AFRICAN BITTER LEAF EXTRACT EFFECT ON EPITHELIUM THICKNESS OF SEMINIFEROUS TUBULES IN WISTAR RATS WITH DIABETES MELLITUS

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ABSTRACT

Introduction: Diabetes mellitus (DM) is a metabolic disease that can cause complications in the form of damage to male reproductive organs, such as decreased sperm motility and cell count, impaired spermatogenesis, and erectile dysfunction. A measurement that can be done to detect male reproductive disorders is to measure the thickness of the seminiferous tubule epithelium. Herbal medicines can be used to support treating diabetes. Studies have claimed that African bitter leaf helps in treating DM and its complications.
Purpose: This study aims to determine the effect of African bitter leaf extract on epithelium thickness of seminiferous tubules in rats with DM.
Method: True Experimental with Post-Test Only Control Group Design using Rattus norvegicus. Rats were divided into five groups, with six rats in each group. Rats were administered with a single dose of alloxan 150 mg/kg BW/IP. Group K is diabetic rats given glibenclamide 0,63 mg/kg BW. Group P0 are diabetic rats given CMC 0,1% 0,7 ml. Group P1 are diabetic rats given African bitter leaf extract 100 mg/kg BW. Group P2 are diabetic rats given African bitter leaf extract 200 mg/kg BW. Group P3 are diabetic rats given African bitter leaf extract 400 mg/kg BW. Treatments were given with oral gavage for 14 days. Rats were dissected, and their testis was made as histological slides. Results are statistically tested with ANOVA.
Results: The average epithelium thickness from group K are 79,61 µm, group P0 are 59,96 µm, group P1 are 71,87 µm, group P2 are 67,31 µm, group P3 are 63,95 µm showed significant result (p=0,000).
Conclusions: African bitter leaf extract affects epithelium thickness of seminiferous tubules, with group K, shows the best result, followed by group P1.

Keywords: African bitter leaf extract, epithelium thickness of seminiferous tubules, diabetes mellitus

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia due to insulin secretion abnormalities (insulin deficiency), insulin action (tissue resistance to insulin), or both.[1] The prevalence of DM in the Indonesian population in 2018 is based on the Indonesian Association of Endocrinologists (PERKENI) 2015, reaching 10.9%.[2]

DM can cause complications in infertility in men due to changes in glucose metabolism in Sertoli cells, disruption of spermatogenesis, inducing cell apoptosis in the testes, erectile dysfunction, and decreased libido.[3] Several studies on fertility rates in the community stated that an increase in DM cases was related to infertility. The incidence of DM in young adults is increasing, and it is feared that it will affect the reproductive function of men in their reproductive age.[4] DM can cause a decrease in the number and motility of sperm, the hormone testosterone, and have a negative impact on testicular histology in the seminiferous tubules in the form of a decrease in tubular epithelial diameter and thickness, decreased number of Sertoli cells, and spermatogenic cells, as well as thickening of the basement membrane.[5] DM can cause oxidative stress.[6] Oxidative stress occurs when levels of ROS (Reactive Oxygen Species) in cells or tissues exceed the limit of antioxidant defenses, which can cause DNA, protein, and lipid damage.[7] Oxidative stress is the main cause of male reproductive disorders. It causes decreased production of the hormone testosterone, LH (luteinizing hormone), FSH (follicle-stimulating hormone), which induces damage to the mitochondrial membrane, and microvascular complications.[6] Microvascular complications include microangiopathy and neuropathy. Microangiopathy causes avascular in seminiferous tubules, resulting in a decreasing number of cells in the epithelium of the seminiferous tubules and leads to a decrease in the thickness of the seminiferous tubule epithelium.[6]

Along with the development of science, many studies have been carried out on the use of herbal medicines to support the treatment of DM, one of which is African leaves (Vernonia amygdalina). African leaves contain antihyperglycemic flavonoids and antioxidants that can reduce blood glucose levels and overcome oxidative stress.[8] Several studies have stated that giving African leaf extracts can improve the histological appearance of the testes of diabetic Wistar rats.[9,10]

However, current research on the effect of African leaf extract on the complications of DM in male infertility, especially from testicular histopathology, is still rarely studied and is less well known by the public. This study was conducted to determine the effect of giving African leaf extract (Vernonia amygdalina) on the thickness of the seminiferous tubular epithelium of Wistar rats with diabetes mellitus.

