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# Stimulus from the activator agent *Solanum melongena L.* on sperm quality through the lipid profile of *Rattus norvegicus* with hyperlipid induction

## Estímulo del agente activador Solanum *melongena L.* sobre la calidad del esperma a través del perfil lipídico de *Rattus norvegicus* con inducción hiperlipídica

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**SUMMARY**

**Introduction:** *A high-fat diet and obesity, promoted by an unhealthy lifestyle, affect the structure of spermatozoa. The study aims to determine the effect of using purple eggplant on improving lipid profile and sperm quality as an alternative to traditional medicine of Rattus norvegicus.* **Methods:** *This type of research is a laboratory experiment using a post-test with a control group design. Twenty-eight Rattus norvegicus were divided into four groups. The number of spermatozoa was calculated by sucking the sperm stock solution using a hemocytometer suction.* **Results:** *The high cholesterol and distilled water (G1), high cholesterol and purple eggplant 100 mg/kg BW (G2), and high cholesterol and purple eggplant 200 mg/kg BW (G3) groups had higher body weight compared to the standard feed and distilled water (G0) group. The G1 group had the highest body weight (136.25±0.901 grams) and cholesterol levels (168.13±1.797 mg/dL). Moreover, G3 group (159.38±1.253 mg/dL) had lower cholesterol levels compared to G2 group (152.25±10.31 mg/dL). The G3 group ($35.00x10^6$±0.500) had the highest mean sperm count, followed by G1, G2, and G0 groups ($33.25x10^6$± 0.675, $32.75x10^6$±0.366, $31.38x10^6$±0.680, respectively).* **Conclusion:** *The purple eggplant extract was able to reduce cholesterol levels and increase sperm count.*

**Keywords:** *Purple eggplant, body weight, cholesterol levels, sperm count*

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## RESUMEN

**Introducción:** *Una dieta alta en grasas y la obesidad, promovida por un estilo de vida poco saludable, afectan la estructura de los espermatozoides. El estudio tiene como objetivo determinar el efecto del uso de berenjena morada en la mejora del perfil lipídico y la calidad del esperma como alternativa a la medicina tradicional de Rattus norvegicus.* **Métodos:** *Este tipo de investigación es un experimento de laboratorio que utiliza pos-prueba con un diseño de grupo de control. Se dividieron veintiocho Rattus norvegicus en cuatro grupos. El número de espermatozoides se calculó succionando la solución madre de esperma usando una succión de hemocitómetro.* **Resultados:** *Los grupos fueron, colesterol alto y agua destilada (G1), colesterol alto y berenjena morada 100 mg/kg de peso corporal (G2), colesterol alto y berenjena morada de 200 mg/kg de peso corporal (G3), los cuales presentaron un mayor peso corporal en comparación con el alimento estándar y los grupos con agua destilada (G0). El grupo G1 tuvo el mayor peso corporal (136,25 ±0,901 gramos) y niveles de colesterol (168,13 ± 1,797 mg /dL). Además, el grupo G3 (159,38 ± 1,253 mg/dL) tuvo niveles de colesterol más bajos en comparación con el grupo G2 (152,25 ± 10,31 mg/dL). El grupo G3 (35,00x$10^6$ ± 0,500) tuvo el recuento medio de espermatozoides más alto, seguido de los grupos G1, G2 y G0 (33,25x$10^6$ ± 0,675, 32,75x$10^6$ ± 0,366, 31,38x$10^6$ ± 0,680, respectivamente).* **Conclusión:** *El extracto de berenjena morada pudo reducir los niveles de colesterol y aumentar el recuento de espermatozoides.*


**Palabras clave**: *Berenjena morada, peso corporal, niveles de colesterol, recuento de espermatozoides.*

## INTRODUCTION

A high-fat diet and obesity, which are encouraged by an unhealthy lifestyle, affect the structure of spermatozoa and the development and health of offspring. Indeed, infertile men have been observed to have improper dietary patterns, including meal omissions, insufficient antioxidant intake, and a high energy density (1). An unhealthy hypercaloric diet, high saturated fat and trans fat intake, a high glycemic index, and low nutritional density may all be associated with increased oxidative stress, the underlying cause of obesity, intestinal dysbiosis, type 2 diabetes, and insulin resistance (2). The metabolic disorders mentioned above are associated with a decline in fertility, primarily due to the generation of oxidative stress, which is regarded as a significant factor contributing to decreased sperm quality and an increased risk of infertility and hormonal and immunological disorders (3).

