International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

The Influence of Sambiloto Leaf Extract (Andrographis Paniculata Nees) on Histopathologic Imaging of The Liver of Male Wistar Rats (Rattus Novergicus) on a High Cholesterol Diet

Conradi Putra Bhakti Leky[1], Derri Riskiyanti Tallo Manafe[2], Anita Lindesni Shinta Amat[3], Efrisca Meliyuita Boru Damanik[4]

[1]Faculty of Medicine and Veterinarian, Universitas Nusa Cendana
[2]Departement of Physiology, Faculty of Medicine and Veterinarian, Universitas Nusa Cendana
[3]Departement of Biochemistry, Faculty of Medicine and Veterinarian, Universitas Nusa Cendana
[4]Departement of Anatomy Pathology, Faculty of Medicine and Veterinarian, Universitas Nusa Cendana

ABSTRACT: Elevated cholesterol levels can lead to various diseases and reduce quality of life, including non-alcoholic fatty liver disease (NAFLD), which poses a growing global health concern. Andrographis paniculata (sambiloto) contains andrographolide, a bioactive compound known to inhibit cholesterol synthesis, suggesting its potential as a herbal alternative to manage hypercholesterolemia. This study aimed to evaluate the effect of sambiloto leaf extract (Andrographis paniculata Nees) on the liver histopathology of male Wistar rats (Rattus norvegicus) fed a high-cholesterol diet. This true experimental laboratory study used a post-test control group design with randomized probability sampling. Rats were divided into six groups: normal control, negative control, positive control (atorvastatin), and three treatment groups receiving sambiloto extract at doses of 200, 400, and 800 mg/kgBW. Liver histopathology was assessed using the NAFLD Activity Score (NAS), and data were analyzed using the Kruskal–Wallis test followed by post hoc LSD analysis. The results showed a significant effect of sambiloto extract on liver histopathological changes ($p = 0.028$, $p < 0.05$), although no significant differences were observed among the different dosage groups. In conclusion, sambiloto leaf extract has a significant influence on improving liver histopathological profiles in male Wistar rats fed a high-cholesterol diet.

KEYWORDS: Sambiloto, Wistar Rats, Hipercholesterolemia, NAFLD activity score

INTRODUCTION

Hypercholesterolemia is a condition characterized by high levels of cholesterol in the blood, which can lead to the narrowing of blood vessels. According to the World Health Organization, hypercholesterolemia is one of the non-communicable diseases responsible for 71% of deaths worldwide.(1) According to the Ministry of Health of the Republic of Indonesia, 43.8% of 96 individuals who visited the Integrated Coaching Post (Posbindu) for non-communicable disease screening in East Nusa Tenggara (NTT) were found to have hypercholesterolemia.(2) One of the consequences of hypercholesterolemia is non-alcoholic fatty liver disease (NAFLD). NAFLD is one of the leading causes of liver disease globally and is associated with various metabolic conditions such as obesity, type 2 diabetes, hyperlipidemia, hypertension, and metabolic syndrome. A study by Buzzetti et al. (2016) also demonstrated that excessive lipid intake in hyperlipidemia can activate inflammatory pathways, leading to inflammation and fibrosis, as seen in NAFLD and NASH.(3) Hypercholesterolemia is believed by the Indonesian population to be treatable through various herbal remedies. One of the plants believed by Indonesians to help manage hypercholesterolemia is Sambiloto (Andrographis paniculata).(4)Various studies conducted by Toppo et al. (2017), Akhtar et al. (2016), and Liu et al. (2020) have shown that Sambiloto extract, specifically andrographolide and its derivatives can help reduce lipid accumulation in the livers of animals fed with a high-fat diet, and can also help regulate metabolic alterations in obese rats back to normal levels.(5–7)

METHOD

The method used in this study was a laboratory experimental study with a true experimental design post-test with control group approach. The research subjects were divided into six groups, namely the normal group (standard feed, CMC, and distilled water ad

