Meditory
(The Journal of Medical Laboratory)
Volume 14 Issue 1 Page 139-155– June 2026
https://doi.org/10.33992/meditory.v13i1.4851

EFFECTS OF JONGI (*Dillenia indica*) LEAF EXTRACT IN RATS FED A HIGH-FAT DIET

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Article History
Received, October 8th, 2025
Revised, November 6th, 2025
Reviewed, May 18th, 2026
Posted, July 20th, 2026

Editor
Putu Nita Cahyawati

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Keywords
Adipocyte, *Dillenia indica*, high-fat diet, obesity, Wistar rats

Abstract

Background: Obesity is a complex metabolic disorder associated with various health risks, including cardiovascular disease, diabetes, and hepatic steatosis. Given the limitations of current pharmacological treatments, there is increasing interest in natural products with anti-obesity potential, such as jongi (*Dillenia indica*).
Objective: This study evaluated the effects of *Dillenia indica* leaf extract in rats fed a high-fat diet.
Methods: Thirty male Wistar rats were divided into five groups: standard diet, high-fat diet, and three others receiving high-fat diets (HFD) supplemented with either 500 mg/kg or 750 mg/kg of *Dillenia indica* extract, or 10 mg/kg of orlistat. HFD was administered to the corresponding groups for 28 days, followed by oral treatment for 14 days. Parameters measured included body weight, liver weight, lipid profiles, and adipocyte area.
Results: The group treated with 750 mg/kg of *Dillenia indica* showed numerically lower liver weight, total cholesterol, LDL, and triglyceride levels compared with the HFD group. However, these differences were not statistically significant. Both *Dillenia indica* treatment groups showed significantly lower mean adipocyte areas than the HFD group ($p < 0.05$), with the lowest mean area observed at 750 mg/kg BW.
Conclusions: *Dillenia indica* leaf extract at a dose of 750 mg/kg BW significantly reduced adipocyte area in HFD-fed rats. However, its effects on body weight, liver weight, and lipid profiles were not statistically significant. Further long-term studies with a broader range of doses are required.

Cite this Article
Bancin NCQ, Widagdo KAJ, Putrika MP, Maharani AC, Dzulfikar AA, Adianingsih OR. Effects of Jongi (Dillenia indica) Leaf Extract In Rats Fed A High-Fat Diet. Meditory J Med Lab. 2026;14(1):139–55.

INTRODUCTION

Obesity is an escalating worldwide health issue marked by unhealthy excess fat buildup that jeopardizes well-being. The World Health Organization reports that obesity rates have almost tripled globally since 1975, with over 650 million adults affected by 2016. Trends indicate a continued rise in both developed and developing countries. This condition is closely linked to various non-communicable diseases, such as type 2 diabetes, heart disease, certain cancers, and joint disorders, significantly impacting illness and death rates worldwide (1–4). Recognized as a chronic disease, obesity is characterized by persistent low-grade inflammation, particularly in white adipose tissue (5). A major complication arising from this metabolic dysfunction is non-alcoholic fatty liver disease (NAFLD), where excessive fat builds up in the liver. With a global prevalence of 20–30% that skyrockets to 70–90% among individuals with obesity and type 2 diabetes, NAFLD is a leading cause of chronic liver disease, firmly establishing obesity as its strongest risk factor (6).

While conventional anti-obesity medications are clinically effective, their use is often limited by the adverse effects. For instance, orlistat, a commonly prescribed weight-loss medication, inhibits both pancreatic and gastric lipases. Although this mechanism successfully reduces the breakdown and absorption of dietary fats, it frequently results in side effects directly related to this reduced absorption, such as reduced uptake of fat-soluble vitamins. Additionally, patients often report gastrointestinal disturbances, including abdominal pain, frequent bowel movements, bowel urgency, and flatulence (7). Furthermore, the increasingly popular glucagon-like peptide-1 receptor agonists (GLP-1 RAs) are associated with prominent gastrointestinal adverse effects, most notably nausea, vomiting, diarrhea, and constipation, which affect up to 60% of patients during the initial treatment phase. The capacity of GLP-1 RAs to decelerate gastric emptying also raises clinical concerns regarding potential intestinal obstruction and perioperative aspiration risk. Beyond gastrointestinal symptoms, GLP-1 RA therapy has been shown to considerably elevate the probability of gallbladder and biliary disorders (8). These tolerability issues and potential long-term complications prompt a continuous search for safer, natural alternatives with anti-obesity potential.

One such candidate is *Dillenia indica* (elephant apple), a plant used in traditional Southeast Asian medicine. The leaves and fruit contain bioactive compounds, such as flavonoids, phenolics, and terpenoids, which have been reported to exhibit antioxidant, antihyperglycemic, and lipid-lowering activities (3,4). Several flavonoids are known to improve insulin sensitivity, reduce obesity-related oxidative stress, improve the redox balance, and thereby ameliorate macronutrient metabolism (9). Recent research suggests that extracts from this plant may influence fat metabolism, block fat-digesting enzymes, and reduce fat cell formation in laboratories and animal studies, pointing to possible anti-obesity benefits (10,11).

Among the various plant parts, the leaves of *Dillenia indica* are of particular interest because they contain several polyphenolic compounds, including naringenin, catechin, epicatechin, syringic acid, vanillic acid, and kaempferol. A previous in vitro study using oleic acid-treated human hepatocellular carcinoma (HepG2) cells reported that a hydroethanolic leaf extract reduced intracellular lipid accumulation and modulated proteins involved in lipid metabolism. These changes included increased expression of sirtuin 1 (SIRT-1), phosphorylated liver kinase B1 (p-LKB-1), phosphorylated AMP-

activated protein kinase (p-AMPK), phosphorylated acetyl-CoA carboxylase (p-ACC), peroxisome proliferator-activated receptor alpha (PPAR-α), and carnitine palmitoyltransferase 1 (CPT-1), as well as decreased expression of sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase (FAS) (12). However, these findings were obtained in a cellular model of NAFLD and do not provide direct evidence of anti-obesity efficacy in vivo.

