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Anti-hyperlipidemic Effect of *Camellia hakodae* Ninh Extract in an *In Vivo* Rat Model

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ABSTRACT

Vietnamese yellow tea, *Camellia hakodae* Ninh (Theaceae), is an endemic species in Vietnam. Its neutral properties and sweet taste is readily harnessed in traditional Vietnamese medicine for the management of heart, kidney, and liver problems. Yellow tea is known to lower blood pressure and lipids but the anti-hyperlipidemic effect of *C. hakodae* has not been studied. This study aimed to assess the blood lipid-lowering effects of the dry extract of *C. hakodae* leaves (CKL-THV) in rats. Wistar rats were induced with exogenous hyperlipidemia using a cholesterol-rich diet and then administered CKL-THV continuously for 4 weeks. The study evaluated the effects of CKL-THV on lipid profiles and the atherogenic index of plasma (AIP). Results indicated that CKL-THV at doses of 0.35 and 1.05 g/kg/day effectively reduced total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) indices compared to the model group after both 2 and 4 weeks. Notably, CKL-THV at a dose of 1.05 g/kg/day significantly ($p < 0.05$) increased HDL-C level compared to the model group. Both doses of CKL-THV significantly lowered the AIP compared to the model group ($p < 0.05$). CKL-THV demonstrated the ability to regulate blood lipid indices and reduce the AIP in a rat model of exogenous hyperlipidemia. These findings suggest that CKL-THV holds potential as an anti-hyperlipidemic agent.

Keywords: Endogenous dyslipidemia, Exogenous hyperlipidemia, Yellow tea, *Camellia hakodae*.

Introduction

Dyslipidemia does not only leads to quantitative changes in lipoprotein concentration but also causes an alteration in the qualitative composition of various lipoprotein subfractions, including high-density lipoprotein particles. Lipid dyslipidemia is a common health concern worldwide that increases the risk of atherosclerosis, a major factor in cardiovascular disease.[1,2] Currently, many different methods are used to treat lipid disorders.[3–5] However, research into new, safer, and more effective treatments remains a high priority, especially in Vietnam with its rich tradition of herbal medicine. *Camellia hakodae* Ninh. (*Camellia* genus), or Vietnamese yellow tea, is a species endemic in Vietnam. According to traditional Vietnamese medicine, yellow tea leaf has antioxidant and anti-cancer effects, lowers blood pressure and lipids, and prevents atherosclerosis.[6] It has neutral properties, and a sweet taste, and is associated with the heart, kidney, and liver meridians. *C. hakodae* has been the subject of only minimal research on its chemical composition and pharmacological effects. A recent study by Nguyen *et al*. identified several pentacyclic triterpene derivatives and their potent cytotoxic activity against various human cancer cells.[7]

To make products that support the treatment of lipid disorders, the pharmacy training Institute at Vietnam Military Medical University has developed a dried extract of *C. hakodae* leaves (CKL-THV) to support the treatment of dyslipidemia disorders. The extract was obtained using an ultrasonic extraction method. To evaluate the efficacy of treatments for lipid disorders via exogenous mechanisms, researchers often employ the classic method pioneered by Nassiri-Asl *et al*.[8] Suitable for screening studies, this method induces hyperlipidemia in rat models through a high-fat and cholesterol-rich diet. In 2013, Thong *et al.* refined this model by modifying the diet, reducing the levels of cholic acid and propylthiouracil. This adjustment helps to mitigate excessive increases in blood lipid levels, enabling a more precise evaluation of the study agent's effects. Moreover, this modified model is more cost-effective than the classic model.[9] Building upon this approach, our research focuses on assessing the impact of CKL-THV on blood lipid and atherogenic indices in experimental rats.

Materials and Methods

Plant Materials

Leaves of *C. hakodae* were collected from Tam Quan commune (21°26′20″N 105°36′3″E), Tam Dao District, Vinh Phuc Province, Vietnam in March 2022. Botanical identification was confirmed by Bui Hong Quang, PhD, at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). To ensure traceability, a voucher specimen (MB-01) was archived at the Department of Pharmacology, Vietnam Military Medical University, Vietnam. The dried leaf extract of *C. hakodae* met standards quality specifications. After harvesting, the *C. hakodae* leaves were washed, dried, ground into powder, and ultrasonically extracted with 96% ethanol. The extract was then concentrated and spray-dried to obtain a dry extract powder (CKL-THV). Based on traditional knowledge and the preparation process,[10] the anticipated human dose of CKL-THV was calculated to be 0.05 g/kg/day. To determine the appropriate dose for rats, dose extrapolation was applied based on the differences in body weight (BW) and metabolism between humans and rats.[11] Using a factor of 7, the human dose was converted into a corresponding dose for rats (0.35 g/kg/day). CKL-THV powder was mixed with distilled water to create suspensions at two concentrations: 0.035 g/ml and 0.105 g/ml. The treatment groups received the suspension by oral administration at a volume of 10 ml/kg, equating to effective doses of 0.35 and 1.05 g/kg/day.

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Equipment
An Evolution 3000 biochemical testing machine (Biochemical Systems International, Italy) was used in the study. Quantification kits including total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were purchased from Erba (Germany). As a positive control, atorvastatin was obtained from STADA (Hanoi, Vietnam).

