Review Article

Efficacy of Terpenoid in Attenuating Aortic Atherosclerosis in Apolipoprotein-E Deficient Mice: A Meta-Analysis of Animal Studies

Han Liu,1 Yang Zhang,2 Siqiao Sun,2 and Shuai Wang1,2

1Department of Respiration, The First Hospital of Jilin University, Changchun, Jilin, China
2Department of Vascular Surgery, The First Hospital of Jilin University, Changchun, Jilin, China

Correspondence should be addressed to Shuai Wang; wang_shuai@jlu.edu.cn

Received 29 April 2019; Revised 10 June 2019; Accepted 17 June 2019; Published 17 July 2019

Academic Editor: Ahmed Abdel-Latif

Copyright © 2019 Han Liu et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. The apolipoprotein E knockout (ApoE -/-) mouse model is well established for the study of terpenoids in the prevention of atherosclerosis. Studies investigating the clinical benefit of terpenoids in humans are scarce. This systematic review and meta-analysis evaluated the effects of terpenoid administration on atherosclerotic lesion area in ApoE -/- mice. Methods. A comprehensive literature search using PubMed, Embase, and the Cochrane Library databases was performed to identify studies that assessed the effects of terpenoids on atherosclerosis in ApoE -/- mice. The primary outcome was atherosclerotic lesion area, and study quality was estimated using SYRCLE’s risk of bias tool. Results. The meta-analysis included 25 studies. Overall, terpenoids significantly reduced atherosclerotic lesion area when compared to vehicle control (P<0.00001; SMD: -0.55; 95% CI: -0.72, -0.39). In terpenoid type and dose subgroup analyses, sesquiterpenoid (P=0.002; SMD: -0.93; 95% CI: -1.52, -0.34), diterpenoid (P=0.01; SMD: -0.30; 95% CI: -0.54, -0.06), triterpenoid (P<0.00001; SMD: -0.66; 95% CI: -0.94, -0.39), tetraterpenoid (P<0.00001; SMD: -1.81; 95% CI: -2.70, -0.91), low dose (P=0.0001; SMD: -0.51; 95% CI: -0.76, -0.25), medium dose (P=0.0001; SMD: -0.48; 95% CI: -0.72, -0.24), and high dose (P=0.002; SMD: -1.07; 95% CI: -1.74, -0.40) significantly decreased atherosclerotic lesion area when compared to vehicle control. PROSPERO register number is CRD42019121176. Conclusion. Sesquiterpenoid, diterpenoid, triterpenoid, and tetraterpenoid have potential as antiatherosclerotic agents with a wide range of doses. This systematic review provides a reference for research programs aimed at the development of terpenoid-based clinical drugs.

1. Introduction

Atherosclerosis is the main cause of cardiovascular disease, which is the leading cause of death globally [1, 2]. Dyslipidemia and oxidative stress are relevant to the pathogenesis of atherosclerosis [3, 4]. Therefore, statin-based lipid-modifying therapies, such as atorvastatin and rosuvastatin, are effective for lowering blood cholesterol levels and providing clinical benefits in patients with cardiovascular disease. However, the morbidity and mortality associated with atherosclerosis remain high [5], and there is an urgent unmet clinical need for novel prevention and treatment strategies [6].

In recent years, studies have shown that natural compounds, such as flavonoids, alkaloids, and terpenoids, attenuate atherosclerosis [7–9]. Terpenoids are a large and diverse class of naturally occurring organic chemicals that are similar to terpenes. Most terpenoids are multicyclic structures with oxygen-containing functional groups. Furthermore, terpenoids have a wide range of pharmacological effects, including antitumor, anti-inflammatory, antiatherosclerotic, and antimalarial activities [10–12]. The majority of studies on the antiatherosclerotic effects of terpenoids have focused on paclitaxel, [13, 14] which is a natural diterpene, and consensus on the antiatherosclerotic effects of other terpenoids has not been reached.

Studies in animals allow for initial investigations on the safety and efficacy of new interventions and provide an important link between basic research and clinical trials. The apolipoprotein E knockout (ApoE -/-) mouse model spontaneously develops atherosclerotic plaques and
is commonly used to mimic the pathophysiological process of atherosclerosis in humans [15, 16]. The present systematic review and meta-analysis evaluated the effects of terpenoid administration on atherosclerotic lesion area in ApoE -/- mice, in an effort to understand the clinical potential of terpenoids as antiatherosclerotic agents.

2. Materials and Methods

2.1. Reporting Standards. This systematic review complies with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The systematic review protocol was prepared using the SYRCLE format for animal intervention studies [17, 18].

2.2. Search Strategy. An experienced information specialist (HL) searched the PubMed, Embase, and Cochrane Library databases from January 2001 to December 2018 using the keywords: “atherosclerosis,” “atherogenesis,” “apolipoproteins e,” “apoE,” “mice,” and “terpenoid” and the following search strategies: (atherosclerosis OR atherogenesis) AND (“apolipoprotein e” OR apoE) AND (mice OR mouse) AND (terpenoid OR hemiterpenoid OR monoterpenoid OR sesquiterpenoid OR diterpenoid OR sesterterpenoid OR triterpenoid OR tetraterpenoid OR polyterpenoid). The reference lists of included and review articles were manually searched to identify additional relevant studies. The search was performed on December 10, 2018, and was restricted to articles published in the English language.