Although previous studies have suggested the anti-obesity potential of *Dillenia indica*, most have focused on fruit or bark extracts, whereas evidence regarding the leaf extract, particularly in high-fat diet (HFD)-induced obesity models, remains limited. To the best of our knowledge, no study has systematically evaluated the histomorphological changes in adipocyte area following administration of *Dillenia indica* leaf extract in an HFD-induced rat model of obesity, which constitutes the novelty of this research. Therefore, this study aimed to evaluate the effects of *Dillenia indica* leaf extract at two different doses (500 and 750 mg/kg BW) in HFD-fed Wistar rats, with a particular focus on adipocyte morphology, lipid profile, liver weight, and body weight changes. The findings were intended to provide preliminary evidence regarding the biological effects of the leaf extract in HFD-fed rats. Since only two doses were evaluated, the present study was not designed to establish a definitive dose–response relationship. Future studies should evaluate a broader dose range and further investigate the underlying mechanisms and safety profile of *Dillenia indica* leaf extract.

MATERIALS AND METHODS

Preparation of *Dillenia indica* Leaf Extracts

Fresh *Dillenia indica* leaves were obtained from a commercial herbal supplier in Bekasi, West Java, Indonesia. *Dillenia indica* L. was identified by Generasi Biologi Indonesia, Gresik, Indonesia (No. 08.114/Genbinesia/IX/2023). Fresh *Dillenia indica* leaves were washed and dried at 40°C for three days. The dried leaves were then ground into powder. Dried *D. indica* leaves (250 g) were soaked in 1.2 L 98% ethanol for 72 hours at room temperature with occasional stirring. The extract was filtered using Whatman No. 42 filter paper to separate the filtrate from the residue. The resulting filtrate was then concentrated using a rotary evaporator at 40°C and 100 rpm. The extraction yield was calculated as the percentage of dry extract weight obtained from the initial dry leaf powder weight. The yield of the *D. indica* extract was 7.2% (w/w) (13).

Animals

This research was conducted at the Animal Laboratory, Faculty of Medicine, Universitas Brawijaya, with the approval of the Health Ethics Committee of the Faculty of Medicine, Universitas Brawijaya (No. 193/EC/KEPK/08/2023). All animals were cared for in accordance with the principles and guidelines of animal care. The experimental animal subjects were white male Wistar rats, 8 weeks old, weighing 150-200 g, kept in separate cages. The treatment of rats began with 7 days of acclimation in a cage maintained at a constant temperature and a standardized 12/12 h light/dark cycle. During this period, the rats were observed for any health issues or behavioral changes and were provided with standard laboratory food and water *ad libitum* (14).

Animal Intervention

Thirty white male Wistar rats were randomly divided into five groups (n = 6 per group) using a lottery method. Each rat was assigned a unique number (1–30), and the numbers were written on identical pieces of paper. The papers were folded, placed in a container, and drawn randomly by an independent researcher to assign each rat to one of five treatment groups. This procedure ensured unbiased group allocation and minimized selection bias (15). The sample size was determined using the Federer formula: (t-1) (n-1) ≥ 15. The groups consisted of a standard diet (normal control), a high-fat diet (HFD), a high-fat diet with 500 mg/kg BW dose of *D. indica* extract (HFD + DI 500), a high-fat diet with 750 mg/kg BW dose of *D. indica* extract (HFD + DI 750), and a high-fat diet with orlistat standard (HFD + Orlistat). To establish the obesity model, rats were fed an HFD for a 28-day induction period prior to treatment, as previously reported to induce weight gain and dyslipidemia in Wistar rats (16). To assess the obesity model, body weight was measured at baseline and after the 28-day HFD induction period. Subsequently, *Dillenia indica* leaf extract at doses of 500 mg/kg BW and 750 mg/kg BW was administered orally using gastric gavage for 14 days (days 29-42). For comparison, one group received orlistat at a dose of 10 mg/kg BW. Orlistat was obtained from commercially available 120 mg capsules (Novel Pharmaceutical Laboratories, Indonesia). Orlistat is a well-established gastric and pancreatic lipase inhibitor widely used in high-fat diet-induced obesity models (17,18). Body weight was measured every week. The details of the treatment administered to each experimental group are presented in **Table 1**.

Table 1. Groups of animal intervention

Group	Treatment
Normal	Standard diet (normal control group)
HFD	High-fat diet only
HFD + DI 500	High-fat diet + 500 mg/kg body weight (BW) *Dillenia indica* extract
HFD + DI 750	High-fat diet + 750 mg/kg BW *Dillenia indica* extract
HFD + Orlistat	High-fat diet + 10 mg/kg BW orlistat (positive control)

High-Fat Diet Preparation

The high-fat diet formulation consisted of quail egg yolk, wheat flour, and standard laboratory feed. The components were administered in proportions of 2 g egg yolk, 4 g wheat flour, and 1 g standard feed per 200 g of body weight per day. These ingredients were mixed with water to form a homogeneous semi-solid mass, which was then shaped into uniform pellets for daily administration (14).

Animal Euthanasia and Tissue Collection

On day 42, all animals were subjected to chloroform inhalation for anesthesia. Once the animals were unresponsive, cervical dislocation was immediately performed as the definitive physical method of euthanasia (19). Subsequently, a midline abdominal incision was made to access and collect liver tissue and blood samples (20). Liver samples were weighed, and blood samples were centrifuged for biochemical analyses.

