

Anti-atherosclerotic potentials of *TD.HLM01* herbal combination against lipid disorders induced by high-fat diet in rabbits

[Potenciales anti-ateroscleróticos de la combinación de hierbas *TD.HLM01* contra los trastornos lipídicos inducidos por una dieta rica en grasas en conejos]

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Abstract

Context: Lipoprotein disorders and atherosclerosis are becoming increasingly common worldwide, especially in low- and middle-income countries. Lipid-lowering drugs such as statins have limitations in treating atherosclerosis due to adverse effects, including increased liver enzymes and rhabdomyolysis. Nature-derived products that regulate dyslipidemia and anti-atherosclerosis of herbal origins are currently of great interest. *TD.HLM01* is an herbal product containing dried medicinal extracts such as *Atractylodes macrocephala* Koidz., *Melicope pteleifolia* (Champ. ex Benth.) T.G.Hartley, *Garcinia oblongifolia* Champ. ex Benth., *Citrus reticulata* Blanco.

Aims: To assess the therapeutic effects of *TD.HLM01* in atherosclerosis rabbits induced by high-fat diet.

Methods: Experimental rabbits were orally administered with a mixture of cholesterol solution mixed in peanut oil and treated with atorvastatin (2.4 mg/kg b.w.) or *TD.HLM01* at two doses (90 and 270 mg/kg/b.w.) for 10 consecutive weeks before the final assessment.

Results: The investigational product significantly improved the serum lipid profile without any liver adverse events, provided protective effects in inflammatory and antioxidant parameters, and lowered aorta atherosclerosis and liver steatosis grading in both histopathological macro- and micro-images.

Conclusions: This study has demonstrated the potential efficacy of *TD.HLM01* *in vivo* in atherosclerosis treatment as a promising complementary product.

Keywords: antioxidant activity; atherosclerosis; dyslipidemia; herbal medicine; rabbit.

Resumen

Contexto: Los trastornos de las lipoproteínas y la aterosclerosis son cada vez más comunes en todo el mundo, especialmente en los países de ingresos bajos y medios. Los fármacos reductores de lípidos, como las estatinas, tienen limitaciones en el tratamiento de la aterosclerosis debido a los efectos adversos, que incluyen el aumento de las enzimas hepáticas y la rabdomiólisis. Los productos derivados de la naturaleza que regulan la dislipidemia y la lucha contra la aterosclerosis de origen vegetal son actualmente de gran interés. *TD.HLM01* es un producto vegetal que contiene extractos medicinales secos como *Atractylodes macrocephala* Koidz., *Melicope pteleifolia* (Champ. ex Benth.) T.G.Hartley, *Garcinia oblongifolia* Champ. ex Benth., *Citrus reticulata* Blanco.

Objetivos: Evaluar los efectos terapéuticos de *TD.HLM01* en conejos con aterosclerosis inducida por una dieta rica en grasas.

Métodos: A los conejos experimentales se les administró por vía oral una mezcla de solución de colesterol mezclada con aceite de maní y se les trató con atorvastatina (2.4 mg/kg de peso corporal) o *TD.HLM01* en dos dosis (90 y 270 mg/kg de peso corporal) durante 10 semanas consecutivas antes de la evaluación final.

Resultados: El producto en investigación mejoró significativamente el perfil de lípidos séricos sin ningún evento adverso hepático, proporcionó efectos protectores en los parámetros inflamatorios y antioxidantes, y redujo la aterosclerosis de la aorta y la clasificación de esteatosis hepática en macro- y micro-ímagenes histopatológicas.

Conclusiones: Este estudio ha demostrado la eficacia potencial *in vivo* de *TD.HLM01* en el tratamiento de la aterosclerosis como un producto complementario prometedor.

Palabras Clave: actividad antioxidante; aterosclerosis; conejo; dislipidemia; medicina herbaria.

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Abbreviation: ALT: alanine transaminase; Apo-A1: apoprotein A1; AST: aspartate transferase; CETP: cholesterol ester transfer protein; CM: chylomicron; CRP: C-reactive protein; CVDs: cardiovascular diseases; ELISA: enzyme-linked immunosorbent assay; eNOS: endothelial nitric oxide synthase; GSH: glutathione; HDL: high-density lipoprotein; HFD: high-fat diet; IDL: intermediate-density lipoprotein; LCAT: lecithin-cholesterol acyltransferase; LDH: lactate dehydrogenase; LDL: low-density lipoprotein; Lp: lipoprotein; MDA: malondialdehyde; MPa: megapascal; NO: nitric oxide; NOS: nitric oxide synthase; RCT: randomized controlled trial; SD: standard deviation; SOD: superoxide dismutase; SR-BI: scavenger receptor class B type I; TC: total cholesterol; *TD.HLM01*: investigational herbal product of Sao Thai Duong Jsc.; TG: triglyceride; TNF- α : tumor necrosis factor-alpha; VLDL: very low-density lipoprotein.

INTRODUCTION

Cardiovascular diseases (CVDs), particularly atherosclerosis, represent a major global health burden, with disproportionately increasing prevalence in low- and middle-income countries (De Backer, 2018). While conventional treatments like statins have proven effective in managing these conditions, their widespread use faces significant challenges (Khawwaja and Hong, 2024). These include adverse effects such as muscle pain, liver dysfunction, and, in rare cases, rhabdomyolysis, as well as cost barriers that particularly affect developing regions (Khawwaja and Hong, 2024). This situation creates an urgent need for alternative therapeutic approaches that combine effectiveness with accessibility.

The search for such alternatives has led to increasing interest in traditional medicine systems, which have long utilized specific plant combinations for treating cardiovascular conditions. In Vietnamese traditional medicine, a particular combination of plants has shown promise. These plants are now formulated as *TD.HLM01*, and comprise *Atractylodes macrocephala* Koidz., *Melicope pteleifolia* (Champ. ex Benth.) T.G.Hartley, *Garcinia oblongifolia* Champ. ex Benth., *Citrus reticulata* Blanco. Traditional practitioners have documented these herbs' potential benefits in managing cardiovascular health, though their precise mechanisms and effectiveness in treating atherosclerosis remain scientifically unexplored (Ji et al., 2015; Testai et al., 2021; Yao et al., 2020).

To understand the potential impact of these herbs, it's essential to first comprehend the complex nature of lipid metabolism and its role in cardiovascular disease. Lipoproteins serve as the body's primary mechanism for transporting lipids through the bloodstream (Feingold et al., 2000). The plasma lipoprotein system includes several distinct components: chylomicrons (CM), chylomicron remnants, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and lipoprotein(a) or Lp(a) (Feingold et al., 2000). Each lipoprotein component plays a specific role in lipid metabolism (Natesan and Kim, 2021). Chylomicrons transport exogenous lipids from the intestine to the liver and peripheral tissues (Natesan

and Kim, 2021). After delivering their lipid cargo, they become chylomicron remnants. The liver then synthesizes VLDL to transport endogenous lipids to other tissues, where they undergo conversion to IDL and, ultimately, LDL. In peripheral tissues, both LDL and HDL participate in complex metabolic processes (Natesan and Kim, 2021). However, while HDL demonstrates protective anti-atherosclerotic properties, chylomicron remnants, VLDL, IDL, LDL, and Lp(a) contribute to atherosclerosis development (Natesan and Kim, 2021). Blood lipid disorders can trigger a cascade of serious health complications, including atherosclerosis, peripheral vascular disease, hypertension, diabetes, and obesity (Natesan and Kim, 2021). Research has shown that these complications primarily result from elevated levels of non-HDL-C lipoproteins (Liu et al., 2022). While controlling atherosclerosis risk factors has contributed significantly to reducing mortality and morbidity rates (Gorelick et al., 2014), the pharmaceutical approach to treatment faces limitations due to serious side effects and high costs (Ohsfeldt et al., 2010).

