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The impact of using Binahong (Anredera Cardifolia) leaf extract on male wistar white rats fed a high-fat diet's liver function and liver histopathology

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ABSTRACT

Worldwide, Nonalcoholic Fatty Liver Disease (NAFLD) is the leading cause of chronic liver disease. Nutritional excess causes adipose depot enlargement and ectopic fat buildup, causing NAFLD. The Binahong plant (Anredera cordifolia) contains saponins, alkaloids, polyphenols, flavonoids, and mono polysaccharides like L-arabinose, D-galactose, L-rhamnose, and D-glucose, which have been shown to help NAFLD. The study examined the liver function and histopathology of male white Wistar rats fed high-fat diets after Binahong leaf extract was administered. Research approach/sample/population: Quantitative research employing lab or experiment formats. We sampled 24 white Wistar rats (Rattus norvegicus). A high-fat diet was the precondition, Binahong leaf extract was the independent variable, and liver function and histological characteristics improved. The research approach begins with test animal acclimation, Binahong leaf extract extraction, phytochemical screening, treatment protocols, examination, and Histopathology preparations. Research data was processed with SPSS. The normality test shows a 2-tailed significance of 0.678 > 0.05, indicating regularly distributed data. SGOT 0.10 and SGPT 0.20 liver function analyses demonstrated homogeneity p values > 0.05. The research found that Binahong leaf extract (Anrederacordifolia Ten. Steenis) has 400mg/BW antioxidants and can restore liver function in high-fat livers.

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INTRODUCTION

Eating the correct amounts of macronutrients (foods that provide energy and tissue repair) without going overboard on these nutrients is the hallmark of a healthy diet, as is ensuring enough water and other micronutrients (López-Gil & Tárraga-López, 2022; Von Braun et al., 2023). The energy required for cellular functions essential for daily operation is supplied by macronutrients, which include carbs, proteins, and fats. The body needs trace levels of

micronutrients (such as vitamins and minerals) to carry out normal metabolic processes, growth, and development (Cena & Calder, 2020).

The liver alone produces several plasma proteins. The liver regulates blood glucose based on hormones and neurological impulses (Bedossa, 2017; Murel, 2017; Ozougwu, 2017; Sharma & Nagalli, 2022). It stores food glucose as glycogen and uses blood glucose for energy. Gluconeogenesis, mainly in the liver, stabilizes fasting blood glucose. The liver metabolizes dietary lipids, generates cholesterol, triglycerides, and lipoproteins, and extracts and processes them (Yip et al., 2021). Aside from breaking down cholesterol into bile acids, the liver also secretes these chemicals into the bile, which helps the body absorb fat-soluble fats and vitamins (Moriles & Azer, 2020).

Toxins and medicines are primarily processed in the liver. Biotransformation converts lipophilic molecules to hydrophilic ones for elimination. The liver regulates blood hormone levels as the primary site of hormone catabolism (Murel, 2017). The liver also has a role in hormone synthesis, such as hepcidin, thrombopoietin, erythropoietin, insulin-like growth factor 1, and vitamin D, a 25-OH prohormone. Laboratory tests can provide a clearer picture of several of these liver functions. They understand the honesty of the soul (Neuschwander-Tetri, 2017; Powell et al., 2021; Upadhyay et al., 2022).

Significant organs like the liver can perform their activities with a substantial reserve capacity. Although liver disease is severe, patients usually function. Damage-detecting tests are needed to diagnose liver disease (Cariello et al., 2010). Plasma activity of liver cell enzymes, which produce patterns during injury, is usually measured. Chronic liver damage generally causes fibrosis; fibrotic indicators may indicate damage (Berumen et al., 2021; Pinzani et al., 2005; Roehlen et al., 2020). Chronic inflammation causes damage, and cytokines modify liver protein synthesis, allowing inflammation detection (not necessarily liver-related) (Parola & Pinzani, 2019). Some proteins are generated large amounts during liver regeneration and neoplasia; these markers may identify liver cell proliferation (Moriles & Azer, 2020).

