Potential lipid-modulating and hepatic protective effects of *Citrus amblycarpa* and *Dimocarpus longan* leaf extracts in high-fat diet-induced rats

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ABSTRACT

Citrus amblycarpa and Dimocarpus longan are minor fruit plants whose leaves have traditionally been used in herbal medicine. In this study, we explored the potential lipid-modulating activity and effects on liver histology of ethanol extracts derived from C. amblycarpa and D. longan leaves, administered either individually or in combination, in a rat model of diet-induced hypercholesterolemia. The leaf extracts were obtained through maceration using 96% ethanol. Hypercholesterolemia was induced by administering a high-fat emulsion in combination with propylthiouracil for 14 days. Following induction, the animals received C. amblycarpa leaf extract (612.5 mg/kg body weight), D. longan leaf extract (200 mg/kg body weight), or a 1:1 combination of both extracts. Total cholesterol levels were measured, and liver tissues were evaluated through histopathological examination. Phytochemical analysis confirmed the presence of several bioactive compounds, including flavonoids, saponins, tannins, alkaloids, terpenoids, and steroids in both extracts. The combination treatment tended to show a greater reduction in total cholesterol levels and more favorable liver histological features compared to the single-extract treatments.

Keywords: *Citrus amblycarpa, Dimocarpus longan, hypercholesterolemia, lipid metabolism, liver histology, medicinal plants*

INTRODUCTION

Hypercholesterolemia is a metabolic disorder characterized by elevated levels of circulating cholesterol, particularly low-density lipoprotein cholesterol (LDL-C), and is closely associated with an increased risk of cardiovascular disease and liver dysfunction. These pathological processes highlight the strong interrelationship between dyslipidemia and liver injury, emphasizing the need for therapeutic strategies that can regulate lipid metabolism while preserving liver integrity. The liver plays a crucial role in regulating lipid metabolism, including processes such as cholesterol synthesis, lipoprotein balance, and bile acid production. When liver function is impaired, these metabolic processes can become disrupted, potentially worsening dyslipidemia and contributing to the development of metabolic conditions such as non-alcoholic fatty liver disease (NAFLD). This condition is closely linked to oxidative stress and excessive lipid accumulation in liver tissues (Delli Bovi *et al.*, 2021). Therefore, preserving both the functional and structural integrity of the liver is essential for maintaining metabolic balance and preventing further disease progression.

Although synthetic lipid-lowering agents such as statins are widely used in clinical practice, their long-term administration may cause adverse effects, including hepatotoxicity and muscle-related complications. As a result, interest in medicinal plants as alternative or

complementary strategies for managing hypercholesterolemia has increased in recent years. Recent experimental studies have demonstrated that plant-derived bioactive compounds, including flavonoids, polyphenols, saponins, and sterols, are capable of modulating lipid metabolism, reducing oxidative stress, and improving liver histological features in high-fat diet-induced animal models (Ward *et al*., 2019). Although medicinal plants are generally perceived as safer alternatives to synthetic drugs, emerging evidence suggests that certain phytochemicals may also produce adverse effects. These may include hepatotoxicity and pro-oxidant activity, particularly when used at high doses or over extended periods. Therefore, it is important to carefully evaluate not only the efficacy but also the safety profile of plant-based therapies before their wider application (Nechchadi *et al.*, 2024).

Minor fruit plants represent an underexplored yet promising source of medicinal compounds. Minor fruits and medicinal plants: emerging sources of nutraceuticals and health-promoting compounds (Remita *et al.*, 2021). Among them, species of the genus *Citrus* have been widely studied due to their rich flavonoid content and their potential to regulate lipid homeostasis. Citrus-derived phytochemicals have been reported to inhibit cholesterol biosynthesis, enhance bile acid excretion, and improve hepatic antioxidant capacity, leading to reductions in serum cholesterol levels and improvements in liver structure (Xu *et al*., 2025; Ding *et al*., 2022).

