EFFECT OF AFRICAN LEAF EXTRACT (Vernonia amygdalina) ON ALLOXAN-INDUCED DIABETES MELLITUS LEYDIG CELL MYTOSIS OF WISTAR RATS

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ABSTRACT

Introduction: Diabetes mellitus can reduce the quality of human life when accompanied by complications, one of which is sexual dysfunction. In diabetics, there is an increase in ROS production which can cause damage to local Leydig cell proliferation so that Leydig cell activity and testosterone secretion decrease. African leaves can be used as a control of blood sugar levels and antioxidants to overcome oxidative stress.
Purpose: To determine the effect of administration of African leaf extract (Vernonia amygdalina) on alloxan-induced Leydig cell mitosis in male rats with diabetes mellitus.
Method: This experimental study used 31 male Rattus norvegicus white rats divided into K, P0, P1, P2, and P3 groups. Each group consisting of six rats and one rat outside the study group was used as a reference. Experimental animals will be induced alloxan with a single dose of 150 mg/kgBW for four days. African leaf extract was administered using a probe to groups P1, P2, P3 at a dose of 100, 200, and 400 mg/kgBW for 14 days. Rats were anaesthetized and euthanized so that the testes could be used to make preparations with HE staining and analysis of preparations using a 400x magnification light microscope. The research data were analyzed using One-Way Annova with $P<0.05$.
Results: The administration of African leaf extract affected increasing the number of mitotic Leydig cells ($P=0.026$). There was a significant increase in the number of mitotic Leydig cells at a dose of 100 mg/kgBW ($P=0.021$), but not effective at a dose of 200 mg/kgBW ($P=0.188$) and 400 mg/kgBW ($P=0.823$).
Conclusion: The administration of African leaf extract at a dose of 100 mg/kgBW has been shown to increase the number of mitotic Leydig cells so that it can be used to improve sexual function in diabetics.

Keyword: Diabetes mellitus, African leaf extract, alloxan

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INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by the pancreas not being able to produce enough insulin or when the body cannot use the insulin produced effectively characterized by a hyperglycemia condition that is an increase in glucose concentration in the blood.[1,2] People who live with diabetes mellitus are also at increased risk of other diseases, including heart, peripheral arterial and cerebrovascular disease, obesity, cataracts, erectile dysfunction, and non-alcoholic fatty liver disease.[1,2]

Globally, an estimated 463 million people have diabetes mellitus by 2019, and there will be an increase of about 578 million people by 2030.[3]

Data obtained from Rikesdas in Indonesia in 2013 estimated the absolute number of people with diabetes mellitus is about 6.9% in the population aged 15 years and above and has increased by 2% in 2018. In East Java province, the population diagnosed with diabetes mellitus was 2.1% or an estimated 605,974 people in 2013. It increased to 2.6% in 2018.4 Diabetes mellitus can cause some complications that can threaten life expectancy and reduce a person's quality of life. One of the complications that can be caused is the failure of sexual function.[5,6] Diabetes mellitus can also interfere with the function of the hypothalamus-pituitary-gonad axis and cause damage to the proliferation of Leydig cells locally; decreased levels of LH and FSH.[7] LH serves to stimulate Leydig cells to produce testosterone hormone. In contrast, FSH binds to intra-testicular testosterone hormone produced by Leydig cells by forming ABP (*androgen binding protein*).[7]

Some researchers prove that the triggering factors of sexual dysfunction, decreased production of testosterone hormone, decreased quality of spermatozoa, and degeneration of Leydig cells in people with diabetes mellitus is an excessive increase in free radicals, resulting in an imbalance between the production of free radicals and the total capacity of antioxidants in the body also called oxidative stress. Oxidative stress can cause Leydig cell activity and testosterone secretion to decrease.[5,6]