2.3. Inclusion and Exclusion Criteria. Inclusion criteria are as follows: (1) study design: original research; (2) animal model: ApoE -/- mice; (3) disease model: atherosclerosis; (4) intervention: terpenoids. Exclusion criteria are as follows: (1) case reports, conference abstracts, review articles, and editorials, (2) missing data, or (3) overlapping or duplicate datasets.

2.4. Study Selection. Two reviewers (YZ and SS) independently examined the titles and abstracts of the articles identified by the literature search to select eligible studies. The full text of potentially relevant articles was retrieved and independently examined by two reviewers (YZ and SW) to determine whether these studies met the inclusion criteria. Disagreements on study selection were resolved by discussion and consensus.

2.5. Data Extraction. Two reviewers (HL and SW) independently extracted data from eligible studies, including the first author’s name, publication year, age of mice, gender, diet, terpenoid dose, duration and route of treatment, control and treatment group sample sizes, location of the atherosclerotic lesion, stain used to assess the atherosclerotic lesion, and atherosclerotic lesion area. Data that were presented graphically in the original publications were extracted using Adobe Photoshop 7.0.

The primary outcome was atherosclerotic lesion area measured as a percentage or a numerical value.

Disagreements on data extraction were resolved by discussion and consensus.

2.6. Quality Assessment. Two investigators (SS and SW) independently assessed the quality of the included studies using SYRCLE’s risk of bias tool, which contains domains evaluating sequence generation, baseline characteristics, allocation concealment, random housing, blinding, random and selective outcome assessments, incomplete outcomes data, and other sources of bias [44]. Publication bias was detected by visual inspection of funnel plots.

Disagreements on quality assessment were resolved by discussion and consensus.

2.7. Data Synthesis and Statistical Analysis. Statistical analyses were performed using Review Manager (RevMan Version 5.3 for Windows Copenhagen: The Nordic Cochrane Centre, The Cochrane Collaboration, 2014). Standardized mean differences (SMD) with 95% confidence intervals (CI) were calculated to reflect the effects of terpenoids or vehicle control on atherosclerotic lesion area. A random-effects model was used to pool studies. Heterogeneity was determined as moderate ($I^2 ≥ 30\%$) or high ($I^2 ≥ 50\%$) using the inconsistency index.

Multiple independent groups in a study (e.g., different terpenoid doses) were considered separate datasets. In eleven studies [19, 20, 24, 26, 28, 33, 34, 38–40, 42], multiple groups that tested different terpenoid doses were compared to a single control group. In order to avoid an artificial increase in sample size in the pooled analysis, the number of animals in the control group for each study was divided by the number of comparator groups.

Subanalyses were conducted to investigate the effects of sesquiterpenoid, diterpenoid, triterpenoid, and tetraterpenoid on the atherosclerotic lesion area.

Sensitivity analyses were conducted to determine whether the findings were robust. $P<0.05$ was considered statistically significant.

3. Results

3.1. Study Selection. The search identified 1,032 articles. Titles and abstracts were screened, and 40 studies were considered potentially eligible for inclusion. After evaluating full-text articles, nine studies were excluded, because outcomes data were not reported [45–53], and six studies were excluded, because multiple interventions were assessed [54–59]. Finally, 25 studies were included in the present meta-analysis [19–43] (Figure 1).

3.2. Study Characteristics. The characteristics of the 25 included studies are described in Table 1. These studies provided 59 datasets and involved 707 animals.

