

MINISTRY OF EDUCATION
AND TRAINING

MINISTRY OF DEFENCE

MILITARY MEDICAL ACADEMY

NGO THI MY BINH

**RESEARCH ON THE TOXICITY AND ANTI-
OBESITY AND LIPID-LOWERING EFFECTS
IN EXPERIMENTS OF DRIED EXTRACT
LEAVES OF *CAMELLIA HAKODAE* NINH**

Speciality: Pharmacology and toxicity

Code: 9 72 01 18

SUMMARY OF DOCTOR OF MEDICINE THESIS

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LIST OF PUBLISHED WORKS
RESEARCH RESULTS OF THE THESIS

1. **Ngo Thi My Binh, Nguyen Hoang Ngan, Nguyen Hong Hanh (2023)**, “Study the sub-chronic toxicity of yeallow tea leaves (*Camellia hakodae* Ninh)”, *Vietnam Medical Journal*, 529 (2), pp. 118 - 123.

2. **Ngo Thi My Binh, Nguyen Hoang Ngan, Nguyen Hong Hanh (2023)**, “Study on the acute toxicity and endogenous hypolipidemic effect of dry extract from yeallow tea leaves (*Camellia hakodae* Ninh) in experimental animal”, *Military Medical Journal*, Vol. 48, Pharmacy Special Issue, pp. 354 - 363.

3. **Ngo Thi My Binh, Nguyen Hoang Ngan, Do Thi Huong Lan, Nguyen Hong Hanh, Nguyen Thanh Ha Tuan (2024)**, “Anti-hyperlipidemic Effect of *Camellia hakodae* Ninh Extract in an In Vivo Rat Model”, *Trop J Nat Prod Res*, 8 (11), p. 9199-9205.

INTRODUCTION

Obesity and dyslipidemia are two global health concerns. As of 2020, approximately 2.6 billion people worldwide were overweight or obese. By 2035, this number is estimated to exceed 4 billion, with obesity rates projected to rise from 14% to 24% of the population, affecting nearly 2 billion adults, children, and adolescents. The combined effects of being overweight, obese, and having dyslipidemia pose a double threat to health, potentially leading to severe and fatal diseases. Currently, various drug classes are used to treat obesity and dyslipidemia, but high costs and numerous side effects hinder their use. Therefore, current research focuses on discovering new, safe, effective, and affordable drugs derived from various medicinal sources.

Camellia hakodae Ninh yellow flower tea is used in folklore as a drink with many uses. The Yellow Flower Tea contains polyphenol compounds, which have been shown to have antioxidant, weight loss, waist reduction, lipid lowering effects... Dried extract leaves of *Camellia hakodae* Ninh (CKL-THV) prepared at the Military Medical Academy, meeting the basic standards. To be able to bring CKL-THV for clinical use, thesis topic: **“Research on the toxicity and anti-obesity and lipid-lowering effects in experiments of dried extract leaves of *Camellia hakodae* Ninh”** was carried out with 2 objective:

1. *Evaluate the acute toxicity and sub-chronic toxicity of CKL-THV in experiment.*

2. *Evaluate the anti-obesity, lipid-lowering effects and some mechanisms of action of CKL-THV in experiment.*

THE NEW MAIN SCIENTIFIC OF THE THESIS

The thesis evaluated CKL-THV preparations with outstanding characteristics, providing scientific value and potential for application compared to previously researched preparations. The thesis evaluated the safety of CKL-THV and anti-obesity and lipid-lowering effects in an in vivo test. In addition, the research of the thesis takes an important step forward when elucidating the mechanism of action of CKL-THV through its ability to inhibit two enzymes, HMG-CoA reductase and cholesterol esterase, on in vitro testing, which previous studies have not fully performed. The combination of biological efficacy assessment and molecular mechanisms provides a comprehensive view, confirming the potential of CKL-THV preparations as a dual mechanism in the treatment of dyslipidemia and obesity.

The research of the thesis opens up the potential for new product development based on CKL-THV powder materials, with high practical applicability in the pharmaceutical field. The successful development of a product of natural origin, combining modern extraction technology and a clear mechanism of action, will contribute significantly to safe and effective treatment for pathologies related to lipid metabolism, meeting the growing needs of the community.

STRUCTURE OF THE THESIS

The thesis consists of 143 pages. Problem setting: 02 pages; Overview: 35 pages; Research subjects and methods: 23 pages; Research results: 35 pages; Discussion: 45 pages; Conclusion: 02 pages; Recommendation: 01 page. The thesis has 156 references (Vietnamese: 36, English: 120); 32 tables, 20 charts, 22 images and 6 appendices.

CHAPTER 1. OVERVIEW

1.1. Overview of obesity and dyslipidemia

1.1.1. Obesity

Obesity is defined as the accumulation of excess or abnormal fat, which can adversely affect health. The pathogenesis of obesity is due to energy imbalance, when the energy introduced into the body exceeds the energy consumed, excess energy will increase reserves in the form of fat, body weight increases, leading to overweight and obesity. The goal of treating obesity is to control weight and reduce the risk of complications and improve health.

Use of anti-obesity drugs is recommended for patients with BMI ≥ 30 kg/m² or BMI ≥ 27 kg/m² with related diseases such as hypertension, type 2 diabetes. As of 2024, there are 7 FDA-approved drugs in the treatment of obesity in adult patients. In Vietnam, there are currently only two drugs approved for treatment: orlistat and liraglutid.

