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The Effect of Giving Nanoherbal of Biwa (Eriobotrya japonica (Thunb.) Lindl. (Thunb.) Lindl.) Leaf on the Quality and Quantity of Spermatozoa And Microstructure in Diabetes Rat (Rattus norvegicus) Induced by Alloxan

Syafruddin Ilyas[1]*, Salomo Hutahaean[1], Elimasni[2], Olvita Mayani[2], Wardah Sawitri Polem[2], Lailatun Nisfa[2], and Herwina Francisca[2]

[1] Biology Departement, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan. Jl. Bioteknologi No. 1 Kampus USU, Padang Bulan, Medan, Indonesia.
[2] Student of Biology Departement, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan. Jl. Bioteknologi No. 1 Kampus USU, Padang Bulan, Medan, Indonesia.

Abstract. One of causes in decreasing quantity and quality of spermatozoon is disease. Increased blood glucose levels can trigger demage of spermatogenesis in the testes, thereby reducing spermatozoa production. Biwa leaves (Eriobotrya japonica (Thunb.) Lindl.) contain triterpenes which are expected to overcome blood glucose levels and quantity of spermatozoa in the cauda epididymis. To investigate this problem, an experimental test of nano herbal of biwa leaves was carried out in a rat model (Rattus norvegicus) diabet. There are five treatments and six replications, it was found that the best dose of 500 mg/kg body weight could improve spermatogenesis so that the number of rat spermatozoa remained normal. It was concluded that nano herbal of biwa leaves can be used as an alternative drug to maintain fertility as seen from spermatogenesis and the number of spermatozoa. The result is analyzed by ANOVA test and further by Post-Hoc-Test (Duncan). The result show the significant differences ($p<0,05$) to quantity and quality of spermatozoon of adult white male mouse (Rattus norvegicus).

Keyword: Nano herbal, Eriobotrya japonica (Thunb.) Lindl. Microstructure, Spermatozoa

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1 Introduction

Diabetes mellitus (DM) is one of the chronic diseases that has become the most important public health problem that cannot be resolved. This increase is expected to increase to more than 580 million by 2035. Indonesia ranks seventh in the list of 10 countries with the largest number of DM sufferers in the world [1]

*Corresponding author at: Biology Departement, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan. Jl. Bioteknologi No. 1 Kampus USU, Padang Bulan, Medan, Indonesia
E-mail address: syafruddin6@usu.ac.id

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Treatment of diabetes mellitus is a chronic treatment of living wells. Treatment of diabetes mellitus, such as the use of insulin and oral antidiabetic drugs, is relatively expensive because it is used for a long time and can cause undesirable effects. Therefore, it is necessary to look for drugs that have few side effects and relatively low prices [2]. One effort to treat diabetes mellitus is to use herbs as an alternative drug that provides fewer side effects and relatively lower prices than conventional drugs that already exist. Research suggests diabetes mellitus is related to male fertility, causing a disruption in hormones that affect spermatogenesis. In addition, there are abnormalities in the process of spermatogenesis itself which is followed by ejaculation disorders. In patients with diabetic tests, there was a significant decrease in testosterone levels accompanied by decreased levels of LH and FSH.