However, most existing studies have largely focused on citrus fruits or peels, while the medicinal potential of citrus leaves, particularly *Citrus amblycarpa*, has received relatively limited attention. This highlights an important research gap, as plant leaves may possess distinct phytochemical compositions and bioactive compounds that differ from those found in fruits (Xu *et al.*, 2025). Similarly, *Dimocarpus longan* is a minor fruit plant traditionally used in Asian herbal medicine. Although the fruit has been widely investigated, recent studies suggest that *D. longan* leaves also possess antihyperlipidemic, antioxidant, and hepatoprotective activities in experimental models of metabolic disorders (Parawestri and Kuswanti, 2025). These biological effects are closely associated with the existence of phenolic constituents and flavonoid compounds that play a role in lipid homeostasis and protection against oxidative cellular damage.

In addition, although individual plant extracts have been reported to exhibit lipid-modulating and antioxidant properties, studies examining the combined use of different plant leaf extracts remain scarce. Given that phytochemicals can interact through multiple biological pathways, their combined use may lead to synergistic effects. However, evidence supporting such combination strategies is still limited and requires further investigation (Nechchadi *et al.*, 2024). Therefore, investigating the combined effects of *Citrus amblycarpa* and *Dimocarpus longan* leaf extracts is of scientific interest, particularly in elucidating their potential synergistic roles in modulating lipid metabolism and improving liver histological features under hypercholesterolemic conditions. Moreover, interactions among various bioactive compounds may lead to synergistic effects by simultaneously targeting different pathways involved in lipid metabolism and liver protection. This multi-target approach is considered advantageous compared to single-compound therapies, as it may improve therapeutic outcomes while minimizing metabolic burden (Liu *et al*., 2022). Therefore, investigating the combined effects of *Citrus amblycarpa* and *Dimocarpus longan* leaf extracts is scientifically relevant, particularly for understanding their potential synergistic roles in modulating lipid metabolism and improving liver histological features under hypercholesterolemic conditions.

MATERIALS AND METHODS

Fresh leaves of *Dimocarpus longan* Lour. and *Citrus amblycarpa* (Hassk.) Ochse were used as plant materials in this study. The plant materials were collected from Kalisegoro Village, Gunungpati, Semarang, Indonesia, and botanical identification was performed at

Universitas Ahmad Dahlan Yogyakarta Indonesia to confirm species authenticity. The collected leaves were cleaned, air-dried, and processed into simplicia before extraction. The research was conducted in 2025 at the Pharmacology and Natural Products Chemistry Laboratory, Faculty of Medcine, Universitas Negeri Semarang, Central Java, Indonesia.

Simvastatin 10 mg tablets were used as the positive control. Standard feed (BR2, Comfeed, Indonesia), distilled water, 96% ethanol, magnesium powder, Dragendorff reagent, Mayer reagent, concentrated hydrochloric acid, 1% ferric chloride, concentrated sulfuric acid, 0.5% sodium carboxymethyl cellulose (Na-CMC), 70% ethanol, propylthiouracil (PTU), lard, and duck egg yolk were used for extraction, phytochemical screening, and hypercholesterolemia induction. All chemicals and reagents were of analytical grade and obtained from Pharmacy Laboratory, Universitas Negeri Semarang, Indonesia.

The main instruments used in this study were a rotary evaporator for solvent removal, an analytical balance (Ohaus, USA) for weighing samples and reagents, a cholesterol analyzer (Nesco Multicheck Tester) for total cholesterol measurement, a drying oven for simplicia preparation, and standard glassware including Erlenmeyer flasks and volumetric flasks (Pyrex, USA). General laboratory utensils such as scissors, filter paper, mortar and pestle, and oral gavage equipment were used as supporting apparatus. This study is a true experimental study using rats as subjects. The design used is a post-test only control group design. The researchers selected the experimental group and control group randomly.

The crude drug powder was extracted using the maceration method with a ratio of 1:10. 500 grams were placed in a dark glass jar with 5L of 96% ethanol solvent, stirred occasionally, then left for 3x24 hours in a dark place until saturated and macerated. The resulting macerate is then filtered through flannel cloth to obtain the filtrate. The filtrate is collected and concentrated using a rotary evaporator at 40°C until a concentrated extract is obtained (Bitwell *et al.* , 2023).

Phytochemical screening test by dissolving 0.5 g of concentrated extract in 10 mL of distilled water, then testing for flavonoids, alkaloids, tannins, saponins, terpenoids, and steroids.

