THE EFFECT OF ADMINISTERING AFRICAN BITTER LEAF EXTRACT ON SPERMATOGENIC CELLS OF HYPERGLYCEMIC WISTAR RATS

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ABSTRACT

Introduction: Diabetes is still a world health problem that can cause many complications. Male infertility is one of the diabetic complications. This condition is caused by oxidative stress in diabetic patients. African bitter leaf is believed to contain an antioxidant compound that can repair male infertility.
Purpose: This study aims to determine the effect of administering African bitter leaf extract on spermatogenic cell count in hyperglycemic Wistar rats.
Method: This study use *Rattus norvegicus* as an animal model, which was administrated with alloxan to induce hyperglycemic. P0 group were given Na CMC 0,1%, Control group were given glibenclamide 0,63/kg bodyweight for 14 days. 100mg/kg bodyweight (P1), 200mg/kg (P2), and 400mg/kg (P3) of African bitter leaf extract were administrated for 14 days. In the end, the animals were sacrificed, and testicle histopathologic sections were made.
Results: Significant result (P<0,05) comparing spermatogonium, primary spermatocyte, and spermatid count between groups. Post-hoc test shows the significant result on comparison of primary spermatocyte and spermatid count between P0 and P1.
Conclusions: There is an effect of bitter leaf extract administration on spermatogenic cell count in hyperglycemic Wistar rats. The best improvement can be observed in the P1 group, administered with 100mg/kg bodyweight African bitter leaf.

Keywords: African bitter leaf, antioxidant, hyperglycemic, spermatogenic cell counts

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INTRODUCTION

Diabetes is still a world health problem, including in Indonesia[1]. Diabetes can cause complications, such as impairment of spermatogenesis in male[2]. Decrease of *Follicle Stimulating Hormone* (FSH) and *Luteinizing hormone* (LH), increase in the amount of *Reactive Oxygen Species* (ROS) and *Advanced Glycation End Products* (AGEs) in hyperglycemic condition causing oxidative stress that impairs spermatogenesis in diabetic male[3]. A cohort study in 1994-2012 shows that 18.499 of 39.516 diabetic males experience infertility[4].

African bitter leaf (*Vernonia amygdalina*) is believed to contain antihyperglycemic and antioxidant effects. African bitter leaf is scrub. It grows in tropical areas such as Africa and Indonesia. It belongs to the family *Asteraceae*. People use African bitter leaf for the side dish, and it contains antibacterial, anti-inflammation, antifungal, antimalarial, anti-cancer, anti-hyperlipidemic effect, and etc[5–7].

African bitter leaf has antioxidant components (flavonoid, alkaloid, steroid, phenolic acid, saponin, tannin, *sesquiterpene lactone*). Those can stabilize free radicals to a more stable structure, chelating with metals and suppress pro-oxidant enzyme[8].

According to this theory, we'd like to study the potential of African bitter leaf in male fertility. Therefore, it can be used as an alternative treatment for diabetic male infertility. This study aims to determine the effect of administering African bitter leaf extract on spermatogenic cells.

METHOD

Materials

The materials used in this study were an ethanolic extract of African bitter leaf, CMC Na 0,1%. The ethanolic extract is given orally using oral gavage once daily.

Animal

The experimental animals used were white male rats (*Rattus norvegicus*). The experimental animals used were 2-3 months old with 150-250 grams bodyweight as many as 31 animals. The animals were adapted to the environment for a week and placed in a cage containing two rats, each separated by wire.

Male Wistar rats were divided randomly into five groups, Control group (K) that were induced to hyperglycemic condition and given Glibenclamide 0,63mg/kg BW/day, Treatment group 0 (P0) that were induced to hyperglycemic condition and given CMC Na 0,1% 0,7ml/day, Treatment group 1 (P1) that were induced to hyperglycemic condition and given an ethanolic extract of African

bitter leaf 100mg/kg BW/day, Treatment group 2 (P2) that were induced to hyperglycemic condition and given an ethanolic extract of African bitter leaf 200mg/kg BW/day, and Treatment group 3 (P3) that were induced to hyperglycemic condition and given an ethanolic extract of African bitter leaf 400mg/kg BW/day,

Experimental procedures

In this study, rats were induced to hyperglycemic conditions by administering a single dose of intraperitoneal injection *alloxan monohydrate* 150mg/kg BW. The rats would be given the treatment for 14 days if, after four days of *alloxan* administration, the blood glucose level is above 200mg/dL. The rats would be sacrificed by anesthesia using *ketamine-xylazine* and cervical dislocation. The testicle will be made into histopathological sections and observed with a light microscope.

