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Original Research Article

The Effects of *Camellia hakodae* Ninh Dried Leaf Extract on Obesity in Rats Induced by a High-Fat Diet

Ngo T. M. Binh[1,2], Nguyen T. H. Tuan[3], Nguyen H. Ngan[1], Nguyen H. Hanh[4], Truong H. A. Huy[1], Dao P. Tien[1], Lu T. Hue[1], Dang H. Giang[1], Trinh D. Toan[1], Pham T. Phuong[5]*

[1] *Department of Pharmacology, Vietnam Military Medical University, 160 Phung Hung, Phuc La, Ha Dong, Hanoi 12108, Vietnam*
[2] *Thai Nguyen University of Medicine and Pharmacy, 284 Luong Ngoc Quyen, Quang Trung, Thai Nguyen City, Thai Nguyen 24117, Vietnam*
[3] *103 Military Hospital, Vietnam Military Medical University, 160 Phung Hung, Phuc La, Ha Dong, Hanoi 12108, Vietnam*
[4] *E Hospital, 87- 89 Tran Cung, Nghia Tan, Cau Giay, Hanoi 11308, Vietnam*
[5] *Vietnam University of Traditional Medicine, 2 Tran Phu, Ha Đong, Hanoi 12107, Vietnam*

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ABSTRACT

Camellia hakodae Ninh (Theaceae), also known as Hakoda Golden Tea, is widely used to have various health benefits, including enhancing energy expenditure, anti-diabetic, antioxidant, anti-cancer, and lipid-lowering properties, and prevention of arterial atherosclerosis. However, studies on obesity treatment in this species have not been conducted. This study aimed to evaluate the effects of *C. hakodae* dried leaf extract (CKL-THV) in the treatment of obesity in an experimental rat model. Obesity was induced in Wistar strain rats with a high-fat diet over 12 weeks. Subsequently, the rats were continuously administered the research product for 8 weeks. The effects of CKL-THV were evaluated in terms of reduction in rat weight, waist circumference, fat weight, Lee obesity index, body fat index, organ weights, and rat blood lipids during the study period. CKL-THV led to a 2.55−3.09% decrease in rat weight, a 1.37−1.66% reduction in Lee obesity index, a decrease in body fat weight, absolute organ (heart, liver, kidney, spleen) weights, reductions in total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and non-HDL cholesterol (HDL-C), and an increase in HDL-C levels. However, CKL-THV did not reduce rat waist circumference, body fat index, or relative organ weights during the study period. These results indicate that CKL-THV at doses of 0.35 and 1.05 g/kg/day has the potential prevent and treat obesity. This is the first study of Vietnamese *C. hakodae* with anti-obesity activity.

Keywords: *Camellia Hakodae*, CKL-THV, Obesity, Lee Obesity Index, Total Cholesterol, Triglycerides.

Introduction

Obesity is recognized by the World Health Organization (WHO) and the American Medical Association (AMA) as a chronic disease that requires long-term management and treatment. Obesity is characterized by excess or abnormal fat accumulation, which can seriously impact health.[1] In 2020, approximately 2.6 billion people worldwide were overweight or obese, and by 2035, it is estimated that the global prevalence will reach over 4 billion individuals. The obesity rate alone is projected to increase from 14% to 24% of the population during the same period, affecting nearly 2 billion adults and children by 2035.[2] The goal of obesity treatment is to control weight and reduce the risk of complications, thus improving health. Both the European Guidelines on Obesity Management in Adults and the Guidelines of the Ministry of Health of Vietnam agree on specific weight loss targets in obesity.

*Corresponding author: Email: thuyphuongydhctvn@gmail.com
Tel: +84983654033

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treatment, recommending a 5−15% reduction in body weight (BW) within 6 months of starting treatment.[1,3,4] Consideration may be given to more than a 20% weight reduction for severe obesity cases (body mass index, BMI ≥ 35 kg/m^2).

As of 2024, the Food and Drug Administration (FDA) has approved several medications for obesity treatment in adults, including lipase inhibitors (orlistat), combination drugs of sympathetic nervous system amines suppressing appetite with anticonvulsants (phentermine/topiramate), opioid receptor antagonists combined with antidepressants (naltrexone/bupropion), *glucagon-like peptide-1* (GLP-1) receptor agonists (liraglutide, semaglutide), melanocortin-4 receptor agonists (setmelanotide), and dual glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 receptor agonists (tirzepatide).[5-7] In Vietnam, only two medications, orlistat and liraglutide 3.0 mg, have been approved for obesity treatment.[1]Current trends in community healthcare emphasize using herbal medicines that nourish the body, limit adverse side effects, and reduce treatment costs. According to traditional medicine, Golden Tea is considered neutral-sweet in taste and associated with the heart, kidney, and gallbladder meridians.[8,9] *Camellia* L. tea varieties have been reported to have various health benefits, including enhancing energy expenditure, anti-diabetic, antioxidant, anti-cancer, and lipid-lowering properties, and prevention of arterial atherosclerosis.[8-12] *Camellia hakodae* Ninh, also known as Hakoda Golden Tea, was discovered by Professors Hakoda and Tran Ninh in the Northeastern mountains of Tam Dao in 1999. This tea species is unique to Tam Dao, Vinh Phuc, Vietnam, and is a valuable medicinal plant that requires effective conservation, development, and utilization. Studies on the chemical

composition and biological activity of *C. hakodae* show that it contains flavonoids,[13] triterpenoids,[14] and has a lowering blood lipids effect.[10] To create a safe and effective product for obesity treatment that is convenient to use, the Vietnam Military Medical Academy has developed a powdered *C. hakodae* dried leaf extract (CKL-THV) that meets quality standards. This study evaluated the supportive effects of CKL-THV in treating obese rats with obesity induced by a high-fat diet.

Materials and Methods

Plant Materials

C. hakodae leaves (compliant with the internal standard) 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, Ph.D., 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. After harvesting, the *C. hakodae* leaves were washed, dried, and ground into powder. The herbal powder was then subjected to ultrasonic extraction using 96% ethanol solvent, and the extract was filtered, concentrated, and spray-dried to obtain dried powder. Based on traditional knowledge, dose extrapolation (the average weight of a person is 50 kg), and the CKL-THV preparation process, the expected human dose based on CKL-THV weight is 0.05 g/kg/day.[15] Based on the differences in BW and metabolism between humans and rats, dose extrapolation was applied to determine the appropriate dose for rats. Using a dose conversion method,[16] the equivalent rat dose (conversion factor of 7) was determined to be 0.35 g/kg/day. CKL-THV powder was uniformly dispersed in distilled water to obtain solutions containing 0.035 and 0.105 g/ml concentrations. The rats in the experimental groups were orally administered the test solutions at a volume of 10 ml/kg BW, equating to effective doses of 0.35 and 1.05 g/kg/day.

Equipment and Chemicals

Equipment employed included an analytical CP224S balance precision scale (Sartorius, Germany), a biochemical analyzer (Biochemical Systems International SpA, Italy), and quantitative kits for TC, TG, and HDL-C. Orlistat 10 mg was supplied by STADA, Vietnam.

Animals

Healthy adult white Wistar rats of both genders weighing 180−200 g were provided by the Experimental Animal Supply Committee, Military Medical Academy, and were kept under laboratory conditions, provided with standard food, and had access to water ad libitum. The study was conducted with the approval of the Scientific Council of the Military Medical University and in compliance with ethical standards in medical research (ethical permission number IACUC-2303/22, issued on March 23, 2022).

