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Original Article

The Effect of Salacca Zalacca Bark Extract on Collagenization and Histopathological of Skin Tissue in Obese Male Mice Wounds

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ABSTRACT

Collagen regulates wound healing in open and closed skin wounds with its fibrillar structure or as a soluble component. Consume antioxidant-rich vegetables like Salak to fight free radicals and heal. The research aimed to determine the effect of Salak bark extract given to obese rats on accelerating the healing of wounds on the back and to examine the histology of the skin tissue. This type of research is quantitative with a true experiment model. The samples used were Wistar white rats (Rattus norvegicus). The treatment groups of rats were all given distilled water and rat pellet feed, but Salak bark extract was given at different doses per day for 14 days, namely the control group: NaCl 0.9%, and the group was given Salak bark extract at the same dose. Varies per group for mg/kg/BW for Group P1: 300, Group P2: 500, and Group P3: 700. The research data was analyzed using SPSS. In the results of research data processing in the normality test, significant values of p > 0.05 were 0.003 in the control group, 0.102 in P1, 0.078 in P2, and 0.200 in P3. A homogeneity value of 5.024 > 0.05 and an ANOVA value of 0.000 < 0.05 indicate promising results. Salak bark extract at 700 mg/kg BW had the highest collagen density in histological pictures of skin tissue. Salak bark extract contains flavonoids, saponins, tannins, alkaloids, and glycosides, which have antioxidant, antimicrobial, and anti-inflammatory properties and aid wound healing, collagenization, and weight loss in obese mice.

Keywords: *Collagen, Histopathology, Salak Bark, Wound Healing, Obesity*

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INTRODUCTION

Skin protects living things against shock, temperature, UV radiation, toxins, and other hazards [1]. The skin protects the body from the outside world. The skin protects against thermal, chemical, and UV stress, water loss, and infections [2, 3]. Various nerve endings allow the skin to control body temperature, increase metabolic function, and synthesize vitamin D [4,5]. Externally, the skin has three layers: epidermis, dermis, and subcutaneous fat [6].

As the body's outermost layer, the skin is vulnerable to external factors [7]. Skin injury or disruption might undermine its integrity. Wounds are skin injuries [8,9]. Physical contact, medical operations, or physiological changes can cause wounds that disturb the body's anatomy [10]. Animal injuries can result from bites, accidents, or sharp object lacerations. Skin wounds harm the dermis. Accidental trauma or surgery can cause wounds, which can be lethal [11, 12].

Wounds can be open or closed depending on their source [13]. The wound opens, the skin is broken, and the underlying tissue is exposed to the outside world, allowing blood to escape. These are wounds that loose skin [14]. The wound will enable microbes in. Abrasion or glazes, lacerations, cuts, punctures, avulsions, penetrations, and gunshot wounds are open wounds. Both incisions are closed, so the skin and underlying tissue are protected. Wounds below the skin do not bleed externally [15]. Closed

wounds include contusions, hematomas, and crush wounds. After an injury, wounds heal spontaneously [11, 16].

Wound healing is a normal tissue response [10,16]. However, wound healing involves complicated interactions between cell types, cytokines, mediators, and the vascular system. To halt bleeding, blood arteries constrict, and platelets aggregate. Then, inflammatory cells, starting with neutrophils, arrive. The mediators and cytokines released by these inflammatory cells stimulate angiogenesis, thrombosis, and re-epithelialization. Fibroblasts construct scaffolding from extracellular components [9, 17,18.

Routine wound healing involves inflammation, proliferation, and remodeling after tissue injury [14,15,17]. Nature uses inflammation to eliminate damaged tissue and prevent invasive infections. Granulation tissue, a network of new capillaries, fibroblasts, and macrophages in loose supporting structures, forms in the wound bed during the proliferative phase. The second phase, epithelialization, lasts 8-21 days following damage. The natural proliferation phase gives us a model for treating wounds to close them. In the final ripening phase, collagen deposition and breakdown are balanced [19].

Wound healing is regulated by collagen, a critical extracellular matrix component [2]. Collagen is the body's main protein [20]. Fibroblasts produce this collagen, which changes shape during wound healing [21]. Chronic inflammation slows wound healing. Due to pro- and anti-inflammatory mediator imbalance, neutrophils and macrophages are recruited, and inflammatory cytokines are overexpressed.

To overcome free radical-induced wound healing issues. Antioxidant consumption is crucial to fight free radicals. Plants contain antioxidants that improve health—many antioxidant-rich plants, including the Salak plant [22, 23].