The ethnomedicinal use of plants offers a promising alternative approach. This traditional knowledge provides a strong foundation for modern scientific investigation. Our research addresses three critical knowledge gaps: (1) the scientific validation of these traditionally used herbs in combination for treating atherosclerosis, (2) the mechanisms by which this combination affects lipid metabolism and inflammatory responses, and (3) the potential of this formulation as a cost-effective alternative or complement to existing treatments. *TD.HLM01* was developed as a standardized formulation that combines these four major herbs in specific proportions based on traditional usage patterns. The present study aims to bridge traditional knowledge with modern scientific understanding by investigating whether these historically observed benefits can be explained through contemporary mechanisms such as lipid regulation, anti-inflammatory effects, and antioxidant activities. The study was conducted to determine the potential therapeutic effects of *TD.HLM01* hard capsules on high-fat diet-induced atherosclerosis in experimental rabbit models. This approach validates traditional knowledge underlying these plants' therapeutic effects, potentially leading to more accessible treatment options for cardiovascular disease.

MATERIAL AND METHODS

Plant material

The preparation of *TD.HLM01* hard capsule was conducted by the Sao Thai Duong Joint Stock Company, Hanoi, Vietnam. Fresh plant materials were cultivated from the company farm in Son La, Viet Nam (21°12'N, 104°46'E) and identified based on Vietnamese Pharmacopeia 5th edition: *Atractylodes macrocephala* Koidz. (rhizomes), *Melicope pteleifolia* (Champ. ex Benth.) T.G.Hartley (leaves and roots), *Garcinia oblongifolia* Champ. ex Benth. (cortex), *Citrus reticulata* Blanco. (cortex) (Vietnamese Ministry of Health, 2017).

Preparation of *TD.HLM01* hard capsule

The fresh plant materials were firstly washed and dried at 70°C then boiled three times at 100°C for 3 hours each with distilled water to collect the extracted liquid (herbs/solution ratio was 1:6). Then, the total amount of extracted liquid was concentrated at 80°C and -0.08 MPa until the humidity had reached under 35%. The extract was completely dried in the next step at 60°C and -0.06 MPa for under 5% of humidity extract. Then, the dried extract product was ground to a fine powder mixed with other excipients (talc, magnesium stearate, among others) and preserved in vacuum bags (storage conditions: temperature (25°C), humidity (under 75%).

From *Atractylodes macrocephala* (rhizomes) (130 mg), *Citrus reticulata* (cortex) (44 mg), *Melicope pteleifolia* (leaves and roots) (200 mg), *Garcinia oblongifolia* (cortex) (200 mg) and excipients were formulated in a hard capsule based on a Vietnamese traditional remedy. After the preparation process, the investigational products were subjected to a quality examination confirmed by the Sao Thai Duong Legal Director (Registration number: 01NC/TCCS/TPT-22). The quality examination conformed to the guidelines of Vietnamese Pharmacopeia 5th edition (Vietnamese Ministry of Health, 2017).

Eight *TD.HLM01* hard capsules were dissolved in distilled water to form an investigational product with a final volume of 66.7 mL before oral administration by gastric tube (1 mL/1000 g body weight). The investigational product was freshly prepared every day before the administration procedure.

Drugs and chemicals

Cholesterol was obtained from NK Chemicals Pte Ltd. Singapore and used as an inducer for lipoprotein disorders and atherosclerosis in rabbits. Atorvastatin 10 mg (STADA) tablets were obtained from Stelapharm, Vietnam, and used as the positive control

compound. Pentobarbital was obtained from Sigma (Aldrich) Chemicals Pvt. Ltd. USA and used as the anesthetic agent. The biochemical analyzer (ERBA Chem., India) and commercial ERBA diagnostic kits were used for serum analysis of biochemistry. ELISA rabbit superoxide dismutase (SOD) kit (MBS1601528), rabbit nitric oxide synthase (NOS) ELISA kit (MBS2600090), rabbit tumor necrosis factor-alpha (TNFα) ELISA kit (MBS2500169), rabbit glutathione (GSH) ELISA kit (MBS2602347), rabbit malondialdehyde (MDA) ELISA kit (MBS2602584) were obtained from MyBioSource, USA for anti-inflammatory and antioxidant activity examinations.

Animals

Both sexes of New Zealand white rabbits, with an average weight of 2.0 ± 0.2 kilograms, were provided by the National Institute of Hygiene and Epidemiology Vietnam. The animals were grouped in separate cages with one animal in each cage and were maintained under standard room conditions ($27 \pm 2^\circ\text{C}$; $80 \pm 10\%$ humidity; 12 h light/dark cycle). After randomized grouping and before initiation of the experiment, animals were allowed to feed on the standard diet and water *ad libitum* and were acclimatized to the laboratory conditions for seven days in the Department of Pharmacology, Hanoi Medical University. All protocols used in this study were approved by the Scientific Board Committee of Hanoi Medical University (reference number: IRB00003121).

Animal study conduct

The anti-atherosclerotic effect was evaluated on the atherosclerosis model using a cholesterol oil mixture in white rabbits (Fan et al., 2015; Zhang et al., 2021b).

Cholesterol oil mixture preparation: 100 mL of peanut oil was heated in a water bath, then 100 g of pure cholesterol was added and stirred steadily until the mixture was completely dissolved; finally, peanut oil was added to make sufficient 320 mL of a mixture enriched in lipids (Pham et al., 2021).

Rabbits were divided into five groups, seven rabbits in each group, and were orally treated for 10 weeks as follows:

- Group 1 (normal control group): Distilled water at a dose of 1 mL/kg b.w.

All experimental animals in high-fat diet groups (groups 2 to 5) were orally administered a cholesterol oil mixture (0.5 g cholesterol/kg b.w. equal to 1.6 mL cholesterol mixture/kg b.w.) about 2 hours prior to any treatment.

- Group 2 (model group): Distilled water at a dose of 1 mL/kg b.w.
- Group 3 (atorvastatin): Atorvastatin at a dose of 2.4 mg/mL/kg b.w.
- Group 4 (*TD.HLM.01* 90 mg/kg): *TD.HLM01* at a dose of 90 mg/mL/kg b.w.
- Group 5 (*TD.HLM.01* 270 mg/kg): *TD.HLM01* at a dose of 270 mg/mL/kg b.w.

Tested doses in this study were selected mainly based on findings from preliminary studies of acute toxicity, sub-chronic toxicity, and antihyperlipidemic activity. Weighing rabbits in all groups was carried out at the beginning of the study and after 4/8/10 weeks of the study. Rabbits in all groups had to be fasted overnight before blood was taken at the following times: the beginning of the study, day 29 (after 4 weeks), and day 57 (after 8 weeks) through marginal ear veins using a 19 gauge butterfly needle. At the end of the study, day 71 (in the 10th week), rabbits were anesthetized with pentobarbital (20 mg/kg, i.v.) and then sacrificed for blood sampling (using cardiac puncture with thoracotomy method), aorta and liver isolation (using abdominal surgery) to evaluate immune parameters.

Assessment of serum biochemical parameters

Quantification of the following parameters: Total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate transferase (AST), alanine transaminase (ALT), total bilirubin, albumin, C-reactive protein (CRP), lactate dehydrogenase (LDH). LDL-C was calculated according to the Friedewald's formula: $LDL-C = TC - (HDL-C) - (TG/2.2)$ (mmol/L) (Vohnout et al., 2008).

Evaluation of inflammation and oxidation indices

At the end of the study, in the 10th week, the serum inflammation and oxidation indices: SOD, MDA, TNF- α , NOS, and GSH were evaluated by ELISA assays using BioTek® ELx808 reader to read the results.

Histopathological examination

A histopathological examination was conducted in a total of five experimental animals in each group for both liver and aorta samples:

- Macroscopic assessment of the aorta: A segment of the aortic arch and thoracic aorta was cut to obtain an 8-cm-length segment (Sudan III staining to assess the macroscopic area, the damaged area of the artery lumen) (Lin et al., 2015; Zhao et al., 2021).