METHOD

This research is true experimental research with a post-test-only control group design. The sample of this study is 32 male Wistar rats (Rattus norvegicus) aged 2-3 months and weighing 150-250 grams which met the inclusion and exclusion criteria. The experimental animals were divided into five groups, namely groups K, P0, P1, P2, and P3, where each group consisted of 6 experimental animals. Two rats outside the research group were added, which were used as a baseline.

The experimental animals were placed in each cage separately. The environmental conditions for raising rats are room temperature (18-26°C), ideal humidity (30%-70%), and normal lighting (light/dark cycle 12/12 hours). The experimental animals were fed pellets and drank water ad libitum until the end of the study. The experimental animal was

adapted for seven days, then a single dose of 150 mg/kg BW of alloxan was induced intraperitoneally. At four days after alloxan induction, blood sugar levels were measured from the tail vein using a glucometer to ensure the rats were hyperglycemic. The treatment according to the group was started, group K was given glibenclamide 0,63 mg/kg BW/PO/day, group P0 was given CMC 0,1% 0,7 ml/PO/day, group P1 was given African leaf extract at a dose of 100 mg/kg BW/PO/day, group P2 was given African leaf extract at a dose of 200 mg/kg BW/PO/day, group P3 was given African leaf extract at a dose of 400 mg/kg BW/PO/day for 14 days. Oral administration to rats using oral gavage. After being treated for 14 days, surgery was performed to extract the testicular organs. The surgery started by giving anesthesia using ketamine-xylazine, then cervical dislocation was carried out to take the testicular organs and made preparations with Hematoxylin-Eosin (HE) staining. Observation of the preparations using a 100x magnification light microscope, photographed with Optilab, and measurement of the thickness of the seminiferous tubule epithelium using the Image Raster program.

Epithelial thickness measurements were carried out on the seminiferous tubules with a nearly round and intact shape. The epithelial thickness of the seminiferous tubules is measured by drawing a line from the edge of the basement membrane to the lumen surface of each seminiferous tubule. Measurements were made four times at different sites in each seminiferous tubule. In one histological slide, 15 seminiferous tubules were counted, and the results for each slide were averaged. Observations were made in five visual fields with three seminiferous tubules in each visual field. The results of the research data were analyzed by a one-way ANOVA test (significant if the $p<0.05$).

RESULTS

Based on the research that has been done, the seminiferous tubule epithelial thickness data were obtained as follows.

Table 1. Comparison of the Average Thickness of Seminiferous Tubule Epithelium in Each Group

Group	Average ± SD (μm)
K: DM rats were given glibenclamide 0,63 mg/kg BW/PO/day	79,61 ± 3,73
P0: DM rats were given CMC 0,1% 0,7 ml/PO/day	59,96 ± 3,75
P1: DM rats were given African leaf extract at a dose of 100 mg/kg BW/PO/day	71,87 ± 3,08
P2: DM rats were given African leaf extract at a dose of 200 mg/kg BW/PO/day	67,31 ± 6,86
P3: DM rats were given African leaf extract at a dose of 400 mg/kg BW/PO/day	63,95 ± 3,18

Table 2. The Result of Analysis of Average Thickness of Seminiferous Tubule Epithelium in Each Group

Group	Significance (p-value)		
	Normality Test	Homogeneity Test	Hypothesis Test
K	0,088	0,085	0,000
P0	0,175		
P1	0,692		
P2	0,411		
P3	0,624		

***Post Hoc* Test (*Bonferroni*)**			
Group		Significance	Result
K	P0	0,000	Significant
	P1	0,108	Non-Significant
	P2	0,002	Significant
	P3	0,000	Significant
P0	K	0,000	Significant
	P1	0,003	Significant

	P2	0,147	Non-Significant
	P3	1,000	Non-Significant
P1	K	0,108	Non-Significant
	P0	0,003	Significant
	P2	1,000	Non-Significant
	P3	0,093	Non-Significant
P2	K	0,002	Significant
	P0	0,147	Non-Significant
	P1	1,000	Non-Significant
	P3	1,000	Non-Significant
P3	K	0,000	Significant
	P0	1,000	Non-Significant
	P1	0,093	Non-Significant
	P2	1,000	Non-Significant

In Table 2, it can be concluded that the results of the data normality test for each group using the Shapiro-Wilk, data are normally distributed with p>0,05. So, it is continued with the homogeneity test using the Levene Test, and the data is homogeneous because p>0.05. The results of data hypothesis testing using ANOVA showed significant results with p<0.05 (p=0.000).

