The Effect Of Giving Salak Skin Extract (*Salacca Zalacca*) On Pancreatic Function And Pancreatic Histopathology Of Male Wistar Strain White Rats In Obesity Model

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Abstract

The most important function of the pancreas is to control the quality of blood sugar with the glycogen process. So that the percentage of sugar in the blood is accelerated by its release from the liver. And liver insulin is stimulated to convert glucose into glycogen and stored in cells, thereby reducing the percentage of sugar in the blood. Salak fruit skin extract contains secondary metabolites such as saponins, tannins, alkaloids, flavonoids, and steroids that help repair pancreatic cells damaged by obesity by stopping pancreatic cell apoptosis. Snake fruit skin has the potential to be a diabetes medicine because it has a mixed content. The type of research used is true experimental with post test only control group design. The results of histopathological observations of pancreatic tissue showed significant differences between the treatment and control groups. The treatment group with a dose of 600 mg/kgbb was different from the control group. Based on the results of the normality test, the significance results were 0.200 in all groups. Data is said to be normally distributed if the p value > 0.05

Keywords: *Salak Peel Extract, Pancreas, Obesity*

Introduction

In the coordination of digestion, the pancreas is a part that performs two important tasks: forming digestive enzymes (also known as exocrine role) and forming a few hormones (also known as endocrine role). The pancreas is in the upper left quadrant of the abdomen, or stomach, with its head or part of the caput attached to the duodenum. The pancreatic duct is important for transporting enzyme products from the pancreas to the duodenum. People have known the pancreas for a long time. The Greek Herophilus who worked as a surgeon in 335–280 BC, first discovered the pancreas. All vertebrates have endocrine and digestive glands called the pancreas. With a yellowish color, the pancreas resembles a sponge.(Roosdiana, A., Sutrisno, Mahdi, C., & Safitri, 2019). In the posterior stomach section, the pancreas gland is close to the duodenum, or small intestine. Often called the islets of Langerhans because its cells are clustered in islands that are exactly like a map. In 1869, it was named after Paul Langerhans, its discoverer. There are about 100,000 islets of Langerhans in each pancreas, with 100 beta islets in each. There are alpha cells in addition to beta cells that produce glucagon, which in turn acts on insulin, increasing the percentage of blood glucose. In addition, somatostatin and PP cells are released from delta cells, which produce pancreatic polypeptide hormones (Uswatun Hasanah, 2013). Belayneh, YM, et al (2009) stated that antidiabetic drugs can be used to treat diabetes, but these drugs are not cheap, difficult to use, and have serious impacts. Diabetic patients with kidney, liver, or heart failure are also not advised to use oral antidiabetic drugs such as biguanides, sulfonylureas, and thiazolidinediones. Therefore, an important treatment option for diabetes mellitus is the use of plants as bioactive compounds that are easy to use, safe, and economical. Salak (*Salacca zalacca*) is one of the medicinal plants. Salak, which belongs to the Arecaceae family, is an endemic plant of Indonesia which is a type of aren (enau), coconut, oil palm, palm, and fern that has low branches. The branches of this plant are covered with densely patterned leaf sheaths and have thorns, which make many new shoots emerge from the trunk, which are almost invisible. Pieces of salak fruit that are very efficacious for traditional medicine are found in the skin of the salak fruit. Salak skin is a waste that is difficult to recycle. However, Nazaruddin and Kristiawati (2000) stated that salak skin has nutrients such as protein percentage, carbohydrate percentage, water percentage to low fat. Phytochemical research is shown by the flesh and skin of salak fruit having flavonoid, tannin, and alkaloid content. Meanwhile, saponins, steroids, and triterpenoids are not found in the skin of this fruit (Sahputra, FM, 2008). According to the above problems, researchers really want to examine the impact of giving snake fruit skin extract (*Salacca zalacca*) on the role of the pancreas and the histopathology of the pancreas of white male wistar rats with obesity models. This test is

felt to be further studied as a treatment using plants that are believed to have a small impact and economical costs than using drugs.