Animals
Adult Wistar rats of both sexes (180−200 g) were procured from the Laboratory Animal Supply Department at Vietnam Military Medical University and were maintained under laboratory conditions. They were fed a standard diet and had unrestricted access to water throughout the study. The research was conducted with the approval of the Scientific Council of the Military Medical University and complied with ethical standards in medical research (ethical permission number IACUC-2203/22, issued on March 22, 2022).

In Vivo Test
The study employed the modified Nassiri's exogenous dyslipidemia model.[8,9] A cholesterol oil mixture was prepared by heating peanut oil (200 mg), adding cholesterol (50 g), stirring until dissolved, and cooling. Cholic acid (5 g) and propylthiouracil (PTU, 2.5 g) were added, followed by peanut oil to a final volume of 500 ml The result was a 10% (w/v) cholesterol oil mixture, 1 ml of which contained 0.1 g of cholesterol, 0.01 g of cholic acid, and 0.005 g PTU. Fifty adult Wistar rats were acclimatized for one week and then randomly divided into five groups of ten. Every morning for 4 weeks, all groups received treatment orally through a specialized blunt-tipped curved needle inserted directly into the stomach:
Control group (G1): distilled water (10 ml/kg BW)
Model group (G2): cholesterol oil mixture (10 ml/kg/day), followed by distilled water (10 ml/kg/day) 2 hours later.
Atorvastatin group (G3): cholesterol oil mixture (10 ml/kg/day), followed by atorvastatin (10 mg/kg/day) 2 hours later.
CKL-THV low dose (G4): cholesterol oil mixture (10 ml/kg/day), followed by CKL-THV extract (0.35 g/kg/day) 2 hours later.
CKL-THV high dose (G5): cholesterol oil mixture (10 ml/kg/day), followed by CKL-THV extract (1.05 g/kg/day) 2 hours later.
Blood samples were collected from the orbital sinus after the rats were fasted overnight. Plasma was isolated by centrifuging the blood at 3000 rpm for 15 minutes at room temperature. Plasma samples were then incubated with specific reagents, followed by photometric determination of blood lipid content including TC, TG, and HDL-C at the beginning of the experiment, after two weeks, and after four weeks. LDL-C and non-HDL-C index were calculated according to the Friedewald formula (Equations 1 and 2):

$$LDL - C = TC - HDL - C - \frac{TG}{2.2} \quad (1)$$

$$Non\ HDL - C = TC - HDL - C \quad (2)$$

The atherogenic index of plasma (AIP) was calculated according to Equation 3:

$$AIP = \log \frac{TG}{HDL-C} \quad (3)$$

Statistical Analysis
The data was processed according to appropriate biomedical statistical methods, considering the data type (parametric or non-parametric), using SPSS 22.0 software. Data were expressed as mean ± standard deviation ($\overline{X}$ ± SD). A one-way ANOVA test was used to compare average values between three or more groups. Post-hoc assessment will be performed using the least significant difference (LSD) test if variances were homogenous, or Dunnett's T3 test if variances are heterogeneous. A statistically significant difference is considered at $p < 0.05$.

Results and Discussion

Total Cholestrol in Rat Blood
After 2 weeks (T2) and 4 weeks (T4) of administering the cholesterol oil mixture, the total cholesterol concentration in the blood of rats in groups G2−G5 increased compared to that of T0 (Figure 1), and the result was statistically significant compared to the control group G1 ($p < 0.01$). In the model group (G2), the cholesterol oil mixture effectively increased TC from 1.91 ± 0.22 mmol/l at baseline to 3.50 ± 0.61 and 4.26 ± 0.55 mmol/l at weeks 2 and 4. Atorvastatin treatment (G3) significantly reduced TC levels compared to the model group (G2) at both week 2 (2.84 ± 0.378 mmol/l decrease) and week 4 (3.31 ± 0.70 mmol/l decrease), demonstrating its effectiveness in lowering cholesterol. Regarding the CKL-THV treatments (G4 and G5), both doses significantly lowered TC levels compared to the model group (G2) after two weeks ($p < 0.05$) and four weeks ($p < 0.01$), suggesting that CKL-THV has a cholesterol-lowering effect. It is noteworthy that there was no statistically significant difference in TC levels ($p > 0.05$) between the atorvastatin and CKL-THV groups throughout the study, indicating that CKL-THV might be as effective as atorvastatin in reducing cholesterol in this model. Thus, the results suggest CKL-THV to be a potential cholesterol-lowering agent.

TG Concentration in Rat Blood
The rat blood TG concentration results are shown in Figure 2. In the model group (G2), consistent with the expected effects of the cholesterol oil mixture, the TG levels increased from 0.77 ± 0.14 to 0.96 ± 0.17 mmol/l between T0 and T4. Compared to the control group G1, TG levels were significantly elevated after 2 weeks ($p < 0.01$) and even more so after 4 weeks ($p < 0.001$). Interestingly, both the atorvastatin (G3) and CKL-THV groups (G4 and G5) showed a significant increase in TG levels after 4 weeks compared to the control group ($p < 0.05$). This is counterintuitive, as these groups were receiving treatments expected to lower TG. Compared to the model group (G2), both CKL-THV doses (0.35 and 1.05 g/kg/day) significantly reduced TG levels after two weeks ($p < 0.05$). The high dose (G5) maintained this effect at week 4 ($p < 0.05$), and the low dose (G4) also showed a significant reduction at week 4 compared to the model group, suggesting that CKL-THV may have a beneficial effect on lowering TG. Like the findings for cholesterol, there was no statistically significant difference in TG levels ($p > 0.05$) between the atorvastatin (G3) and CKL-THV groups (G4 and G5) throughout the study, again indicating that CKL-THV might be comparable to atorvastatin in reducing TG in this model.