1.1.2. Dyslipidemia

Dyslipidemia is a primary or secondary metabolic disorder characterized by an abnormality in the concentration and/or function of one or more types of plasma lipids and lipoproteins. By modern standards, dyslipidemia is diagnosed when plasma lipid and/or lipoprotein levels do not reach the recommended target values for prophylaxis of atherosclerotic cardiovascular disease. Major Heart Associations around the world have identified LDL-C as a primary target in the treatment of dyslipidemia. The lower the LDL-C levels, the lower the cardiovascular risk.

Currently, there are many classes of drugs approved in the treatment of dyslipidemia with different mechanisms such as: statins,

fibrates, niacin, cholesterol absorption inhibitors (ezetimib), bile acid binders, PCSK9 inhibitors, omega-3 fatty acids, bempedoic acid, inclisiran... These drugs help reduce LDL-C, triglycerides, increase HDL-C and are especially effective in reducing the risk of cardiovascular disease.

1.2. Several models study anti-obesity, lipid-lowering effects

1.2.1. In vivo models of obesity induction

There are many models of obesity in laboratory animals such as using an energy-rich diet, acting on the hypothalamus, or causing animals to become genetically obese. Among them, the model of using energy-rich diets, especially high-fat diets (HFD), is the most commonly used. Causing obese animals using the HFD diet is a popular method in anti-obesity drug screening trials because of its ease of implementation and high obesity effectiveness. However, because the diet was high in fat, the rat quickly lost their appetite, had to constantly change their diet but still had to ensure their fat content to maintain the rat's obesity. In the study of the thesis, we used the HFD diet to induce an obese rat model in the evaluation of the anti-obesity effect of CKL-THV.

1.2.2. Models of hyperlipidemia in vivo

Inducing endogenous hyperlipidemia in experimental animals, agents such as poloxamer P407, Tween 80, Triton WR-1339 can be used. In particular, the P407 usage model is a common model applied to many studies to rapidly evaluate the lipid-disorder-modulating effects of a new drug or a new product, especially drugs that inhibit HMG-CoA reductase. The model of exogenous hyperlipidemia using a mixture of cholesterol oils by Nassiri et al is a classic and quite popular model for studying drugs with lipid-lowering effects. Nguyen

Trong Thong and his colleagues improved this model, adjusted some components in the cholesterol oil mixture, and created a model that is highly feasible, cheaper and has many similar characteristics to dyslipidemia in humans. Therefore, this method has been selected by us for use in research evaluating the lipid-lowering effects of CKL-THV by exogenous mechanisms.

1.2.3. Models to study the mechanism of action in vitro

Cholesterol biosynthesis is divided into several stages, with catalysis by multiple enzymes, both HMG-CoA reductase and cholesterol esterase enzymes are strongly associated with dyslipidemia and obesity through the regulation of lipid metabolism in the body. To evaluate the inhibitory effect of these 2 enzymes, modern analytical methods such as high-performance liquid chromatography and liquid chromatography combined with mass spectrometry can be applied. UV-Vis spectroscopy is a popular and accessible method in laboratories, with relatively simple equipment and low investment costs.

1.3. Some medicinal herbs have anti-obesity and lipid-lowering effects

In recent decades, medicinal herbs with anti-obesity and lipid-lowering effects have been widely studied. Based on scientific database sources in Science direct, Medicine/Pubmed and Google Scholar, a 2021 systematic study compiled worldwide 451 plant species in 329 genera and 110 families with anti-obesity effects. The main plant families used are Conpositae (34 genera and 43 species) and Leguminosae (26 genera and 43 species). Analysis of the used parts of the above medicinal herbs shows that leaves (24%) and fruits

(18%) are the two most used parts, decoction processing method (42%) is the main method.

1.4. *Camellia hakodae* yellow flower tea and dried extract leaves of *Camellia hakodae*

Camellia hakodae Ninh yellow flower tea is a tree endemic to Tam Dao National Park, Vietnam. According to Traditional Medicine, Yellow Flower Tea has a peaceful, sweet and fragrant taste, attributed to the three sutras: Mind, Kidney and Can. The main active chemical ingredient in Yellow Flower Tea is polyphenols. Some studies have proven that polyphenols in Yellow Flower Tea also have anti-cancer effects, inhibiting tumor formation, growth and metastasis, antibacterial, anti-inflammatory, hypoglycemic, weight loss and lipid lowering.

Regarding toxicity, many studies show that preparations extracted from leaves or flowers from different species of Yellow Flower Tea are quite safe. Dry extract of leaves *Camellia hakodae* Ninh is prepared at the Military Medical Academy, extracted by ultrasonic extraction method with 96% ethanol solvent, then prepared dried tall powder by spray drying method.

CHAPTER 2. RESEARCH OBJECTS AND METHODS

2.1. Subjects of study

2.1.1. *Research material*: High dried leaves of yellow flower tea prepared at the Military Medical Academy, meeting the base standard

2.1.2. *Research animals*: Swiss white rat, both breeds, healthy, weight 18 ± 2.0 g. White rat Wistar strain both breeds, healthy, weight 180 ± 20 g; 100-120 g.

