The Effect Of Giving Salak Skin Extract (*Salacca Zalacca*) On The Reduction Of Cholesterol Levels And Histopathology Of The Testis Of Wistar Male White Rats In An Obesity Model

Albert Prawira[1], Purwanti[2], Sri Wahyuni Nasution[3], Felanda Ahsanu Nadia[4], Rico Alexander*[5]

[1,2,3,4,5] Master of Biomedical Science Study Program, Faculty of Medicine, Dentistry, and Health Sciences, Prima Indonesia University, North Sumatra.

*E-mail : ricoalexander@unprimdn.ac.id

ABSTRACT

This study aims to evaluate the effect of snake fruit skin extract (*Salacca zalacca*) on reducing cholesterol levels and histopathological features of testes in male Wistar strain white rats with obesity model. This study uses a quantitative experimental method with a true experiment design. Male Wistar rats (*Rattus novergicus*) were induced to eat high cholesterol in the form of quail egg yolk to increase body weight to obesity. The results showed that snake fruit skin extract with doses of 400 mg/KgBW and 600 mg/KgBW was effective in reducing total cholesterol levels in obese rats, with cholesterol levels less than 54 mg/dl. Histopathological observations of the testes showed improvements in testicular structure in the group given snake fruit skin extract, especially at a dose of 600 mg/KgBW which was close to normal conditions. The conclusion of this study is that snake fruit skin extract has the potential as a cholesterol – lowering agent and improves testicular function in obese rats. This study requires further research to perfect research for the development of herbal medicines based on snake fruit skin extract for the treatment of high cholesterol and reproductive disorders due to obesity.

Keywords: *Salak Skin Extract, Cholesterol, Obesity*

INTRODUCTION

Cholesterol is one of the most abundant and important molecules in the body, creating functional membranes by influencing fluidity and allowing cells to biosynthesize a variety of other important molecules. Cholesterol is present both inside and outside cells, as it is an

essential component of all cell membranes, but it and other nonpolar substances are transported in the plasma via lipoprotein particles (classified by hydration density) that are otherwise insoluble in blood (Cox, R.A., & Garcia-Palmieri, 2011). Low-density lipoprotein (LDL) is the main carrier of cholesterol to peripheral tissues. LDL consists of cholesterol, protein, and a phospholipid shell with a cholesteryl ester core and triglycerides. All components of LDL are susceptible to oxidation to produce oxidized LDL (OxLDL). OxLDL has been associated with various pathologies (Brown et al., 1996). Because of the network between plasma cholesterol percentage and greater chance of disease, cholesterol diets are becoming more popular among the public. It is important to know how much cholesterol is in your diet because animal foods such as eggs, meat, dairy products, and others contribute between 20 and 25 percent of total body cholesterol. For proper food labeling, laboratories must provide food manufacturers with nutritional information. Previous studies have explained that snake fruit skin not only provides healthy functions, but also provides essential nutrients. The content of snake fruit skin is vitamins and minerals including vitamin A, vitamin C, potassium, and iron. Vitamin C is a powerful antioxidant that protects several body cells from oxidative damage (Joshua & Sinuraya, 2018). The inability of the skin to maintain its structural and physiological integrity is known as skin aging. The primary source of phytochemicals, which are compounds with a broad spectrum of biological activity, are plants. A growing variety of plant extracts and phytochemicals have shown promising results as anti-aging agents through ongoing scientific research efforts (Girsang et al., 2019).

LITERATURE REVIEW

Salak is one of the main commodities and is an icon of the city of Padangsidempuan. As an area with an icon of a city producing salak fruit, the city of Padangsidimpuan has the availability of land to develop salak commodities covering an area of 212.48 ha. The largest salak plantation in Padangsidimpuan City is located in Lubuk Raya Village with an area of 80 Ha and Tinjoman Village with an area of 52 Ha (BPS Padangsidimpuan). So far, the use of salak fruit has only been limited to the fruit part which is directly sold by farmers to collectors. In addition, in recent years there have been several industrial centers, both home-based and larger scale above home-based, which process salak fruit into various products. Salak skin can function as herbal medicine to prevent and treat diseases. The bioactive components in the skin of this fruit can be beneficial for health. It is known that salak fruit skin has antioxidant, antimicrobial, antidiabetic, anticancer, antihypertensive, anticholesterol, antihyperuricemia, and immune system maintenance properties. The results of phytochemical screening of snake fruit skin showed that there were solutions such as saponins, phenols, tannins, flavonoids, triterpenoids, and alkaloids. Flavonoids in snake fruit skin are one of the compounds that have