- Microscopic evaluation of the ascending aorta (aortic segment prior to the coronary artery). The assessment of aorta microscopic injuries was based on the grading classification below (Fan et al., 2015): The grading scale ranged from 0 to 4, where Grade 0 represented normal microscopic structure. Grade 1 indicated a foam cell-rich lesion composed of macrophages and covered by a few smooth muscle cells on the top. Grade 2 showed a fibrous plaque containing a lipid core in the center. Grade 3 was characterized by a fibrous plaque containing a large necrotic core in the center, covered by a thin fibrous cap. Grade 4 represented advanced lesions showing marked calcification.
- Macroscopic assessment of the liver
- Liver microscopic evaluation: Liver steatosis was determined based on multiple parameters according to the classification system of Fu et al. (2009): The inflammatory assessment used a 0-4 scale: No inflammation (Grade 0); focal collections of mononuclear inflammatory cells (Grade 1); diffuse infiltrates of mononuclear inflammatory cells (Grade 2); focal collections of polymorphonuclear cells in addition to mononuclear cell infiltrates (Grade 3); and diffuse infiltrates of polymorphonuclear cells in the parenchymal or lobular area (Grade 4). The severity of liver steatosis was graded as: none (Grade 0); less than 30% (Grade 1); 30-50% (Grade 2); 51-75% (Grade 3); and greater than 75% under low power microscope detection (Grade 4). Hepatocellular ballooning was classified as absent (Grade 0), mild (Grade 1), or marked (Grade 2). Perisinusoidal fibrosis was categorized as absent (Grade 0), up to 33% (Grade 1), 34-66% (Grade 2), or greater than 66% (Grade 3). Overall fibrosis was assessed as none (Grade 0); either pericentral/pericellular or focal portal fibrosis (Grade 1); bridging fibrosis from central to central or central to portal regions (Grade 2); or cirrhosis (Grade 3).

Statistical analysis

The data were expressed as the mean $\pm$ standard deviation (SD) and statistical analysis was carried out employing student's T-test. In the analysis of histological grading, nonparametric tests (Mann-Whitney U test) were used. Both analyses were performed using SPSS 26. The p-value < 0.05 was considered to be statistically significant.

Table 1. Effect of *TD.HLM01* on serum lipid profiles.

Study stage	Lipid index (mmol/L)	Normal control (1)	Model (2)	Atorvastatin (2.4 mg/kg) (3)	<i>TD.HLM01</i> (90 mg/kg) (4)	<i>TD.HLM01</i> (270 mg/kg) (5)
Beginning of study	TC	2.77 ± 0.54	2.67 ± 0.41	2.87 ± 0.45	2.79 ± 0.65	2.78 ± 0.63
	TG	1.09 ± 0.11	1.05 ± 0.16	1.01 ± 0.10	1.01 ± 0.36	0.98 ± 0.08
	HDL-C	0.58 ± 0.16	0.57 ± 0.16	0.53 ± 0.11	0.71 ± 0.19	0.74 ± 0.16
	LDL-C	1.69 ± 0.41	1.62 ± 0.40	1.88 ± 0.45	1.62 ± 0.54	1.60 ± 0.51
4 th week	TC	3.67 ± 0.64	34.26 ± 4.59 ^{c,***}	25.35 ± 6.39 ^{c,Δ}	28.51 ± 3.77 ^c	26.66 ± 5.28 ^c
	TG	1.35 ± 0.35	2.04 ± 0.67 ^{a,*}	1.99 ± 0.48 ^b	2.01 ± 0.53 ^b	2.19 ± 0.58 ^b
	HDL-C	0.67 ± 0.18	1.51 ± 0.47 ^{b,***}	1.25 ± 0.39 ^b	2.10 ± 0.60 ^c	1.75 ± 0.56 ^b
	LDL-C	2.38 ± 0.62	31.82 ± 4.53 ^{c,***}	23.19 ± 6.08 ^Δ	25.49 ± 3.68 ^{c,Δ}	23.92 ± 5.25 ^{c,Δ}
8 th week	TC	3.70 ± 0.59	38.60 ± 8.79 ^{c,***}	27.80 ± 5.36 ^{c,Δ}	41.66 ± 10.08 ^c	39.16 ± 10.14 ^c
	TG	1.25 ± 0.51	3.52 ± 0.82 ^{c,***}	2.91 ± 0.84 ^b	2.64 ± 0.59 ^{b,Δ}	3.63 ± 1.02 ^c
	HDL-C	0.77 ± 0.20	1.33 ± 0.40 ^{b,**}	1.83 ± 0.54 ^c	1.69 ± 0.37 ^b	1.90 ± 0.48 ^{b,Δ}
	LDL-C	2.36 ± 0.56	35.67 ± 8.56 ^{c,***}	24.65 ± 5.51 ^{c,Δ}	38.77 ± 10.29 ^c	35.61 ± 10.23 ^c
End of study (10 th week)	TC	3.64 ± 0.40	45.35 ± 8.63 ^{c,***}	32.52 ± 6.73 ^{c,ΔΔ}	38.92 ± 9.83 ^c	33.79 ± 8.55 ^{c,Δ}
	TG	1.35 ± 0.32	4.77 ± 1.07 ^{c,***}	2.65 ± 0.70 ^{b,ΔΔΔ}	3.01 ± 0.57 ^{b,ΔΔ}	3.17 ± 0.81 ^{c,ΔΔ}
	HDL-C	0.61 ± 0.13	1.49 ± 0.36 ^{c,***}	2.01 ± 0.45 ^{c,Δ}	1.99 ± 0.59 ^b	2.81 ± 0.69 ^{c,ΔΔΔ}
	LDL-C	2.42 ± 0.47	41.69 ± 8.55 ^{c,***}	29.30 ± 6.67 ^{c,Δ}	35.56 ± 10.00 ^c	29.54 ± 8.98 ^{c,Δ}

Data are expressed as the mean ± standard deviation (n = 7).

*p<0.05, **p<0.01, ***p<0.001 statistically significant differences compared to normal control group.

^Δp<0.05, ^{ΔΔ}p<0.01, ^{ΔΔΔ}p<0.001 statistically significant differences between Groups 3, 4, and 5 compared to the model group.

^ap<0.05, ^bp<0.01, ^c p<0.001 statistically significant differences compared to the beginning of the study.

TC: total cholesterol; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

RESULTS

Assessment of serum biochemical parameters

A high-lipid diet significantly induced the total cholesterol (TC), triglyceride (TG), and LDL-C levels when compared to all treatment groups at the end of the study, with the results observed at the beginning (p<0.001). The results showed notable changes in all those parameters of atorvastatin and *TD.HLM01* (270 mg/kg) in comparison with the model group. *TD.HLM01* (90 mg/kg) only showed efficacy on TG as compared to the model group (p<0.01). HDL-C levels of all treatment groups were dramatically increased as compared to the beginning moment of the study (p<0.001). *TD.HLM01* (90 mg/kg) did not show any improvement in HDL-C when compared with a model group, but significant increases were observed in the *TD.HLM01* (270 mg/kg) after 8 and 10 weeks (p<0.05 and p<0.001, respectively). HDL-C increment was also exhibited in the atorvastatin group after 10 weeks of treatment compared to the model group (p<0.05) (Table 1).

Significant increases in AST and ALT levels were observed in all treatment groups after 10 weeks of treatment, with a notable improvement of AST observed in atorvastatin and *TD.HLM01* groups compared with the model group (p<0.001 and p<0.05, respectively). There were no considerable differences in albumin concentrations between all study groups and between time points. In comparison with the beginning of the study, total bilirubin concentrations significantly increased after eight weeks in the model group (p<0.01). After 10 weeks, that aspect was also exhibited in groups 3, 4, and 5 with p<0.01, p<0.01 and p<0.05, respectively (Table 2).