2.1.3. *Research equipment*: Biochemical testing machine Biochemical Systems International Srl (Italy), Humancout 30TS hematology analyzer (Germany), UV-VIS Hitachi UH5300 ultraviolet-visible spectrometer (Japan), Analytical scale with sensitivity 0.1 mg; Technical scale (Sartorius, Germany), Hettlite Pro micro pipette set (Hettich, Germany) ...

2.1.4. *Drugs and chemicals*: Triton-X100, Acetonitrile, Poloxamer 407, Propofol 1%, Ultra-pure water, cholesterol esterase, Atorvastatin 10mg, Orlistat 120mg, Kit to evaluate the inhibitory effect of HMG-CoA reductase enzyme.

2.2. Research methods

- + Acute toxicity assessment: Litchfield Wilcoxon method
- + Sub-chronic toxicity assessment: guidance from the Ministry of Health
- + Evaluation of anti-obesity effects: model of causing animal obesity with a diet rich in HFD fat.
- + Evaluation of endogenous lipid-lowering effects: model of hyperlipidemia by P-407
- + Evaluate the exogenous lipid-lowering effect: model of hyperlipidemia with cholesterol oil mixture.
- + Evaluate the mechanism of action in vitro: UV-Vis method

2.3. **Data processing method**: SPSS 22.0 medical statistics software

CHAPTER 3. RESEARCH RESULTS

3.1. Results of toxicological studies

3.1.1. *Acute toxicity:*

Mice in groups were given CKL-THV to the highest dose that could be given orally to rat through a curved obtuse needle of 12g/kg body weight. Closely monitored rats in groups for 72 hours after ingestion of the preparation and throughout 7 days after ingestion of the preparation, Mice in all groups were normal, silky hairy, showed no dyspnea or cyanosis, and ate, exercised, and excreted normally. No abnormalities were seen, no rat died during the study period. LD50 did not known.

3.1.2. *Sub-chronic toxicity*

- * General condition and rat weight: Rat in all study group were normal, silky hair, eating, movement, excretion normal. CKL-THV did not affect rat weight.

- * On haematological criteria: CKL-THV does not affect the number of ampulla, white blood cells, platelets, hemoglobin content, hematocrit, mean red blood cell volume.

- * On biochemical indicators: CKL-THV does not affect AST, ALT, albumin, total cholesterol, triglycerides and creatinine activity.

- * On liver, spleen, and kidney organs: CKL-THV does not affect the macroscopic and microscopic structures of liver, spleen, and rat kidneys.

3.2. Results of anti-obesity and lipid-lowering effects studies

3.2.1. *Anti-obesity effects*

- * Rat weight: CKL-THV reduces weight by 2.55 – by 3.09 % after 8 weeks. At the end of the trial, CKL-THV reduced rat weight at the treatment group to the model group significantly ($p < 0.001$).

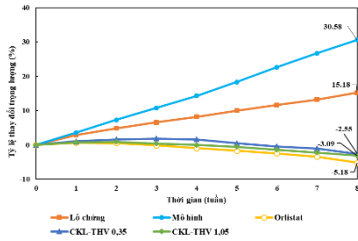


Chart 3. 1. Rat weight changes in groups

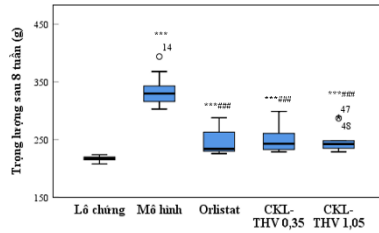


Chart 3. 2. Rat weights in study groups after 8 weeks

* Rat abdominal circumference: CKL-THV has not reduced measurements of rat abdominal circumference during the study period. However, at the end of the trial, CKL-THV reduced rat abdominal circumference measurements in the treatment group compared to the significant model group ($p < 0.01$).

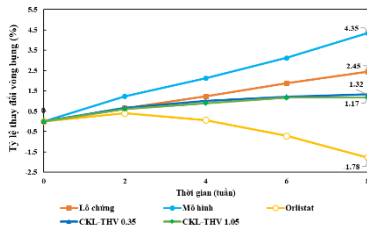


Chart 3. 3. Rat abdominal circumference alterations

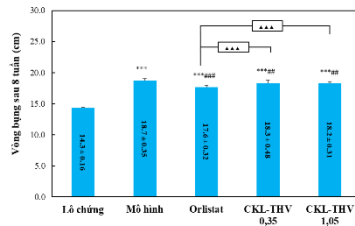


Chart 3. 4. Rat clumsy rings in study groups after 8 weeks

* Lee's index: CKL-THV did not reduce rat abdominal circumference measurements during the study period. However, at the end of the trial, CKL-THV reduced rat abdominal circumference measurements in the treatment group compared to the significant model group ($p < 0.01$).

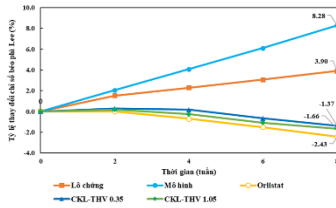


Chart 3. 5. Change in Lee obesity index in groups

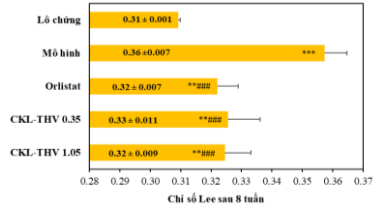


Chart 3. 6. Lee obesity index in study groups after 8 weeks

* Fat weight and fat index: CKL-THV reduced fat weight in the treatment group compared to the significant model group ($p < 0.05$). However, CKL-THV did not affect fat index in the study groups.