Liver function declines in chronic liver disease (Anderson et al., 2015; Bellentani, 2017; Berumen et al., 2021; Koutoukidis et al., 2021). It produces clotting factors and other proteins, detoxifies toxic metabolic products, and excretes bile. This illness induces fibrosis and cirrhosis by inflaming, destroying, and regenerating liver parenchyma (Berumen et al., 2021; Pinzani et al., 2005; Roehlen et al., 2020). Cirrhosis, the last stage of chronic liver disease, disrupts hepatic architecture, forms many nodules, reorganizes blood vessels, neo-angiogenesis, and deposits extracellular matrix. Fibrosis and cirrhosis are caused by the recruitment of stellate cells and fibroblasts, while liver stem cells regenerate parenchyma (Sharma & Nagalli, 2022).

NAFLD causes most chronic liver disease. Worldwide, NAFLD is the leading cause of chronic liver disease (Hallsworth & Adams, 2019). NAFLD causes metabolic dysfunction (Powell et al., 2021). NAFLD causes liver cells to store too much fat. This accumulation in liver cells is called hepatic steatosis or fatty liver. There are numerous cell fat storage types. NAFLD involves droplet-stored fat (primarily triglycerides). These drops vary in size but are usually enormous. Because of this, they fill the cell, pushing the others to the limits. This is macro vesicular steatosis (Francque et al., 2021).

The injured liver creates new, healthy tissue to mend itself. Continued injury may exhaust the liver's ability to generate healthy tissue and eliminate harm (Francque et al., 2021). Thus, scar tissue will accumulate. This scar tissue is fibrosis. Inflammatory and profibrogenic macrophages may cause liver fibrosis and persistent inflammation. This condition must be addressed immediately to prevent further damage (Ross et al., 2021).

The Binahong plant is one of several natural chemicals and medicinal plants that have shown promise in treating nonalcoholic fatty liver disease (NAFLD). The medicinal plant Binahong, scientifically known as Anredera cordifolia, is well-liked by the Indonesian people. Most found bonded chain components in the Binahong plant are mono polysaccharides such as L-

arabinose, D-galactose, L-rhamnose, and D-glucose, as well as alkaloids, polyphenols, flavonoids, and saponins (Djamil et al., 2017). This plant's leaves, stems, tubers, and flowers contain antimicrobial flavonoid chemicals. Flavonoids are direct antibiotics with a broad target. Binahong leaves include phenolic compounds, ascorbic acid, and antioxidants. Binahong leaves include flavonoids that reduce inflammation and saponins that prevent germs from growing in wounds, preventing infection, increasing fibroblast cells, and stimulating collagen synthesis (Duke et al., 2022; Adeeyo et al., 2021). The purpose of this study was to examine the effects of administering an extract from the leaves of the Anderera cardifolia plant on the histopathological appearance and liver function in male Wistar white rats (Rattus norvegicus) that had been given a high-fat diet.

RESEARCH METHOD

This type of experimental quantitative research uses mice as test animals (Notoatmodjo, 2022). The number of samples used was 24 male Wistar rats (Rattus norvegicus) for each of the six experimental groups. Grouping of test animals was carried out randomly into 4 test groups. Variables refer to characteristics or attributes that can be measured (Suwarno & Nugroho, 2023). The independent variable is the administration of Binahong (Anderera cardifolia) leaf extract, the dependent variable is the improvement of liver function and histopathological features, and the precondition variable is a high-fat diet. The acclimatization process for test animals lasted seven days in the laboratory at the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. This study utilized various tools and materials to analyze the effects of dragon fruit on blood cholesterol levels in experimental animals. Data was analyzed using SPSS (Ghozali, 2018), Kolmogorov-Smirnov test, one-way ANOVA, and Post Hoc Test with LSD technique. The study also included food and drinks for experimental animals.