LDL-C Concentration in Rat Blood
LDL-C concentration was calculated using the Friedewald formula (Equation 1) with the results shown in Figure 3. After administering the cholesterol oil mixture for 2 weeks, the blood LDL-C concentration in all groups (G2−G5) showed a statistically significant increase compared to the control group ($p < 0.01$). The significance level reached $p < 0.001$ after 4 weeks. In the model group (G2), LDL-C increased from 0.17 ± 1.07 to 2.15 ± 0.67 mmol/l (week 2) and 2.89 ± 0.61 mmol/l (week 4). However, both atorvastatin (G3) and CKL-THV (G4 and G5) were effective in reducing blood LDL-C compared to the model group (G2). The change was statistically significant with $p < 0.05$ after 2 weeks and $p < 0.001$ after 4 weeks. There was no difference in LDL-C concentrations between the atorvastatin and CKL-THV groups during the study period ($p > 0.05$).

HDL-C Concentration in Rat Blood
No statistically significant change in HDL-C levels ($p > 0.05$) were found in any group throughout the study compared to the control group (Figure 4). This suggests that neither the cholesterol oil mixture nor atorvastatin (G3) or CKL-THV groups (G4 and G5) had a major effect on HDL-C in this model. Interestingly, the high dose of CKL-THV (G5, 1.05 g/kg/day) specifically increased HDL-C levels compared to the model group (G2) after 2 weeks ($p < 0.05$). Atorvastatin (G3) increased HDL-C levels compared to the model group, but only at week 4 ($p < 0.05$). There was no statistically significant difference in HDL-C levels ($p > 0.05$) between the atorvastatin and CKL-THV groups throughout the study. Hence, both CKL-THV and atorvastatin may have some influence on HDL-C levels. Since HDL is considered "good" cholesterol, its increase with high-dose CKL-THV (G5, 1.05 g/kg/day) is a promising finding.

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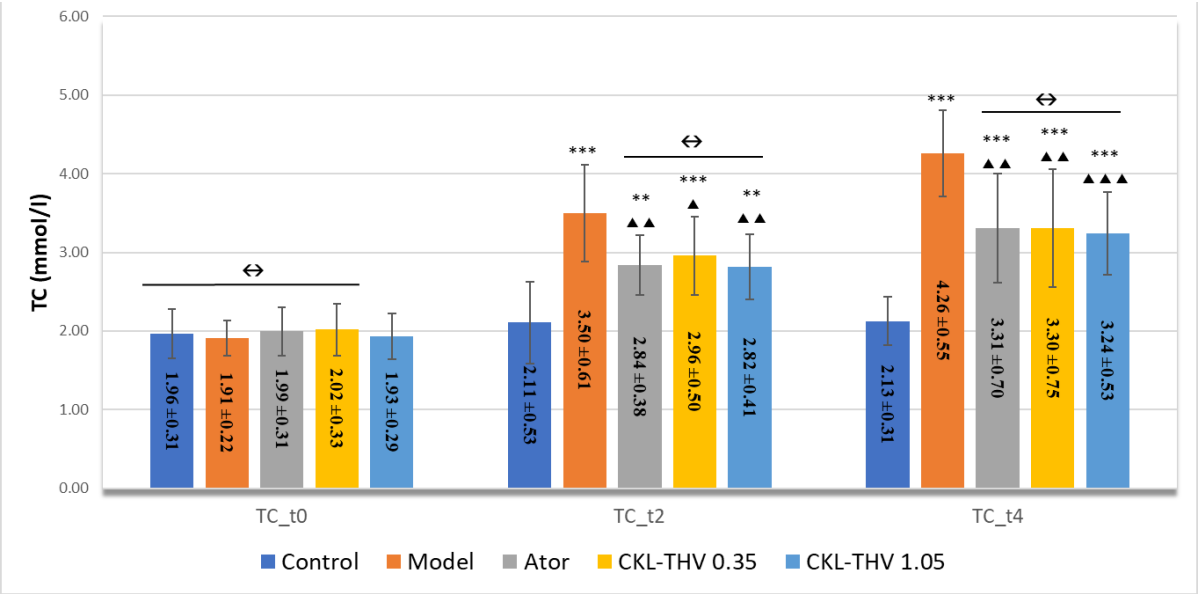

Figure 1: TC concentration (mmol/l). Control group (G1): distilled water (10 ml/kg BW). Model group (G2): cholesterol oil (10 ml/kg) then distilled water (10 ml/kg/day) 2 hours later. Ator (atorvastatin) group (G3): cholesterol oil mixture (10 ml/kg/day), then atorvastatin (10 mg/kg/day) 2 hours later. CKL-THV 0.35 (G4): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (0.35 g/kg/day) 2 hours later. CKL-THV 1.05 (G5): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (1.05 g/kg/day) 2 hours later. n = 10, ± SD.