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

libitum); the negative control group (standard feed, CMC, distilled water ad libitum, and hypercholesterol diet); the positive control group (atorvastatin, standard feed, CMC, distilled water ad libitum, and hypercholesterol diet); treatment group 1 (sambiloto extract solution at a dose of 200 mg/kgBW, standard feed, CMC, distilled water ad libitum, and hypercholesterol diet); treatment group 2 (sambiloto extract solution at a dose of 400 mg/kgBW, standard feed, CMC, distilled water ad libitum, and hypercholesterol diet); and treatment group 3 (sambiloto extract solution at a dose of 800 mg/kgBW, standard feed, CMC, distilled water ad libitum, and hypercholesterol diet). All research subjects underwent body weight measurement to assess eligibility through inclusion criteria, as well as cholesterol level measurement before and after treatment. Data homogeneity was tested using Levene's test, and data normality was tested using the Shapiro–Wilk test. The bivariate test used to assess changes in liver histopathology, namely liver steatosis in the form of NAFLD Activity Scoring, was the Kruskal–Wallis test and the post hoc test used was LSD (Least Significant Difference).

RESULT

Table I. Total Cholesterol Levels Of Subjects

Animal Test Group	Mean cholesterol levels (mg/dL)		
	Day 7	Day 21	Day 36
Normal Group	48,95	28,23	16,88
Negative Control	48,95	94,18	73,97
Positive Control	36,75	84,51	25,06
Treatment Group 1	21,03	104,59	18,39
Treatment Group 2	52,10	81,11	39,81
Treatment Group 3	49,41	81,77	17,39
Total	42,03	79.07	31,92
Normal value : 10-54 mg/dL			

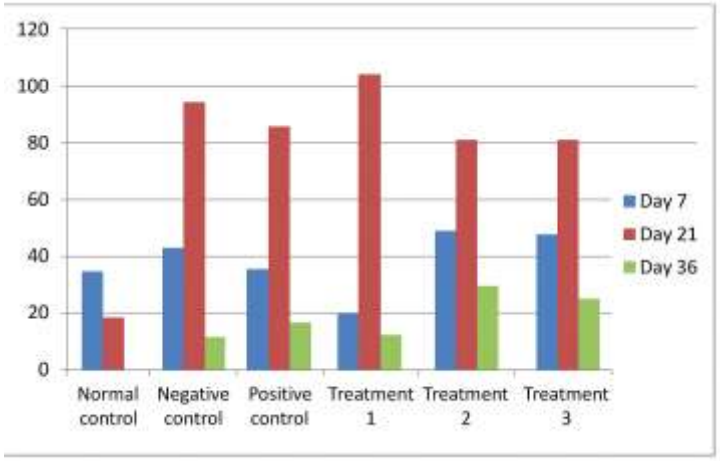

Figure 1: Subject's Cholesterol Change

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

Administration of a high-cholesterol diet for two weeks in five groups of subjects showed an increase in total cholesterol levels in all experimental groups, except for the normal control group. After 14 days of cholesterol administration, the rats were given treatments according to their respective groups. All experimental groups showed a decrease in cholesterol levels.

Table II. Mean *NAFLD Activity Score* Value Of Subjects

Test Groups	Mean Steatosis Value	Mean Inflammation Value	Mean Cell Ballooning Value	Mean NAS Value
Normal Control	6.125%	0,75	0	1,5
Negative Control	47,56%	1,4	2	3,2
Positive Control	7,76%	0,25	0	1,25
Treatment 1	10,92%	0	0	1
Treatment 2	13,12%	0,4	0,4	1,8
Treatment 3	12,962%	0,75	0,75	1,5

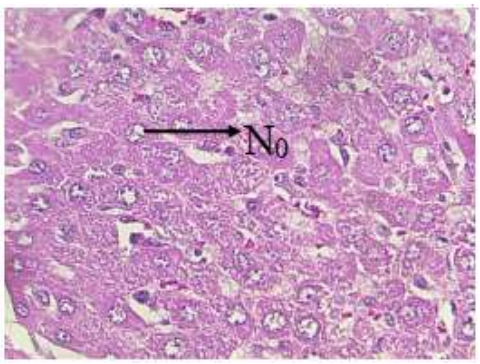

Figure 2: Normal Control Group (400x Magnification)
Description: N0: Normal Hepatocyte Cell

In the normal group, which was given only standard feed, minimal hepatic steatosis was observed, indicated by an average NAFLD Activity Score of 1.5.