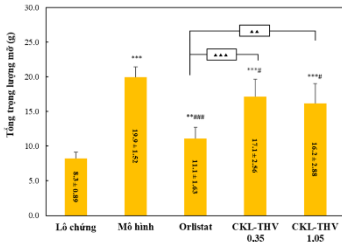


Chart 3. 7. Fat weight in study groups after 8 weeks

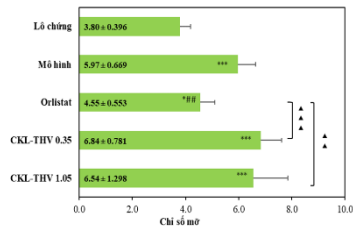


Chart 3. 8. Fat index in study groups after 8 weeks

* Organ weight: CKL-THV reduces the absolute weight of heart, liver, kidney, and spleen organs at all groups compared to model group ($p < 0.001$). However, only the relative weight of the hepatic organs was reduced in the groups compared to the model group ($p < 0.05$)

* Blood lipid indices: CKL-THV reduced rat TC, TG, LDL-C, non-HDL-C indices at the treatment group to the model group. Increases HDL-C levels comparable to orlistat.

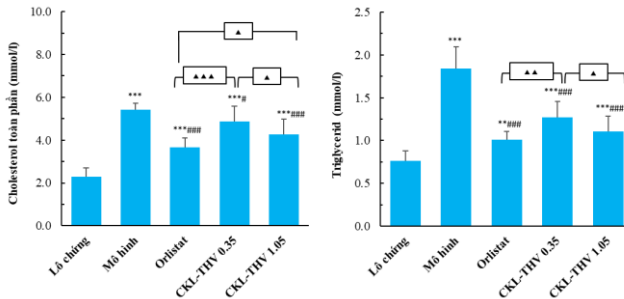


Chart 3. 9. TC and TG concentrations in groups

CKL-THV reduced rat TC, TG concentration at the treatment group to the model group.

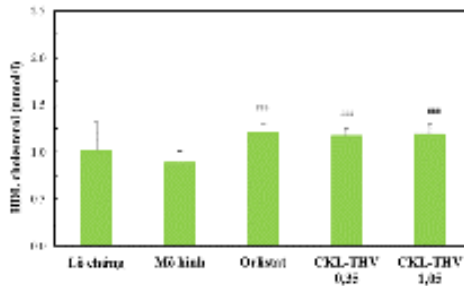


Chart 3.10. HDL-C concentrations in groups

CKL-THV increases HDL-C levels at the treatment group.

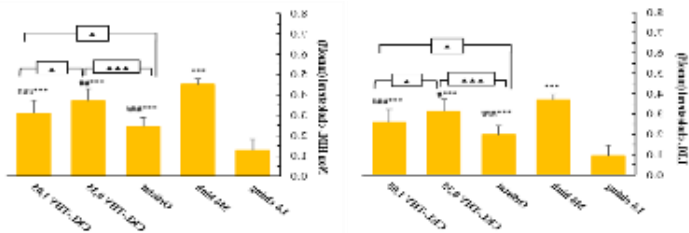


Chart 3. 10. LDL-C and non HDL-C concentrations in groups

CKL-THV reduced rat LDL-C, non HDL-C concentration at the treatment group to the model group

3.2.2. Lipid-lowering effect

* Effects on endogenous hyperlipidemia model:

Table 3. 16. Effect of CKL-THV on blood lipid concentrations

Concentrations (n = 10, $\bar{X} \pm SD$)		Model (2)	Ator (3)	CKL- THV 0,6 (4)	CKL- THV 1,8 (5)
TC	Concentrations (mmol/l)	2,76 $\pm$ 0,42	2,12 $\pm$ 0,31 ^{##}	2,03 $\pm$ 0,37 ^{##}	1,98 $\pm$ 0,55 ^{##}
	% change from Gr (2)	-	↓ 23,2 %	↓ 26,5 %	↓ 28,3 %
TG	Concentrations (mmol/l)	1,04 $\pm$ 0,14	0,84 $\pm$ 0,10 ^{##}	0,82 $\pm$ 0,09 ^{##}	0,86 $\pm$ 0,09 ^{##}
	% change from Gr (2)	-	↓ 19,2 %	↓ 21,2 %	↓ 17,3 %
HDL	Concentrations (mmol/l)	0,57 $\pm$ 0,09	0,56 $\pm$ 0,08	0,53 $\pm$ 0,11	0,53 $\pm$ 0,11
	% change from Gr (2)	-	↓ 1,8 %	↓ 7,0 %	↓ 7,0 %
LDL	Concentrations (mmol/l)	1,72 $\pm$ 0,33	1,17 $\pm$ 0,30 ^{##}	1,13 $\pm$ 0,30 ^{##}	1,06 $\pm$ 0,46 ^{##}
	% change from Gr (2)	-	↓ 32,0 %	↓ 34,3 %	↓ 38,4 %
Non HDL	Concentrations (mmol/l)	2,19 $\pm$ 0,37	1,56 $\pm$ 0,29 ^{###}	1,51 $\pm$ 0,31 ^{###}	1,45 $\pm$ 0,47 ^{##}
	% change from Gr (2)	-	↓ 28,8 %	↓ 31,1 %	↓ 33,8 %

CKL-THV reduced rat blood TC, TG, LDL-C and non-HDL-C indices in the treatment group compared to the model group ($p < 0.01$). There is no difference in HDL-C index.

