


## **The Effect of Giving Salak (*Salacca Zalacca*) Bark Extract on Liver Function and Histopathological Features of the Liver of Male Wistar White Rats Obesity Model**

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| Article Info  | ABSTRACT   |
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| <p><b>Article history:</b><br/>Received January 15, 2024<br/>Revised January 24, 2024<br/>Accepted February 10, 2024</p> <hr/> <p><b>Corresponding Author:</b><br/><b>Ade Arhami</b><br/>Master Study Program in Biomedical Sciences, Faculty of Medicine, Dentistry and Health Sciences, Prima Indonesia University, Medan<br/>Email:<br/>adearhami@unprimdn.ac.id</p> | <p>Nonalcoholic fatty liver disease (NAFLD) Lifestyle factors contribute to fatty liver disease. Physical activity, weight loss, and antioxidant-rich components like Salak herb are used to minimize liver disease. The study examined how Salak bark extract affected liver function in white male Wistar rats, an obesity model, and histopathology. An actual experiment or laboratory experimental design is used in this quantitative research. Four experimental groups of 24 mice were employed. Research data was analyzed with SPSS 25.0. Normality test findings reveal 2-tailed significance of <math>0.991 &gt; 0.05</math>. The One-Way ANOVA test at a 95% confidence level for the SGOT liver function analysis data showed the same variance, 0.233 (<math>p &gt; 0.05</math>) and 0.718 (<math>p &gt; 0.05</math>) for the SGPT liver function analysis. Histopathological studies showed snake fruit extract at 300mg/BW scored 3 (Hydropic degeneration), 500mg/BW scored 2 (Parenchymatous degeneration), and 700mg/BW scored 1 (Normal). The phytochemical screening of alkaloids, flavonoids, saponins, tannins, and glycosides demonstrated that Salak bark extract's secondary metabolite concentration contains antioxidant-effective active components. The study demonstrated no inflammation, cell improvement, necrosis, or fat at 700 mg/BW.</p> <p><b>Keywords:</b><br/>Salak Bark Extract, Liver, NAFLD, Obesity</p> <p>This article is licensed under a <a href="https://creativecommons.org/licenses/by-sa/4.0/">Creative Commons Attribution 4.0 International License</a>.</p> <div style="text-align: center;"></div> |

### **1. INTRODUCTION**

Modern kids and teens are absorbed in technology and digital media. Television viewing time and content are linked to health issues and adverse effects. Even in young children, excessive TV viewing, especially after supper, is linked to obesity. According to a study, the growing usage of digital media, including interactive and social media, poses health hazards to children and adolescents. Adverse effects on sleep, attention, and learning; high depression rates; exposure to erroneous, inappropriate, or harmful content and contact; privacy compromise; and obesity [1], [2].

Excess fat builds up in the subcutaneous fat tissue and eventually spreads to other parts of the body, causing the disease known as obesity. From a medical standpoint, obesity is a form of malnutrition brought on by the chronic overconsumption of bad foods. A rise in total cholesterol levels  $> 200$  mg/dL is one of the health concerns experienced by obese persons [3].

A person is considered obese if their body weight has grown due to the accumulation of extra fat. The operational definition for adults is a body mass index (BMI) of 30 or more, calculated as weight in kilograms divided by height in meters squared. According to Perri et al. (2020), people are considered "overweight" if their body mass index (BMI) is 25 or more but lower than 30 [4].

Twenty to thirty-five percent of children and adolescents are overweight or obese in both industrialized and developing nations. Due to the correlation between being overweight or obese and severe health issues like cancer, type 2 diabetes, and cardiovascular disease (including heart attacks and strokes), and type 2 diabetes, this is an important matter to address [5].

Factors in adipose tissue (such as inflammatory compounds, adipocytokines, and immune cells), signaling molecules, microbiome, ambient chemicals, food components, metabolites, and several genes combine to cause obesity [6], [7]. Furthermore, urbanization, sociodemographics (income, poverty, and education), environmental factors, and social inheritance all have significant roles in the societal and individual development of obesity. The risk of obesity is increased by cultural practices and attitudes, media consumption, inactivity, lack of physical activity, and malnutrition. Finally, obesity has become more common in recent human history due to evolution [8].

People who are overweight are more likely to develop metabolic syndrome and its associated complications, which can increase their risk of death. These complications include type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), high blood pressure, high cholesterol, chronic kidney disease, cardiovascular disease (CVD), obstructive sleep apnea, osteoarthritis, and cancers (such as breast, colon, and prostate) [7].

The leading cause of liver disease globally is currently nonalcoholic fatty liver disease (NAFLD), which occurs when the liver accumulates extra fat as a result of obesity [9], [10]. Two new worldwide public health crises, alcoholic liver disease (ALD) and nonalcoholic fatty liver disease (NAFLD), share the common thread of excessive hepatic fat buildup as their underlying cause [11].

Metabolic diseases, including insulin resistance, often include non-alcoholic fatty liver disease (NAFLD), which shows up clinically as dysfunction and illness of the endothelial cells in the liver, pancreas, and heart [12], [13]. The worldwide rise in food consumption, per capita income, sedentary lifestyle, body mass index, and excess calories has led to nonalcoholic fatty liver disease (NAFLD), a public health concern [14]. Nonalcoholic fatty liver disease (NAFLD) causes liver cells to store too much fat. Energy regulation is vital in the liver. However, the liver should not store excess energy as fat [15], [16]. The liver stores glycogen but not fat. Fat droplets in more than 5% of liver cells are aberrant. NAFLD patients have above 5% liver cells with fat droplets [17].