Evaluation of inflammation and oxidation indices

The indicators of inflammation and cell destruction, such as LDH, CRP, and TNF-α were significantly increased in the model group compared to the normal control group (p<0.05). In contrast, no notable difference was observed in either *TD.HLM01* treated groups. Oxidative markers such as SOD, GSH, and MDA were adversely altered in the model group in comparison with the normal control group (p<0.01

and $p < 0.001$, respectively). *TD.HLM01* exhibited potential effects with significant changes in SOD and GSH compared to the model group ($p < 0.001$ and $p < 0.01$). There were also significant NOS improve-

ments in both *TD.HLM01*-treated groups increased in the *TD.HLM01* groups when compared with the model group ($p < 0.001$) (Table 3).

Table 2. Effect of *TD.HLM01* on biochemical liver parameters.

Study stage	Liver index	Normal control (1)	Model (2)	Atorvastatin (2.4 mg/kg) (3)	<i>TD.HLM01</i> (90 mg/kg) (4)	<i>TD.HLM01</i> (270 mg/kg) (5)
Beginning of study	AST	48.57 ± 9.18	48.86 ± 8.86	50.00 ± 10.03	53.57 ± 13.89	49.71 ± 8.83
	ALT	42.43 ± 7.44	41.14 ± 8.09	44.57 ± 10.44	41.14 ± 9.28	47.57 ± 6.37
	Albumin	3.61 ± 0.37	3.49 ± 0.30	3.46 ± 0.40	3.34 ± 0.24	3.23 ± 0.31
	Total bilirubin	10.74 ± 0.60	10.31 ± 0.86	10.44 ± 0.57	10.50 ± 0.82	10.86 ± 0.81
4 th week	AST	42.57 ± 9.73	64.86 ± 15.13 **	75.00 ± 9.71 ^c	55.00 ± 17.28	51.00 ± 13.13
	ALT	54.00 ± 11.40	65.14 ± 15.94 ^a	89.00 ± 13.72 ^{b,Δ}	69.86 ± 16.94 ^a	63.00 ± 14.67 ^a
	Albumin	3.31 ± 0.12	3.30 ± 0.17	3.37 ± 0.19	3.23 ± 0.13	3.10 ± 0.31
	Total bilirubin	10.19 ± 0.83	11.57 ± 1.63	10.29 ± 0.56	11.60 ± 0.81 ^a	11.27 ± 0.62
8 th week	AST	40.43 ± 5.88	110.57 ± 24.49 ^{c,***}	77.14 ± 23.33 ^{a,Δ}	63.00 ± 18.23 ^{ΔΔ}	99.57 ± 11.28 ^c
	ALT	52.71 ± 12.23	120.29 ± 20.07 ^{c,***}	105.29 ± 21.27 ^c	93.43 ± 15.24 ^{c,Δ}	104.00 ± 14.32 ^c
	Albumin	3.26 ± 0.30	3.03 ± 0.33 ^b	3.03 ± 0.34	3.09 ± 0.18 ^a	3.10 ± 0.33
	Total bilirubin	10.50 ± 0.89	15.50 ± 3.06 ^{b,***}	11.46 ± 1.77 ^Δ	13.86 ± 3.57	13.24 ± 2.52
End of study (10 th week)	AST	39.29 ± 8.14	145.86 ± 24.31 ^{c,***}	86.29 ± 22.72 ^{b,ΔΔΔ}	112.71 ± 18.08 ^{c,Δ}	118.29 ± 21.25 ^{c,Δ}
	ALT	53.14 ± 13.92	108.43 ± 32.89 ^{c,***}	103.43 ± 28.70 ^b	124.00 ± 26.78 ^c	125.86 ± 27.13 ^c
	Albumin	3.39 ± 0.36	3.34 ± 0.24	3.30 ± 0.29	3.01 ± 0.38	3.03 ± 0.35
	Total bilirubin	10.94 ± 1.07	16.06 ± 2.81 ^{b,***}	14.53 ± 1.79 ^b	15.59 ± 3.52 ^b	15.37 ± 2.85 ^a

Data are expressed as the mean ± standard deviation (n = 7). The measurements: AST and ALT were in IU/L, albumin was in g/dL, and total bilirubin was in μmol/L.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant differences compared to normal control group.

^Δ $p < 0.05$, ^{ΔΔ} $p < 0.01$, ^{ΔΔΔ} $p < 0.001$ statistically significant differences between Groups 3, 4, and 5 compared to the model group.

^a $p < 0.05$, ^b $p < 0.01$, ^c $p < 0.001$ statistically significant differences compared to the beginning of the study.

AST: aspartate transferase; ALT: alanine transaminase.

Table 3. Effect of *TD.HLM01* on anti-inflammatory and antioxidant parameters.

Parameter	Normal control (1)	Model (2)	Atorvastatin (2.4 mg/kg) (3)	<i>TD.HLM01</i> (90 mg/kg) (4)	<i>TD.HLM01</i> (270 mg/kg) (5)
LDH (IU/L)	162.86 ± 47.69	254.71 ± 77.83 ^a	168.00 ± 43.82 ^Δ	138.43 ± 19.61 ^{ΔΔ}	106.43 ± 26.56 ^{ΔΔΔ}
CRP (mg/L)	0.101 ± 0.06	0.439 ± 0.133 ^{***}	0.244 ± 0.07 ^{ΔΔ}	0.367 ± 0.115	0.361 ± 0.078
TNFα (pg/mL)	21.54 ± 3.36	34.65 ± 9.72 ^{**}	24.63 ± 4.71 ^Δ	21.63 ± 6.13 ^Δ	23.76 ± 5.22 ^Δ
NOS (ng/mL)	0.258 ± 0.081	0.173 ± 0.054 ^a	0.375 ± 0.121 ^{ΔΔ}	0.596 ± 0.141 ^{ΔΔΔ}	0.505 ± 0.143 ^{ΔΔΔ}
SOD (ng/mL)	65.36 ± 6.65	49.64 ± 11.43 ^{**}	146.16 ± 35.44 ^{ΔΔΔ}	80.03 ± 13.83 ^{ΔΔΔ}	78.89 ± 16.23 ^{ΔΔΔ}
GSH (μg/mL)	178.13 ± 33.99	91.00 ± 16.27 ^{***}	145.73 ± 33.46 ^{ΔΔ}	139.24 ± 28.24 ^{ΔΔ}	149.30 ± 37.48 ^{ΔΔ}
MDA (nmol/mL)	9.81 ± 2.49	23.31 ± 5.47 ^{***}	15.61 ± 4.54 ^Δ	18.83 ± 4.87	17.06 ± 2.99 ^Δ

Data are expressed as the mean ± standard deviation (n = 7).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ statistically significant differences compared to normal control group.

^Δ $p < 0.05$, ^{ΔΔ} $p < 0.01$, ^{ΔΔΔ} $p < 0.001$ statistically significant differences between Groups 3, 4, and 5 compared to the model group. LDH: lactate dehydrogenase; CRP: C-reactive protein; TNFα: tumor necrosis factor-α; NOS: nitric oxide synthase; SOD: superoxide dismutase; GSH: glutathione; MDA: malondialdehyde.

Histopathological examination

Histopathological examination of the normal control group showed the typical aorta and liver morphology with no mononuclear inflammatory cell infiltration or fatty lipid streak. In contrast, the aorta and liver samples of the model control group exhibited

atherosclerotic plaque, foam cell formation, and macrophage infiltration – hallmarks of atherosclerosis diseases. Rabbits treated with *TD.HLM01* in groups 4 and 5 showed significant improvement with fewer lipid cores observed in the aorta and low grade of liver steatosis (Tables 4 and 5, Figs. 1 and 2).