Histological Analysis of Adipocyte Area

Adipose tissue samples were rinsed with 0.9% physiological saline and fixed in 10% neutral-buffered formalin for 24 hours. Tissue dehydration was carried out using a graded ethanol series, followed by clearing with xylene for 30 minutes. Samples were embedded in paraffin blocks and sectioned at 3–5 μm thickness using a rotary microtome. Sections were mounted on slides and stained with hematoxylin and eosin (H&E) following standard protocols (20). Histological evaluation was conducted under a light microscope at 100× magnification. For each animal, five random microscopic fields were captured from different regions of the adipose tissue section. Adipocyte cross-sectional area was measured using ImageJ software (version 1.53, National Institutes of Health, USA). Before measurement, the ImageJ scale was calibrated using a stage micrometer (1 mm = 1000 μm) to express adipocyte area in square micrometers (μm²). At least 50 adipocytes per animal (approximately 10 per microscopic field) were manually outlined and measured, resulting in a minimum of 300 adipocytes analyzed per experimental group (n=6 animals per group) (21). Histological examination was performed by two observers who were blinded to the treatment groups.

Body Weight Measurement

Obesity development was evaluated using weekly body weight measurements recorded with a digital top-loading analytical balance (Ohaus Corp., Parsippany, NJ, USA).

Lipid Profile Measurement

The lipid profile, comprising total cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels, was measured in blood plasma using a LipidPro® meter (Infopia Co., Ltd., Anyang, South Korea). Blood samples (approximately 1 mL) were collected from the orbital sinus and transferred into a microtube. The samples were then centrifuged at 4000 rpm for 10 minutes to separate the plasma for analysis (23).

Organ Weight Measurement and Fixation

The liver, located in the abdominal cavity, was carefully excised and immediately weighed. Subsequently, the liver was fixed in formalin-saline solution (24).

Statistical Analysis

Data are presented as mean ± standard deviation. Prior to statistical analysis, data normality and homogeneity of variance were assessed using the Shapiro–Wilk test and Levene's test, respectively. As the data met the assumptions of normality and homogeneity of variance ($p > 0.05$), differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical analyses were performed using SPSS version 20, with statistical significance set at $p < 0.05$ (25).

RESULTS AND DISCUSSIONS

Effect of *Dillenia indica* Leaf Extract on Liver Weight in HFD-Fed Rats

After the administration of *Dillenia indica* leaf extract and subsequent euthanasia, the liver from each rat was excised and weighed individually. **Figure 1** illustrates the liver

weight profile across groups, highlighting the effect of *Dillenia indica* extract on liver weight. The group treated with 750 mg/kg of *D. indica* extract (HFD + DI 750) showed the lowest average liver weight (7.62 ± 1.33 g). This value was lower than in the orlistat-treated group (HFD + Orlistat, 8.50 ± 1.13 g) and the group that received 500 mg/kg extract (HFD + DI 500, 8.67 ± 1.21 g), both of which displayed liver weights approximating that of the normal control (8.31 ± 1.15 g). In contrast, rats fed a HFD without treatment showed the highest average liver weight (9.23 ± 1.68 g).

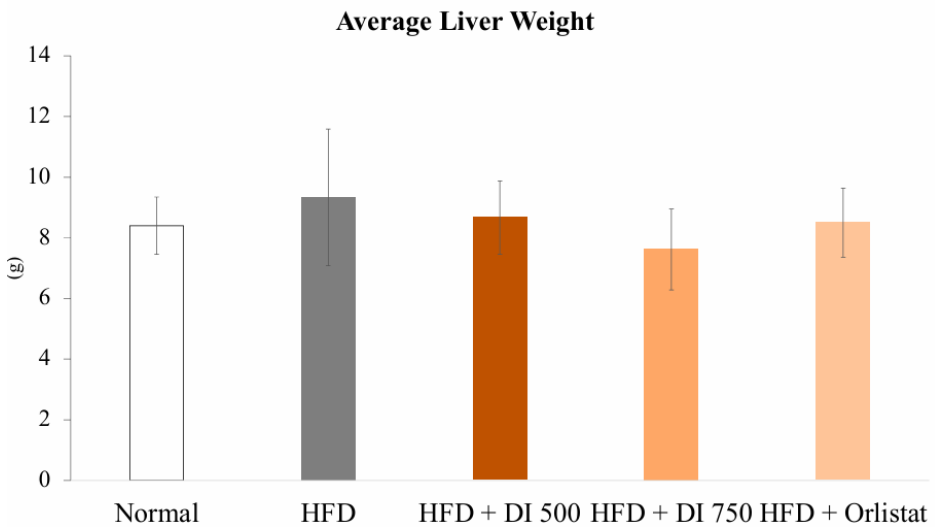

Figure 1. Effect of *Dillenia indica* Leaf Extract on Liver Weight.
Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

While the precise molecular mechanisms were not directly investigated in this study, we hypothesize, based on previous in vitro studies, that the numerically lower liver weight may be related to the ability of the extract to modulate the SIRT-1/phosphorylated liver kinase B1 (p-LKB-1)/AMPK signaling cascade, which regulates lipid metabolism by suppressing lipogenesis and promoting fatty acid oxidation. The extract may also reduce HMG-CoA reductase (HMGCR) activity, thereby decreasing cholesterol synthesis, while increasing the expression of PPAR-α, a nuclear receptor involved in lipid oxidation (12). Although these pathways were not evaluated in the present study, previous findings suggest that they may contribute to the numerically lower liver weight observed following *Dillenia indica* treatment. Similarly, the numerically lower liver weight observed in the orlistat group may be related to its known mechanism as a lipase inhibitor, which limits dietary fat absorption and may reduce hepatic lipid accumulation (25). However, the differences in liver weight among groups were not statistically significant.