Literature review

A study called pancreatic histopathology examines the tissues and structures of the pancreas under a microscopic view. In this case, pancreatic histopathology involves looking at the shape of pancreatic tissue using staining systems such as Hematoxylin-Eosin (HE) to understand changes in structure and function associated with diabetes mellitus, pancreatitis, and other medical conditions. In this study, pancreatic histopathology was used to determine the histopathological appearance of how certain substances, such as ethanol extract from neem leaves, affected pancreatic slices from white rats fed a high-fat diet. The study also helped understand how these substances affect the structure and function of pancreatic tissue, as well as their potential use in the treatment and care of pancreatic-related health conditions.(A. Petersmann et al., 2019). Unusual or excessive fat accumulation can cause health hazards known as obesity. More than 25 points of body mass index (BMI) is considered overweight, and more than 30 points is considered obese. More than 4 million people died each year due to being overweight or obese in 2017, according to the global disease criteria. This shows that the problem has spread to epidemic proportions. (WHO, 2022). Ideally, a coordinated classification of obesity would have the following characteristics: be based on practical measurements that are widely available to providers regardless of setting; accurately reflect health hazards (prognosis); and be able to be used to determine strategies and treatment purposes

Methods

The type of research used is True experimental, using the Post Test Only Control Group Design research preparation, which is a research model that focuses on observing the control group and treatment after an activity is given. The test material in the study was male white rats (Rattus norvegicus) of the Wistar strain weighing 200-300 grams aged 2-3 months. Researchers determined that rats (Rattus norvegicus) were used as research materials because animals with characteristics and physiology resembling humans have become one of the dominant animals chosen in biomedical science research. To make salak skin extract, 20 kilograms were collected. To make salak skin extract, the blended salak skin was weighed 150 grams and extracted with 900 milliliters of 70% ethanol liquid. The extract was left for about five days (watered every day), and then filtered using filter paper (hasi). After extracting 4.86

grams of known extract, its concentration was divided into three different levels: 300 mg/kgBW, 500 mg/kgBW, and 700 mg/kgBW. Feeding high-fat diet to mice, high cholesterol for a week. The feed given is like quail egg yolk. The impact of exogenous feeding is that the percentage of cholesterol is increased so that it is in a state of obesity. High-fat, high-cholesterol food is distributed for 14 days before starting treatment with snake fruit skin flower extract. This study used test animals in the form of white rats (Rattus norvegicus) male Wistar strain with a body weight of 200gr to 300gr. The test animals were divided into 4 groups. The sample calculation was based on the Ferderer formula for 4 groups and the results obtained were 6 per group, so that the total sample in this study was 24 rats. The control group was only given standard pellet feed and distilled water, while the treatment group was given a high-fat diet and snake fruit skin extract (*Salacca zalacca*) with different doses, namely 200mg/KgBB, 400mg/KgBB, and 600mg/KgBB. The following are the characteristics of the test animals. The collection and assessment of histopathological observation data were examined microscopically. The test results were tabulated and then changed to the analyst's findings and presented descriptively. Furthermore, the final research data analysis used SPSS (Statistics of Package for Social Science) 25.0. for windows. Analyzed the data using the Kolmogorov-Smirnov test approach ($p > 0.05$) in testing data normality. In order to test the significance of each test group, it was carried out using the one-way variance analysis technique or One Way ANOVA at a 95% confidence level ($p < 0.05$). Further analysis or testing using the Post Hoc Test with the LSD technique. The operational definitions of variables are shown in Table 1.

Table 1. Operational Definitions

Variables	Operational Definition	Methods and Measuring Tools
Salak Fruit Skin Extract	Snake fruit skin extract (*Salacca zalacca*) is snake fruit skin extracted using 70% ethanol. Ethanol extract of snake fruit skin is given once a day for 14 days to test animals orally.	Measuring with the help of a needle and given by the remaceration method using a blunt probe

Obese Rats	Male white Wistar rats (*Rattus novergicus*) obesity model fed high-fat diet in the form of quail egg yolk until reaching an index value of >0.3 according to the Lee obesity index. The Lee index value includes the result of dividing the root of body weight in grams multiplied by ten by the naso-anal length in millimeters.	Digital scales

Acclimatization is an activity to adjust to a new environment, climate, condition, or atmosphere. Before the activity was given, all male Wistar rats went through an acclimatization process for seven days in the Laboratory of the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of North Sumatra. The rats were given time to adapt to the new environment, as well as their food and drink. The provision of feed and drink to the rats was carried out according to their standard needs (ad libitum).