Nutrient metabolism and metabolic waste excretion occur in the liver, the biggest solid organ, gland, and essential organ. Its primary job is to regulate the flow and safety of digestive system-absorbed chemicals before they reach the circulatory system [18]. The liver, your body's chemical factory, conducts several complex processes to keep you healthy. Most nutrients absorbed after eating travel through the portal vein from the intestines to the liver. As the first filter and processor of nutrients, the liver is crucial [19], [20]. The liver handles sugar, protein, and fat. After transformation, the liver releases sugar, fat, and protein for energy and growth. After eating, the liver processes any excess energy source and stores it in the liver and elsewhere in the body, such as in adipose tissue, until it is needed again [21]. The liver is vital to health due to its multiple functions and advantages [22], [23].

Lifestyle variables, including high-calorie consumption and little exercise, are linked to fatty liver disease. Excess liver fat disrupts all liver functions and must be treated immediately [24]. Weight loss treats NAFLD across the illness spectrum [20]. Regardless of how it is done, weight loss will improve liver blood tests (liver enzymes), liver fat, liver inflammation, and scar tissue or fibrosis. Weight loss affects liver repair depending on the degree >5% weight loss is needed to reduce liver fat, 7–10% to alleviate liver inflammation, and >10% to ameliorate fibrosis/scarring, but lower reductions may benefit [25]. In addition to physical activity, weight loss is accompanied by antioxidant-rich foods like Salak (*Salacca zalacca*).

Salak is a unique palm fruit from Indonesia, Malaysia, Thailand, and Brunei Darussalam. The fruit is creamy white and tastes like honey. The skin is reddish brown with snake scales. Salak fruit is antioxidant-rich. Traditional medicine uses Salak, a common snake fruit. This fruit has antidiabetic, antioxidant, and anticancer effects. Salak may be used as an antidiabetic due to its polyphenols, including epicatechin, caffeic acid, gallic acid, and ferulic acid [26].

Salak peel extract has the highest antioxidant capacity (DPPH scavenging activity) of tropical fruits, according to Pujiwati et al. [27]. Thus, researchers want to know if Salak bark extract (*Salacca zalacca*) improves liver function and histological results in obese mice. In light of the above, the current study's overarching goal is to determine, through histopathological analysis, whether or not white male Wistar rats (*Rattus norvegicus*) with obesity exhibit improved liver function after receiving Salak bark extract (*Salacca zalacca*).

## 2. METHOD

This research is experimental quantitative research employing an actual experiment or laboratory experimental design [28]. The experimental groups had 24 Wistar rats and 6 test animals. Test animals were randomly divided into four groups. Research variables are factors, events, conditions, treatments, or actions the researcher believes may affect experiment outcomes [29]. The precondition variable was a high cholesterol diet to create obesity, the independent variable was Salak bark extract (*Salacca zalacca*), and the dependent variable was liver function and improvement of histological characteristics.

Various instruments were utilized in this study, such as rat cages, Ohaus scales, glass jars, rotary evaporators, blenders, stirrers, test tubes, stopwatches, gloves, masks, sonde syringes with blunt tips, blood capillary pipettes, spectrophotometers, HDL and LDL quantitation kits, precipitation buffers, colorimetric enzymatic kits, hematocrit capillary pipettes, and ependorf tubes. At the same time, the ingredients include snake fruit (*Salacca zalacca*) skin, alcohol, distilled water, 96 percent ethanol, and other similar substances. An HE coloring agent (xylol, graded alcohol 100%, 96%, 80%, 70%, hematoxylin, cosin), tissue, adhesive, sodium chloride, formalin 10%, graded alcohol 70%, 80%, 90%, xylol, paraffin, and other substances.

Animal House, Faculty of Mathematics and Natural Sciences, University of North Sumatra, was the site of the seven-day acclimatization period for the research animals. After that, we made Salak bark extract, tested its phytochemical and antioxidant properties, and put mice through their paces on a high-fat, high-cholesterol diet for a week. Then, we varied the dosages given to each group—the control group, groups 1, 2, and 3—using 300, 500, and 700 ml of Salak bark extract, respectively. The next step was to check the liver's function. Then, we prepared the tissue for histopathology. Lastly, we used a microscope set at 400x magnification to observe the tissue. Histopathological changes in the rat liver were assessed using the Manja Roenigk scoring model (Normal = score 1, Parenchymatous Degeneration = score 2, Hydropic degeneration = score 3, and Necrosis = score 4)

The research data was analyzed using SPSS 25.0 for Windows. The Kolmogorov-Smirnov test ( $p > 0.05$ ) assessed data normality. The significance between groups was tested using a one-way analysis of variance (One-way ANOVA) with a 95% confidence level ( $p < 0.05$ ) [30]. The Post Hoc Test with LSD was used for further analysis.

### 3. RESULTS AND DISCUSSION

This study employed male Wistar white rats (*Rattus norvegicus*) weighing 160-200g as its research participants. Animals were split into four groups for the experiment: one group received only distilled water, another received Salak bark extract at doses of 300mg/kgBW, a third received 500mg/kgBW, and the fourth received 700mg/kgBW. A total of 24 mice were used in this investigation, with six mice per group determined using the Ferderer formula.

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. Due to liver parenchymal injury, ALT and AST are liver functional status indicators. The following are the mice's average body weight and abdomen circumference after 14 days of duck egg yolk-high-fat diet.