RESULTS AND DISCUSSIONS

Result

Liver Function Observation Results

Table 1. Data body and liver before and after high-fat diet

Group	AVG BW – AC		SGOT levels (IUL)		SPGT levels (IUL)	
	H0	H14	H0	H14	H0	H14
Treatment P0	171,0 gr -11,9 cm	294,5 gr -16,58 cm	190,0 ± 12,8	128,0 ± 22,9	91,75 ± 9,06	46,0 ± 12,7
Treatment P1	175,0 gr -12,53 cm	293,5 gr -15,35 cm	192,5 ± 32,9	89,0 ± 40,1	94,25 ± 6,8	26,5 ± 6,18
Treatment P2	179,5 gr -11,5 cm	310,5 gr -15,03 cm	202,25 ± 22,78	80,6 ± 16,2	108,25 ± 11,44	24,3 ± 8,16
Treatment P3	175,2 gr - 12,3 cm	301,8 gr - 15,38 cm	192,75 ± 10,04	69,0 ± 4,5	106,25 ± 14,08	22,3 ± 19,5

Note: Average (AVG), Body Weight (BW) and Abdominal Circumference (AC), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT)

Liver function can be assessed by serum aminotransferase or transaminase activity. Aminotransferase indicates liver damage well. If both rise, the liver is damaged. Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) are the two aminotransferases. Since they can indicate liver parenchymal injury, ALT and AST are liver function indicators.

Table 1 shows the mice's body weight and belly circumference, measured before treatment and 14 days after eating duck egg yolk-based high-fat diets. The average rat's BW and LP increased from 293.5 grams to 310.5 grams on day 14, with the highest waist circumference being 16.58 cm. The test animals were randomly separated into four groups after acclimation and eating a high-fat, cholesterol-rich diet. Each group had six mice. Each mouse's tail was identified with a waterproof marker. Control mice received only distilled water. In the treatment group,

mice were given Binahong (*Anderera cardifolia*) leaf extract fluid at different doses (Group P1 dose 200 mg/BW 1 ml, Group P2 dose 300 mg/BW 1 ml, Group P3 dose 400 mg/BW 1 ml) for 14 days, then terminated under anesthesia and laparotomies to remove the liver and measure SGOT and SPGT.

Regular mouse SGPT and SGOT values are 17.5 - 30.2 and 45.7 - 80.8 (IU/L), respectively. The table above shows that all mice had impaired liver function because their SGPT and SGOT values were above 30.2 and 80.8 (IU/L). From the data above, it can be observed that the entire group had well above normal SGOT and SGPT readings on day 0, 14 days after the acclimation period and ingestion of a high-fat, cholesterol diet. The P2 group had the highest average SGOT and SGPT values before treatment with Binahong (*Anderera cardifolia*) adap extract, with 202.25 ± 22.78 and 108.25 ± 11.44, respectively.

After 14 days, mice in the control group received only distilled water, as seen in Table 1. Mice were administered liquid Binahong (*Anderera cardifolia*) leaf extract at various doses, resulting in average SGOT values in treatment groups P3 (69 ± 4.5) and P2 (80.6 ± 16.2). The SGOT value for group P1 was 89 ± 40.1, whereas the SGPT values reported average results: 26.5 ± 6.18, P2 24.3 ± 8.16, and P3 22.3 ± 19.5. The control group had abnormal liver function, with SGOT values of 128 ± 22.9 and SGPT values of 46 ± 12.7. After an acclimation period and a high-fat, cholesterol-rich diet for 14 days, Binahong (*Anderera cardifolia*) extract restored liver function in male Wistar rats (*Rattus norvegicus*).