an important role in carrying out various pharmacological functions. For years, flavonoids have been used as basic ingredients for pharmaceutical and cosmetic products. However, most flavonoids do not mix with water, with the conclusion that they cannot be used well in oral form and are not effective as drugs (E. Susilowati S., A. Rahmadani, L. Meylina, 2020). High-fat meals produce larger chylomicron particles due to the increased amount of triglycerides carried, while fasting meals produce smaller chylomicron particles (Feingold, 2022).

VLDL

VLDL particles are made by the liver and contain apolipoproteins B-100, CI, C-II, C-III, and E. Apo B-100 is the core protein, and one molecule of it is present in each VLDL particle. The size of VLDL particles is only about that of a chylomicron, and varies with the amount of triglyceride ingested. Triglyceride production in the liver causes the secreted VLDL particles to be larger (Feingold, 2022).

LDL

Most of the cholesterol in circulation is carried by LDL. Small dense LDL particles have a lower affinity for the LDL receptor, which causes them to remain in the circulation longer. In addition, LDL more readily binds to intra-arterial proteoglycans and traps the arterial wall. Finally, small dense LDL particles are more readily oxidized, which may increase uptake by macrophages (Feingold, 2022).

IDL

Cholesterol-enriched IDL particles are created when VLDL triglycerides are lost from muscle and adipose tissue (Feingold, 2022) argue that the content of IDL particles is apolipoprotein B-100 and E and is pro-atherogenic.

HDL (High – Density Lipoprotein)

High-density lipoprotein particles are enriched with cholesterol and phospholipids, and apolipoproteins AI, A-II, A-IV, CI, C-II, C-III, and E associate with HDL particles. Each HDL particle contains a large amount of Apo AI protein, which is a core structural protein (Feingold, 2022) argue that one potential function of HDL particles is to transport cholesterol and other compounds in the peripheral system to the liver (i.e., reverse cholesterol transport). The testis consists of interstitial cells (Leydig cells), where testosterone is produced, and seminiferous tubules, where spermatozoa develop. The seminiferous tubules are long U-shaped tubules with their distal ends leading to the superior central and posterior regions of the testis at the rete testis, which has flat cuboidal epithelium. The seminiferous tubules form

a U-turn at the periphery of the testis. This is clinically important because a biopsy of the midtestis produces severe obstruction and destruction, but a biopsy of the testis at the periphery will not, regardless of how extensive it is. The rete testis protects fluid from the epididymis from entering the seminiferous tubules. Vasectomy increases the pressure to the point of epididymal rupture, but does not affect the testis or spermatogenesis, because of the valve-like effect of the rete testis. The seminiferous tubules loop peripherally into the tunica albuginea of the tunica vasculosa (Silver, 2018).

METHODS

The type of testing used is quantitative experimental. Quantitative experimental is the use of true experiment design or laboratory experiment. True experiment is an experimental test that is carried out seriously and carries out external variable maintenance so that experimental activities are not affected. The use of pre-test and post-test group design for knowledge and analysis of the effects before and after administration of snake fruit skin extract (*Zalacca salacca*) on reducing cholesterol percentage and histopathological depiction of testes of male Wistar rats (*Rattus norvegicus*) suffering from obesity.

The test sample used was a male rat (*Rattus norvegicus*) of the Wistar strain measuring 200 to 300 grams and aged 2 to 3 months. The selection of male rats of the Wistar strain as test material was because rats have a composition and physiology that are mostly similar to humans and are the dominant animals chosen in biomedical research.