Three studies used sesquiterpenoid as the intervention [19–21], nine studies used diterpenoid as the intervention [22–30], ten studies used triterpenoid as the intervention [31–40], and three studies used tetraterpenoid as the intervention [41–43].
| Study                        | Terpenoid                  | Age | Sex  | Diet  | Study length | Dose | Route | Location of lesion area | Analysis                        | Staining  | Groups and sample size |
|-----------------------------|----------------------------|-----|------|-------|--------------|------|-------|-------------------------|---------------------------------|-----------|------------------------|
| Jiang et al., 2016 [19]     | Sesquiterpenoid, artesunate| 4W  | ?    | HFD   | 24W          | 1.5 mg/kg/d, 5 mg/kg/d, 15 mg/kg/d | i.p. | Thoracic aorta | En face (longitudinal)           | Oil red O | Control=6, Sesquiterpenoid low=6, Sesquiterpenoid medium=6, Sesquiterpenoid high=6 |
| Lopez-Franco et al., 2006 [20] | Sesquiterpenoid, parthenolide | 12W | Male | HFD   | (1) 10W (2) 20W | 4 mg/kg/d, 10 mg/kg/d, 2 mg/kg/d | i.p. | Aortic root | Cross-sectional               | Oil red O | Control=6, 6, Sesquiterpenoid low=4, Sesquiterpenoid medium=9, Sesquiterpenoid high=8 |
| Wang et al., 2016 [21]      | Sesquiterpenoid, patchouli | 8W  | Female | HFD | 10W          | 40 mg/kg/d | IG   | (1) Thoracic and abdominal aorta (2) Aortic root | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=10, 10, Sesquiterpenoid treatment=10, 10 |
| Li et al., 2018 [22]        | Diterpenoid, pseudolaric acid B | 8W  | Male | HFD   | 4W           | 5 mg/kg/d | IG   | (1) Thoracic and abdominal aorta (2) Aortic root | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=12, 12, Diterpenoid treatment=12, 12 |
| Liu et al., 2012 [23]       | Diterpenoid, ginkgolide B  | 8W  | Male | HFD   | 8W           | 20 mg/kg/d | IG   | (1) Thoracic aorta (2) Aortic sinus | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=3, 10, Diterpenoid treatment=3, 10 |
| Liu et al., 2015 [24]       | Diterpenoid, cryptotanshinone | 6W  | Male | HFD   | 16W          | 15 mg/kg/d, 45 mg/kg/d | IG   | Aortic root to iliac branches (2) Aortic sinus | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=6, 6, Diterpenoid low=6, 6, Diterpenoid medium=6, 6, Diterpenoid high=6, 6 |
| Steffens et al., 2005 [25]  | Diterpenoid, delta-9-tetrahydrocannabinol | 10W | Male | HFD   | 6W           | 1 mg/kg/d | IG   | Aortic root | Cross-sectional | Sudan IV | Control=8, Diterpenoid treatment=6 |
| Tang et al., 2011 [26]      | Diterpenoid, tanshinone IIa | 6W  | Male | NCD   | 20W          | 10 mg/kg/d, 30 mg/kg/d, 90 mg/kg/d | IG   | (1) Aortic arch (2) Aortic root | (1) En face (longitudinal) (2) Cross-sectional | (1) Oil red O (2) HE | Control=10, 10, Diterpenoid low=10, 10, Diterpenoid medium=10, 10, Diterpenoid high=10, 10 |
| Study | Terpenoid | Age | Sex | Diet | Study length | Dose Route | Location of lesion area | Analysis | Staining | Groups and sample size |
|-------|-----------|-----|-----|------|--------------|------------|-------------------------|---------|----------|-----------------------|
| Xu et al., 2011 [27] | Diterpenoid, tanshinone IIA | 6W | Male | HCD | 16W | 10 mg/kg/d, 30 mg/kg/d | IG | (1) Aortic root to iliac branches (2) Aortic sinus | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=6, 6 Diterpenoid low=6, 6 Diterpenoid high=6, 6 |
| Xu et al., 2012 [28] | Diterpenoid, tanshinone IIA | 6W | Male | HCD | 16W | 30 mg/kg/d | IG | Aortic roots | Cross-sectional | Oil red O | Control=6 Diterpenoid treatment=6 |
| Zhao et al., 2016 [29] | Diterpenoid, tanshinone IIA | 8W | Male | HFD | 8W | 30 mg/kg/d | IG | Thoracic aorta | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=8, 8 Diterpenoid treatment=9, 8 |
| Zhou et al., 2015 [30] | Diterpenoid, retinoic acid | 8W | Male | HFD | 8W | 2 mg/kg/d | i.p. | Aortic sinus | Cross-sectional | Oil red O | Control=10 Diterpenoid treatment=10 |
| Buus et al., 2011 [31] | Triterpenoid, oleanolic acid | 12W | Male | HFD | 8W | 100 mg/kg/d | IG | Aortic root to thoracic aorta | (1) Aortic root to abdominal aorta (2) Aortic root | (1) En face (longitudinal) (2) Cross-sectional | Oil red O | Control=8, 8 Triterpenoid =10 |
| Chen et al., 2012 [32] | Triterpenoid, corosolic acid | 8W | Male | HFD | 12W | 10 mg/kg/d | Chow | (1) Aortic root to abdominal aorta (2) Aortic root | (1) En face (longitudinal) (2) Cross-sectional | (1) Sudan IV (2) HE | Control=8, 8 Triterpenoid =8, 8 |
| Gu et al., 2013 [33] | Triterpenoid, celastrol | 8W | Male | HFD | 4W | 1 mg/kg/d, 2 mg/kg/d | i.p. | Aortic sinus | Cross-sectional | Oil red O | Control=5 Triterpenoid low=5 Triterpenoid high=5 |
| Gui et al., 2016 [34] | Triterpenoid, betulin | 6W | Male | HFD | 12W | 20 mg/kg/d, 40 mg/kg/d | Chow | (1) Aortic root to iliac branches (2) Aortic sinus | (1) En face (longitudinal) (2) Cross-sectional | (1) Sudan IV (2) Oil red O | Control=6, 6 Triterpenoid low=8, 8 Triterpenoid high=6, 6 |
| Jia et al., 2014 [35] | Triterpenoid, notoginsenoside R1 | 9W | Male | HFD | 8W | 25 mg/kg/d | i.p. | Aortic root | Cross-sectional | HE | Control=9 Triterpenoid treatment=9 |
| Li et al., 2011 [36] | Triterpenoid, ginsenoside-Rd | 6W | ? | HFD | 12W | 20 mg/kg/d | i.p. | Aortic root to iliac branches | En face (longitudinal) | Oil red O | Control=10 Triterpenoid=10 |
### Table 1: Continued.

