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Histopathological Profile of Hepatic Steatosis in High Fat Diet-Fed Rats Treated with *Rhodomyrtus Tomentosa* Leaf Ethanolic Extract

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ABSTRACT

Background: A high-fat diet can increase cholesterol levels and trigger fat accumulation in the liver, causing steatosis. Karamunting leaves (*Rhodomyrtus tomentosa*) contain flavonoids, saponins, and phenolic compounds that have antioxidant potential and can modulate lipid metabolism. However, the effects of its extract on liver histology in animals induced with a high-fat diet have not been widely studied. **Objective:** This study aimed to determine the effects of various doses of ethanol extract of *Rhodomyrtus tomentosa* leaves on liver steatosis in rats induced with a high-fat diet. **Methods:** An experimental study with a posttest-only control group design was conducted using 25 male Wistar rats divided into five groups: a negative control group (Na-CMC 0.5% 500 mg), a positive control group (simvastatin 0.9 mg/kg BW), and three treatment groups administered Karamunting leaf extract at doses of 200, 400, and 800 mg/kg body weight. After induction with a high-fat diet, the rats received treatment. The liver tissue was then processed into histological sections using hematoxylin and eosin (H&E) staining, examined microscopically, and analyzed using ImageJ software. The collected data were analyzed using ANOVA. **Results:** The highest percentage of steatosis was found in the negative control group (1.56%) and the lowest in the positive control group (0.44%). The treatment groups showed steatosis values of 1.02% (E200), 0.54% (E400), and 0.81% (E800). The ANOVA results (p-value 0.078) showed no significant differences between the groups. **Conclusion:** There was no significant effect of various doses of *Rhodomyrtus tomentosa* leaves ethanol extract on hepatic steatosis in rats induced with a high-fat diet.

Keywords: High-fat diet; *Rhodomyrtus tomentosa*; Hepatic steatosis

INTRODUCTION

In today's modern era, Indonesians have a strong preference for fast food. Fast food is food that can be easily and quickly prepared as a substitute for home-cooked meals. This type of food often contains additives such as preservatives, flavor enhancers, and sweeteners, and has a very high fat content[1] . High fat intake causes an increase in total cholesterol levels in the blood, which can ultimately lead to narrowing and blockage of the coronary artery walls. High fat consumption also affects *Low Density Lipoprotein* (LDL) and triglyceride levels, which can lead to various serious health problems.[2] The liver is a central organ in lipid metabolism, producing very low-density lipoproteins (VLDL). If there is excess cholesterol in the body, it can inhibit the liver's ability to clear cholesterol. This can cause a spike in cholesterol and affect a person's health. Steatosis is a liver disease triggered by excessive synthesis of liver triglycerides.[3]

Indonesia is renowned for its high biodiversity, especially in terms of plants. Plants in Indonesia are abundant, particularly agricultural products, spices, and medicinal plants. This diversity is supported by Indonesia's geographical conditions, which have a tropical climate with frequent rainfall throughout the year.[4] Karamunting leaves (*Rhodomytrus tomentosa*) are one of the medicinal plants that are often the focus of research, both domestically and abroad. Karamunting are a plant native to Kalimantan that works well as a medicine. Karamunting plants contain more than 100 compounds that have medicinal value, especially in the leaves and fruit.[5]

Karamunting leaves are known to contain various phenolic chemical compounds, including alkaloids, flavonoids, tannins, saponins, and terpenoids. Karamunting leaves and fruit have very high flavonoid content.[6,7] Traditionally, the leaves of this plant have been used for various purposes, including as an antidiabetic, antibacterial against Staphylococcus aureus, anti-inflammatory, and its flowers are used as an anticholesterol and triglyceride.[8]