Salak has many pharmacological uses due to its antioxidant content. They are often eaten for their sweetness. Its inherent sugar, fiber, minerals, and vitamins make it a healthy fruit. Phytochemical studies of this fruit found flavonoids, phenolics, glycosides, gallic acid, quercetin, chlorogenic acid, epicatechin, proanthocyanidins, lycopene, and ß-carotene. Pharmacological tests on the fruit's flesh and skin have shown antioxidant, anti-inflammatory, anticancer, and antidiabetic properties.

Based on this background, this researcher tested and analyzed the effect of salak bark extract (Salacca zalacca) on collagenization, wound healing, and histopathological appearance in obese white male Wistar rats (*Rattus norvegicus*).

METHOD

This research is experimental quantitative research employing an actual experiment or laboratory experimental design [24]. Variables are traits or traits that may be measured or seen that vary among persons or organizations. In this study, the Independent Variable was salak bark extract (*Salacca zalacca*), the Dependent Variable was wound healing and histopathological picture, and the Precondition Variable was the rat's backcut wound.

Tools used are macerators, analytical scales, glass stirring rods, glass funnels, vacuum rotary evaporators, cups, measuring cups, glass beakers, ovens, water baths, autoclaves, incubators, laminar air flow, test tubes, petri dish tube racks, L rods, round tube needle, dropper pipette, micropipette, vortex, caliper, microscope, object glass, and hot plate. The test materials were Salak peel, 0.9% NaCl, distilled water, 70% ethanol, spirit, and Mayer's reagent.

The study utilized a sample of 24 male Wistar rats (Rattus norvegicus) weighing 160-200 grams and aged 2-3 months. The rats were randomly divided into six test groups, each comprising six.

Acclimation is adapting to a new climate, environment, or atmosphere. Before treatment, all male Wistar strains were acclimatized for seven days at the Animal House, Faculty of Mathematics and Natural Sciences, University of North Sumatra. To adjust, mice were given time, food, and drink (ad libitum). In this research, mice are housed in groups in lab cages for acclimatization. Plastic (30 cm x 20 cm x 10 cm) rat cages have tiny wire mesh. During the investigation, a daily change of 0.5–1 cm thick rice husks covered the cage bottom. The room temperature was 25–27 °C, the humidity was 35–50%, and the lighting was controlled to cycle 12 h light/12 h dark. Mice ate regular mouse pellets and drank

distilled water freely.

Remaceration produces salak peel extract. Peel 20 kg of Salak and gather the peel. Recipe for Salak bark extract: Blended Salak skin is weighed around 150 g and soaked in 900 ml of 70% ethanol solution for five days (ground daily). The extract was filtered using filter paper (first filter result), and the rest was extracted in 2 days with 600 cc of 70% ethanol and filtered again. Next, the solution from the first and second filters is collected and evaporated in a vacuum evaporator at 700°C until a quarter of the initial volume, then dried in the oven at 400°C until it becomes a thick extract. 4.86 g of the known extract was split into 300 mg/KgBB, 500 mg/KgBW, and 700 mg/KgBW concentrations.

To determine the tannin content, boil 1 gram of extract in 10 milliliters of water for 5 minutes, strain the mixture, and add 3 or 4 drops of ferric chloride. It indicates the presence of catechol tannins if it is blue-green (green-black) and pirogalo tannins if it is blue-black. One gram of sample extract, some solid hydrochloric acid, and fifteen minutes in a water bath are all needed to determine the flavonoid concentration. A reddish or yellowish hue indicates a favorable result when flavonoids (flavone, chalcone, and aurone) are detected.

In the Alkoloid Content Test, 2 grams of sample extract are placed in a test tube, dripped with 5 mL of 2 N HCl, heated, 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 residue. 2 grams of sample extract was added to a test tube with 2 mL of ethyl acetate and agitated for the Steroid/Terpenoid Content Test. The ethyl acetate layer was dropped onto a drop plate to dry. After drying, two drops of acetic acid and one drop of concentrated sulfuric acid were added. Terpenoids are present if they become red or yellow. Steroids are present if they turn green. For the Saponin Content Test, place 1 gram of sample extract in a test tube, add 10 ml of boiling water, calm, and shake vigorously 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[25].

Measure the absorbance of a 70 ppm vitamin C solution to test antioxidant activity in wavelength. Combine 1 mL of this solution with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide and incubate at 50 °C for 20 minutes. After incubation, 1 ml of TCA was added, centrifuged at 3000 rpm for 10 minutes, put into a 10 ml measuring flask, added 1 ml of distilled water and 0.5 ml of FeCl3, added enough oxalic acid to the limit mark, and measured absorption with a UV-Vis spectrophotometer calibrated from 400-800 to obtain the maximum wavelength.