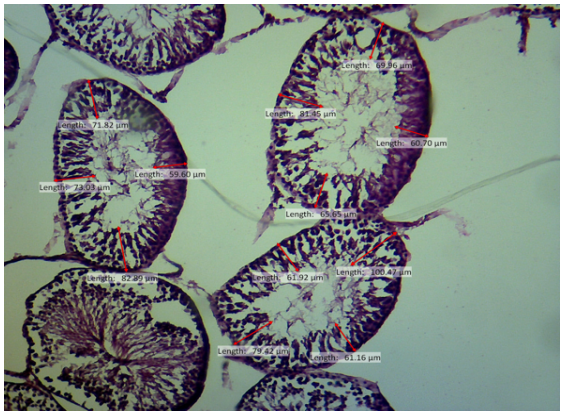

Figure 1. Microscopic Observation of the Seminiferous Tubules of Group K. 100x enlargement and HE staining.

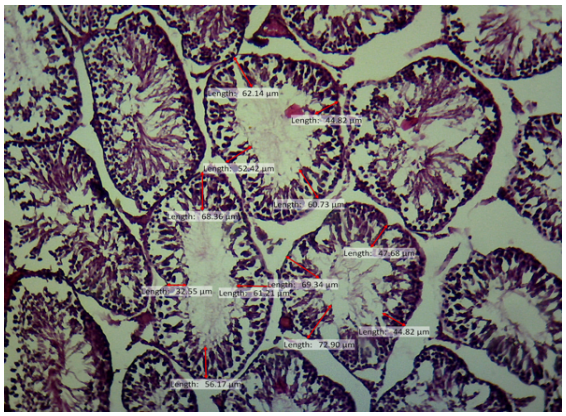

Figure 2. Microscopic Observation of the Seminiferous Tubules of Group P0. 100x enlargement and HE staining.

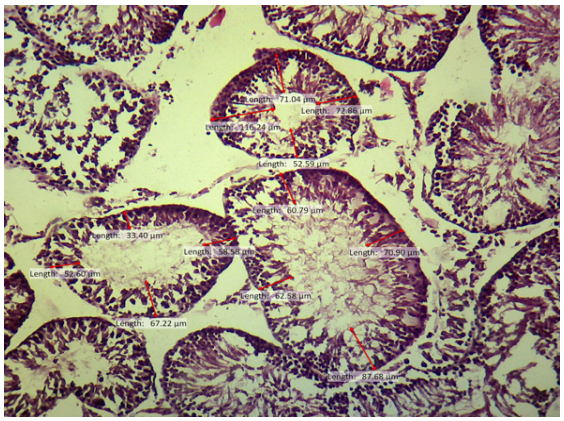

Figure 3. Microscopic Observation of the Seminiferous Tubules of Group P1. 100x enlargement and HE staining.

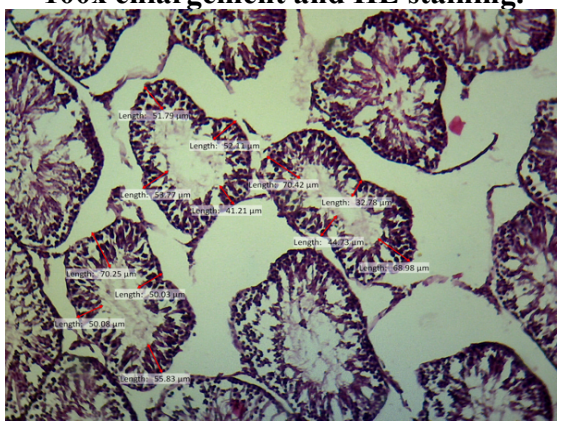

Figure 4. Microscopic Observation of the Seminiferous Tubules of Group P2. 100x enlargement and HE staining.

