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The Effects of Low Carbohydrate Diet and Salak Pondoh (*Salacca edulis Reinw*) Peel Extract on Body Weight, Obesity Index, Visceral Fat Mass and Cholesterol Levels in Male Obese Rats and Dyslipidemia

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KEYWORDS

Salak Pondoh peel, Body Weight, Obesity Index, Cholesterol Levels, Visceral Fat Mass

ABSTRACT:

Introduction: Obesity is a condition characterized by excessive fat accumulation, and its prevalence continues to rise each year. This condition is associated with changes in cholesterol levels. Obese patients often fail to reduce their body weight (BW) through diet management only. Salak Pondoh peel extract (EPP) contains bioactive compounds with potential anti-obesity effects and the ability to improve hypercholesterolemia.

Objectives: This study aimed to evaluate the effects of Low Carbohydrate Diet (LCD) and EPP on body weight, obesity index, cholesterol levels, and visceral fat mass in male Wistar rats with obesity and dyslipidemia.

Methods: Thirty male Wistar rats were used in this study, which aged five weeks old and weighed 100-150 g. They were randomized and divided into six groups. Rats in negative control (NC), positive control (PC), and treatment groups (T1-3) were induced by feeding a high-fat high fructose (HFHFr) for 28 days, while normal (N) group received standard food throughout the study. During treatment periods, NC group received an LCD and distilled water without treatment, PC group received an LCD and orlistat, T1-3 were given an LCD combined with 150, 300 and 600 mg/kg BW/day. The data were analyzed using repeated measures Analysis of Variance (ANOVA), One-Way ANOVA, and Bonferroni post hoc tests.

Results: After 28 days of intervention, we observed that LCD+150 mg/kg BW/day of EPP significantly reduced obesity index ($p<0.001$) and has the lowest of visceral fat mass compared to other treatment groups, whereas LCD+ 300 mg/kg BW/ day of EPP significantly reduced body weight ($p=0.019$), and cholesterol levels ($p=0.043$).

Conclusions: The combination of a low-carbohydrate diet and Salak Pondoh peel extract demonstrates significant potential as a natural alternative therapy for obesity and dyslipidemia.

1. Introduction

Obesity is defined as an excessive accumulation of fat, which can negatively impact overall health (Trandafir et al., 2022). It is associated with an increased risk of several diseases, such as type 2 diabetes mellitus, hypertension, dyslipidemia, and various types of cancer, which can increase mortality rates (Abdelaal et al., 2017). The prevalence of obesity in Indonesia has risen significantly, as reported by Indonesian Basic Health Research, Riskesdas showing an increase from 19.1% in 2007 to 35.4% in 2018 (Kemenkes RI, 2018). By 2030, it is predicted that 1 in 5 women and 1 in 7 men will be obese, with a mortality rate predicted to reach 2.8 million people (Lobstein and Jewell, 2022).

Obesity is often linked to the consumption of high-calorie foods and drinks, coupled with a lack of physical activity, which slows down the body's energy expenditure (Arifani and Setyaningrum, 2021). Excessive energy intake leads to fat accumulation in adipose tissue. When fat accumulates in subcutaneous adipose tissue, skeletal muscle, and metabolic organs, it can cause an increase in body weight (BW). However, fat accumulation in visceral adipose tissue is particularly problematic, as it reduces adiponectin levels and disrupts triglyceride oxidation. This can result in adipose tissue dysfunction, further increasing BW and promoting long-term obesity (Fonseca et al., 2018). Obesity is also closely related to dyslipidemia, with increased body mass index (BMI) found in 60-70% of patients with lipid metabolism disorders (Khutami et al., 2022; Trandafir et al., 2022).

Long-term use of synthetic weight loss drugs can cause various side effects, such as nausea, vomiting, diarrhea, gastrointestinal disturbances, and impaired absorption of fat-soluble vitamins like vitamins A, D, E, and K (Marso et al., 2016; Tak and Lee, 2021). In recent years, LCD has gained popularity, contrasting with the low-fat dietary guidelines in place in America since the 1970s. LCD has been shown to significantly reduce body mass and BMI (Chawla, 2020). However, there is still debate among experts about the effectiveness of these diets, as dietary management and physical activity alone often fail (Ruban et al., 2019).

Salak Pondoh, a fruit produced domestically with an annual output of 1.4 million tons, is widely available. However, the potential benefits of its peel are underutilized (Kementan, 2020). Salak peel contains several active compounds such as alkaloids, polyphenolics, flavonoids, quinones, tannins, triterpenoids, and steroids, which offer numerous health benefits, including the ability to reduce cholesterol and blood sugar levels in rats.

2. Objectives

As there are no studies examining the effect of an LCD combined with EPP on body health, this study aimed to evaluate the effectiveness of this combination in reducing body weight, obesity index, cholesterol levels and visceral fat mass in obese male rats and dyslipidemia.