In Vivo Test

The study was conducted in two phases.

Phase 1: Establishing the obesity rat model

Male white Wistar rats weighing 100−125 g were fed a high-fat diet (HFD) for 12 weeks. After 12 weeks, the Lee obesity index in rats (equivalent to BMI in humans) was calculated using formula 1.[17]

$$\text{Lee obesity index} = \frac{\sqrt[3]{W}}{L} \quad (1)$$

where W is the rat weight (g) and L is the body length of the rat from the nose to the anus (cm). Rats with Lee index values of 0.310 or higher were considered obese.

Phase 2: Experiment

Forty obese rats from phase 1 and 10 normal male white Wistar rats (180−200 g) were included in phase 2. The animals were divided into 5 groups, each consisting of 10 individuals, and were subjected to the following daily dietary regimens for 8 weeks.

Group 1, physiological control: normal rats fed a standard diet and given 10 ml/kg of distilled water daily.

Group 2, model: obese rats fed the HFD and given 10 ml/kg of distilled water daily.

Group 3, reference: obese rats fed the HFD and given orlistat solution at a dose of 50 mg/kg/day (0.05 g/kg/day).

Group 4, treatment group 1: obese rats fed the HFD and given CKL-THV solution at a dose of 0.35 g/kg/day.

Group 5, treatment group 2: obese rats fed the HFD and given CKL-THV solution at a dose of 1.05 g/kg/day.

The BW of rats and weight change ratios were determined and monitored weekly from the beginning of phase 2. The body length of the rats was determined following light anesthesia using 1.5 mL of 1% w/v propofol solution administered via intraperitoneal injection. The body length was measured from the tip of the nose to the anus using a measuring tape accurate to 0.1 cm. Measurements were taken at the beginning of phase 2 of the experiment (t0), and after 2 weeks (t2), 4 weeks (t4), 6 weeks (t6), and 8 weeks (t8).

The waist circumference and circumference change ratios were determined after measuring the body length. The rats were positioned ventrally, and the maximum circumference of the abdomen was measured with a tape measure at the same time points as for the body length.

The Lee obesity index and index change ratios were calculated using formula 1 every 2 weeks based on the BW and length of the rats.

Blood lipid parameters were determined on the final day of the experiment. Blood samples were collected from the orbital sinus of the rats. Lipid parameters, including TC, TG, and HDL, were measured. LDL-C and non-HDL-C indexes were calculated using the Friedewald formula 2 and formula 3.[10,18]

$$\text{LDL} - \text{C} = \text{TC} - \text{HDL} - \text{C} - \frac{\text{TG}}{2.2} \quad (2)$$

$$\text{Non HDL} - \text{C} = \text{TC} - \text{HDL} - \text{C} \quad (3)$$

Organ weights were determined after the rats were euthanized. The major organs (liver, spleen, kidneys, heart) were excised, rinsed with 0.9% cold saline, dried with paper towels, and weighed using an analytical balance. Relative organ weight was calculated using formula 4.

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Body weight}} \times 100\ (\%) \quad (4)$$

Fat weight and body fat index were determined from subcutaneous and intra-abdominal fat tissues surrounding the organs, dissected using scissors, rinsed with 0.9% cold saline, dried with paper towels, and weighed on an analytical balance. The body fat index of the rats was calculated using formula 5.

$$\text{Body fat index} = \frac{\text{Fat weight}}{\text{Body weight}} \times 100\ (\%) \quad (5)$$

Statistical Analysis

Data was analyzed using biostatistical methods, employing Statistical Package for the Social Sciences (SPSS) software version 22.0. Results are expressed as $\bar{X}$ ± standard deviation (SD). One-way ANOVA was used to compare mean values among three or more groups. Post hoc analysis was conducted using the least significant difference (LSD) test if variances were homogeneous, or Dunnett's T3 if variances were heterogeneous. Statistical significance was accepted at $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to the control Group 1; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ compared to the model Group 2; and $^{\blacktriangle}p < 0.05$, $^{\blacktriangle\blacktriangle}p < 0.01$, and $^{\blacktriangle\blacktriangle\blacktriangle}p < 0.001$ compared to the orlistat Group 3).

Results and Discussion

Obesity Model Induction

After 12 weeks of feeding 50 rats an HFD, the weight and length of all the rats were measured. Subsequently, the Lee obesity index was

The relative energy values of nutrients in the HFD were the following:

Nutrients	Energy values (Kcal)	Percentage (%)
Proteins	641.0	14.26
Carbohydrates	1693.4	37.67
Fat	2161.4	48.08

Total 4495.8 100

Table 1: Summary of research parameters at the start of phase 2.

Group	Research Indicators ($\bar{X}$ ± SD)			
	BW (g)	Nose to anus length (cm)	Abdominal circumference (cm)	Lee obesity index
Control	188.9 ± 7.78	19.3 ± 0.21	13.9 ± 0.19	0.298 ± 0.003
Model	257.3 ± 18.47 ***	19.3 ± 0.25	17.9 ± 0.55 ***	0.330 ± 0.006 ***
Orlistat	257.2 ± 22.07 ***	19.3 ± 0.31	17.9 ± 0.30 ***	0.330 ± 0.006 ***
CKL-THV 0.35	257.1 ± 25.07 ***	19.3 ± 0.23	18.0 ± 0.39 ***	0.330 ± 0.010 ***
CKL-THV 1.05	257.2 ± 22.33 ***	19.2 ± 0.27	18.0 ± 0.26 ***	0.330 ± 0.009 ***

The p-values were assessed using one-way ANOVA, with post hoc Dunnett T3 tests, comparing the parameters of weight, length, waist circumference, and Lee index among the study groups at the start of the experiment.

Table 2: Weights of organs in experimental rats.

Group	Organ weights (g)				Relative organ to BWs (%)			
	Heart	Liver	Kidneys	Spleen	Heart	Liver	Kidneys	Spleen
Control	0.61 ± 0.054	5.22 ± 0.908	1.34 ± 0.264	0.53 ± 0.092	0.28 ± 0.024	2.40 ± 0.410	0.62 ± 0.124	0.24 ± 0.042
Model	1.03 ± 0.079 ***	9.45 ± 1.280 ***	2.29 ± 0.178 ***	0.95 ± 0.111 ***	0.31 ± 0.028 *	2.83 ± 0.457 *	0.69 ± 0.090	0.29 ± 0.047
Orlistat	0.64 ± 0.073 ###	5.57 ± 0.896 ###	1.33 ± 0.160 ###	0.52 ± 0.075 ###	0.26 ± 0.023 ###	2.31 ± 0.458 #	0.55 ± 0.083 #	0.21 ± 0.037 ##
CKL-THV 0.35	0.75 ± 0.048 ***###▲▲▲	6.68 ± 0.771 **###▲	1.65 ± 0.274 *###▲▲	0.64 ± 0.216 ###▲	0.30 ± 0.032 ▲▲	2.68 ± 0.313	0.66 ± 0.111 ▲	0.26 ± 0.097
CKL-THV 1.05	0.72 ± 0.043 ***###▲▲	5.91 ± 1.091 ###	1.43 ± 0.372 ###	0.58 ± 0.106 ###	0.29 ± 0.024 ▲	2.39 ± 0.508 #	0.58 ± 0.167	0.23 ± 0.045

The p-values were assessed using one-way ANOVA with post hoc LSD tests to compare the weights (relative weights) of the organs among the experimental groups.

calculated, and 40 rats with a Lee index greater than 0.301 were selected for inclusion in the experimental phase 2 of the study. The control group for this phase consisted of 10 healthy rats, weighing 180−200 g. Table 1 summarizes some research parameters for the rats in the study groups at the commencement of phase 2.