Effect of *Dillenia indica* Leaf Extract on Lipid Profile in HFD-Fed Rats

The total cholesterol (TC) levels observed in each treatment group are shown in **Figure 2**. The lipid profile analysis showed that the HFD group had the highest TC level (55.83 ± 8.2 mg/dL), which was significantly elevated compared to the normal control (42.17 ± 6.4 mg/dL, p <0.05). Notably, the HFD + DI 750 group, which received 750 mg/kg BW of *Dillenia indica* extract, showed a numerical reduction in TC (43.83 ± 4.79 mg/dL), approaching the level of normal control. This suggests that higher doses of the extract showed numerically lower total cholesterol levels. In addition, both the HFD + DI 500 and

HFD + Orlistat groups also showed reductions in TC level (51.33 ± 8.1 and 53.17 ± 9.21 mg/dL, respectively), when compared to the HFD group. However, these reductions were less pronounced than those observed in HFD 750. These results show that the 750 mg/kg dose produced numerically lower total cholesterol levels than the HFD group, although the difference was not statistically significant. The lipid-modulating effects may be mediated by flavonoids present in the extract. When consumed, flavonoids are metabolized into aglycones, which are more lipophilic and can interact with cell membranes, thereby enhancing their bioavailability and biological activity in target tissues (26). These findings are aligned with the study by Khan *et al.* (2019), which reported that *Dillenia indica* fruit extract (ethanolic or aqueous) significantly reduced total cholesterol levels by 19-25% in rats fed with high-fat or high-cholesterol diets, indicating its potential as a cholesterol-lowering agent (27).

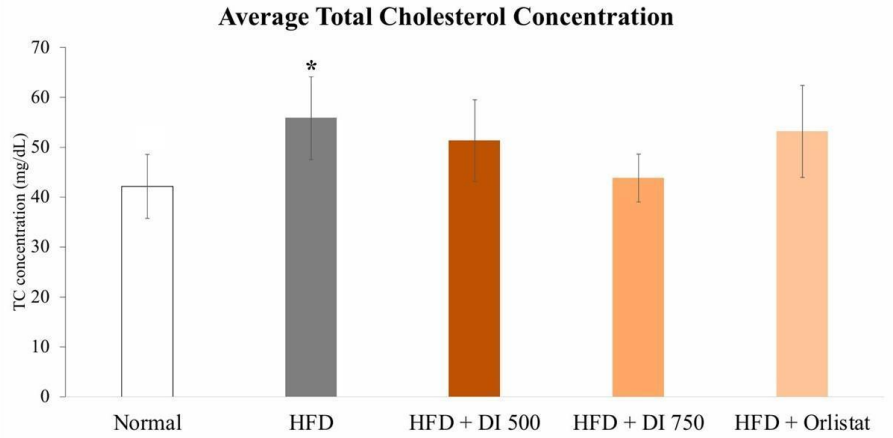

Figure 2. Effect of *Dillenia indica* Leaf Extract on Total Cholesterol

* p <0.05 vs normal group. Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

Changes in high-density lipoprotein (HDL) levels among the groups are displayed in **Figure 3**. The highest HDL concentration was observed in the HFD group (28.03 ± 14.5 mg/dL). HDL levels varied among groups. However, no clear treatment-related improvement was observed. In comparison, Khan *et al.* (2019) demonstrated that the ethanol extract of *Dillenia indica* increased HDL levels by 14%, while the aqueous extract maintained HDL concentrations in rats fed a cholesterol-enriched diet (27).

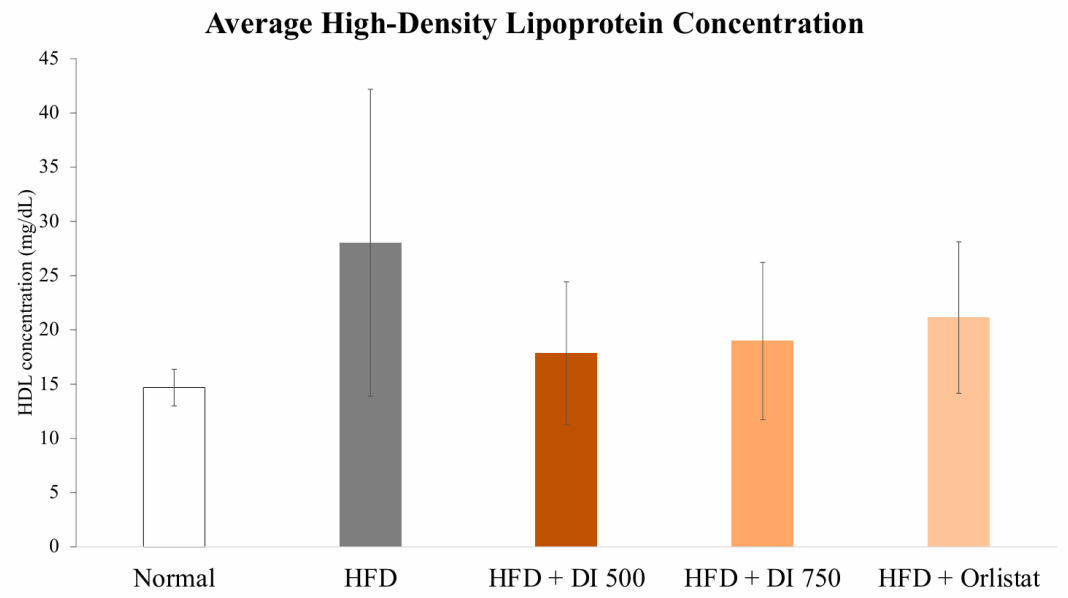

Figure 3. Effect of *Dillenia indica* Leaf Extract on High-Density Lipoprotein (HDL)
Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