3. Methods

The equipment used in this study for the animal subjects included 12 cages, each equipped with a feeding and drinking area, measuring cylinders, a rat's gauge feeding tube, and handscoon. The tools used for extract preparation included black cloth, a grinder, a cabinet dryer, a rotary evaporator (IKA RV 10), a blower oven (Binder FD 56), and a digital scale. To measure body weight and obesity index, a digital scale (Taffware) and a measuring tape (Butterfly) were used. For blood sampling and dissection, microhematocrit tubes, vacutainer tubes, syringes, scalpels, and a dissection board were employed.

Materials

Fresh Salak Pondoh (*Salacca edulis Reinw*) fruit peel was sourced from Wonokerto Village, Sleman, and Yogyakarta. The materials for grinding and extraction, including 70% ethanol and sterile water, were provided by the Phytochemistry Laboratory of Setia Budi University (USB) Surakarta. Standard feed, HFHFr feed, and LCD feed were obtained from the Integrated Laboratory Technical Implementation Unit of Universitas Sebelas Maret.

Preparation of Salak Pondoh Peel Extract

Fresh Salak Pondoh peel were dried under indirect sunlight (closed by black cloth) for 7 days. The dried fruit peel was grounded and extracted by soaking 1 kg of fine peel powder in 6 liters of 70% ethanol for 5 days, with intermittent shaking. The solution was then filtered to obtain the filtrate. The remaining residue was re-dissolved in 4 liters of the same solvent for 2 days, and then the solution was filtered. The filtrates from 2x filtrations were concentrated using a rotary evaporator at 70 rpm and 50-56°C of temperature. Afterward, the filtrate was dried using an oven at 40°C to form a thick extract. The final product was stored in a glass bottle in the refrigerator until ready for use.

Analysis of Bioactive Compounds Using GC-MS

The bioactive compounds in the Salak Pondoh peel extract (EPP) were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The GC-MS instrument was operated according to the standard procedures of the Research and Testing Laboratory at Gadjah Mada University, Yogyakarta. The analysis was conducted using the Thermo Fisher Scientific Trace 1310 Gas Chromatograph and the Thermo Scientific ISQ LT Single Quadrupole Mass Spectrometer. The samples were extracted with ethanol as the solvent and centrifuged at 9,500 rpm for 3 minutes. The injector temperature was set to a maximum of 325/350°C. The results were identified using the GC-MS library provided by the Research and Testing Laboratory of Gadjah Mada University, Yogyakarta.

Research Design

This study was a laboratory experimental study with a pre-posttest design for measuring body weight, obesity index, and cholesterol levels, and a post-test only design for measuring visceral fat mass. The subjects were male Wistar rats, 5 weeks old, with a body weight approximately 100-150 g, and in good health. Each group consisted of 4 rats, with an additional 10% added to the minimum sample size to account for potential dropout, resulting in 5 rats per group (Ilyas et al., 2017).

Obesity Rat Modelling

This study was approved by The Research Ethics Committee of Faculty of Medicine, Universitas Sebelas Maret, with ethical approval number 39/UN27.06.11/KEP/EC/2024. The rats were maintained at the Integrated Laboratory Technical Implementation Unit of Universitas Sebelas Maret using polypropylene cages, each cage contained 2-3 rats. The cages were placed in a room with a temperature of 22-27°C, 40-70% of humidity, and 12 h dark and light cycles Environmental conditions remained consistent throughout the adaptation, modelling, until treatment periods, and the cages were cleaned every day (Devina et al., 2023).

The rats were first adapted for five days by being fed standard BR-2 feed and allowed to drink ad libitum. After the adaptation period, the rats were randomly assigned into six groups: the normal group (N), negative control (NC), positive control (PC), treatment 1 (T1), treatment 2 (T2), and treatment 3 (T3). Rats in NC, PC, T1, T2, and T3 was induced by feeding the rats with a HFHFr diet for 28 days, following the method by Sundari (2022). The HFHFr diet consisted of 56.64% fat and 10% fructose. The normal groups were only fed standard feed throughout the study. The food was provided twice daily, in the morning and evening.

Preparation of a Low-Carbohydrate Diet

The low-carbohydrate feed formulation was based on 50 grams of chicken egg yolk, 30 grams of ganyong starch flour, 10 grams of pollard, and 10 grams of catfish, following the research by Sanjaya (2023), who made a pellet formulation based on ganyong starch flour, catfish, and red bean flour. In this study, the formulation was modified by replacing some of ingredients with chicken egg yolk and pollard to adjust the carbohydrate and calorie content. The formula calculation was done using Microsoft Excel.

The preparation of the LCD began with processing the catfish fillets. The catfish meat, chicken egg yolk, ganyong starch flour, and pollard were thoroughly mixed to ensure even distribution. The mixture was then placed in a baking tray and dried using an oven to prevent spoilage.

