Majalah Obat Tradisional (Trad. Med. J.), October-December 2025
Vol. 30(3), p 330-338
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 22-11-2024
Revised : 24-04-2025
Accepted : 05-06-2025

# In Vivo Antihyperlipidemic Activity of *TD.HLM01* – A Vietnamese Traditional Remedy in Experimental Models

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## ABSTRACT

One of the primary risk factors for cardiovascular disease nowadays is dyslipidemia. For many years, multi-herbal preparations have been the standard treatment approach for treating dyslipidemia. The purpose of this study was to assess the effects of *TD.HLM01* hard capsule on experimental rodent models of endogenous and exogenous dyslipidemia. To create hyperlipidemia in the endogenous hyperlipidemia paradigm, mice were first given *TD.HLM01* hard capsule therapy and intraperitoneal injections of poloxamer-407. In the exogenous model, rats were given a diet of oil-cholesterol mixture orally and treated with *TD.HLM01* hard capsules for four consecutive weeks. Serum lipid parameters were evaluated in both experiments. The results indicated that *TD.HLM01* significantly reduced TC, TG, and non-HDL-C levels in the experimental models of exogenous hyperlipidemia and endogenous hyperlipidemia. *TD.HLM01* improved the serum LDH levels and ameliorated the elevated AST and ALT levels in cholesterol-induced hyperlipidemia rats. This study has demonstrated the potential efficacy of *TD.HLM01* in hyperlipidemia experimental models *in vivo*.

**Keywords:** dyslipidemia; oil-cholesterol diet; poloxamer–407; rodent; traditional medicine

## INTRODUCTION

Polyherbal formulations represent a traditional therapeutic approach that utilizes the synergistic effects of multiple medicinal herbs to optimize therapeutic outcomes in the treatment of cardiovascular diseases (Adegbola et al., 2017). Approximately 8% of the global population relies on herbal medications as part of their primary healthcare regimen, a number that is experiencing rapid growth worldwide (Welz et al., 2018). Dyslipidemia, defined by elevated levels of lipids in the blood, such as cholesterol, triglycerides, and diminished high-density lipoprotein cholesterol (HDL-C), is a critical health issue (Rader & Kathiresan, 2018). This condition serves as a significant risk factor for cardiovascular disease (CVD), which remains the leading cause of mortality globally. Dyslipidemia is intricately linked to the pathogenesis of CVD conditions, including atherosclerosis, myocardial infarction, and cerebrovascular accidents. Currently, statins are the most commonly utilized lipid-lowering agents due to their efficacy in reducing plasma lipid levels (US Preventive Services Task Force, 2022). However, these medications are associated with several adverse effects, including hepatotoxicity, rhabdomyolysis, and skeletal muscle injury, which have constrained their broader application. Consequently, there is a pressing need to identify and develop effective and natural alternatives that can regulate lipid metabolism. In recent years, traditional Vietnamese medicine has garnered increased attention for its potential in treating metabolic syndrome and has become a prevalent therapeutic approach for managing symptoms in patients with dyslipidemia. *TD.HLM01* hard capsules have been produced based on a traditional Vietnamese remedy that aims to treat blood lipid disorders. The main constituents of *TD.HLM01* combination are *Rhizoma Pinelliae, Rhizoma Atractylodis macrocephalae, Rhizoma Gastrodiae elatae, Pericarpium Citri reticulatae perenne, Poria, Radix et Rhizoma Glycyrrhizae, Folium et Radix Euodiae Leptae,* and *Cortex Garciniae*. To provide scientific evidence of its efficacy when combined in a formulation in treating dyslipidemia, the present study aimed to assess the effects of *TD.HLM01* hard capsules on serum lipid profiles of dyslipidemia experimental animals.

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DOI: 10.22146/mot.101779 | *Majalah Obat Tradisional, 30(3), 2025*

## MATERIALS AND METHODS

### Plant materials

The preparation *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 and identified based on the Vietnamese Pharmacopeia 5th edition (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 to under 5% humidity. Then, the dried extract product was ground to a fine powder, mixed with other excipients (talc, magnesium stearate, corn starch), and preserved in vacuum bags (storage conditions: temperature (25°C), humidity (under 75%)).

A 750 mg extracted powder was mainly produced from the major constituent herbs and excipients; then, it was formulated in a hard capsule as listed in Table I. These materials complied with the standards of Vietnamese Pharmacopeia V and standard basis 01NC/TCCS/TPT-22 confirmed by Sao Thai Duong Legal Director (Vietnamese Ministry of Health, 2017).

### *Experimental animals*

The animals for the experiments were Wistar rats (weighing 160–220 g) and Swiss mice (weighing 25 – 35 g), which were provided by the National Institute of Hygiene and Epidemiology. Seven days before the experiments and during the experimental period, the animals were housed under standard conditions (temperature between 23 and 25 °C, humidity of 55 ± 1.5%, a 12 h dark-night cycle, and free, unlimited, all-time access to water and pellet food) in the Laboratory of the Department of Pharmacology, Hanoi Medical University. This study was approved by the Scientific Board Committee of Hanoi Medical University (ref number: IRB00003121) with approval number 6807/QĐ-ĐHYHN.

### *Drugs and chemicals*

Propylthiouracil (Rieserstat®) 50mg, Cholesterol (Lot S107115, Merck, Germany), Acid Cholic (Sigma (Aldrich) Chemicals Pvt. Ltd. USA), Poloxamer 407 (Sigma (Aldrich) Chemicals Pvt. Ltd. USA), Atorvastatin 10mg (Stellapharm J.V. Co., Ltd.).