Phytochemical Test

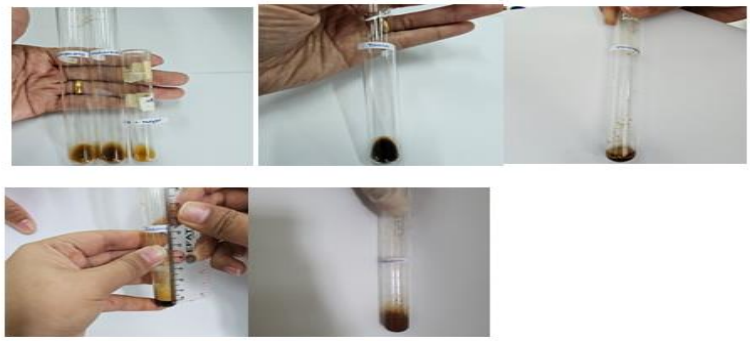


Figure 1. Phytochemical test of Binahong leaf extract

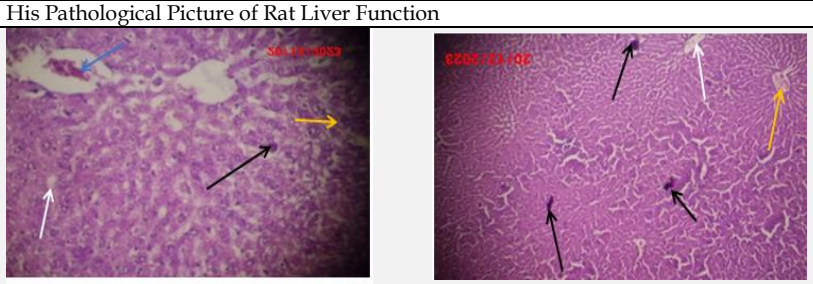
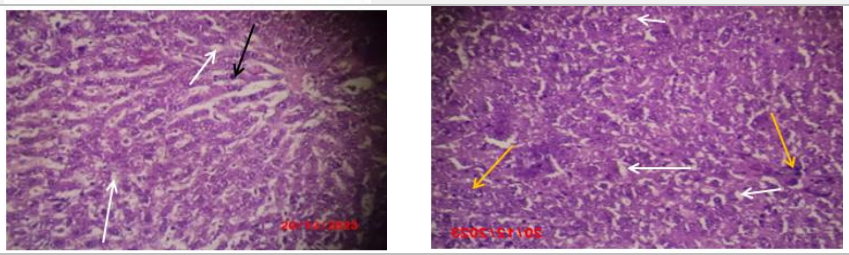
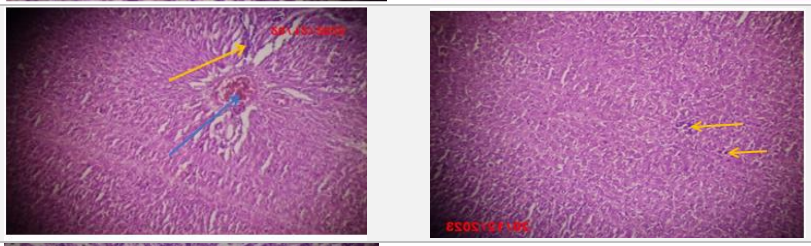
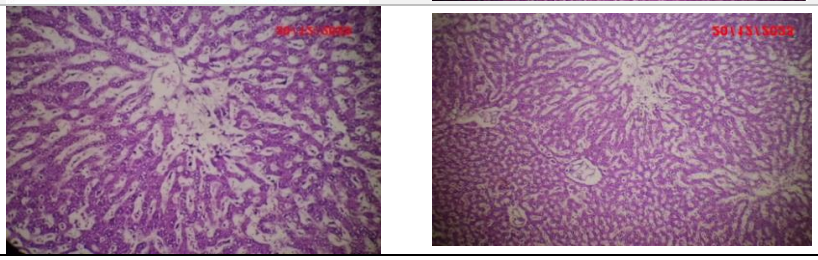
Phytochemical tests were conducted using the dosage in the treatment group to determine if any active components in Binahong leaf extract could affect liver repair in rat samples. Using a color reagent to observe the testing reaction is the basis of the phytochemical screening procedure. Positive results were observed when chemicals from Binahong Leaf extract were tested for alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids, suggesting that these compounds include secondary metabolite content. On the other hand, secondary metabolites are not present in glycosides. Variations in plant growth environments are another potential source of this. In this way, it can be concluded that Binahong Leaf extract (*Anrederacordifolia Ten. Steenis*) has efficacious compounds as antioxidants.