The Intervention in Male Rats with Obesity

After the rats became obese, rats in the NC group were fed a low-carbohydrate diet (LCD) and distilled

water. The PC group received LCD and 12.3 mg/kg BW orlistat (Novel Pharmaceutical Laboratories). The T1 – T3 were given LCD and EPP at a dose of 150, 300, and 600 mg/kg BW/day.

Measurements of Body Weight, Obesity Index, Cholesterol Levels, and Visceral Fat Mass

Body weight (g) was measured using a Taffware digital scale. Naso-anal length was determined by measuring the distance from the tip of the nose to the anus. After obtaining these measurements, the obesity index was calculated using the Rohrer index formula (g/cm³) as follows: [(Body weight (g) / naso-anal length (cm)³) × 10³]. Rats were considered obese if their Rohrer index was ≥ 30 g/cm³ (Putro et al., 2022).

To measure total cholesterol levels, 2 mL of blood was drawn from the retroorbital vein and analyzed using the enzymatic photometric assay (cholesterol oxidase-peroxidase amino antipyrine or CHOP-PAP method) with a Microlab 300 photometer. At the end of the intervention, the rats were sacrificed to obtain visceral fat mass. This was measured by collecting fat from the epididymal and retroperitoneal areas and weighing it on a digital scale.

Statistical Analysis

Numerical data were presented as mean and standard deviation (SD) using SPSS for Windows version 25.0 software. The Shapiro Wilk test and the Levene test were used to test the data normality and the homogenity of variants, respectively. A paired t-test were used to determine the difference between before and after obesity induction. The difference in mean feect among the six groups was repeated sequentially, so a repeated measure ANOVA test was performed, followed by Bonferroni post hoc test. If the parametric test did not fulfill the requirements, the Friedman test was conducted, follow by the Wilcoxonn test. The p-value <0.05 was considered as statistically significant.