| Study                     | Terpenoid                        | Age | Sex | Diet   | Study length | Dose          | Route | Location of lesion area | Analysis                        | Staining     | Groups and sample size |
|---------------------------|----------------------------------|-----|-----|--------|--------------|---------------|-------|------------------------|---------------------------------|-------------|------------------------|
| Liu et al., 2016          | Triterpenoid, ilexgenin A        | 8W  | Male| HFD    | 16W          | 12 mg/kg/d    | Chow  | Aortic sinus           | Cross-sectional                 | HE          | Control=10 Triterpenoid=10 |
| Messner et al., 2011      | Triterpenoid, ursolic acid       | 8W  | Male| HFD    | 24W          | 0.9 mg/kg/d   | Drink | Aortic root to iliac branches | En face (longitudinal) | Sudan IV | Control=5 Triterpenoid low=4 Triterpenoid high=5 |
| Tang et al., 2018         | Triterpenoid, celosins           | 12W | Male| HFD    | 4W           | 10 mg/kg/d    | Chow  | Aortic root to iliac branches | En face (longitudinal) | Oil red O | Control=9 Triterpenoid low=9 Triterpenoid medium=10 Triterpenoid high=10 |
| Zhou et al., 2016         | Triterpenoid, compound K         | 8W  | Male| HFD    | 8W           | 1 mg/kg/d     | i.p.   | (1) Aortic root to abdominal aorta (2) Aortic root | (1) En face (longitudinal) (2) Cross-sectional | Oil red O (1) HE | Control=6,6 Triterpenoid low=6,6 Triterpenoid medium=6,6 Triterpenoid high=6,6 |
| Dwyer et al., 2001        | Tetraterpenoid, lutein           | 12W | Female| NCD    | 8W           | 2000 mg/kg/d  | Chow  | Aortic root            | Cross-sectional                  | Oil red O | Control=7 Tetraterpenoid=9 |
| Han et al., 2015          | Tetraterpenoid, lutein           | 8W  | Male| HFD    | 24W          | 25 mg/kg/d    | Chow  | Aortic sinus           | Cross-sectional                  | Oil red O | Control=3 Tetraterpenoid low=3 Tetraterpenoid medium=3 Tetraterpenoid high=3 |
| Zou et al., 2017          | Tetraterpenoid, astaxanthin      | 6W  | Male| NCD    | 12W          | 500 mg/kg/d   | Chow  | Aortic sinus           | Cross-sectional                  | Oil red O | Control=6 Tetraterpenoid treatment=6 |

Note: NCD, normal-chow diet; HCD, high-cholesterol diet; HFD, high-fat diet; IG, intragastric; i.p., intraperitoneal injection; HE, hematoxylin and eosin; ? = not reported.
Terpenoid administration was initiated in 4-week-old mice in one study [19], in 6-week-old mice in seven studies [24, 26–28, 34, 36, 43], in 8-week-old mice in ten studies [21–23, 29, 30, 32, 33, 37, 38, 42], in 9-week-old mice in one study [35], in 10-week-old mice in two studies [25, 40], and in 12-week-old mice in four studies [20, 31, 39, 41].

The duration of terpenoid treatment varied from four weeks to 24 weeks.

Route of administration of terpenoid treatment was in drinking water in one study [38] and in the chow in seven studies [32, 34, 37, 39, 41–43], via an intragastric route in ten studies [21–29, 31] and via intraperitoneal injection in seven studies [19, 20, 30, 33, 35, 36, 40].

Terpenoid doses varied among different studies. It mainly ranged from 1 to 100 mg/kg/d. In addition, 500 and 2000 mg/kg/d were used in two studies [41, 43].

All studies reported an aortic-root or -sinus lesion area. Furthermore, ten studies [20, 25, 27, 30, 33, 35, 37, 41–43] reported cross-sectional aortic lesion area, five studies [19, 31, 36, 38, 39] reported longitudinal aortic lesion area (Table 1), and ten studies [21–24, 26, 28, 29, 32, 34, 40] reported both cross-sectional and longitudinal aortic lesion areas.

3.3. Quality Assessment. Assessment of study quality is presented in Figure 2. A total of 19 (73.1%) studies were randomized, but the risks of bias due to allocation concealment and blinding were unclear. Sixteen studies had no missing outcomes data. The risk of selective outcomes reporting was unclear in nine studies. Across studies, the risk of bias from other sources was low.

