent cause of mortality globally. In 2020, it was anticipated that 21.13% of individuals aged 30–79 have an increased carotid plaque [2]. AS is distinguished by lipid accumulation, chronic low-grade inflammation,

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and endothelial dysfunction [3]. Moreover, the pathogenesis of atherosclerosis encompasses oxidative modification of lipoproteins, immune cell activation, and alterations in the extracellular matrix [4]. Currently, the commonly used drugs are statins for anti-AS. Despite their widespread use, statins are associated with adverse effects such as abnormal liver function, muscle toxicity, and cognitive dysfunction [5]. The primary focus has consistently been on developing strategies to mitigate the risk of atherosclerosis.

Increasing evidence showed that abnormal lipid metabolism is the most critical risk factor for atherosclerosis [6]. Hyperlipidemia is closely associated with dietary patterns, notably those high in fats and cholesterol, and is directly correlated with elevated serum lipid levels [7]. Total cholesterol (TC), triglycerides (TG), or low-density lipoprotein (LDL) are elevated; high-density lipoprotein (HDL) is reduced and is considered a risk factor [8].

Epidemiological research indicates that dietary habits are a critical risk factor for developing pathological conditions such as atherosclerosis [9]. While pharmacological interventions have demonstrated efficacy in managing atherosclerosis, they are often associated with side effects and high costs, prompting interest in alternative or complementary strategies. Compelling data indicate that dietary components may directly impact the development of atherosclerosis, either by themselves or by affecting established risk factors, including plasma lipids, blood pressure, and plasma glucose levels [10]. Multiple studies have demonstrated that supplements such as omega-3 fatty acids, plant sterols, antioxidants (including vitamins C and E), and dietary fiber may effectively lower the risk of atherosclerosis through various mechanisms [11]. Dietary supplements have gained increasing attention due to their potential anti-inflammatory, lipid-lowering, and antioxidative properties, which are critical in atherosclerosis management. However, research exploring the role of food-derived supplements in managing atherosclerosis remains limited, with most existing studies concentrating on isolated nutrients or pharmacological agents, thereby overlooking the potential synergistic effects of multiple bioactive components. This underexplored area represents a critical research gap, particularly given the potential of dietary supplements to offer safer, more accessible, and cost-effective solutions. Thus, consuming safe and efficacious functional foods represents a practical and accessible approach to prophylactic and treating vascular diseases. The health supplement EsV3 is mainly formulated with Eckol, kefir soy milk, VD3, and so on, which are beneficial for improving AS. This project will investigate the prophylactic and therapeutic impacts of EsV3 on atherosclerotic in ApoE$^{-/-}$ mice.

Materials and methods

Materials

Experimental animals

Male C57BL/6 mice (20 ± 2 g) and ApoE$^{-/-}$ mice (20 ± 2 g), both six weeks old, were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd, license No. SCXK (Beijing) 2021–0006. All animals were of specific pathogen-free (SPF) grade. The feeding temperature was 20 ~ 25℃, relative humidity 50% ~ 60%, and free drinking and feeding. Experiments were started after one week of acclimatization feeding.

Drugs and reagents

HYDROX Co., Ltd. (Saitama, Japan) and Eiho Technology Co., Ltd. (Wuhan, China) supplied EsV3. EsV3 is a mixture of Soy milk kefir, eckol, fish oil, Vitamin D3, and trehalose in 81.33, 2.21, 6.2, 2.1, and 8.16%. A high-fat diet with 1.25% cholesterol, purchased from Rat-Lab (Wuhan) Biotechnology Co. Atorvastatin Tablets (Pfizer Pharmaceutical Co., Ltd.). TC, TG, LDL-C, and HDL-C assay kits (Nanjing Jiancheng Biologicals, item numbers: A111-1–1, A110-1–1, A113-1–1, A112-2–1, respectively).

Experimental instruments

Carbon dioxide incubator (Thermo, USA: 367743–17216), SW-CJ-2FD ultra-clean bench (Shanghai Boxun Company), multi-functional enzyme marker (BIO-Tek Synergy H1, USA).

Experimental grouping

ApoE$^{-/-}$ mice on high-fat diet were randomly assigned to the model group, atorvastatin group, EsV3 high-dose prophylactic administration group (P-EsV3-H), EsV3 low-dose prophylactic administration group (P-EsV3-L), EsV3 high-dose therapeutic administration group (EsV3-H), EsV3 low-dose therapeutic administration group (EsV3-L), eight mice in each group. C57BL/6 mice were used as a control group. EsV3 administration groups are a general term for EsV3 prophylactic and therapeutic administration groups.

Model preparation

Atherosclerosis research frequently utilizes ApoE$^{-/-}$ mice fed a high-fat diet [12]. ApoE$^{-/-}$ mice were given a high-fat diet daily for four weeks in order to establish hyperlipidemia (Mouse serum was assayed for TC, TG, HDL-C, and LDL-C, and a significant difference between the control group and the model group was recognized as a successful hyperlipidemia model), and P-EsV3-H and P-EsV3-L groups were administered continuously during

the modeling period. In the fourth week of the modeling period, blood was collected from the eye socket, and the serum was separated and prepared for use.

The EsV3 therapeutic administration group and the atorvastatin group were started as soon as it was clear that hyperlipidemia had occurred. EsV3 was administered by mixing it into the feed, and atorvastatin was administered by intragastrical. The dose of EsV3 was 0.36 g/kg for the low-dose prophylactic and therapeutic groups and 1.8 g/kg for the high-dose prophylactic and therapeutic groups. 5 mg/kg was administered by intragastrical to the atorvastatin group. 16 weeks were administered to the prophylactic group and 12 weeks to the therapeutic and atorvastatin groups. The mice's body weight and food intake were monitored weekly throughout the trial. All mice were anesthetized with 0.3% sodium pentobarbital and then sacrificed by cervical dislocation.

Indicator testing

General observation

Body weight and food intake were measured weekly for each group of mice, followed by statistical analysis to evaluate the effects of treatment on these parameters.

Concentration of serum lipid parameters

Serum TC, TG, LDL-C, and HDL-C levels were determined using assay kits from Nanjing Jiancheng Bioengineering Institute, Jiangsu, China.