After determining the maximum wavelength, the operating time is tested to determine when the reaction is most stable and read the absorbance at the 1st to 30th minute.

The antioxidant activity of Salak peel ethanol extract was measured using 25 mg of extract diluted in 25 ml of ethanol p.a in a 25 ml measuring flask at 1000 ppm. Add 20 µl, 40 µl, 60 µl, 80 µl, and 100 µl of stock solution to test tubes at concentrations of 2 ppm, four ppm, six ppm, eight ppm, and ten ppm, followed by 1 ml of phosphate buffer. 1 ml K3Fe (CN)6 1% and 0.2 N (pH 6.6). Incubate 50°C for 20 minutes. Following incubation, one cc of 10% TCA solution was added and centrifuged for 10 minutes at 3000 rpm. After centrifuging, pipette it into a 10 ml measuring flask and add 0.5 ml of 0.1% FeCl3 and distilled water to the mark. Then, the most excellent wavelength absorption is calculated.

Test Animal Preparation Mice were fed a high-fat diet daily for 14 days to promote obesity. The feed is duck egg yolk. Duck eggs are utilized as high-fat diet feed because their yolks contain 2,118.75 mg/100 g of cholesterol, compared to 1,881.30 mg/100 g for free-range chicken eggs and 1,274.50 mg/100 g for chicken eggs race. Additionally, duck eggs include 9.30-11.80% protein, 11.40-13.52% fat, and 1.50-1.74% sugar. Give 2 mL/200 gBB orally after homogenizing raw duck egg yolk. This food is fatty. This high-fat meal was provided for 14 days before Salak bark extract treatment.

After the acclimatization phase, the test animals were randomly separated into four groups. Six mice made up each group. This set of groups includes the Control Group (P-0): The rats were given rat pellet feed and 0.9% NaCl per day for 14 days. In Treatment Group I (P-1), the rats were given distilled water daily and a 300mg/kg BB dose of salak bark extract. In Treatment Group II (P-2), the rats were given 500mg/kg BW of salak bark extract along with distilled water per day per head for 14 days.

Finally, in Treatment Group III (P-3), the rats were given 700mg/kg BW of salak bark extract along with distilled water daily.

Wound healing period observation. A wound's day can be determined by the absence of erythema, edema, and new tissue or closure since the rat's back incision. Every day following wound formation, translucent tracing paper was used to track wound healing. A permanent marker is used to trace wound boundaries on transparent paper. The wound area is determined on graph paper. Tracing paper was laid on graph paper (2 mm^2) to count coiled squares. The percentage of wound healing equals the initial wound length minus the day of observation wound length divided by the initial wound length and multiplied by 100%. Observations were done for up to 14 days because the usual wound healing process takes between 3 and 14 days to reach the proliferation phase. I am inhaling excess technical chloroform that killed all mice after 14 days.

After anesthetizing mice with 0.1mg/200gBW ketamine xylazine, histopathological samples were taken. Histopathological studies used mouse skin samples from healed lesions in a 10% formalin buffer solution. Healed skin samples were sectioned at 5 µm thickness and stained with H&E. The materials were maintained in a 40% PFA pot at room temperature and delivered to the University of North Sumatra Histopathology Laboratory for preparation. Olympus BX51® light microscopes with 100x and 400x magnification watched the wound edge preparations during wound healing, while Olympus DP20® microscope cameras captured photographs.

Re-capitalization revealed histopathological findings. Epithelial thickness score with Score 1 (Reepithelialization of 0-25% of the wound part of normal), Score 2 (25-50%), Score 3 (50-75%), and Score 4 (>75%).

The statistical package SPSS 25.0 for Windows was used for data analysis. We used the Kolmogorov-Smirnov test technique to examine the data normality test ($p > 0.05$). A one-way analysis of variance technique, also known as One-way ANOVA, was used to investigate the significance between groups at a 95% confidence level ($p < 0.05$) [26]. After that, the Post Hoc Test with the LSD technique was used for any further testing or analysis.

RESULTS

The results of the 14-day study were obtained by providing treatment to four groups of test animals. The control group received 0.9% Nacl, treatment group 1 received 300mg/kgBW Salak bark extract, treatment group 2 received 500mg/kgBW, and treatment group 3 received 700mg/kgBW. A sterile scalpel with blade no. 4 made ± 2 cm long incisions on the rat's back, reaching the subcutaneous or hypodermis area.