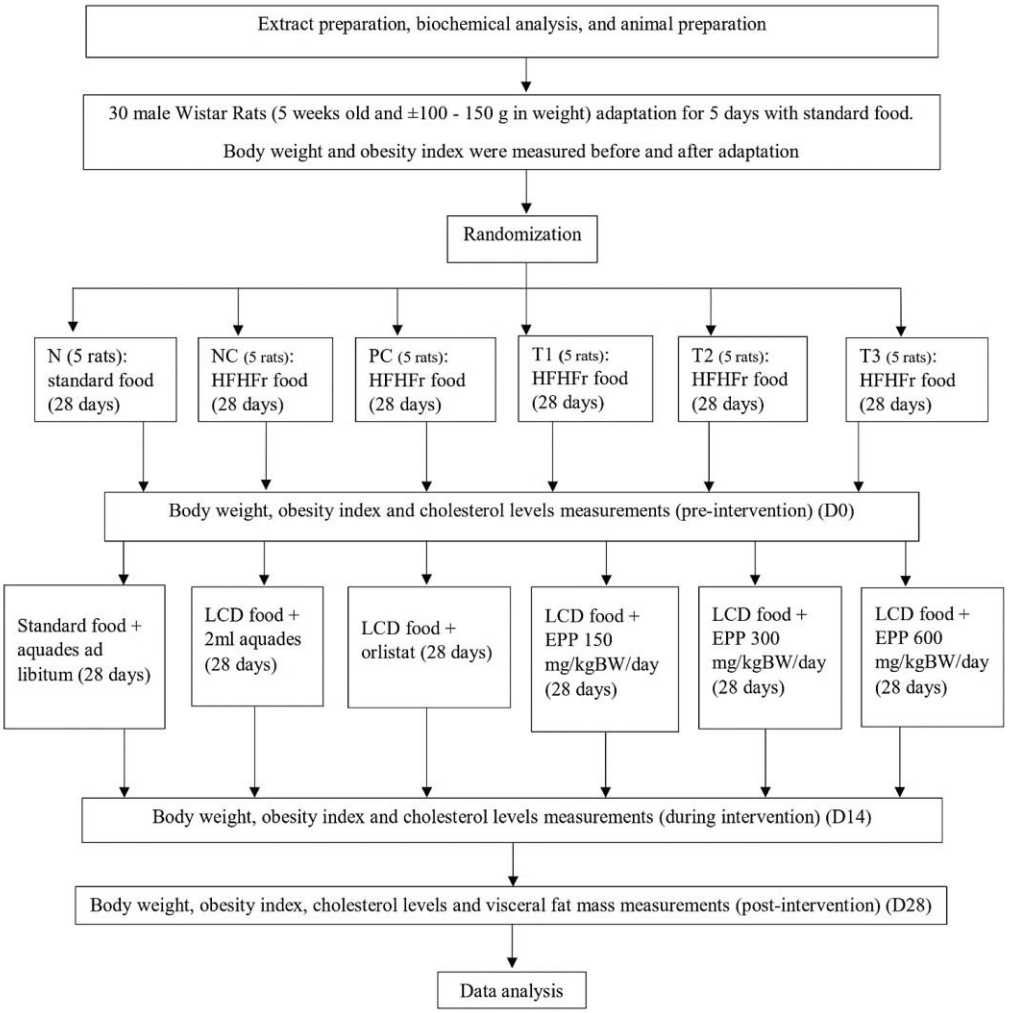

Figure 1. A Schematic Diagram Illustrating the Study Design and Timeline

N: normal group; NC: negative control (obese rats); PC: positive control (obese rats treated with 12.3 mg/kgBW/day orlistat); T1: treatment group 1 (obese rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obese rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obese rats treated with LCD + 600 mg/kgBW/day EPP); D0: day 0 (pre-intervention); D14: day 14 (during intervention); D28: day 28 (post-intervention).

4. Results

Bioactive Compounds in EPP

Bioactive compounds in EPP, including n-hexadecanoic acid and its derivatives, erythritol, and stevioside, were detected through GC-MS analysis. These compounds may potentially help in improving obesity and dyslipidemia. Glycerin was also detected in EPP.

Effect of HFHFr Administration on Body Weight and Obesity Index in Rats

There were significant (p=0.001) BW changes and significant (p<0.001) obesity index changes among all groups after 28 days administration of HFHFr diet (Table 1).

Effect of LCD and EPP Administration on Body Weight

T2 group showed a significant reduction in BW (22.2 ± 20.89 g) after 28 days of intervention. This BW reduction was higher than the BW reduction in NC group (0.4±14.65 g), but was not higher than the BW reduction in PC group (29.2±6.87 g) (Table 2).

BW among the rat groups was significantly different before the intervention (p=0.001) (Table 2). The BW in the PC group was significantly lower than the NC group (p=0.005) (b), while the BW in the T3 group was significantly higher than the PC group (p=0.001) (c). After 28 days of intervention BW in the NC, PC, T1, T2, and T3 was decrease. The BW in the T2 group significantly decreased (p<0.05) and was significantly lower than the N group (a) (Figure 2).

Effect of LCD and EPP Administration on Obesity Index

T1, T2, and T3 groups showed a significant reduction in obesity index (p<0.05) after 28 days of intervention. The obesity index reduction in T1 group was higher than the obesity index reduction in N and NC groups (Table 3).

The NC, PC, T1, T2, and T3 groups all showed a significant decrease in the obesity index after 28 days of intervention. The PC group experienced the greatest reduction, followed by the T1, T2, T3, and NC groups (Table 3). The obesity index in the T1 and T2 groups were significantly lower than the N and NC groups (a,b) (Figure 3).

Effect of LCD and EPP Administration on Cholesterol Levels

T2 group showed a significant reduction in cholesterol levels (37.26±33.94 g) after 28 days of intervention. This cholesterol levels reduction was higher than the cholesterol levels in the PC group (Table 4).

The T1 and T2 groups showed a decrease in mean cholesterol levels after 28 days of intervention. The reduction in these groups was more significant compared to the NC and PC groups. However, the mean total cholesterol level in the T3 group increased higher than the N group. There was a significant difference in cholesterol levels among the groups after 28 days of intervention (p=0.031) (Figure 3).

Table 1. Mean Body Weight and Obesity Index of Rats before and After Obesity Induction

Obesity Parameters	Group	Before Induction	After Induction	Δ	p[a]
BW (g)	N	160.4 ± 5.12	252.0 ± 15.79	91.6 ± 16.13	<0.001*
	NC	154.2 ± 7.36	267.6 ± 17.09	113.4 ± 12.66	<0.001*
	PC	157.2 ± 7.04	223.8 ± 18.30	66.6 ± 15.72	0.001*
	T1	148.6 ± 5.94	253.4 ± 16.13	104.8 ± 13.75	<0.001*
	T2	151.6 ± 7.36	254.8 ± 17.10	103.2 ± 14.39	<0.001*

	T3	156.4 ± 7.40	275.8 ± 13.82	119.4 ± 14.79	<0.001*
	p^b	0.125	0.001*	<0.001*	
Index Obesity (g/cm^3)	N	25.89 ± 1.03	28.00 ± 1.24	2.10 ± 1.02	0.010*
	NC	26.44 ± 1.26	30.59 ± 0.53	4.15 ± 1.59	0.004*
	PC	26.95 ± 1.20	30.62 ± 0.53	3.67 ± 1.59	0.007*
	T1	25.92 ± 1.80	30.66 ± 0.68	4.73 ± 1.97	0.006*
	T2	26.89 ± 1.08	30.88 ± 0.65	3.99 ± 1.54	0.004*
	T3	26.57 ± 1.72	30.63 ± 0.63	4.06 ± 1.73	0.006*
	p^b	0.741	<0.001*	0.211	

Source: Primary data (2024). p^a) simple paired t test; p^b) One-Way ANOVA; *)p<0.05; Δ: difference before and after induction; N: normal group; NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1: treatment group 1(obesse rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obesse rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obesse rats treated with LCD + 600 mg/kgBW/day EPP).