Research conducted by Khalilah et al in 2021 proved that administering ethanol extract from karamunting leaves can reduce total cholesterol and glucose in rats fed a high-fat diet. The results of the study showed that the best dose to produce this effect was 800 mg/kgBW. These findings provide evidence that administering the right dose has the potential to lower cholesterol and blood sugar levels. This is in line with research conducted by Anggraeni et al in 2021, which proved that the administration of ethanol extract from karamunting leaves has an effect on lowering cholesterol.[9,10] In order for the use of traditional medicine to be accepted by the community and health services, scientific evidence is needed that the medicine is beneficial and safe for use in humans. Based on a study conducted by Hidayati in 2011, the administration of ethanol from karamunting leaf extract (Rhodomyrtus tomentosa) did not affect the histology of the liver at doses of 10, 20, and 40 mg/kgBW.[11]

Based on the above description and the limited studies on the effect of ethanol from karamunting leaf extract on liver histology in rats, the researcher aims to investigate the effect of varying doses of ethanol extract from karamunting leaves (*Rhodomyrtus tomentosa*) on histological steatosis in rats induced by a high-fat diet.

METHODS

This study was a laboratory experimental study with a posttest-only control group design to evaluate the effect of varying doses of ethanol extract of karamunting leaves (*Rhodomyrtus tomentosa*) on the appearance of liver steatosis in Wistar rats induced by a high-fat diet. The study was conducted at the Biomedical Laboratory, Faculty of Medicine, University of Jambi. The experimental animals used were male Wistar rats aged 2.5–3 months weighing 160–180 grams, which first underwent a one-week adaptation period with standard feed and ad libitum water. After the adaptation period, the rats were fed a high-fat diet for four weeks to induce hypercholesterolemia. After feeding the high-fat diet for 4 weeks, the rats' blood was examined to see whether they had developed hyperlipidemia or not. Rats that developed hyperlipidemia were used as samples for the study.

The mouse samples were divided into five groups, namely a negative control group given 0.5% Na-CMC, a positive control group given simvastatin, and three treatment groups each receiving ethanol extract of karamunting leaves at doses of 200 mg/kgBW, 400 mg/kgBW, and 800 mg/kgBW. Karamunting leaf extract was obtained through a maceration process using 96% ethanol, then evaporated to a thick extract before being administered orally via a feeding tube every day for two weeks.

After all treatments were completed, prior to organ sampling, the rats were first anesthetized using ketamine–xylazine in a ratio of 8:1, then euthanized by cervical dislocation. Surgery was then performed to remove the liver, which was then processed into histological preparations. Liver samples were fixed in 10% formalin, processed using the paraffin block method, then cut to a thickness of 4–5 µm and stained using Hematoxylin–Eosin. The finished preparations were observed under a 40x magnification light microscope, then analyzed using ImageJ or Fiji software to calculate the percentage of liver cells undergoing steatosis according to the steatosis degree assessment criteria.

The data obtained were analyzed descriptively to describe the mean and distribution of steatosis grades in each group, then inferential analysis was performed using a one-way ANOVA test to determine the significant differences between treatment groups. A p-value < 0.05 was considered statistically significant.

RESULTS

A. Average Cholesterol Levels in Rats Induced by a High-Fat Diet Before and After Administration of Karamunting Extract in the Treatment and Control Groups

The following data shows the results before and after high-fat diet induction in male Wistar rats.