The test animals were fed duck egg yolk-based high-fat, high-cholesterol diets daily until they grew obese. Lee's principle was used to calculate obese mice's body weight using digital scales [27].

Table 1. Body Weight Measurement Results

Parameter	Groups	Average Weight After Treatment	
		High Fat Diet	14 D + Salak Bark Extract
Body Weight (Gr)	Control	318	309
	P1	320	263
	P2	322	259
	P3	318	247
Naso-anal Length (mm)	Control	204	209
	P1	201	226
	P2	203	227
	P3	203	225
Lee index	Control	0.33	0.32
	P1	0.34	0.28
	P2	0.33	0.28
	P3	0.33	0.27

Table 2. Average Wound Healing

		Treatment Group			
Days		C	P1	P2	P3
H2	cm	1.987	1.788	1.262	1.353
	%	0,05	0,46	36.90	1,36
H4	cm	1.870	1.688	1.428	1.523
	%	0,29	0,67	28.61	1,02
H6	cm	1.704	1.598	1.338	1.433
	%	0,64	0,84	1,38	1,19
H8	cm	1.683	1.592	1.071	1.133
	%	0,68	0,86	1,95	1,81
H10	cm	1.650	1.502	0,66	1.023
	%	0,74	24.89	2,20	48.87
H12	cm	1.623	0,68	0,35	0,37
	%	0,81	50.73	3,12	73.67
H14	cm	1.582	0,33	0,17	0,14
	%	0,90	3,21	87.61	89.99

Salak bark extract included Alkaloids, Flavonoids, Saponins, Tannins, and Glycosides

after phytochemical screening. Contains no steroids/triterpenoids. So obese Wistar rats (*Rattus Norvegicus*) receive Salak bark extract to repair cuts.

The Kolmogorov-Smirnov test was maintained as a normal test utilizing these data. The Kolmogorov-Smirnov test was kept as a normalcy test using these data. The data is said to be regularly distributed if the p-value is more significant than 0.05 and not normally distributed if the p-value is less than 0.05 [26].

Table 3. Normality Test Result

Groups	Kolmogorov-Smirnov[a]		
	Statistic	df	Sig.
Control	.401	6	.003
P1	.299	6	.102
P2	.308	6	.078
P3	.223	6	.200*

**. This is a lower bound of the true significance.*
a. Lilliefors Significance Correction

Based on Kolmogorov-Smirnov normalcy test results. The significant values were 0.003 in the control group, 0.102 in P1, 0.078 in P2, and 0.200 in P3. A p-value > 0.05 indicates typically distributed data. This implies that the data is normally distributed.

We tested group homogeneity with the Levene test at 5%. When making decisions, data is homogeneous if the significance value is more significant than 0.05 and not homogeneous if it is less than 0.05 [28].

Table 4. Homogeneity Test Results

Levene static	**df1**	**df2**	**Sig**
5.024	3	20	.009

The Levene homogeneity test findings are in the table above. The significant column probability is 5.024. The considerable probability value is more significant than 0.05, indicating that the control group, treatment group-1, treatment group-2, and treatment group 3 are homogeneous or have the same variance.

The research data passed normality and homogeneity tests and had homogeneous variance. Hence, a One-way ANOVA test was performed to compare trial group effectiveness.

Table 5. One-Way ANOVA Test Results

Groups	**Sum**	**df**	**Mean**	**F**	**Sig**
Between	2.210	3	.737	1067.7	.000
In	.014	20	.001		
Total	2.224	23			

The One-Way Anova test in Table 5 yields a significance value of 0.000 or < 0.05 [28]. These statistics show a significant difference between the control and treatment groups. Whether groups differ significantly is determined by the LSD Post Hoc Test. The Post Hoc LSD test analysis in this study indicated a significance value of 0.000 or less than 0.05, indicating significant differences between groups.

Table 6. LSD Post-Hoc Test Results

Groups		**Mean difference**	**Sig**
Control	Treatment 1	.35333*	.000
	Treatment 2	.70000*	.000
	Treatment 3	.75667*	.000
P1	Control	35333*	.000
	Treatment 2	.34667*	.000
	Treatment 3	.40333*	.000
P2	Control	.70000*	.000
	Treatment 1	.34667*	.000
	Treatment 3	.05667*	.000
P3	Control	.75667*	.000
	Treatment 1	.40333*	.000
	Treatment 2	.05667*	.000