Table 1. Average body weight and abdominal circumference of mice before and after a high-fat diet

| Average Body Weight and Abdominal Circumference |                             |                             |
|---|-----------------------------|-----------------------------|
| Group   | Before a high-fat diet (H0) | After a high-fat diet (H14) |
| P0  | 170 gr - 11,9 cm            | 293,5 gr - 16,59 cm         |
| P1  | 174 gr - 12,54 cm           | 294,5 gr - 15,35 cm         |
| P2  | 179,5 gr - 11,5 cm          | 311,5 gr - 15,04 cm         |
| P3  | 175,16 gr - 12,2 cm         | 301,83 gr - 15,39 cm        |

Mice in the control group got nothing except purified water. In the meantime, Salak bark extract (*Salacca Zalacca*) was administered to the mice in the treatment group at varying doses (300 mg/BW 1 ml for Group P1, 500 mg/BW 1 ml for Group P2, and 700 mg/BW 1 ml for Group P3) throughout the duration. Table 2 shows the results of measuring the SGOT and SPGT levels after 14 days of the mice being terminated under anesthesia and a laparotomy being performed to remove the liver.

Table 2. Average SGOT-SGPT

| Average SGOT – SGPT |            |            |            |            |
|---------------------|------------|------------|------------|------------|
| Group               | H0         |            | H14        |            |
|                     | SGOT (U/L) | SGPT (U/L) | SGOT (U/L) | SGPT (U/L) |
| P0                  | 207.83     | 101.83     | 137.83     | 56.83      |
| P1                  | 188.83     | 99.50      | 88.83      | 37.67      |
| P2                  | 181.67     | 98.83      | 78.50      | 29.00      |
| P3                  | 190.17     | 97.67      | 71.50      | 27.00      |

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. Before treatment with Salak bark extract (*Salacca Zalacca*), the P0 group had the highest average SGOT and SGPT values of 207.83 and 101.83, respectively.

After 14 days, mice in the control group received only distilled water, as seen in Table 2. SGOT readings were regular in mice given liquid Salak bark extract (*Salacca Zalacca*) at varying doses, with treatment group 3 (P3) having a value of 71.50 and treatment group 2 (P2) having 78.50. P1 had an SGOT of 88.83, which is close to normal. The P1 37.67, P2 29.00, and P3 27.00 groups had average SGPT values. The control group had abnormal liver function with SGOT 137.83 and SGPT 56.83. After an acclimation period and a high-fat, cholesterol-rich diet for 14 days, Salak bark extract (*Salacca Zalacca*) restored liver function in male Wistar rats (*Rattus norvegicus*).

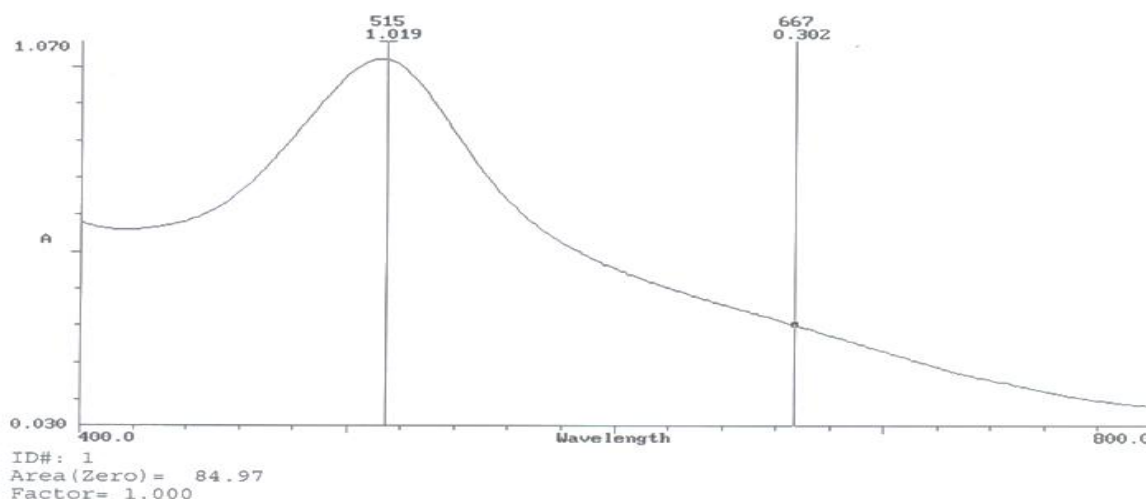
The phytochemical screening aimed to identify any components in the salak bark extract that may enhance liver function in the obesity model *Rattus Norvegicus* (Wistar rats). A test tube with 2 grams of Salak bark extract was dripped with 5 mL of 2 N HCl, boiled, cooled, and divided into 3 1 mL test tubes. Reagents are added to each tube. If Mayer's reagent precipitates white or yellow, alkaloids are present. Wagner's reagent detects alkaloids if a brown precipitate appears. The Dragendrof reagent contains alkaloids and gives an orange precipitate. Second, 1 gram of Salak peel extract was placed in a test tube, 10 mL of boiling water was added and boiled for 5 minutes, and 3-4 drops of FeCl<sub>3</sub> were added to the filtrate. Catechol tannins are present if the color is blue-green (green-black) or blue and black—pirogalo tannin. Concentrated HCl was added to a test tube with 1 gram of Salak (Salacca Zalacca) bark extract's three flavonoids and cooked for 15 minutes in a water bath. Red or yellow indicates flavonoids (flavone, chalcone, aurone). After adding 10ml of hot water to a test tube with 1 gram of extract, it was cooled and shaken violently for 10 seconds. Saponin is present if the foam is 1-10 cm high in 10 minutes and does not dissolve after adding one drop of 2 N HCl. The phytochemical test revealed that salak bark extract (Salacca Zalacca) included alkaloids, glycosides, tannins, saponins, and flavonoids.