Histological analysis

Liver tissues were fixed, sectioned, and subjected to hematoxylin and eosin (H&E) staining. The morphological characteristics of the liver were subsequently examined under a light microscope. To evaluate hepatic lipid accumulation, lipid droplets in liver sections were quantified following staining with H&E staining. Lipid droplets, appearing as clear vacuoles within hepatocytes due to the extraction of lipids during the staining process, were identified and outlined manually or using automated segmentation tools in Image-Pro Plus software. Images were imported into the software, and a threshold was set to differentiate lipid droplet vacuoles from surrounding stained cellular structures. The total area of lipid droplets was quantified using the "area measurement" tool, and the percentage of droplet area relative to the total tissue area in each field was calculated.

Atherosclerotic lesion analysis

The entire brachiocephalic artery was excised from its origin at the aortic arch to its bifurcation into the right common carotid artery. The tissue was fixed in 10% formalin for 10 min, followed by Oil Red O staining to quantify lipid accumulation [13]. The aortic plaque area was quantified using Image Pro Plus software, which calculated the ratio of plaque area to total vessel area by measuring both parameters. The "area measurement" tool was used to trace and quantify the cross-sectional area of the atherosclerotic plaque within the vessel lumen. The outer boundary of the vessel was outlined to determine the total vessel cross-sectional area. Finally, the plaque-to-vessel area ratio was calculated as the plaque area divided by the total vessel area.

Detection of MDA, SOD and GSH

Serum MDA (A003-1–2, Nanjing Jiancheng Biologicals), SOD (A001-1–2, Nanjing Jiancheng Biologicals) and GSH (A005-1–2, Nanjing Jiancheng Biologicals) assay kits were used to detect oxidative indicators following the provided instructions.

Statistical processing

GraphPad Prism 8 statistical software was used. The data for measures conforming to normal distribution were expressed as $x \pm s$. Statistical comparisons between two groups were performed using the student's t-test, while one-way analysis of variance (ANOVA) was employed for comparisons involving multiple groups. For comparisons involving multiple groups, a one-way ANOVA was performed. In instances where only two groups were compared, such as between the prevention and treatment groups within the EsV3 treatment, a two-tailed unpaired T-test was employed. Specifically, the P-EsV3-L group was compared with the EsV3-L group, and the p-EsV3-H group was compared with the EsV3-H group. A p-value of less than 0.05 was considered statistically significant.

Results

Effects of EsV3 on blood lipids of ApoE$^{-/-}$ mice

Four weeks after modeling, the lipid levels of mice in each group were compared. The TC levels in the mice serum in the model group were considerably higher ($P < 0.001$) compared to the control group. There was a trend toward lower TC in the P-EsV3 groups compared to the model group, but it was insignificant. At the end of the 16th week of modeling, TC levels were significantly higher ($P < 0.001$) in the model group compared with the control group. TC levels were significantly lower in both atorvastatin and EsV3 administration groups compared to the model group. There was no difference in the EsV3 administration group compared to atorvastatin, suggesting that EsV3 acts similarly to atorvastatin in TC level regulation (Table S1,2,3, Fig. 1A-B).

The TG levels in the mice serum in the model group were significantly higher ($P < 0.001$) compared to the control group in the fourth week of modeling. Both