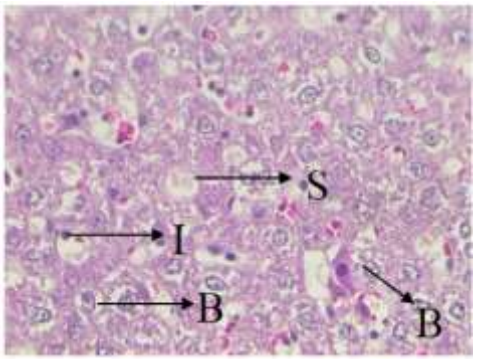

Figure 3: Negative Control Group (400x Magnification)
Description: N0: Normal Hepatocyte Cell | S: Steatotic Hepatocyte Cell | I: Inflammatoric Hepatocyte Cell | B: Ballooning Hepatocyte Cell

In the negative control group, which was given a hypercholesterol diet, moderate hepatic steatosis was observed, indicated by an average NAFLD Activity Score of 3.2.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

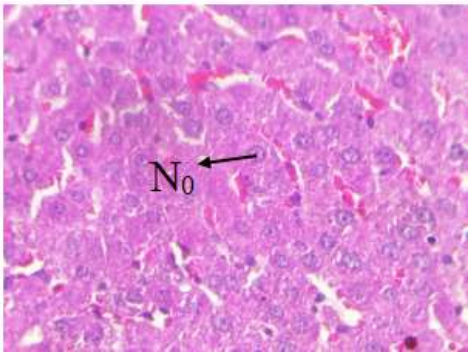

Figure 4: Positive Control Group (400x Magnification)
Description: N0: Normal Hepatocyte Cell

In the positive control group, which was given a hypercholesterol diet followed by atorvastatin administration, minimal hepatic steatosis was observed, indicated by an average NAFLD Activity Score of 1.25.

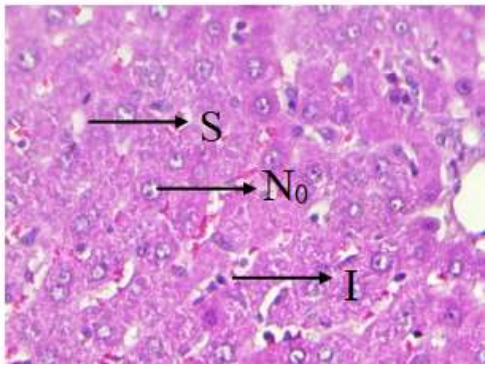

Figure 5: Treatment Group 1 (400x Magnification)
Description: N0: Normal Hepatocyte Cell | S: Steatotic Hepatocyte Cell | I: Inflammatoric Hepatocyte Cell

In treatment group 1, which was given a hypercholesterol diet followed by sambiloto extract at a dose of 200 mg/kgBW, minimal hepatic steatosis was observed, indicated by an average NAFLD Activity Score of 1.

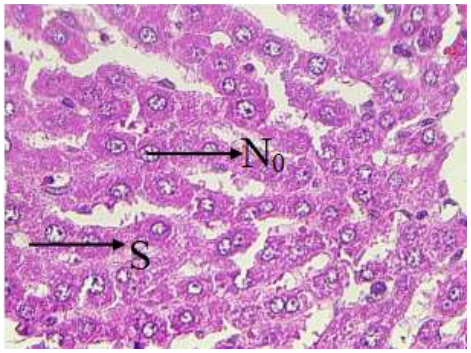

Figure 6: Treatment Group 2 (400x Magnification)
Description: N0: Normal Hepatocyte Cell | S: Steatotic Hepatocyte Cell

In treatment group 2, which was given a hypercholesterol diet followed by sambiloto extract at a dose of 400 mg/kgBW, minimal hepatic steatosis was observed, indicated by an average NAFLD Activity Score of 1.8.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

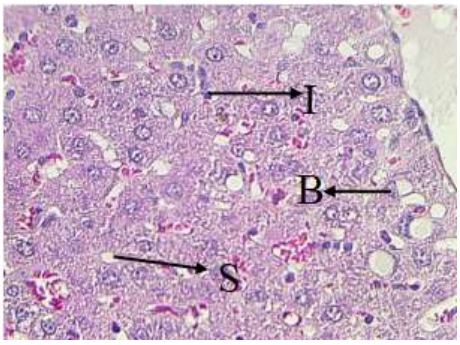

Figure 7: Treatment Group 3 (400x Magnification)
Description: S: Steatotic Hepatocyte Cell | I: Inflammatoric Hepatocyte Cell | B: Ballooning Hepatocyte Cell

In treatment group 3, which was given a hypercholesterol diet followed by sambiloto extract at a dose of 800 mg/kgBW, minimal hepatic steatosis was observed, indicated by an average NAFLD Activity Score of 1.5.