Table 4. Effect of *TD.HLM01* on aorta histopathological examination.

Group	Treatment (n = 5)	Aorta histopathology grade				
		0	1	2	3	4
1	Normal control	5	0	0	0	0
2	Model**	0	0	5	0	0
3	Atorvastatin* ^Δ	1	3	1	0	0
4	<i>TD.HLM01</i> (90 mg/kg b.w.)** ^Δ	0	4	1	0	0
5	<i>TD.HLM01</i> (270 mg/kg b.w.) ^Δ	2	2	1	0	0

*p<0.05, **p<0.01 statistically significant differences compared to Group 1.
^Δ p<0.05 statistically significant differences between Groups 3, 4, and 5 compared to Group 2.

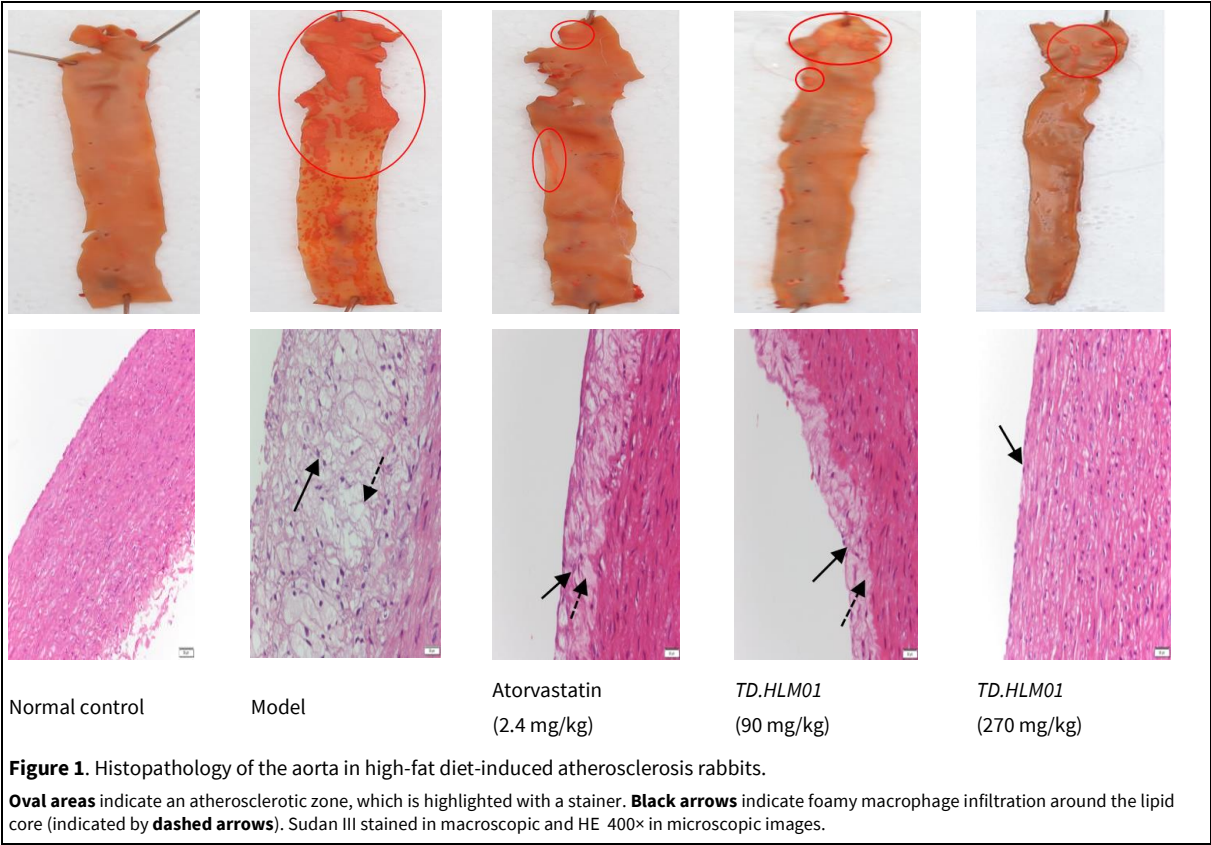


Table 5. Effect of *TD.HLM01* on liver histopathological examination.

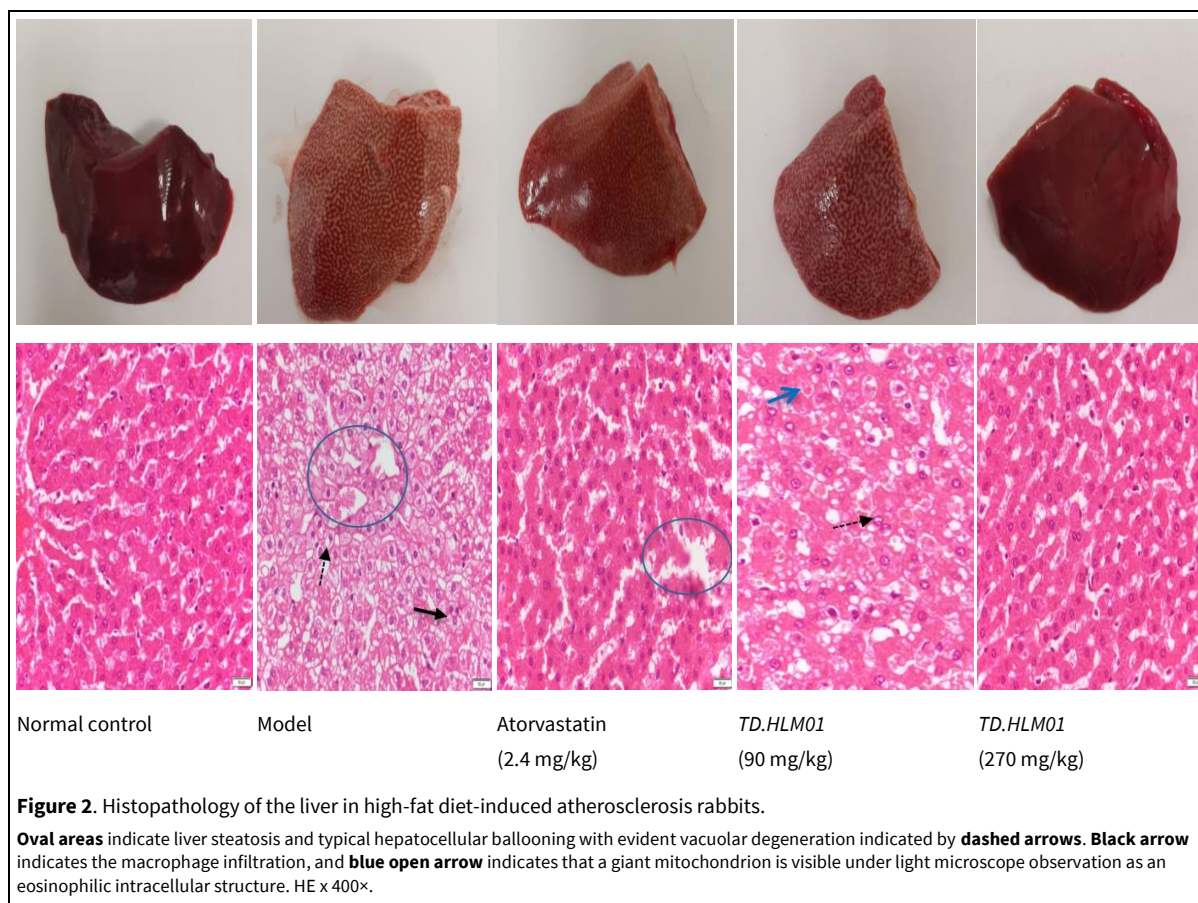
Liver steatosis index	Group	Treatment (n = 5)	Grade				
			0	1	2	3	4
Inflammatory	1	Normal control	3	2	0	0	0
	2	Model*	0	4	1	0	0
	3	Atorvastatin	1	1	2	1	0
	4	<i>TD.HLM01</i> (90 mg/kg)	1	2	1	1	0
	5	<i>TD.HLM01</i> (270 mg/kg)*	0	2	3	0	0
Steatosis	1	Normal control	2	3	0	0	0
	2	Model**	0	0	1	3	1
	3	Atorvastatin ^Δ	1	3	0	1	0
	4	<i>TD.HLM01</i> (90 mg/kg) ^Δ	0	3	1	1	0
	5	<i>TD.HLM01</i> (270 mg/kg)	2	2	0	0	1
Cytologic ballooning	1	Normal control	2	3	0		
	2	Model**	0	0	5		
	3	Atorvastatin ^Δ	2	2	1		
	4	<i>TD.HLM01</i> (90 mg/kg)	0	3	2		
	5	<i>TD.HLM01</i> (270 mg/kg)	2	1	2		
Perisinusoidal fibrosis	1	Normal control	3	2	0	0	
	2	Model*	0	4	1	0	
	3	Atorvastatin ^Δ	3	2	0	0	
	4	<i>TD.HLM01</i> (90 mg/kg) ^Δ	3	2	0	0	
	5	<i>TD.HLM01</i> (270 mg/kg)	0	5	0	0	
Fibrosis	1	Normal control	2	3	0	0	
	2	Model*	0	1	4	0	
	3	Atorvastatin	0	3	2	0	
	4	<i>TD.HLM01</i> (90 mg/kg)	0	3	1	1	
	5	<i>TD.HLM01</i> (270 mg/kg)*	0	1	4	0	