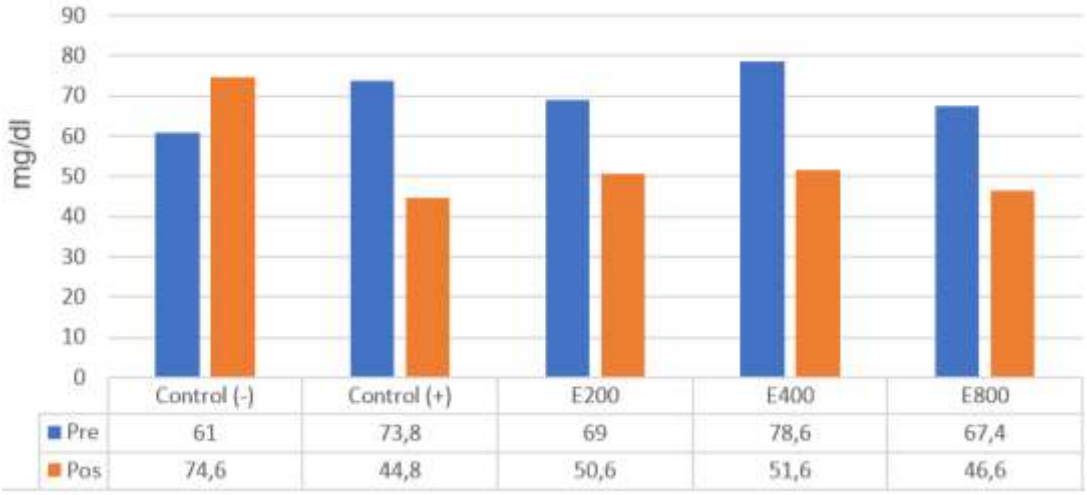

Figure 1. Cholesterol Levels Before and After Treatment

Based on the bar chart in Figure 1, the total cholesterol levels of rats induced with a high-fat diet showed different changes in each group after treatment. The negative control group experienced an increase in cholesterol levels from 61 mg/dL to 74.6 mg/dL. In contrast, the positive control group with simvastatin 0.9 mg/kgBW showed a decrease from 73.8 mg/dL to 44.8 mg/dL. Administration of karamunting leaf extract at various doses also showed a consistent decrease, namely at a dose of 200 mg/kgBW from 69 mg/dL to 50.6 mg/dL, at a dose of 400 mg/kgBW from 78.6 mg/dL to 51.6 mg/dL, and at a dose of 800 mg/kgBW from 67.4 mg/dL to 46.6 mg/dL. high-fat diet showed different changes in

B. Histopathological Features of Steatosis

Histopathological examination of liver tissue was performed to evaluate the morphological features of hepatic steatosis in each experimental group. Representative liver histopathological images of the negative control, positive control, and Karamunting leaf extract treatment groups are presented in Figure 2. Histopathological observation showed steatotic changes in the liver tissue across the experimental groups, characterized by macrovesicular and microvesicular lipid vacuoles within hepatocytes. The degree of histological involvement was mild across all groups. The negative control group showed steatotic changes following

high-fat diet induction, while steatotic changes were also observed in the positive control and Karamunting leaf extract-treated groups. Among the extract-treated groups, the E200, E400, and E800 groups showed varying amounts of steatotic involvement on histopathological examination. Overall, the histopathological findings demonstrated only mild hepatic steatosis across the experimental groups, consistent with the low proportion of affected hepatocytes observed in the quantitative assessment.

