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ADMINISTRATED OF SNAKE WOOD *(Strychnos lucida)* TO DECREASED ABNORMALITY SPERMATOZOA OF MICE *(Mus musculus)* DIABETES MELLITUS MODELING

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ABSTRACT

Diabetes mellitus, known as diabetes or blood sugar disease, is a group of chronic diseases characterized by increased blood sugar level (hyperglycemia). Prolonged hyperglycemia conditions will cause an increase in ROS (Reactive Oxygen Spesies) production by the mitochondria, which then cause damage to the mitochondrial membrane resulting in loss of function of the mitochondrial membrane potential and can induce sperm cells apoptosis. This study aims to determine effect of kayu ular (*Strychnos lucida*) steeping on decreasing spermatozoa abnormalities of mice (*Mus musculus*) diabetes mellitus model. The result showed that administration of kayu ular steeping at concentration of 2,6gr/50ml was signicantly reduced ($P<0.05$) spermatozoa abnormality. This dose is the most effective concentration to decreased the spermatozoa abnormality of mice. This though to be due to the active compounds found in steeping kayu ular (*Strychnos lucida*) which can counteract free radicals due to diabetes mellitus that decreasing spermatozoa abnormalities.

Keywords: *diabetes mellitus, abnormalities sperm, strychnos lucida.*

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INTRODUCTION

Indonesia is currently the seventh ranked country with the largest number of DM sufferers in the world after China, India and America. Total DM sufferers in Indonesia based on WHO data, currently there are around 8 million people, and it is estimated that the number will exceed 21 million people in 2025 (Kemenkes, 2012). Not only are those over 50 years old susceptible to infection, the productive age group around 20-30 years is also susceptible to developing DM. This is caused by changing lifestyles, because people consume various types of food (Yang et al, 2010). Diabetes Mellitus (DM), also known as diabetes or blood sugar disease, is a group of chronic diseases characterized by increased blood sugar levels (hyperglycemia) as a result of disturbances in the body's metabolic system, when the pancreas is unable to produce the hormone insulin as needed. Body (IDF, 2015). Prolonged hyperglycemia will cause an increase in the production of ROS (Reactive Oxygen Species) by the mitochondria, which causes damage to the mitochondrial membrane so that the potential function of the mitochondrial membrane is lost and induces sperm cell apostosis (Yang et al, 2010). Other research has revealed the negative impact of diabetes on fertility and generally diabetics experience impaired sexual function due to low levels of the hormone testosterone. Low testosterone

hormone causes decreased attraction, loss of libido, decreased sexual activity and affects the occurrence of erections (Rachmadi, 2008).

One of the plants used by the community as a traditional medicine is snake wood which has the scientific name Strychnos lucida. The chemical compounds contained in snake wood are alkaloids (brusina, striknina), tannins, saponins (triterpenoids/steroids), flavonoids (Gusmalina, 2015). The seeds and wood parts of this plant contain alkaloids which have microbial properties and act as antioxidants which are useful for maintaining the health of spermatozoa. These antioxidants can remove free radicals and reduce oxidative stress, so that the amount of ROS and antioxidants is in a balanced state (Lukacinova et al, 2008). Antioxidants are able to counteract free radicals by neutralizing free radicals and inhibiting lipid peroxidation so that the hormones of spermatozoa formation can be maintained to optimize the formation of spermatozoa cells. To prove this, it is necessary to conduct a study to examine the effect of infusion of snake wood (S. lucida) on the reduction of spermatozoa abnormalities in mice (Mus musculus) a model of diabetes mellitus. This study aims to determine the effect of giving snakewood infusion on the reduction of spermatozoa abnormalities in mice with diabetes mellitus.

METHOD

The research was conducted in an experimental laboratory. The research was conducted on 12 February - 12 March 2019, at the Zoology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University. This study used an experimental method using a completely randomized design (CRD) consisting of 5 treatments and 3 repetitions.