Visual inspection of a funnel plot revealed substantial publication bias (Figure 3).

3.4. Effect of Terpenoids on Atherosclerotic Lesion Area. The effect of terpenoids on atherosclerotic lesion area was reported for 59 datasets obtained from 25 studies (n=434, ApoE-/- mice administered terpenoid; n=273, ApoE-/- mice administered vehicle control). The meta-analysis demonstrated that overall terpenoids significantly reduced atherosclerotic lesion area when compared to vehicle control (P<0.00001; SMD: -0.55; 95% CI: -0.72, -0.39). There was no evidence of heterogeneity between studies (I²=0%; Figures 4 and 5).

Subgroup analyses were conducted to investigate the effects of terpenoid type and dose on atherosclerotic lesion area. In terpenoid type subgroup analyses, sesquiterpenoid (n=59, ApoE-/- mice administered sesquiterpenoid; n=38, ApoE-/- mice administered vehicle control) significantly reduced atherosclerotic lesion area when compared to vehicle control (P=0.002; SMD: -0.93; 95% CI: -1.52, -0.34); there was evidence of moderate heterogeneity between studies (I²=31%). Diterpenoid (n=184, ApoE-/- mice administered diterpenoid; n=121, ApoE-/- mice administered vehicle control) significantly reduced atherosclerotic lesion area when compared to vehicle control (P=0.01; SMD: -0.30; 95% CI: -0.54, -0.06), triterpenoid (n=167, ApoE-/- mice administered triterpenoid; n=98, ApoE-/- mice administered vehicle control; P=0.00001; SMD: -0.66; 95% CI: -0.94, -0.39), and tetraterpenoid (n=24, ApoE-/- mice administered tetraterpenoid; n=16, ApoE-/- mice administered vehicle control) significantly reduced atherosclerotic lesion area when compared to vehicle control (P<0.0001; SMD: -0.54; 95% CI: -0.74, -0.34).

Two studies used female animals [21, 41], 21 studies used male animals [20, 22–35, 37–40, 42, 43], and the gender of the animals was not reported in two studies [19, 36].

Mice received normal chow diet in three studies [26, 41, 43], a high-cholesterol diet in two studies [27, 28], and a high-fat diet in 20 studies [19–25, 29–40, 42].
Random sequence generation (selection bias)
Allocation concealment (selection bias)
Blinding of participants and personnel (performance bias)
Blinding of outcome assessment (detection bias)
Incomplete outcome data (attrition bias)
Selective reporting (reporting bias)
Other biases

Low risk of bias
Unclear risk of bias
High risk of bias

Figure 2: Risk of bias and quality assessment score (%) for studies included in the meta-analysis.

Sensitivity analysis that substituted the fixed effect model for the random effects model did not change the overall findings (SMD -0.55 (-0.72, -0.39) vs. -0.58 (-0.69, -0.46) and SMD -0.55 (-0.72, -0.39) vs. -0.57 (-0.68, -0.45)).

4. Discussion

The use of animal models provides a valuable approach to preclinical research, which informs treatment strategies for human diseases. Previous evidence from experiments in animals suggests that natural terpenoids have potential benefits for the treatment of atherosclerosis. However, parameters such as type and age of animal, sample size, housing conditions, and length of follow-up vary across studies. A synthesis and quantitative analysis of the data from animal models that accounts for these sources of heterogeneity may provide insight into the benefits of terpenoids as clinically desirable therapeutic agents in atherosclerosis. Therefore, we performed this systematic review and meta-analysis to evaluate the effects terpenoid administration on atherosclerotic lesion area in ApoE -/- mice. Findings showed that terpenoid administration significantly reduced aortic atherosclerosis lesion area compared to vehicle control.

The terpenoid family constitutes several members, including hemiterpenoid, monoterpenoid, sesquiterpenoid, diterpenoid, sesterterpenoid, triterpenoid, tetraterpenoid, and polyterpenoid [60]. In subanalyses stratified by number of isoprene groups, sesquiterpenoid, diterpenoid, triterpenoid, and tetraterpenoid significantly reduced aortic atherosclerosis lesion area compared to vehicle control in ApoE -/- mice.

To the authors’ knowledge, this systematic review and meta-analysis is the first to evaluate the effects of terpenoids on atherosclerosis in ApoE -/- mice. Findings are expected to provide a scientific basis for clinical trials of terpenoids in cardiovascular diseases.

There was no heterogeneity between studies in the overall analysis, but there was a moderate degree of heterogeneity between studies in the analysis of sesquiterpenoid and high dose group. Potential sources of heterogeneity include age
Figure 4: A forest plot of the effects of different terpenoids types on atherosclerotic lesion area. Subgroup analyses evaluated the effects of sesquiterpenoid, diterpenoid, triterpenoid, and tetraterpenoid. SD, standard deviation; CI, confidence interval; Std, standard; IV, inverse variance.
Figure 5: A forest plot of the effects of different terpenoids doses on atherosclerotic lesion area. Subgroup analyses evaluated the effects of low dose (≤10 mg/kg/d), medium dose (>10 mg/kg/d, ≤50 mg/kg/d), and high dose (>50 mg/kg/d). SD, standard deviation; CI, confidence interval; Std, standard; IV, inverse variance.
and sex of mice and diet administered, each of which can influence the progression of atherosclerosis [1, 61]. Furthermore, method of measurement can affect the assessment. Ten studies reported cross-sectional aortic lesion area, five studies reported longitudinal aortic lesion area, and ten studies reported both cross-sectional and longitudinal aortic lesion area.