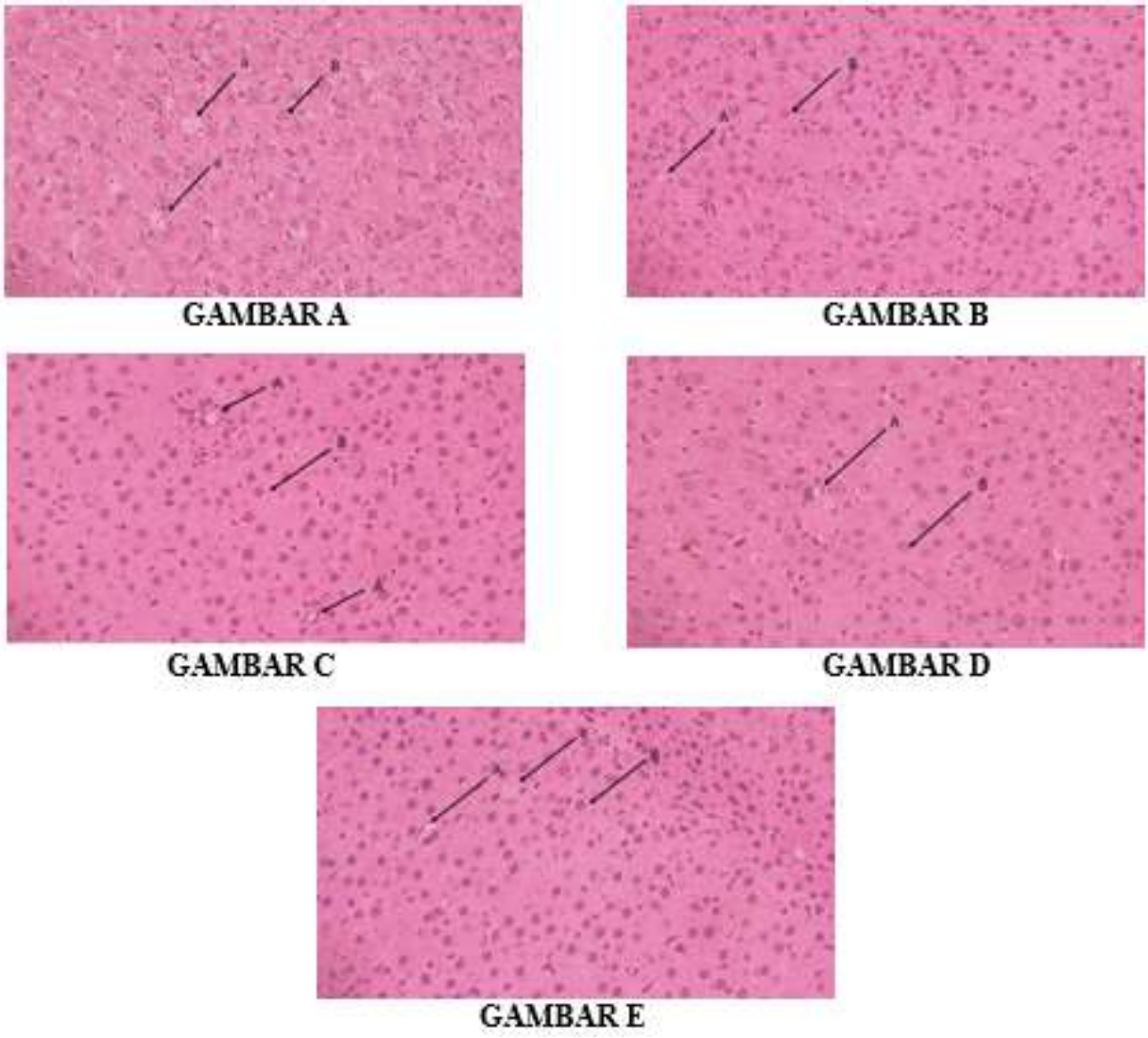

Figure 2. Representative histopathological features of liver tissue in rats induced by a high-fat diet. (A) Negative control group (K−); (B) Positive control group treated with simvastatin 0.9 mg/kgBW (K+); (C) Karamunting leaf extract 200 mg/kgBW (E200); (D) Karamunting leaf extract 400 mg/kgBW (E400); (E) Karamunting leaf extract 800 mg/kgBW (E800)

C. Percentage and Degree of Steatosis in the Liver of Mice Induced by a High-Fat Diet After Administration of Karamunting Extract in the Treatment and Control Groups

Table 1. Overview of the Average Percentage of Steatosis from Liver Histology in Rats Induced by a High-Fat Diet

Variable	Steatosis Percentage (%)	Grade
Negative Control	1.56	0
Positive Control	0.44	0
E200	1.02	0
E400	0.54	0
E800	0.81	0

Based on Table 1, it shows that the percentage of steatosis in the livers of rats induced by a high-fat diet varied, with the lowest percentage of steatosis found in the positive control group at 0.44%, while the highest percentage of steatosis was found in the negative control group at 1.56%..