* Effects on exogenous hyperlipidemia model:

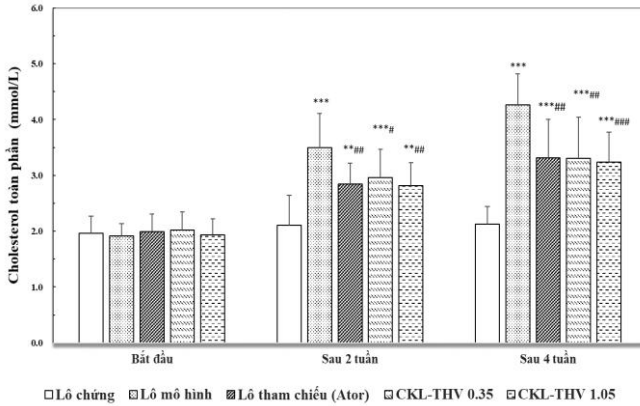


Chart 3. 12. TC concentrations in groups

After 2 weeks, CKL-THV reduced TC index in the treatment group to the model group.

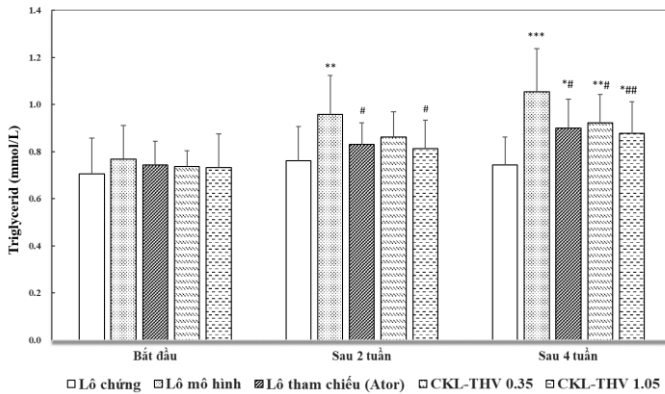


Chart 3. 13. TG concentrations in groups

After 4 weeks, CKL-THV reduced TG in all groups.

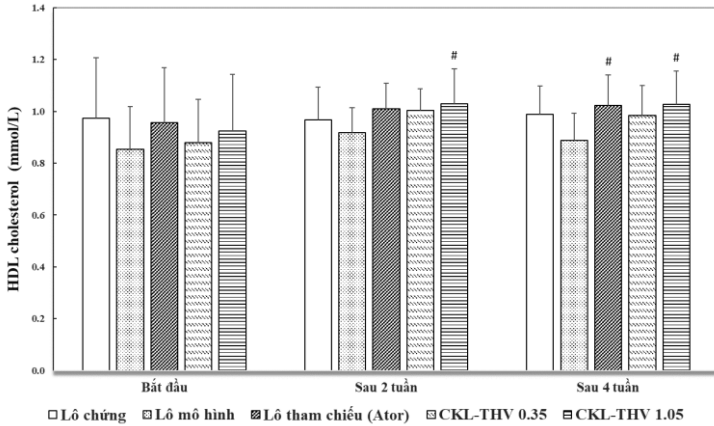


Chart 3. 14. HDL-C concentrations in groups
CKL-THV dose of 1.05 g/kg/24h increases HDL-C from week 2

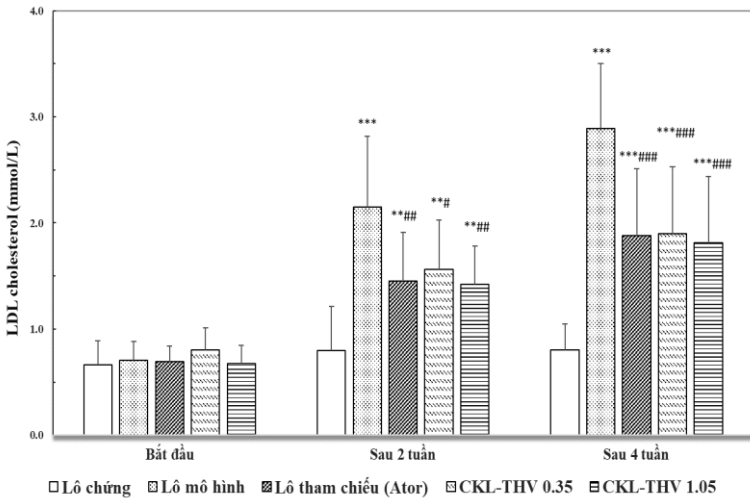


Chart 3. 15. LDL-C concentrations in groups
CKL-THV reduced LDL-C index in the treatment group to the model group after 2 weeks and 4 weeks.