LDH (Lactate dehydrogenase). Malondialdehyde (MDA) and glutathione (GSH) were measured using an ELISA kit (Cloud-Clone Corp., USA) and a BioTek® ELx808 Reader.

### *Study methods*

All methods were carried out in accordance with ARRIVE guidelines and the National Institutes of Health guide for the care and use of laboratory animals.

*Exogenous dyslipidemia model in rats*

Dyslipidemia was induced in rats by oral administration of a cholesterol mixture (cholesterol 10%, cholic acid 1%, propylthiouracil (PTU) 0.5%, and peanut oil added to precisely 1mL) for 4 weeks (Ba Tuyen et al., 2021; Cunha et al., 2021). Adult *Wistar* rats (both sexes, weighing 190 ± 30 g) were divided into five groups, 10 rats per group. Rats were treated by gastric tube twice a day, at least two hours apart:

- Group 1 (normal control group): distilled water 1 mL/100 g b.w twice a day.
- Group 2 (model group): cholesterol mixture 10 mL/kg b.w/day and then distilled water 1 mL/100 g b.w.
- Group 3 (atorvastatin group): cholesterol mixture 10 mL/kg b.w/day and then atorvastatin at the dose of 10 mg/kg b.w/day.
- Group 4 (*TD.HLM01*): cholesterol mixture 10 mL/kg b.w/day and then TD.HLM01 at the dose of 0.18 g/kg/day.
- Group 5 (*TD.HLM01*): cholesterol mixture 10 mL/kg b.w/day and then TD.HLM01 at the dose of 0.54 g/kg/day.

Rat body weight was recorded at baseline, after 2 weeks, and after 4 weeks. On the 15th day and the 29th day, rats were fasted overnight. Blood was collected to analyze serum concentrations of total cholesterol (TC), triglyceride (TG), and HDL–C. At the end of the study, aspartate transaminase (AST), alanine aminotransaminase (ALT), albumin, total bilirubin, creatinine, LDH, MDA, and GSH were also quantified. Non-HDL-C concentration was calculated using the formula: Non-HDL-C = TC - (HDL-C) (mmol/L).

*Endogenous dyslipidemia model in mice*

The poloxamer 407 (P-407) induced dyslipidemia model was described by Millar et al. (Korolenko et al., 2016). In the experimental design, mice were randomly divided into five groups of ten animals, each as below:

- Group 1 (normal control group): Mice were given oral distilled water 1 ml/100 g b.w/day and then injected i.p. with 0.9% NaCl 10 ml/kg b.w on the 7th day.

**Table I. Composition and standards of medicinal plants and excipients in *TD.HLM01***

| No. | Medicinal material | Standards | Quantity |
|---|---|---|---|
| 1 | Rhizoma Pinelliae | Vietnamese Pharmacopeia V | 66 mg |
| | Rhizoma Atractylodis macrocephalae | Vietnamese Pharmacopeia V | 130 mg |
| | Rhizoma Gastrodiae elatae | Vietnamese Pharmacopeia V | 44 mg |
| | Pericarpium Citri reticulatae perenne | Vietnamese Pharmacopeia V | 44 mg |
| | Poria | Vietnamese Pharmacopeia V | 44 mg |
| | Radix et Rhizoma Glycyrrhizae | Vietnamese Pharmacopeia V | 22 mg |
| | Folium et Radix Euodiae Leptae | Vietnamese Pharmacopeia V | 200 mg |
| | Cortex Garciniae | 01NC/TCCS/TPT-22 | 200 mg |
| | Total of dry extract of medicinal herbs: | | 750 mg |
| 2 | Corn starch | Vietnamese Pharmacopeia V | 60 mg |
| 3 | Talc | Vietnamese Pharmacopeia V | 20 mg |
| 4 | Magnesium stearate | Vietnamese Pharmacopeia V | 10 mg |
| 5 | Gelatin capsule shell size 00 | Manufacturer's standard | 01 |

- Group 2 (model group): Mice were given oral distilled water 1 ml/100 g b.w/day; then injected i.p 2% P-407 at the dose of 200 mg/kg b.w on the 7$^{th}$ day.
- Group 3 (atorvastatin group): Mice were given oral atorvastatin at the dose of 100 mg/kg b.w/day; then injected i.p 2% P-407 at the dose of 200 mg/kg b.w on the 7$^{th}$ day.
- Group 4 (*TD.HLM01*): Mice were given oral TD.HLM01 at the dose of 0.36 g/kg/day; then injected i.p 2% P-407 at the dose of 200 mg/kg b.w on the 7$^{th}$ day.
- Group 5 (*TD.HLM01*): Mice were given oral TD.HLM01 at the dose of 1.08 g/kg/day; then injected i.p 2% P-407 at the dose of 200 mg/kg b.w on the 7$^{th}$ day.

The *TD.HLM01* doses tested in mice were adjusted using body surface area conversion factors (Center for Drug Evaluation and Research (FDA), 2005). Blood was collected at 24 hours after i.p injection of P-407 and analyzed for serum lipids such as TG, TC, and HDL-C. Non-HDL-cholesterol (non-HDL-C) was estimated with the formula: Non-HDL-C = TC - (HDL-C).

***Statistical analysis***

All data were analyzed using SPSS version 26. Data was shown as mean values and expressed as the mean ± standard deviation (SD). Statistical analysis was carried out using the student's t-test. The p-values of < 0.05 were considered to be statistically significant.

## RESULTS

After the four-week experiment period, all rats were still alive and gained normal weight. No adverse effect was observed, and there was no notable change in vital signs, skin, fur, or daily behaviors.