Spermatogenic Cell Counts

Spermatogenic cell counts were done using a light microscope with 400x zoom. Spematogonium, primary spermatocyte, and spermatid were counted by the average cell counts in 5 random fields of view.

Data Analysis

Data obtained from this study were tested for normality with *the Shapiro-Wilk* test and homogeneity by *Levene's test*. If the normality and homogeneity results show normal and homogenous data, the ANOVA test and *Bonferroni* (*posthoc* test) are used for the hypothesis test. Data shows significant if $P < 0,05$.

RESULTS

The histopathological section of seminiferous tubule from the experimental animals can be observed in figure 1.

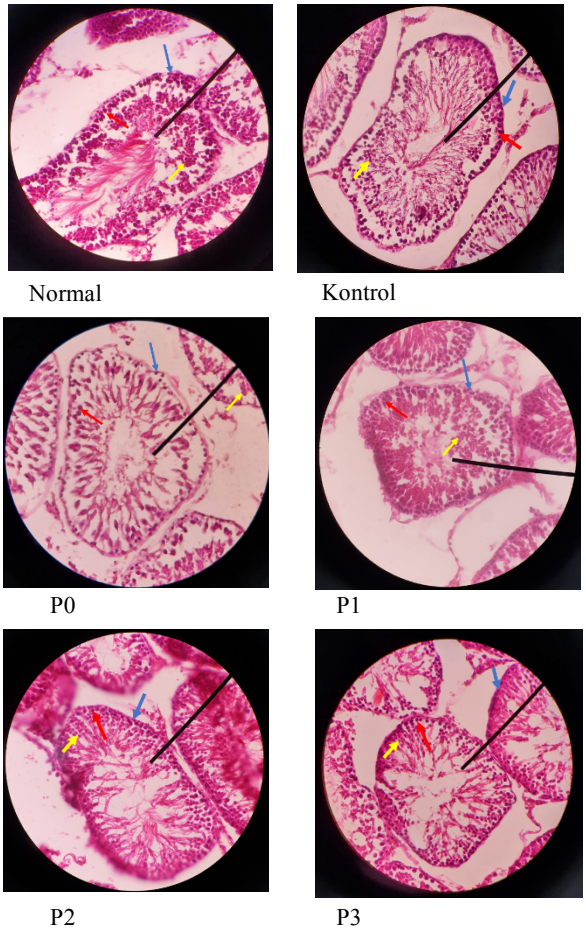

Figure 1. Histopathological section of seminiferous tubule from the experimental animals observed with a light microscope with 400x zoom. Spermatogonia (blue arrow), primary spermatocyte (red arrow), spermatid (yellow arrow)

A comparison of spermatogenic cell count is described in table 1. In this study, the hypothetical test is conducted using ANOVA and continued with *the posthoc* test *Bonferroni*. ANOVA test, which compares all groups (K: P0:P1:P2:P3), shows significant results. It means that there are differences in spermatogenic cell count (spermatogonia, primary spermatocyte, and spermatid) between treatment groups. P0 group has the lowest average cell count compared to other groups [Table 1].

P*ost-hoc* test using *Bonferroni* shows insignificant results compared to spermatogonium [Table 2] but shows significant results in comparison of primary spermatocyte and spermatid between P0 and P1 [Table 3 and 4].