One of the leading causes of disruption of the process of spermatogenesis in the testes is the presence of free radicals (4). Free radicals can also inhibit the process of spermatogenesis because Leydig cells are disrupted to reduce levels of testosterone hormone secretion. The negative impact of free radicals on reproductive health can be overcome by giving antioxidants (5,6). Additionally, while sperm produces reactive oxygen species (ROS), an imbalance between ROS and antioxidants will harm sperm associated with male infertility, peroxyl radicals, and peroxynitrite (7).

Food is the primary source of cholesterol. Dietary cholesterol is initially transported from the small intestine to the liver, where it is then redistributed to the organs that require it. The Scavenger receptor B type 1 (SR-B1) is a high-density lipoprotein (HDL) receptor on the cell surface that mediates the uptake of HDL-cholesterol ester (HDL-CE). Additionally, less than half of cholesterol is synthesized de novo. The synthesis of cholesterol is a multi-step enzymatic process. The synthesis of cholesterol begins with the transport of acetyl-CoA from the mitochondria to the cytosol. Hydroxy-methyl-glutaryl-CoA (HMG-CoA) is formed through a series of reactions and is then converted to mevalonate by HMG-CoA reductase. Finally, a series of steps will result in the formation of cholesterol (8,9). Cholesterol is a critical component of mammalian plasma membranes, where it is required for the proper permeability and fluidity of the membrane. Hypercholesterolemia is a risk factor for noninsulin-dependent diabetes, osteoarthritis, some types of cancer, and certain reproductive and metabolic disorders. Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low-density lipoprotein and blood cholesterol. Cholesterol is a steroid lipid found in the cell membranes and transported in the blood plasma of all animals (10).

Many studies have been conducted to identify natural substances that can protect the body from oxidative stress, such as the use of antioxidant compounds found in fruits, vegetables, and

grain plants (11-13). Antioxidants are needed to protect the body from free radical attack through protecting enzymes that repair DNA damage, therefore increasing our body's ability to regenerate itself (14). One of them is purple eggplant (*Solanum melongena L*), one of Indonesia's medicinal plants. Purple eggplant contains alkaloid compounds in the form of glycosides, namely solanine, tomatin, and solasodine. Solasodine is a steroidal glycoalkaloid compound contained in purple eggplant and is thought to have an antifertility effect (15). The administration of purple eggplant ethanol extract could significantly reduce the percentage of rat spermatozoa motility compared to controls. Solasodine inhibits the expression of luteinizing hormone (LH) and spermatogenesis in mice (16) having high antioxidant content. Purple eggplant contains anthocyanin pigments which act as antioxidants. Anthocyanins are part of phenolic compounds, which are classified as flavonoids. Sadivola stated that the dominant anthocyanin content in purple eggplant skin is delphinidin 3-rutisinode, a purple pigment. Anthocyanin pigments are more stable under acidic conditions than basic and neutral conditions and are unstable with hot light and certain metals. Microencapsulation is one way to increase the stability of an anthocyanin compound (delphinidin 3-rutisinode) (17). This study aimed to determine the effect of using purple eggplant on the improvement of lipid profile and sperm quality as an alternative to traditional medicine.

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## METHODS

### Research Type and Design

This study was a laboratory experiment using a post-test research design with a control group (*post-test control group design)*. This study used experimental animals in the form of white male rats *(Rattus norvegicus)* Wistar strain. A total of 28 *Rattus norvegicus* were divided into four groups, including G0, G1, G2, and G3. G0 was only given standard feed and distilled water. G1 was only fed high cholesterol and distilled water diet. G2 was fed a high cholesterol diet and given purple eggplant with 100 mg/kg BW. G3 was fed a high-cholesterol diet with purple eggplant treatment with 200 mg/kg BW. This study was carried out for 4 weeks.

### Test Animals

The test animals selected were *Rattus norvegicus* Wistar under the inclusion criteria in the form of *Rattus norvegicus* Wistar strain, 3-4 months old, bodyweight of 150-200 g, healthy condition and no macro anatomical abnormalities, and never get any treatment. Exclusion criteria were sick rats and dead rats during the study.

### Procedure for Making Purple Eggplant Extract

Fresh purple eggplant was dried in an oven at 70 °C for 24 hours and then mashed with a blender, macerated with 96 % ethanol until the macerated was clear. The results of the macerate are put into a vacuum rotatory evaporator until a thickened extract is obtained, then proceed with making a suspension at a dose of 100 mg/kg, BW and 200 mg/kg, BW, diluted by adding distilled water.