***Effect of TD.HLM01 on serum lipid levels in the exogenous dyslipidemia model***

After 2 weeks of treatment, atorvastatin tended to decrease levels of TG, TC, and Non-HDL-C and increase the HDL-C level as compared with the model group, but no significant difference was observed ($p > 0.05$). The groups were treated with *TD.HLM01* at the dose of 0.54 g/kg had considerably lower levels of TC, TG, and Non-HDL-C ( $p < 0.05$), while the HDL-C level was not increased as compared to the model group ($p > 0.05$). No differential change was observed between the groups treated with *TD.HLM01* at the dose of 0.18 g/kg and the model group ($p > 0.05$). *A*fter 4 weeks of treatment, atorvastatin and *TD.HLM01* at the two doses had a notable decrease in levels of TG ($p < 0.05$ & $p < 0.001$, respectively) as compared to the model group. However, in terms of the HDL-C level, no significant difference was observed between these groups and the model group ($p > 0.05$). Atorvastatin and *TD.HLM01* at the dose of 0.54 g/kg significantly reduced TC and Non-HDL-C levels ($p < 0.05$ and $p < 0.01$); *TD.HLM01* at the dose of 0.18 g/kg tended to reduce TC and Non-HDL-C levels as compared to the model group ($p > 0.05$) (Table I).

At the end of the study, the animals in the model group had a significant increase in AST and ALT as compared to the normal control group ($p < 0.01$ and $p < 0.001$, respectively). Atorvastatin and *TD.HLM01* at the two doses tended to attenuate AST and ALT as compared to the model group, but no significant difference was observed ($p > 0.05$). No significant change was observed in albumin, total bilirubin, and creatinine assessments among all groups. (Table II)

In the model group, LDH and MDA concentrations had a considerable increase ($p < 0.01$ and $p < 0.001$, respectively), and GSH was

**Table II. Effect of *TD.HLM01* on lipid levels in cholesterol induced hyperlipidemia**

| Group | TG | TC | HDL-C | Non-HDL-C |
|---|---|---|---|---|
| **Lipid levels in cholesterol induced hyperlipidemia after 2 weeks of treatment** | | | | |
| Normal control | 1,25 ± 0,16 | 1,12 ± 0,23 | 0,75 ± 0,16 | 0,49 ± 0,16 |
| Model | 3,28 ± 0,87*** | 1,13 ± 0,23 | 0,83 ± 0,25 | 2,45 ± 0,75*** |
| Atorvastatin 10 mg/kg | 2,81 ± 0,86 | 1,04 ± 0,26 | 0,94 ± 0,14 | 1,88 ± 0,77 |
| *TD.HLM01* 0.18 g/kg | 3,26 ± 0,48 | 0,98 ± 0,23 | 0,79 ± 0,17 | 2,46 ± 0,43 |
| *TD.HLM01* 0.54 g/kg | 2,50 ± 0,55$^{+}$ | 0,94 ± 0,15$^{+}$ | 0,70 ± 0,13 | 1,80 ± 0,55$^{+}$ |
| **Lipid levels in cholesterol induced hyperlipidemia after 4 weeks of treatment** | | | | |
| Normal control | 1,30 ± 0,13 | 1,08 ± 0,25 | 0,71 ± 0,12 | 0,59 ± 0,16 |
| Model | 4,11 ± 0,95*** | 1,40 ± 0,27* | 0,85 ± 0,15* | 3,26 ± 0,91*** |
| Atorvastatin 10 mg/kg | 2,89 ± 0,68$^{++}$ | 1,10 ± 0,28$^{+}$ | 0,84 ± 0,20 | 2,05 ± 0,65$^{++}$ |
| *TD.HLM01* 0.18 g/kg | 3,84 ± 0,65 | 0,85 ± 0,24$^{+++}$ | 0,74 ± 0,13 | 3,11 ± 0,70 |
| *TD.HLM01* 0.54 g/kg | 3,28 ± 0,62$^{+}$ | 0,74 ± 0,23$^{+++}$ | 0,88 ± 0,21 | 2,40 ± 0,73$^{+}$ |

Values are mean ± SD (n = 10). *, **, *** compared with normal control group ($p < 0.05$, $p < 0.01$, $p < 0.001$). $^{+}$, $^{++}$, $^{+++}$ compared with model group ($p < 0.05$, $p < 0.01$, $p < 0.001$).

significantly reduced ($p < 0.001$) as compared to the normal control group. In groups treated with *TD.HLM01* (0.18 g/kg and 0.54 g/kg), there were substantial reductions in LDH as compared with the model group ($p < 0.05$ and $p < 0.001$, respectively), had a tendency to lower MDA level and increase in the level of GSH but had non-significant changes as same as the model group. (Table III)

***Effects of TD.HLM01 on lipid levels in Poloxamer 407 induced dyslipidemia***

The serum lipid levels were significantly elevated in the P-407 control as compared to the normal control ($p < 0.001$). Atorvastatin and *TD.HLM01* at the two doses showed a considerable decrease in TC and Non-HDL-C levels and tended to increase HDL-C levels compared to the model group. No differential change was observed between *TD.HLM01* of both doses and the atorvastatin group ($p > 0.05$). *TD.HLM01* at the two doses tended to reduce TG level as compared to the model group, but no significant difference was observed ($p > 0.05$) and was statistically significant compared to the atorvastatin group ($p < 0.01$). (Figure 1)