Results

This study used test animals in the form of white rats (Rattus norvegicus) male Wistar strain with a body weight of 200gr to 300gr. The test animals were divided into 4 groups. The sample calculation was based on the Ferderer formula for 4 groups and the results obtained were 6 per group, so that the total sample in this study was 24 rats. The control group was only given standard pellet feed and distilled water, while the treatment group was given a high-fat diet and snake fruit skin extract (*Salacca zalacca*) with different doses, namely 200mg/KgBB, 400mg/KgBB, and 600mg/KgBB. The following are the characteristics of the test animals are shown in Table 2.

Table 2. Characteristics of Test Animals

Component	Group K	Group P1	Group P2	Group P3
Types of Rats	*Rattus norvegicus*white wistar strain			

Gender	Male			
General Conditions	White fur color, healthy and active			
Average Initial Body Weight	294gr	245gr	281gr	283gr
Average Final Weight	306gr	314gr	331gr	313gr

Table1-Characteristics of Test Animals

Based on the data obtained, before the high-fat diet was given, the Lee index value in the treatment group was 0.29. This value is smaller than <0.3 or is not yet included in the obesity condition. After consuming a high-fat diet in the form of quail egg yolk for 14 days, the body weight and nasoanal length of the mice were recalculated to determine the Lee index value. In the treatment group, the Lee index value changed to 0.33. Based on these data, it can be concluded that the test animals in the treatment group were in an obese condition before the snake fruit skin extract (*Salacca zalacca*) was given. Researchers also conducted phytochemical screening tests on snake fruit skin extract (*Salacca zalacca*) to see the content of secondary metabolite compounds in the extract, which can be used to improve pancreatic function in obese white rats (Rattus norvegicus) Wistar strain. The following are the screening results obtained in Table 3.

Table 3. Phytochemical Tests

Secondary Metabolites	Color	Results
Flavonoid	Red	+
Saponins	Yellow and foamy	+
Tannin	turquoise	+
Alkaloid	Yellow	+
Steroid	Green	+

Table2-Phytochemical Test

Based on the results of the normality test that has been carried out using the Kolmogorov-Smirnov Test. obtained a significance result of 0.200 in all groups. Data is said to be normally distributed if the p value> 0.05. Therefore, it can be concluded that the data is normally distributed. After the data is known to be normally distributed, the homogeneity test is

continued using the Levene test to determine whether each variant of the population group of this study is the same or homogeneous. The homogeneity test of results are shown in Table 4.

Table 4. Homogeneity Test Results

Levene static	**df1**	**df2**	**Sig**
1,781	3	20	.183

The results of the homogeneity test using the Levene test can be seen in the table above. The probability value in the significance column is 0.183. The probability value of significance obtained is greater than 0.05, so it can be concluded that the control group, treatment group 1, treatment group 2, and treatment group 3 come from populations that have the same variance or are homogeneous.

Histopathological Observation Results

The following is a histopathological image of the pancreatic tissue of each treatment group are shown in Figure 1:

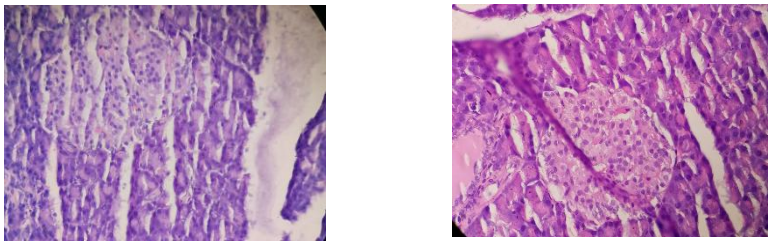

Figure 1. Histopathology of Pancreas Group (K)

The control group had a normal histological picture of the pancreas. There was no inflammatory cell infiltration, necrosis or cell degeneration. The results of histopathological observations of the pancreas in the control group showed that the organ was in normal shape because it was not given a high-fat diet with a magnification of 4x4. The histopathology of pancreas treatment 1 (200 mg/KgBW) are shown in Figure 2.