2 Material and Methods

This research study used healthy and fertile rats (Rattus norvegicus) and aged 8-11 weeks weighing 150-200 g, healthy, fertile (had given birth once) as many as 30 animals, induced using 150 mg / kg alloxan, peritoneal injection until the blood sugar level in rats rises, causing the test animals to have diabetes. Biwa is a plant that has anti-diabetes and antioxidant properties. To get the optimal therapeutic effect used as nano herbal preparations [3,4,5]. Nano biwa leaf herbal preparations in the form of Self Nano-Emulsifying Drug Delivery System (SNEDDS). The active ingredient of biwa is filled with ethanol, evaporated and obtained thick. it is standardized according to Indonesian Herbal Pharmacopoeia. Rats (Rattus norvegicus) which had been induced by aloxan and had an increase in blood sugar levels were given nano herbal biwa leaf (Eriobotrya japonica (Thunb.) Lindl.) using gavage needles orally for 2 weeks by dividing groups into five groups of rats, each of them six receiving a treatment schedule following. Group I: Normal control (physiological salts). Group II: Control treated with Alloxan (150 mg / kg, peritoneal injection / ip). Group III: Alloxan (150 mg / kg.ip) + nano biwa leaf herb (250 mg / kg, orally / po), Group IV: Alloxan (150 mg / kg.ip) + nano biwa leaf herb (500mg / kg, po), Group V: Alloxan (150 mg / kg.ip) + Standard drug (Glibenclamide) (5 mg / kg, po) Nano herbal extracts of biwa leaf and standard drug glibenclamide (5 mg / kg) and physiological salts are given with the help of a gavage needle. Group I functioned as a normal control, which received physiological saline (0.9%) for 14 days. Group II to Group V are diabetic control mice. Group III to Group V (which previously received alloxan) were given fixed-dose biwa leaf herbal nano (250 mg / kg, po), (500 mg / kg, po) and standard drug Glibenclamide (5 mg / kg) for 14 consecutive days participate. After 2 weeks of treatment the test animals were dissected and their testicles were taken and spermatozoa were observed in rats.

TFormatting table, graphic content, Equation and reference.

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3 Result and Discussion

Eriobotrya japonica (Thunb.) Lindl. is one of herbs that has a potential to be researched in the health sector, this is because previous research has shown that Eriobotrya japonica (Thunb.) Lindl. has very diverse properties, one of which is diabetes mellitus**.**

3.1 Motility of Spermatozoa

The results of the analysis showed that nano herbal biwa leaf (*Eriobotrya japonica (Thunb.) Lindl.*) given to alloxan-induced mice affected the Motility of Spermatozoa.

Table 1. The mean percentage of motility spermatozoa

Groups	Motility Spermatozoa
C-	$83,1 \pm 2,22^{b}$
C+	$74,1 \pm 7,13^{a}$
T1	$80,6 \pm 1,2^{b}$
T2	$81,5 \pm 1,64^{b}$
T3	$80,8 \pm 1,72^{b}$

Note: C-: normal control , C+: aloksan control, T1: 250mg/kg BW, T2: 500 mg/kg BW, T3: *Glibenclamide* 5 mg/kg BW.

Based on table 1 there were significant differences in rat motulity sperm. We can have a conclution that T2 dose 500 mg/kg BW have a best motility sperm when compared to other group and. While mean, group T3glibenclamide has a result close to group C+. According to Satriyasa [6], a decrease and an increase in the quantity of sperm can be caused by disruption of tissue growth and development, so the number of spermatogenic cells decreases because spermatogenic cells are cells that are actively dividing. improvement in sperm quality can occur due to the presence of hypoglycemic active compounds so as to reduce blood sugar levels of diabetes mice. The cytotoxic substance is triterpenoid glycoside. a decrease in the number of spermatids due to triterpenoid glycoside is thought to occur through several mechanisms, one of which is the disruption of sertoli cell function which causes the supply of lactate and pyruvate to decrease

3.2 Viablity of Spermatozoa

The results of the analysis showed that nano herbal biwa leaf (*Eriobotrya japonica (Thunb.) Lindl.*) given to alloxan-induced mice affected the Vialibity of Spermatozoa.

Table 2. The mean percentage of viability spermatozoa

Groups	Viability Spermatozoa (%) ± SD
C-	$83,3 \pm 1,75^{c}$
C+	$79,3 \pm 0,53^{a}$
T1	$80,3 \pm 1,03^{ab}$
T2	$82,3 \pm 1,96^{bc}$
T3	$81,3 \pm 2,16^{abc}$

Note: C-: normal control , C+: aloksan control, T1: 250mg/kg BW, T2: 500 mg/kg BW, T3: *Glibenclamide* 5 mg/kg BW.