Rats in the model group, those receiving orlistat, and those treated with CKL-THV all exhibited a Lee index greater than 0.310 (Table 1). The weights, waist circumferences, and Lee indexes of these groups were significantly different from those of the control group ($p < 0.001$). There were no significant differences in the lengths of the rats among the five study groups ($p > 0.05$). Obese rats in the model group, those receiving orlistat, and those treated with CKL-THV showed similarities in weight, length, waist circumference, and Lee index ($p > 0.05$).

Impact of CKL-THV on Rat Weight

The weight of rats in both the control and the model groups increased over time (Figure 1). Notably, the model group exhibited a significantly higher rate of weight change than all other groups, with a weight increase of 30.58% from the beginning of phase 2. Rats treated with orlistat and those receiving CKL-THV tended to show a decrease in weight, with the CKL-THV group experiencing a 2.55−3.09% reduction in weight after 8 weeks.

Assessing the weight of the rats at the end of the study yielded the results in Figure 2. At the end of the experiment (after 8 weeks), there was a statistically significant difference in the weight of rats in the control group compared to all other groups ($p < 0.001$). A comparison of the weight of rats in the model group with those receiving orlistat and CKL-THV also revealed statistically significant differences ($p < 0.001$). However, no significant difference in weight was observed between the groups treated with orlistat and CKL-THV.

Impact of CKL-THV on Waist Circumference

The waist circumference of rats in both the model and control groups increased over time (Figure 3). The model group demonstrated a 4.35% increase in waist circumference compared to that at the start of Phase 2. Only the rats treated with orlistat showed a 1.78% reduction in waist measurement after 8 weeks. The CKL-THV samples did not result in a decrease in waist circumference. A specific evaluation of waist circumference at the end of the experiment yielded the results in Figure 4.

At the end of the experiment, the waist circumference of rats in the control group was smaller than that of the model group and those treated with orlistat and CKL-THV, with a statistically significant difference ($p < 0.001$). Compared to the model group, the orlistat and CKL-THV groups exhibited a statistically significant reduction in

waist circumference. Compared with the orlistat group, CKL-THV groups at doses of 0.35 and 1.05 g/kg/day showed significant differences in waist circumference ($p < 0.001$).

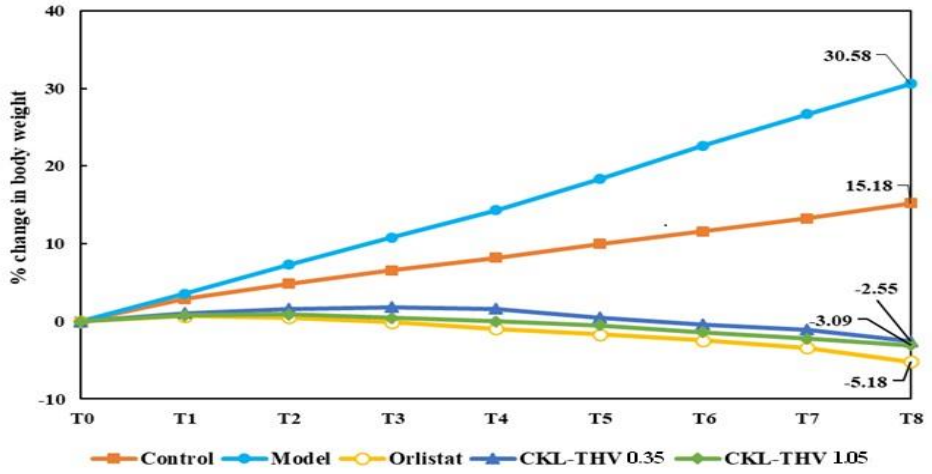

Figure 1: Change in rat weight across experimental groups over time.

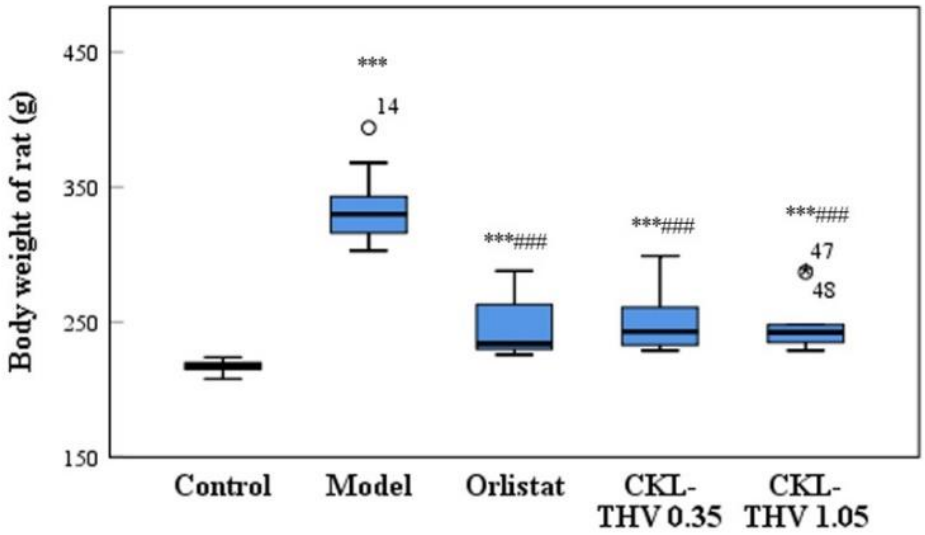

Figure 2: Rat weight after phase 2. The p-value was evaluated using the Mann-Whitney test to compare the weight of rats between groups at the end of the experiment.

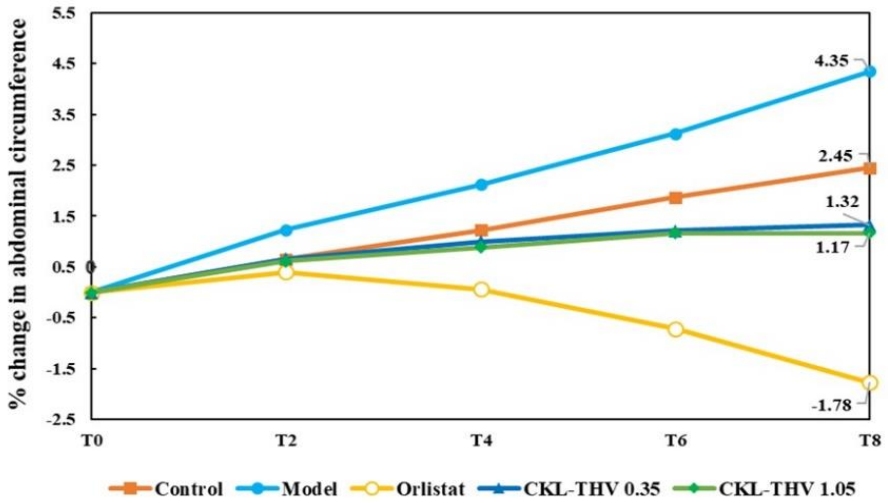

Figure 3: Changes in rat waist circumference across experimental groups over time.