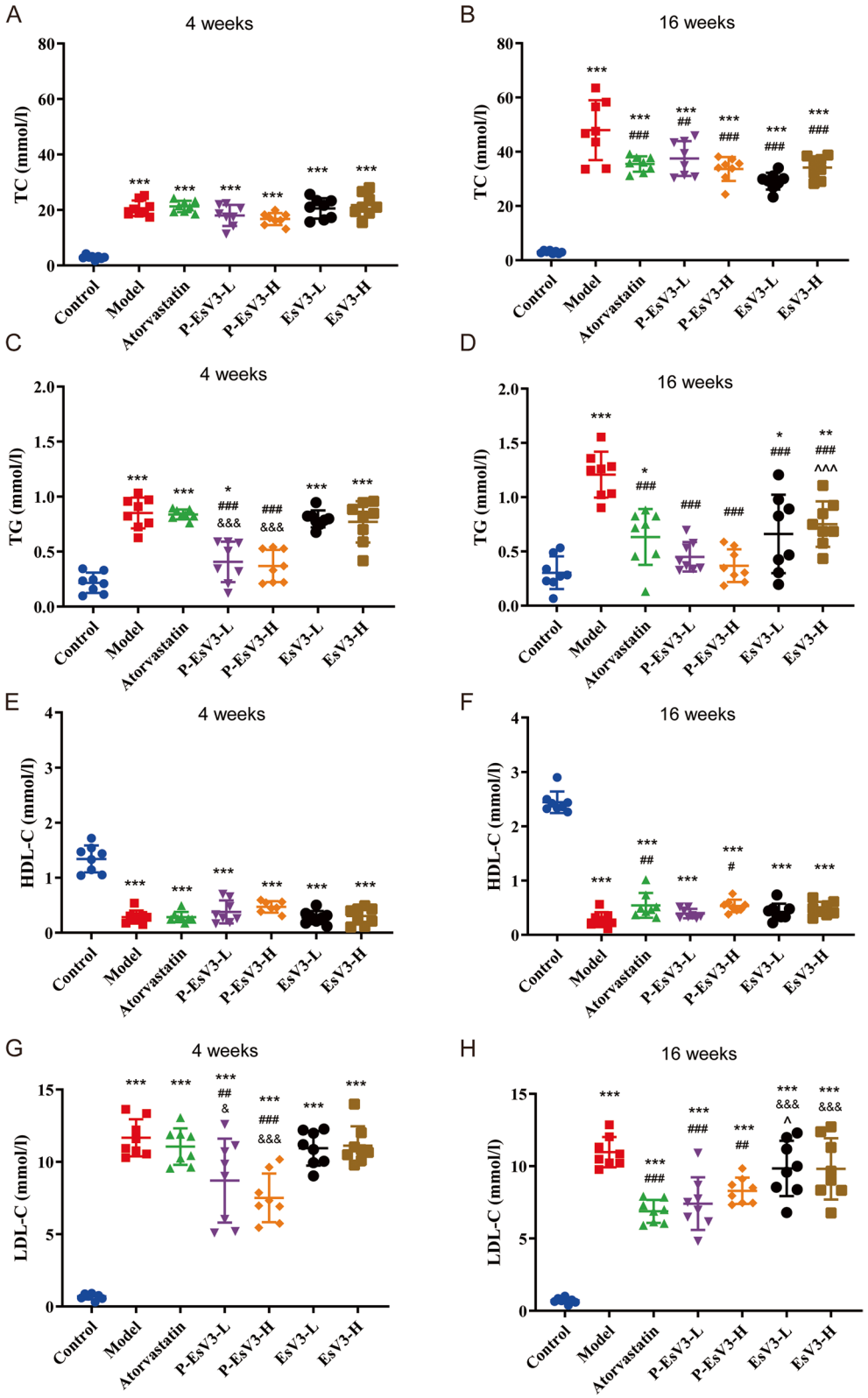

Fig. 1 Comparison of lipid (serum TC, TG, LDL-C, HDL-C) levels among groups at week 4 and week 16 ($n=8$). **A**, **E**. TC; **B**, **F**. TG; **C**, **G**. HDL-C; **D**, **H**. LDL-C. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. control group. $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ vs. model group. $^{\&}$ $p<0.05$, $^{\&\&}$ $p<0.01$, $^{\&\&\&}$ $p<0.001$ vs. atorvastatin. $^{\wedge}$ $p<0.05$ vs. P-EsV3 groups. For comparisons involving multiple groups, a one-way ANOVA was performed. A two-tailed unpaired t-test was performed to compare the P-EsV3-L group with the EsV3-L group

the P-EsV3-H and P-EsV3-L administration significantly inhibited the increase in TG levels compared to the model group ($p < 0.001$). This finding indicated that EsV3 prophylactic administration groups could improve hypertriglyceridemia in ApoE$^{-/-}$ mice. At the end of the 16th week of modeling, compared with the control group, TG levels were significantly higher ($P < 0.001$) in the model group ($P < 0.001$) [12]. TG levels were significantly reduced in all EsV3 administration groups compared to the model group and had similar effects as atorvastatin. In addition, the regulation of TG by P-EsV3-H was superior to that of EsV3-H when compared between the therapeutic and prophylactic groups of EsV3 ($P < 0.001$), suggesting that the EsV3 prophylactic group was more effective in regulating TG (Table S1,2, Fig. 1C-D).

HDL-C levels were significantly lower at both the fourth week and 16th week of modeling compared to the control group ($P < 0.001$). At week 16 of modeling, HDL-C levels were significantly upregulated in the P-EsV3-H group and the atorvastatin group compared with the model group, suggesting that the EsV3 high-dose prophylactic dosing group had a similar effect as atorvastatin on HDL-C regulation (TableS1,2, Fig. 1E-F).

The LDL-C levels in the mice serum in the model group were significantly higher ($P < 0.001$) compared to the control group in the fourth week of modeling. There was a significant decrease in LDL-C content in the P-EsV3 groups compared to the model group, suggesting that the EsV3 prophylactic dosing groups could regulate LDL-C content during hyperlipidemia. At the end of the 16th week of modeling, compared with the control group, LDL-C levels were significantly higher ($P < 0.001$) in the model group ($P < 0.001$). Compared with the model group, serum LDL-C levels were significantly lower ($P < 0.001$) in the P-EsV3 and atorvastatin groups. The EsV3 prophylactic administration group had a similar effect on LDL-C regulation as the atorvastatin group. In addition, the regulation of LDL-C by P-EsV3-L was superior to that of EsV3-L when compared between the therapeutic and prophylactic groups of EsV3 ($P < 0.001$), suggesting that the EsV3 prophylactic group was more effective in regulating LDL-C (Table S1,2, Fig. 1G-H).

In summary, EsV3, as a preventive and therapeutic agent, significantly improved TC, TG, LDL-C, and HDL-C levels in atherosclerotic mice, with effects comparable to atorvastatin. EsV3 prophylactic administration groups were superior to the therapeutic groups in regulating TG and LDL-C.

Effects of EsV3 on body weight of ApoE$^{-/-}$ mice

The initial body weight of each group was not significantly different from that of the control group. After 16 weeks of modeling, the body weight of the model group increased significantly compared with the control group ($P < 0.001$). Compared with the model group, body weight decreased in all dosing groups, with the atorvastatin group significantly down-regulating the body weight of mice ($P < 0.05$). There was no significant difference between the EsV3 prophylactic and therapeutic groups (Fig. 2A-B). These results show that weight loss in the EsV3-treated groups suggests that EsV3 may have a potential weight loss effect.

Effects of EsV3 on food intake in ApoE$^{-/-}$ mice

The results showed that the food intake of all groups of mice tended to increase and then decrease. Meanwhile, there was no significant variation in mouse food consumption between groups (Fig. 2C). This indicates that the EsV3 groups had no significant effect on intake, indicating its safety.

EsV3 reduced aortic plaque formation in ApoE$^{-/-}$ mice

Compared with the control group, mice in the model group showed a large distribution of aortic oil-red lipid droplets and a significantly higher percentage of aortic plaque area ($P < 0.001$). The EsV3 administration groups (P-EsV3-L, P-EsV3-H, EsV3-L, and EsV3-H) significantly reduce the aortic plaque area ($P < 0.05$, $P < 0.01$ or $P < 0.001$) (Fig. 3A-B). The atorvastatin group significantly reduced the aortic plaque area compared with the model group ($P < 0.001$). Compared with the atorvastatin group, there was no significant difference in the effect of both the prophylactic and therapeutic groups, indicating that the inhibitory effect of the administered group on atherosclerotic plaque formation was comparable to that of atorvastatin. No significant difference between EsV3 prophylactic and therapeutic groups on the aortic plaque area. The above results suggest that EsV3 has a significant inhibitory effect on the formation of atherosclerotic aortic plaque lesions.