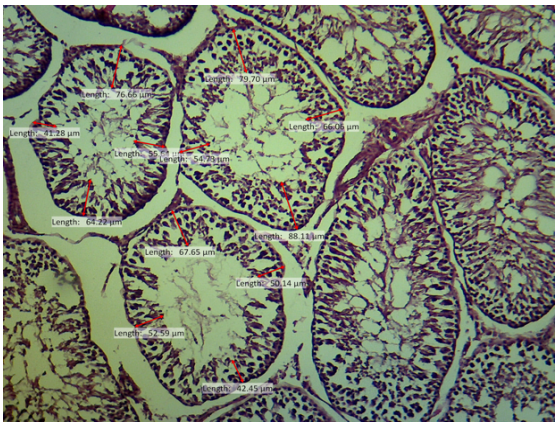

Figure 5. Microscopic Observation of the Seminiferous Tubules of Group P3. 100x enlargement and HE staining.

DISCUSSION

A decrease in the thickness of the seminiferous tubule epithelium is one of the complications of DM, which indicates a disturbance in the male reproductive organs.[5] The lowest average epithelial thickness was obtained from the P0 group, this is due to alloxan induction which works by damaging pancreatic beta cells through glucokinase inhibition and oxidative stress mechanisms resulting in DM.[11,12] Then it causes a decrease in the thickness of the seminiferous tubular epithelium due to a decrease in the number of spermatogenic cells and Sertoli cells, which are the main constituent components of the epithelium of the seminiferous tubules through the occurrence of cell apoptosis.[13] In Figure 2, it is found that the seminiferous tubules tend not to be round, and the epithelium is shrinking. This damage occurs through mechanisms such as endocrine system disorders, oxidative stress, and microvascular complications.14 Endocrine system disorders in the form of decreased levels of LH and FSH hormones resulting in a decrease in the amount of testosterone which affects the spermatogenesis process.[15,16] Oxidative stress causes damage to sperm DNA, mitochondrial DNA fragmentation, and induction of cell apoptosis resulting in a decrease in the number of sperm cells.[13]

The highest mean epithelial thickness was obtained from group K, where glibenclamide is a generic drug commonly used in the treatment of DM. In Figure 1, the seminiferous tubules still tend to be round, and the epithelium is more intact. Hypothesis test results showed significant results (p=0.000), which means an effect of African leaf extract on the seminiferous tubular epithelial thickness of Wistar rats with DM. Comparing the groups' given African leaf extract, it was found that the P1 group had the highest value, followed by P2, then P3 with the lowest. This shows that the administration of African leaf extract at a dose of 200 mg/kg BW and 400 mg/kg BW still gives a repair effect, but a dose of 100 mg/kg BW can still provide a better repair effect. In the Post Hoc test, there was a significant difference between P1 and P0 with p=0.003. This shows that the administration of African leaf extract at a dose of 100 mg/kg BW effectively provides a repair effect on the seminiferous tubules. This statement is supported by previous studies that state that African leaf extract at a dose of 100 mg/kg BW can provide corrective effects and not cause side effects.[10,17] Administration of high doses of African leaf extract such as 250 and 500 mg/kg BW can be detrimental because the antioxidant content that exceeds normal capacity can cause cytotoxic effects.[8,17] The toxic effect causes a decrease in diameter, epithelial thickness, and density of seminiferous tubules and a decrease in sperm count.[10,17]

The mechanism of action of African leaf extracts is related to their antioxidant and antidiabetic properties, which mainly work by regenerating damaged pancreatic beta cells to reduce high blood glucose levels and overcome oxidative stress.[8] Flavonoids work as anti-hyperglycemic through several mechanisms, namely, alpha-glucosidase inhibition, increasing GLUT4 translocation, inhibition of glucose-6-phosphatase hepatic activity, regeneration of pancreatic beta cells, increasing insulin

secretion, and increasing GLUT2 action in pancreatic beta cells.[18,19] Flavonoids are thought to be able to increase the sensitivity of insulin receptors in tissues in DM. Flavonoids also have antioxidant properties related to the phenolic -OH group, which can capture free radicals to become neutral.[11]

CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that giving African leaf extract can provide an effect in the form of an increase in the thickness of the seminiferous tubule epithelium, with a concentration of 100 mg/kg BW showing the highest results.

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