3.2.3. Mechanism of action in vitro

* Mechanism of HMG-CoA reductase enzyme inhibition

Table 3.17. Percent inhibition of HMG-CoA reductase enzyme

Concentradion ($\mu\text{g/ml}$)	% inhibitor
CKL-THV 12,5	$26,5 \pm 1,31$
CKL-THV 25	$38,1 \pm 3,06$
CKL-THV 37,5	$50,0 \pm 4,55$
CKL-THV 50	$64,6 \pm 9,26$
Pravastatin	$85,6 \pm 12,92$

When increasing CKL-THV concentration from 12.5 $\mu\text{g/ml}$ to 50 $\mu\text{g/ml}$, the percent inhibition of HMG-CoA reductase enzyme increased linearly from 26.5% to 64.6%.

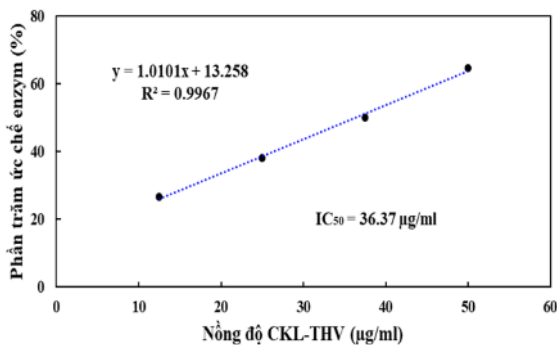


Chart 3. 18. Linear route between CKL-THV concentration and % inhibition of HMG CoA reductase

Concentration inhibiting 50% of HMG-CoA reductase enzyme activity of CKL-THV $\text{IC}_{50} = 36.37 \mu\text{g/ml}$.

* Mechanism of cholesterol esterase enzyme inhibition

Table 3.18. Percent inhibition of cholesterol esterase enzyme

Concentradion ($\mu\text{g/ml}$)	% inhibitor
CKL-THV 20	$14,4 \pm 1,28$
CKL-THV 50	$21,7 \pm 1,53$
CKL-THV 100	$36,9 \pm 2,66$
CKL-THV 150	$47,2 \pm 0,63$
CKL-THV 200	$60,4 \pm 3,17$

When increasing CKL-THV concentration from 20 $\mu\text{g/ml}$ to 200 $\mu\text{g/ml}$, the percent inhibition of cholesterol esterase enzyme increased linearly from 14.4% to 60.4%.

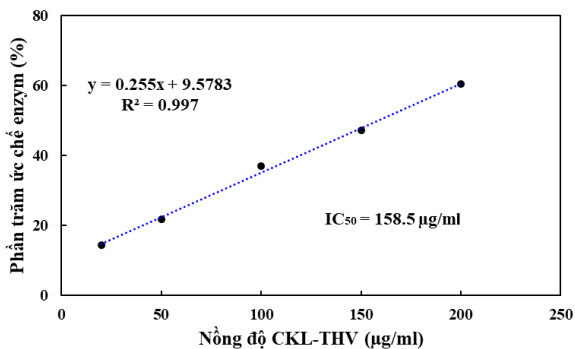


Chart 3. 20. Linear route between CKL-THV concentration and % inhibition of cholesterol esterase

Concentration inhibiting 50% of cholesterol esterase enzyme activity of CKL-THV $\text{IC}_{50} = 158.5 \mu\text{g/ml}$.

CHAPTER 4. DISCUSS

4.1. Toxicological studies

4.1.1. Acute toxicity

For 72 hours after oral administration of CKL-THV and throughout the following 7 days, mice in all groups were normal, silky-hairy, with no signs of dyspnea or cyanosis; normal eating, movement and excretion activities. There were no dead mice or any unusual signs or poisoning. Mice were taken at a dose of 12.0 g/kg body weight (20 times the expected human treatment dose converted to rat) without any deaths, nor were any abnormalities seen. Therefore, the LD50 of CKL-THV cannot be determined. The dose level of 12.0 g/kg is a very high dose level, allowing to confirm that the study sample was safe in an experimental white mice acute toxicity test. With reference to the oral acute toxicity classification, CKL-THV was assigned to group 5 (LD50 >5000 mg/kg), which is a substantially non-toxic type.

4.1.2. Sub-chronic toxicity

The results of the study on general condition, hematological index, blood biochemistry and macroscopic, microscopic liver, kidney, and rat spleen showed that CKL-THV did not affect the general condition, body weight development, hematopoietic functions, liver, kidney, and rat spleen studies when using dose levels of 0.35 g/kg/24h and 1.05 g/kg/24h, continuously for 90 days. When compared with the results of sub-chronic toxicity studies of other Yellow Flower Tea preparations such as *Camellia euphlebia*, we found that CKL-THV is safer. Our results are equivalent to the study by Nguyen Hong Hanh et al. on *Camellia hakodae* leaf extract.

Camellia hakodae Yellow Flower Tea leaf products, whether dried extracts or extracts, are highly safe in experimental studies.

4.2. Anti-obesity, lipid-lowering effects

4.2.1. Anti-obesity effects

We successfully built an obese rat model using a high-fat HFD diet. Experimental animals using the HFD diet increased their total energy intake. Saturated fats from ingredients such as lard, butter, whole milk powder, and condensed milk have the ability to increase LDL cholesterol levels and accumulate visceral fat, thereby leading to obesity.