## DISCUSSION

Hypercholesterolemia, characterized by elevated serum lipoproteins and reduced HDL-C levels, is a major contributing factor to the development of atherosclerotic disease. The primary cause of hypercholesterolemia is excessive dietary cholesterol intake. These conditions can disrupt lipoprotein metabolism, increase body weight, and elevate levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterol (LDL-C) while decreasing high-density lipoprotein cholesterol (HDL-C) levels in the serum. Nassiri and colleagues administered a mixture containing 10 g of cholesterol, 10 g of cholic acid, and 3 g of PTU in 100 mL of peanut oil to rats over four weeks (Andreadou et al., 2020; Ba Tuyen et al., 2021). Rats receiving 10 mL of this mixture per kilogram per day showed a 316.2 % increase in cholesterol as compared to the control group. The inclusion of cholic acid and PTU increases cholesterol absorption and reduces its conversion to bile acids, resulting in a more stable and uniform model. Ba Tuyen P, colleagues, and some researchers later refined this model by reducing the amounts of cholic acid and PTU (Andreadou et al., 2020; Ba Tuyen et al., 2021; Rajesh & Patil, 2024). This improved model consistently increased cholesterol levels in Vietnamese rat strains and has been successfully used by several researchers in Vietnam. We conducted our study using this improved model under similar conditions.

The therapeutic effects of the *TD.HLM01* hard capsules are attributed to a combination of herbal medicines that enhance lipid metabolism. In treated mice, a reduction in cholesterol levels was observed, likely due to decreased cholesterol absorption or increased utilization of triglycerides. These effects of *TD.HLM01* hard capsules are a combination of some herbal medicines that improve lipid metabolism. Firstly, *Rhizoma Atractylodis macrocephalae* was administered orally to high-fat-diet-induced dyslipidemia rats to confirm its effect *in vivo* and demonstrate an inhibitory effect of *Rhizoma Atractylodis macrocephalae* on adipogenesis through the reduction of an adipogenic factor, phospho-Akt (J.-H. Wang et al., 2015). This finding aligns closely with the research outcomes reported by Bo Li et al. (Li et al., 2024). Secondly, hesperidin in *Rhizoma Atractylodis macrocephalae* has been reported

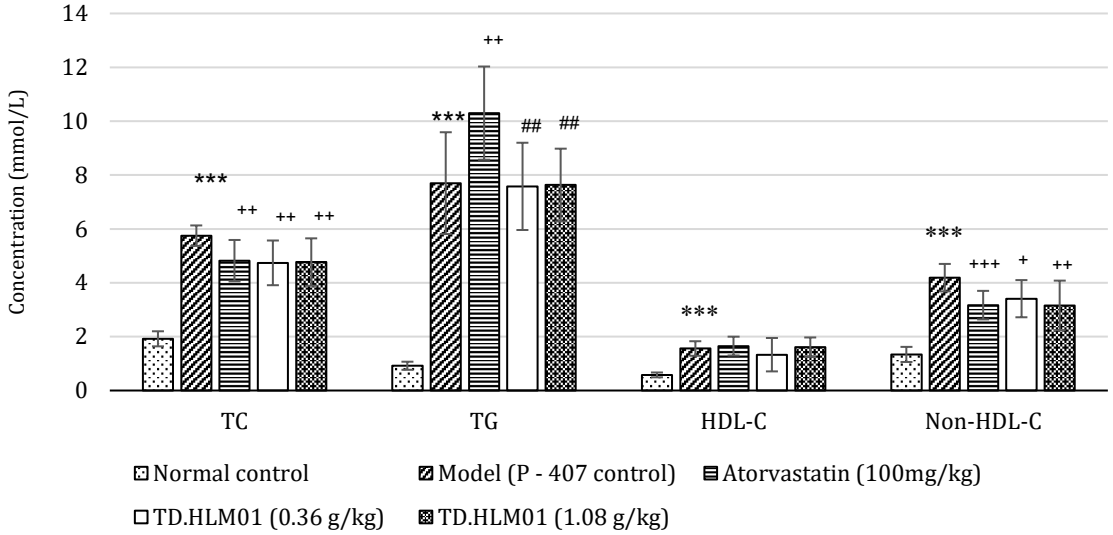


**Figure 1. Effect of *TD.HLM01* on lipid levels in Poloxamer 407 induced dyslipidemia**

Values are mean ± SD (n = 10). *** compared with normal control group ($p < 0.001$); $^{+}$, $^{++}$, $^{+++}$ compared with model group ($p < 0.05$, $p < 0.01$, $p < 0.001$); $^{\#\#}$ compared with atorvastatin group ($p< 0.01$).

**Table III. Effects of *TD.HLM01* on hepatic enzymes concentration and liver, kidney function after 4 weeks**

| Group (n=10) | AST | ALT | Albumin | Total bilirubin | Creatinine |
|---|---|---|---|---|---|
| Normal control | 76.10 ± 12.39 | 31.80 ± 4.05 | 3.03 ± 0.32 | 10.22 ± 0.52 | 80.90 ± 9.87 |
| Model | 108.70 ± 12.73*** | 47.20 ± 11.54** | 3.09 ± 0.30 | 10.42 ± 0.58 | 81.30 ± 8.10 |
| Atorvastatin 10 mg/kg | 96.60 ± 14.57** | 45.50 ± 13.97** | 3.05 ± 0.37 | 10.15 ± 0.78 | 81.80 ± 5.53 |
| *TD.HLM01* 0.18 g/kg | 101.30 ± 17.68** | 40.20 ± 11.50* | 3.03 ± 0.16 | 10.88 ± 0.83 | 83.30 ± 6.82 |
| *TD.HLM01* 0.54 g/kg | 101.30 ± 17.68** | 41.90 ± 7.75*** | 3.06 ± 0.23 | 10.87 ± 0.89 | 84.30 ± 7.44 |

Values are mean ± SD (n = 10). *, **, *** compared with normal control group ($p < 0.05$, $p < 0.01$, $p < 0.001$).