*p<0.05, **p<0.01 statistically significant differences compared to Group 1.
^Δ p<0.05 statistically significant differences between Groups 3, 4, and 5 compared to Group 2.

DISCUSSION

The pathogenesis of atherosclerosis is thought to involve an imbalance in lipid metabolism, as well as genetic and environmental factors (Jebari-Benslaiman et al., 2022). A high-fat diet promotes hyperlipidemia and arterial spasms, leading to altered hemodynamics of the carotid artery. Many studies have been conducted using experimental models to investigate the development of atherosclerosis from a high-fat diet. To study pathophysiology and develop therapeutic modalities, appropriate experimental animals are necessary. Rabbits have been widely used in the experimental study of atherosclerosis because rabbits are highly sensitive to a cholesterol-rich diet

and accumulate large amounts of cholesterol in their plasma. They also provide information on factors contributing to the progression and regression of this disease that may be extrapolated to humans (Fan et al., 2015). Unlike mice and rats, lipid metabolism in rabbits is similar to that in humans: Plasma cholesterol ester transfer protein (CETP) activity, apoB-containing LP particle composition, efficient absorption of cholesterol, rapid development of severe hypercholesterolemia, and consequently aortic atherosclerosis. However, there are some marked differences between rabbits and humans in lipoprotein metabolism: Rabbits’ hepatic lipase activity is naturally low, so HDL concentrations are



rapidly increased (Fan et al., 2015). That aspect was also observed in this study, showing a significant increase in HDL concentration in the treatment groups compared with the normal control group. In this study, a high-cholesterol diet was successfully used as a method to induce atherosclerosis in rabbits, leading to a dramatic change in both serum lipid profiles and aorta histopathological images. This phenomenon is characterized by cholesterol accumulation, macrophage infiltration, increased connective tissue components, and thrombus formation (Jebari-Benslaiman et al., 2022).

The results demonstrated that *TD.HLM01* reduced serum triglyceride in both groups using doses of 90 mg/kg and 270 mg/kg, thereby reducing the tendency of atherosclerosis compared to the model group. Increasing the dose expressed noticeable improvements in serum TC, LDL-C, and HDL-C levels observed in a dose-dependent manner with *TD.HLM01* treatment groups. The potentials of *TD.HLM01* hard capsule improves lipid metabolism and anti-atherosclerotic activity, which can be attributed to the combination of herbal constituents. *Atractylodes macrocephala*, one component of *TD.HLM01* contains sesquiterpenoids and alkynes, which are considered to have biological effects on lipid disorders and atherosclerosis (Li et al., 2024;

Zhang et al., 2021a). The main anti-hyperlipidemia active components of AM are its volatile and liposoluble components, which may enhance serum HDL-C by increasing the expression of the RCT-related proteins Apo-A1, LCAT, and SR-BI (Li et al., 2024). *Citrus reticulata* has been utilized as a versatile medicinal herb with vast cardiovascular benefits in Asian nations for centuries. Accumulating evidence suggests that *Citrus reticulata* and its components are effective in preventing cardiovascular diseases such as atherosclerosis, myocardial infarction, myocardial ischemia, arrhythmia, cardiac hypertrophy, heart failure, and hypertension. Studies showed that the two most bioactive components of *Citrus reticulata* were flavonoids and volatile oils (Zou et al., 2022). *Citrus reticulata* extracts, enriched with polymethoxy flavonoids, have been identified as a prospective therapeutic treatment for metabolic disorders and circulatory system diseases due to their remarkable anti-inflammatory, antioxidant, and anti-apoptotic activity (Choi et al., 2015; Guo et al., 2016). Flavonoids and volatile oils, including hesperidin, hesperetin, naringin, and naringenin act separately or in concert to counteract lipid peroxidation, thrombosis, inflammatory responses, as well as endothelial dysfunction (Choi et al., 2015). *Garcinia oblongifolia* plants contain a broad range of biologically active

metabolites with relevant therapeutic properties in anti-inflammation, antioxidant activities, and lipid-related and cardiovascular disorders (Espirito Santo et al., 2020). The study results indicated that *Garcinia oblongifolia* in the investigational product remedy might share the same characteristics as other *Garcinia* sp. Dyslipidemia induced by a high-fat diet in this study had a typical pathological aspect of non-alcoholic fatty liver disease with AST:ALT ratio lower than 1.5 but higher than 0.8 (indicating the more advanced disease), increased TG and low HDL-C (Sattar et al., 2014). Using either atorvastatin or *TD.HLM01* did not show improvement in liver enzymes as compared to the normal control group, which might be due to pathological reasons. In all treatment groups, slight increases in total bilirubin were observed, but all of them showed no adverse effects on the albumin production process of the liver. Besides that, the rabbit's vital signs and daily behavior were also monitored throughout the study without any mortality or adverse events observed, indicating the safety of *TD.HLM01* at the investigated doses in rabbits.

In recent decades, atherosclerosis and other lipid-related cardiovascular diseases have threatened the healthcare system worldwide because of its complications and high death rate (Roth et al., 2017). Oxidative stress, characterized by the overproduction of reactive oxygen species (ROS) and oxidized LDL, has a pivotal role in the progression of atherosclerosis, a major cause of coronary heart disease and stroke. An antioxidant may act through a variety mechanism of actions to facilitate atherosclerosis treatment, including inhibition of LDL oxidation, increment of antioxidant capacity, reduction of generated ROS, improvement of endothelial dysfunction and vasodilation, the promotion of NO bioavailability, prevention of atherosclerotic plaque formation, prevention of mononuclear inflammatory cell infiltration and suppression of foam cell formation (Malekmohammad et al., 2019). The primary objective of this study is to evaluate the anti-atherosclerotic effects of the investigational product across various mechanisms, including almost some aspects of antioxidant activity. The antioxidant capacity was evaluated by quantifications of GSH and SOD, which are two of the free radicals scavenging enzymes that contribute to total biophysical antioxidant capacity and the lipid peroxidation by-product MDA (Nair and Nair, 2017). Treatment with *TD.HLM01* significantly restored the levels of GSH and SOD compared to the normal control group, and it might be more efficient when used in higher dose according to the notable change in the MDA level of group 5 (*TD.HLM01* 270 mg/kg) compared to the model group which may be correlated to the potent therapeutic effect of the investigation product resulting in reducing ROS and maintaining antioxidant

capacity. Endothelial nitric oxide synthase (eNOS) enzyme, which is highly expressed in the endothelial cells of cardiac vasculature, contributes to maintaining the endothelial-myocardial interactions (Li et al., 2017). In the present study, eNOS was evaluated as an index to directly estimate the improvement in endothelial function and vasodilation and, indirectly, the production of nitric oxide. Both doses of *TD.HLM01* significantly increased the NOS level compared to the model group, which indicated that it may possess a potential cardiovascular protective effect.