Figure 4 presents the low-density lipoprotein (LDL) concentrations observed among the experimental groups. The LDL concentration was highest in the HFD group (13.85 ± 2.34 mg/dL). In contrast, the normal control group maintained lower LDL levels (9.48 ± 1.50 mg/dL), consistent with normal lipid metabolism under standard dietary conditions. Meanwhile, rats treated with *Dillenia indica* extract showed reductions in LDL levels that were numerically lower in both the HFD + DI 500 group (10.93 ± 2.95 mg/dL) and the HFD + DI 750 group (10.75 ± 4.81 mg/dL) compared to the untreated HFD group. This suggests a trend toward lower LDL concentrations in extract-treated groups compared with the HFD group. This observation is consistent with the findings of Khan *et al.* (2019), who reported that *Dillenia indica* fruit extract significantly reduced LDL concentrations in hyperlipidemic rats (from 47 to 39 mg/dL) across various lipid-rich dietary conditions (27). Similarly, the HFD + Orlistat group showed a numerical reduction in LDL levels (11.02 ± 3.94 mg/dL).

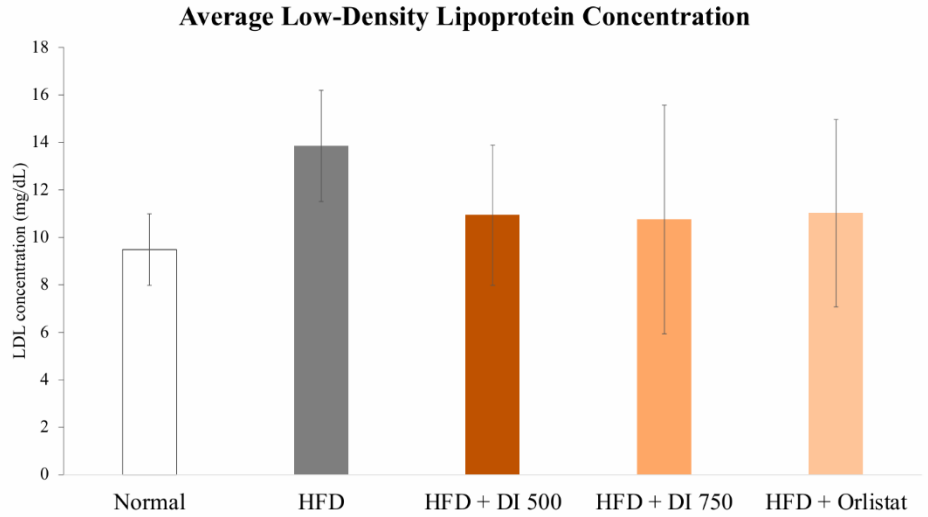

Figure 4. Effect of *Dillenia indica* Leaf Extract on Low-Density Lipoprotein (LDL)

Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

The triglyceride (TG) levels in the HFD group and treatment groups are illustrated in **Figure 5**. TG levels in the treatment groups demonstrated a downward trend compared to the HFD group (62.00 ± 17.33 mg/dL). HFD + DI 500 showed a moderate reduction in TG levels (51.00 ± 15.81 mg/dL), whereas a greater reduction was seen in HFD + DI 750 (44.17 ± 13.13 mg/dL). The lowest TG level was observed in the HFD + Orlistat-treated group (36.60 ± 6.34 mg/dL). The HFD + DI 750 group showed a greater numerical reduction in triglyceride levels than the HFD + DI 500 group. However, a dose-response relationship cannot be established from the present data. Similarly, Khan *et al.* (2019) found that *Dillenia indica* fruit extract reduced triglyceride levels by 29-32% in hyperlipidemic animal models, supporting its lipid-lowering potential and aligning with the downward trend observed in the current study (27). Nonetheless, the HFD + Orlistat group showed the lowest TG level, while the *Dillenia indica* groups showed numerical downward trends that were not statistically significant.

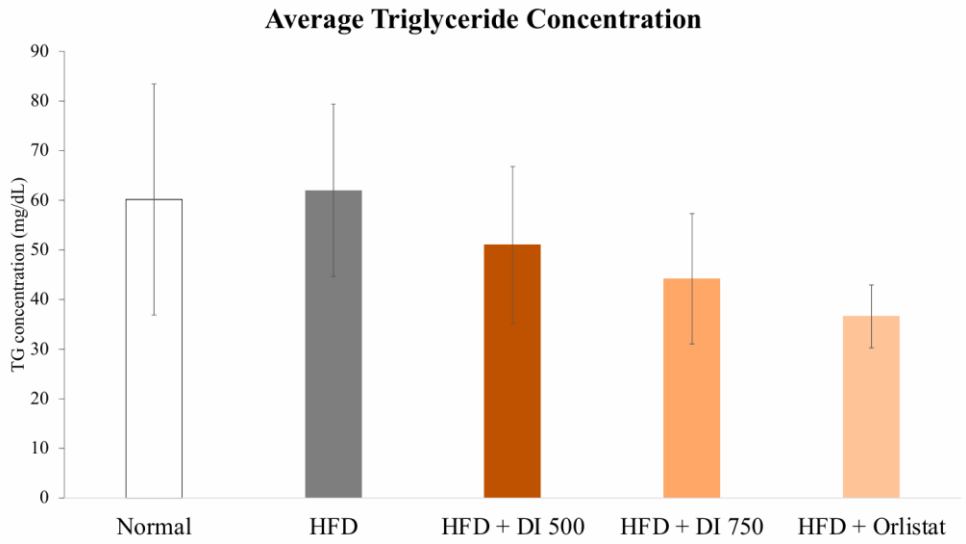

Figure 5. Effect of *Dillenia indica* Leaf Extract on Triglyceride (TG)

Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

Effect of *Dillenia indica* Leaf Extract on Body Weight in HFD-Fed Rats

After the 28-day induction period, the mean body weight of the HFD-fed rats increased from 183.4 ± 27.8 g at baseline to 244.2 ± 28.5 g, representing a 33.1% increase. The normal-diet group showed a 51.0% increase during the same period. Therefore, although the HFD-fed rats gained weight, body-weight data alone were insufficient to conclusively confirm the establishment of obesity before treatment. **Figure 6** summarizes the changes in mean body weight during the study period. All groups showed an increase in body weight. However, no statistically significant differences were observed among the groups. The HFD + DI 500 group showed numerically lower weight gain than the HFD group, whereas the HFD + DI 750 group showed slightly greater weight gain than the HFD + DI 500 group. These findings indicate numerical variation among groups but do not demonstrate a significant effect of *Dillenia indica* leaf extract on body weight.