**Table IV. Effects of *TD.HLM01* on the levels of oxidation indices in the blood of rats after 4 weeks**

| Groups (n=10) | LDH (U/L) | MDA (nmol/ml) | GSH (µg/ml) | Groups (n=10) |
|---|---|---|---|---|
| Normal control | 555,10 ± 112,36 | 24,39 ± 6,28 | 87,83 ± 14,90 | Normal control |
| Model | 748,50 ± 120,79** | 51,00 ± 10,94*** | 45,45 ± 11,93*** | Model |
| Atorvastatin 10 mg/kg | 559,60 ± 120,42$^{++}$ | 38,36 ± 10,13$^{+}$ | 57,60 ± 11,35$^{+}$ | Atorvastatin 10 mg/kg |
| *TD.HLM01* 0.18 g/kg | 583,30 ± 137,93$^{+}$ | 44,95 ± 10,88 | 51,79 ± 12,73 | *TD.HLM01* 0.18 g/kg |
| *TD.HLM01* 0.54 g/kg | 498,30 ± 120,11$^{+++}$ | 42,59 ± 12,08 | 55,12 ± 8,65 | *TD.HLM01* 0.54 g/kg |

Values are mean ± SD (n = 10). **, *** compared with normal control group ($p < 0.05$, $p < 0.01$, $p < 0.001$); $^{+}$, $^{++}$, $^{+++}$ compared with model group ($p < 0.05$, $p < 0.01$, $p < 0.001$).

to possess significant anti-inflammatory, analgesic, antifungal, antiviral, and antioxidant activities (S.-H. Yang et al., 2023). Furthermore, *Pericarpium Citri reticulatae perenne* contains flavonoids such as narirutin, hesperidin, didymin, nobiletin, tangeretin, and heptamethoxyflavone, which have been shown to significantly reduce TC and LDL-C levels in hyperlipidemic rats induced by fat emulsion (H. Xiong et al., 2019; Yu et al., 2019). *Citri reticulatae Pericarpium* also contains hesperidin that ameliorates hypercholesterolemia and fatty liver by inhibiting cholesterol synthesis and absorption while regulating retinol-binding protein, cutaneous fatty acid-binding protein, and heart fatty acid-binding protein mRNA (Zou et al., 2022). Thirdly, an extract from *Cortex Garciniae*, considered a potential anti-obesity treatment, was extracted, with approximately half of its composition consisting of hydroxycitrate (Semwal et al., 2015). Oral administration of

(-) hydroxycitrate has been shown to suppress hepatic fatty acid synthesis and lipid accumulation in rats. The ingestion of hydroxycitrate orally has the capability to impede the synthesis of fatty acids and the accumulation of lipids in the liver of rats, resulting in a tendency towards reduced lipid levels in the body (Mohan et al., 2015).

An endogenous hyperlipidemia model was developed using intraperitoneal injection of P-407 at 200 mg/kg, a preferred polyether-based surfactant due to its rapid onset and lower toxicity. P-407 induces dose-dependent hypercholesterolemia and hypertriglyceridemia by inhibiting lipoprotein lipase, stimulating HMG-CoA (3-hydroxy-3-methylglutaryl CoA) reductase, and increasing hepatic cholesterol content (Itodo, 2023). Statins, chosen as the reference standard for their efficacy, inhibit HMG-CoA reductase, reducing serum total cholesterol and lowering LDL levels by decreasing its precursors, VLDL and IDL. Among the statins, atorvastatin is the more cost-effective option for high-intensity therapy (Davies et al., 2016). Due to its safety, P-407-induced hyperlipidemia models in rats and mice are commonly conducted with a standard dose of 0.5 g/kg. This study used a lower dose of 0.2 g/kg to facilitate the evaluation and comparison of treatment effects. Significant hyperlipidemia was achieved within 24 hours of intraperitoneal injection, with triglyceride levels increasing 8.4-fold, total cholesterol (TC) and non-HDL cholesterol levels tripling, and HDL cholesterol levels rising 2.7-fold. The result indicated that *TD.HLM01* (0.36 g/kg/day) significantly lowered TC and non-HDL cholesterol levels in dyslipidemic mice while slightly increasing HDL cholesterol levels. The observation that a threefold increase in *TD.HLM01* dosage (from 0.18 to 0.54 g/kg in rats; 0.36 to 1.08 g/kg in mice) did not produce significantly enhanced effects on most serum lipids, liver, and kidney parameters warrants careful consideration, with several possible pharmacological and physiological factors. *TD.HLM01* is a traditional remedy, combining a variety of herbs, its active compounds likely act on various biological targets involved in lipid metabolism (e.g., HMG-CoA reductase, lipoprotein lipase, cholesterol absorption transporters) (Arefhosseini et al., 2022; Feng et al., 2018; Gogoi et al., 2015; Nguyen et al., 2023; F. Xiong et al., 2021; L. Yang et al., 2024). The lower dose (0.18 g/kg or 0.36 g/kg) might already be sufficient to saturate these key targets, such as HMG-CoA and lipid absorption in the gut (Engelking et al., 2012; Gesto et al., 2020). Once saturated, adding more drug cannot increase the effect mediated by that specific pathway (Witte et al., 2018). In addition, the transport mechanisms responsible for absorbing the active compounds from the gut could become saturated at higher oral doses (X.-X. Wang et al., 2017). This means a threefold increase in administered dose might not lead to a proportional increase in the concentration of active compounds in the bloodstream and target tissues. Moreover, most of the lipid parameters reached approximately their normal levels, except for TG, which may indicate the ceiling effect of active compounds or the potentially different potencies and mechanisms, less potent compounds requiring much higher doses to show a significant additional effect (Bhattaram et al., 2002). Further investigations need to be conducted to clarify the dose-dependent manner of *TD.HLM01*, while the starting dose of the product should be used in lower doses in other studies based on these findings.