### Procedure for Making High-Fat Feed

In this study, high-fat feeding was made from quail egg yolks, referring to the previous study. The yolk of the quail is separated from the white of the egg. Giving quail egg yolk to rats by induced using a gastric probe as much as 1.5 mL/head was carried out every morning for 4 weeks except for the negative control rat group, which was not induced by quail egg yolk**.**

### Blood Sampling Procedure

The blood samples of experimental animals were taken from the hearts of rats. Before sampling, the experimental animals were fasted at night (12 hours fast). During fasting, the experimental animals were given water *ad libitum*. Animal sacrifices were made in the morning, starting with physical euthanasia techniques. To draw blood can be done directly by inserting a syringe directly into the heart and aspirating slowly.

**Sperm Sampling Procedure**

Spermatozoa collection of male rats begins with animal sacrifice, which is carried out in the morning, begins with physical euthanasia techniques, and then dissects their reproductive organs. The cauda epididymis was then removed and placed in a petri dish containing 0.9 % sodium chloride. Additionally, the cauda epididymis was inserted into a watch glass containing 1 ml of 0.9 percent NaCl, and the proximal part of the cauda was cut slightly with scissors and the cauda was pressed slowly until secretory fluid emerged and was suspended in 0.9 percent NaCl. The suspension of spermatozoa from the cauda epididymis obtained can be used for observations, including motility, number, and morphology of spermatozoa.

**Sperm Count Procedure**

To determine the number of spermatozoa, the following method was used. The number of spermatozoa was calculated by sucking the sperm stock solution using a hemocytometer suction pipette to the 0.5 mark, then the physiological NaCl solution was sucked up to the 101 marks, and the pipette was shaken. Discard a few drops on tissue paper, then put them in a counting chamber which has been closed with a coverslip and has been prepared in a microscope, then examined under a microscope. Calculated using the formula for the number of spermatozoa counted (s) x dilution x 1 ml NaCl = s x 20,000 = million/$mm^3$.

**Sperm Motility Calculation**

The rat sperm was taken from the *cauda epididymis* by slashing and pressing gently. One drop of sperm was placed on an object glass, plus one drop of 0.9 % NaCl physiological solution, mixed evenly and covered with an object glass. The percentage of motile spermatozoa was calculated in one field of view using a light microscope at a magnification of 100 times by estimating progressively moving spermatozoa from the entire field of view and the estimated area, then multiplied by 100 %. Assessment is done by calculating the percentage of spermatozoa whose movement is progressively forward compared to those observed (moving and immobile).

**Data Processing and Analysis**

Data obtained from observations on sperm count, body weight, and cholesterol levels were previously carried out. From the dependent and independent variables, normality and homogeneity were carried out with the Shapiro-Wilk test because the number of samples was <50; if the data distribution was normal, then the average was homogeneous with the one-way ANOVA test. If the result means $p<0.005$, the results were continued with the LSD test using Post Hock. If the data were not normal and not homogeneous, it would be tested with the Kruskal Walis test, followed by the Mann-Whitney Test. The results of data processing are displayed in the form of tables and graphs.

## RESULTS

During the study, rats were weighed every week for 4 weeks. The weighing was done using a digital scale. The results of rat body weight, cholesterol level, and sperm count in each group are summarized in Table 1. The body weight, cholesterol level, and sperm count of rats were normally distributed ($p >0.05$).

The results showed that the high-fat diet was successful in increasing the body weight of rats. The bodyweight of the G1, G2, and G3 groups was higher than the G0 group, which was only given standard feed. The G1 group showed the highest increase in body weight. Whilst G0 and G1 groups were the lowest and the highest cholesterol levels, respectively. Moreover, the G3 group had lower cholesterol levels compared to the G2 group. In addition, the G3 group had the highest mean sperm count, followed by G1, G2, and G0 groups (Table 1).

The microscopic observations, including sperm count, viability, and morphology, in male *Rattus norvegicus* Wistar strain with an objective lens magnification of 100x, had different results in each treatment. On sperm viability examination (Figure 1), G0 showed the sperm still alive (the

Table 1

The results of rat body weight, cholesterol level, and sperm count (N=28)

| Group | Weight (Grams)* | Normality Test | Cholesterol level (mg/dL)* | Normality Test | Sperm Count*# | Normality Test |
|---|---|---|---|---|---|---|
| G0 | 131.38±0.822 | 0.945 | 129.38±2.719 | 0.210 | 31.38±0.680 | 0.603 |
| G1 | 136.25±0.901 | 0.976 | 168.13±1.797 | 0.446 | 33.25±0.675 | 0.351 |
| G2 | 130.13±0.675 | 0.626 | 159.38±1.253 | 0.959 | 32.75±0.366 | 0.408 |
| G3 | 129.88±0.766 | 0.720 | 152.25±10.31 | 0.454 | 35.00±0.500 | 0.273 |

*Mean±SD

#Value multiplied by 106 mL

G0: Group was only fed standard feed and aquades
G1: Group was only fed high cholesterol and aquades
G2: Group was fed a diet high cholesterol and purple eggplant 100mg/kg, BW
G3: Group was fed a diet high cholesterol and purple eggplant 200mg/kg, BW

yellow arrow) because the sperm did not absorb the red color in the eosin negrosin reagent. At the same time, the red arrows indicate sperm that died because they absorbed the red color of the eosin-negrosin reagent. G1 to G3 only showed dead sperm.