Effect of EsV3 on the histopathological morphology of the liver in hyperlipidemic mice

HE staining of liver tissue showed that the hepatocytes in the control group were round and full in shape, with intact nuclei in a central position, clear cell borders, no apparent lipid droplets in the cytoplasm, and a striated arrangement with no narrowing of the hepatic sinusoids. Compared with the control group, the hepatocytes in the model group were more prominent, with many fat vacuoles of different sizes in the cytoplasm, and the hepatic sinusoids were narrowed or disappeared. Lipid droplets were significantly reduced in the EsV3 administration groups and the atorvastatin group, and their pathological changes were significantly reduced compared with the model group, with a reduced degree of hepatocyte

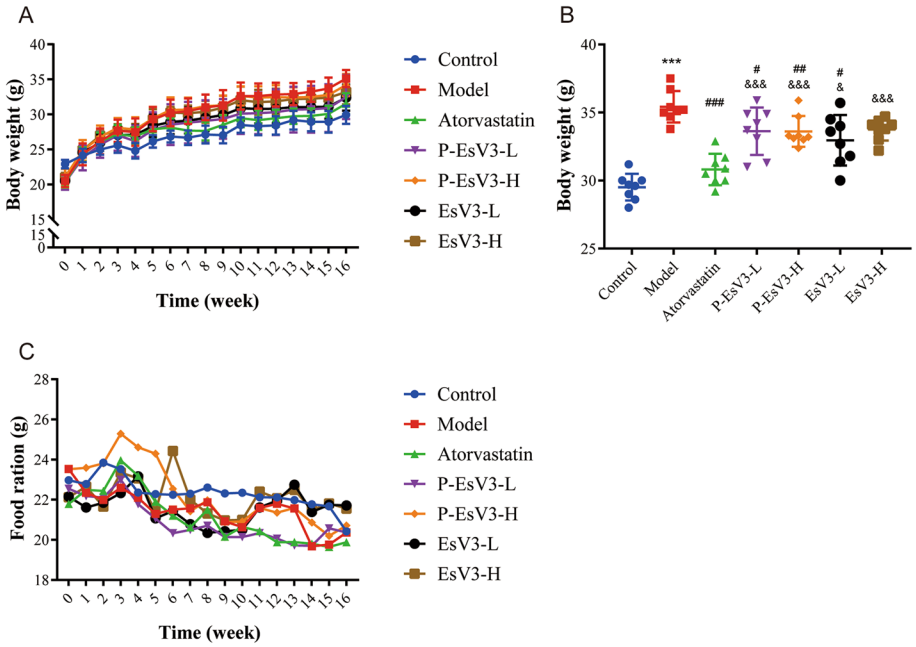

Fig. 2 Effect of EsV3 treatment on body weight and feeding. **A**. Body weight of EsV3 in hyperlipidemic mice ($n=8$). **B**. Body weight of EsV3 in hyperlipidemic mice at 16 weeks ($n=8$). **C**. Effect of EsV3 on feeding in hyperlipidemic mice ($n=8$). * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. control group. $^{\#}$ $p<0.05$, $^{\#\#}$ $p<0.01$, $^{\#\#\#}$ $p<0.001$ vs. model group. $^{\&}$ $p<0.05$, $^{\&\&}$ $p<0.01$, $^{\&\&\&}$ $p<0.001$ vs. atorvastatin. For comparisons involving multiple groups, a one-way ANOVA was performed

steatosis, significantly fewer fat vacuoles, and reduced hepatic sinusoidal stenosis. Normal hepatocytes could be seen, and the degree of hepatic sinusoidal stenosis was restored. The P-EsV3-H and EsV3-H showed improvements closer to those of the atorvastatin group, indicating comparable inhibition of hepatic lipid droplets to that of the atorvastatin group in Fig. 4.

EsV3 regulated the levels of oxidant-related markers in serum samples

The oxidative markers evaluated in this study included malondialdehyde (MDA), superoxide dismutase (SOD), and total glutathione (T-GSH) [14]. MDA, a byproduct of lipid peroxidation, is a critical indicator of oxidative damage and disrupted lipid metabolism in atherosclerosis. Accordingly, we quantified the levels of these oxidative markers in serum samples from atherosclerotic mice. Compared with the control group, serum MDA levels were significantly higher ($P<0.001$), and SOD and GSH levels were significantly lower ($P<0.01$, $P<0.001$) in the model group, suggesting that oxidative stress occurred. Serum MDA levels were significantly lower ($P<0.001$), and SOD and GSH levels were significantly higher ($P<0.01$) in the atorvastatin group compared with the model group. Compared with the model group, the MDA level of EsV3 was significantly reduced in EsV3 administration groups ($P<0.001$), which was similar to the effect of the atorvastatin group (Fig. 5A). Serum SOD levels exhibited an upward trend in EsV3 administration groups relative to the model group (Fig. 5B). Similarly, both P-ESV3-H and EsV3 treatment groups exhibited significantly higher GSH levels relative to the model group ($P<0.001$) (Fig. 5C). These findings indicate that EsV3 partly suppressed the development of atherosclerosis by exerting its antioxidant properties.

Discussion

This study highlights the beneficial effects of EsV3 in an ApoE$^{-/-}$ mouse model of atherosclerosis. The primary findings demonstrate that oral administration of EsV3, either as a preventive measure or a therapeutic intervention, reduced serum levels of TG, TC, and LDL-C while increasing serum levels of HDL-C. Additionally, EsV3 administration groups resulted in diminished aortic plaque formation and decreased hepatic lipid droplet accumulation. These results underscore the potential of EsV3 as a promising dietary supplement for the management of atherosclerosis.