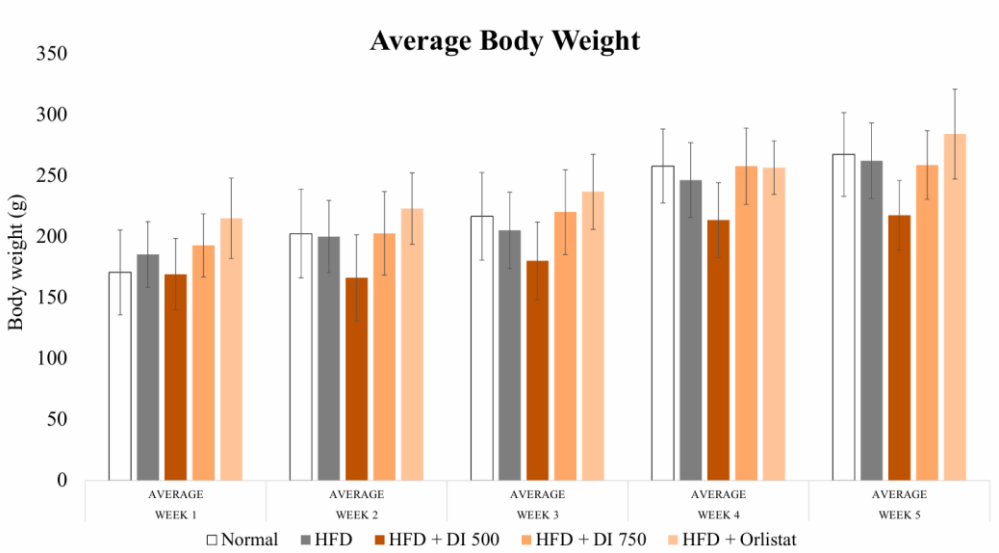

Figure 6. Effect of *Dillenia indica* Leaf Extract on Average Body Weight
The average body weight data of rats from each group were recorded over a five-week period. Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

This finding suggests variability in the treatment response. However, further studies across a wider dose range are required to determine whether a dose-dependent effect exists (28). While previous research by Kuddus *et al.* (2020) demonstrated that *Dillenia indica* fruit extract significantly reduced body weight in HFD-fed rats by down-regulating pro-adipogenic transcription factors and lipid-metabolizing enzymes, the leaf extract in the present study did not produce a comparable statistically significant reduction (29). This divergence may reflect differences in the composition and concentrations of bioactive compounds between plant parts. Thus, although the findings from the previous study support the anti-obesity potential of the fruit extract, the present study provides only limited evidence for the effect of the leaf extract on body weight because the observed difference was not statistically significant. The HFD + Orlistat group showed one of the greatest increases in body weight, which was unexpected considering the established role of orlistat as a lipase inhibitor. Because food intake, energy expenditure, body composition, and treatment-related stress were not assessed, the unexpected body weight findings in the orlistat group should be interpreted cautiously. Overall, the HFD + DI 500 group showed only a numerical trend toward lower weight gain compared with the HFD group, as none of the treatments produced a statistically significant effect on total body weight.

Effect of *Dillenia indica* Leaf Extract on Fat Histology in HFD-Fed Rats

The histological analysis of adipose tissue provides insight into structural changes associated with obesity and the effects of various treatments. Histological findings of adipose tissue structure in each group are presented in **Figure 7**. In the normal group, adipocytes appeared as small, uniformly distributed fat cells with clear cellular boundaries, consistent with typical healthy adipose tissue in normal rats. On the other hand, the HFD-only group, which received a HFD without any treatment, showed a larger mean adipocyte area than the normal group. This adipocyte hypertrophy is recognized as a primary characteristic of diet-induced obesity that can trigger local inflammation and systemic metabolic dysfunction (30). The cells appeared swollen and more irregular in shape,

consistent with adipocyte hypertrophy typically observed in diet-induced obesity. The group given HFD and *Dillenia indica* extract at 750 mg/kg showed a relatively smaller adipocyte area compared to the group that received only a HFD, although there were still signs of enlargement. Similarly, the group given HFD and *Dillenia indica* extract at a dose of 500 mg/kg showed moderate improvement, with adipocytes slightly smaller than in the HFD control group, suggesting that the extract may limit adipocyte hypertrophy. Meanwhile, the group given HFD and Orlistat showed adipocyte morphology comparable to that of the normal control group.