Synthetic drugs are popularly used to treat dyslipidemia, with a high concern about their impacts on the liver, particularly statins, the most effective agent in lipid-related disorders treatment (B. Wang et al., 2025). The model groups in both experiments showed significantly elevated lipids (TC, TG, Non-HDL-C), and, in the rat model, AST and ALT were significantly elevated compared to the normal control. This aligns with the known link between dyslipidemia and liver stress/damage (Alruhaimi et al., 2024). Atractylenolide I (AO-I), the principal active compound in *Rhizoma Atractylodis macrocephalae*, has demonstrated the ability to normalize ALT and AST levels in liver injury (Gao et al., 2023). Consequently, assessments were carried out to evaluate antioxidant efficacy and liver and kidney functionality. However, it was hoped that with further evidence-based studies, this product would become a mainstream medicine with fewer adverse effects on the liver.

Oxidative stress is an early event in the evolution of hyperlipidemia, and appropriate support for enhancing antioxidant supply in higher lipid subjects may help prevent the course of the disease (Vekic et al., 2023). In the present study, LDH, MDA, and GSH activities were measured to assess the modulation of oxidative stress-related enzymes in cholesterol-induced hyperlipidemic rats. *TD.HLM01* treatment significantly lowered LDH in both dose groups and tended to improve MDA/GSH towards normal levels, alongside lipid improvements. This indicated a strong correlation: the dyslipidemic state promotes oxidative stress and cellular damage, and the treatment's ability to ameliorate hyperlipidemia is associated with reduced oxidative stress. LDH, an important enzyme in the anaerobic metabolic pathway,

has been demonstrated to be related to the occurrence of cardiovascular diseases (Zhu et al., 2022). LDH's link to cardiovascular risk makes its reduction by *TD.HLM01* particularly relevant. This finding indicated the potential of *TD.HLM01* in the preventive treatment of hyperlipidemia and other clinical hyperlipidemia-related disorders.

In summary, evaluating lipids, liver enzymes, kidney function, and oxidative stress markers concurrently for *TD.HLM01* provides a novel, integrated view of its effects and preliminary safety profile in modulating both endogenous and exogenous lipid metabolism pathways, marking the scientific rationale for the combination of medicinal herbs in cases of mixed dyslipidemia and other hyperlipidemia-related disorders in clinical settings. Moreover, this study was conducted to reveal the potential of *TD.HLM01* in both endogenous and exogenous pathways of lipid metabolism, providing data for its usage in mixed high lipid level cases. By bridging traditional medicine with modern pharmacological research, *TD.HLM01* hard capsule positions it as a promising candidate for an alternative therapeutic approach for hyperlipidemia, with potential implications for the prevention of cardiovascular events in subsequent clinical applications.

## CONCLUSION

Our results demonstrate that the *TD.HLM01* hard capsule effectively treated hyperlipidemia in experimental models, significantly reducing elevated TC, TG, and non-HDL-C levels in both Poloxamer-407-induced mice and cholesterol-fed rats. While both tested doses showed efficacy compared to the untreated model groups, the higher dose did not consistently produce significantly better results across all measured blood lipid or liver parameters compared to the lower dose, suggesting a possible plateau in the dose-response effect within this range. These findings provide preclinical support for *TD.HLM01* as a potentially effective traditional medicine for managing dyslipidemia, warranting further investigation into its mechanisms and clinical potential.

## ACKNOWLEDGEMENT

The authors acknowledge and thank the HMU staffs for their helpful advice and technical support. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

## REFERENCES

Adegbola, P., Aderibigbe, I., Hammed, W., & Omotayo, T. (2017). Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: A review. *American Journal of Cardiovascular Disease*, *7*(2), 19–32.

Alruhaimi, R. S., Hussein, O. E., Alnasser, S. M., Germoush, M., Alotaibi, M., Hassanein, E. H. M., Mohtadi, M. E., & Mahmoud, A. M. (2024). Oxidative Stress, Inflammation, and Altered Lymphocyte E-NTPDase Are Implicated in Acute Dyslipidemia in Rats: Protective Role of Arbutin. *Pharmaceuticals*, *17*. https://doi.org/10.3390/ph17101343

Andreadou, I., Schulz, R., Badimon, L., Adameová, A., Kleinbongard, P., Lecour, S., Nikolaou, P., Falcão-Pires, I., Vilahur, G., Woudberg, N., Heusch, G., & Ferdinandy, P. (2020). Hyperlipidaemia and cardioprotection: Animal models for translational studies. *British Journal of Pharmacology*, *177*(23), 5287–5311. https://doi.org/10.1111/bph.14931

Arefhosseini, S., Tutunchi, H., Nomi-Golzar, S., Mahboob, S., Pouretedal, Z., & Ebrahimi-Mameghani, M. (2022). The effect of hydroxy citric acid supplementation with calorie-restricted diet on metabolic, atherogenic and inflammatory biomarkers in women with non-alcoholic fatty liver disease: A randomized controlled clinical trial. *Food & Function*, *13*(9), 5124–5134. https://doi.org/10.1039/d1fo03685h