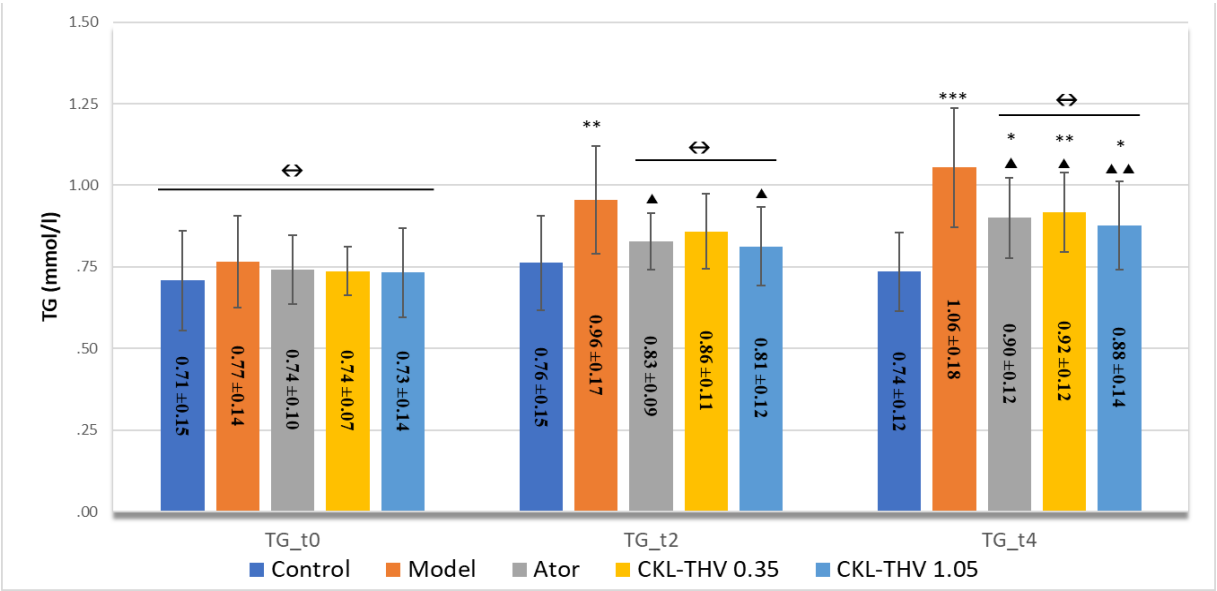

Figure 2: TG concentration (mmol/l). Control group (G1): distilled water (10 ml/kg BW). Model group (G2): cholesterol oil (10 ml/kg), then distilled water (10 ml/kg/day) 2 hours later. Ator (atorvastatin) group (G3): cholesterol oil mixture (10 ml/kg/day), then atorvastatin (10 mg/kg/day) 2 hours later. CKL-THV 0.35 (G4): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (0.35 g/kg/day) 2 hours later. CKL-THV 1.05 (G5): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (1.05 g/kg/day) 2 hours later. n = 10, ± SD.

Non-HDL-C Concentration in Rat Blood

Non-HDL-C concentrations were calculated using the Friedewald formula (Equation 2), with the results shown in Figure 5. As expected, in the model group (G2), the cholesterol oil mixture significantly increased non-HDL cholesterol levels (2.58 ± 0.66 mmol/l) compared to the control group (G1, 1.06 ± 0.22 mmol/l). This effect became stronger over time, with $p < 0.01$ after 2 weeks and $p < 0.001$ by week 4. Both CKL-THV treatments (G4 and G5) and atorvastatin (G3) were effective in reducing non-HDL-C levels compared to the model group (G2). This reduction was statistically significant at both the 2-week ($p < 0.01$) and 4-week ($p < 0.001$) time points. When comparing these treatments, there was no statistically significant difference in non-HDL-C levels ($p > 0.05$). This suggests that CKL-THV might be as effective as atorvastatin in reducing non-HDL-C in this model. Overall, the results are promising for CKL-THV as a potential therapeutic agent for lowering non-HDL-C, a major contributor to atherosclerosis.

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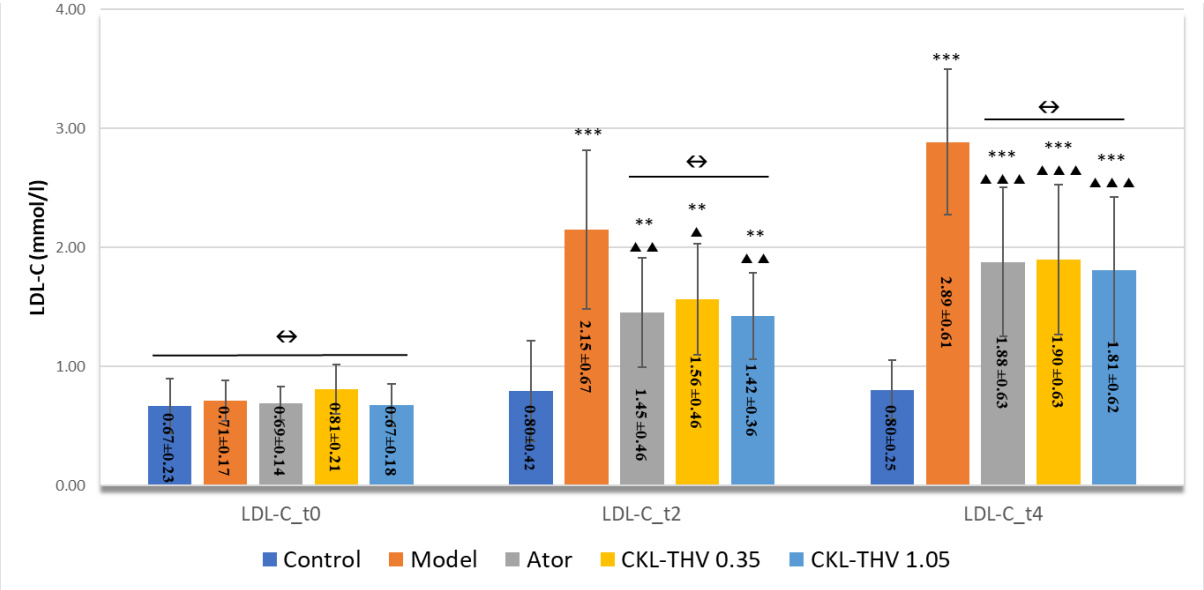