Table 1. Comparison of spermatogenic (spermatogonia, primary spermatocyte, and spermatid) average cell count between treatment groups

Treatment Group	Average cell count ± SD		
	Spermato-gonia	Primary spermatocyte	Spermatid
K	32.56 ± 8.429	24.60 ± 6.950	52.88 ± 7.946
P0	25.80 ± 3.952	17.00 ± 3.391	41.64 ± 10.624
P1	41.08 ± 13.111	32.40 ± 12.602	64.36 ± 7.055
P2	35.72 ± 5.661	27.80 ± 6.760	55.24 ± 10.716
P3	27.52 ± 6.190	22.60 ± 3.435	51.24 ± 4.412

Table 2. Hipothetical test of spermatogonia cell count between groups

Group	P (<0,05)
K : P0 : P1 : P2 : P3	0,047*
K : P0	1,000**
K : P1	1,000**
K : P2	1,000**
K : P3	1,000**
P0 : P1	0,074**
P0 : P2	0,674**
P0 : P3	1,000**
P1 : P2	1,000**
P1 : P3	0,156**
P2 : P3	1,000**

* : Statistically significant

** : Statistically insignificant

Table 3. Hipothetical test of primary spermatocyte cell count between groups

Group	P (<0,05)
K : P0 : P1 : P2 : P3	0,043*
K : P0	1,000**
K : P1	1,000**
K : P2	1,000**
K : P3	1,000**
P0 : P1	0,038*
P0 : P2	0,325**
P0 : P3	1,000**
P1 : P2	1,000**
P1 : P3	0,501**
P2 : P3	1,000**

* : Statistically significant

** : Statistically insignificant

Table 4. Hipothetical test of spermatid cell count between groups

Group	P (<0,05)
K : P0 : P1 : P2 : P3	0,008*
K : P0	0,492**
K : P1	0,450**
K : P2	1,000**
K : P3	1,000**
P0 : P1	0,004*
P0 : P2	0,197**
P0 : P3	0,888**
P1 : P2	1,000**
P1 : P3	0,239**
P2 : P3	1,000**

* : Statistically significant

** : Statistically insignificant

DISCUSSION

This study is determined to compare spermatogenic cell count in alloxan-induced hyperglycemic *Rattus norvegicus* and given glibenclamide (K), CMC Na 0,1% (P0), ethanolic extract of African bitter leaf 100mg/kgbw (P1), 200mg/kgbw (P2), 400mg/kgbw (P3) for 14 days.

Diabetes causes impairment in male fertility by interfering with LH & FSH secretion, increasing ROS production from the polyol-sorbitol pathway, AGEs formation, and protein kinase C. This will cause a decrease in testosterone hormone level, an increase in sperm DNA damage, and sperm apoptosis[3,9].

A previous study showed that a certain amount of African bitter leaf extract could increase spermatogenic cell count, repair seminiferous tubule microarchitecture, and increase the live death ratio of sperm[10]. African bitter leaf contains an alkaloid, flavonoid, saponin, tannin, steroid, and sesquiterpene lactone, which contain antioxidant activity by preventing free radical formation, suppressing pro-oxidant enzymes, and stimulating antioxidant enzymes[11,12].

African bitter leaf extract can lower blood glucose level by regenerating pancreatic β-cell which secrete insulin, suppressing serum caspase-3 which cause apoptosis of pancreatic β-cell and increase *Glucose Transporter*-2 (GLUT-2) expression which gives the signal to secrete insulin[5,13].

ANOVA test shows significant result in comparison of spermatogenic cell count between all groups, which means that there are differences in spermatogenic cell count (spermatogonia, primary spermatocyte, and spermatid)

The posthoc test shows insignificant results in comparison to spermatogonia cell count. This may happen because spermatogonia have the highest amount of *Superoxide Dismutase* enzyme, which has antioxidant activity. So, spermatogonia have the highest tolerance level to oxidative stress[14]. Although the posthoc test shows an insignificant result, there is an increased average cell count in P1, P2, P3, and K groups compared to P0.

P0 group has the lowest spermatogenic cell count, showing the damage of spermatogenic cells because of oxidative stress. Administration of African bitter leaf extract in the P1, P2, and P3 groups cause improvement in spermatogenic cell count compared to P0. P1 shows the closest cell count compared to normal rats. This result is in line with a previous study by Saalu et al. [10].