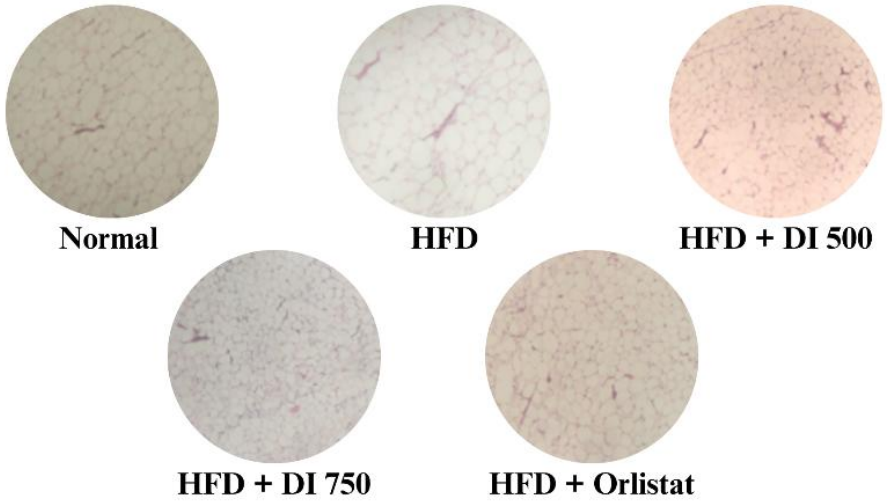

Figure 7. Representative histological images of adipose tissue.
Magnification = 100x. Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

This suggests that Orlistat, as a well-known lipase inhibitor, was associated with smaller adipocyte area. The findings of Poret *et al.* (2018) highlight that chronic consumption of a HFD can induce adipocyte hypertrophy and inflammation in visceral adipose tissue, particularly in obesity-prone rats, thereby increasing the risk of obesity-related comorbidities. In the present study, histological analysis revealed significant adipocyte enlargement in HFD-fed rats compared with normal controls, suggesting hypertrophy due to excessive lipid accumulation. These findings support the proposed link between increased adipocyte size and inflammation in the progression of obesity, as described by Poret *et al.* (2018), and reinforce the idea that targeting adipocyte morphology may be an effective strategy to mitigate diet-induced obesity (32). In line with this, the present study showed that both doses of *Dillenia indica* leaf extract were associated with significantly lower mean adipocyte areas than the HFD group.

Effect of *Dillenia indica* Leaf Extract on Adipocyte Area in HFD-Fed Rats

Figure 8 compares the average adipocyte area across groups, while **Figure 9** shows the morphometric analysis of adipocyte area using ImageJ software. The highest average adipocyte area was observed in the high-fat diet control group (4272.00 μm²), followed by the normal control (3478.09 μm²), HFD + DI 500 (3252.70 μm²), HFD + Orlistat (2633.16 μm²), and the HFD + DI 750 group had the lowest value (2047.37 μm²). One-way ANOVA

indicated a significant difference among the groups ($p < 0.05$). Tukey's HSD test showed that the HFD + DI 500, HFD + DI 750, and HFD + Orlistat groups had significantly lower mean adipocyte areas than the HFD group ($p < 0.05$).

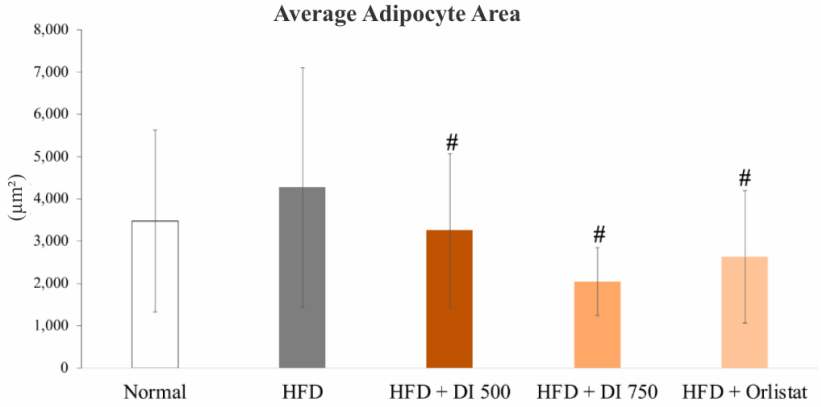

Figure 8. Effect of *Dillenia indica* Leaf Extract on Adipocyte Area.
#p < 0.05 vs HFD group. Abbreviations: HFD: high-fat diet; HFD + DI 500: HFD + *Dillenia indica* 500 mg/kg; HFD + DI 750: HFD + *Dillenia indica* 750 mg/kg