One of the plants that can lower blood sugar levels and free radical levels in diabetes mellitus is by utilizing the African leaf plant (*Vernonia amygdalina*).[8,9] The content of flavonoids in African leaves serves as a controller of blood sugar levels. It can improve the antioxidant system in the body, so it can help overcome oxidative stress.[10] Results from previous research, the administration of ethanol

extract of African leaves at a dose of 100 mg/kg in normal Sprague-Dawley male rats was shown to significantly increase the diameter of seminiferous tubules and tubular cross-sectional area to affect Leydig cell activity and spermatogenesis process.[11]

However, research on the use of African leaf extract as an infertility treatment in diabetes mellitus patients, especially against Leydig cell activity, is still slight. There are still many people who do not know the benefits of African leaf extract.[12] Research was carried out to determine the effect of giving *Vernonia amygdalina* extract on the mitotic Leydig cells in male rats with diabetes mellitus induced by alloxan.

METHOD

This research is True Experimental research with a post-test-only control group design. This experimental study was conducted using white male rats (Rattus norvegicus) Wistar strain aged 2-3 months with a weight of 150-250 grams. The sample size was determined using Federer's formula and obtained a large sample of at least five rats per group. The sample of 31 white male rats was divided into K, P0, P1, P2, and P3 groups, each group consisting of six mice and one average rat is used as the baseline.

The experimental animals will be induced intraperitoneally with single-dose alloxan 150 mg/kg BW for four days. After the fourth day, all the rats re-measured their blood glucose levels through the tail veins. Then the African leaf extract was given using oral gavage in groups P1, P2, P3 with a dose of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW for 14 days. Rats are anaesthetized and euthanized to take the testicular organ for making microscope slides using HE staining and light microscope slide analysis using 400x microscopic zoom.

Measurement of mitotic Leydig cell with indicator for the number of adult Leydig cells counted in the interstitial area between the seminiferous tubules. The evaluation of mitotic Leydig cells will be determined by randomly selecting five different fields of view and then adding and calculating the average number of cells. The research data were analyzed using One-Way ANOVA with $P < 0.05$.

RESULTS

Based on the research that has been done obtained the results of the research as follows.

Table 1. Comparison of Average Number of Mitotic Leydig Cells All Groups

Treatment	Number of Mitotic Leydig Cells ± Elementary Average
K: Group of rats given glibenclamide 0.63 mg/kg.	29,6±8,44
P0: The group of rats given CMC 0.1% as much as 0.7 ml/oral/day.	15,6±5,22
P1: Groups of rats given African leaf extract at a dose of 100 mg/kg BW	28,6±7,63
P2: Groups of rats given African leaf extract at a dose of 200 mg/kg BW	22,8 ±4,66
P3: Groups of rats given African leaf extract at a dose of 400 mg/kg BW	18,8± 5,40