Analysis of Histopathological Images of Liver Function

Referring to the data in Table 2, the microscopic image of the Control group (P0) mice liver reveals inflammatory cell infiltration (yellow arrow), necrosis (black arrow), congestion (blue arrow), and fatty degeneration (white arrow). With a score of 4, they are indicating the presence of necrosis within the liver cells. Liver-rich deterioration (white arrow) and inflammatory cell infiltration (yellow arrow) were visible in the first treatment group (P1). This category receives a 3, meaning that hydropic degeneration or fatty tissue alterations have occurred in the liver cells. Pictures from the second treatment group (P2) showed rich

deterioration (white), congestion (blue arrow), and inflammatory cell infiltration (yellow arrow) in the liver. Parenchymatous degeneration or bleeding in the liver cells constitutes changes that result in a score of 2 for this group. Lastly, we have the third treatment group (P3). Everything appears in order: the cells show signs of improvement, inflammation is not apparent, and neither fat nor necrosis is visible. Also, this picture is considered typical, with a score of 1.

Table 2. Histopathological picture of rat liver function

No	Group	His Pathological Picture of Rat Liver Function
1	Control	
2	Treatment 1 (P1)	
3	Treatment 2 (P2)	
4	Treatment 3 (P3)	

From the histopathological picture above, the control group (P-0) given rat pellets + distilled water/day/tail for 14 days had the worst liver function picture with a score of 4 due to fatty degeneration, congestion, necrosis, and inflammatory cell infiltration. Treatment Group I (P-1) received rat pellets + Binahong (*Anderera cardifolia*) leaf extract at 200 mg/BW 1ml and distilled water/day/head for 14 days and had hydrophic degeneration with a score of 3. or fatty liver cells with widespread liver fatty degeneration and inflammatory cell infiltration.

Group 2 (P-2) was fed rat pellets + binahong (*Anderera cardifolia*) leaf extract at 300mg/BW 1ml and administered distilled water/day/head for 14 days. Histopathological results showed parenchymatous degeneration with a score of 2. or hepatic cell hemorrhage. Treatment Group 3 (P-3) fed rat pellets + Binahong (*Anderera cardifolia*) leaf extract at 400mg/BW 1ml and distilled water/day/head for 14 days had average outcomes (score 1), no inflammation,

improving cells, and no necrosis or fat. The researchers used SPSS to evaluate liver function data after histopathological pictures.

Normality test

Table 3. Kolmogorov-smirnov normality test

One-Sample Kolmogorov-Smirnov Test		
N		Unstandardized Residual
		24
Normal Parameters[a,b]	Mean	,000000
	Std. Deviation	.55783524
Most Extreme Differences	Absolute	.147
	Positive	.147
	Negative	-.089
Kolmogorov-Smirnov Z		.720
Asymp. Sig. (2-tailed)		.678

a. Test distribution is Normal.
b. Calculated from data.

When determining whether data is normal or abnormal, normalcy testing employs the One-Sample Kolmogorov Smirnov Test, where a significance value larger than 0.05 indicates normalcy and a significance value smaller than 0.05 indicates abnormality. According to the data in the table, the data follows a normal distribution since the 2-tailed significance result is 0.678 > 0.05.