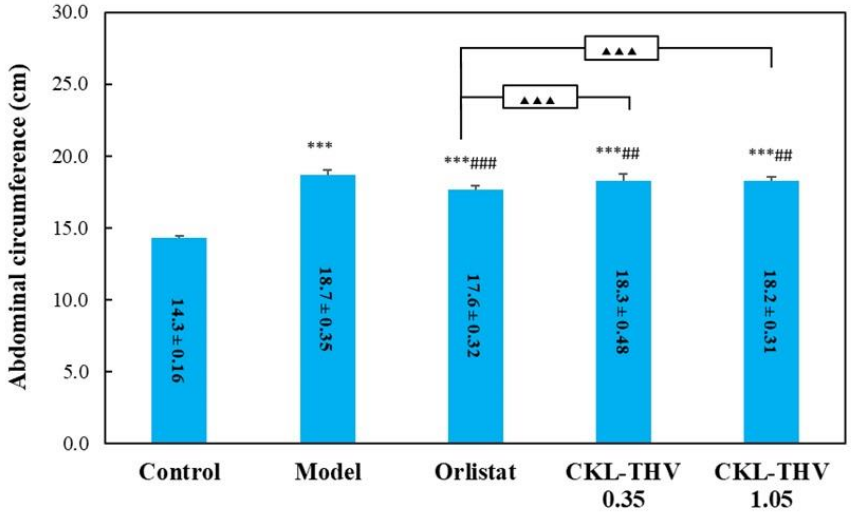

Figure 4: Waist circumference of rats in experimental groups after phase 2. The p-value was evaluated using one-way ANOVA with post hoc LSD tests to compare the waist circumference of rats across the experimental groups at the end of the experiment.

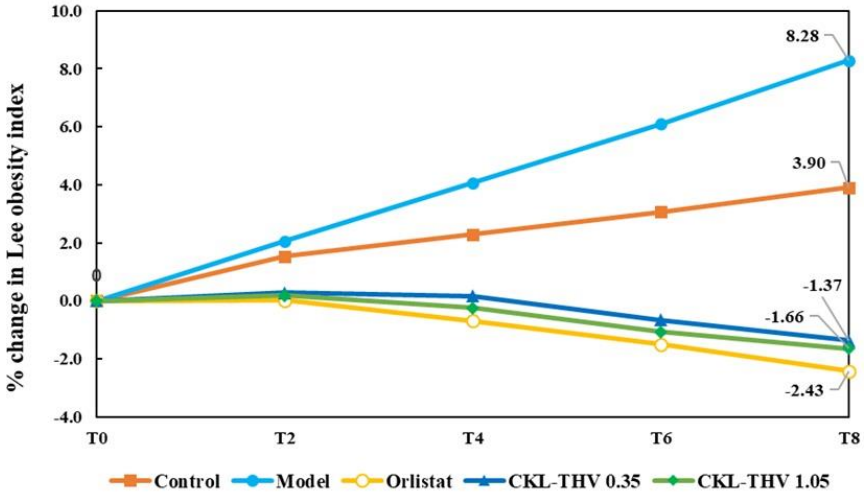

Figure 5: Changes in Lee obesity index of rats across experimental groups over time.

Impact of CKL-THV on the Lee Obesity Index and Body Fat Index

The Lee obesity index in rats from the control and model groups increased over time by 3.90% and 8.28%, respectively (Figure 5). Groups treated with orlistat and CKL-THV showed a tendency to decrease their Lee index. After 8 weeks, the orlistat group exhibited a reduction of 2.43% in the Lee obesity index, while the CKL-THV groups showed reductions ranging from 1.34% to 1.66% (Figure 5).

At the end of the experiment, there was a statistically significant difference in the Lee obesity index between the control group and the model group ($p < 0.001$), as well as between the control group and the other groups ($p < 0.01$). The Lee obesity index in the orlistat and CKL-THV groups decreased compared to the model group ($p < 0.001$). However, no significant differences were observed when comparing the orlistat and CKL-THV groups at doses of 0.35 and 1.05 g/kg/day (Figure 6).

Compared to the control group, the fat weight in the experimental groups showed statistically significant differences. The orlistat group significantly reduced fat weight compared to the model group ($p < 0.001$). The CKL-THV groups also reduced fat weight compared to the model group ($p < 0.05$). Significant differences in total fat weight were found when compared with the orlistat group, with $p < 0.001$ for the CKL-THV group at 0.35 g/kg/day and $p < 0.01$ at 1.05 g/kg/day (Figure 7).

The model group showed a notable increase in the body fat index compared to the control group, with a statistically significant difference ($p < 0.001$). In the orlistat group, the body fat index decreased compared to the model group ($p < 0.001$) but remained elevated compared to the control group ($p < 0.05$). The CKL-THV groups showed differences in fat density compared to the control group ($p < 0.01$). However, the body fat index remained high compared to the model group, with no significant difference observed ($p > 0.05$). There were significant differences in the body fat index between the orlistat group and the CKL-THV groups at 0.35 g/kg/day ($p < 0.001$) and 1.05 g/kg/day (Figure 8).

Impact of CKL-THV on Organ Weights

Compared to the control group, the heart, liver, kidneys, and spleen weights in the model group were significantly higher ($p < 0.001$). In the orlistat group, the weights of the heart, liver, kidneys, and spleen decreased (Table 2) compared to the model group ($p < 0.001$) and were comparable to those in the control group ($p > 0.05$). The CKL-THV groups showed reduced heart, liver, kidneys, and spleen weights compared to the model group ($p < 0.001$). The CKL-THV group at a

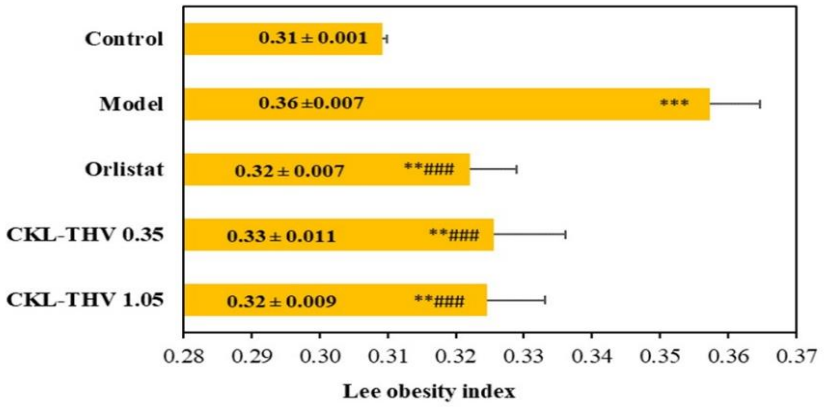

Figure 6: Lee obesity index of rats in experimental groups after phase 2. Note: The p-values were evaluated using one-way ANOVA with post hoc Dunnett T3 tests to compare the Lee indexes among the corresponding groups.