CKL-THV reduced the weight of obese rat by 2.55 to 3.09%. CKL-THV contains polyphenols, which help enhance energy metabolism in the body, regulate lipid synthesis and regulate the intestinal flora. In addition, polyphenols can also regulate hormones such as ghrelin and leptin, thereby controlling appetite and supporting weight loss.

In addition, polyphenols have also been shown to help improve energy expenditure, prevent the maturation of adipocytes into adipocytes, and increase the expression of adiponectin (a hormone secreted by adipose tissue in the body. secreted by the body, plays a role in regulating glucose levels and breaking down fatty acids). Although it did not reduce rat abdominal circumference measurements during the study period, at the end of the trial, CKL-THV reduced rat abdominal circumference measurements in treatment group compared to the significant model group.

The Lee index of rat using CKL-THV decreased from 1.37 to 1.66% compared to the start of the official test. Lee's index is evaluated depending on the rat's weight and body length

measurements. According to the results in graph 3.2, after 8 weeks, the weight of rat in the groups using CKL-THV was reduced compared to the model group. Meanwhile, the study rat were all adults, and the rat length changed insignificantly during the study period. Due to a decrease in rat body weight while a negligible change in length resulted in a decrease in Lee's index.

Researching the fat index, we have only evaluated the effect of CKL-THV on the general body fat index, but we have not evaluated the effect on different parts of the body fat such as the epididymal fat index, retroperitoneal fat index... The groups using CKL-THV had differences in the general body fat index ($p < 0.01$), however, when compared to the model group, no differences were observed.

The weight of organs in the orlistat and CKL-THV groups was reduced compared to the model group ($p < 0.001$). The reduction in organ weight was associated with a reduction in body weight of rat treated with orlistat or CKL-THV. The relative weight of the viscera reflects how much the viscera change in the body relative to the overall change in body weight. As total body weight increases, the absolute weight of the organs may increase, but the relative weight of the organs is unlikely to change. CKL-THV does not greatly affect the relative weight of the heart, liver, kidney, and spleen organs of obese rat. Using high doses of CKL-THV (1.05 g/kg/24h) only reduced the relative weight of liver organs compared to the model group ($p < 0.05$), while the other organs remained unchanged.

Polyphenols in Yellow Flower Tea have been shown to activate AMPK (mono phosphate activated protein kinase 5' adenosine) leading to reduced cholesterol, fatty acid synthesis and triglyceride formation. Group using CKL-THV also reduced TC, TG, LDL-C,

non-HDL-C indices, increasing HDL-C compared to the statistically significant model pgroup.

4.2.2. Lipid-lowering effect

The results of the endogenous lipid-lowering effect study of CKL-THV (table 3.16) showed that, CKL-THV reduced mice blood lipid indices (TC, TG, LDL and non-HDL) with statistical significance. Specifically, compared to the model group, the group using CKL-THV at dose of 0.6 g/kg/24h reduced TC by 26.5%, TG by 21.2%, LDL by 34.3% and HDL by 31.1% non-HDL. Group using CKL-THV at dose of 1.8 g/kg/24h reduced TC by 28.3%; Reduced TG by 17.3%, LDL by 38.4% and HDL by 33.8% compared to the model group.

According to the exogenous pathway, compared to the model group, in rat, CKL-THV at dose of 0.35g/kg/24h reduced the non-HDL-C ($p<0.01$), TC and LDL-C ($p<0.05$) indices after 2 weeks. There was a slight but not significant decrease in the TG index ($p<0.05$). However, by week 4, all of the above indicators had a statistically significant decrease compared to the model group.

The results of studies on the lipid-lowering effects of CKL-THV in the endogenous and exogenous pathways are fully consistent with previous studies on Yellow Flower Tea. Preparations extracted from different species of Yellow Flower Tea have also been shown to reduce TC, TG and LDL-C concentrations such as high water extract and high ethanol extract from *Camellia euphlebia* flowers, ethanol extract of *Camellia nitidissima* flowers, high flower extract and high *Camellia flava* leaf extract. Studies on the chemical composition of Yellow Flower Tea show that the main compounds similar to green tea such as phenolics, flavonoids, saponins, polysaccharids, amino

acids and essential oils all appear in tea flower and leaf samples. Among them, polyphenols are the most important compounds with antioxidant, anti-cancer, anti-inflammatory, weight loss and lipid-lowering effects. Experimentally, *Camellia hakodae* Ninh Yellow Flower Tea has only been studied and used as a mixture of aqueous leaf extract with gynostemma mater in obese rat. *Camellia hakodae* yellow flower tea, whether extracted or dried, used alone or in combination, has the effect of lowering blood lipids.

4.2.3. Mechanism of action in vitro

* HMG-CoA reductase enzyme inhibition:

CKL-THV has the ability to inhibit the activity of the HMG-CoA reductase enzyme and this inhibition ability is related to the concentration of the solution. Based on the decrease in NADPH absorbance in the reactions, we proceeded to calculate the percent inhibition of the HMG-CoA reductase enzyme by CKL-THV at different concentrations. When increasing CKL-THV concentration from 12.5 µg/ml to 50 µg/ml, the percent inhibition of HMG-CoA reductase enzyme also increased linearly from 26.5% to 64.6%.