Flavonoid estimation: The flavonoid test is performed using three tests, namely the Wilstater test, the Bate-Smith test, and the 10% NaOH test. Wilstater Test add 1mL of extract to a test tube and add 0.1g of Mg and five drops of concentrated HCl. If a red or orange color forms, then flavonoids are present. 10% NaOH Test add a few drops of 10% NaOH solution to 1 mL of extract. A positive flavonoid reaction is indicated by a change in color to orange or orange (Saputra *et al.,* 2024).

Saponin estimation: A total of 2 mg of extract is placed in a test tube and 10 mL of hot water is added, then heated to boiling and filtered. The filtrate is placed in a test tube, shaken for ±10 seconds, left for 10 minutes, then 1 mL of HCl 2 mL is added. Saponin is positive if foam forms and remains for 15 minutes (Mohlakoana and Moteetee, 2021).

Tannin estimation: Approximately 2 mg of concentrated extract is heated for about 5 minutes, then a few drops of 1% FeCl3 are added. The extract shows a positive tannin reaction if the solution turns greenish brown or blackish blue (Suryani *et al.,* 2025).

Alkaloids estimation: The extract is filtered with filter paper, and reacted with 3 drops of concentrated HCl, then 5 drops of Mayer's reagent are added. The presence of alkaloids is indicated by the formation of a white precipitate in the Mayer's test (Settaluri *et al.,* 2024).

Terpenoids and steroids estimation: The 0.2 g extract is added to glacial acetic acid until submerged, then let it sit for approximately 15 minutes. Six drops of the sample solution are placed in a test tube using a pipette, then 2–3 drops of H□SO□ are added. The presence of triterpenoids is indicated by a brown or violet-

purple color change, while the presence of steroids is indicated by a blue-green color (Yulianti *et al.,* 2025).

In vivo study

Induction of hypercholesterolemia

A high-fat diet was formulated using a combination of lard and duck egg yolk as the primary fat sources, in a 3:1 ratio (150 mL lard and 50 g duck egg yolk). The mixture was thoroughly homogenized to produce a uniform emulsion. This emulsion was then administered orally to the experimental animals at a dose of 4 mL per rat per day, divided into two administrations, for 14 consecutive days to induce a hyperlipidemic state (Larasati *et al*., 2024) (Pehlivanović Kelle *et al*., 2024).

To further enhance the induction of hypercholesterolemia, propylthiouracil (PTU) was co-administered. PTU acts by suppressing thyroid hormone synthesis, thereby slowing lipid metabolism and promoting lipid accumulation. The PTU solution was prepared by dissolving the compound in distilled water to obtain a 1.25% (w/v) concentration. The freshly prepared solution was administered orally at a dose of 1 mL per rat per day for 14 days (Jin *et al*., 2024). This combined approach has been widely employed in experimental studies to establish a stable and reproducible hyperlipidemic model.

Extract dosage determination: The extract doses used in this study were selected based on previous research demonstrating their biological activity in experimental models. Unlike simvastatin, which is a single, well-defined compound, plant extracts are composed of complex mixtures of bioactive constituents. As a result, higher doses are generally required to achieve comparable biological effects. Therefore, the selected doses were considered suitable for evaluating the potential lipid-modulating activity of the extracts in vivo (Parawestri and Kuswanti, 2025; Rahib *et al*., 2024).

Cholesterol level measurement: Measurements were performed using a Nesco Multicheck Tester. Total cholesterol levels were measured before high-fat feed induction (pretest) and on the seventh and fourteenth days of treatment (posttest) (Kelle *et al.,* 2024).

