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The Effect of Moringa Oleifera Extract on Sperm Quality and Quantity of Diabetes Wistar Rat

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ABSTRACT

Diabetes mellitus is a condition in which blood glucose levels increase in a person's body which can result in chronic disorder. The effect of diabetes on a male reproductive system can affect sperm quality and disrupt the spermatogenesis process. Moringa oleifera leaves are rich in antioxidants such as flavonoids, vitamin A, vitamin E, and vitamin C, and contain selenium which helps lower blood sugar levels. This study was conducted to determine the effect of administering *Moringa oleifera* leaf extract on the quality and quantity of sperms in Wistar rats with diabetes mellitus. This research is an experimental study using *pre-and post-randomized* controlled group design. This study examined blood sugar levels, density, motility, and viability of sperm cells. This experimental test was divided into 4 groups: one as a control group and three groups as treatment groups induced with alloxan and given ethanol extract of Moringa leaves with doses of 250, 500, and 750mg/kgBB. The results showed that the ethanol extract of Moringa leaves showed significant improvements in various doses (P-value ≤ 0.05).

1. Introduction

According to the WHO, diabetes is a serious chronic disease that occurs when the pancreas cannot produce enough insulin (a hormone that regulates blood sugar or glucose), or the body cannot effectively use the insulin produced by insulin. Judging from the records of 220 countries in the world, the number of diabetes sufferers is estimated to increase from 415 million in 2015 to 642 million in 2040.[1,2] Indonesia ranks seventh in the world after China, India, the United States, Brazil, Russia and Mexico. Based on data (Rikesdas, 2013), the prevalence of diabetes in Indonesia increased from 5.7% in 2007 to 6.9% or around 9.1 million in 2013.[3,4]

The effects of diabetes on male reproduction can affect sexual behavior disorders, sperm quality, and also ejaculation. In diabetes, there will be disturbances in the spermatogenesis and oligozoospermic processes. This diabetes condition will cause damage to the endothelium of the blood vessels causing microangiopathy which interferes with the provision of nutrition through the blood vessels to the tissues that form spermatozoa[5,6]

Leaves of Moringa (Moringa Oleifera) is a plant that is easily available in Indonesia and is one of the plants that can be used as traditional medicine.[7]

Moringa Oleifera leaves are rich in antioxidants such as flavonoids, vitamin A, vitamin E, and vitamin C, and contain selenium which helps lower blood sugar levels. Based on the results of the phytochemical test, Moringa leaves show that the

presence of alkaloids and steroids / triterpenoids play a positive role in reducing blood sugar levels [8]

Based on the description above, the researchers are interested in knowing the effect of giving Moringa leaf extract on the quality and quantity of sperm in Wistar rats with diabetes mellitus.[9,10]

2. Methods

This study is an experimental study using Wistar rats as experimental animals, using a pre and post randomized controlled group design. The research was carried out at the Riwandi Animal House Laboratory which was conducted from September 2020 to October 2020. The samples used were Moringa Oleifera leaves taken from the yards of residents of Tualang Village, Perbaungan District, Serdang Bedagai Regency.[2]

In this study, the tools and materials used were oval needle (Gavage), 1 ml syringe, minor set, stirring rod, scale, blender, measuring flask, Erlenmeyer flask, Neubauer Chamber counting room, light microscope, dropper pipette, micro pipette, object glass, cover glass, pot jar, aluminum foil, rotary evaporator, glucometer, Moringa Oleifera leaf extract, Wistar rat, standard feed, 96% ethanol, eosin nigrosin dye, PBS, safranin stainnly, crystal violet, aquadest, methanol, NaCl, and Alloxan.[3]

How to make moringa leaves extract

Weigh collected fresh moringa leaves and wash thoroughly under running water. The process of extracting Moringa leaves uses the maceration method, using 96% ethanol as a solvent.

Then use a blender to crush the Moringa oleifera leaves to get Moringa leaf powder. Put the Moringa leaf powder in the jar, and add 6L of 96% ethanol. Then closed and left for 48 hours protected from the sun. The mixture is filtered to obtain macerate.

The pulp was macerated with 96% ethanol using the same procedure. Maceration was carried out until clear maserate was obtained with a digital shaker with a speed of 50 rpm. The maserate that has been obtained is then evaporated for 2 hours with a rotary evaporator.