In the leaves of *Camellia hakodae* Ninh Yellow Flower Tea, Nguyen Thanh Tuyen and colleagues isolated and identified 7 types of flavonoids, 9 pentacyclic triterpene derivatives and two new ursane-type triterpene derivatives. Flavonoid compounds were shown to bind to the enzyme HMG-CoA reductase. Several triterpenes compounds have also been studied and shown to inhibit HMG-CoA reductase. Thus, *Camellia hakodae* Ninh is considered a potential species, which inhibits the enzyme HMG-CoA reductase, thereby inhibiting cholesterol synthesis in the treatment of dyslipidemia. Based on the HMG-CoA reductase inhibition percentage of CKL-THV solutions,

we determined a concentration that inhibits 50% of IC₅₀ enzyme activity of 36.37 µg/ml. This value contributes to the demonstration that CKL-THV has the ability to reduce endogenous cholesterol synthesis in the body, which has positive implications for the treatment of dyslipidemia.

* Cholesterol esterase enzyme inhibition:

The results in table 3.18 and chart 3.20 show: When increasing CKL-THV concentration from 20 µg/ml to 200 µg/ml, the percentage inhibition of cholesterol esterase enzyme also increased linearly from 14.4% to 60.4%. Based on the linear regression equation, we determined the IC₅₀ value of CKL-THV to be 158.5 µg/ml. Research results on chemical composition show that the leaves of *Camellia hakodae* Ninh Yellow Flower Tea contain 7 types of flavonoids. This is a group of substances representing the polyphenol group. Several studies have demonstrated the inhibitory effect of cholesterol esterase enzymes of polyphenol-containing preparations. As such, CKL-THV can be considered as a potential preparation, which has a good inhibitory effect on cholesterol esterase enzymes, contributing to lower cholesterol absorption, thereby helping to control blood cholesterol and aid in fighting obesity.

The results of the evaluation of the mechanism of action of inhibition of HMG-CoA reductase and cholesterol esterase enzymes in the above in vitro assays contribute to provide further evidence of the mechanism of action of CKL-THV for the treatment of dyslipidemia and anti-obesity.

CONCLUSIONS

1. CKL-THV is safe in experimental acute toxicity and sub-chronic toxicity testing.

- CKL-THV is safe in acute toxicity testing in white rat. The maximum dose level used was 12.0 g/kg/24h without any rat dying, nor any abnormal symptoms, so the LD50 dose could not be determined.

- CKL-THV is safe in sub-chronic toxicity testing in white rats. CKL-THV at dose levels of 0.35 and 1.05 g/kg/24h did not affect the overall development of rat, did not affect hematological, blood biochemical indicators and liver function, kidney and spleen of rat.

2. CKL-THV had the effect of supporting the treatment of obesity and lowering blood lipids

- CKL-THV doses of 0.35 g/kg/24h and 1.05g/kg/24h are effective in supporting the treatment of obesity in experimental animals in in vivo testing. Reduced obese rat by 2.55% to 3.09% by weight, equivalent to orlistat and independent of dose; Reduced the abdominal circumference of rat compared to the model group. However, in the same group, no reduction in abdominal circumference measurements of obese rats was observed during the study period; Reduction in body fat weight, independent of the dose used. However, it does not reduce the body's fat index; Reduces Lee's obesity index by 1.37% to 1.66%, this effect is equivalent to orlistat and does not depend on the dose; Reduces absolute weight of heart, liver, kidney, spleen organs, the effectiveness varies with dose. However, only a decrease in the relative weight of liver organs was recorded when using CKL-THV at a dose of 1.05 g/kg/24h; Reduces blood lipid index

TC, TG, LDL-C, non-HDL-C and increases HDL-C index. This effect is poor orlistat and the effect varies with dose.

- CKL-THV has lipid-lowering effects on experimental animals in endogenous and exogenous pathways in in vivo testing.

- + CKL-THV doses of 0.6 and 1.8 g/kg/24h reduced TC, TG, LDL-C and non-HDL-C indexes in white rat causing endogenous hyperlipidemia with P-407.

- + CKL-THV doses of 0.35 and 1.05g/kg/24h reduced TC, TG, LDL-C, HDL-C indexes earlier and stronger than TG in rats, causing hyperlipidemia with a mixture of cholesterol oils. CKL-THV dose of 1.05 g/kg/24h causes a significant increase in HDL-C levels.

- CKL-THV has the mechanism of inhibition of HMG-CoA reductase and cholesterol esterase enzymes in in vitro testing. CKL-THV's ability to inhibit HMG-CoA reductase ($IC_{50} = 36.37 \mu\text{g/ml}$) is stronger than its ability to inhibit cholesterol esterase ($158.5 \mu\text{g/ml}$).

RECOMMENDATIONS

Based on the results of research achieved on the toxicity and anti-obesity and lipid-lowering effects of high dried leaves of *Camellia hakodae* Ninh, some recommendations are made as follows:

- 1- Further research on the mechanism of anti-obesity effect of CKL-THV on in vitro testing of inhibitory effect on pancreatic lipase enzym.

- 2- Study of the anti-obesity and dyslipidemic effects of CKL-THV preparations in the clinical setting.

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