D. Comparison of Hepatic Steatosis among Treatment Groups

Table 2. The Effect of Karamunting Leaf Extract on Steatosis in Liver Histology in Rats Induced by a High-Fat Diet

	Sum of Squares	df	Mean Square	F	Sig
Between Groups	0.157	4	0.039	2.741	0.078
In Group	0.318	20	0.016		
Total	0.476	24			

Based on Table 2, it shows that the significance value in the bivariate test using ANOVA is 0.078, so it can be concluded that there is no significant effect of karamunting leaf extract administration on the steatosis pattern in the histology of the liver of rats induced by a high-fat diet.

DISCUSSION

A. Effect of Karamunting Leaf Extract on Total Cholesterol Levels

This study showed that the total cholesterol levels of rats induced by a high-fat diet showed different changes in each group after treatment. The negative control group experienced an increase in cholesterol from 61 mg/dL to 74.6 mg/dL, confirming that a high-

fat diet increases cholesterol without intervention. In contrast, the positive control group given simvastatin showed a significant decrease from 73.8 mg/dL to 44.8 mg/dL, consistent with evidence that simvastatin effectively lowers cholesterol through HMG-CoA reductase inhibition as well as antioxidant and anti-inflammatory activities[12,] .

In the karamunting extract treatment group, cholesterol levels decreased at all doses. A dose of 200 mg/kgBW reduced levels from 69 mg/dL to 50.6 mg/dL, E400 from 78.6 mg/dL to 51.6 mg/dL, and E800 from 67.4 mg/dL to 46.6 mg/dL. This pattern indicates an increase in the hypolipidemic effect with increasing doses, which is thought to be related to the content of flavonoids, phenolics, and anthocyanins that have antioxidant activity and the potential to inhibit HMG-CoA reductase as mechanism similar to natural statins[13,14,15]

B. Histopathological Changes in Hepatic Steatosis

Histopathological examination of liver tissue showed mild steatotic changes across all experimental groups, characterized by macrovesicular and microvesicular steatosis in hepatocytes. Macrovesicular steatosis is characterized by the presence of large lipid vacuoles within the hepatocyte cytoplasm that may displace the nucleus toward the periphery, whereas microvesicular steatosis is characterized by multiple small lipid vacuoles distributed within the cytoplasm. These morphological findings represent intracellular lipid accumulation in hepatocytes following exposure to a high-fat diet.

Despite the presence of steatotic changes in the representative liver sections, the overall histological involvement was mild. Quantitative assessment showed that the proportion of steatotic hepatocytes was less than 5% in all experimental groups; therefore, all groups were classified as grade 0 steatosis. The negative control group showed the highest percentage of steatosis, whereas the positive control group treated with simvastatin showed the lowest percentage. Among the Karamunting leaf extract-treated groups, variations in steatotic involvement were observed, with the E400 group showing a histological percentage closest to that of the positive control group.

However, the histopathological differences observed among the groups should be interpreted cautiously. Although numerical differences in the percentage of steatosis were present, all groups remained within the same histological grade, indicating that the overall severity of hepatic steatosis was low. Furthermore, statistical analysis showed no significant difference in hepatic steatosis among the experimental groups (p=0.078). Therefore, the representative histopathological images primarily demonstrate mild hepatic steatotic involvement and should not be interpreted as evidence of a definitive treatment effect of Karamunting leaf extract.

The mild degree of steatosis observed in this study may also explain why morphological differences among the treatment groups were limited. Since the proportion of

affected hepatocytes remained below the threshold for grade 1 steatosis, differences between the negative control, positive control, and Karamunting extract groups were mainly numerical rather than differences in histological grade. Thus, although the histopathological findings provide visual support for the presence of hepatic steatosis, they should be interpreted together with the quantitative and statistical findings.