Table III. LSD Analysis of Normal Group with Negative Control Group, Positive Control Group, Treatment Group 1, Treatment Group 2, Treatment Group 3

Normal Control	Vs.	Negative Control	P = 0.003
	Vs.	Positive Control	P = 0.447
	Vs.	Treatment Group 1	P = 0.274
	Vs.	Treatment Group 2	P = 0.732
	Vs.	Treatment Group 3	P = 0.343

The analysis results presented in Table III show a significant difference between the normal control group and the negative control group, while the normal group showed no difference in liver histopathological features compared to the other treatment groups.

Table IV. LSD Analysis of Negative Group with Positive Control Group, Treatment Group 1, Treatment Group 2, Treatment Group 3

Negative Control	Vs.	Positive Control	P = 0.000
	Vs.	Treatment Group 1	P = 0.000
	Vs.	Treatment Group 2	P = 0.004
	Vs.	Treatment Group 3	P = 0.000

The analysis results in Table IV show a significant difference between the negative control group and all other groups, namely the normal control group, the positive control group, treatment group 1, treatment group 2, and treatment group 3.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

Table V. LSD Analysis of Positive Group with Treatment Group 1, Treatment Group 2, Treatment Group 3

Positive Control	Vs.	Treatment Group 1	$P = 0.762$
	Vs.	Treatment Group 2	$P = 0.134$
	Vs.	Treatment Group 3	$P = 0.920$

The analysis results in Table 4 show that there was no significant difference between the positive control group and treatment groups 1, 2, and 3.

Table VI. LSD Analysis of Treatment Group 1 with Treatment Group 2, Treatment Group 3

Treatment Group 1	Vs.	Treatment Group 2	$P = 0.134$
	Vs.	Treatment Group 3	$P = 0.920$

The analysis results in Table VI show no significant difference in the liver histopathological features of rats in treatment group 1 compared to treatment groups 2 and 3.

Table VII. LSD Analysis of Treatment Group 2 with Treatment Group 3

Treatment Group 2	Vs.	Treatment Group 3	$P = 0.185$

The analysis results in Table 6 show that there was no significant difference in the liver histopathological features of rats in treatment group 2 compared to treatment group 3.

DISCUSSION

Based on the bivariate analysis results, the significance value of p = 0.033 in Table 4.3 indicates that sambiloto extract has an effect that causes changes in the histopathological features of white rats given a hypercholesterol diet. This finding is consistent with the study conducted by Jong FHH et al.[10].

The normal control group had a low level of fatty liver disease, as this group did not receive any treatment in the form of a hypercholesterol diet. Consequently, few hepatic steatosis markers such as steatosis, inflammation, and cell ballooning were observed in the liver tissue. However, a small degree of steatosis was still present, with the average steatosis level in the normal group reaching 6.125%. This may occur because rats naturally possess a normal amount of fat within liver tissue, and it may also be influenced by the standard chow diet provided to the rats.[11]

In the negative control group, moderate hepatic steatosis was observed, indicated by an average NAS score of 3.2. This shows that the level of steatosis in the negative control group was higher than in the other groups. Histologically, the negative control group exhibited an average hepatic steatosis of 47.56%, with an average of 1.4 inflammatory cells and 2 ballooning cells. This occurred because the rats in the negative control group were given a hypercholesterol diet, leading to increased fat accumulation in the liver tissue.

In the positive control group, the NAS score was 1.3, indicating a low degree of hepatic steatosis. The average percentage of steatotic hepatocytes was 7.76%, the average number of inflammatory cells was 0.25, and no ballooning cells were observed. This result was due to the administration of a hypercholesterol diet followed by treatment with atorvastatin, a drug belonging to the statin class.