Body weight loss (bw) measurement in experimental rats

The experimental rats used in this study were obtained from "Sudarno *Mus musculus* Farm" Susukan Ungaran Semarang, Indonesia. Before conducting body weight loss (bw) measurement in experimental on rat, an ethical clearance test was conducted at the Faculty of Medicine, Semarang State University No. 112/KEPK/FK/KLE/2025. The study involved a total of 30 rats, which were divided into 5 groups, each containing 6 rats: the negative control group (Control - Na-induced rat), the positive control group (Control + Simvastatin 10 mg induced rat), and the three treatment groups receiving *Dimocarpus longan* leaf extract, *Citrus amblycarpa* leaf extract, or a combination of both extracts. Body weight (BW) was monitored periodically in all experimental rats to evaluate the overall metabolic impact of high-fat diet (HFD) induction and to assess the safety as well as the metabolic effects of the administered plant extracts.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by LSD post hoc test to determine significant differences between groups (Kim, 2017).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical testing aims to identify the presence or absence of biologically active compounds or secondary metabolite content in 96% ethanol extracts of *Dimocarpus longan* leaf and *Citrus amblycarpa* leaf. After phytochemical screening, it was found that extract of *Dimocarpus longan* and *Citrus amblycarpa* leaf contain secondary metabolites: flavonoids, alkaloids, saponins, tannins, steroids, and terpenoids. The results of phytochemical screening of 96% ethanol extracts of longan and Citrus amblycarpa leaves can be seen in Tables 1 and 2.

Body weight loss (bw) measurement in experimental rats

Table 3 presents the changes in body weight (BW) for each experimental group across three time points: Day 1, Day 14, and Day 21. The measurements were conducted to assess the metabolic impact of the high-fat diet and propylthiouracil (PTU) treatment, as well as the effects of *Dimocarpus longan* and *Citrus amblycarpa* leaf extracts on body weight loss.

The **negative control group (Control -)** showed an increase in body weight from 209 g on Day 1 to 244.3 g on Day 14. By Day 21, the body weight slightly decreased to 231.2 g, resulting in a total weight loss of 13.1 g. The **positive control group (Control +),** which was treated with Simvastatin 10 mg, showed a similar pattern, with body weight increasing from 207.5 g on Day 1 to 242 g on Day 14, followed by a decrease to 215 g on Day 21, leading to a total weight loss of 27 g.

Treatment groups: LOLE (*Dimocarpus longan* leaf extract), rats treated with LOLE exhibited an increase in body weight from 202.2 g on Day 1 to 238.2 g on Day 14, and a slight decrease to 222.6 g on Day 21, resulting in a total weight loss of 15.6 g. LILE (*Citrus amblycarpa* leaf extract); The LILE group showed the lowest weight loss, with a minimal increase from 194.6 g on Day 1 to 224.6 g on Day 14, followed by a decrease to 221.4 g on Day 21, leading to a total weight loss of just 3.2 g. Combine (Combination of LOLE and LILE): The combination group showed an increase in body weight from 201 g on Day 1 to 237 g on Day 14, followed by a decrease to 213.6 g on Day 21, resulting in a total weight loss of 23.4 g.

The overall mean body weight loss across all groups was 18.57 g, with the highest loss observed in the positive control group (Simvastatin-treated), followed by the combination treatment group. This suggests that the combination of both plant extracts may have a more moderate effect on weight loss compared to Simvastatin, while the LILE treatment resulted in minimal weight loss.

The results show that both plant extracts, whether used alone or in combination, did not cause significant weight loss compared to the positive control group (Simvastatin). The slight weight loss observed in the LILE group (3.2 g) might be due to the bioactive compounds in *Citrus amblycarpa* leaves, which seem to help regulate lipid metabolism without leading to considerable weight loss, in contrast to Simvastatin, which typically causes more noticeable weight reduction as part of its intended pharmacological effect (Nechchadi *et al*., 2024). The weight loss observed can largely be attributed to the metabolic and hormonal changes induced by PTU, which inhibits thyroid hormone production, thereby slowing down metabolism and affecting lipid metabolism (Singh *et al*., 2020). Additionally, the combination of *Dimocarpus longan* and *Citrus amblycarpa* extracts led to moderate weight loss, suggesting that these extracts may work together synergistically to affect lipid metabolism. However, more research is needed to determine whether this combination could bring about more significant metabolic changes or help with weight management in the long term.

Changes in body weight are widely recognized as a key physiological parameter in experimental models of hyperlipidemia and metabolic disorders, as they reflect shifts in energy balance, lipid metabolism, and general health condition (Nechchadi *et al*., 2024; Liu *et al*., 2022). In hypercholesterolemic animal models, both high-fat diet induction and pharmacological agents such as propylthiouracil (PTU) can markedly influence metabolic rate, lipid accumulation, and body weight patterns (Jin *et al*., 2024). Therefore, monitoring body weight is essential not only to confirm the successful establishment of the disease model but also to differentiate between true lipid-lowering effects and non-specific outcomes, such as toxicity or cachexia (Kelle *et al*., 2024). In addition, body weight evaluation is an important component in preclinical studies to assess the safety profile of therapeutic agents, including plant-derived extracts. Significant weight loss may indicate potential adverse effects, whereas relatively stable body weight suggests better tolerability (Rahib *et al*., 2024). Previous studies have also shown that monitoring body weight provides

valuable supportive information in interpreting metabolic changes and evaluating treatment efficacy in models of hyperlipidemia and liver injury (Nechchadi *et al*., 2024; Liu *et al*., 2022).