1. Inclusion criteria:
    a. Male white rats of the Wistar strain (*Rattus norvegicus*)
    b. Weight 200-300gr
    c. Aged 2 to 3 months
    d. Have a healthy physical condition
    e. No anatomical abnormalities were found
    f. Never been used as a sample for other research
2. Exclusion criteria:
    a. Male white rats of the Wistar strain died during the experimental period.
    b. During the trial, Wistar strain male white rats developed deformities.

Variables refer to characteristics or attributes obtained by measurement or observation and many variations between people or organizations studied. The variables in this study are the objects of research observation, in this case, namely the administration of salak fruit skin extract, the percentage of cholesterol that decreases and the histopathology of the testes of male wistar rats (*Rattus norvegicus*) suffering from obesity.

a. Vvariable X (Free):	Administration of snake fruit skin extract (*Zalacca salacca*)
b. Variable Y (Dependent):	Percentage of Cholesterol lowered Histopathological picture of testis
c. Controlled Variables:	High cholesterol diet to cause obesity

Phytochemical testing is a type of initial research to determine the content of active solutions found in plants until they can function as a treatment during healing in various diseases. Phytochemical testing is carried out to understand the content of cholesterol-lowering solutions in male wistar rats in the essence of snake fruit skin (*Zalacca salacca*). The phytochemical screening test is as follows:

a. Tannin Content Test

Put 1 gram of essence into the test tube and add 10 mL. Then the water is heated for 5 minutes followed by the addition of 3 to 4 drops of FeCl3 filtrate, if it changes to green-blue (green-black) it indicates positive for the appearance of catechol tannin, but if it changes to blue-black it indicates positive for the appearance of pyrogallo tannin.

b. Flavonoid Content Test

Insertion of 1 gram of sample extract into a test tube followed by adding concentrated HCl heated for 15 minutes at the top of the water bath. If it forms a red or yellow color, it indicates positive flavonoids (flavones, chalcones and aurones).

c. Alkaloid Content Test

The introduction of 2 grams of sample extract into a test tube followed by dripping 5 mL of hot 2 N HCl. Then allowed to cool and divided into 3 test tubes containing 1 mL. the addition of each tube contains reagents. Each reagent containing Mayer is added, positive for alkaloid content if a white or yellow precipitate is formed. When Wagner reagent is added, positive for alkaloids if a brown precipitate is formed. When Dragendorf reagent is added, it has alkaloids if an orange precipitate is formed.

d. Steroid/Terpenoid Content Test

Put 2 grams of sample essence in a test tube, followed by the addition of 2 mL of ethyl acetate until shaken. Taking the ethyl acetate layer and dropping it on the plate until dry. When it is dry, add 2 drops of acetic acid hydrate and 1 drop of concentrated sulfuric acid. If it forms red or yellow, it produces a positive terpenoid. If it forms green, it produces a positive steroid.

e. Saponin Content Test

Put 1 gram of sample extract into a test tube, and add 10 ml of hot water which is then allowed to cool until shaken vigorously for 10 seconds. Positive results have saponins if the formation of foam with a height of 1 to 10 cm is approximately 10 minutes and if the addition of 1 drop of 2 N HCl, the foam is there.

Acclimatization is a procedure to adjust to a new place, weather, condition, or situation. Before being given the action, all male Wistar strains went through an acclimatization procedure for up to seven days in the Laboratory of the Department of Pharmacology, Faculty of Medicine, University of North Sumatra. The mice were given time to adjust to a new place, including food and drink (ad libitum).

RESULTS

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 200 mg/KgBW, 400 mg/KgBW, and 600 mg/KgBW. The following are the characteristics of the test animals can see been at Table 1:

Table 1. 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	255 gr	248 gr	264 gr	264 gr
Average Final Weight	318 gr	284 gr	295 gr	294 gr

Phytochemical Test Results

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 testicular function in obese white rats (*Rattus norvegicus*) Wistar strain. The following are the screening phytochemical test results obtained can be seen at Table 2.