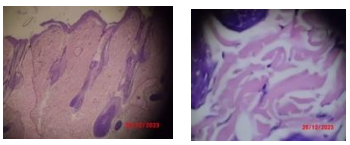
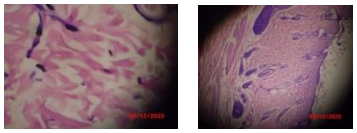
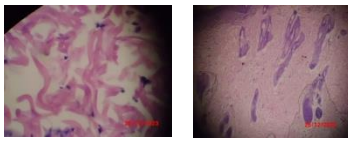
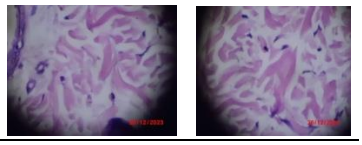
Histopathological investigations were made with Olympus BX51® light microscopes with 100x and 400x magnification, and the wound edge preparations were watched during wound healing while Olympus DP20® microscope cameras captured photographs. This observation examined cell structure and shape, notably collagen density, in each cut wound specimen in the treatment group with 0.9% NaCl, Salak bark extract at 300mg/KgBW, 500mg/KgBW, and 700mg/KgBW. Daily morning doses of *Salacca* zalacca are given.

Routine wound healing involves inflammation, proliferation, and remodeling after tissue injury. Collagen, a crucial extracellular matrix component, regulates wound healing [29]. Fibroblasts produce collagen and other wound-healing proteins during proliferation. Histopathological analysis of cut wounds treated with 0.9% Nacl and Salak bark extract (Salacca Zalacca) demonstrated collagen density and quantity differences in Table 7.

In obese male Wistar white rats (Rattus norvegicus), Salak bark extract (Salacca Zalacca) can affect collagen density in incision wound healing. Collagen density differences between the control and treatment groups prove this. The microscopic appearance of treated

histological skin tissue is shown in Table 7.

Table 7. Histopathological Results

Groups	Image of Skin Tissue
Control (Nacl 0.9%)	
Treatment 1 (300mg/KgBW)	
Treatment 2 (500mg/KgBW)	
Treatment 3 (700mg/KgBW)	

Weak collagen development was seen in the control group that received 0.9% Nacl because the skin tissue was still inflamed. Salak bark extract (*Salacca Zalacca*) treatment microscopic images show collagen-filling tissues of varying densities. The collagen density in the histopathology of obese male white rats (Rattus norvegicus) of the Wistar strain cannot be separated from Salak (Salacca Zalacca) skin extract compounds.

DISCUSSION

Treatment groups 1 through 3 had significantly higher incision wound healing rates than the control group (20.92%, 76.56%, 87.61%, and 89.99%, respectively) on the last day of the research. After comparing the healing rates of the control group, treatment 1, and treatment 2, treatment three resulted in the best results.

Group 3, which received 700 mg/kg of Salak (*Salacca Zalacca*) extract, had the maximum collagen density, according to histological examinations of skin tissue. The histopathology of obese white male Wistar rats (Rattus norvegicus) skin tissue shows a high collagen density indistinguishable from the compound content of Salak (*Salacca Zalacca*) skin extract.

The secondary metabolite content of salak peel extract, which includes tannins, alkaloids, saponins, and flavonoids, speeds up wound healing, collagenization, and weight loss by neutralizing free radicals caused by dermapen scars and obesity. The results show that tannins, alkaloids, flavonoids, saponins, and oxidative stress can all work together to boost antioxidant activity, which speeds up wound healing and aids weight loss.

This study's findings suggest that snakebite, a naturally occurring substance, could have medicinal applications due to its accessibility [29,30]. Salak bark has excellent potential as a wound-healing agent for those who are overweight.

CONCLUSION

Based on the measurement of the wound length, the research found that white rats (Rattus norvegicus) Wistar strain, which is obese, benefited from the application of salak bark extract (Salacca Zalacca) at doses of 300, 500, and 700mg/KgBW to speed up the healing process as cuts. 14 days. The salak (Salacca Zalacca) bark extract group had shorter wounds than the control group.

Histopathological analysis of skin samples reveals a statistically significant variation in collagen density between the two groups (control and treated). Collagen density was highest in treatment group 3, which received 700 mg/KgBB of Moringa flower extract.

The results show that obese mice can heal their wounds faster and have more collagen density after receiving salak bark extract at 300, 500, and 700 mg/kgBW, with 700 mg/kgBW being the most effective.

Researchers found that secondary metabolite compounds in salak bark extract include flavonoids, saponins, tannins, alkaloids, and glycosides. These compounds have antioxidant, antimicrobial, and anti-inflammatory properties, aiding wound healing, collagen formation, and weight loss in obese mice.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest regarding the results of using test animals, the

research site, and the costs incurred by each author in this research.

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