Lipid-lowering effects of *Dimocarpus longan* and *Citrus amblycarpa* leaf extracts in hypercholesterolemic rats

Before conducting the lipid-lowering effects on rat, an ethical clearance test was conducted at the Faculty of Medicine, Semarang State University No. 112/KEPK/FK/KLE/2025. Total cholesterol levels of rats were measured to evaluate the lipid-lowering potential of *Dimocarpus longan* leaf extract, *Citrus amblycarpa* leaf extract, and their combination in hypercholesterolemic rats.

Table 4 shows the total cholesterol levels in the experimental rats at three different time points: Day 1, Day 14, and Day 21, following treatment with various interventions. These measurements were taken to evaluate the lipid-lowering effects of *Dimocarpus longan* leaf extract, *Citrus amblycarpa* leaf extract, and their combination, in comparison to the control groups.

The negative control group (treated with CMC-Na) showed a gradual increase in total cholesterol levels from 124.4 mg/dL on Day 1 to 209.2 mg/dL on Day 14, and then slightly decreased to 199.8 mg/dL on Day 21. The mean cholesterol level at Day 21 was 177.80 ± 46.49 mg/dL. The positive control group (treated with Simvastatin 10 mg) showed a decrease in total cholesterol levels, from 127.4 mg/dL on Day 1 to 217 mg/dL on Day 14, and then a significant drop to 153 mg/dL by Day 21, with a mean cholesterol level of 165.80 ± 46.15 mg/dL. This demonstrates the expected cholesterol-lowering effect of Simvastatin. The group treated with *Dimocarpus longan* leaf extract (LOLE) showed a similar trend to the positive control group, with a slight decrease in cholesterol from 125.4 mg/dL on Day 1 to 215.8 mg/dL on Day 14, and a more pronounced reduction to 168.4 mg/dL on Day 21. The mean cholesterol level for this group was 169.87 ± 45.23 mg/dL, indicating a moderate effect in lowering cholesterol. The *Citrus amblycarpa* leaf extract (LILE) treated group demonstrated a decrease in total cholesterol levels from 127.6 mg/dL on Day 1 to 215.2 mg/dL on Day 14, followed by a reduction to 166.8 mg/dL on Day 21, with a mean of 169.87 ± 43.87 mg/dL. This suggests that *Citrus amblycarpa* leaves also have a lipid-lowering effect, though not as pronounced as the Simvastatin or LOLE treatments. The combination of LOLE and LILE resulted in a reduction of total cholesterol from 126.6 mg/dL on Day 1 to 219.6 mg/dL on Day 14, and a significant decrease to 161.6 mg/dL on Day 21, with a mean value of 169.27 ± 46.98 mg/dL. This suggests that the combined effect of both plant extracts may have a synergistic action in lowering cholesterol levels.

The results indicate that the treatments, especially the positive control (Simvastatin) and the combination of LOLE and LILE, showed a significant difference compared to the negative control group, with a p-value < 0.05. This suggests that the treatments were effective in lowering cholesterol levels when compared to the control group. The results in Table 4 highlight the lipid-lowering potential of both *Dimocarpus longan* and *Citrus amblycarpa* leaf extracts, whether used individually or in combination. While Simvastatin showed the greatest reduction in cholesterol levels, the combination of both plant extracts (LOLE and LILE) also displayed a promising effect. This could be due to the synergistic action of their bioactive compounds. These findings align with previous studies, which suggest that phytochemicals in medicinal plants such as flavonoids, saponins, and terpenoids can regulate lipid metabolism by inhibiting cholesterol production and boosting its excretion (Liu *et al*., 2022; Nechchadi *et al*., 2024).