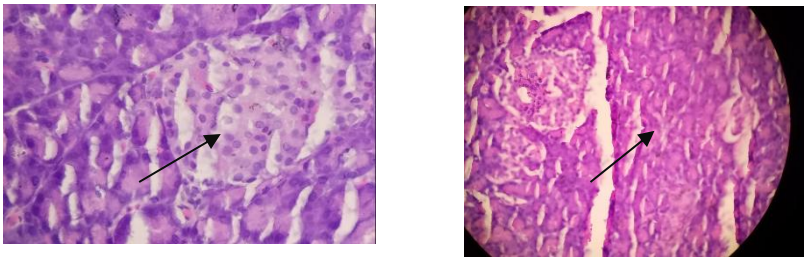

Figure 2. Histopathology of Pancreas Treatment 1 (200mg/KgBW)

Treatment 1 which was given a high-fat diet and salak skin extract at a dose of 200mg/KgBW showed a difference in shape because the pancreas organ had been exposed to the consumption of a high-fat diet that changed the histological structure of the rat pancreas. There were slight changes in the endocrine cells in the Islets of Langerhans, with some cells showing signs of karyoexis. Exocrine cells were still in good condition, but there were early indications of damage with a magnification of 10x10.

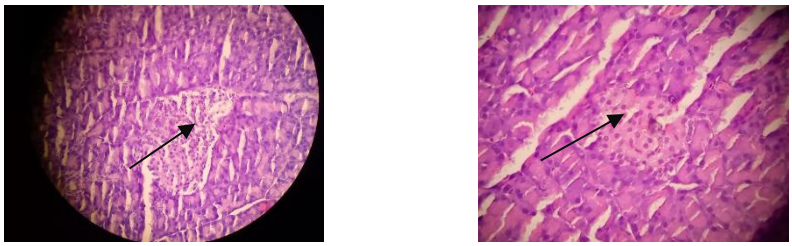

Figure 3. Histopathology of Pancreas Treatment 2 (400mg/KgBW)

Treatment 2 given salak skin extract with a dose of 400mg/KgBW showed improvement in the histological structure of the pancreas. There was an increase in damage with clear apoptosis in exocrine cells. The islets of Langerhans showed more cells undergoing necrosis and atrophy, as well as morphological changes in the cell nucleus with a magnification of 20x20. The histopathology of pancreas treatment 2 (400 mg/KgBW) are shown in Figure 3.

Figure 4. Histopathology of Pancreas Treatment 3 (600mg/KgBW)

Treatment 3 which was given a high-fat diet and snake fruit skin extract with a dose of 600mg/KgBW showed a histological structure of the pancreas that was close to the control

group with magnification 40x40. The histopathology of pancreas treatment 3 (600 mg/KgBW) are shown in Figure 4.