Procedure

1. Preparation of Test Animals

   The model animals used in this study were 15 male mice two months old and weighing 20 grams. Prior to being used as experimental animals, all mice were acclimatized for seven days in a mouse cage made of a box-shaped plastic container with a little rice husk as bedding, feed and ram wire as a cover. The body weight of mice (M. musculus) was weighed at the start of the study

2. Preparation of Streptozotocin Solution and Injection

   Prior to injection, streptozotocin (STZ) was dissolved in citrate buffer with a pH of 4.5 (5.25 g of citrate buffer solution (Na2HNO4) was weighed and added to 22.5 ml of distilled water), homogenized then added 39.8 mg of streptozotocin (STZ) to obtain the final concentration of streptozotocin (STZ) solution in solution was 22.5 mg/ml STZ. Streptzotocin solution (STZ) was injected intraperitoneally as much as 0.1 ml per head. Mice with glucose levels exceeding 200 mg/dl were considered diabetic using a glucometer (Easy Touch GCHb) (Dalimartha, 2007).

2. Making snake wood infusion

   Refining snake wood plants, can be done in the following way:

   • Snake wood that has been cut, grinded using a smoothing tool until it becomes snake wood powder.

   • After obtaining snake wood powder, the process of making snake wood infusion is carried out. Snake wood powder was weighed as much as 0.66 grams, 1.3 grams and 2.6 grams, then each was put into a beaker glass.

   • 150 ml of distilled water is heated on a hot plate for ± 15 minutes until it boils, then remove and pour into a glass beaker containing 50 ml of snakewood each.

   • After that it is stirred and allowed to stand for 15 minutes (until warm), then filtered using Whatman filter paper to obtain snake wood infusion.

   Dosage of Snake Wood Stew

   The dose of using a snakewood infusion is 50 ml, then the conversion is carried out from humans to animal models of mice with a calculation of 0.0026 x 50 ml = 0.13 ml/head/day. Based on the above results, the dose/volume of administration used was 0.13 ml/head/day with the concentration of snake wood infusion divided into, concentration I: 0.65 gr/50 ml, concentration II: 1.3 gr/50 ml, and concentration III: 2.6 gr/50 ml.

3. Treatment Group

   This study used a completely randomized design (CRD) consisting of 15 mice divided into five groups of 3 mice each. Three groups other than the negative control, namely the positive control group, treatment I, treatment II and treatment III, were induced by STZ 0.1 ml/head/day for five days. Then after STZ induction, 12 mice were examined for their blood sugar levels on the 6th or 7th day. Measuring blood glucose levels was carried out by taking blood from mice through the tail which was previously cleaned

with alcohol. Then the blood is dripped on a glucometer strip (Easy Touch GCHb) and put in the glucometer to read the glucose level. Mice are said to be diabetic if their blood glucose levels reach ≥200 mg/dl (Dalimartha, 2007). The infusion of snakewood infusion was carried out in the morning and evening for 14 days for each treatment. Different treatment was given to each group, namely group (P1) was given a concentration of 0.65 gr/50 ml snake wood infusion, group (P2) was given a concentration of 1.3 gr/50 ml snake wood infusion and (P3) was given a concentration of 2 concentrations of snake wood. .6 gr/50 ml with a dose/volume of 0.13 ml/head/day in the three treatment groups.

4. Collection of spermatozoa

On the 15th day after administration of snake wood infusion, the mice were terminated/sacrificed by way of neck dislocation of mice, then dissected in the lower abdomen. Spermatozoa samples were taken from the epididymis, which is exactly 1 cm below the cauda epididymis. In that place it is clamped and then cut. The cut parts were excreted by chopping the sperm, then 2 drops of 0.9% NaCl were added and stirred to make it homogeneous and then used to observe the motility and morphology of spermatozoa (Fibullah, 2015).

5. Observation of Spermatozoa Morphology

Calculation of the number and morphology of spermatozoa was carried out using the method according to (Suparni, 2009). The number of spermatozoa is determined as follows:

a. The spermatozoa suspension was first made homogeneous.
b. Next, 10 µl of spermatozoa samples were taken.
c. The spermatozoa mixture was put into the Haemocytometer counting chamber boxes, the number of spermatozoa was in boxes A, B, C, D, and counted under a microscope with 400x magnification.
d. The spermatozoa in the 25 small squares in the main rectangle in the haemocytometer counting chamber are added up to determine the number of spermatozoa, then divided by the Haemocytometer correction factor. The results of this division are the total number of spermatozoa and million per mL/ejaculate. The formula for the number of calculated spermatozoa (s) x 200,000 = million/ml. Determine the morphology of spermatozoa, a homogeneous sperm sample was dripped using a pipette on a glass object as much as 1 drop, then dripped with eosin dye solution, then covered with a glass cover.