The level of antioxidant activity test determined is IC<sub>50</sub> < 50 µg/ml = Powerful, IC<sub>50</sub> 50-100 µg/ml = Strong, IC<sub>50</sub> 101-150 µg/ml = Medium, IC<sub>50</sub> > 150 µg/ml = Weak. The test starts by determining the wavelength, operating time, and antioxidant activity of the ethanol extract of Salak peel.

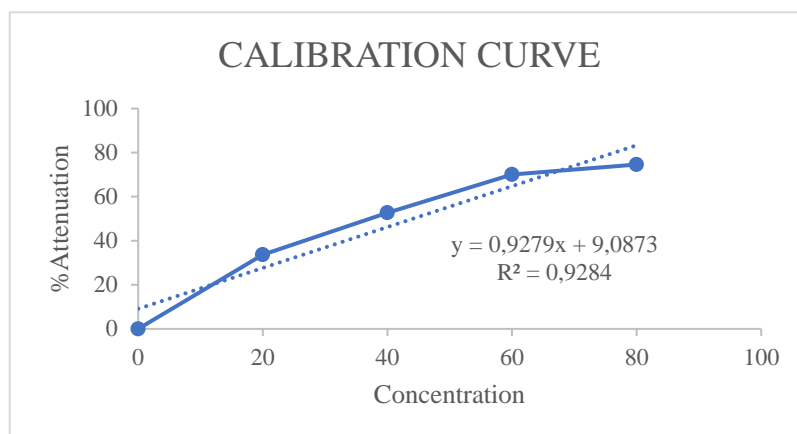
To make 50 mL of 0.5 mM DPPH solution (200 ppm), 10 mg of powder is dissolved in methanol. Pipette 1 mL of DPPH standard solution to measure its maximum absorption wavelength. Place in a 5 mL volumetric flask. Methanol was added to the mark to make a 40-ppm solution. Maximum wavelength was obtained with a UV-Vis spectrophotometer (400–800 nm). Maximum wavelength 515 nm. To create the extract test solution, weigh 10 mg of thick extract and dissolve it in 10 mL methanol. She obtained a 1000-ppm solution. Take 0.1, 0.2, 0.3, and 0.4 mL from the 1000 ppm extract solution. Then, 1 ml of 200 ppm DPPH solution at each concentration and methanol to the mark (5 mL volumetric flask). Concentrations were 20, 40, 60, and 80 ppm. After 30 minutes, absorbance was measured at 515 nm with a UV-Vis spectrophotometer. Using DPPH to determine free radical scavenging in test samples provided diverse results. The result is 33.6604% at 20 ppm, 52.6987% at 40 ppm, 70.0686% at 60 ppm, and 74.5892% at 80 ppm. Calculate the IC<sub>50</sub> for DPPH trapping results. This reveals that plant extracts lower DPPH activity by 50%. Based on the table, 44.0931 ppm indicates very significant antioxidant activity. The test sample turns dark purple when DPPH is added and yellowish when the extract is dampened.