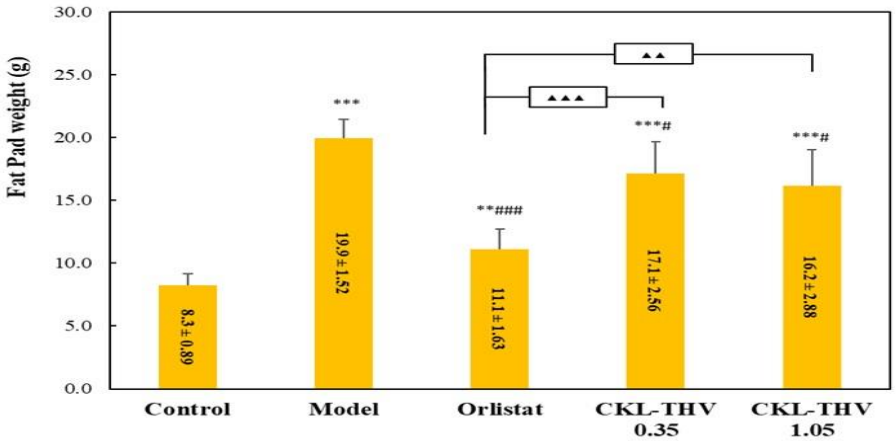

Figure 7: Body fat weight of rats in experimental groups after phase 2. The p-values were evaluated using one-way ANOVA with post hoc Dunnett T3 tests to compare the weight of fat tissue among the corresponding groups.

dose of 0.35 g/kg/day differed from the control group in heart weight ($p < 0.001$), liver weight ($p < 0.01$), and kidney weight ($p < 0.05$), while spleen weight was comparable ($p > 0.05$). When comparing this group with the orlistat group, significant differences were also observed in heart weight ($p < 0.001$), liver weight ($p < 0.05$), kidney weight ($p < 0.01$), and spleen weight ($p < 0.05$). The CKL-THV group at a dose of 1.05 g/kg/day only showed a difference in heart weight compared to the control group ($p < 0.01$) and the orlistat group ($p < 0.01$) (Table 2). The liver, kidneys, and spleen weights were comparable to the control and orlistat groups ($p > 0.05$).

In terms of relative weight, compared to the control group, only the relative weights of the heart and liver in the model group increased significantly ($p < 0.05$), while the relative weights of the kidneys and spleen did not show significant changes ($p > 0.05$) (Table 2). In comparison with the model group, the orlistat group reduced the relative weights of the heart ($p < 0.001$), liver ($p < 0.05$), kidneys ($p < 0.05$), and spleen ($p < 0.01$). The CKL-THV groups showed no differences in the relative weights of the organs compared to the control group ($p > 0.05$). Compared to the model group, only the CKL-THV group at a dose of 1.05 g/kg/day showed a difference in the relative weight of the liver ($p < 0.05$), while other organs did not change (Table 2). However, significant differences were noted when comparing the relative weights of organs between the CKL-THV and orlistat groups. Specifically, the CKL-THV group at a dose of 0.35 g/kg/day exhibited differences in the relative weights of the heart ($p < 0.01$) and kidneys ($p < 0.05$) compared to the orlistat group. The CKL-THV group at a dose of 1.05 g/kg/day only showed a difference in the relative weight of the heart compared to the orlistat group ($p < 0.05$) (Table 2).

Impact of CKL-THV on Blood Lipid Levels

At the end of the experiment, the TC and TG levels in the model group were significantly higher than those of the control group ($p < 0.001$). In the orlistat group, both TC and TG levels showed a significant decrease compared to the model group ($p < 0.001$) and were nearly equivalent to the control group ($p > 0.05$). The TC and TG levels in both CKL-THV groups decreased compared to the model group ($p < 0.001$) but remained elevated compared to the control group ($p < 0.001$) (Figure 9).

There were statistically significant differences in TC and TG levels between the orlistat group and the CKL-THV groups at a dose of 0.35 g/kg/day, with p-values of $p < 0.001$ and $p < 0.01$, respectively. The CKL-THV group at a dose of 1.05 g/kg/day differed from the orlistat group in TC levels ($p < 0.05$) but was equivalent in TG levels ($p > 0.05$). A comparison between the two CKL-THV groups revealed statistically significant differences ($p < 0.05$) in both TC and TG levels (Figure 9).

Both the orlistat and CKL-THV groups exhibited statistically significant increases in HDL-C levels compared to the model group ($p < 0.001$) and were comparable to the control group ($p > 0.05$). No significant differences in HDL-C levels were observed between the CKL-THV groups and the orlistat group (Figure 10).

At the end of the experiment, both LDL-C and non-HDL-C levels in the model group were significantly higher than those in the control group ($p < 0.001$). In the orlistat group, both LDL-C and non-HDL-C levels showed a statistically significant decrease compared to the model group ($p < 0.001$). The CKL-THV groups exhibited increased levels of LDL-C and non-HDL-C compared to the control group ($p <$

0.001) and a statistically significant decrease compared to the model group (Figure 11). The CKL-THV group at a dose of 0.35 g/kg/day reduced both

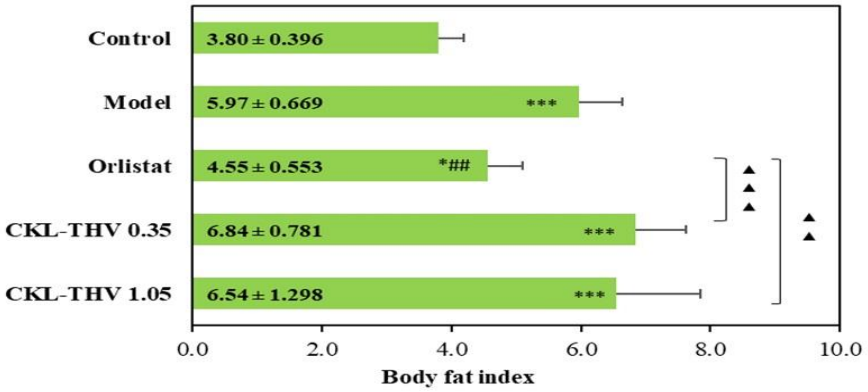

Figure 8: Body fat index of rats in experimental groups after phase 2. The p-values were evaluated using one-way ANOVA with post hoc Dunnett T3 tests to compare the body fat indexes among the corresponding groups.

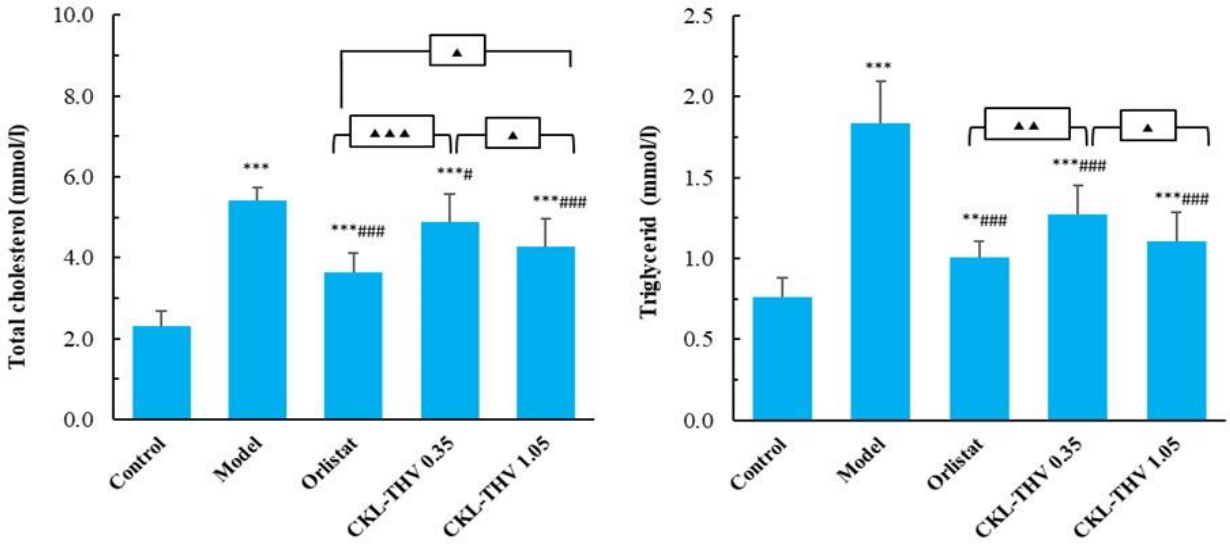

Figure 9: TC and TG levels in rats from experimental groups after phase 2. The p-values were evaluated using one-way ANOVA with post hoc LSD tests to compare the total cholesterol and triglyceride levels among the rat groups.