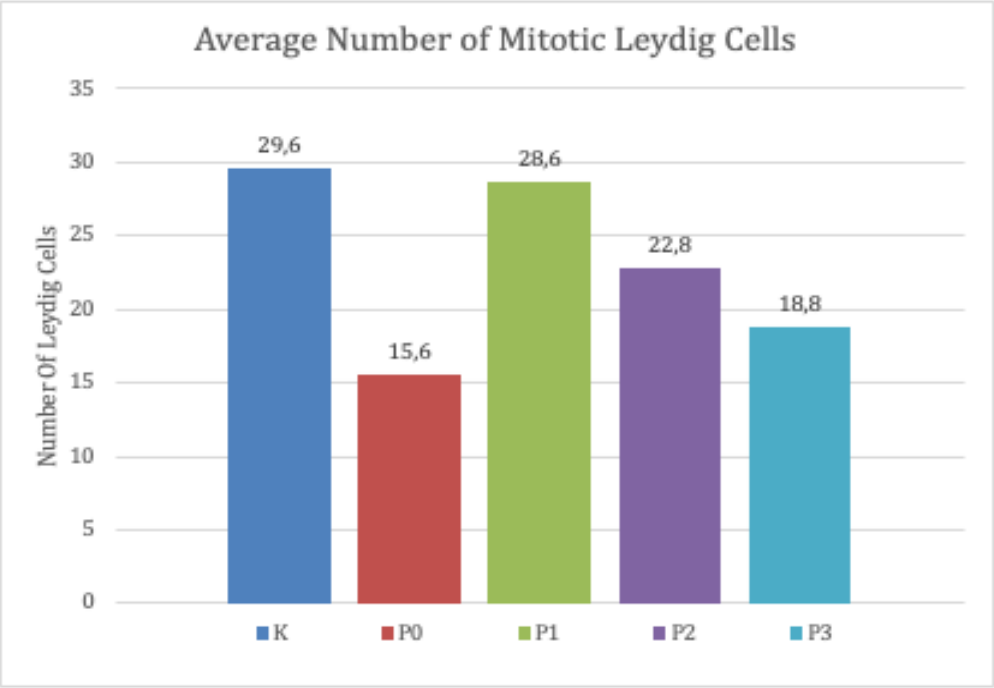

Figure 1. Comparison of Average Number of Mitotic Leydig Cells Between Groups

Data analysis results in Table 1 and Figure 1 Indicates that group K has the highest number of mitotic Leydig cells, the second in group P1, the third in group P2, the fourth group P3, and the fifth is the group given CMC 0.1%.

Table 2. Leydig Cell Mythical Number of Data Hypothesis Results

Test results		P	Description
Hypothesis test		0,026	Significant
		Post-hoc Analysis	
K	P0	0,008	Significant
	P1	0,655	Insignificant
	P2	0,128	Insignificant
	P3	0,013	Significant
P0	K	0,008	Significant
	P1	0,021	Significant
	P2	0,188	Insignificant
	P3	0,823	Insignificant
P1	K	0,655	Insignificant
	P0	0,021	Significant
	P2	0,270	Insignificant
	P3	0,034	Significant
P2	K	0,128	Insignificant
	P0	0,188	Insignificant
	P1	0,270	Insignificant
	P3	0,270	Insignificant
P3	K	0,013	Significant
	P0	0,823	Insignificant
	P1	0,034	Significant
	P2	0,270	Insignificant

Data analysis results in Table 1 and Figure 1 indicate that group K has the highest mitotic Leydig cells. The second is group P1, the third in group P2, the fourth group P3, and the fifth group with a given CMC of 0.1%.

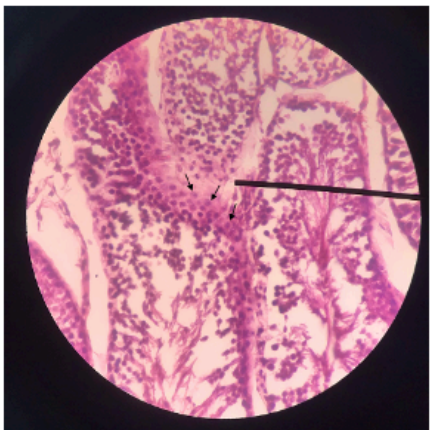

Figure 2. Leydig Cells in the Interstitial Tissue of the Control Group using 400x microsopic zoom

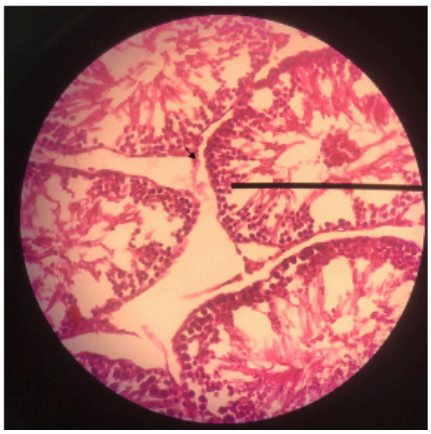

Figure 3. Leydig Cells in the Interstitial Tissue of the P0 Group using 400x microsopic zoom