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TEST SETUP
Orion AquaMate 8000 UV-Vis v1.006 2W2V292312
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Scanning          6:55pm 1Nov23
Test Name         DPPH[Saved]
Measurement Mode  Absorbance
Start Wavelength  400.0nm
Stop Wavelength   800.0nm
Sample Positioner Manual 6
Scan Speed        Fast
Interval          1.0nm
ID# (0=OFF)      1
Auto Print        Off
Auto Save Data    Off
```



Graph 1. Wavelength of Antioxidant Activity Test


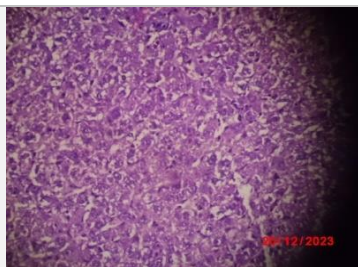
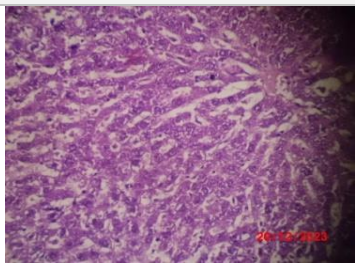
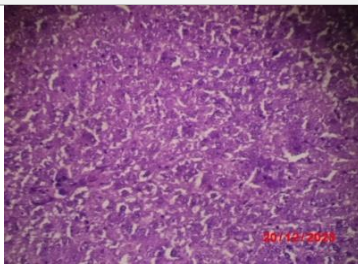


Graph 2. Antioxidant Activity Test Calibration Curve

Histological testing involves cutting and placing the fixed liver in a plastic specimen pot. Hematoxylin-eosin stained the samples. Histopathological procedures involved fixing the liver in 10% Neutral Buffer Formalin, cutting it, and inserting it in a plastic specimen holder. Next, 70%, 80%, and 90% absolute alcohol I and absolute II were used for 2 hours each to dehydrate. After clarifying with xylol, the product is printed in paraffin blocks and refrigerated. A microtome cut the paraffin blocks into 5-6  $\mu\text{m}$  thick strips. The sliced products are floated in 60°C warm water to stretch and prevent tissue folding. After removal, the preparation was stained with Hematoxylin and Eosin (HE) in an object glass. It was then studied under a 400x microscope. Table 3 shows histological differences in liver function improvements treated with distilled water and Salak bark extract (*Salacca Zalacca*).

The Control Group (P-0), fed only 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 1 (P1) was fed rat pellets + Salak bark extract (*Salacca Zalacca*) at 300mg/BW 1ml and given distilled water/day/head for 14 days. Histopathological results showed hydrophic degeneration or fatty in liver cells with extensive and widespread fatty degeneration and inflammatory cell infiltration. Treatment Group 2 (P2) received rat pellets + Salak bark extract (*Salacca Zalacca*) at 500mg/BW 1ml and distilled water/day/head for 14 days. Histopathological results showed parenchymatous degeneration or bleeding in liver cells, scoring 2. The Treatment Group 3 (P3) group, fed rat pellets + Salak bark extract (*Salacca Zalacca*) at 700mg/BW 1ml and given distilled water/day/head for 14 days, had average results (scoring 1), no inflammation, cell cells improved, necrosis and fat were not visible.

Table 3. Liver Histopathology

| No | Group   | Histopathology In Improving Liver Function  |   |
|----|---|---|---|
| 1  | Control<br>(Aquades)  |  |  |
| 2  | Treatment 1 (P1)<br>Dosage<br>300mg/KgBB<br>Salak bark<br>extract |  |  |

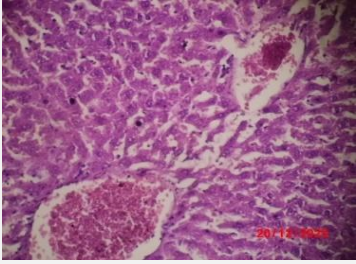
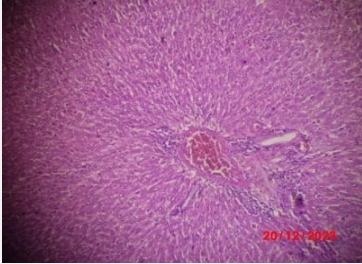
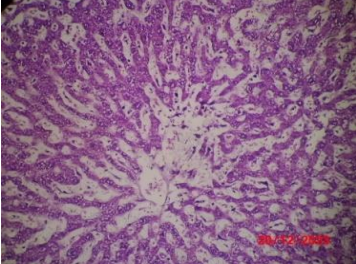

|   |   |   |   |
|---|---|---|---|
| 3 | Treatment 2 (P2)<br>Dosage<br>500mg/KgBB<br>Salak bark<br>extract |  |  |
| 4 | Treatment 3 (P3)<br>Dosage<br>700mg/KgBB<br>Salak bark<br>extract |  |  |

Table 4 displays the outcomes of a normality test that used the One-Sample Kolmogorov-Smirnov test. The test considered data to be expected if the significance value was more significant than 0.05 and data to be abnormal if it was smaller than 0.05 [30]. The data for this investigation follows a normal distribution, as seen in the table above, where the 2-tailed significance results are  $0.991 > 0.05$ .

Table 4. Kolmogorov-Smirnov Normality Test

|                                  |                | Unstandardized Residual |
|----------------------------------|----------------|-------------------------|
| N                                |                | 24                      |
| Normal Parameters <sup>a,b</sup> | Mean           | 0E-7                    |
|                                  | Std. Deviation | 6.06876642              |
|                                  | Absolute       | .089                    |
| Most Extreme Differences         | Positive       | .060                    |
|                                  | Negative       | -.089                   |
| Kolmogorov-Smirnov Z             |                | .437                    |
| Asymp. Sig. (2-tailed)           |                | .991                    |

The results of the homogeneity test can be seen in Table 5. It can be seen that the research data variables for the P0, P1, P2, and P3 groups all come from the same population with the same variance, which is 0.233 ( $p > 0.05$ ) for the liver function analysis data. SGOT on day 14 of Salak bark extract treatment, and 0.718 ( $p > 0.05$ ) SGPT on day 14 of Salak bark extract treatment for liver function.

Table 5. Homogeneity of Variances ANOVA Results

| Results Category | Levene Statistic | df1 | df2 | Sig. |
|------------------|------------------|-----|-----|------|
| SGOT             | 1.550            | 3   | 20  | .233 |
| SGPT             | .454             | 3   | 20  | .718 |

Table 6. ANOVA Test Results SGOT and SGPT

| Results Category |                | Sum of Squares | df | Mean Square | F      | Sig. |
|------------------|----------------|----------------|----|-------------|--------|------|
| SGOT             | Between Groups | 16166.667      | 3  | 5388.889    | 31.862 | .000 |
|                  | Within Groups  | 3382.667       | 20 | 169.133     |        |      |
|                  | Total          | 19549.333      | 23 |             |        |      |
| SGPT             | Between Groups | 3337.458       | 3  | 1112.486    | 35.993 | .000 |
|                  | Within Groups  | 618.167        | 20 | 30.908      |        |      |
|                  | Total          | 3955.625       | 23 |             |        |      |