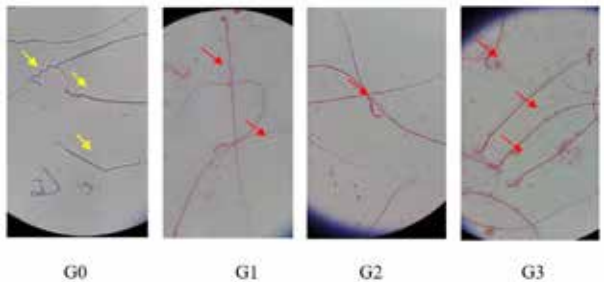


Figure 1. Examination of sperm viability with an objective lens magnification of 100x.

## DISCUSSION

This study demonstrated that giving a high-fat diet for 4 weeks could increase the body weight of rats and cholesterol levels compared to the control group. The administration of purple

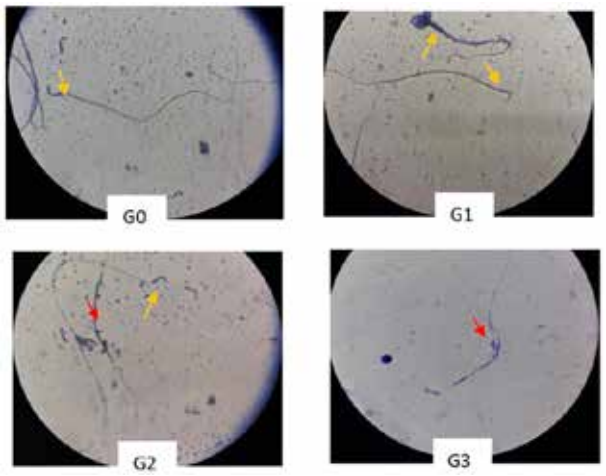


Figure 2. Sperm morphology examination with an objective lens magnification of 100x.

Figure 2 shows the results of the sperm morphology examination. The normal sperm shapes from head to tail (yellow arrows) were shown in G0 and G1. G2 had the sperm in normal and abnormal forms, which only the sperm body (red arrow). While G3 had the sperm with only body shape (red arrow).

eggplant at 200 mg/kg BW was able to reduce cholesterol levels and increase the sperm count. Several diseases have been treated with eggplant, including asthma, bronchitis, diabetes, rheumatoid arthritis, and hypercholesterolemia (18). The clinical use of eggplant is due to its phenolic and alkaloid. The primary phenolic compounds in the skin and pulp of eggplant are delphinidin (an anthocyanin) and chlorogenic acid (a phenolic acid) (19). Moreover, delphinidin has exhibited inhibitory properties against the α-amylase enzyme, and thereby it may be useful in the treatment of diabetes and its complications such as overweight and obesity, and cardiovascular disease (20). It has been suggested that chlorogenic acids exhibit anti-obesity and anti-hyperlipidemic activities by alleviating the levels of free fatty acids and triglycerides (TG) (21). Additionally, several studies have reported the pharmacologic aspects of eggplant, such as anti-oxidant (22), obesity (23), hyperlipidemia (18). Hyperlipidemia is characterized by abnormally high levels of lipids in the blood, including fat, cholesterol, and TGs. It is widely recognized as the primary risk factor for cardiovascular disease and is one of the leading causes of death worldwide.

A previous study also showed increased high cholesterol levels after getting feed with a high fat composition (24). Eggs and egg products account for 25 % of daily total cholesterol intake in children and adults in the United States (25). Some studies related to man have shown the association of BMI with reproductive parameters like poor semen quality (26), decreased sperm concentration (27), decreased number of normal motile sperm cells (28), increased ROS, and increased DNA fragmentation index (29).