Ba Tuyen, P., Huyen, T. T., Hang, D. T. T., & Thi Van Anh, P. (2021). A Novel Herbal Medicine for Dyslipidemia: Assessments in Experimental Models. *Evidence-Based Complementary and Alternative Medicine : eCAM*, *2021*, 5529744. https://doi.org/10.1155/2021/5529744

Bhattaram, V., Graefe, U., Kohlert, C., Veit, M., & Derendorf, H. (2002). Pharmacokinetics and bioavailability of herbal medicinal products. *Phytomedicine : International Journal of Phytotherapy and Phytopharmacology*, *9 Suppl 3*, 1–33. https://doi.org/10.1078/1433-187X-00210

Center for Drug Evaluation and Research (FDA). (2005). *Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*. Pharmacology and Toxicology.

Cunha, L. F., Ongaratto, M. A., Endres, M., & Barschak, A. G. (2021). Modelling

hypercholesterolaemia in rats using high cholesterol diet. *International Journal of Experimental Pathology*, *102*(2), 74–79. https://doi.org/10.1111/iep.12387

Davies, J. T., Delfino, S. F., Feinberg, C. E., Johnson, M. F., Nappi, V. L., Olinger, J. T., Schwab, A. P., & Swanson, H. I. (2016). Current and Emerging Uses of Statins in Clinical Therapeutics: A Review. *Lipid Insights*, *9*, 13–29. https://doi.org/10.4137/LPI.S37450

Engelking, L. J., McFarlane, M., Li, C., & Liang, G. (2012). Blockade of cholesterol absorption by ezetimibe reveals a complex homeostatic network in enterocytes[S]. *Journal of Lipid Research*, *53*, 1359–1368. https://doi.org/10.1194/jlr.M027599

Feng, S., Dai, Z., Liu, A. B., Huang, J., Narsipur, N., Guo, G., Kong, B., Reuhl, K., Lu, W., Luo, Z., & Yang, C. S. (2018). Intake of stigmasterol and β-sitosterol alters lipid metabolism and alleviates NAFLD in mice fed a high-fat western-style diet. *Biochimica Et Biophysica Acta. Molecular and Cell Biology of Lipids*, *1863*(10), 1274–1284. https://doi.org/10.1016/j.bbalip.2018.08.004

Gao, Y., Xin, D., Liang, X.-D., & Tang, Y. (2023). Effect of a combination of Atractylodes macrocephala extract with strychnine on the TLR4/NF-κB/NLRP3 pathway in MH7A cells. *Experimental and Therapeutic Medicine*, *25*(2), 91. https://doi.org/10.3892/etm.2023.11791

Gesto, D., Pereira, C. M. S., Cerqueira, N., & Sousa, S. (2020). An Atomic-Level Perspective of HMG-CoA-Reductase: The Target Enzyme to Treat Hypercholesterolemia. *Molecules*, *25*. https://doi.org/10.3390/molecules25173891

Gogoi, A., Gogoi, N., & Neog, B. (2015). Dubious anti-obesity agent HCA from garcinia: A systematic review. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*.

Itodo, M. (2023). *Antilipidemic Potentials of Moringa Oleifera Root Extract in Poloxamer 407- Induced Hyperlipidemia*. https://doi.org/10.5281/zenodo.10438987

Korolenko, T. A., Johnston, T. P., Tuzikov, F. V., Tuzikova, N. A., Pupyshev, A. B., Spiridonov, V. K., Goncharova, N. V., Maiborodin, I. V., & Zhukova, N. A. (2016). Early-stage atherosclerosis in poloxamer 407-induced hyperlipidemic mice: Pathological features and changes in the lipid composition of serum lipoprotein fractions and subfractions. *Lipids in Health and Disease*, *15*(1), 16. https://doi.org/10.1186/s12944-016-0186-7

Li, B., Jiang, X.-F., Dong, Y.-J., Zhang, Y.-P., He, X.-L.-S., Zhou, C.-L., Ding, Y.-Y., Wang, N., Wang, Y.-B., Cheng, W.-Q., Jiang, N.-H., Su, J., Lv, G.-Y., & Chen, S.-H. (2024). The effects of *Atractylodes macrocephala* extract BZEP self-microemulsion based on gut–liver axis HDL/LPS signaling pathway to ameliorate metabolic dysfunction-associated fatty liver disease in rats. *Biomedicine & Pharmacotherapy*, *175*, 116519. https://doi.org/10.1016/j.biopha.2024.116519

Mohan, S. K., Veeraraghavan, V. P., & Jainu, M. (2015). Effect of pioglitazone, quercetin and hydroxy citric acid on extracellular matrix components in experimentally induced non-alcoholic steatohepatitis. *Iranian Journal of Basic Medical Sciences*, *18*(8), 832–836.