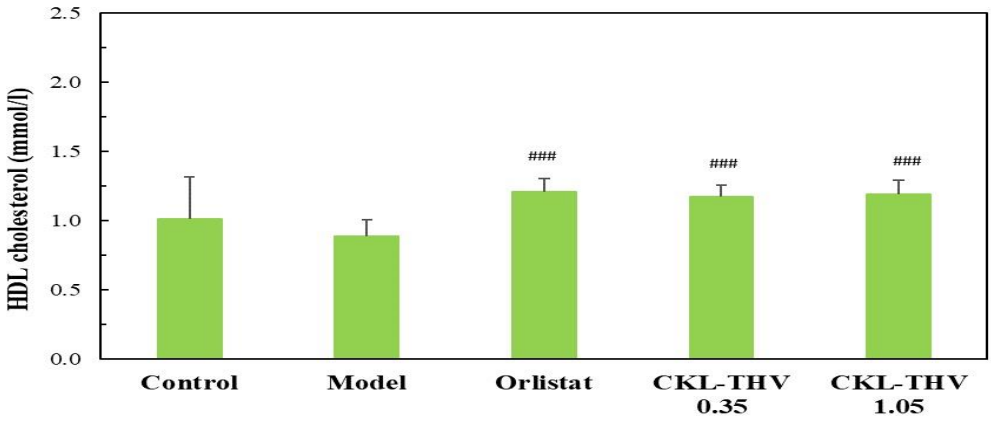

Figure 10: HDL-C levels in rats from experimental groups after phase 2. The p-values were assessed using one-way ANOVA with post hoc Dunnett T3 tests to compare the mean levels of HDL-C among the rat groups.

LDL-C and non-HDL-C levels compared to the model group, with significance levels of $p < 0.05$ and $p < 0.01$, respectively. The CKL-THV group at a dose of 1.05 g/kg/day also reduced both LDL-C and non-HDL-C levels compared to the model group, with a significance

level of $p < 0.001$ (Figure 11). There were statistically significant differences in LDL-C and non-HDL-C levels between the orlistat group and the CKL-THV groups at doses of 0.35 and 1.05 g/kg/day, with significance levels of $p < 0.001$ and $p < 0.05$, respectively. The CKL-THV group at a dose of 1.05 g/kg/day resulted in a greater reduction in LDL-C and non-HDL-C levels compared to the dose of 0.35 g/kg/day ($p < 0.05$) (Figure 11).

In experimental studies, to evaluate the effectiveness of obesity treatment, it is necessary to research on animal models with induced obesity. There are several methods to cause obesity in animals, such as overfeeding, high-energy diets, interventions targeting the hypothalamus (surgical or chemical), or genetic predisposition to obesity.[19-21] Among these, inducing obesity through high-energy diets is commonly applied. HFD containing 45% to 60% fat is typically used to induce obesity in rodents,[22,23] with study durations ranging from 8 to 27 weeks.[24]

In this study, rats were induced to obesity using a high-energy HFD due to its common use, feasibility, and effectiveness.[19] Numerous studies have shown that Wistar rats are prone to obesity from high-energy diets.[25] Therefore, in this study, an HFD designed to induce obesity in
rats was formulated, with a total energy value of 4495.8 kcal and a fat composition of 48.08%. This fat-rich diet increases energy intake, leading to an imbalance in dietary intake and energy expenditure, thus resulting in obesity. After several weeks on the HFD, rats may develop characteristics similar to humans with metabolic syndrome, such as obesity, hyperglycemia, hyperlipidemia, hypertension, and endothelial damage associated with cardiovascular diseases.[25]

Our study was conducted in two phases. In phase 1, the experimental rats were fed an HFD for 12 weeks to induce obesity. Subsequently, in phase 2, the 8-week experimental phase, the obese rats continued to receive the HFD while also being administered either the study formulation or the positive control drug, orlistat, daily. The anti-obesity effects were then evaluated based on the research criteria. Orlistat was chosen as the positive control drug for the reference group of rats. It has been FDA-approved since 1999 for obesity treatment, but was only officially used in Vietnam in 2008. It is a dehydro derivative of lipstatin, a compound that inhibits lipase, derived from the bacterium *Streptomyces toxytricini*. Lipases hydrolyze fats into monoglycerides and fatty acids. Orlistat selectively and strongly inhibits pancreatic lipase, thus preventing the breakdown of dietary triglycerides into absorbable free fatty acids.[1] The drug does not affect other intestinal enzymes such as trypsin, chymotrypsin, pancreatic amylase, cholesterol esterase, and carboxylesterase. Consequently, carbohydrate and protein components in the diet are absorbed normally.

The parameters used to assess the obesity status of the rats included BW, length from nose to anus, waist circumference, and the Lee obesity index. Like the BMI index used to diagnose obesity in humans, the Lee index is used to diagnose obesity in rats. A rat is considered obese when its Lee index exceeds 0.310.[17]

The results in Table 1 indicate successful induction of obesity in rats using an HFD. The rats in the model group, those receiving orlistat, and those receiving CKL-THV were equivalent in weight, length, waist circumference, and Lee obesity index. After 12 weeks on the HFD, the average Lee index of the rats in the four groups was 0.330, which differed significantly from the control group ($p < 0.001$). Additionally, other research parameters, such as weight and waist circumference, also showed statistically significant differences when comparing these four groups with the control group of normal rats. The average length of the rats did not change significantly between groups ($p > 0.05$) because the rats in the study were mature and stabilized in length. Thus, the rats entering the main experimental phase were obese and met the study criteria.

Weight gain is a common sign of obesity. A study by Mariana *et al.* indicated that most research on obesity induced by high-energy diets considers the increase in total BW as the primary parameter for evaluating the progression of obesity.[24] Therefore, weight reduction is the primary goal in obesity treatment.

The results of our study on the percentage change in BW of the rats across the research groups over time showed that the control group, fed a normal diet, continued to grow normally, increasing by 15.18% after 8 weeks of study. In contrast, the model group exhibited a significant increase in BW, with a 30.58% weight gain after 8 weeks compared to the beginning of the main experimental phase. Thus, maintaining the HFD had a strong impact on weight change in the experimental rats. The HFD in this study was formulated with a high fat content (48.08%), which may increase neuropeptide Y (NPY), a hormone that stimulates appetite, thus encouraging the rats to eat more. NPY increases food intake and reduces thermogenic activity, leading to weight gain and obesity.[26]

Although the rats were maintained on the HFD, the groups using the positive control orlistat and CKL-THV showed a reduction in weight over time. The orlistat group exhibited a weight decrease of 5.18% after 8 weeks, a reduction greater than that observed in the CKL-THV groups. However, at the end of the experiment, no significant difference was found between the orlistat group and the CKL-THV groups regarding rat weight. Orlistat does not specifically affect satiety mechanisms or appetite. It inhibits pancreatic lipase, thus preventing the hydrolysis of fats into monoglycerides and fatty acids.[1,27] This results in a 30% reduction in fat absorption.[7] The decreased absorption of fats leads to lower caloric intake, thereby causing weight loss. CKL-THV at doses of 0.35 and 1.05 g/kg/day resulted in weight reductions of 2.55% to 3.09% compared to the start of phase 2. Compared with the final week of the experiment, the weights of the rats in both CKL-THV groups differed significantly from those of the model group ($p < 0.001$). The effect of CKL-THV was comparable to that of orlistat ($p > 0.05$).