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

In treatment group 1, the NAS score was 1, with an average of 10.92% steatotic cells, no inflammatory cells, and no ballooning cells. This finding suggests that rats given a hypercholesterol diet followed by sambiloto extract at a dose of 200 mg/kgBW experienced a reduction in hepatic fat accumulation. This aligns with the theory that andrographolide extract in sambiloto can decrease the expression of sterol regulatory element-binding proteins (SREBP), which are key components in the biosynthesis of cholesterol, fatty acids, and triglycerides. The downregulation of SREBP leads to decreased cellular lipid accumulation, thereby reducing lipid deposition in the liver and lowering the NAS score.[12]

In treatment group 2, the NAS score was 1.8, with 13.12% steatotic cells, an average of 0.4 inflammatory cells, and an average of 0.4 ballooning cells. This indicates that andrographolide in sambiloto extract at a dose of 400 mg/kgBW also reduced hepatic fat accumulation, as reflected by a significantly lower NAS score compared to the negative control group. However, there was no difference in NAS scores between treatment groups 2 and 1. This may be attributed to the higher initial and final cholesterol levels in treatment group 2, as shown in Figure 4.1. Such data may suggest that the hepatic steatosis process occurred more rapidly or extensively in group 2, as elevated intrahepatic triglyceride levels resulting from high cholesterol may induce insulin resistance, thereby accelerating hepatic de novo lipogenesis.[13]

In treatment group 3, the NAS score was 1.5, with an average steatosis level of 12.962%, an average of 0.75 inflammatory cells, and an average of 1.5 ballooning cells. These results indicate that sambiloto extract at a dose of 800 mg/kgBW was able to reduce hepatic fat accumulation, though it was less effective than in treatment group 1. This may also be due to the high initial cholesterol levels in the animals of treatment group 3, where elevated hepatic triglycerides could accelerate fat accumulation induced by insulin resistance.[13]

Hepatic steatosis is caused by an increased intake of cholesterol. Elevated cholesterol levels lead to an increase in triglycerides, which are produced through the esterification of glycerol and free fatty acids. These triglycerides can accumulate in the form of macrovesicular or microvesicular vesicles, which can be observed in histopathological examinations. Triglycerides may be obtained through dietary intake, such as a hypercholesterol diet.[3]

The andrographolide content in sambiloto has an inhibitory effect on cholesterol and fatty acid synthesis in metabolism. This inhibitory effect occurs through the downregulation of sterol regulatory element-binding proteins (SREBP), which are essential components in the biosynthesis of cholesterol, fatty acids, and triglycerides. SREBP functions as a transcription factor that regulates lipid biosynthesis and adipogenesis by controlling the expression of several enzymes required for the synthesis of cholesterol, fatty acids, triacylglycerols, and phospholipids.[14]

Andrographolide has also been shown to reduce the expression of HMG-CoA reductase (HMG-CoAR). A decrease in HMG-CoAR expression reduces the conversion of HMG-CoA into mevalonate, an essential component in cholesterol synthesis.[15] Through the inhibition of cholesterol, fatty acid, triacylglycerol, and phospholipid synthesis, cholesterol accumulation decreases, resulting in a reduction of fatty acids in liver tissue. Consequently, hepatic steatosis decreases, which can be detected through NAS scoring.[16]

In this study, increasing the dose of sambiloto extract did not result in a greater improvement in hepatic steatosis. However, this does not necessarily indicate the absence of significant differences between doses. Certain factors, such as variations in cholesterol levels among treatment groups, may have influenced the results. Differences in blood cholesterol levels can affect the rate of fat accumulation in liver tissue, leading to variations in the progression of steatosis between groups and consequently affecting the activity of andrographolide in sambiloto.[13]

CONCLUSION

There was a significant effect of Andrographis paniculata (sambiloto) extract administration on the liver histopathological features of male Wistar rats (Rattus norvegicus) with hypercholesterolemia. Differences in histopathological findings were observed between rats treated with sambiloto extract and those that did not receive sambiloto or atorvastatin (negative control group). However, no significant differences were found between the sambiloto-treated groups and the positive control group (atorvastatin), nor among the sambiloto-treated groups receiving different doses.