Atherosclerosis is characterized by the development of fibrofatty lesions within the arterial walls. These lesions are marked by abnormal lipid accumulation, enhanced inflammatory cell infiltration, matrix deposition, and proliferation of smooth muscle cells [15, 16]. TC plays a central role in the pathogenesis of atherosclerosis, a

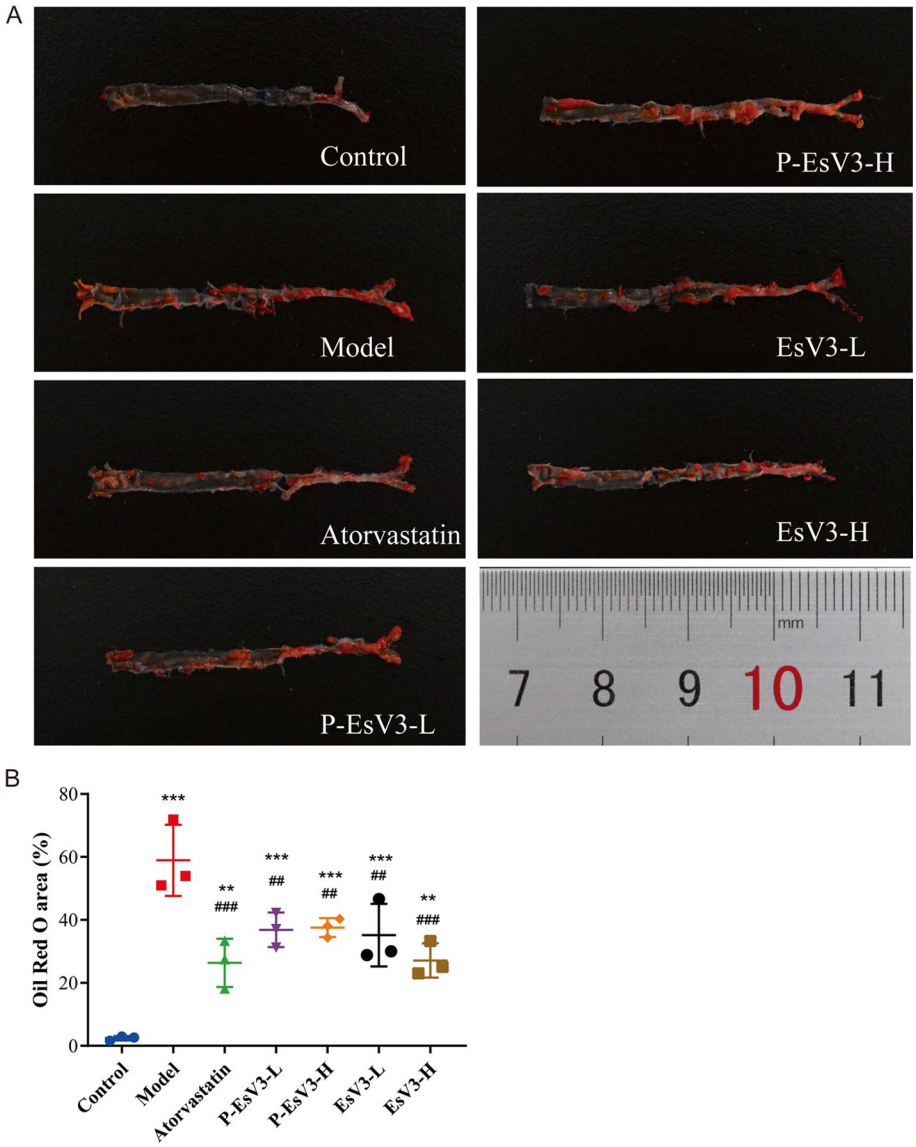

Fig. 3 EsV3 inhibited plaque formation in the aorta of ApoE$^{-/-}$ mice. **A**-**B**. Atherosclerotic plaque lesion formation of EsV3 in hyperlipidemic mice ($n = 3$). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ vs. model group. For comparisons involving multiple groups, a one-way ANOVA was performed

condition characterized by lipid accumulation within the arterial walls, leading to plaque formation and vascular obstruction. Elevated TC levels contribute to atherogenesis by promoting the deposition of cholesterol in the arterial intima [17]. TG plays a crucial role in the formation and advancement of atherosclerosis, primarily via being linked to triglyceride-rich lipoproteins (TRLs), such as very-low-density lipoprotein (VLDL) and chylomicrons [18]. HDL-C has a preventive function in the development of atherosclerosis by participating in reverse cholesterol transport (RCT). Dysfunctional HDL-C may lose its ability to provide protection, leading to oxidative stress and inflammation. As a result, its effectiveness in preventing the advancement of atherosclerosis is reduced [19]. High levels of LDL-C and triglycerides play a vital role in the formation of atherosclerosis [20]. Excess LDL in the blood is taken up and modified by endothelial cells to form oxidized LDL (ox-LDL). Ox-LDL activates endothelial cells and macrophages, induces an inflammatory response, and prompts the transformation of macrophages into foam cells, further accelerating lipid deposition in the arterial wall [21]. In addition, hyperlipidemia can further exacerbate the progression of thrombosis [22]. In conclusion, there is a close and complex