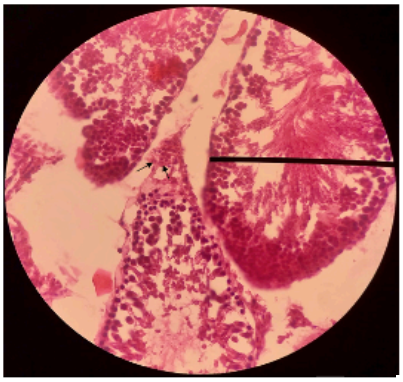

Figure 4. Leydig Cells in the Interstitial Tissue of the P1 Group using 400x microsopic zoom

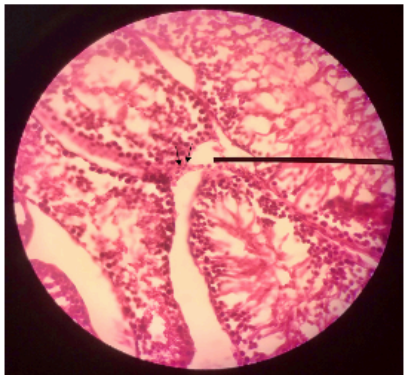

Figure 5. Leydig Cells in the Interstitial Tissue of the P2 Group using 400x microsopic zoom

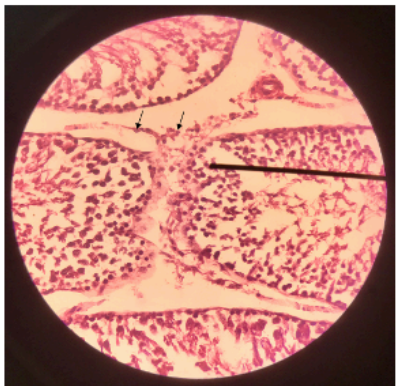

Figure 6. Leydig Cells in the Interstitial Tissue of the P3 Group using 400x microsopic zoom

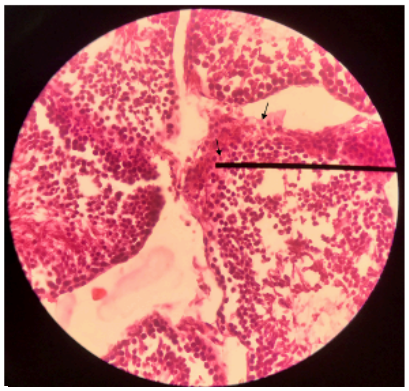

Figure 7. Leydig Cells in the Interstitial Tissue of the Normal Rat using 400x microsopic zoom

In the picture above, it can be seen that the mitotic number of Leydig cells in the P0, P2 and P3 groups decreased when compared to the normal rat testicular. In addition, the epithelium of the seminiferous tubules also looks thinner, and the area around or between the seminiferous tubules looks smaller. Histologically, in group K and group P1, there was only a slight difference in the number of mitotic Leydig cells compared with normal rat testicular.

DISCUSSION

In people with diabetes mellitus shows the formation of ROS that can cause increased production of free radicals in the body. If not handled appropriately, there will be oxidative stress. Oxidative stress can cause Leydig cell activity to decrease, leading to loss of testicular interstitial tissue density and reduced volume of Leydig cells per interstitial space.[13.14]

The results of the data analysis are in table 1. It shows that in the P0 group, there

was a decrease in the average number of mitotic Leydig cells when compared to the other experimental groups. This is due to the induction of alloxan, resulting in decreased production, insulin secretion, and damage to pancreatic beta cells. The histological picture in the P0 group shows that there is damage to the testicular tissue and Leydig cells. The stromal tissue around the seminiferous tubules looks smaller compared to the testes of normal rats.

In the P1, P2, and P3 groups, there was an increase in the number of mitotic Leydig cells compared to the P0 group. The P1 group (giving a dose of 100 mg/kg BW of African leaf extract) has a P-value = 0.021, which means there is a significant difference compared to the P0 group. In this group, the mean number of mitotic Leydig cells was 28.6 ± 7.63, where the mean number of mitotic Leydig cells in this group was not much different compared to the K group with an average of 29.6 ± 8.44. It can be concluded that the effective dose of using African leaf extract is 100 mg/kg BW because it can increase the number of mitotic Leydig cells and can be used as an antidiabetic.