Afterward, a comparison was made to determine if the three groups under study or observation had significantly different average liver function findings. Based on the test findings, the SGOT and SGPT liver function have a significance value of 0.00 at a 95% confidence level ( $p < 0.05$ ). This shows that the average values of the various sample groups differ significantly. Based on the data, it can be concluded that the three groups had different average percentages of liver function.

Table 7. Post Hoc Test Results SGOT

Dependent Variable: SGOT

LSD

| (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|------------|------------|-----------------------|------------|------|-------------------------|-------------|
|            |            |                       |            |      | Lower Bound             | Upper Bound |
| K          | P1         | 49.000*               | 7.509      | .000 | 33.34                   | 64.66       |
|            | P2         | 59.333*               | 7.509      | .000 | 43.67                   | 75.00       |
|            | P3         | 66.333*               | 7.509      | .000 | 50.67                   | 82.00       |
| P1         | K          | -49.000*              | 7.509      | .000 | -64.66                  | -33.34      |
|            | P2         | 10.333                | 7.509      | .184 | -5.33                   | 26.00       |
|            | P3         | 17.333*               | 7.509      | .032 | 1.67                    | 33.00       |
| P2         | K          | -59.333*              | 7.509      | .000 | -75.00                  | -43.67      |
|            | P1         | -10.333               | 7.509      | .184 | -26.00                  | 5.33        |
|            | P3         | 7.000                 | 7.509      | .362 | -8.66                   | 22.66       |
| P3         | K          | -66.333*              | 7.509      | .000 | -82.00                  | -50.67      |
|            | P1         | -17.333*              | 7.509      | .032 | -33.00                  | -1.67       |
|            | P2         | -7.000                | 7.509      | .362 | -22.66                  | 8.66        |

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

Table 8. Post Hoc Test Results SGPT

Dependent Variable: SGPT

LSD

| (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|------------|------------|-----------------------|------------|------|-------------------------|-------------|
|            |            |                       |            |      | Lower Bound             | Upper Bound |
| K          | P1         | 19.167*               | 3.210      | .000 | 12.47                   | 25.86       |
|            | P2         | 27.833*               | 3.210      | .000 | 21.14                   | 34.53       |
|            | P3         | 29.833*               | 3.210      | .000 | 23.14                   | 36.53       |
| P1         | K          | -19.167*              | 3.210      | .000 | -25.86                  | -12.47      |
|            | P2         | 8.667*                | 3.210      | .014 | 1.97                    | 15.36       |
|            | P3         | 10.667*               | 3.210      | .003 | 3.97                    | 17.36       |
| P2         | K          | -27.833*              | 3.210      | .000 | -34.53                  | -21.14      |
|            | P1         | -8.667*               | 3.210      | .014 | -15.36                  | -1.97       |
|            | P3         | 2.000                 | 3.210      | .540 | -4.70                   | 8.70        |
| P3         | K          | -29.833*              | 3.210      | .000 | -36.53                  | -23.14      |
|            | P1         | -10.667*              | 3.210      | .003 | -17.36                  | -3.97       |
|            | P2         | -2.000                | 3.210      | .540 | -8.70                   | 4.70        |

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

The results of additional tests utilizing the Bonferroni Post Hoc Test may be seen in Tables 7 and 8. The average percentage of SPOG/SPGT liver function in white rats (*Rattus norvegicus*) of the Wistar strain is marked with an asterisk "\*" in the comparison between groups I and J, indicating a difference between the two groups.

## Discussion

In addition to supporting metabolism, immunity, digestion, detoxification, and vitamin storage, the liver performs various other vital tasks in the human body. Due to its numerous roles and advantages, the liver is a vital organ for human health. As a general rule, a sedentary lifestyle and eating more calories than the body needs are the root causes of non-alcoholic fatty liver disease (NAFLD). Consequently, being overweight or obese is the leading risk factor for this condition [31].

For NAFLD of all types, losing weight is the best treatment. According to Vilar-Gomez et al. (2015), there will be improvements in liver function, fibrosis, inflammation, and fat content and testing when weight is lost, regardless

of the method used [25]. The Salak plant (*Salacca zalacca*) is one of many antioxidant-rich substances that, when combined with exercise, can lead to weight loss.

The exotic Salak fruit (*Salacca zalacca*) is a member of the palm family and is endemic to Southeast Asian countries like Brunei, Darussalam, Indonesia, Malaysia, and Thailand—a great deal of the antioxidants found in Salak fruit come from Saleh et al. (2018). The typical snake fruit, Salak (*Salacca zalacca*), has many traditional medical applications. Some of this fruit's purported medical benefits include its ability to lower blood sugar levels, fight cancer, and act as an antioxidant. Some research suggests that the bioactive components found in Salak, including polyphenols like epicatechin, gallic acid, caffeic acid, and ferulic acid, may have traditional uses as an antidiabetic [26].