Table 2 shows that there were significant differences in rat viability sperm. We conclude that group T2 (dose 500 mg/kg BW have a best percentagce viability sperm if compered to others groups. The percentage of T2 was 82,3%, mean while T1 (dose 250mg/kg BW) has a result close to C+. According to Zirkin and Vassilos [7], a decrease in the amount of viability can also be caused by the secretion of the hormone testosterone by leydig cells. The hormone testosterone plays a role in maintaining the survival of spermatozoa in the epididymis. Decreased secretion in

mice will result in spermatozoa survival. The secretion of the hormone testosterone will be inhibited because the mice have diabetes milletus. According to Ilyas [8], viability is the survival of spermatozoa when it has been removed from the reproductive tract of male animals. The living spermatozoa head does not absorb color (transparent head), while the dead will be red due to the entry of the dye into the spermatozoa head due to damage to the spermatozoa head cell membrane

3.3 Morphology Spermatozoa

The results of the analysis showed that nano herbal biwa (Eriobotrya japonica (Thunb.) Lindl.) leaf given to alloxan-induced mice affected the morphology spermatozoa can be seen in table)

Table 3. The mean percentage of morphology spermatozoa.

Groups	Normal Spermatozoa (%) ± SD
C-	$82,6 \pm 2,94^{b}$
C+	$73,5 \pm 6,89^{a}$
T1	$76,6 \pm 8,38^{ab}$
T2	$81,5 \pm 1,64^{b}$
T3	$80,8 \pm 1,83^{b}$

Note: C-: normal control , C+: aloksan control, T1: 250mg/kg BW, T2: 500 mg/kg BW, T3: *Glibenclamide* 5 mg/kg BW.

Table 3 shows that there were significant differences in rat viability sperm. We conclude that group T2 (dose 500 mg/kg BW) have a best percentagce viability sperm 81,5% if comperaced to others groups. Group T2 have a close result to C- mean while group T3 (Glibenclamide) have a close result to group C+. The treatment of the positive control group has the lowest morphological proportion, this is thought to be because the mice have diabetes mellitus, a spermatzoa morphological disorder caused by a decrease in the ability of antioxidant defense through the reductase pathway, changes in protein properties due to an increase in ROS caused by high blood sugar levels, resulting in damage. DNA which ultimately causes morphological abnormalities of spermatozoa (Pramudito, 2009).[8]

3.4 Quanity of Spermatozoa

The results of the analysis showed that nano herbal biwa (Eriobotrya japonica (Thunb.) Lindl.) leaf given to mice that had been induced alloxan affected the sperm quantity in mice can be seen in the following figure 1

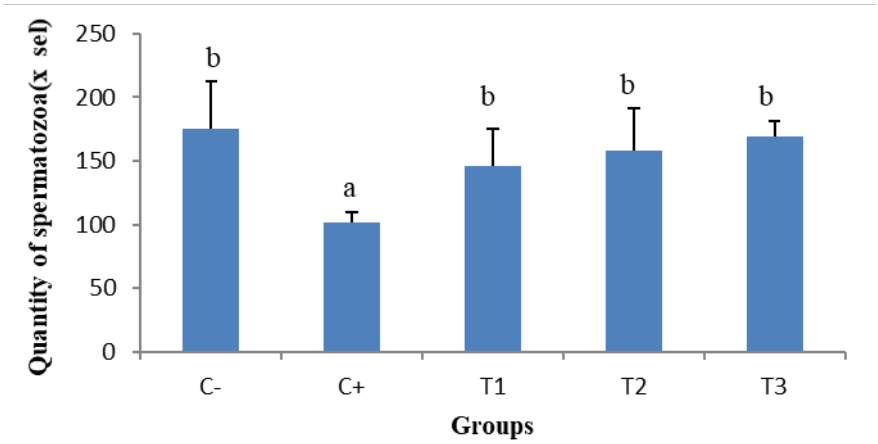

Figure 1. Quantity of spermatozoa. Note:C-: normal control , C+: aloksan control, T1: 250mg/kg BW, T2: 500 mg/kg BW, T3: *Glibenclamide* 5 mg/kg BW.