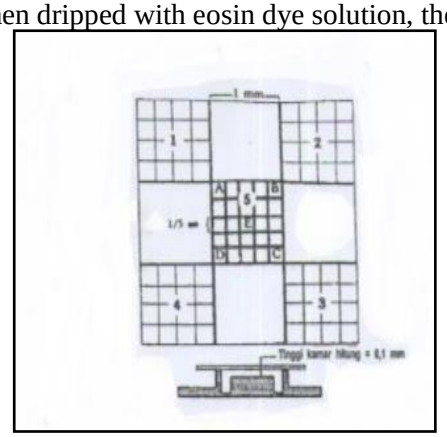

Figure 1. Haemocytometer Count Chamber

The sample was allowed to stand for a few moments and then observed under a microscope with a magnification of 400x evenly in 5 fields of view, to determine the percentage of normal and abnormal spermatozoa. The characteristics of normal spermatozoa are the shape of the head like a fishing hook and a long, straight tail, while the abnormal spermatozoa have an irregular head shape shaped like a banana or irregular (amorphous), too crooked and the shape of the tail is not straight, or no tail or only a tail (no tail). head).

Data analysis

Data from observations of spermatozoa abnormalities were analyzed using ANOVA ($p<0.05$). If there is an effect, it is continued with the least significant difference test (LSD) to find out the difference in each treatment.

DISCUSSION RESULT

The results showed that the higher the concentration of snakewood given, the decrease in spermatozoa abnormalities in mice with diabetes mellitus. Based on the results of a one-way Analysis of Variance (ANOVA) using the SPSS 22.0 program, it was shown that the administration of wood infusion had a significant effect ($p<0.05$) on decreasing spermatozoa abnormalities in mice with diabetes mellitus.

Table 1. Average Spermatozoa Abnormalities in Mice Diabetes Mellitus Model after Infusion of Snake Wood (S. lucida).

Concentration Wood Stew Snakes (0.13 ml/head/day)	**Sperm abnormality (µl) (Mean ± SD)**
Control -	23,67 ± 14,8[a]
Control +	77,33 ± 25,0[b]
0,66 gr/50 ml	59,33 ± 21,5[ab]
1,3 gr/50 ml	50,00 ± 19,6[ab]
2,6 gr/50 ml	38,00 ± 16,7[c]

Note: superscripts with different letters show significant differences ($P<0.05$).

The results of further tests with the least significant difference test (LSD) showed that the average decrease in spermatozoa abnormality in diabetes mellitus mice after being given snake wood infusion in the control group of mice was significantly different ($p <0.05$) with a decrease in spermatozoa abnormalities in the group of mice that were given wood infusion snake concentration 0.66gr/50ml, 1.3gr/50ml and 2.6gr/50ml.

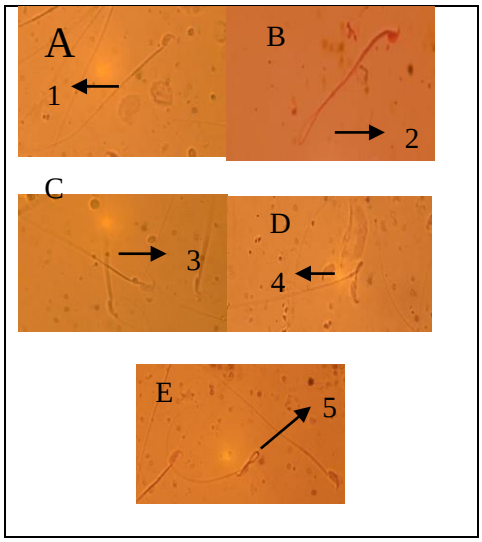

Figure 3. Morphological images of mouse spermatozoa. Description: (1) Normal spermatozoa, (2) Spermatozoa with a coiled tail, (3) Spermatozoa that still have cytoplasmic droplets, (4) Head spermatozoa without a tail body, (5). Spermatozoa with crooked necks. 400x magnification.