Figure 3: LDL-C concentration (mmol/l). Control group (G1): distilled water (10 ml/kg BW). Model group (G2): cholesterol oil (10 ml/kg), then distilled water (10 ml/kg/day) 2 hours later. Ator (atorvastatin) group (G3): cholesterol oil mixture (10 ml/kg/day), then atorvastatin (10 mg/kg/day) 2 hours later. CKL-THV 0.35 (G4): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (0.35 g/kg/day) 2 hours later. CKL-THV 1.05 (G5): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (1.05 g/kg/day) 2 hours later. n = 10, ± SD.

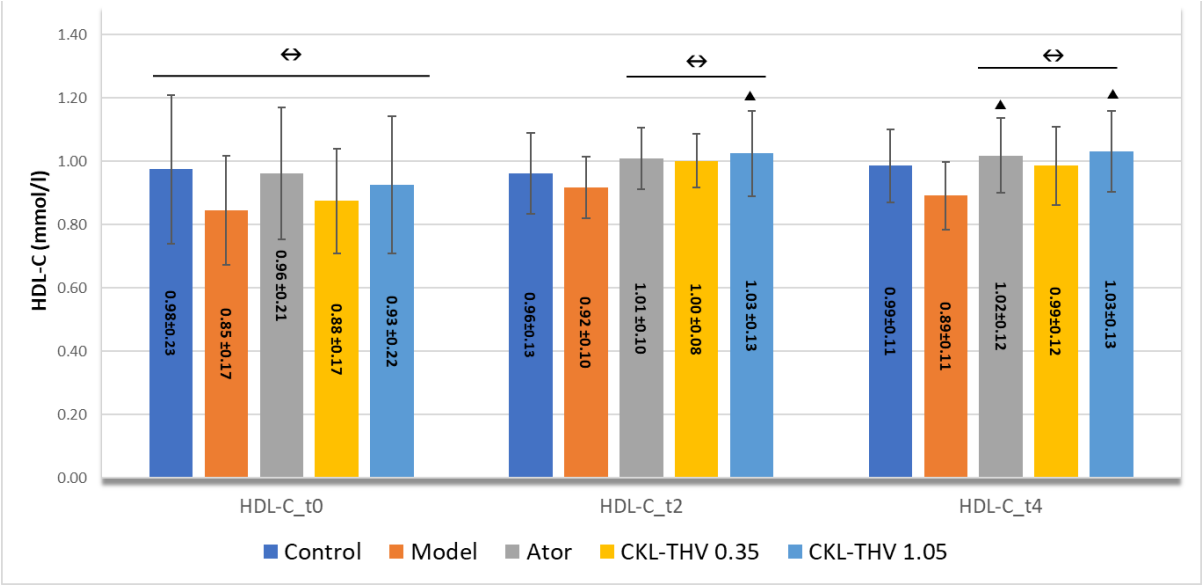

Figure 4: HDL-C concentration (mmol/l). Control group (G1): distilled water (10 ml/kg BW). Model group (G2): cholesterol oil (10 ml/kg), then distilled water (10 ml/kg/day) 2 hours later. Ator (atorvastatin) group (G3): cholesterol oil mixture (10 ml/kg/day), then atorvastatin (10 mg/kg/day) 2 hours later. CKL-THV 0.35 (G4): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (0.35 g/kg/day) 2 hours later. CKL-THV 1.05 (G5): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (1.05 g/kg/day) 2 hours later. n = 10, ± SD.

AIP Index

The AIP index is used to assess atherogenicity, the tendency to develop atherosclerosis or plaque buildup in arteries. Calculated according to Equation 3, a higher AIP indicates a greater risk of atherosclerosis. The model group (G2) showed the expected effects of the cholesterol oil mixture, with an increasing AIP over time (Figure 6). Compared to the control group (G1), AIP was significantly elevated after 2 weeks ($p < 0.01$) and even more so after 4 weeks ($p < 0.001$). Like the results for TG, there was a statistically significant increase ($p < 0.05$) in AIP observed in both atorvastatin (G3) and CKL-THV groups (G4 and G5) after 4 weeks compared to the control group. This is despite the potential cholesterol-lowering effects of these treatments. Compared to the model group (G2), both CKL-THV doses (0.35 and 1.05 g/kg/day) significantly reduced AIP levels after two weeks ($p < 0.05$) and maintained this effect at week 4 ($p < 0.01$) (Figure 6). This suggests a potential benefit of CKL-THV in reducing atherogenic risk. In addition, there was no statistically significant difference in AIP levels ($p > 0.05$) between the atorvastatin and CKL-THV groups throughout the study. This again suggests CKL-THV might be comparable to atorvastatin in reducing atherogenic risk in this model.