Research steps

Group I (P0) = consisted of 6 adult male rats that were not treated (control group), namely not alloxan induced and not given Moringa leaf extract, only fed and drank ad libitum. Group II (P1) = consisted of 6 adult male rats that were injected intraperitoneally with alloxan at a dose of 130mg / kgBW / day and given 250mg / kgBW Moringa leaf extract. Group III (P1) = consisted of 6 adult male rats that were injected intraperitoneally with alloxan at a dose of 130mg / kgBW / day and given 500mg / kgBW Moringa leaf extract. Group IV (P3) = consisted of 6 adult male rats that were injected intraperitoneally with alloxan at a dose of 130mg / kgBB / day and given 750mg / kgBB of Moringa leaf extract. On the last day of the study, sperm and blood sugar levels were examined. To determine the quantity and quality of sperm, several testes of experimental animals were evaluated by placing part of the vas deferens in a dish stained with NaCl solution so that the sperm did not die. Furthermore, massage is carried out using tweezers on the vas deferens to get the required sperm cells. The sperm cells that have come out of the vas deferens are taken using tweezers and then placed in an object glass and covered with a deck glass. Furthermore, it is observed under a microscope using four fields of view.

3. Results and Discussion

Phytochemical screening results

As a start of this research, the ethanol extract of Moringa leaves (EEDK) that has been tested can be examined for its phytochemical content qualitatively through phytochemical screening. The results of phytochemical screening on the ethanol extract of Moringa leaves can be seen in the following table.[4]

From the table data above, it can be seen that the ethanol extract of Moringa leaves has phytochemical content in the form of alkaloids, tannins, saponins, triterpenoids, and polyphenols.[5]

Then, the analysis was continued on the parameters in mice including blood sugar levels, density, motility, and viability of sperm cells[6]

Prior to the inferential statistical test of these parameters, the data normality analysis was carried

out using the Shapiro-Wilk analysis as shown in the following table.

From the table data above, it can be seen that all parameters are measured at male wistar rats as experimental animals normally distributed. This can be seen of the P value of each parameter in each group > 0.05.

Check rats blood sugar levels

Evaluation of blood sugar levels in rats was carried out with 3 different times, namely before induction, after induction, and after being given treatment according to the group (day 14). Each blood sugar level was analyzed by One Way Anova. The results of the One Way Anova analysis can be seen in the following table.[7,8]

Data are presented in the form of Mean ± SD; EEDK: Moringa Leaf Ethanol Extract; * P value is obtained from One-Way Anova analysis; ** Different superscripts in the same column show significant differences at p value <0.05 according to the Games-Howell post-hoc test

From the table data above, it can be seen that the blood sugar levels of the rats in each group both before and after induction were not found to be any different statistically significant. This is reflected in the value of P > 0.05. However, the blood sugar levels of the rats after being treated were given the extract to 3 groups different shows that are statistically significant differences both to control and other doses. This can be seen from the results of the analysis of the post hoc test games-howell marked by superscript in the table.[8]

Rat sperm examination

Evaluation of rat sperm was carried out on rat epididymal fluid samples using 3 parameters including: density, motility, and viability of sperm cells. Each these parameters are analyzed by One Way Anova and the results of the analysis are shown in the following table.[9,10]

Data are presented in the form of Mean ± SD; EEDK: Moringa Leaf Ethanol Extract; * P value is obtained from One-Way Anova analysis; ** Different superscripts in the same column show significant differences at p values <0.05 based on the Tukey HSD post-hoc test

From the table data above, it can be seen that the data giving ethanol extract from Moringa leaves at various doses shows a significant improvement on the parameters of good sperm examination with other doses as well as against control. This can be seen from the P value of the results One Way Anova analysis which is <0.05 and the results of the Tukey HSD Post Hoc Test analysis marked via superscript on the table.[10]

The results of this study where the ethanol extract of Moringa leaves can improve the blood sugar levels of rats and prevented sperm damage in alloxan-induced diabetic rats. Increasing the dose of Moringa leaf ethanol extract significantly decreased the blood sugar levels of the rats and increased both the density, motility and viability of sperm cells.[11]