Spermatozoa with different damage, including; coiled tail spermatozoa, spermatozoa that still have cytoplasmic droplets, spermatozoa with a head without a tail body and spermatozoa with a crooked neck shape. The damage to the spermatozoa is thought to be caused by the presence of free radicals in diabetic mice. The presence of free radicals in the reproductive organs causes tissue changes in the testes which are characterized by damage to the mitochondrial membrane of Leydig cells so that the process of spermatogenesis becomes disrupted. Disruption of the process of spermatogenesis will cause the quality of the sperm produced to decrease (Zhang, 2007). In addition, free radicals can also damage the integrity of DNA in the nucleus of spermatozoa so that it can induce cell apoptosis which can affect the concentration/amount of spermatozoa, also resulting in changes in the morphology of spermatozoa during

the process of spermatogenesis (Faranita, 2009). The plasma membrane of spermatozoa contains large amounts of phospholipids and unsaturated fatty acids, which are very susceptible to attack by free radicals, especially hydroxyl radicals, so that ROS can easily penetrate into the plasma membrane. The hydroxyl radicals will cause a chain reaction called lipid peroxidation. The end result of this chain reaction is the breakdown of the fatty acid chains into compounds that are toxic to spermatozoa cells (Zulfa, 2006). Gived snake wood infusion which contains active tannin compounds has the potential to reduce blood sugar levels. In addition, tannins can increase glucose transport by activating the insulin-mediated signaling pathway. Saponins function as anti-diabetics because they are able to regenerate the pancreas, thereby causing an increase in the number of pancreatic beta cells which can increase insulin secretion. Giving snakewood infusion has an effect on reducing abnormal spermatozoa in mice with diabetes mellitus. The decrease in spermatozoa abnormalities in the positive control was lower when compared to the decrease in abnormalities in the group of mice that were given snakewood infusion. This shows that the active compounds contained in snake wood can ward off free radicals in diabetes mellitus thereby reducing spermatozoa abnormalities.

The active compounds of triterpenoids (saponins) in snake wood cause the secretion of the hormone testosterone to take place properly. This hormone plays an important role at one stage of the process of division of germ cells for the formation of spermatozoa (Ascobat, 2008). The process of glycolysis in the process of testosterone hormone secretion will produce energy in the form of adenosine triphosphate (ATP) which is utilized by spermatozoa as an energy source in the process of movement so that they remain motile and to maintain their vitality (Souhoka, 2009). The highest type of spermatozoa abnormality is tail curl. This is thought to be due to the process of spermatogenesis not going well due to the presence of free radicals. After being treated with snake wood infusion, it was seen that there was a decrease in spermatozoa abnormalities. The decrease in spermatozoa abnormality is thought to be due to the active compounds contained in snake wood. Flavonoids found in snake wood are antioxidant compounds that can fight free radicals. The mechanism of the effect of flavonoids as antioxidants is to provide an acidic environment in the medium, regenerate the main antioxidants, deactivate metal peroxide contaminants, capture O2, bind O2 and convert it into the triplet form of O2. Flavonoid compounds are also one of the phytochemicals that can inhibit the activity of the aromase enzyme which plays a role in converting androgens (testosterone) to estrogens resulting in an increase in the hormone testosterone (Purnawati, 2006).

Flavonoid compounds also have estrogen-like activity which is thought to suppress the function of the anterior pituitary to secrete FSH (Folicle Stimulating Hormone) and LH (Luteinizing Hormone) [16]. FSH and LH hormones have a very important role in the process of spermatogenesis. The LH hormone functions to stimulate the Leydig cells to produce the hormone testosterone in the testes. Meanwhile, the function of FSH stimulates testicular growth and enhances the production of androgen-binding protein (ABP) by Sertoli cells. The increase in ABP causes a high concentration of testosterone which is important for the formation and maturation of spermatozoa in the process of spermatogenesis. The results of this study indicate that the content of active compounds from snake wood in the form of alkaloids and saponins also act as antioxidants that can fight free radicals. Free radicals are unpaired molecules. This unpaired molecule will damage the spermatozoa through a mechanism called oxidative stress. which occurs due to an imbalance between free radicals and antioxidants in the body (Hariyatmi, 2004). Biologically, the notion of antioxidants are compounds that can counteract or reduce the negative effects of oxidants. Antioxidants work by donating an electron to oxidant compounds to prevent oxidation reactions, so that oxidative stress and cell damage do not occur (Sayuti, 2015. In addition, the alkaloids contained in snake wood can increase the activity of the ATP-ase enzyme in the spermatozoa cell membrane which is thought to improve the physiological condition of spermatozoa which are damaged due to hyperglycemia.

CONCLUTION

Based on the results obtained, it can be concluded that the infusion of snakewood (Strychnos lucida) with three different concentrations had an effect on reducing spermatozoa abnormalities in mice models of diabetes mellitus. The concentration that was most effective in reducing mice spermatozoa abnormality was 2.6gr/50ml.

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