The histological picture in the P1 group showed an increase in the number of mitotic Leydig cells and reduced levels of damage to testicular tissue compared to the P0 group and had a histological picture that is not much different from the testicles of normal rats. This is per previous research that is the administration of African leaf extract at a dose of 100 mg/kg BW can increase the diameter of the seminiferous tubules and the cross-sectional area of the tubules to increase the activity of the mitotic number of Leydig cells.[11.15]

In the group giving African leaf extract at a dose of 200 mg/kg BW and 400 mg/kg BW, it was seen that there was an increase in the number of mitotic Leydig cells, but there was no significant difference when compared to the P0 group with P= 0.188 and P= 0.823. In both groups, the histological picture was found to decrease the number of mitotic Leydig cells compared to normal rat testicular. The decrease in the number of mitotic Leydig cells was caused by the administration of African leaf extract with too high a toxic effect on the testicular of rats.[12.15]

This is in accordance with the research conducted by Saalu et al., the administration of African leaf extract with a dose of 200 mg/kg BW can significantly decrease concentration, motility, normal sperm morphological percentage, and is not significant in increasing levels of the LH hormone which can cause interference with Leydig cell function.[15] Based on previous research, African leaf extracts at

doses of 250 mg/kg BW, 300 mg/kg BW, and 500 mg/kg BW causing an average decrease in seminiferous tubules damage to the interstitial tissue, which affected the number of mitotic Leydig cells.[11,12]

This is due to one of the antioxidant compounds in African leaf extract, an alkaloid. Although the mechanism of the effect of alkaloids is not yet known more clearly, it is believed that alkaloids can release metabolites that will bind to cell molecules and cross-link DNA, causing cytotoxicity in the testicular of rats.[11.15]

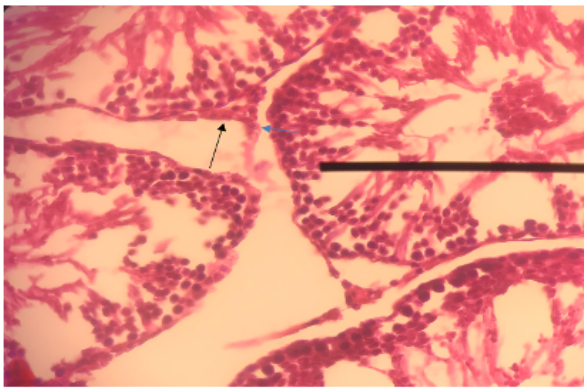

Figure 8. Testicular Histology of Group P0.
Black Arrow: Shows Tissue Damage Around the Seminiferou Tubules.
Blue Arrow: Indicates A Decreased Number Of Leydig Cell Mitosis In Interstital Tissue.

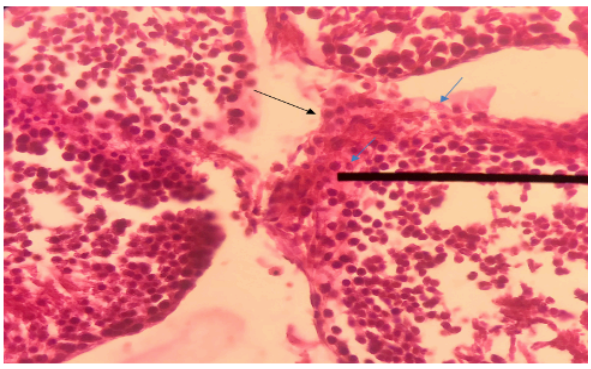

Figure 9. Histology of Normal Rat Testicular.
Black Arrow: Indicates the Tissue Around the Seminiferou Tubules is Wider When Compared to Group P0.
Blue Arrow: Indicates Number Mitotic of Leydig Cells i Interstital Tissue.

CONCLUSION

Based on the results of this study, it can be concluded that the administration of Vernonia amygdalina extract at 100 mg/kg BW is proven effective in the increase of mitotic Leydig cells. Hence, it could be utilized to fix out sexual dysfunction in diabetes mellitus patients.

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