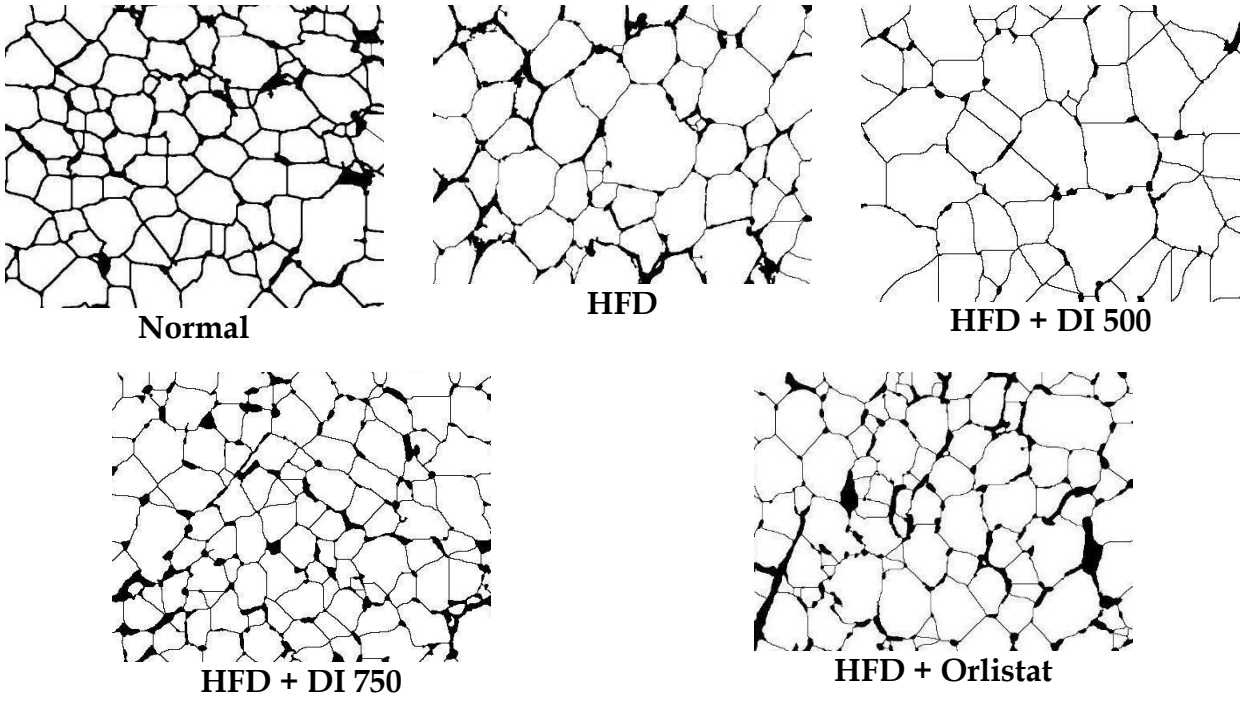

Figure 9. Representative Measurement of Adipocyte Area Using ImageJ

These results indicate that administration of *Dillenia indica* leaf extract at a dose of 750 mg/kg BW resulted in the lowest adipocyte area among all experimental groups. This reduction may be associated with the bioactive compounds present in *Dillenia indica,* such as flavonoids that may inhibit adipocyte hypertrophy or promote lipid metabolism. Although most flavonoids are water-soluble, they possess some lipophilicity, which may allow their uptake by adipose tissue. For instance, quercetin and its metabolites have been detected in white adipose tissue of rats, suggesting that long-term intake of flavonoid-rich

supplements could result in the accumulation of anti-adipogenic concentrations of these compounds in adipose tissues (33). A possible dose-related effect was observed, as the 750 mg/kg dose produced a greater reduction in adipocyte area than the 500 mg/kg dose. Because the present study strictly evaluated histomorphological changes, we can only hypothesize the underlying mechanisms based on existing literature. Based on García-Barrado *et al.* (2020), dietary flavonoids (including quercetin and anthocyanins) have been reported in preclinical studies to attenuate chronic inflammation in adipose tissue by modulating key signalling pathways, such as nuclear factor-kappa B (NF-κB), mitogen-activated protein kinases (MAPKs), and the NLR family pyrin domain containing 3 (NLRP3) inflammasome. In addition, several flavonoids have been shown to inhibit adipocyte differentiation by downregulating the expression of the proadipogenic transcription factors PPARγ and CCAAT/enhancer binding protein α (C/EBPα) (34). Therefore, we hypothesize that the reduction in adipocyte area observed following *Dillenia indica* leaf extract administration may be associated with these previously documented anti-inflammatory and anti-adipogenic mechanisms.

CLINICAL IMPLICATION

The findings of this study suggest that *Dillenia indica* leaf extract, particularly at a dose of 750 mg/kg body weight, may affect adipocyte morphology. Although it did not significantly reduce overall body weight, liver weight, total cholesterol, LDL, or triglyceride levels, a significant reduction in adipocyte area was observed. These findings support further studies to evaluate the effects, mechanisms of action, optimal dosage, and long-term safety of *Dillenia indica* leaf extract.

LIMITATIONS

This study has several limitations. First, only two doses of *Dillenia indica* leaf extract were evaluated, which limits the ability to establish a comprehensive dose- response relationship. Second, chloroform was used to induce unconsciousness before cervical dislocation. Chloroform is classified as an unacceptable agent according to American Veterinary Medical Association (AVMA) Guidelines (2020). Future studies should adopt euthanasia procedures recommended by current international guidelines, such as CO_2 inhalation or the use of inhaled anesthetics like isoflurane, followed by an appropriate secondary physical method to ensure death (35). Third, the molecular mechanisms underlying the observed anti-obesity effects were not directly investigated. Consequently, any involvement of specific signaling cascades (such as AMPK, SIRT1, PPARα/PPARγ, NF-κB, MAPK, oxidative stress, and inflammatory cytokines) remains purely hypothetical based on prior literature. Further studies are needed to evaluate a broader range of doses and to explore the molecular pathways underlying the biological activity of *Dillenia indica* leaf extract.

CONCLUSIONS

Dillenia indica leaf extract (750 mg/kg BW) significantly reduced mean adipocyte area in HFD-fed rats. However, changes in body weight, liver weight, and lipid profiles remain non-significant numerical trends. Consequently, its broader systemic anti-obesity efficacy

cannot be definitively concluded, necessitating further long-term studies to evaluate molecular mechanisms, safety, and optimal dosing.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTOR CONTRIBUTIONS

N.C.Q.B. designed the experimental protocol, conducted the study, analyzed the data, and drafted the manuscript. K.A.J.W. and M.P.P. contributed to experimental execution, data collection, and assisted in writing the manuscript draft. A.C.M. and A.A.D. participated in animal handling, laboratory analyses, data interpretation, and writing the manuscript draft. O.R.A. supervised the research, provided critical revisions, and contributed to manuscript preparation.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the Ministry of Education, Culture, Research, and Technology of Indonesia through the Student Creativity Program, as well as Universitas Brawijaya, for providing financial support and access to essential laboratory facilities.

FUNDING

This research was funded by the Directorate General of Learning and Student Affairs under the Student Creativity Program (PKM).

DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors used ChatGPT to assist in improving the language and grammar of the manuscript. The authors reviewed and verified the content to ensure accuracy and integrity grity.

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