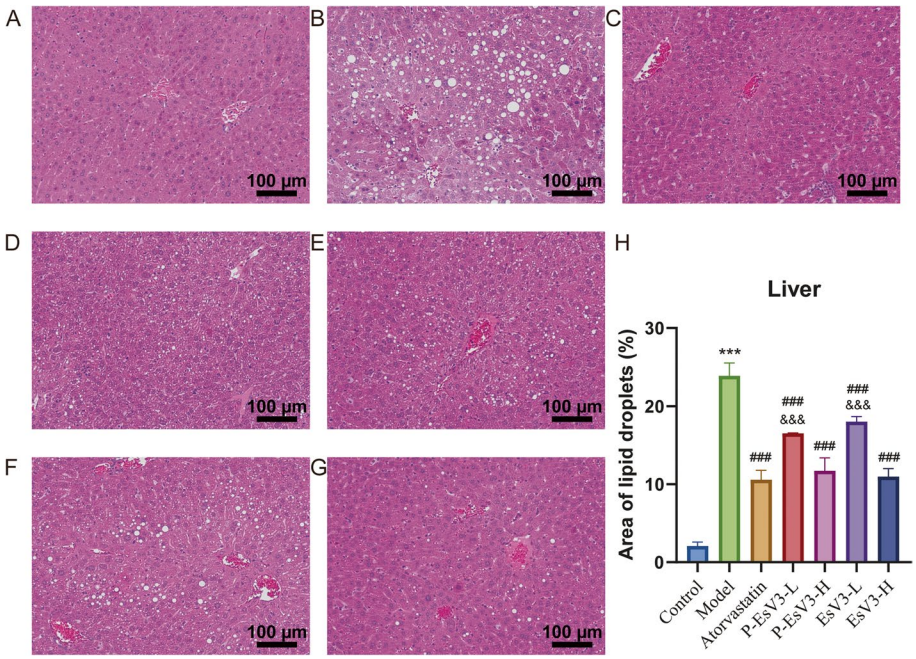

Fig. 4 Effect of EsV3 on liver histopathology in hyperlipidemic mice (HE, 100x). **A**. Control; **B**. Model; **C**. Atorvastatin; **D**. P-EsV3-L; **E**. P-EsV3-H; **F**. EsV3-L; **G**. EsV3-H. **H**. Lipid droplet area ratio in hepatocytes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ vs. model group. $^{\&}$ $p < 0.05$, $^{\&\&}$ $p < 0.01$, $^{\&\&\&}$ $p < 0.001$ vs. atorvastatin. For comparisons involving multiple groups, a one-way ANOVA was performed

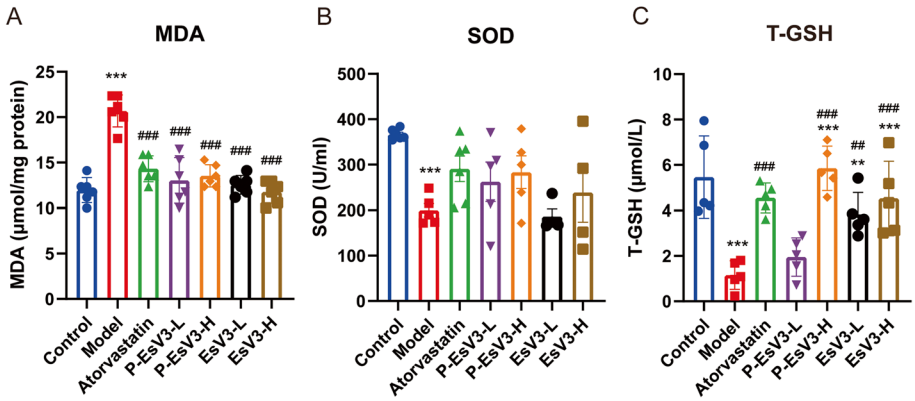

Fig. 5 Effect of EsV3 on antioxidant markers in ApoE$^{-/-}$ mice. **A**. MDA concentration in serum. Activities of SOD (**B**) and T-GSH (**C**) in serum. Results were presented as means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control group. $^{\#}$ $p < 0.05$, $^{\#\#}$ $p < 0.01$, $^{\#\#\#}$ $p < 0.001$ vs. model group. For comparisons involving multiple groups, a one-way ANOVA was performed

interaction between hyperlipidemia and atherosclerosis, and effective control of blood lipid levels is essential for preventing and treating atherosclerosis.

Atorvastatin is a selective methylglutaryl coenzyme A reductase inhibitor. It has been widely used in the clinic for its apparent effects in lowering blood lipids, inhibiting atherosclerosis, and improving vascular endothelial function [23]. In addition, atorvastatin has pleiotropic benefits. These effects include anti-inflammatory and antioxidant activities, which help improve the function of the endothelium and reduce inflammation in blood vessels. Together, these processes reduce the advancement of atherosclerosis and decrease the occurrence of cardiovascular events [24]. However, some patients experience muscle-related adverse effects, including myalgia, muscle weakness, or rhabdomyolysis, as well as statin intolerance, manifesting as abnormal liver function and gastrointestinal discomfort [25]. Although there have

been substantial attempts to decrease the impact of atherosclerotic cardiovascular disease with atorvastatin and other medications, a considerable amount of risk remains.

Dietary patterns play a critical role in the development and progression of hyperlipidemia, with diets high in saturated fats and cholesterol strongly linked to elevated blood lipid levels [26]. Additionally, a high intake of refined carbohydrates and added sugars is associated with increased triglyceride levels, further amplifying the risk of hyperlipidemia [27]. ApoE$^{-/-}$ mice are often used as animal models to investigate hyperlipidemia and atherosclerosis since they possess a hereditary inclination towards lipid dysregulation. Without functioning ApoE, these animals have a reduced ability to remove chylomicron and VLDL remnants, leading to increased cholesterol levels in the blood and the spontaneous formation of atherosclerotic plaques. The atherogenic process is expedited when a high-fat diet is paired with it, leading to a further elevation in plasma levels of total cholesterol, triglycerides, and LDL-C [28].

Therefore, the role of diet in hyperlipidemia and atherosclerosis should be considered. Dietary supplements have shown potential in preventing and treating hyperlipidemia, thereby improving AS. The health supplements have great potential for preventing and treating atherosclerosis with few side effects and significant efficacy [29]. For example, apigenin reduced miR33 and TLR-4/NF-κB p65 pathway levels, increased ABCA1, decreased lipid accumulation, and lessened muscle cells and macrophages in the atherosclerotic regions of LPS-challenged ApoE$^{-/-}$ mice [30]. The health supplement EsV3 contains Eckol, kefir soy milk, VD3, and fish oil. Eckol, extracted from native wakame using ethanol and purified by fractionation, was added to EsV3—the fractionated product contained phlorotannins identified as Eckol. Eckol prevents thrombus formation by inhibiting thrombin activity. In addition, it inhibits smooth muscle cell proliferation and plaque formation [31]. Kefir soy milk contains β-poly globulin from soy protein, which acts on the liver and induces the production of FGF21 [32, 33]. FGF21 maintains the homeostasis of blood cholesterol to prevent the development of AS. The soy milk-kefir diet significantly enhanced the excretion of neutral sterols and bile acids via feces. This dietary intervention markedly decreased male hamsters' serum non-HDL cholesterol to HDL cholesterol ratio [34]. Fish oil is rich in Omega-3 unsaturated fatty acids, which are beneficial for stabilizing the lipid profile. Research has shown that dietary supplementation with fish oil significantly improves the endothelial-dependent dilation of coronary blood vessels in heart transplant recipients. However, this intervention does not influence endothelial-independent vasodilation [35]. EsV3 may exert its anti-atherosclerotic effects mainly through eckol, kefir soy milk, and fish oil.