Glycosides, alkaloids, flavonoids, saponins, and tannins have been identified in different sections through phytochemical screening. Tests for glycosides, alkaloids, saponins, tannins, and flavonoids all came back positive, suggesting that salak bark extract (*Salacca Zalacca*) has secondary metabolites in these substances. This proves that the active chemicals in salak bark extract (*Salacca Zalacca*) have antioxidant properties.

After 14 days of a high-fat diet consisting of quail egg yolk, the rats were tested for their body weight and belly circumference before they were given Salak bark extract. Afterward, the levels of SGPT and SGOT were assessed once more in the mice. All mouse groups showed aberrant liver function because SGPT and SGOT values were more significant than 30.2 (IU/L) and 80.8 (IU/L), respectively. The P0 group had the highest average SGOT value (207.83) and SGPT value (101.83), respectively, before treatment with Salak bark extract (*Salacca Zalacca*).

The mice in the control group received only distilled water after 14 days of treatment. Results of SGOT values showing average results, namely in treatment group 3 (P3) with a value of 71.50 and treatment group 2 (P2) with a value of 78.50, were seen in the treatment group of mice that were administered liquid Salak bark extract (*Salacca Zalacca*) at varied doses. The SGOT for the P1 group was 88.83, considered near to normal. At the same time, the P1 group had SGPT values of 37.67, the P2 group had 29.00, and the P3 group had 27.00. On the other hand, the control group's abnormally high SGOT and SGPT readings indicated abnormal liver function. After acclimating to a high-fat, cholesterol-rich diet for 14 days, male Wistar rats (*Rattus norvegicus*) treated with Salak bark extract (*Salacca Zalacca*) for 14 days showed a marked improvement in liver function.

Histological analysis confirms this, showing signs of fatty degeneration, congestion, necrosis, infiltration, and cell inflammation in the Control Group (P0), which received nothing more than rat pellets and distilled water daily for 14 days. This group also had the worst liver function picture, scoring a 4.

After 14 days of treatment with rat pellets and Salak bark extract (*Salacca Zalacca*) at a dosage of 300 mg/BW/1 ml, along with 1 liter of distilled water per day per head, the histopathological results showed changes in the liver cells, including hydrophilic degeneration, extensive and widespread fatty degeneration, and inflammatory cell infiltration, yielding a score of 3.

For 14 days, members of Treatment Group 2 (P2) were administered 500 mg/BW of Salak bark extract in rat pellets and 1 ml of distilled water daily per head. Histopathological examinations revealed alterations manifesting as parenchymatous degeneration or hemorrhage, yielding a score of 2. hepatic cells.

Treatment Group 3 (P3), on the other hand, had average outcomes (score 1), no inflammation, cells began to improve, no apparent necrosis or fat, and was given distilled water daily per head in addition to rat pellets and Salak bark extract at a dose of 700 mg/BW 1 ml for 14 days. After acclimating to a high-fat, cholesterol-rich diet for 14 days, male Wistar rats (*Rattus norvegicus*) treated with Salak bark extract for 14 days showed a marked improvement in liver function.

#### 4. CONCLUSION

Based on the histological picture, the Control Group (P-0) had the worst liver function picture with a score of 4 due to fatty degeneration, congestion, necrosis, and inflammatory cell infiltration. This group was given rat pellets and distilled water daily per head for 14 days.

After 14 days of treatment with rat pellets and Salak bark extract (*Salacca Zalacca*) at a dosage of 300 mg/BW/1 ml, along with 1 liter of distilled water per day per head, the histopathological results showed changes in the liver cells, including hydrophilic degeneration, extensive and widespread fatty degeneration, and inflammatory cell infiltration, yielding a score of 3.

Histopathological findings showed changes in the form of parenchymatous degeneration or bleeding in liver cells, resulting in a score of 2 in Treatment Group 2 (P2), which was administered rat pellets + Salak bark extract (*Salacca Zalacca*) at a dose of 500mg/BW 1ml and distilled water/day/head for 14 days. After 14 days of treatment with rat pellets and Salak bark extract (*Salacca Zalacca*) at a dose of 700 mg/BW/1 ml and a daily dose of distilled water per head, results were expected (score 1), inflammation was not observed, cells began to recover, and no signs of necrosis or fat were detected.

Therefore, after an acclimation period and a diet rich in fat and cholesterol for 14 days, the test rats were treated with Salak bark extract (*Salacca Zalacca*) for 14 days, and the results showed a significant improvement in liver function compared to the control group. The extract of Salak bark (*Salacca Zalacca*) is the source of the secondary metabolites found in this substance. This proves that the active chemicals in Salak bark extract (*Salacca Zalacca*) have antioxidant properties. Additionally, male white Wistar rats on a high-fat diet improved liver function after administering Salak bark extract (*Salacca Zalacca*).



The study's primary recommendation is that future studies comparing the effects of snake fruit bark extract on the liver function of obese mice should be considered. This study could be a reference for researchers interested in studying this topic. *Salacca zalacca*, or Salak bark extract, requires more excellent study because it is more practical for future studies, safer to use, and easier to mass-produce, particularly in North Sumatra and other regions where it is grown.