C. Effect of Karamunting Leaf Extract on Hepatic Steatosis

In this study, the lowest percentage of steatosis was found in the positive control group (0.44%) that was given simvastatin 0.9 mg/kgBW. Simvastatin has a protective effect against liver steatosis not only through cholesterol reduction, but also by increasing the activity of antioxidant enzymes such as SOD and GPx, reducing oxidative stress, and inhibiting the NF-κB inflammatory pathway that plays a role in the development of steatosis [35]. In addition, simvastatin improves sinusoid endothe function and reduces hepatic stellate cell activation, thereby reducing lipid accumulation and inflammation in the liver tissue .

Conversely, the highest percentage of steatosis was found in the negative control group that was given 0.5% Na-CMC as a solvent. Na-CMC is inert and does not affect lipid metabolism, so this group was fully exposed to the effects of a high-fat diet that triggers the accumulation of triglycerides and cholesterol in hepatocytes (37). A high-fat diet is known to increase fatty acid synthesis, inhibit β-oxidation, decrease VLDL secretion, and increase ROS, which causes oxidative stress, inflammation, and mitochondrial dysfunction, thereby exacerbating hepatocellular injury[16,17] .

Unlike the treatment groups that received antioxidant or hypolipidemic interventions, the negative control group lacked compensatory mechanisms to suppress these processes. Therefore, steatosis appeared most severe, and Na-CMC as a carrier did not affect cholesterol reduction or histological improvement[18] .

D. Comparison of the Effects of Karamunting Leaf Extract among Treatment Groups

This study found that administering varying doses of ethanol extract from karamunting leaves to rats induced with a high-fat diet had no significant effect on the severity of hepatic steatosis. All groups remained at the lowest degree of steatosis (grade 0). However, the 400 mg/kgBW dose group showed a percentage of steatosis that was close to that of the positive control group that received simvastatin. Karamunting leaf extract (Rhodomyrtus tomentosa) contains flavonoids, tannins, saponins, triterpenoids, and phenolic compounds that have antioxidant activity and are able to modulate lipid metabolism. These compounds have the potential to suppress lipogenesis, increase β-oxidation, and reduce triglyceride accumulation in hepatocytes, thereby maintaining the liver tissue at a low histological grade despite exposure to a high-fat diet.

Previous phytopharmacological studies have also shown that the phenolic and flavonoid content of R. tomentosa can suppress lipid formation through the regulation of inflammatory pathways and increased antioxidant activity. However, the effectiveness of histological changes is influenced by the dose, duration of administration, and bioavailability of the active compounds. The study by Wang et al. reported that the modulation of lipid metabolism by R. tomentosa phenolic extract was only evident at certain concentrations and durations (19), while Situmorang's study showed that low doses or short-term interventions did not produce significant histological changes[19] .

CONCLUSION

In the negative control group, total cholesterol levels increased after a high-fat diet. In the positive control group (simvastatin administration), cholesterol levels decreased. In the treatment group with karamunting extract, all doses showed a decrease in total cholesterol levels. The smallest percentage of steatosis in the livers of rats induced by a high-fat diet was found in the positive control group, while the largest percentage of steatosis was found in the negative control group. Statistically, a sig. value >0.078 was obtained, which means that there was no significant effect of karamunting leaf extract on steatosis in the histology of the livers of rats induced by a high-fat diet.

RECOMMENDATIONS

Future researchers are advised to add a pretest before treatment so that the changes that occur can be compared more comprehensively. It is hoped that students can utilize the results of this study as learning materials and references, as well as develop similar studies with more complete designs so that the results obtained are more comprehensive.