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The key blood lipid parameters such as TC, TG, HDL-C, and particularly LDL-C are examined in assessing dyslipidemia. LDL-C has long been regarded as a therapeutic target for cardiovascular diseases and lipid disorders. According to a global epidemiological study, elevated plasma LDL-C levels ranked 11th as a risk factor for mortality in 2007, rising to 8th place by 2019.[1] LDL-C levels can be directly measured or calculated using the Friedewald formula based on concentrations of TC, TG, and HDL-C, provided that the TG level is < 4.5 mmol/l.[12] Also derived from the Friedewald formula, the non-HDL level represents the difference between TC and HDL-C. This index reflects the cholesterol content of atherosclerotic apoB-containing lipoproteins such as LDL-C, very-low-density lipoprotein cholesterol (VLDL-C), remnant chylomicrons, and Lp(a).[13] Both LDL-C and non-HDL-C levels are closely associated with cardiovascular disease risk. A recent study even suggests that elevated non-HDL-C may pose a higher risk of cardiovascular disease than LDL-C.[13] Hence, to evaluate the effects of the research product CKL-THV, TC, TG, and HDL-C, as well as the inferred values of LDL-C and Non-HDL-C were analyzed.

In this study, the research findings concerning blood lipid indices (TC, TG, LDL-C, non-HDL-C) in the model group (G2) indicate the successful establishment of an exogenous lipid dyslipidemia model in rats subjected to a cholesterol-rich diet. Wistar rats exhibited dyslipidemia shortly after 2 weeks of consuming the cholesterol oil mixture. Specifically, compared to the control group, the model group displayed significant increases in TC, LDL-C, and non-HDL-C concentrations ($p < 0.001$), along with elevated TG concentrations ($p < 0.01$). By the 4th week, all the blood lipid parameters had escalated significantly compared to the control group, with a significance level of $p < 0.001$. Consumption of a diet supplemented with excess cholesterol augments the cholesterol content on the endoplasmic reticulum membrane of liver cells, resulting in reduced low-density lipoprotein receptor (LDL-R) and consequent elevation of LDL-C in plasma. The cholic acid in the cholesterol oil mixture enhances cholesterol absorption while diminishing bile acid synthesis from free cholesterol. Free cholesterol undergoes esterification for storage, leading to LDL-R degradation and subsequent elevation of cholesterol and LDL in the bloodstream. Furthermore, the antithyroid drug propylthiouracil exacerbates the increase in serum cholesterol levels. The treatment groups, in addition to the cholesterol-rich diet, also received either atorvastatin or CKL-THV. Comparison with the control group revealed an increase in the blood lipid indices in these groups, albeit with varying degrees of statistical significance. Notably, TC, LDL-C, and non-HDL-C levels increased to a greater extent and earlier than TG. Throughout the 4-week study period, the HDL-C index exhibited minimal change compared to the control group.

Atorvastatin, a statin derivative, is commonly employed as a positive control drug in research models evaluating lipid-lowering effects.[14] In this study, rats were administered atorvastatin at a dosage of 10mg/kg/day, equivalent to the effective human dose. Analysis of the group receiving atorvastatin revealed, compared to the model group, reductions in blood lipid indices starting from the second week of treatment, including TC, LDL-C, non-HDL-C ($p < 0.01$), and TG ($p < 0.05$). By the fourth week, significant decreases in TC, LDL-C, non-HDL-C ($p < 0.001$), and TG ($p < 0.05$) were exhibited. Moreover, HDL-C levels demonstrated a progressive increase over time, with statistical significance observed only in the fourth week ($p < 0.05$). These findings align entirely with previous studies elucidating the mechanism of atorvastatin in inhibiting hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase enzyme activity, thereby impeding cholesterol synthesis in liver cells, reducing LDL-C and TG levels, and marginally elevating HDL-C.[14]

Treatment groups receiving CKL-THV at doses of 0.35 and 1.05 g/kg/day demonstrated reductions in TC, TG, LDL-C, and non-HDL-C indices over the 4 weeks. At the 2-week mark, compared to the model group, the low CKL-THV dose led to reductions in non-HDL-C ($p < 0.01$), TC, and LDL-C ($p < 0.05$), with a slight decrease in TG ($p < 0.05$). By week 4, all the indices LDL-C and non-HDL-C ($p < 0.001$), TC ($p < 0.01$), and TG ($p < 0.05$) decreased significantly compared to the model group. Thus, at a dosage of 0.35 g/kg/day, CKL-THV reduced blood lipid indices, with TC, LDL-C, and non-HDL-C showing