REFERENCES

1. World Health Organization. Noncommunicable diseases (Internet). 2021 (cited 2021 Aug 5);1. Available from: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

International Journal of Current Science Research and Review
ISSN: 2581-8341
Volume 08 Issue 10 October 2025
DOI: 10.47191/ijcsrr/V8-i10-21, Impact Factor: 8.048
IJCSRR @ 2025
www.ijcsrr.org

2. Kemenkes.RI. Profil Penyakit Tidak Menular Tahun 2016. 2016.
3. Buzzetti E, Pinzani M, Tsochatzis EA. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism (Internet) 2016;65(8):1038–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26823198
4. Yuliani RD. Tanaman Obat yang bisa digunakan untuk melarutkan kolesterol jahat (Internet). Dinkes Kulon Progo2020 (cited 2021 Jun 30); Available from: https://dinkes.kulonprogokab.go.id/detil/511/tanaman-obat-yang-bisa-digunakan-untuk-melarutkan-kolesterol-jahat
5. Toppo E, Darvin SS, Esakkimuthu S, Nayak MK, Balakrishna K, Sivasankaran K, et al. Effect of two andrographolide derivatives on cellular and rodent models of non-alcoholic fatty liver disease. Biomed Pharmacother 2017;95:402–11.
6. Akhtar MT, Sarib MSBM, Ismail IS, Abas F, Ismail A, Lajis NH, et al. Anti-diabetic activity and metabolic changes induced by Andrographis paniculata plant extract in obese diabetic rats. Molecules 2016;21(8).
7. Liu YT, Chen HW, Lii CK, Jhuang JH, Huang CS, Li ML, et al. A diterpenoid, 14-deoxy-11, 12-didehydroandrographolide, in andrographis paniculata reduces steatohepatitis and liver injury in mice fed a high-fat and high-cholesterol diet. Nutrients 2020;12(2).
8. University JH. The Rat (Internet). (cited 2021 May 3);Available from: http://web.jhu.edu/animalcare/procedures/rat.html
9. Nistiar F, Racz O, Lukacinova A, Hubkova B, Novakova J, Lovasova E, et al. Age dependency on some physiological and biochemical parameters of male Wistar rats in controlled environment. J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng 2012;47(9):1224–33.
10. Jong FHH, Gunawan A, Santoso MWA, Anjani S, Tirthaningsih NW, Basori A. Effects of Sambiloto Ethanol Extract on Fatty Liver, SGOT/SGPT Levels and Lipid Profile of Wistar Strain White Rat (Rattus norvegicus) Exposed to High-Fat Diet. Folia Medica Indones (Internet) 2018;54(2):89. Available from: https://e-journal.unair.ac.id/FMI/article/view/8856
11. Yaligar J, Gopalan V, Kiat OW, Sugii S, Shui G, Lam BD, et al. Evaluation of Dietary Effects on Hepatic Lipids in High Fat and Placebo Diet Fed Rats by In Vivo MRS and LC-MS Techniques. PLoS One (Internet) 2014;9(3):10. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0091436#authcontrib
12. Ding L, Li J, Song B, Xiao X, Huang W, Zhang B, et al. Andrographolide prevents high-fat diet-induced obesity in C57BL/6 mice by suppressing the sterol regulatory element-binding protein pathway. J Pharmacol Exp Ther 2014;351(2):474–83.
13. Hydes T, Alam U, Cuthbertson DJ. The Impact of Macronutrient Intake on Non-alcoholic Fatty Liver Disease (NAFLD): Too Much Fat , Too Much Carbohydrate , or Just Too Many Calories ? Distribution of Excess Energy Amongst. 2021;8(February).
14. Bertolio R, Napoletano F, Mano M, Maurer-stroh S, Fantuz M, Zannini A, et al. Mechanical Cues and Lipid Metabolism. Nat Commun (Internet) 2019;1–11. Available from: http://dx.doi.org/10.1038/s41467-019-09152-7
15. Rajaratinam H, Nafi SNM. Andrographolide is an Alternative Treatment to Overcome Resistance in ER-Positive Breast Cancer via Cholesterol Biosynthesis Pathway. Malaysian J Med Sci 2019;26(5):6–20.
16. Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol (Internet) 2014;20(42):15539–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25400438

Cite this Article: Bhakti Leky, C.P., Tallo Manafe, D.R., Shinta Amat, A.L., Damanik, E.M.B. (2025). The Influence of Sambiloto Leaf Extract (Andrographis Paniculata Nees) on Histopathologic Imaging of The Liver of Male Wistar Rats (Rattus Novergicus) on a High Cholesterol Diet. International Journal of Current Science Research and Review, 8(10), pp. 5092-5099. DOI: https://doi.org/10.47191/ijcsrr/V8-i10-21