CKL-THV contains polyphenols that help enhance energy metabolism, regulate lipid synthesis, and modulate gut microbiota. Additionally, polyphenols can regulate hormones such as ghrelin and leptin, thereby controlling appetite and aiding weight loss.[28] Some polyphenols, such as catechins, are believed to have high biological activity in reducing BW. A study by Chen *et al.* on the use of high-dose catechins extracted from tea in obese women showed significant weight loss effects after two weeks of treatment without any adverse effects.[29] Compared with other studies, the leaves of *C. hakodae* in mixed formulations also have weight-loss effects. Research by Hanh *et al.* demonstrated that a mixture of extracts of *C. hakodae* leaves and *Gynostemma pentaphyllum* at doses of 12 and 24 g/kg/day had weight loss effects in obese white rats, comparable to atorvastatin at a dose of 15 mg/kg/day.[30] Thus, the weight loss effect of the CKL-THV formulation in our study is scientifically substantiated.

In addition to BW, the change in waist circumference of the rats in the study groups was also chosen as a basis for evaluating the anti-obesity effects of CKL-THV. Waist circumference was measured after 2, 4, 6, and 8 weeks of CKL-THV administration. The results shown in Figure 3 indicate that in the model group, the waist circumference of the rats increased by 4.35% by the end of the 8-week trial. The rats in the model group were fed an HFD that stimulates lipogenesis and reduces lipolysis. When excess fat is not utilized, the body stores it in adipose cells in the abdomen, increasing the waist circumference.

The group of rats receiving orlistat showed a reduction in waist circumference of 1.78% after the 8-week trial. Meanwhile, the groups using CKL-THV did not show a significant reduction in waist circumference. However, at the end of the trial, CKL-THV did significantly reduce the waist circumference of the rats compared to the model group ($p < 0.01$). A comparison between the CKL-THV groups and the orlistat group indicated a significant difference in waist circumference ($p < 0.001$). Thus, the polyphenols in CKL-THV may help reduce abdominal fat by limiting fat absorption from food and by affecting gut microbiota.

To evaluate the anti-obesity effects of CKL-THV, the body fat index and the Lee obesity index were assessed. The Lee index is defined as the ratio of the cube root of BW to the length from the nose to the anus of the rat. The body fat index is the percentage of total fat mass relative to the BW of the experimental rats. These indices have been employed to evaluate the effects of formulations in the treatment of obesity in several studies.[31-33]

The results shown in Figures 6 and 7 indicate that, at the end of the trial, the Lee obesity index and body fat index in the model group significantly increased compared to the control group ($p < 0.001$). The

increased caloric and fat intake from the HFD leads to the storage of excess energy as fat. The HFD increases visceral fat mass, thereby elevating the body fat index. Additionally, since the length of the rats did not change significantly throughout the trial, the Lee index of the rats also increased. Both CKL-THV groups showed differences in the Lee obesity index and body fat index compared to the control group (p

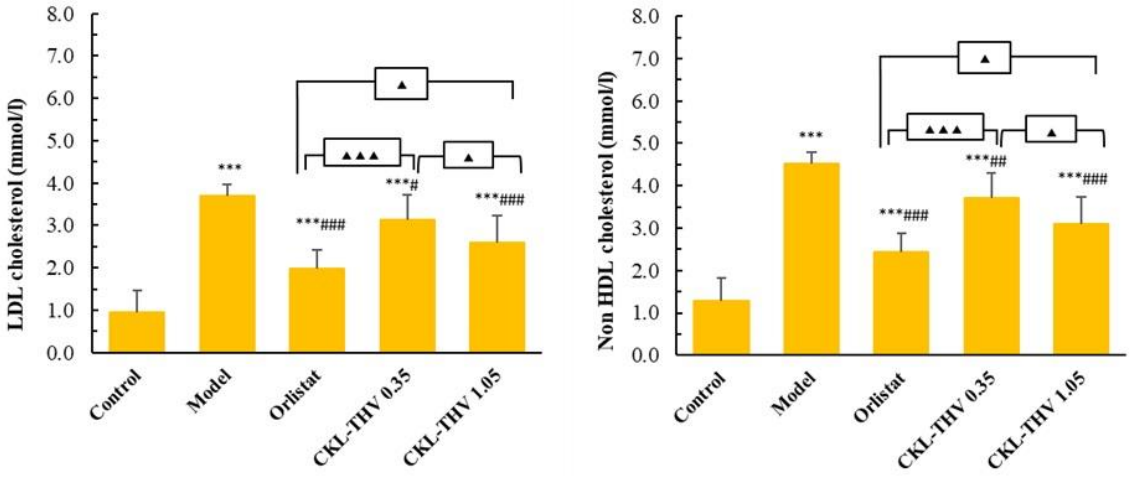

Figure 11: LDL-C and non-HDL-C levels in rats from experimental groups after phase 2. The p-values were assessed using one-way ANOVA with post hoc LSD tests to compare the mean levels of LDL-C and non-HDL-C among the experimental groups.

< 0.01 to p < 0.001). However, when compared to the model group, only the change in the Lee index was statistically significant (p < 0.001). The Lee index is dependent on the measurements of weight and body length of the rats. According to the results shown in Figure 2, the weight of the rats in the CKL-THV groups decreased compared to the model group. Meanwhile, the length of the rats changed insignificantly during the study period, as the rats were already mature. The decreasing rat weight while the length remained unchanged led to a reduced Lee index. A deeper evaluation of the changes in the Lee obesity index of the rats across the study groups over time (Figure 5) revealed that the Lee index in the model group increased by 8.28% over the trial. In contrast, the orlistat groups showed a decrease of 2.43%, while the CKL-THV groups exhibited a decrease in the Lee index ranging from 1.37% to 1.66%. In the model group, the increase in the Lee index over time reflects the progression of obesity. Rats exposed to the HFD were more susceptible to increased fat absorption, fat accumulation, waist circumference, and BW. The Lee indexes of the rats taking CKL-THV decreased by 1.37% to 1.66% over the 8-week trial.

Despite the reduction in fat weight in the CKL-THV groups compared to the model group (Figure 7), no statistically significant difference in body fat index was observed between the CKL-THV groups and the model group. CKL-THV did not reduce the body fat index of the rats as effectively as orlistat. The consumption of high-energy food leads to weight gain and abdominal fat accumulation.[25] The HFD provides excess calories primarily from saturated fatty acids, promoting fat accumulation in the body, particularly in visceral fat tissues. These tissues play a crucial role in fat storage and are associated with metabolic disorders. When consuming an HFD, adipocytes increase in size and number, thereby increasing the mass of these fat tissues. The CKL-THV groups showed a reduction in body fat weight (p < 0.05) due to the high polyphenol content in the product. Polyphenols have been shown to enhance energy expenditure, inhibit pre-adipocytes' maturation into adipocytes, and increase adiponectin expression, a hormone secreted by adipose tissue that regulates glucose levels and fatty acid breakdown.[34] However, this reduction in fat weight by CKL-THV was not sufficient to significantly alter the body fat index, as observed with orlistat.