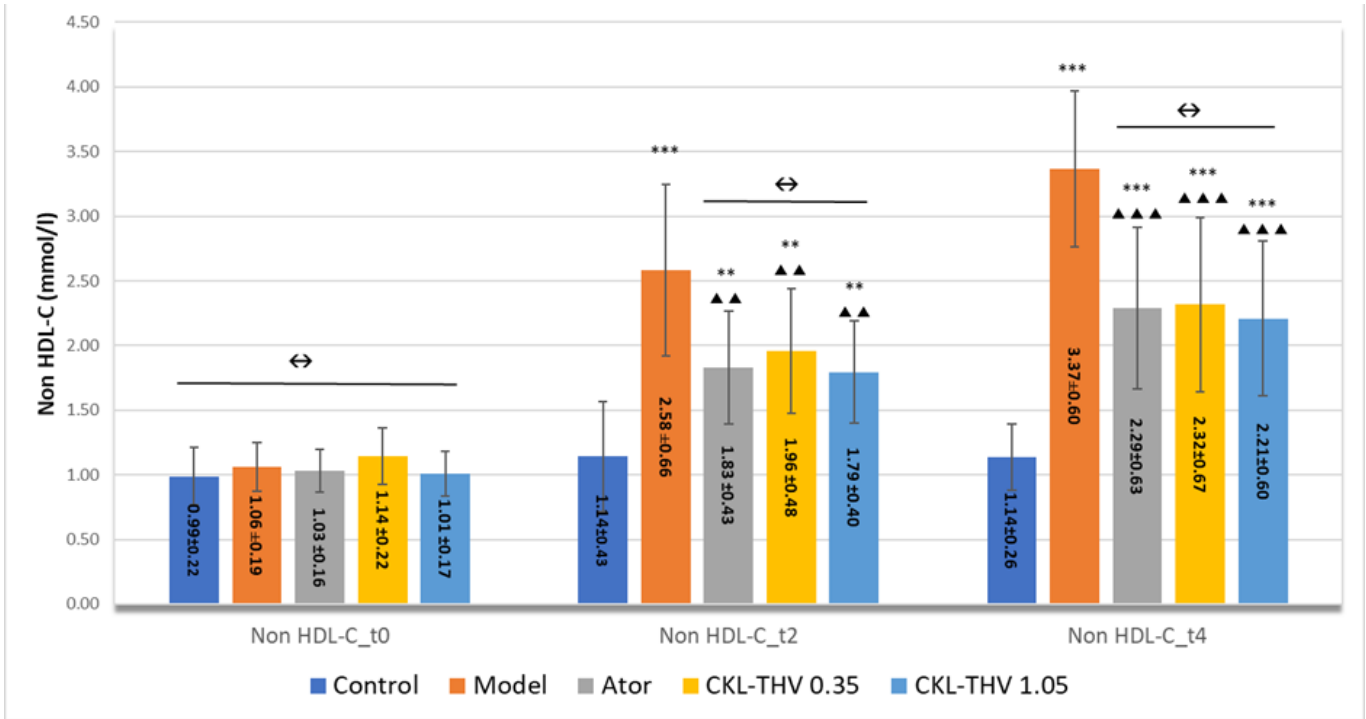

Figure 5: Non-HDL-C concentration (mmol/l). Control group (G1): distilled water (10 ml/kg BW). Model group (G2): cholesterol oil (10 ml/kg), then distilled water (10 ml/kg/day) 2 hours later. Ator (atorvastatin) group (G3): cholesterol oil mixture (10 ml/kg/day), then atorvastatin (10 mg/kg/day) 2 hours later. CKL-THV 0.35 (G4): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (0.35 g/kg/day) 2 hours later. CKL-THV 1.05 (G5): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (1.05 g/kg/day) 2 hours later. n = 10, ± SD.

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earlier and more pronounced decreases than TG. This dosage did not impact the HDL-C index in the rats throughout the study.

Conversely, in the group receiving a CKL-THV dose of 1.05 g/kg/day, the blood lipid indices TC, LDL-C, and non-HDL-C ($p < 0.01$), and TG ($p < 0.05$) exhibited significant changes compared to the model group after the second week. By the fourth week, the reduction trend in these indices intensified: TC, LDL-C, and non-HDL-C ($p < 0.001$), TG ($p < 0.01$). Notably, the average HDL-C index of rats increased significantly compared to the model group at both 2 and 4 weeks, with a statistical significance level of $p < 0.05$. Thus, with a dose of 1.05 g/kg/day, CKL-THV reduced all blood lipid indices, with TC, LDL-C, and non-HDL-C exhibiting stronger decreases than TG. This dosage positively affected the HDL-C index, augmenting levels of "good" cholesterol in the blood of throughout the study. However, when comparing blood lipid indices between groups receiving CKL-THV doses of 0.35 and 1.05 g/kg/day, no statistically significant differences were found ($p > 0.05$).