One-Way ANOVA Test

Table 4. Results of the ANOVA test of homogeneity of variances

Results Category	Levene Statistics	Significance
SGOT	4.913	0.10
SGPT	4.140	0.20

Source: SPSS (Statistics of Package for Social Science) 25.0. for windows

Table 5. ANOVA test results

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
SGOT	Between Groups	10033.458	3	3344.486	6.710	.003
	Within Groups	9968.167	20	498.408		
	Total	20001.625	23			
SGPT	Between Groups	2153.792	3	717.931	13.965	.000
	Within Groups	1028.167	20	51.408		
	Total	3181.958	23			

Data from SGOT liver function analysis on day 14 during treatment with Binahong leaf extract is 0.10 (p>0.05), and data from SGPT liver function analysis on day 14 during treatment with Binahong leaf extract is 0.20 (p>0.05), indicating that the research variables P0, P1, P2, and P3 have a homogeneous or similar variance. Regarding SGOT liver function, the significance value at a 95% confidence level (p < 0.05) is 0.003, while SGPT liver function is 0.00. As a result, the average values of the various sample groups differ significantly. Results in rejecting Ho concluded that the three groups do not have very different average percentages of liver function.

Post Hoc Test

Table 6. Post hoc test results data

Multiple Comparisons							
LSD							
Dependent	(I)	(J)	Mean	Std.	Sig.	95%	Confidence

Variable	Groups	Groups	Difference (I-J)	Error		Interval	
						Lower Bound	Upper Bound
SGOT14	P0	P1	34.833*	12.889	.014	7.95	61.72
		P2	43.167*	12.889	.003	16.28	70.05
		P3	54.833*	12.889	.000	27.95	81.72
	P1	P0	-34.833*	12.889	.014	-61.72	-7.95
		P2	8.333	12.889	.525	-18.55	35.22
		P3	20.000	12.889	.136	-6.89	46.89
	P2	P0	-43.167*	12.889	.003	-70.05	-16.28
		P1	-8.333	12.889	.525	-35.22	18.55
		P3	11.667	12.889	.376	-15.22	38.55
	P3	P0	-54.833*	12.889	.000	-81.72	-27.95
		P1	-20.000	12.889	.136	-46.89	6.89
		P2	-11.667	12.889	.376	-38.55	15.22
SGPT14	P0	P1	19.500*	4.140	.000	10.86	28.14
		P2	21.667*	4.140	.000	13.03	30.30
		P3	23.667*	4.140	.000	15.03	32.30
	P1	P0	-19.500*	4.140	.000	-28.14	-10.86
		P2	2.167	4.140	.606	-6.47	10.80
		P3	4.167	4.140	.326	-4.47	12.80
	P2	P0	-21.667*	4.140	.000	-30.30	-13.03
		P1	-2.167	4.140	.606	-10.80	6.47
		P3	2.000	4.140	.634	-6.64	10.64
	P3	P0	-23.667*	4.140	.000	-32.30	-15.03
		P1	-4.167	4.140	.326	-12.80	4.47
		P2	-2.000	4.140	.634	-10.64	6.64

*. The mean difference is significant at the 0.05 level.

Based on the six known outcomes of further tests, the Post Hoc Bonferroni Test was used. In the Wistar strain white rats (*Rattus norvegicus*), the average SPOG/SPGT liver function percentage was marked with a star "*" when comparing groups I and J. This difference was observed across all groups. Using SPSS for Windows, we administered the LSD Post Hoc Test to the groups.

Discussion

Male Wistar rats (Rattus norvegicus) weighing 160-200 grams and 2-3 months old were used in this study. Six test animals were in each group. This investigation utilized Binahong (Anderera cardifolia) leaf extract on 24 Wistar rats per group. Binahong (Anredera cordifolia) is a medicinal Basellaceae plant. Binahong is antibacterial, gastroprotective, antiviral, antidiabetic, anti-inflammatory, wound healing, and antioxidant. Flavanols, glycosides, alkaloids, flavonoids, saponins, and steroids were found in phytochemical screening (Nxumalo et al., 2020). Secondary metabolites from Binahong Leaf extract (Anrederacordifolia Ten. Steenis) were found in alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. Thus, Binahong Leaf extract has antioxidant-active components.