From the figure we can seen that the highest sperm quantity in the C-treatment with sperm counts $174{,}83\ X\ 10^5$, and then group T2 (dose 500 mg/kg BW) have a quantity $158{,}00\ X\ 10^5$. The increase in the number of spermatozoa cells is thought to be due to the content contained in them that can fight free radicals, this compound is thought to be able to maintain the integrity of the plasma membrane by protecting spermtozoa from exposure to free radicals (Hemachand, 2003). In addition, the number of spermatozoa produced depends on the spermatogenesis process in the seminiferous tubules [9].

According to Satriyasa [6], a decrease and an increase in the quantity of sperm can be caused by disruption of tissue growth and development, so the number of spermatogenic cells decreases because spermatogenic cells are cells that are actively dividing. improvement in sperm quality can occur due to the presence of hypoglycemic active compounds so as to reduce blood sugar levels of diabetes mice. The cytotoxic substance is triterpenoid glycoside. a decrease in the number of spermatids due to triterpenoid glycoside is thought to occur through several mechanisms, one of which is the disruption of Sertoli cell function which causes the supply of lactate and pyruvate to decrease.

3.5 Seminiferous Tubule Diameter

The results of the analysis showed that nano herbal biwa (Eriobotrya japonica (Thunb.) Lindl.) leaf given to alloxan-induced mice affected the Seminiferous Tubule Diameter can be seen at figure 2.

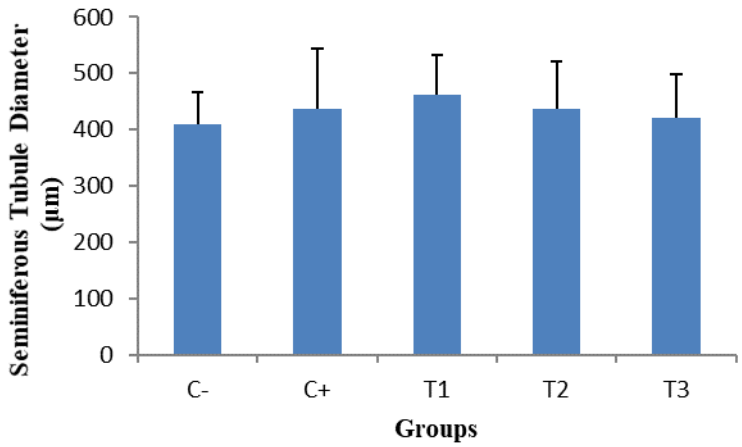

Figure 2. Seminiferous Tubule Diameter. Note:C-: normal control , C+: aloksan control, T1: 250mg/kg BW, T2: 500 mg/kg BW, T3: *Glibenclamide* 5 mg/kg BW.

Figure 2 shows the results of the analysis that the herbal nano biwa (Eriobotrya japonica (Thunb.) Lindl.) leaf that there were not significant differences in rat viability sperm given to alloxan-induced mice affected the diameter of the seminiferous tubules, The longest diameter was in T1 and the smallest diameter was in C+. This is what causes the difference in volume in each testis caused by differences in the treatment given. The diamter graph of seminiferous tubules can be seen below

According to Hughes and Acerini [10], the testes are the main glands in the male reproductive system which are responsible for the production of male gametes or spermatozoa (spermatogenesis) and synthesis of male hormones or androgens (steroidogenesis). A pair of testicles, located in the inguinal, are stored in the scrotal sac. In mammals, the testes descend and exit the abdominal cavity (peritoneal) to the extracorporeal position and finally enter the scrotum

(inguinoskrotal). This process is known as the descending testiculorum which is controlled by androgens. In this position the temperature of the testes becomes lower than the body temperature required for spermatogenesis. According to Nelsen (1953) in Yurnadi [11], the size of the seminiferous tubule diameter is determined by the interaction between the Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH). This interaction is determined by FSH hormone levels, where if FSH levels are low even if not produced, the LH will not be able to maintain the normal size of the diameter of the seminiferous tubules so that the tubules will shrink. Thus the FSH hormone which is still in normal levels is sufficient to maintain the diameter size of the seminiferous tubules.

4 Conscultion

Nano-E. japonica administration was significantly different in each treatment ($p<0.05$), but not significantly different in Seminiferous Tubule Diameter t ($p>0.05$). The best use dose is the T2 group (500 mg / kg WB)

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