Atherosclerosis is a chronic inflammatory vascular disease based on circulating monocytes infiltrating tissues, including the aorta and liver. The potential antigens released from apoptotic cells in the plaques can promote the progression of atherosclerosis, and impaired apoptotic cell clearance can sustain atherogenesis (Kong et al., 2022). In this study, the anti-inflammatory effect of *TD.HLM01* in biochemical examination and TNF α levels, which is a central cytokine in inflammatory reactions, were consistent with the histopathological changes in the aorta and liver. Treatment with *TD.HLM01* significantly maintained the LDH and TNF α levels compared to the model group, and no notable changes were observed compared to the normal control group. CRP levels, however, showed no significant improvement. In the micro-histopathological images of both the aorta and liver, significant improvements were observed when comparing treatment groups with the model group, but macrophage presence at different levels was evidently observed, indicating the exact pathology of atherosclerosis. Histopathologic sections of the model group clearly showed the typical morphology of atherosclerosis with a lipid core, foamy macrophages, and liver steatosis with hepatocellular ballooning and infiltration of mononuclear cells. The results also demonstrated the protective effects of *TD.HLM01* when was assessed by the histopathology grading of the aorta and liver.

Overall, mild atherosclerosis in aorta images with low levels of liver steatosis grading and the improvement in all other aspects may be accepted as abundant evidence for the potential therapeutic effects of *TD.HLM01* in atherosclerosis preventive treatment. The investigational product offers ease of production and a favorable safety profile. It was the first step in future trials evaluating the safety and efficacy of *TD.HLM01* hard capsule on volunteers, providing further evidence of its potential applications in clinical settings.

CONCLUSION

The *TD.HLM01* hard capsules, administered at both doses, significantly prevented the progression of

atherosclerosis in a rabbit model. Treatment with *TD.HLM01* hard capsules effectively improved the serum lipid profiles without any adverse effects on liver function, while ameliorating the organ injuries in the histo-architecture of the aorta and liver. These findings suggest that *TD.HLM01* may serve as an effective traditional medicine and warrant further study for its safety and efficacy in clinical practice.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

- Choi BK, Kim TW, Lee DR, Jung WH, Lim JH, Jung JY, Yang SH, Suh JW (2015) A polymethoxy flavonoids-rich *Citrus aurantium* extract ameliorates ethanol-induced liver injury through modulation of AMPK and Nrf2-related signals in a binge drinking mouse model. *Phytother Res* 29(10): 1577–1584. <https://doi.org/10.1002/ptr.5415>
- De Backer G (2018) Prevention of cardiovascular disease: Much more is needed. *Eur J Prevent Cardiol* 25: 1083 - 1086. <https://doi.org/10.1177/2047487318770297>
- Espirito Santo BLS do, Santana LF, Kato Junior WH, de Araújo F de O, Bogo D, Freitas K de C, Guimarães R de CA, Hiane PA, Pott A, Filiú WF de O, Arakaki Asato M, Figueiredo P de O, Bastos PRH de O (2020) Medicinal potential of *Garcinia* species and their compounds. *Molecules* 25: 4513. <https://doi.org/10.3390/molecules25194513>
- Fan J, Kitajima S, Watanabe T, Xu J, Zhang J, Liu E, Chen YE (2015) Rabbit models for the study of human atherosclerosis: From pathophysiological mechanisms to translational medicine. *Pharmacol Ther* 146: 104–119. <https://doi.org/10.1016/j.pharmthera.2014.09.009>
- Feingold KR, Anawalt B, Blackman MR, Boyce A, Chrousos G, Corpas E, WW de Herder, Dhatariya K, Dungan K, Hofland J, Kalra S, Kaltsas G, Kapoor N, Koch C, Kopp P, Korbonits M, Kovacs CS, Kuohung W, Laferrère B, Levy M, McGee EA, McLachlan R, New M, Purnell J, Sahay R, Shah AS, Singer F, Sperling MA, Stratakis CA, Trencle DL, Wilson DP (2000) Introduction to lipids and lipoproteins. South Dartmouth (MA): MDText.com, Inc. <https://www.ncbi.nlm.nih.gov/books/NBK305896/>
- Fu J-F, Fang Y-L, Liang L, Wang C-L, Hong F, Dong G-P (2009) A rabbit model of pediatric nonalcoholic steatohepatitis: The role of adiponectin. *World J Gastroenterol* 15(8): 912–918. <https://doi.org/10.3748/wjg.15.912>
- Gorelick PB, Goldstein LB, Ovbiagele B (2014) New guidelines to reduce risk of atherosclerotic cardiovascular disease. *Stroke* 45(4): 945–947. <https://doi.org/10.1161/STROKEAHA.114.004560>
- Guo J, Tao H, Cao Y, Ho C-T, Jin S, Huang Q (2016) Prevention of obesity and type 2 diabetes with aged *Citrus* peel (chenpi) extract. *J Agric Food Chem* 64(10): 2053–2061. <https://doi.org/10.1021/acs.jafc.5b06157>
- Jebari-Benslaïman S, Galicia-García U, Larrea-Sebal A, Olaetxea JR, Alloza I, Vandenbroeck K, Benito-Vicente A, Martín C (2022) Pathophysiology of atherosclerosis. *Int J Mol Sci* 23(6): 3346. <https://doi.org/10.3390/ijms23063346>
- Ji G, Chen R, Zheng J (2015) Macrophage activation by polysaccharides from *Atractylodes macrocephala* Koidz through the nuclear factor-κB pathway. *Pharm Biol* 53(4): 512–517. <https://doi.org/10.3109/13880209.2014.929152>
- Khatiwada N, Hong Z (2024) Potential benefits and risks associated with the use of statins. *Pharmaceutics* 16(2): 214. <https://doi.org/10.3390/pharmaceutics16020214>
- Kong P, Cui ZY, Huang XF, Zhang DD, Guo RJ, Han M (2022) Inflammation and atherosclerosis: Signaling pathways and therapeutic intervention. *Sig Transduct Target Ther* 7: 131. <https://doi.org/10.1038/s41392-022-00955-7>
- Li B, Chen X, Wu H, Su J, Ding Y, Zhang Z, Rong M, Dong Y, He X, Li L, Lv G, Chen S (2024) The anti-hyperlipidemia effect of *Atractylodes macrocephala* rhizome increased HDL via reverse cholesterol transfer. *Heliyon* 10(7): e28019. <https://doi.org/10.1016/j.heliyon.2024.e28019>
- Li F, Zong J, Zhang H, Zhang P, Xu L, Liang K, Yang L, Yong H, Qian W (2017) Orientin reduces myocardial infarction size via eNOS/NO signaling and thus mitigates adverse cardiac remodeling. *Front Pharmacol* 8: 926. <https://doi.org/10.3389/fphar.2017.00926>
- Lin Y, Bai L, Chen Y, Zhu N, Bai Y, Li Q, Zhao S, Fan J, Liu E (2015) Practical assessment of the quantification of atherosclerotic lesions in apoE^{-/-} mice. *Mol Med Rep* 12(4): 5298–5306. <https://doi.org/10.3892/mmr.2015.4084>
- Liu T, Zhao D, Qi Y (2022) Global trends in the epidemiology and management of dyslipidemia. *J Clin Med* 11(21): 6377. <https://doi.org/10.3390/jcm11216377>
- Malekmohammad K, Sewell RDE, Raffieian-Kopaei M (2019) Antioxidants and atherosclerosis: Mechanistic aspects. *Biomolecules* 9(8): 301. <https://doi.org/10.3390/biom9080301>
- Nair A, Nair BJ (2017) Comparative analysis of the oxidative stress and antioxidant status in type II diabetics and nondiabetics: A biochemical study. *J Oral Maxillofac Pathol* 21(3): 394–401. https://doi.org/10.4103/jomfp.JOMFP_56_16
- Natesan V, Kim S-J (2021) Lipid metabolism, disorders and therapeutic drugs - Review. *Biomol Ther (Seoul)* 29(6): 596–604. <https://doi.org/10.4062/biomolther.2021.122>
- Ohsfeldt RL, Gandhi SK, Fox KM, Bullano MF, Davidson M (2010) Medical and cost burden of atherosclerosis among patients treated in routine clinical practice. *J Med Econ* 13(3): 500–507. <https://doi.org/10.3111/13696998.2010.506348>
- Pham TP, Pham TVA, Dang TTH, Nguyen TT, Pham QB (2021) Effects of Hamo NK hard capsule on experimental atherosclerosis model. *J Med Res* 141 E8 (5): 95–103. <https://doi.org/10.52852/tcnycy.v141i5.216>
- Roth GA, Johnson C, Abajobir A, Abd-Allah F, Abera SF, Abyu G, Ahmed M, Aksut B, Alam T, Alam K, Alla F, Alvis-Guzman N, Amrock S, Ansari H, Ärnlöv J, Asayesh H, Atey TM, Avila-Burgos L, Awasthi A, Banerjee A, Barac A, Barnighausen T, Barregard L, Bedi N, Belay Ketema E, Bennett D, Berhe G, Bhutta Z, Bitew S, Carapetis J, Carrero JJ, Malta DC, Castañeda-Orjuela CA, Castilló-Rivas J, Catalá-López F, Choi J-Y, Christensen H, Cirillo M, Cooper L, Criqui M, Cundiff D, Damasceno A, Dandona L, Dandona R, Davletov K, Dharmaratne S, Dorairaj P, Dubey M, Ehrenkranz R, El Sayed Zaki M, Faraon EJA, Esteghamati A, Farid T, Farvid M, Feigin V, Ding EL, Fowkes G, Gebrehiwot T, Gillum R, Gold A, Gona P, Gupta R, Habtewold TD, Hafezi-Nejad N, Hailu T, Hailu GB, Hankey G, Hassen HY, Abate KH, Havmoeller R, Hay SI,