Male obesity has a less well-documented effect on reproduction than female obesity does. Several studies, however, indicate that overweight and obese men have lower sperm quality and fertility. Male obesity is suspected to alter sperm parameters, particularly sperm concentration, total progressively motile sperm count (29), total sperm count, total motile sperm count sperm morphology, and DNA fragmentation (30). However, the magnitude of this increase was small and had only a minor clinical significance, as there was no correlation between increased ROS production and decreased sperm DNA integrity or motility. Additionally, increased BMI was found to be significantly associated with a decrease in sperm concentration, serum testosterone, and serum estradiol (31). In obese men, excessive ROS production and abnormal hormonal regulation result in suboptimal sperm quality. It is hypothesized that these patients' oxidative stress is caused by dysregulation of adipocytokine and ROS generation (32). The excessive production of ROS may be a result of obese men's increased metabolic rates and maintenance of hemostasis. Additionally, elevated levels of ROS and temperature in the testicles may denature enzymes involved in spermatogenesis. Increased scrotal skin temperature was associated with a decrease in sperm concentration (27).

The decrease in spermatozoa viability in the purple eggplant extract treatment group was influenced by the presence of alkaloid compounds in purple eggplant, namely solasodine which can interfere with the permeability of the spermatozoa membrane. The decrease in sperm viability is caused by disruption of the permeability of the spermatozoa membrane which can later interfere with the transportation of nutrients needed for the movement of spermatozoa (33), besides that the permeability of the spermatozoa membrane is closely related to cell metabolism and plays a role in the formation of energy so that in this case the permeability of the spermatozoa membrane is closely related to the motility and viability of spermatozoa.

The relationship between lipids and sperm production is complex and unknown in its etiology. In humans, the cholesterol content of sperm varies significantly, even between ejaculates (34). In the meantime, the amount of cholesterol in sperm membranes is directly proportional to the sperm morphology (35) and fertility potential (34). For several mammalian species, the lipid composition of the sperm plasma membrane has been determined (36). In comparison to other cell types, spermatozoa have a unique lipid composition. Spermatozoa contain a greater proportion of neutral lipids, particularly a high concentration of diacylglycerol (DAG) (37,38). Recently, the lipid component (O-acyl)-ϖ-hydroxy-fatty acids (OAHFA) with a carbon chain length of up to 52 was identified in spermatozoa for the first time. It was found in

the head of sperm rather than the tail region (38).

However, few studies are examining the detailed relationships between serum cholesterol or other lipid profiles and sperm quality. This cross-sectional study, like our previous ones, had some limitations (39,40). These included the lack of semen volume and total sperm count data in the initially designed protocol of the program, absence of detailed records of endocrine levels and lipid contents of semen, and lack of abstinence duration records for evaluation in the study. Moreover, the lipid-related pathological conditions, such as familial hypercholesterolemia or ApoE genotype, were not explored in our study because of the initially designed data requirement. The semen samples of our study were collected using home-collection kits; the quality may not be equal to that resulting from the on-site collection. Further studies, including those assessing the lipid content of semen and performing measurement of lipid-related nuclear receptors such as liver X receptors (LXRs) (41), peroxisome proliferator-activated receptors (PPARs), small heterodimer partner (SHP) (42), 27, and retinoid X receptors (RXRs) (43), should be designed further to explore the relationships between lipids and semen quality (34).

Reduced motility, intracellular enzyme damage, and damage to the structure of the plasma membrane are all characteristics of spermatozoa damaged by lipid peroxidation (44). Additionally, as spermatozoa mature in the epididymis, the composition of the components that comprise the spermatozoa plasma membrane changes. The plasma membrane of the spermatozoa will lose some cholesterol, increasing the ratio of unsaturated fatty acids to cholesterol (45).

The increase in abnormalities in the treatment group was caused by the purple eggplant extract containing tannins. Tannins function to bind proteins and ions contained in spermatozoa membranes so that the tyrosine enzyme and phosphorylation process in the spermatozoa membranes are disrupted and ultimately cause morphological abnormalities of spermatozoa (46). The increase in morphological abnormalities of spermatozoa can be caused by damage in the seminiferous tubules and when the spermatozoa leave the seminiferous tubules and during their journey through the epididymis. The male reproductive system in normal conditions maintains a balance between ROS production and antioxidant activity. However, excessive ROS production in sperm or seminal plasma can impair the antioxidant defense mechanisms of the sperm or seminal plasma, resulting in oxidative stress. It is well established that oxidative stress damages sperm chromatin/DNA. Antioxidant therapy is widely accepted to improve sperm quality and male fertility by reducing oxidative stress. The findings of this study using purple eggplant corroborate those of Sabeti et al. (47).

## CONCLUSION

Giving a high-fat diet for 4 weeks was able to increase the bodyweight of rats and cholesterol levels compared to the control group. At the same time, the administration of purple eggplant at 200 mg/kg, BW could reduce cholesterol levels and increase the sperm count.

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