**Study Limitations.** This meta-analysis was associated with several limitations. First, the relevance of our findings to humans is limited by species specific differences in lipoprotein metabolism and vascular physiology [62]. The ApoE -/- mouse model is well established for studying atherosclerosis, and the principal characteristics and progression of atherosclerosis in ApoE -/- mice and human subjects appear similar [15,16]; however, there are differences in pathogenesis. Specifically, the location of the atherosclerotic plaque may differ due to variations in heart rate, blood pressure, and hemodynamics. Atherosclerotic plaque builds up in the root of the aorta and in the brachiocephalic artery in ApoE -/- mice and the coronary artery, the carotid artery, the iliac artery, and the arteries of the lower limb in humans. Second, age is a risk factor in progression of atherosclerosis; therefore, due to differences in life cycle, the natural history of atherosclerotic disease in mice cannot be directly translated to humans. Third, patients usually present to the clinic with advanced atherosclerosis, but the included studies administered terpenoids to mice before disease had progressed and were therefore evaluating early prevention rather than benefit of treatment in advanced disease. Fourth, other animal models of atherosclerosis, such as LDLR -/- mice, rabbits, and hamsters, were not included in this meta-analysis. Fifth, only one parameter, atherosclerotic lesion area, was used to evaluate the effects of terpenoids on atherosclerosis. Other parameters, such as low density lipoprotein (LDL), high density lipoprotein (HDL), total cholesterol (TC), triglycerides (TG), and body mass index (BMI), were not considered. [63] Sixth, the sample sizes of some included studies were relatively small. Seventh, hemiterpenoid, monoterpenoid, and polyterpenoid were not assessed in the analysis. Further investigations in animal models using larger sample sizes are warranted to determine if terpenoids are beneficial for the treatment of atherosclerosis in humans.

The present meta-analysis revealed that terpenoid administration is effective for attenuating aortic atherosclerosis in ApoE -/- mice. In particular, sesquiterpenoid, diterpenoid, triterpenoid, and tetraterpenoid have a potential therapeutic effect with a wide range of doses. Large scale, prospective, and well-designed animal studies are needed to enhance our knowledge of the mechanism of terpenoids for the treatment of atherosclerosis. Randomized controlled trials in humans are required to confirm that terpenoids have clinical benefit as antiatherosclerotic agents.

**Data Availability**

The data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Conflicts of Interest**

The authors declare that they have no conflicts of interest.

**Authors’ Contributions**

Han Liu conducted the analysis and wrote the manuscript; Yang Zhang and Siqiao Sun collected and performed a preliminary analysis of references; Shuai Wang revised the manuscript and approved it for submission.

**References**

[1] D. Mozaffarian, J. Benjamin, S. Go et al., “Heart disease and stroke statistics-2015 update: a report from the American Heart Association,” Circulation, vol. 131, no. 4, pp. 29–322, 2015.

[2] Z. Yang, J. Liu, J. Ge, L. Chen, Z. Zhao, and W. Yang, “Prevalence of cardiovascular disease risk factor in the Chinese population: the 2007-2008 China National Diabetes and Metabolic Disorders Study,” European Heart Journal, vol. 33, no. 2, pp. 213–220, 2012.

[3] V. M. Victor, M. Rocha, E. Solá, C. Bañuls, K. García-Malpartida, and A. Hernández-Mijares, “Oxidative stress, endothelial dysfunction and atherosclerosis,” Current Pharmaceutical Design, vol. 15, no. 26, pp. 2988–3002, 2009.

[4] S. Koba and T. Hirano, “Dyslipidemia and atherosclerosis,” Nihon Rinsho, vol. 69, pp. 138–143, 2011.

[5] J. Logue, H. M. Murray, P. Welsh et al., “Obesity is associated with fatal coronary heart disease independently of traditional risk factors and deprivation,” Heart, vol. 97, no. 7, pp. 564–568, 2011.

[6] T. J. Ford, D. Corcoran, and C. Berry, “Stable coronary syndromes: Pathophysiology, diagnostic advances and therapeutic need,” Heart, vol. 104, no. 4, pp. 284–292, 2018.

[7] A. Pirillo and A. L. Catapano, “Berberine, a plant alkaloid with lipid- and glucose-lowering properties: from in vitro evidence to clinical studies,” Atherosclerosis, vol. 243, no. 2, pp. 449–461, 2015.