Improvement of spermatogenic cell count in P2 and P3 is not as good as P1. This may be caused by alkaloid bioactivation in a larger dosage—the bioactivation cause released metabolites which cause DNA cross-link, cytotoxicity and increasing pro-oxidant activity[15].

CONCLUSION

Based on this research, it is concluded that there are differences in spermatogenic cell count of hyperglycemic rats administered with African bitter leaf extract 100mg/kg BW, 200mg/kg BW, dan 400mg/kg BW for 14 days. Administration of 100mg/kg BW ethanolic bitter leaf extract shows the best improvement in spermatogenic cell count.

REFERENCES

1. International Diabetes Federation. IDF Diabetes Atlas Ninth. Dunia : IDF. 2019. 168 p.
2. Ding GL, Liu Y, Liu ME, Pan XJ, Guo MX, Sheng JZ, et al. The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian J Androl. 2015;17(6):948–53.
3. Rhee SY, Kim YS. The role of advanced glycation end products in diabetic vascular complications. Diabetes Metab J. 2018;42(3):188–95.
4. Glazer CH, Bonde JP, Giwercman A, Vassard D, Pinborg A, Schmidt L, et al. Risk of diabetes according to male factor infertility : a register-based cohort study. 2017;32(7):1474–81.
5. Yeap SK, Ho WY, Beh BK, Liang WS, Ky H, Your AHN, et al. Vernonia amygdalina, an ethnoveterinary and ethnomedical used green vegetable with multiple bioactivities. J Med Plants Res. 2010;4(25):2787–812.
6. Oyeyemi IT, Akinlabi AA, Adewumi A, Aleshinloye AO, Oyeyemi OT. Vernonia amygdalina : A folkloric herb with anthelminthic properties. Beni-Suef Univ J Basic Appl Sci [Internet]. 2018;7(1):43–9. Available from: https://doi.org/10.1016/j.bjbas.2017.07.007
7. Kadiri O, Olawoye B. Vernonia amygdalina : An Underutilized

Vegetable with Nutraceutical Potentials – A Review Turkish Journal of Agriculture - Food Science and Technology Vernonia amygdalina : An Underutilized Vegetable with Nutraceutical Potentials – A Review. 2016;(September).

8. Audu SA, Taiwo AE, Ojuolape AR. A Study Review of Documented Phytochemistry of Vernonia amygdalina (Family Asteraceae) as the Basis for Pharmacologic Activity of Plant Extract. J Nat Sci Res. 2012;2(7):1–8.
9. Shaikh H, Shrivastava VK, Amir M. Diabetes Mellitus and Impairment of Male Reproductive Function: Role of Hypothalamus Pituitary Testicular Axis and Reactive Oxygen Species. 2016;8(1):41–50.
10. Saalu LC, Akunna GG, Ajayi JO. Modulating role of bitter leaf on spermatogenic and steroidogenesis functions of the rat testis. Am J Biochem Mol Biol. 2013;3(3):314–21.
11. Alara OR, Nour A, Olalere OA. PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF Vernonia amygdalina : A REVIEW PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF Vernonia amygdalina : A REVIEW. 2017;(September).
12. Oxide A, Ms S, Fo A, Momoh S, Et F, Vd S, et al. Evaluation of antioxidant and cytotoxic properties of Vernonia amygdalina. 2018;4(4):10–5.
13. Atangwho IJ, Ebong PE, Egbung GE, Obi AU. Extract of Vernonia amygdalina Del. (African bitter leaf) can reverse pancreatic cellular lesion after alloxan damage in the rat. Aust J Basic Appl Sci. 2010;4(5):711–6.
14. Celino FT, Yamaguchi S, Miura C, Ohta T, Tozawa Y, Iwai T, et al. Tolerance of spermatogonia to oxidative stress is due to high levels of Zn and Cu/Zn superoxide dismutase. PLoS One. 2011;6(2):1–11.
15. Saalu LC, Kpela T, Benebo AS, Oyewopo AO, Anifowope EO, Oguntola JA. The dose-dependent testiculoprotective and testiculotoxic potentials of Telfairia occidentalis Hook f. leaves extract in rat. Int J Appl Res Nat Prod. 2010;3(3):27–38.