Table 2. Phytochemical Tests

Secondary Metabolites	Color	Results
Flavonoid	Red	+

Saponins	Yellow and foamy	+
Tannin	turquoise	+
Alkaloid	Yellow	+
Steroid	Green	+

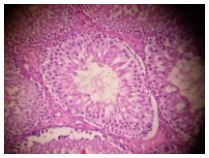
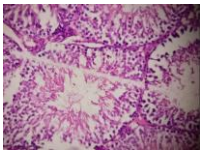
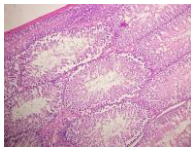
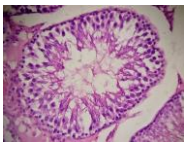
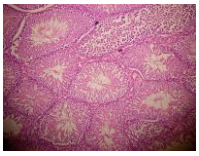
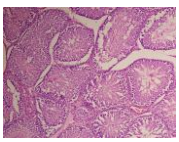
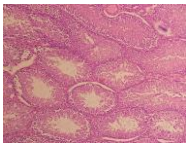
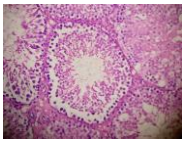
Description: (+) = Contains the tested compound group

(-) = Does not contain the tested compound

Reporting Research Results

Histopathological observations were conducted using a light microscope with 400x magnification. The purpose of this observation was to see the structure and morphology of the cells in each testicular tissue specimen in the control group and the treatment group given snake fruit skin extract (*Salacca zalacca*) at a dose of 200 mg/KgBB, 400 mg/KgBB, and 600 mg/KgBB. The administration of snake fruit skin extract (*Salacca zalacca*) was carried out every day in the morning. The following is a histological picture of the testicular tissue of each treatment group can be seen at Table 3.

Table 3. Histopathological Description of Testicular Tissue

No	Group	Histopathological Image of Testicular Tissue	
1	Control		
2	Treatment 1 (200mg/KgBW)		
3	Treatment 2 (400mg/KgBW)		
4	Treatment 3 (600mg/KgBW)		

The results of histopathological observations showed different cell appearances. The control group fed standard pellets and distilled water had normal testicular histology and was included in the score category 10, namely normal tubular epithelium, complete spermatogenesis, and spermatozoa cells ≥10. The histopathology of the testes in the control group was in normal form because they were not given a high-fat diet, so it was used as a reference to describe other groups and as a comparison with the treatment group given a high-fat diet and salak skin extract (*Salacca zalacca*)

DISCUSSION

The purpose of this study is to analyze and test the effectiveness of providingsnake fruit skin extract (*Salacca zalacca*) in lowering cholesterol levels and improving testicular function in obese male wistar rats (*Rattus norvegicus*) and how the histopathology of the testes is. This study used 24 white rats (*Rattus norvegicus*) of the wistar strain as experimental animals, which were divided into 4 different groups. The first group was 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 andsnake fruit skin extract (*Salacca zalacca*)with different doses, namely 200mg/KgBB, 400mg/KgBB, and 600mg/KgBB. The study began by preparing the test animals with a high-fat diet for 14 days. After the high-fat diet, body weight and length were recalculated to determine whether the mice were obese or not. The results showed that after consuming a high-fat diet, the mice had a Lee index value of 0.29 in treatment groups 1, 2 and 3. These results indicate that the mice are included in the obesity category. Obesity is a complex health problem resulting from a combination of causes and individual factors such as behavior and genetics. Behaviors can include physical activity, inactivity, diet, medication use, and other exposures (Kayode, 2021). Obesity is by far the biggest risk factor contributing to the burden of chronic disease. Obesity occurs because of high cholesterol levels which are considered to have a negative impact on health. Cholesterol has many functions, but cholesterol can also be harmful to the body if allowed to reach abnormal blood concentrations. When cholesterol levels are out of balance it can cause various Health conditions (Huff T, Boyd B, 2022). The negative effects of obesity also affect male infertility (Craig, JR, Jenkins, TG, Carrell, DT, & Hotaling, 2017). This happens due to oxidative stress that occurs in obesity conditions. Research has found that oxidative stress, a condition characterized by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system, is an emerging factor in male infertility (Cito, G., Becatti, M., Natali, A., Fucci, R., Picone, R., Cocci, A., ... & Coccia, 2020). Oxidative stress is an important factor for the development of male infertility due to the very high rate of cell division and

mitochondrial oxygen consumption in testicular tissue and the comparable level of unsaturated fatty acids in this tissue compared to other tissues. In addition, the oxygen tension level is low due to the weakness of the testicular arteries, therefore, there is severe cell competition for oxygen. These conditions make testicular tissue and the male reproductive system very vulnerable to oxidative stress (Asadi et al., 2021).3