In this study, EsV3 was classified into prophylactic and therapeutic administration, and their modulatory effects on atherosclerosis were systematically evaluated. Dietary modifications that are more conducive to the prevention of atherosclerosis [10]. The effects observed in the prophylactic and therapeutic administration were comparable concerning food intake and changes in body weight. EsV3 administration groups had comparable effects to the atorvastatin group. These findings suggest that EsV3 exerts no significant impact on appetite and can be safely administered. Weight loss is known to positively influence lipid metabolism and overall health, particularly in the context of atherosclerosis. Studies have demonstrated that weight reduction can lead to decreased LDL-C levels and improved lipid profiles, thereby mitigating cardiovascular risk factors [36]. In our study, EsV3-treated groups experienced weight loss without significant changes in appetite, indicating that EsV3 may facilitate weight reduction through mechanisms independent of caloric intake. Furthermore, weight loss has been associated with reduced systemic inflammation, a critical factor in the pathogenesis of atherosclerosis [37]. By promoting weight loss, EsV3 may not only improve lipid metabolism but also attenuate inflammatory processes, potentially enhancing the prognosis of atherosclerotic conditions. In conclusion, the observed weight reduction in EsV3-treated groups suggests that EsV3 may have therapeutic significance by positively affecting lipid metabolism and reducing systemic inflammation, thereby contributing to improved overall health status in the context of atherosclerosis.

EsV3 administration groups demonstrated significantly greater efficacy in lipid regulation compared to the model group. Moreover, EsV3 administration groups have a similar effect to atorvastatin. Specifically, EsV3 prophylactic administration was more effective than EsV3 therapeutic administration regarding TG and LDL-C levels. Compared with the model group, the EsV3 administration groups inhibited atherosclerotic plaque formation with an effect comparable to that of atorvastatin. The findings from the EsV3 prophylactic administration regarding blood lipid levels indicate that regular consumption of EsV3 may reduce the risk of atherosclerosis. Therapeutic administration demonstrated that EsV3 consumption following arterial plaque formation ameliorated plaque accumulation.

Lipid oxidative damage is crucial to hyperlipidemia and atherosclerosis. ROS-induced oxidative stress increases lipid peroxidation, accelerating plaque development and destabilization [38]. The modulation of MDA, SOD, and GSH by EsV3 suggests that EsV3 may have potential

in the prophylactic and therapeutic of atherosclerosis through the regulation of lipid oxidative damage. Studies have demonstrated that eckol, a polyphenolic compound found in Ecklonia stolonifera, exerts significant anti-hyperlipidemic effects by inhibiting HMG-CoA reductase activity, thereby reducing cholesterol biosynthesis. Additionally, eckol's potent antioxidant properties help mitigate oxidative stress, which is closely linked to inflammation and dyslipidemia [39]. These findings offer a mechanistic basis for the lipid-lowering and anti-inflammatory effects observed in our study. Moreover, Wang et al. highlighted the role of soy isoflavones and their metabolites, which are abundant in kefir soy milk, in regulating lipid metabolism and inflammation. Their study emphasized that soy isoflavones activate key molecular pathways, including AMP-activated protein kinase (AMPK) signaling, which enhances lipid catabolism and reduces lipogenesis. Additionally, the anti-inflammatory effects of soy isoflavones involve the suppression of nuclear factor kappa B (NF-κB) signaling, leading to decreased production of pro-inflammatory cytokines such as TNF-α and IL-6 [40]. By integrating these findings, we propose that the synergistic actions of eckol and kefir soy milk in EsV3 contribute to its observed effects on lipid metabolism and inflammation. The above results suggest that EsV3 prophylactic administration may help improve abnormalities in lipid markers and reduce plaque formation in the aorta. Overall, EsV3 had significant effects in inhibiting atherosclerosis.

We acknowledge the lack of a long-term efficacy assessment as a key limitation. While our study provides valuable insights into the short-term effects of EsV3. Future studies are necessary to evaluate the sustained efficacy and safety of EsV3 over extended periods. Additionally, we note other potential limitations, such as the relatively small sample size and the limited depth of mechanistic studies, highlighting the need for more comprehensive research to elucidate the mechanisms linking EsV3's bioactive components to the observed outcomes. These limitations underline the need for further research, including large-scale, long-term studies and mechanistic investigations, to confirm and extend our findings.

Conclusion

In this study, based on the successful construction of ApoE$^{-/-}$ hyperlipidemia model mice and atherosclerosis model mice, EsV3 preventive administration and therapeutic administration were carried out, blood lipid detection, body weight determination, aortic oil red staining and liver HE staining were performed. The study found that EsV3 administration groups were found to slow the development of hyperlipidemia, with significant effects on all indicators of lipid levels and an effect comparable to that of the positive drug atorvastatin. EsV3 administration groups also reduced aortic plaque formation, decreased hepatic lipid droplet production, and delayed weight gain. EsV3 has good preventive and therapeutic effects on hyperlipidemia and atherosclerotic-like lesions.

Abbreviations

AS	Atherosclerosis
ApoE	Apolipoprotein E
TC	Total cholesterol
TG	Triglycerides
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
P-EsV3-H	EsV3 high-dose prophylactic administration group
P-EsV3-L	EsV3 low-dose prophylactic administration group
EsV3-H	EsV3 high-dose therapeutic administration group
EsV3-L	EsV3 low-dose therapeutic administration group

Supplementary Information

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Supplementary Material 1.

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Not applicable.

Authors' contributions

Yaze Wang designed and performed the experiments and wrote the manuscript. Hifumi Ohishi and Rongji Wu supplied drugs. Hui Liu and Rong Xu revised the manuscript. All authors have read and approved the final manuscript. All authors reviewed the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All experimental protocols were approved by the Institutional Animal Care and Use Committee, Tongji Medical College, Huazhong University of Science and Technology (Approval number: WHYDSW20220930). All methods were carried out in accordance with relevant guidelines and regulations, and all methods were reported in accordance with ARRIVE guidelines.

Consent for publication

Not applicable.

Competing interests

Hifumi Ohishi reports being employee of HYDROX Co., Ltd. (Japan), and Rongji Wu reports being employee of Eiho Technology Co., Ltd. (China), which provided EsV3 for this study.