[8] L. Xiao, L. Liu, X. Guo et al., “Quercetin attenuates high fat diet-induced atherosclerosis in apolipoprotein E knockout mice: A critical role of NADPH oxidase,” Food and Chemical Toxicology, vol. 105, pp. 22–33, 2017.

[9] X. Zhang, M. Liu, L. Qiao et al., “Ginsenoside Rb1 enhances atherosclerotic plaque stability by skewing macrophages to the M2 phenotype,” Journal of Cellular and Molecular Medicine, vol. 22, no. 1, pp. 409–416, 2018.

[10] L. Cao, X. Zhang, F. Cao et al., “Inhibiting inducible miR-223 further reduces viable cells in human cancer cell lines MCF-7 and PC3 treated by celastrol,” BMC Cancer, vol. 15, no. 1, article 873, 2015.

[11] R. Gallily, Z. Yekhtin, and L. O. Hanuš, “The anti-inflammatory properties of terpenoids from cannabis,” Cannabis and Cannabinoid Research, vol. 3, no. 1, pp. 282–290, 2018.

[12] Z. Shi, Y. Chen, C. Lu et al., “Resolving neuroinflammation, the therapeutic potential of the anti-inflammatory drug family of artemisinin,” Pharmacological Research, vol. 136, pp. 172–180, 2018.

[13] M. Brodmann, K. Keirse, D. Scheinert et al., “Drug-coated balloon treatment for femoropopliteal artery disease: the IN.PACT global study de novo in-stent restenosis imaging cohort,” JACC: Cardiovascular Interventions, vol. 10, no. 20, pp. 2113–2123, 2017.
O. López-Franco, P. Hernández-Vargas, G. Ortiz-Muñoz et al., “Tanshinone II A attenuates vascular inflammation in ApoE−/− mice fed a high cholesterol diet,” Archives of Biochemistry and Biophysics, vol. 515, no. 1-2, pp. 72–79, 2011.

D. Zhao, L. Tong, L. Zhang, H. Li, Y. Wan, and T. Zhang, “Tanshinone II A stabilizes vulnerable plaques by suppressing RAGE signaling and NF-κB activation in apolipoprotein-E-deficient mice,” Molecular Medicine Reports, vol. 14, no. 6, pp. 4983–4990, 2016.

W. Zhou, J. Lin, H. Chen, J. Wang, Y. Liu, and M. Xia, “Renin acid induces macrophage cholesterol efflux and inhibits atherosclerotic plaque formation in apoE-deficient mice,” British Journal of Nutrition, vol. 114, no. 4, pp. 509–518, 2015.

N. H. Buis, N. C. Hansson, R. Rodriguez-Rodriguez, E. Stankovic, M. R. Andersen, and U. Simonsen, “Antiatherogenic effects of oleanolic acid in apolipoprotein E knockout mice,” European Journal of Pharmacology, vol. 670, no. 2-3, pp. 319–526, 2011.

H. Chen, J. Yang, Q. Zhang, L. Chen, and Q. Wang, “Cocorosic acid ameliorates atherosclerosis in apolipoprotein E-deficient mice by regulating the nuclear factor-κB signaling pathway and inhibiting monocyte chemoattractant protein-1 expression,” Circulation Journal, vol. 76, no. 4, pp. 993–1003, 2012.

L. Gu, W. Bai, S. Li et al., “Celasol prevents atherosclerosis via inhibiting LOX-1 and oxidative stress,” PLoS ONE, vol. 8, no. 6, Article ID e65477, 2013.

Y.-Z. Gui, H. Yan, F. Gao, C. Xi, H.-H. Li, and Y.-P. Wang, “Betulin attenuates atherosclerosis in apoE−/− mice by up-regulating ABCA1 and ABCG1,” Acta Pharmacologica Sinica, vol. 37, no. 10, pp. 1337–1348, 2016.

C. Jia, M. Xiong, P. Wang et al., “Notoginsenoside R1 attenuates atherosclerotic lesions in ApoE deficient mouse model,” PLoS ONE, vol. 9, no. 6, Article ID e99849, 2014.

J. Li, Z. Xie, Y. Tang, J. Zhou, and Y. Guan, “Ginsenosides-Rd, a purified component from panax notoginseng saponins, prevents atherosclerosis in apoE knockout mice,” European Journal of Pharmacology, vol. 652, no. 1-3, pp. 104–110, 2011.

C. Liu, J. Zhao, Y. Liu et al., “A novel pentacyclic triterpenoid, ilxegnin A, shows reduction of atherosclerosis in apolipoprotein E deficient mice,” International Immunopharmacology, vol. 40, pp. 115–124, 2016.

B. Messner, I. Zeller, C. Ploner et al., “Ursolic acid causes DNA damage, P53-mediated, mitochondria- and caspase-dependent human endothelial cell apoptosis, and accelerates atherosclerotic plaque formation in vivo,” Atherosclerosis, vol. 219, no. 2, pp. 402–408, 2011.