DISCUSSION

This study was conducted to test and analyze the effectiveness of snake fruit skin extract (Salacca zalacca) on the pancreas function of obese male white rats (Rattus norvegicus) Wistar strain, as reviewed from serum amylase and lipase levels, and how the histopathology is. The sample in this study was male white rats (Rattus norvegicus) Wistar strain weighing 200-300gr and aged 2-3 months. The sample determination used the Ferderer formula for 4 groups and the overall results were 24 rats which would be divided into 4 different groups. The first group as the control group, in this group the rats were only given regular pellet feed and distilled water. The treatment group was given a high-fat diet and snake fruit skin extract with different doses, namely 200mg/KgBB, 400mg/KgBB, and 600mg/KgBB. Obesity is a pathological condition of excess fat that accumulates in the tissues under the skin and spreads to organs and tissues throughout the body. In terms of health, obesity is a malnutrition caused by excessive consumption of unhealthy foods over a long period of time. Obese patients have health problems (Khutami, C., Sumiwi, SA, Khairul Ikram, NK, & Muchtaridi, 2022). Being overweight or obese is also a risk factor for diabetes. Obesity is related to diabetes, which is caused by the body's inability to regulate blood sugar levels with insulin in response to food intake and is positively associated with increased obesity rates. Organs involved in the development of diabetes mellitus and obesity include the pancreas (ß cells and a cells) (Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, KB, Ostolaza, H., 2020). Excess fat due to unhealthy food consumption and not balanced with sufficient physical activity causes damage to the pancreas. This condition can be overcome by taking antidiabetic drugs. However, the available antidiabetic drugs are expensive, ineffective, and have serious side effects. Thus, the use of bioactive compounds derived from plants that are effective, easily accessible, safe, and cheaper is an important treatment option for Diabetes Mellitus and obesity (Belayneh, Y.M., Birhanu, Z., Birru, E.M., & Getenet, nd). One of the plants that can overcome diabetes is the snake fruit. Researchers suspect that there is an effect of giving snake fruit skin extract (Salacca zalacca) on improving pancreatic function in obese male white rats (Rattus norvegicus) of the Wistar strain. Researchers conducted a trial on male white

rats (Rattus norvegicus) of the Wistar strain to prove this suspicion. The study began by giving preconditioning treatment in the form of a high-fat diet to induce obesity in the test animals. The high-fat diet given was in the form of quail egg yolk for 14 days. The parameters used to confirm that the rats were obese were using the Lee Index. After a high-fat diet, the rats' body weight and nasoanal length were recalculated to determine whether the rats were obese or not. The results showed that after consuming a high-fat diet, the rats got a Lee index value of 0.33 in treatment group 1 of 0.33, treatment group 2 of 0.34 and 0.33 in treatment group 3, or it can be said that the rats were obese. The test animals were then given treatment in the form of snake fruit skin extract (Salacca zalacca) according to the dose for each group. This research procedure was conducted for 14 days and produced data that needed to be processed and tested first, so it was necessary to do some data analysis in the form of normality, homogeneity, and significance tests. The normality test data was obtained with the help of SPSS using the Kolmogorov-smirnov test. The results showed that the data for each group was normally distributed with a significance value of 0.200 in all test groups for testing serum lipase and amylase levels. So it can be concluded that the data is normally distributed, or can represent the population. The normally distributed data were then tested for homogeneity using the Levene test to determine whether the data came from a population with the same variance. The results obtained showed a significance value of 0.183 for lipase levels and 0.366 for amylase levels. The probability value of significance obtained was greater than 0.05, so it can be concluded that the results of observations of lipase and amylase levels in the control group, treatment group 1, treatment group 2, and treatment group 3 were homogeneous or came from the same population. The normally distributed and homogeneous data were then tested for effectiveness and significance using the One-Way Anova test. The results of the One-way Anova test on the results of observations of lipase and amylase levels showed a significance value of 0.000 or greater than 0.05. Based on these data, it can be concluded that there is a significant difference between the control group, treatment group 1, treatment group 2, and treatment group 3 so that a further post-hoc LSD test is needed. The Post-hoc LSD test was conducted to analyze the difference in average total cholesterol levels between groups.

Conclusion

Snake fruit skin extract therapy (Salacca zalacca) can improve pancreatic function by reducing serum lipase and amylase levels in obese white rats (Rattus norvegicus) Wistar strain. Administration of snake fruit skin extract (Salacca zalacca) at a dose of 600 mg/kgBW has an effect on improving pancreatic function in obese white rats (Rattus norvegicus) Wistar strain, which can be seen through serum lipase and amylase levels that are close to the levels of the control group as a reference group.

Snake fruit skin extract (Salacca zalacca) contains secondary metabolites in the form of saponins, tannins, alkaloids, flavonoids, and steroids which help repair pancreatic cells damaged by obesity by inhibiting pancreatic cell apoptosis.

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