- Horino M, Hotez PJ, Jacobsen K, James S, Javanbakht M, Jeemon P, John D, Jonas J, Kalkonde Y, Karimkhani C, Kasaiean A, Khader Y, Khan A, Khang Y-H, Khera S, Khoja AT, Khubchandani J, Kim D, Kolte D, Kosen S, Krohn KJ, Kumar GA, Kwan GF, Lal DK, Larsson A, Linn S, Lopez A, Lotufo PA, El Razek HMA, Malekzadeh R, Mazidi M, Meier T, Meles KG, Mensah G, Meretoja A, Mezgebe H, Miller T, Mirrahimov E, Mohammed S, Moran AE, Musa KI, Narula J, Neal B, Ngalesoni F, Nguyen G, Obermeyer CM, Owolabi M, Patton G, Pedro J, Qato D, Qorbani M, Rahimi K, Rai RK, Rawaf S, Ribeiro A, Safiri S, Salomon JA, Santos I, Santric Milicevic M, Sartorius B, Schutte A, Sepanlou S, Shaikh MA, Shin M-J, Shishehbor M, Shore H, Silva DAS, Sobngwi E, Stranges S, Swaminathan S, Tabarés-Seisdedos R, Tadele Atnafu N, Tesfay F, Thakur JS, Thrift A, Topor-Madry R, Truelsen T, Tyrovolas S, Ukwaja KN, Uthman O, Vasankari T, Vlassov V, Vollset SE, Wakayo T, Watkins D, Weintraub R, Werdecker A, Westerman R, Wiyongse CS, Wolfe C, Workicho A, Xu G, Yano Y, Yip P, Yonemoto N, Younis M, Yu C, Vos T, Naghavi M, Murray C (2017) Global, regional, and national burden of cardiovascular diseases for 10 causes, 1990 to 2015. *J Am Coll Cardiol* 70(1): 1–25. <https://doi.org/10.1016/j.jacc.2017.04.052>
- Sattar N, Forrest E, Preiss D (2014) Non-alcoholic fatty liver disease. *BMJ* 349: g4596. <https://doi.org/10.1136/bmj.g4596>
- Testai L, De Leo M, Flori L, Polini B, Braca A, Nieri P, Pistelli L & Calderone V (2021) Contribution of irisin pathway in protective effects of mandarin juice (*Citrus reticulata* Blanco) on metabolic syndrome in rats fed with high fat diet. *Phytother Res* 35(8): 4324–4333. <https://doi.org/10.1002/ptr.7128>
- Vietnamese Ministry of Health (2017) Vietnamese Pharmacopeia, 5th edn. Ha Noi: Medical Publishing House Co., Ltd.
- Vohnout B, Vachulová A, Blazíček P, Dukát A, Fodor G, Lietava J (2008) Evaluation of alternative calculation methods for determining LDL cholesterol. *Vnitr Lek* 54(10): 961–964.
- Yao Q, Gao Y, Lai C, Wu C, Zhao C, Wu J, Tang D (2020) The phytochemistry, pharmacology and applications of *Melicope pteleifolia*: A review. *J Ethnopharmacol* 251: 112546. <https://doi.org/10.1016/j.jep.2020.112546>
- Zhang W, Zhao Z, Chang L, Cao Y, Wang S, Kang C, Wang H, Zhou L, Huang L, Guo L (2021a) *Atractylodes* rhizoma: A review of its traditional uses, phytochemistry, pharmacology, toxicology and quality control. *J Ethnopharmacol* 266: 113415. <https://doi.org/10.1016/j.jep.2020.113415>
- Zhang Y, Fatima M, Hou S, Bai L, Zhao S, Liu E (2021b) Research methods for animal models of atherosclerosis (Review). *Mol Med Rep* 24(6): 871. <https://doi.org/10.3892/mmr.2021.12511>
- Zhao L, Zhang S, Su Q, Li S (2021) Effects of withdrawing an atherogenic diet on the atherosclerotic plaque in rabbits. *Exp Ther Med* 22(1): 751. <https://doi.org/10.3892/etm.2021.10183>
- Zou J, Wang J, Ye W, Lu J, Li C, Zhang D, Ye W, Xu S, Chen C, Liu P, Liu Z (2022) Citri Reticulatae Pericarpium (Chenpi): A multi-efficacy pericarp in treating cardiovascular diseases. *Biomed Pharmacother* 154: 113626. <https://doi.org/10.1016/j.biopha.2022.113626>

AUTHOR CONTRIBUTION:

Contribution	Nguyen TT	Nguyen TTH	Duong TN	Nguyen HT	Nguyen THL	Bui TVA	Dang TTH	Dinh TTH	Trinh VQ	Pham XP	Pham TVA
Concepts or ideas	x			x	x						x
Design	x	x		x	x					x	x
Definition of intellectual content	x	x								x	x
Literature search			x	x	x	x	x	x	x	x	x
Experimental studies	x	x	x	x	x	x	x	x	x		x
Data acquisition	x	x	x			x	x	x			
Data analysis							x				
Statistical analysis							x		x		
Manuscript preparation	x								x		x
Manuscript editing						x		x	x		x
Manuscript review	x	x	x	x	x	x	x	x	x		

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