REFERENCES

1. *Alfora D, Saori E, Fajriah LN. Scientific Journal of Health The effect of fast food consumption on adolescent nutrition. J Ilm Kesehat. 2023;2(1):43–9.*
2. *Sahara LI, Adelina R. Analysis of Fat Intake on Blood Fat Profile Related to Coronary Heart Disease (CHD) Incidence in Indonesia: A Literature Study. Binawan University Journal of Food, Health, and Nutrition. 2021;1(2):48–60.*
3. *Heeren J, Scheja L. Metabolic Fatty Liver Disease and Lipoprotein Metabolism. Mol Metab [Internet]. 2021;50(April):101238. Available from: https://doi.org/10.1016/j.molmet.2021.101238*
4. *Ministry of Environment and Forestry. Biodiversity and Bioprospecting Development Potential in Indonesia. 2023.*
5. *Ernawati S, Rahayu SE, Suprihatin, Yenisbar. Medicinal Potential of Karamunting (Rhodomyrtus tomentosa). Vol. 1, UNAS Press. 2019. 6–6 pp.*
6. *Ministry of Health. Indonesian Herbal Pharmacopoeia. 2017.*
7. *RI K. 2018 National Riskesdas Report.pdf [Internet]. Balitbangkes Publishing Agency. 2018. p. 156. Available from: https://repository.badankebijakan.kemkes.go.id/id/eprint/3514/1/Laporan Riskesdas 2018 Nasional.pdf*
8. *Nursamsiar N. The Effect of Extraction Methods on the Antioxidant Activity of Karamunting Leaf Extract (Rhodomyrtus Tomentosa (Aiton) Hassk) Using the PPH Method. Farmasyifa Scientific Journal of Pharmacy. 2022;5(2):183–91.*
9. *Anggraeni LN, Fakhruddin F, Irawan Y. The Effect of Ethanol Extract of Karamunting Leaves (Rhodomyrtus tomentosa (Ait.) Hassk.) on Cholesterol and Triglyceride Levels in Hyperlipidemic White Rats. Jurnal Borneo*

Cendekia. 2021;5(1):96–104.
10. *Wida Ekaputri Hz T, Khalilah N, Nofri Enis R. The Effect of Ethanol Extract of Karamunting Leaves (Rhodomyrtus tomentosa) on Total Cholesterol and Blood Glucose Levels in Male Rats (Rattus norvegicus) Induced by a High-Fat Diet. Jmj. 2022;10(2):306–13.*
11. *Vaja R, Rana M. Drugs and the liver. Anaesth Intensive Care Med. 2023;24(9):536–42.*
12. *Latif WD, Aswad M, Bahar MA. Comparison of Atorvastatin (. Journal of Clinical Pharmacy Sciences. 2022;*
13. *Zaidan S, Abdillah S, Arfian N, Arozal W. Hypolipidemic effects of brown seaweed extract (Sargassum crassifolium) in vivo. -. 2024;*
14. *Antioxidant Lipid Supplements in Cardiovascular Risk Factors: A Systematic Review and Meta-Analysis. Nutrients. 2024;16(14):2213.*
15. *Bravo M, Ramirez T, Fernandez I, De la Peña A, Prieto J, Medina JF. Statins improve hepatic endothelial dysfunction and steatosis in experimental non-alcoholic steatohepatitis. Sci Rep [Internet]. 2019;9:20166. Available from: https://www.nature.com/articles/s41598-019-56366-2*
16. *Wang X, Li Y, Zhang Z, et al. A high-fat diet exacerbates hepatic steatosis through modulation of lipid metabolism and inflammation in mice. Nutrients [Internet]. 2023;15(7):1582. Available from: https://doi.org/10.3390/nu15071582*
17. *Fan J, Chen L, Hu J, et al. Lipid metabolism and non-alcoholic fatty liver disease: The role of hepatic lipid toxicity and inflammation. Front Physiol [Internet]. 2022;13:826349. Available from: https://doi.org/10.3389/fphys.2022.826349*
18. *Gao M, Xu Y, Qiu J, Zhang Y, He P. Changes in gut microbiota and hepatic lipid metabolism in rats fed a high-fat diet due to sodium carboxymethyl cellulose. Front Nutr. 2021;8:715322.*
19. *Wang J, Sun H, Zhang X, et al. The Effect of Simvastatin on Gut Microbiota and Lipid Metabolism in Hyperlipidemic Mice Induced by a High-Fat Diet. Front Pharmacol [Internet]. 2020;11:522. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC7201051/*