The results in Table 2 indicate that, in the model group, the absolute weights of the heart, liver, kidneys, and spleen increased compared to the control group (p < 0.001). The HFD causes an increase in BW and fat accumulation. Consequently, the metabolic activities and functions of organ systems also change. The weights of the organs in the orlistat and CKL-THV groups decreased compared to the model group (p < 0.001). The reduction in organ weight corresponds to the decrease in BW of rats treated with either orlistat or CKL-THV. Compared with the orlistat group, the effect of CKL-THV at a dose of 0.35 g/kg/day in reducing the heart, liver, kidneys, and spleen weights was statistically significant. However, at a higher dose of 1.05 g/kg/day, the

liver, kidneys, and spleen weights decreased to levels comparable to the orlistat group, with a notable difference only in the reduction of heart weight. Thus, CKL-THV effectively reduces the weights of the heart, liver, kidneys, and spleen in obese rats. CKL-THV at a dose of 1.05 g/kg/day demonstrated effects comparable to orlistat in reducing the weights of the liver, kidneys, and spleen.

The relative weight of organs reflects the degree of change in organ weights compared to the overall change in BW. When assessing the relative weights of organs in the model group compared to the control group, statistically significant differences were only observed for the heart and liver. The weights of the kidneys and spleen increased compared to the control group, but did not yield statistically significant differences. However, compared to the model group, differences in the relative organ weights were not observed in the CKL-THV group at a dose of 0.35 g/kg/day. The higher dose of CKL-THV (1.05 g/kg/day) resulted in a significant reduction in the relative weight of the liver compared to the model group (p < 0.05), while the weights of other organs remained unchanged. Thus, CKL-THV does not significantly affect the relative weights of the heart, liver, kidneys, and spleen in obese rats.

An HFD significantly alters the blood lipid profiles of rats. The results shown in Figures 9, 10, and 11 indicate that, in the model group, HFD increases TC, TG, LDL-C, and non-HDL-C, while reducing HDL-C statistically significantly compared to the control group (p < 0.001). The reasons for these changes can be explained as follows:

Increase in TC: When consuming an HFD, the liver increases cholesterol synthesis to produce lipoproteins such as LDL and *very low-density lipoprotein* (VLDL) that transport cholesterol and TG, leading to elevated TC levels. Additionally, the HFD reduces cholesterol secretion from the body, contributing to high blood cholesterol levels.

Increase in TG: The HFD elevates TG levels in the blood through multiple mechanisms. Fatty acids from food are absorbed and metabolized in the liver into TG, which are then encapsulated in VLDL and released into the bloodstream. The HFD stimulates the liver to produce more VLDL while reducing the activity of lipoprotein lipase, an enzyme that breaks down lipids in the blood, leading to TG accumulation. Furthermore, the HFD induces insulin resistance, increasing TG production in the liver. Consequently, elevated TG levels can lead to metabolic disorders. Additionally, the impact of

obesity on lipid metabolism depends on the distribution of fat tissue, with increased visceral and upper body subcutaneous fat correlating with elevated TG levels.[35]

Increase in LDL-C and non-HDL-C: The HFD stimulates the liver to produce more LDL-C and VLDL to transport cholesterol and TG, resulting in increased levels of these substances. Additionally, the HFD reduces excretion of LDL-C, leading to higher levels in the blood. Non-HDL-C, which includes LDL, VLDL, and intermediate-density lipoproteins (IDL), is the sum of all lipoproteins, excluding HDL. Therefore, the concentration of non-HDL-C also rises in the blood.

Decrease in HDL-C: The HFD negatively impacts levels of HDL-C, considered to be the "good" cholesterol in the blood. An excessive intake of fats, particularly saturated fats, can lead to insulin resistance, increasing the body's resistance to insulin. This condition diminishes the liver's ability to regulate and synthesize lipoproteins, including HDL, which is responsible for transporting excess cholesterol from tissues back to the liver. Furthermore, the HFD reduces the activity of the enzyme lecithin–cholesterol acyltransferase (LCAT), which plays a crucial role in forming HDL from other lipoproteins. When LCAT levels decline, the synthesis of HDL is adversely affected, resulting in lower HDL-C levels in the blood. Additionally, the increase in visceral fat due to the HFD can reduce the metabolism and excretion of cholesterol via HDL, further contributing to lower HDL levels in the liver. These changes reflect lipid metabolism disorders and insulin resistance caused by the HFD, potentially leading to an increased risk of cardiovascular diseases and other metabolic disorders.

The study results indicate that orlistat significantly reduced TC, TG, LDL-C, and non-HDL-C levels while increasing HDL-C compared to the model group ($p < 0.001$). Thus, orlistat has a positive effect on improving blood lipid profiles in obese rats. Orlistat is a pancreatic lipase inhibitor that prevents the breakdown of fats in the intestine, thereby reducing lipid absorption from food. When fats are not fully digested and absorbed, levels of blood lipids such as TC, TG, LDL-C, and non-HDL-C decrease. This helps reduce fat accumulation in the body and lowers the risk of cardiovascular diseases. Additionally, orlistat increases levels of HDL-C, which plays a role in transporting excess cholesterol from tissues and blood vessels to the liver for excretion, thereby reducing the risk of atherosclerosis. The increase in HDL-C and reduction in TC, TG, LDL-C, and non-HDL-C indicate that orlistat is very effective in improving blood lipid profiles and reducing cardiovascular risk factors, providing positive outcomes for obesity treatment.

The groups taking CKL-THV also showed statistically significant reductions in TC, TG, LDL-C, and non-HDL-C, along with increased HDL-C compared to the model group. CKL-THV is a preparation rich in polyphenols, which are plant compounds with strong antioxidant properties that have anti-inflammatory effects and reduce oxidative stress, thereby improving cardiovascular function. Polyphenols have been shown to activate AMP-activated protein kinase (AMPK), leading to reductions in cholesterol, fatty acid synthesis, and TG formation.[34] The research results further demonstrate statistically significant differences in TC, TG, LDL-C, and non-HDL-C levels between the CKL-THV and orlistat groups, while HDL-C levels were equivalent between these groups. When comparing the two CKL-THV doses of 0.35 and 1.05 g/kg/day, differences were observed in TC, TG, LDL-C, and non-HDL-C levels. Thus, the effects of CKL-THV in reducing TC, TG, LDL-C, and non-HDL-C in obese rats are dose-dependent and weaker than those of orlistat, while the effect of increasing HDL-C is comparable. However, this study has limitations; food consumption and retention time in the rat's stomach have not been evaluated.

Conclusion

In a rat model of obesity induced by a high-fat diet, CKL-THV at doses of 0.35 and 1.05 g/kg/day exhibited obesity-preventing effects by reducing BW, absolute body fat weight, Lee obesity index, absolute weights of the heart, liver, kidneys, and spleen, and blood lipid levels of TC, TG, LDL-C, and non-HDL-C, while increasing HDL-C. In contrast, neither dose of CKL-THV reduced waist circumference or the body fat index. The dose of 1.05 g/kg/day effected better improvement in obesity indices than 0.35 g/kg/day during the 8-week trial. These results suggest that *C. hakodae* dried leaf extract can be used to develop a potential medicinal source for preventing and treating obesity.

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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