Y. Tang, H. Wu, B. Shao, Y. Wang, C. Liu, and M. Guo, “Celosins inhibit atherosclerosis in ApoE−/− mice and promote autophagy flow,” Journal of Ethnopharmacology, vol. 215, pp. 74–82, 2017.

L. Zhou, Y. Zheng, Z. Li et al., “Compound K attenuates the development of atherosclerosis in ApoE−/− mice via LXRx activation,” International Journal of Molecular Sciences, vol. 17, no. 7, Article ID E1054, 2016.

J. H. Dwyer, M. Navab, K. M. Dwyer et al., “Oxigenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study,” Circulation, vol. 103, no. 24, pp. 2922–2927, 2001.

H. Han, W. Cui, L. Wang et al., “Lutein prevents high fat diet-induced atherosclerosis in ApoE-deficient mice by inhibiting NADPH oxidase and increasing PPAR expression,” Lipids, vol. 50, no. 3, pp. 261–273, 2015.

T. Zou, S. Zhu, F. Luo et al., “Effects of astaxanthin on reverse cholesterol transport and atherosclerosis in mice,” BioMed
[44] C. R. Hooijmans, M. M. Rovers, R. B. M. De Vries, M. Leenaars, M. Ritskes-Hoitinga, and M. W. Langendam, “SYRCLE’s risk of bias tool for animal studies,” *BMC Medical Research Methodology*, vol. 14, no. 1, article no. 43, 2014.

[45] Y. Bao, L. Wang, Y. Xu et al., “Salvianolic acid B inhibits macrophage uptake of modified low density lipoprotein (mLDL) in a scavenger receptor CD36-dependent manner,” *Atherosclerosis*, vol. 223, no. 1, pp. 152–159, 2012.

[46] S. Bechor, N. Zolberg Relevy, A. Harari et al., “9-cis-β-carotene increased cholesterol efflux to HDL in macrophages,” *Nutrients*, vol. 8, no. 7, article 435, 2016.

[47] P. Fernández-Robredo, L. M. Sádaba, A. Salinas-Alamán, S. Recalde, J. A. Rodríguez, and A. García-Layana, “Effect of lutein and antioxidant supplementation on VEGF expression, MMP-2 activity, and ultrastructural alterations in apolipoprotein E-deficient mouse,” *Oxidative Medicine and Cellular Longevity*, vol. 2013, Article ID 213505, 11 pages, 2013.

[48] A. J. Guri, S. A. Misyak, R. Hontecillas et al., “Abscisic acid through the ERK signaling pathway,” *Biochemistry*, vol. 70, no. 9, pp. 1298–1308, 2005.

[49] A. Katsumata, M. Kimura, H. Saigo et al., “Changes in esculeoside a content in different regions of the tomato fruit during maturation and heat processing,” *Journal of Agricultural and Food Chemistry*, vol. 59, no. 8, pp. 4104–4110, 2011.

[50] Q. Li, W. Zhao, X. Zeng, and Z. Hao, “Ursolic acid attenuates atherosclerosis in ApoE−/− mice: role of LOX-1 mediated by ROS/NF-kappaB pathway,” *Molecules*, vol. 23, no. 5, Article ID E1101, 2018.

[51] X. Liu, C. Y. Guo, X. J. Ma et al., “Anti-inflammatory effects of tanshinone IIA on atherosclerotic vessels of ovariecetomized ApoE−/− mice are mediated by estrogen receptor activation and through the ERK signaling pathway,” *Cellular Physiology and Biochemistry*, vol. 35, no. 5, pp. 1744–1755, 2015.

[52] J. J. Yoon, Y. J. Lee, B. H. Han et al., “Protective effect of betulinic acid on early atherosclerosis in diabetic apolipoprotein-E gene knockout mice,” *European Journal of Pharmacology*, vol. 796, pp. 224–232, 2017.

[53] A. Harari, R. Abecassis, N. Relevy et al., “Prevention of atherosclerosis progression by 9-cis-β-carotene rich alga dunaliella in apoE-deficient mice,” *BioMed Research International*, vol. 2013, Article ID 169517, 7 pages, 2013.

[54] G. Liu, B. Wang, J. Zhang, H. Jiang, and F. Liu, “Total panax notoginsenosides prevent atherosclerosis in apolipoprotein E-knockout mice: role of downregulation of CD40 and MMP-9 expression,” *Journal of Ethnopharmacology*, vol. 126, no. 2, pp. 350–354, 2009.

[55] N. Relevy, D. Harats, A. Harari et al., “Vitamin A-deficient diet accelerated atherogenesis in apolipoprotein E−/− mice and dietary β-carotene prevents this consequence,” *BioMed Research International*, vol. 2015, Article ID 758723, 9 pages, 2015.

[56] D. W. Christianson, “Structural and chemical biology of terpenoid cyclases,” *Chemical Reviews*, vol. 117, no. 17, pp. 11570–11648, 2017.

[57] A. Zmysłowski and A. Szterk, “Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols,” *Lipids in Health and Disease*, vol. 16, no. 1, article 188, 2017.