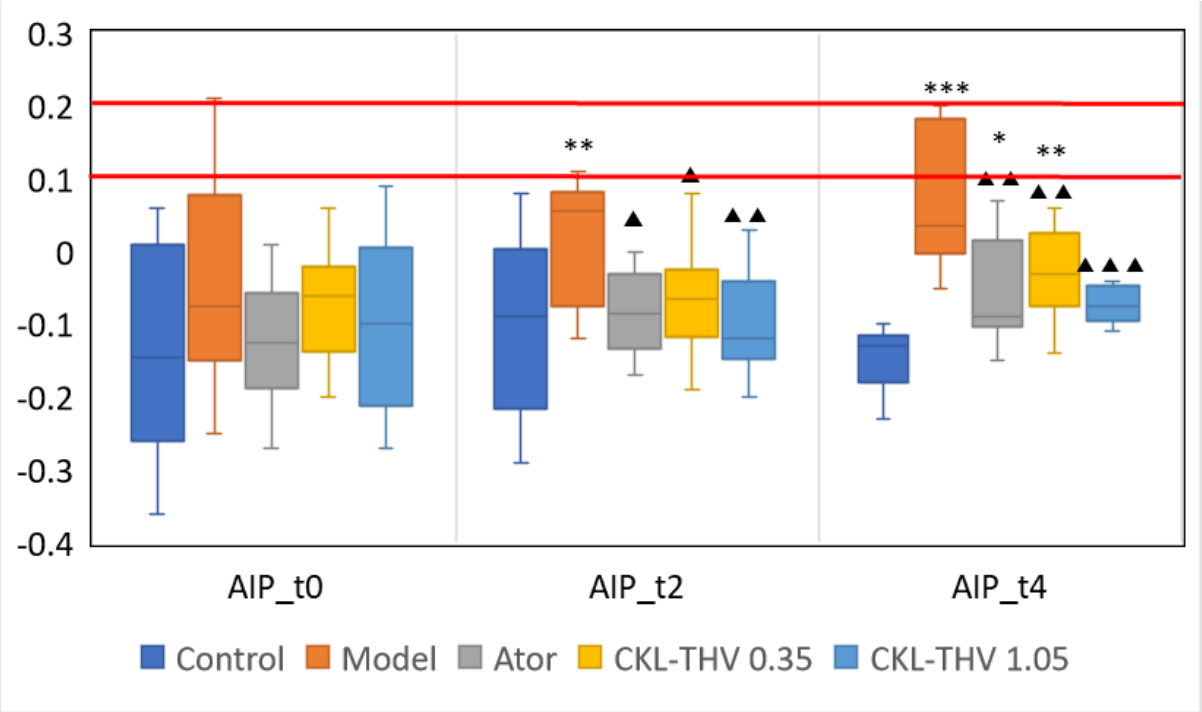

Figure 6: AIP index. Control group (G1): distilled water (10 ml/kg BW). Model group (G2): cholesterol oil (10 ml/kg), then distilled water (10 ml/kg/day) 2 hours later. Ator (atorvastatin) group (G3): cholesterol oil mixture (10 ml/kg/day), then atorvastatin (10 mg/kg/day) 2 hours later. CKL-THV 0.35 (G4): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (0.35 g/kg/day) 2 hours later. CKL-THV 1.05 (G5): cholesterol oil mixture (10 ml/kg/day), then CKL-THV extract (1.05 g/kg/day) 2 hours later. n = 10, ± SD.

These findings are fully aligned with prior studies. Tran *et al.* demonstrated a 35% reduction in blood cholesterol levels with yellow tea.[15] Similarly, Hanh *et al.* illustrated that a blend of yellow tea leaf extract and *Gynostemma pentaphyllum* decreased TC and TG levels in obese white mice after 2 weeks.[16] Furthermore, the recent investigation into the lipid-lowering effect of CKL-THV, based on endogenous mechanisms, revealed that CKL-THV doses of 0.6 and 1.8 g/kg/day significantly reduced BW and TC indices (by 26.5% and 28.3%, respectively), lowered TG (by 21.2% and 17.3%, respectively), and decreased HDL-C (by 31.1% and 33.8%, respectively) in Wistar rats.[17] When comparing the blood lipid indices of the CKL-THV groups with the atorvastatin group, no statistically significant difference was observed. Thus, the lipid-lowering effect of CKL-THV via an exogenous mechanism is equivalent to that of atorvastatin at a dose of 10 mg/kg/day, irrespective of the CKL-THV dosage (0.35 or 1.05 g/kg/day). This outcome aligns with the cholesterol-lowering efficacy of the dried extract of *C. hakodae* leaves.

Dyslipidemia is a risk factor for cardiovascular events.[18] Alongside individual blood lipid parameters, assessments are increasingly reliant on biomarkers like AIP capable of predicting atherosclerosis. AIP reflects the ratio between atherogenic lipoproteins (TG) and anti-atherogenic lipoproteins (HDL-C). Elevated AIP indicates heightened cardiovascular risk.[18,19] In this study, rats in the model group exhibited an elevation in AIP after 2 weeks of consuming the cholesterol oil mixture, escalating further by week 4. This increase in AIP signifies an elevated risk of coronary artery disease due to elevated TG and unchanged HDL-C. Conversely, in the groups receiving atorvastatin and CKL-THV, AIP decreased significantly after 2 weeks ($p < 0.05$) and even more significantly after 4 weeks ($p < 0.01$) compared to the model group. This reduction signifies a decrease in atherosclerotic lipoproteins (TG) and an increase in anti-atherogenic lipoproteins (HDL-C). These results suggest the effectiveness of CKL-THV in mitigating coronary artery disease risk in dyslipidemic rats fed a cholesterol-rich diet.

Conclusion

This study demonstrated that CKL-THV at doses of 0.35 and 1.05 g/kg/day exhibited efficacy in reducing TC, TG, LDL-C, and non-HDL-C in the exogenous hyperlipidemia rat model induced with a cholesterol oil mixture. CKL-THV at a dose of 1.05 g/kg/day appears to reduce TC, TG, LDL-C, and non-HDL-C in rat blood more strongly than the lower dose of 0.35 g/kg/day, albeit without a statistically significant difference. Furthermore, the higher CKL-THV dose demonstrated the additional benefit of increasing HDL-C levels. In conclusion, Vietnamese yellow tea (*C. hakodae* leaf) effectively reduced the atherogenic index, potentially decreasing the risk of coronary artery disease. These findings suggest that CKL-THV holds potential for further exploration and development as an anti-hyperlipidemic agent.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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