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References

1. Liu H, et al. Lycopene Reduces Cholesterol Absorption and Prevents Atherosclerosis in ApoE(-/-) Mice by Downregulating HNF-1α and NPC1L1 Expression. J Agric Food Chem. 2021;69(35):10114–20.

2. Song P, et al. Global and regional prevalence, burden, and risk factors for carotid atherosclerosis: a systematic review, meta-analysis, and modelling study. Lancet Glob Health. 2020;8(5):e721–9.
3. Minelli S, Minelli P, Montinari MR. Reflections on Atherosclerosis: Lesson from the Past and Future Research Directions. J Multidiscip Healthc. 2020;13:621–33.
4. Li L, et al. Recent advance in treatment of atherosclerosis: Key targets and plaque-positioned delivery strategies. J Tissue Eng. 2022;13:20417314221088508.
5. Jarcho JA, Keaney JF Jr. Proof That Lower Is Better–LDL Cholesterol and IMPROVE-IT. N Engl J Med. 2015;372(25):2448–50.
6. Pirillo A, et al. The Interplay of Lipids, Lipoproteins, and Immunity in Atherosclerosis. Curr Atheroscler Rep. 2018;20(3):12.
7. Mozaffarian D, Capewell S. United Nations' dietary policies to prevent cardiovascular disease. BMJ. 2011;343: d5747.
8. Hu C, Li L. The application of resveratrol to mesenchymal stromal cell-based regenerative medicine. Stem Cell Res Ther. 2019;10(1):307.
9. Sandoval-Gallegos EM, et al. In Vitro Bioaccessibility and Effect of Mangifera indica (Ataulfo) Leaf Extract on Induced Dyslipidemia. J Med Food. 2018;21(1):47–56.
10. Riccardi G, et al. Dietary recommendations for prevention of atherosclerosis. Cardiovasc Res. 2022;118(5):1188–204.
11. Demonty I, et al. Continuous dose-response relationship of the LDL-cholesterol-lowering effect of phytosterol intake. J Nutr. 2009;139(2):271–84.
12. Emini Veseli B, et al. Animal models of atherosclerosis. Eur J Pharmacol. 2017;816:3–13.
13. Kisoh K, et al. Estimating drug efficacy with a diet-induced NASH model in chimeric mice with humanized livers. Biomedicines. 2021;9(11):1647.
14. Aycan I, et al. Thymoquinone treatment against acetaminophen-induced hepatotoxicity in rats. Int J Surg. 2014;12(3):213–8.
15. Libby P, et al. Atherosclerosis Nat Rev Dis Primers. 2019;5(1):56.
16. Jayachandran M, Qu S. Harnessing hyperuricemia to atherosclerosis and understanding its mechanistic dependence. Med Res Rev. 2021;41(1):616–29.
17. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell. 2015;161(1):161–72.
18. Nordestgaard BG, Varbo A. Triglycerides and cardiovascular disease. Lancet. 2014;384(9943):626–35.
19. Rader DJ, Hovingh GK. HDL and cardiovascular disease. Lancet. 2014;384(9943):618–25.
20. Libby P. Mechanisms of acute coronary syndromes and their implications for therapy. N Engl J Med. 2013;368(21):2004–13.
21. Libby P. The changing landscape of atherosclerosis. Nature. 2021;592(7855):524–33.
22. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233–41.
23. Stone NJ, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014;63(25 Pt B):2889–934.
24. Ridker PM, Lüscher TF. Anti-inflammatory therapies for cardiovascular disease. Eur Heart J. 2014;35(27):1782–91.
25. Ridker PM, et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. N Engl J Med. 2017;377(12):1119–31.
26. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA. 2002;288(20):2569–78.
27. Mensink RP, et al. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. Am J Clin Nutr. 2003;77(5):1146–55.
28. Getz GS, Reardon CA. Animal models of atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32(5):1104–15.
29. Li TT, et al. The mechanisms of traditional Chinese medicine underlying the prevention and treatment of atherosclerosis. Chin J Nat Med. 2019;17(6):401–12.
30. Al-Khayri JM, et al. Flavonoids as potential anti-inflammatory molecules: a review. Molecules. 2022;27(9):2901.
31. Kim YC, et al. Hepatoprotective constituents of the edible brown alga Ecklonia stolonifera on tacrine-induced cytotoxicity in Hep G2 cells. Arch Pharm Res. 2005;28(12):1376–80.
32. Mitchell JH, Collins AR. Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men–a pilot study. Eur J Nutr. 1999;38(3):143–8.
33. Di Cagno R, et al. Synthesis of isoflavone aglycones and equol in soy milks fermented by food-related lactic acid bacteria and their effect on human intestinal Caco-2 cells. J Agric Food Chem. 2010;58(19):10338–46.
34. Liu JR, et al. Hypocholesterolaemic effects of milk-kefir and soyamilk-kefir in cholesterol-fed hamsters. Br J Nutr. 2006;95(5):939–46.
35. Fleischhauer FJ, Yan WD, Fischell TA. Fish oil improves endothelium-dependent coronary vasodilation in heart transplant recipients. J Am Coll Cardiol. 1993;21(4):982–9.
36. Klop B, Elte JW, Cabezas MC. Dyslipidemia in obesity: mechanisms and potential targets. Nutrients. 2013;5(4):1218–40.
37. Malekmohammad K, Bezsonov EE, Rafieian-Kopaei M. Role of Lipid Accumulation and Inflammation in Atherosclerosis: Focus on Molecular and Cellular Mechanisms. Front Cardiovasc Med. 2021;8: 707529.
38. Kattoor AJ, et al. Oxidative Stress in Atherosclerosis. Curr Atheroscler Rep. 2017;19(11):42.
39. Yoon NY, et al. Anti-hyperlipidemic effect of an edible brown algae, Ecklonia stolonifera, and its constituents on poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. Arch Pharm Res. 2008;31(12):1564–71.
40. Wang S, et al. Anti-obesity molecular mechanism of soy isoflavones: weaving the way to new therapeutic routes. Food Funct. 2017;8(11):3831–46.

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