Nguyen, T. H., Ly, H. H. V., Nguyen, T. M., Diem Le, T. T., Phung, T. M., Nejatbakhsh, R., Nguyen, K. T., & Tran, T. T. T. (2023). The effects of *Glycyrrhiza Glabra* extract in high-fat diet-induced obese male mice: A controlled experimental study. *Obesity Medicine*, *44*, 100525. https://doi.org/10.1016/j.obmed.2023.100525

Rader, D. J., & Kathiresan, S. (2018). Disorders of Lipoprotein Metabolism. In J. L. Jameson, A. S. Fauci, D. L. Kasper, S. L. Hauser, D. L. Longo, & J. Loscalzo (Eds.), *Harrison's Principles of Internal Medicine* (20th ed.). McGraw-Hill Education. accessmedicine.mhmedical.com/content.aspx?aid=1183992782

Rajesh, O., & Patil, V. (2024). Review Article on Animal Models in Preclinical Research in Hyperlipidemia. *International Journal of Pharmaceutical Sciences Review and Research*, *84*, 71–75. https://doi.org/10.47583/ijpsrr.2024.v84i01.011

Semwal, R., Semwal, D., Vermaak, I., & Viljoen, A. (2015). A comprehensive scientific overview of Garcinia cambogia. *Fitoterapia*, *102*, 134–148. https://doi.org/10.1016/j.fitote.2015.02.012

US Preventive Services Task Force. (2022). Statin Use for the Primary Prevention of Cardiovascular Disease in Adults: US Preventive Services Task Force Recommendation Statement. *JAMA*, *328*(8),

746–753. https://doi.org/10.1001/jama.2022.13044

Vekic, J., Stromsnes, K., Mazzalai, S., Zeljkovic, A., Rizzo, M., & Gambini, J. (2023). Oxidative Stress, Atherogenic Dyslipidemia, and Cardiovascular Risk. *Biomedicines*, *11*(11), Article 11. https://doi.org/10.3390/biomedicines11112897

Vietnamese Ministry of Health. (2017). *Vietnamese Pharmacopeia* (Fifth edition). Medical Publishing House Co., Ltd.

Wang, B., Huang, S., Li, S., Deng, Y., Li, Z., Wang, Y., Shi, X., Zhang, W., Shi, L., Wang, X., & Tang, X. (2025). Hepatotoxicity of statins: A real-world study based on the US Food and Drug Administration Adverse Event Reporting System database. *Frontiers in Pharmacology*, *15*. https://doi.org/10.3389/fphar.2024.1502791

Wang, J.-H., Bose, S., Kim, H.-G., Han, K.-S., & Kim, H. (2015). Fermented Rhizoma Atractylodis Macrocephalae alleviates high fat diet-induced obesity in association with regulation of intestinal permeability and microbiota in rats. *Scientific Reports*, *5*(1), 8391. https://doi.org/10.1038/srep08391

Wang, X.-X., Liu, G.-Y., Yang, Y.-F., Wu, X., Xu, W., & Yang, X. (2017). Intestinal Absorption of Triterpenoids and Flavonoids from Glycyrrhizae radix et rhizoma in the Human Caco-2 Monolayer Cell Model. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*, *22*. https://doi.org/10.3390/molecules22101627

Welz, A. N., Emberger-Klein, A., & Menrad, K. (2018). Why people use herbal medicine: Insights from a focus-group study in Germany. *BMC Complementary and Alternative Medicine*, *18*(1), 92. https://doi.org/10.1186/s12906-018-2160-6

Witte, W. E. A., Danhof, M., Graaf, P., & Lange, E. D. (2018). The implications of target saturation for the use of drug–target residence time. *Nature Reviews Drug Discovery*, *18*, 82–84. https://doi.org/10.1038/nrd.2018.234

Xiong, F., Liang, H.-X., Zhang, Z.-J., Mahmud, T., Chan, A. S. C., Li, X., & Lan, W.-J. (2021). Characterization, Antioxidant and Antitumor Activities of Oligosaccharides Isolated from Evodia lepta (Spreng) Merr. By Different Extraction Methods. *Antioxidants*, *10*(11), 1842. https://doi.org/10.3390/antiox10111842

Xiong, H., Wang, J., Ran, Q., Lou, G., Peng, C., Gan, Q., Hu, J., Sun, J., Yao, R., & Huang, Q. (2019). Hesperidin: A Therapeutic Agent For Obesity. *Drug Design, Development and Therapy*, *13*, 3855–3866. https://doi.org/10.2147/DDDT.S227499

Yang, L., Qin, S.-H., & Zi, C.-T. (2024). Research progress of Gastrodia elata Blume polysaccharides: A review of chemical structures and biological activities. *Frontiers in Chemistry*, *12*, 1395222. https://doi.org/10.3389/fchem.2024.1395222

Yang, S.-H., Zhu, J., Wu, W.-T., Li, J.-M., Tong, H.-L., Huang, Y., Gong, Q.-F., Gong, F.-P., & Zhong, L.-Y. (2023). Rhizoma Atractylodis Macrocephalae—Assessing the influence of herbal processing methods and improved effects on functional dyspepsia. *Frontiers in Pharmacology*, *14*, 1236656. https://doi.org/10.3389/fphar.2023.1236656

Yu, J.-J., Su, J., Yan, M.-Q., Lou, Z.-H., & Lyu, G.-Y. (2019). [Correlation between lipid-lowering efficacy and components of Pericarpium Citri Reticulatae]. *Zhongguo Zhong Yao Za Zhi = Zhongguo Zhongyao Zazhi = China Journal of Chinese Materia Medica*, *44*(15), 3335–3342. https://doi.org/10.19540/j.cnki.cjcmm.20190523.304

Zhu, W., Ma, Y., Guo, W., Lu, J., Li, X., Wu, J., Qin, P., Zhu, C., & Zhang, Q. (2022). Serum Level of Lactate Dehydrogenase is Associated with Cardiovascular Disease Risk as Determined by the Framingham Risk Score and Arterial Stiffness in a Health-Examined Population in China. *International Journal of General Medicine*, *15*, 11–17. https://doi.org/10.2147/IJGM.S337517

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. *Biomedicine & Pharmacotherapy*, *154*, 113626. https://doi.org/10.1016/j.biopha.2022.113626