Research Procedures

Acclimatization is a procedure to adjust to a new place, weather, condition, or situation. Before being given the action, all male Wistar strains went through an acclimatization procedure for up to seven days in the Laboratory of the Department of Pharmacology, Faculty of Medicine, University of North Sumatra. The mice were given time to adjust to a new place, including food and drink (ad libitum).

CONCLUSION

1. Snake fruit skin extract (*Salacca zalacca*) with a dose of 400 mg/KgBB and 600 mg/KgBB is effective in reducing total cholesterol levels in obese white wistar rats. This is evident from the cholesterol levels of both groups which are less than 54mg/dl.
2. The results of histopathological observations of testicular tissue in treatment group 3 were: snake fruit skin extract (*Salacca zalacca*)with a dose of 600 mg/KgBW experienced the most significant improvement and approached the control group compared to the other groups.
3. The results of the phytochemical tests carried out showed that there were secondary metabolite compounds insnake fruit skin extract (*Salacca zalacca*) including flavonoids, saponins, tannins, alkaloids, and steroids. These compounds help lower total cholesterol levels and improve testicular function in obese white rats (*Rattus norvegicus*) Wistar strain.

ACKNOWLEDGEMENT

The authors thank Universitas Prima Indonesia for supporting this project, the heads of study programs, and the Faculty of Medicine, Dentistry, and Health Sciences. We also thank the supervising lecturers for their invaluable advice. The author would like to thank the Pharmacology Laboratory, Nanomedicine Laboratory, Faculty of Pharmacy, and the University of North Sumatra, which greatly helped this research.

REFERENCES

Adjeng, A. . N. . (2020). Phytochemical screening and evaluation of liquid soap preparations of 96% ethanol extract of salak pondoh fruit skin as an antioxidant. Pharmaceutical Science and Health, 2(5), 21–24.

Aralas S, Mohamed M, FABM (2009). Antioxidant properties of selected salak (*Salacca zalacca*) varieties in Sabah. Malaysia. Nutr Food Sci, 3(39), 243–250.

Arundhana AI, Hadi H, JM (2013). Sedentary behavior as a risk factor for obesity in elementary school children in Yogyakarta City and Bantul Regency. J Gizi Dan Diet Indones, 1, 71–80.

Asadi, A., Ghahremani, R., Abdolmaleki, A., & Rajaei, F. (2021). Role of sperm apoptosis and oxidative stress in male infertility: A narrative review. In International Journal of Reproductive BioMedicine. https://doi.org/10.18502/ijrm.v19i6.9371

Brown, A. J., Dean, R. T., & Jessup, W. (1996). Free and esterified oxysterol: Formation during copper-oxidation of low density lipoprotein and uptake by macrophages. Journal of Lipid Research, 37(2), 320–335. https://doi.org/10.1016/s0022-2275(20)37619-7

Chareoansiri R, KR (2009). Sugar profiles and soluble and insoluble dietary fiber contents of fruits in Thailand markets. Int J Food Sci Nutr, 60, 126–139.

Cito, G., Becatti, M., Natali, A., Fucci, R., Picone, R., Cocci, A., ... & Coccia, M.E. (2020). Redox status assessment in infertile patients with non-obstructive azoospermia undergoing testicular sperm extraction: A Prospective Study. Andrology, 8(2), 364-371.

Cox, R.A., & Garcia-Palmieri, M.R. (2011). (2011). Cholesterol, triglycerides, and associated lipoproteins.

Craig, J.R., Jenkins, T.G., Carrell, D.T., & Hotaling, J.M. (2017). Obesity, male infertility, and the sperm epigenome. Fertility and Sterility, 4(107), 848–859.