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## REFERENCES

- [1] M. Cernikova, D. Smahel, and M. F. Wright, "Children's Experiences and Awareness about Impact of Digital Media on Health," *Health Commun.*, vol. 33, no. 6, pp. 664–673, 2018, doi: 10.1080/10410236.2017.1298197.
- [2] U. Ansari and F. Naz, "Impact of Digital Media Communication on Children Health: Analyses Using Open Data Barometer' in Education System," *Pjer*, vol. 4, no. 1, pp. 416–437, 2021.
- [3] C. Khutami, S. A. Sumiwi, N. K. Khairul Ikram, and M. Muchtaridi, "The Effects of Antioxidants from Natural Products on Obesity, Dyslipidemia, Diabetes and Their Molecular Signaling Mechanism," *Int. J. Mol. Sci.*, vol. 23, no. 4, p. 2056, Feb. 2022, doi: 10.3390/ijms23042056.
- [4] World Health Organization, "Obesity and overweight," World Health Organization. Accessed: Oct. 21, 2023. [Online]. Available: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight>
- [5] W. Kiess *et al.*, "Clinical aspects of obesity in childhood and adolescence," *Obes. Rev.*, vol. 2, no. 1, pp. 29–36, 2001, doi: 10.1046/j.1467-789x.2001.00017.x.
- [6] H. Morinaga *et al.*, "Obesity Accelerates Hair Thinning By Stem Cell-centric Converging Mechanism," *Nature*, vol. 595, no. 7866, pp. 266–271, 2021, doi: 10.1038/s41586-021-03624-x.
- [7] S. A. Polyzos, J. Kountouras, and C. S. Mantzoros, "Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics," *Metabolism*, vol. 92, no. xxxx, pp. 82–97, 2019, doi: 10.1016/j.metabol.2018.11.014.
- [8] W. Elger, W. Kiess, A. Körner, A. Schrock, M. Vogel, and C. Hirsch, "Influence of overweight/obesity, socioeconomic status, and oral hygiene on caries in primary dentition," *J. Investig. Clin. Dent.*, vol. 10, no. 2, p. e12394, 2019, doi: 10.1111/jicd.12394.
- [9] B. A. Neuschwander-Tetri, "Non-alcoholic fatty liver disease," *BMC Med.*, vol. 15, no. 1, pp. 1–6, 2017, doi: 10.1186/s12916-017-0806-8.
- [10] E. E. Powell, V. W. S. Wong, and M. Rinella, "Non-alcoholic fatty liver disease," *Lancet*, vol. 397, no. 10290, pp. 2212–2224, 2021, doi: 10.1016/S0140-6736(20)32511-3.
- [11] S. K. Asrani, H. Devarbhavi, J. Eaton, and P. S. Kamath, "Burden of liver diseases in the world," *J. Hepatol.*, vol. 70, no. 1, pp. 151–171, 2019, doi: 10.1016/j.jhep.2018.09.014.
- [12] E. L. Anderson *et al.*, "Childhood energy intake is associated with nonalcoholic fatty liver disease in adolescents," *J. Nutr.*, vol. 145, no. 5, pp. 983–989, 2015, doi: 10.3945/jn.114.208397.
- [13] A. B. Engin and A. Engin, *Obesity and Lipotoxicity*, vol. 960. Ankara, Turkey: Springer International Publishing, 2017. doi: 10.1007/978-3-319-48382-5.
- [14] S. Mitra, A. De, and A. Chowdhury, "Epidemiology of non-alcoholic and alcoholic fatty liver diseases," *Transl. Gastroenterol. Hepatol.*, vol. 5, pp. 1–17, 2020, doi: 10.21037/TGH.2019.09.08.
- [15] S. Bellentani, "The epidemiology of non-alcoholic fatty liver disease," *Liver Int.*, vol. 37, no. October 2016, pp. 81–84, 2017, doi: 10.1111/liv.13299.
- [16] P. Bedossa, "Pathology of non-alcoholic fatty liver disease," *Liver Int.*, vol. 37, no. October 2016, pp. 85–89, 2017, doi: 10.1111/liv.13301.
- [17] S. M. Francque *et al.*, "Non-alcoholic fatty liver disease: A patient guideline," *JHEP Reports*, vol. 3, no. 5, p. 100322, 2021, doi: 10.1016/j.jhepr.2021.100322.
- [18] J. C. Ozougwu, "Physiology of the liver," *Int. J. Res. Pharm. Biosci.*, vol. 14, no. 8, pp. 13–24, 2017, doi: 10.1016/0002-9343(54)90342-3.
- [19] N. Méndez-Sánchez *et al.*, "Current trends of liver cirrhosis in Mexico: Similarities and differences with other world regions," *World J. Clin. Cases*, vol. 6, no. 15, pp. 922–930, 2018, doi: 10.12998/wjcc.v6.i15.922.
- [20] D. A. Koutoukidis *et al.*, "The effect of the magnitude of weight loss on non-alcoholic fatty liver disease: a systematic review and meta-analysis," *Metabolism*, vol. 115, no. February, p. 154455, 2021, doi: 10.1016/j.metabol.2020.154455.
- [21] S. M. Francque *et al.*, "Non-alcoholic fatty liver disease: A patient guideline," *JHEP Reports*, vol. 3, no. 5, 2021, doi: 10.1016/j.jhepr.2021.100322.
- [22] L. C. Bertot and L. A. Adams, "The natural course of non-alcoholic fatty liver disease," *Int. J. Mol. Sci.*, vol. 17, no. 5, 2016, doi: 10.3390/ijms17050774.
- [23] M. L. Petroni, L. Brodosi, E. Bugianesi, and G. Marchesini, "Management of non-alcoholic fatty li V er di S ease".
- [24] K. Hallsworth and L. A. Adams, "Lifestyle modification in NAFLD/NASH: Facts and figures," *JHEP Reports*, vol. 1, no. 6, pp. 468–479, 2019, doi: 10.1016/j.jhepr.2019.10.008.
- [25] E. Vilar-Gomez *et al.*, "Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis,"

- Gastroenterology*, vol. 149, no. 2, pp. 367-378.e5, 2015, doi: 10.1053/j.gastro.2015.04.005.
- [26] M. S. M. Saleh *et al.*, "Salacca zalacca: A short review of the palm botany, pharmacological uses and phytochemistry," *Asian Pac. J. Trop. Med.*, vol. 11, no. 12, pp. 645–652, 2018, doi: 10.4103/1995-7645.248321.
- [27] Y. Pujiawati, L. Khotijah, and I. K. G. Wiryawan, "Screening of antioxidant activities and their bioavailability of tropical plants," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1182, no. 1, 2023, doi: 10.1088/1755-1315/1182/1/012083.
- [28] S. Notoatmodjo, *Metodologi Penelitian Kesehatan*, 3rd ed. Jakarta: Rineka Cipta, 2022.
- [29] B. Suwarno and A. Nugroho, *Kumpulan Variabel-Variabel Penelitian Manajemen Pemasaran (Definisi & Artikel Publikasi)*, 1st ed. Bogor: Halaman Moeka Publishing, 2023.
- [30] I. Ghozali, *Aplikasi Analisis Multivariate dengan Program IBM SPSS 25*. Semarang, 2018.
- [31] A. Mazzotti, M. Turchese, C. A. S. Sasdelli, L. Brodosi, and G. Marchesini, "Pathophysiology of Nonalcoholic Fatty Liver Disease: Lifestyle-Gut-Gene Interaction," *Dig. Dis.*, vol. 34, no. 1, pp. 3–10, 2016, doi: 10.1159/000447275.