Mice were measured before receiving Binahong leaf extract. Duck egg yolk-fed mice's body weight and belly circumference after 14 days of high-fat diet. After that, mice were tested for SGPT and SGOT again. All groups of mice had impaired liver function because SGPT and SGOT values were above 30.2 and 80.8 (IU/L). The P2 group had the highest average SGOT and SGPT values before treatment with Binahong (Anderera cardifolia) adap extract, with 202.25 ± 22.78 and 108.25 ± 11.44, respectively.

After 14 days of control group work, the mice only received water. In contrast, rat groups received Anderera cardifolia fluid with varying doses. SGOT showed average results for group 3 (P3) with a value of 69±4.5 and group 2 (P2) at 80.6±16.2. For group P1, the SGOT value is expected (89±40.1). SGPT values with average results were found in groups P1 (26.5±6.18),

P224.3±8.16, and P3 (22.3±19.5). The control group did not achieve normal heart function with SGOT 128±22.9 and SGPT 46±12.7 values.

Accordingly, the histopathological picture shows that the Control Group (P-0), which was only given rat pellets + distilled water/day/head for 14 days, had the worst liver function picture with a score of 4 due to fatty degeneration, congestion, necrosis, and inflammatory cell infiltration. Treatment Group I (P-1) received rat pellets + Binahong leaf extract at 200 mg/BW 1ml and distilled water/day/head for 14 days and had hydrophic degeneration with a score of 3. or fatty liver cells with widespread liver fatty degeneration and inflammatory cell infiltration. Treatment Group 2 (P-2) was fed rat pellets + Binahong leaf extract at 300mg/BW 1ml and given distilled water/day/head for 14 days.

Histopathological results showed parenchymatous degeneration or liver cell bleeding, scoring 2. Treatment Group 3 (P-3) fed rat pellets + Binahong leaf extract at 400mg/BW 1ml and distilled water/day/head for 14 days had average outcomes (score 1), no inflammation, and no visible inflammation. Necrosis and fat disappeared as cells improved. Thus, Binahong extract administered to male Wistar rats (Rattus norvegicus) for 14 days restored liver function following an acclimation period and a high-fat, cholesterol diet. This study's smaller sample size of 24 mice (6 animals/group) may affect the outcomes. The number of samples utilized affects research because it reduces generalization errors.

CONCLUSION

The study's findings indicate that the histopathological picture reveals fatty degeneration, congestion, necrosis, and inflammatory cell infiltration in the Control Group (P-0), which received only rat pellets and distilled water per day per head for 14 days. This group had the worst liver function picture, scoring 4 out of 5. Treatment group I (P-1) received distilled water daily per head for 14 days in addition to rat pellets and Binahong leaf extract (Anderera cardifolia) at 200 mg/BW 1ml. Hepatocellular alterations manifest as hydrophilic or fatty degeneration, with extensive dissemination throughout the liver and inflammatory cells infiltrating the tissue, as shown in the histological image with a score of 3. For 14 days, rats in Treatment Group 2 (P-2) were given distilled water daily and Binahong (Anderera cardifolia) leaf extract at 300 mg/BW 1 ml. Histopathological results showed changes in parenchymatous degeneration, and the group received a score of 2. for bleeding in the liver's cells. Treatment Group 3 (P-3) rats received rat pellets and Binahong leaf extract at 400 mg/BW 1 ml for 14 days. The rats showed no inflammation, cells started to improve, and there was no visible fat or necrosis. The group also received distilled water daily. The results showed that following an acclimation period and a high-fat, cholesterol-rich diet for 14 days, male Wistar rats (Rattus norvegicus) treated with Binahong extract for 14 days significantly restored liver function to normal. The substances in question contain secondary metabolites derived from the Binahong Leaf extract (Anrederacordifolia Ten. Steenis). So, it is safe to say that the antioxidant from Binahong leaf extract has several powerful chemicals. Additionally, male Wistar white rats improved liver function on a high-fat diet after administering Binahong leaf extract.

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