Table 2. Mean BW of Rats Before and After Intervention

Group	BW (g) (Mean ± SD)		ΔBW	p^a
	Before Intervention	After Intervention		
N	252.0 ± 15.79	296.8 ± 25.05	44.8 ± 14.65	<0.001*
NC	267.6 ± 17.09	267.2 ± 53.12	-0.4 ± 20.48	0.347
PC	223.8 ± 18.3	194.6 ± 18.4	-29.2 ± 6.87	0.001*
T1	253.4 ± 16.13	237.7 ± 33.24	-15.7 ± 20.3	0.062
T2	254.8 ± 171	232.6 ± 24.43	-22.2 ± 20.89	0.019*
T3	275.8 ± 16.36	253.2 ± 27.54	-22.6 ± 21.51	0.204
p^b	0.001*	<0.001*	<0.001*	

Source: Primary data (2024). p^a) simple paired t test; p^b) One-Way ANOVA; *) p<0.05. ΔBW: difference BW before and after intervention; N: normal group; NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1: treatment group 1 (obesse rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obesse rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obesse rats treated with LCD + 600 mg/kgBW/day EPP).

Table 3. Mean Obesity Index of Rats Before and After Intervention

Group	Obesity Index (g/cm^3) (Mean ± SD)		ΔObesity Index	p^a
	Before Intervention	After Intervention		
N	28.0 ± 1.24	27.04 ± 1.52	-0.9 ± 1.83	0.143
NC	30.59 ± 0.53	26.72 ± 3.31	-4.1± 2.92	0.034*
PC	30.62 ± 18.3	19.81 ± 0.94	-10.81 ± 0.64	<0.001*
T1	30.66 ± 0.68	21.14 ± 1.95	-9.52 ± 1.50	<0.001*
T2	30.88 ± 0.65	21.84 ± 2.29	-9.04 ± 2.54	<0.001*
T3	30. 23 ± 0.71	25.75 ± 1.00	-4.88 ± 1.28	0.002*
p^b	<0.001*	<0,001*	<0,001*	

Source: Primary data (2024). p^a) simple paired t test; p^b) One-Way ANOVA; *)p<0.05; ΔObesity index: difference obesity index before and after intervention; N: normal group; NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat) ; T1: treatment group 1(obesse rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obesse rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obesse rats treated with LCD + 600 mg/kgBW/day EPP).

Table 4. Mean Cholesterol Levels of Rats Before and After Intervention

Group	Cholesterol Levels (mg/dL) (Mean±SD)		Δ Cholesterol Levels	p[a]
	Before Intervention	After Intervention		
N	78.04 ± 18.48	80.04 ± 7.33	2.0 ± 13.29	0.958
NC	92.22 ± 13.61	79.74± 14.74	-12.48±15.60	0.202
PC	123.68 ± 16.43	100.38±23.36	-22.3 ± 29.29	0.026*
T1	128.88 ± 16.09	100.98 ±3.66	-25.9 ±14.69	0.002*
T2	129.18 ± 29.15	91.92 ±11.45	-37.26±33.94	0.043*
T3	92.00 ± 12.36	99.08 ± 12.11	7.08± 17.90	0.577
p[b]	<0.001*	0.031*	0.033	

Source: Primary data (2024). p[a]) simple paired t test; p[b]) One-Way ANOVA; *)p<0.05; ΔCholesterol levels: difference cholesterol levels before and after intervention; N: normal group; NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat) ; T1: treatment group 1(obesse rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obesse rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obesse rats treated with LCD + 600 mg/kgBW/day EPP).