Histological profile of rat liver

Histological examination of the rat liver was conducted at the "Central Research & Diagnostic Laboratory of Satwa Sehat Animal Clinic, Malang, East Java, Indonesia." Histopathological analysis of the liver was performed to assess the number of cells undergoing apoptosis, karyolysis, and

karyorrhexis. The cell count was performed using *ImageRaster*. This observation was carried out using a light microscope (Nikon Eclipse model Ei) with the assistance of an Optilab SIGMA MTN020 connected to a computer. The data shown in Tables 5, 6, and 7, along with the histological images in Figure 1, offer valuable insights into how *Dimocarpus longan* (LOLE) and *Citrus amblycarpa* (LILE) leaf extracts, both individually and in combination, affect apoptosis, karyorrhexis, and karyolysis in rat liver tissue.

Table 5 presents the average percentage of apoptosis in liver cells across different experimental groups. The results indicate Control (-) 23.24% , Control (+) 14.42%, LOLE: 15.47%, LILE 22.66% and Combination 16.54%. Apoptosis, or programmed cell death, serves as an important marker of cellular damage. The higher apoptosis rate in the negative control group (23.24%) reflects natural cell turnover and damage, which is typical in untreated animals under stress. On the other hand, the positive control group (Simvastatin) showed a reduction in apoptosis (14.42%), highlighting the protective role of Simvastatin in preventing liver cell death. Both *Dimocarpus longan* (LOLE) and *Citrus amblycarpa* (LILE) extracts resulted in moderate levels of apoptosis (15.47% and 22.66%, respectively), suggesting their potential in regulating liver cell death. The combination of both extracts led to a slightly lower apoptosis rate (16.54%), indicating a possible synergistic effect of the two extracts in protecting liver cells. These results are consistent with previous studies that suggest plant-derived compounds can modulate apoptosis by influencing oxidative stress and inflammatory pathways (Liu *et al*., 2022; Nechchadi *et al*., 2024).

Table 6 shows the average percentage of karyorrhexis, which refers to the fragmentation of the cell nucleus during cell death: Control (-) 20.79%, Control (+) 23.74%, LOLE 22.81%, LILE 26.65%, Combination 20.80%. Karyorrhexis indicates the breakdown of the nucleus, a key feature of necrosis or severe apoptosis. The highest rate of karyorrhexis was observed in the LILE group (26.65%), suggesting significant cellular damage or stress. In contrast, the combination group showed a lower percentage (20.80%), similar to the control negative group, which suggests that combining both plant extracts may help reduce nuclear damage. These results indicate that LOLE and LILE have distinct effects on liver cells, and their combination might offer protective benefits against severe cell damage. These findings align with studies showing that plant compounds, such as flavonoids and terpenoids, can support cellular integrity by reducing nuclear fragmentation (Liu *et al*., 2022; Nechchadi *et al*., 2024).

Table 7 presents the average percentage of karyolysis, a process where the cell nucleus dissolves due to severe damage: Control (-) 16.67%, Control (+) 24.00%, LOLE 25.42%, LILE 23.41%, Combination 23.21%. Karyolysis, the dissolution of the cell nucleus, is a clear indication of irreversible cell damage, often observed in necrotic cells. The control positive group (Simvastatin-treated) exhibited the highest karyolysis rate (24.00%), signifying significant liver cell damage, as anticipated from the pharmacological effects of Simvastatin. The treatment with both LOLE and LILE resulted in moderate karyolysis levels (25.42% and 23.41%, respectively), indicating that these extracts might help reduce more severe cell damage. The combination group showed slightly lower karyolysis (23.21%), suggesting that combining both extracts may provide enhanced protection against irreversible cell damage. These findings are in line with previous studies, which indicate that plant-derived bioactive compounds can help mitigate necrotic damage in liver tissue by modulating oxidative stress and lipid metabolism (Liu *et al*., 2022; Nechchadi *et al*., 2024).

The histological images in Figure 1 provide visual confirmation of the findings. The images display liver tissue from various experimental groups, including: Control (-), The liver appears relatively normal with no significant damage. Control (+), There is some hepatocyte damage and lipid accumulation, which reflects the effects of Simvastatin.

LOLE and LILE, Both groups show mild hepatocyte injury, but also signs of tissue regeneration, suggesting that the plant extracts may help protect the liver. Combination, This group shows the least amount of damage and the most tissue recovery, indicating that the combination of LOLE and LILE may have synergistic protective effects. The combination of *Dimocarpus longan* and *Citrus amblycarpa* leaf extracts shows promising potential in reducing liver cell damage and supporting liver regeneration, as demonstrated by the lower levels of apoptosis, karyorrhexis, and karyolysis.

Histopathological examination of liver tissues revealed distinct cellular alterations among the experimental groups. The positive control group (K+) showed marked hepatocellular damage, characterized by increased occurrence of apoptosis, karyorrhexis, and karyolysis, indicating severe cellular degeneration (Delli Bovi *et al.*, 2021). In contrast, the treatment groups receiving *Dimocarpus longan* and *Citrus amblycarpa* extracts showed improvements in liver architecture, characterized by fewer necrotic cells and a more uniform hepatocyte arrangement. Notably, the combination treatment group exhibited the most well-preserved hepatic structure, indicating a potential protective effect against hepatocellular damage. These observations are in line with previous studies suggesting that plant-derived bioactive compounds can mitigate liver injury by reducing oxidative stress and enhancing cellular morphology (Rahib *et al.*, 2024).

Overall, these findings suggest that the combination of *Dimocarpus longan* and *Citrus amblycarpa* extracts may provide a protective effect against hepatocellular damage, potentially through the synergistic action of their bioactive compounds. This interpretation is supported by previous studies indicating that plant-derived antioxidants can enhance cellular resilience to oxidative stress and improve tissue integrity (de Aquino *et al*., 2023).

CONCLUSION

The ethanol extracts of *Citrus amblycarpa* and *Dimocarpus longan* leaves demonstrated potential lipid-modulating effects in hypercholesterolemic rats, with the combination treatment showing greater effectiveness than individual extracts. Improvements in liver histological features were also observed in the treated groups. Nevertheless, it is important to note that lipid evaluation in this study was limited to total cholesterol measurements. Further investigation including additional lipid parameters such as LDL-C, HDL-C, and triglycerides is necessary. Moreover, the lack of biochemical liver function markers (e.g., ALT and AST) means that the hepatoprotective potential cannot yet be fully established. In summary, these findings suggest that *C. amblycarpa* and *D. longan* leaves may serve as promising complementary sources for managing hypercholesterolemia, although further studies are needed to confirm their efficacy and safety.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1: Phytochemical screening of 96% ethanol extract of *Dimocarpus longan* leaf

Phytochemicals	Reagent	Result	Indicator
Flavonoid			
1. Wilstätter Test	Mg Powder +	+	Red or Orange
2. NaOH 10% Test	Concentrated HCl NaOH 10%	+	Yellow
Alkaloid	HCl 2N + Mayer	+	Deposits Appear
Saponin	Hot Water + HCL 2N	+	Foam 1-10 cm after shaking
Tannin	$FeCl_3$	+	Dark Green or Dark Blue
Steroid & Terpenoid	Glacial Acetic Acid + H_2SO_4	+	Bluish green

+: **Presence of secondary metabolites**

Table 2: Phytochemical screening of 96% ethanol extract of *Citrus amblycarpa* leaf

Phytochemicals	Reagent	Result	Indicator
Flavonoid			
1. Wilstätter Test	Mg Powder +	+	Red or Orange
2. NaOH 10% Test	Concentrated HCl NaOH 10%	+	Yellow
Alkaloid	HCl 2N + Mayer	+	Deposits Appear
Saponin	Hot Water + HCL 2N	+	Foam 1-10 cm after shaking
Tannin	$FeCl_3$	+	Dark Green or Dark Blue
Steroid & Terpenoid	Glacial Acetic Acid + H_2SO_4	+	Bluish green

+: **Presence of secondary metabolites**

Table 3: Body weight loss (bw) measurement

Group	Day-1	Day-14	Day-21	BW Loss
Control (-)	209	244.3	231.2	13.1
Control (+)	207.5	242	215	27.0
LOLE	202.2	238.2	222.6	15.6
LILE	194.6	224.6	221.4	3,2
Combine	201	237	213.6	23,4
Mean	**202.86**	**237.22**	**220.76**	**18.57**