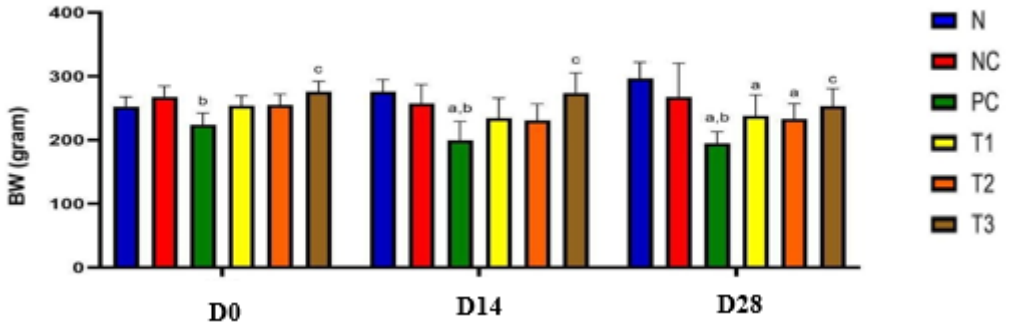

Figure 2. Mean Body Weight on Day D0, D14, and D28 of the Intervention

Source: Primary data (2024). N: normal group; NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1: treatment group 1 (obese rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obese rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obese rats treated with LCD + 600 mg/kgBW/day EPP); D0: day 0 (pre-intervention); D14: day 14 (during intervention); D28: day 28 (post-intervention). "a" indicates a significant mean difference compared to the N group. "b" indicates a significant mean difference compared to the NC group. "c" indicates a significant mean difference compared to the PC group (Bonferroni Post Hoc Test).

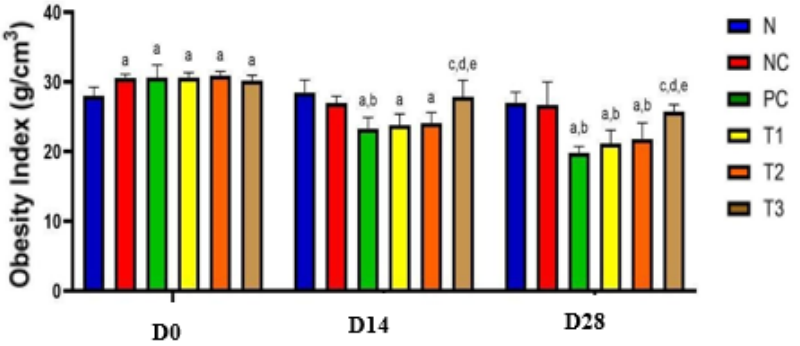

Figure 3. Mean Obesity Index on D0, D14, and D28 of the Intervention

Source: Primary data (2024). N: normal group; NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1: treatment group 1 (obese rats treated with LCD + 150

mg/kgBW/day EPP); T2: treatment group 2 (obese rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obese rats treated with LCD + 600 mg/kgBW/day EPP); D0: day 0 (pre-intervention); D14: day 14 (during intervention); D28: day 28 (post-intervention). "a" indicates a significant mean difference compared to the N group. "b" indicates a significant mean difference compared to the NC group. "c" indicates a significant mean difference compared to the PC group. "d" indicates a significant mean difference compared to the T1 group. "e" indicates a significant mean difference compared to the T2 group (Bonferroni Post Hoc Test).

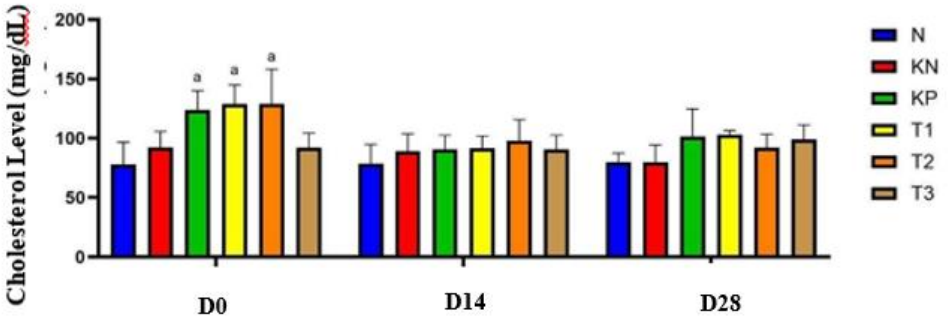

Figure 4. Mean Cholesterol Levels on D0, D14, and D28 of the Intervention

Source: Primary data (2024). N: normal group NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1: treatment group 1 (obese rats treated with LCD + 150 mg/kgBW/day EPP); T2: treatment group 2 (obese rats treated with LCD + 300 mg/kgBW/day EPP); T3: treatment group 3 (obese rats treated with LCD + 600 mg/kgBW/day EPP); D0: day 0 (pre-intervention); D14: day 14 (during intervention); D28: day 28 (post-intervention). ΔTotal cholesterol: the difference between pre- and post-intervention levels. "a" indicates a significant mean difference compared to the N group.

Effect of LCD and EPP Administration on Visceral Fat Mass

The mean visceral fat mass in the N and NC groups was higher compared to the PC, T1, T2, and T3 groups. A significant difference was observed between the groups, with a p-value <0.001. The PC group (1.14 ± 0.26) had the lowest visceral fat mass, followed by T1 (1.69 ± 0.51), T2 (2.01 ± 0.55), and T3 (2.56 ± 0.90) (Table 5). Significant differences were found between the NC and PC group ($p < 0.001$) as well as between the NC and T1 group ($p = 0.004$), as determined by the Bonferroni post hoc test (Figure 5).

Table 5. Mean Visceral Fat Mass of Rats at the End of the Intervention

Group	Visceral fat mass (g) (Mean ± SD)	*p*
	After 28 days of the intervention	
N	3.13 ± 0.86	<0,001*
NC	3.99 ± 1.44	
PC	1.14 ± 0.26	
T1	1.69 ± 0.51	
T2	2.01 ± 0.55	
T3	0.90	

Source: Primary data (2024). N (normal group), NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1 (obese rats treated with a low-calorie diet (LCD) and 150 mg/kg BW/day of EPP), T2 (obese rats treated with an LCD and 300 mg/kg BW/day of EPP), and T3 (obese rats treated with an LCD and 600 mg/kg BW/day of EPP); *) indicate a statistically significant difference ($p < 0.05$).

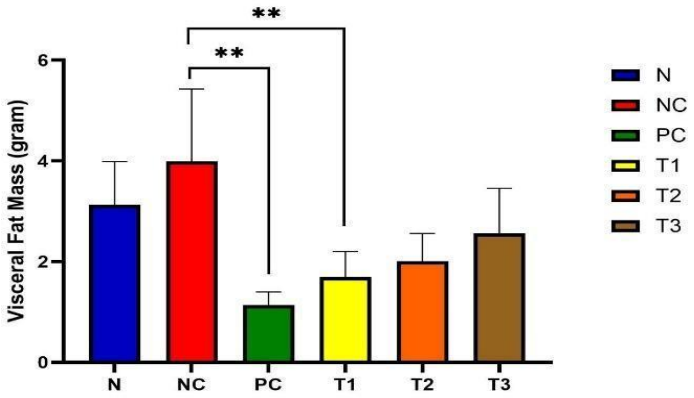

Figure 5. Mean Visceral Fat Mass at the End of the Intervention

Source: Primary data (2024). N (normal group), NC: negative control (obesse rats treated with LCD); PC: positive control (obesse rats treated with LCD + 12.3 mg/kgBW/day orlistat); T1 (obese rats treated with a low-calorie diet (LCD) and 150 mg/kg BW/day of EPP), T2 (obese rats treated with an LCD and 300 mg/kg BW/day of EPP), and T3 (obese rats treated with an LCD and 600 mg/kg BW/day of EPP). (**) shows indicate significant differences between the PC and NC groups, as well as between the T1 and NC groups, with $p \leq 0.01$.

5. Discussion

Table 1 shows that all groups of rats induced with obesity experienced an increase in body weight (BW) and Obesity Index ($p < 0.05$). The highest BW increase was observed in the T3 group, with a gain of 119.4 ± 14.79 g, while the lowest was in the PC group, with an increase of 66.6 ± 15.72 g. A significant difference in BW increase was noted across the groups after 28 days of induction ($p = 0.001$).

The highest increase in the Obesity Index occurred in the T1 group, at 4.73 ± 1.97 g/cm³, whereas the lowest increase was in the N group, at 2.10 ± 1.02 g/cm³. A significant difference in the Obesity Index increase was also observed across the groups after 28 days of induction ($p < 0.001$). The obesity induction method referred to a previous study by Sundari et al. (2022), which demonstrated that obesity induction could increase the average BW of rats over 30 days. Administering a high-fat, high-fructose diet (HFHFr) led to high caloric intake and increased lipid peroxidation, resulting in elevated total cholesterol levels (Khanna, 2022; Yustisia et al., 2022).

The increase BW and Obesity Index in the PC group was the lowest among induced groups. It could be attributed to factors such as genetic variability, differences in basal metabolism, hormonal influences, gut microbiota conditions, and unmeasured environmental factors (Amanda et al., 2021; Corrigan et al., 2020).

After 28 days of administering an LCD combined with EPP at doses of 150, 300, and 600 mg/kg BW/day, the mean BW of the T1, T2, and T3 groups decreased. Similarly, the NC group, which received only an LCD, also showed a reduction in mean BW, although the reduction was less than the intervention groups.

The BW reduction in rats receiving an LCD only might be due to increased diuresis, glycogen depletion, and reduced calorie intake. Other studies have also noted that LCDs can trigger increased resting or postprandial energy expenditure and reduce levels of ghrelin and leptin. LCDs are particularly beneficial for obese individuals, as they tend to promote caloric intake from proteins, which provide sustained energy and help avoid rapid hunger (Ulusoy-Gezer and Rakıcıoğlu, 2024; Corrêa and Tabak, 2024).

The reduction in mean body weight (BW) observed in this study could be attributed to the bioactive compound n-hexadecanoic acid found in EPP. Molecular docking studies on *Bipolaris axonopicola* have shown that n-hexadecanoic acid binds with higher affinity to the human lipase enzyme compared to orlistat therapy A decrease in lipase enzyme activity accelerates weight loss by reducing fat absorption (Goyal et al., 2023).