Control (-)= CMC-Na-induced rat; Control (+)= Simvastatin 10 mg induced rat; LOLE= *Dimocarpus longan* leaf extract induced rat; LILE= *Citrus amblycarpa* leaf extract induced rat; Combine= Rat induced with a mixture of *Dimocarpus longan* leaf extract and *Citrus amblycarpa* leaf extract in a 50:50 ratio

Table 4: The results of the total cholesterol level measurements

Group	Day-1	Day-14	Day-21	Mean±SD
Control (-)	124.4	209.2	199.8*	177.80 ± 46.49
Control (+)	127.4	217	153	165.80 ± 46.15
LOLE	125.4	215.8	168.4*	169.87 ± 45.23
LILE	127.6	215.2	166.8*	169.87 ± 43.87
Combine	126.6	219.6	161.6*	169.27 ± 46.98

Note: The results differed significantly from those of the positive control group (+), with a significance level of *p < 0.05

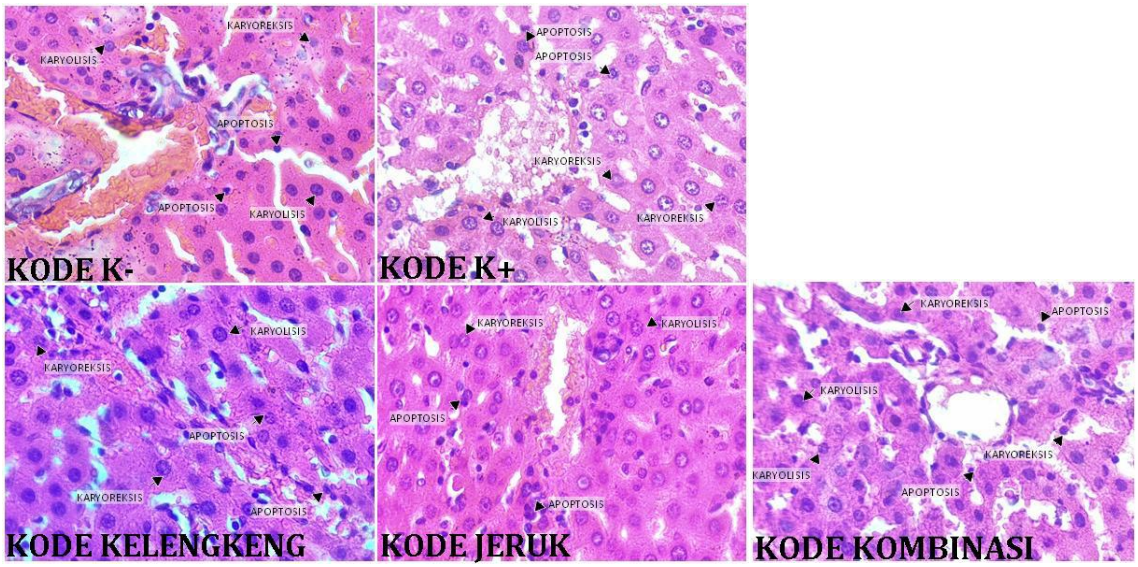

Figure 1: Histological profile of rat liver

KODE K- = Control (-)=CMC-Na-induced rat; KODE K+ = Control (+)=Simvastatin 10 mg induced rat; KODE KELENGKENG= LOLE= *Dimocarpus longan* leaf extract induced rat; KODE JERUK= LILE=*Citrus amblycarpa* leaf extract induced rat; KODE KOMBINASI= Combine= Rat induced with a mixture of *Dimocarpus longan* leaf extract and *Citrus amblycarpa* leaf extract in a 50:50 ratio

Table 5: The average measurement of apoptosis percentage

Group	The average percentage of apoptosis (%)
Control (-)	23,24
Control (+)	14,42
LOLE	15,47
LILE	22,66
Combine	16,54

Table 6: The average measurement of karyorrhexis percentage

Group	The average percentage of karyorrhexis (%)
Control (-)	20,79
Control (+)	23,74
LOLE	22,81
LILE	26,65
Combine	20,80

Table 7: The average measurement of karyolysis percentage

Group	The average percentage of karyolysis (%)
Control (-)	16,67
Control (+)	24,00
LOLE	25,42
LILE	23,08
Combine	23,21