This study showed that administering LCD + EPP for 28 days significantly reduced the obesity index in the T1, T2, and T3 groups ($p<0.05$). Among these, the T1 group (21.14 ± 1.95 g/cm³) was the closest mean

obesity index to the PC group, with a mean reduction -9.52±1.50 g/cm³. This was the most significant decrease compared to the T2 and T3 groups. The obesity index serves as a proxy measure of adiposity, representing the BW of the experimental animal relative to its height (Alqarni et al., 2023).

The reduction in BW and the obesity index observed in this study may not solely be due to n-hexadecanoic acid but also to the erythritol content in EPP. Erythritol is known to suppress appetite, reduce energy intake, and regulate GLP-1 levels. Previous studies have shown that administering erythritol for 16 weeks to rats on a high-fat diet reduced BW and white fat accumulation compared to control groups. Erythritol stimulates the secretion of gut hormones such as GLP-1, CCK, and PYY, which are involved in satiety regulation. Another study demonstrated that supplementing a high-fat diet with 5% erythritol resulted in less weight gain and higher energy expenditure compared to other groups (Mazi and Stanhope, 2023).

A decrease in total cholesterol levels was observed in the NC, PC, T1, and T2 groups. Among these, the T2 group had cholesterol levels closest to the NC group and within the normal range (46–92 mg/dL). A study by Nuranti et al. (2015) on dyslipidemic subjects induced by a high-fat diet showed that administering salak skin extract at doses of 210, 420, and 840 mg/kg BW, as well as standard simvastatin intervention (0.013 mg/20 g BW), significantly lowered cholesterol levels to 167.67 ± 9.07, 175.33 ± 14.84, 154.33 ± 10.41, and 136.67 ± 14.84 mg/dL, respectively. The phenolic compounds in salak skin extract act as antioxidants, reducing hepatic and plasma cholesterol levels by inhibiting HMG-CoA synthesis and enhancing the activity of cholesterol 7-α hydroxylase (CYP7). These antioxidants also mitigate oxidative stress, reducing lipid peroxidation and preventing hypercholesterolemia and high triglyceride Levels (Nuranti et al., 2015).

The presence of stevioside compounds in EPP may also contribute to the reduction in cholesterol levels. Stevioside has been shown to increase LDL receptor (LDLR) expression in human hepatocellular carcinoma (HepG2) cells by inhibiting HMG-CoA reductase (HMGCR), a mechanism similar to simvastatin (Ilias et al., 2021).

In this study, visceral fat mass was measured only once at the end of the intervention phase. Visceral fat, also known as intra-abdominal fat, is located within the abdominal cavity and surrounds organs such as the liver, kidneys, and intestines. It accounts for approximately 20% of total body fat. High visceral fat accumulation is commonly observed in individuals with dyslipidemia and is characterized by increased abdominal circumference, indicative of central obesity. Visceral fat mass is strongly correlated with BMI and is a key marker of cardiovascular disease risk (Ozato et al., 2019).

The reduction in visceral fat mass observed in this study could result from decreased fat absorption, weight loss, and enhanced fat oxidation through physical activity. Effective visceral fat reduction involves mobilizing stored fat, breaking it down, and increasing metabolism to prevent fat cell enlargement and further fat accumulation (El-Zayat et al., 2019).

This study found that the T1 and T2 groups, which received lower doses of EPP, demonstrated better outcomes in body weight, obesity index, lipid profile, and visceral fat mass compared to the T3 group, which received the highest dose of EPP. Glycerin was detected in the GC-MS analysis of EPP, which may explain why the T3 dose did not perform as well as the lower doses. In the T3 group, the concentration of certain compounds, including glycerin, was higher than in the other groups. Previous research has shown that glycerol supplementation can increase triacylglycerol (TAG) and total cholesterol levels (Andrade et al., 2014).

Further studies are needed to better understand the active compounds and their concentrations in EPP to optimize the dosing and enhance its effectiveness in managing obesity and dyslipidemia. Our limitation of this study is that there were significant differences in baseline measurements of body weight, obesity index, and cholesterol levels between the groups, which may have influenced the variability in the final results.

6. Conclusion

The combination of a low-carbohydrate diet (LCD) and EPP at a dose of 150 mg/kg BW/day effective to reduce obesity index and visceral fat mass, whereas the combination of LCD and EPP at a dose of 300 mg/kgBW/ day effective to reduce body weight and cholesterol levels in male white rats with obesity and dyslipidemia.

This study highlights the potential of LCD and EPP as alternative therapies for managing obesity and dyslipidemia. However, further research is needed to identify the active compounds in EPP and their optimal concentrations for effectively treating obesity and dyslipidemia.

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