In this study, it was reported that Moringa leaves have a protective effect against the testes and an anti-diabetic effect. This is due to the phytochemical content of moringa leaves in the form of kaempferol which can improve glycolysis, glucose uptake, glycogen system, activation of AMP activated protein kinase, and the expression of GLUT-4. Through improving blood sugar levels, Moringa leaf extract can provide additional protection to the testicular tissue. In addition, Moringa leaf extract also has antioxidant benefits so that it can reduce further damage to testicular tissue considering that diabetigenic compounds can damage testicular tissue[12]

Table 1 Results of Phytochemical Screening of Moringa Leaves Ethanol Extract (EEDK)

Compound group	Reagent name	Result
Alkaloids	Meyer	+
	Dragendroff	+
	Bouchart	+
Tannins	Hot water + $FeCl_3$ 10%	+
Saponins	Hot water + HCL 2N	+
Flavonoids	Concentrated HCL + Mg powder	–
Triterpenes / steroids	Lieberman - burchat	+
Sugar Glycosides	LP molish	–
Non sugar glycosides	Lieberman Burchard	–
Anthraquinone Glycosides	CCl_4 + dilute ammonia	–
Polyphenol	$FeCl_3$ 1%	+

Table 2. Results of Data Normality Analysis with Shapiro-Wilk

Parameter	Group	P value	Interpretation
Blood Sugar Levels Before Induction	EEDK control	0.242	Normal
	EEDK dose 250 mg / kgBW	0.472	Normal
	EEDK dose 500 mg / kgBW	0.299	Normal
	EEDK dose 750 mg / kgBW	0.420	Normal
Blood Sugar Levels After Induction	EEDK control	0.079	Normal
	EEDK dose 250 mg / kgBW	0.727	Normal
	EEDK dose 500 mg / kgBW	0.235	Normal
	EEDK dose 750 mg / kgBW	0.198	Normal
Blood Sugar Levels After Treatment	EEDK control	0.717	Normal
	EEDK dose 250 mg / kgBW	0.332	Normal
	EEDK dose 500 mg / kgBW	0.228	Normal
	EEDK dose 750 mg / kgBW	0.562	Normal
Sperm Density	EEDK control	0.279	Normal
	EEDK dose 250 mg / kgBW	0.904	Normal
	EEDK dose 500 mg / kgBW	0.535	Normal
	EEDK dose 750 mg / kgBW	0.476	Normal
Motility	EEDK control	0.266	Normal
	EEDK dose 250 mg / kgBW	0.283	Normal
	EEDK dose 500 mg / kgBW	0.262	Normal
	EEDK dose 750 mg / kgBW	0.105	Normal
Viability	EEDK control	0.964	Normal
	EEDK dose 250 mg / kgBW	0.593	Normal
	EEDK dose 500 mg / kgBW	0.293	Normal
	EEDK dose 750 mg / kgBW	0.992	Normal

Table 3 Comparison of Sugar Levels Rat Blood on Each Treatment Group

Treatment group	Blood sugar levels (mg / dl)		
	Before induction	After induction	After treatment **
Control	82.50 ± 7.05	372.00 ± 11.52	391.50 ± 10.75[a]
EEDK 250 mg / kgBW	77.00 ± 5.72	383.50 ± 25.64	318.75 ± 34.25[b]
EEDK 500 mg / kgBW	73.75 ± 7.32	350.00 ± 19.32	240.25 ± 22.69[c]
EEDK 750 mg / kgBW	71.50 ± 4.80	345.00 ± 36.17	197.25 ± 5.25[d]
P* value	**0.130**	**0.148**	**0.001**

Table 4 Comparison of the results of rat sperm examination in each treatment group

Treatment group	Sperm Inspection Parameters **		
	Density (x10⁶ / ml)	Motility (%)	Viability (%)
Control	12.65 ± 3.33[a]	43.73 ± 1.44[a]	46.19 ± 2.10[a]
EEDK 250 mg / kgBW	32.89 ± 1.35[b]	55.82 ± 1.15[b]	66.18 ± 2.76[b]
EEDK 500 mg / kgBW	43.05 ± 1.81[c]	63.85 ± 1.67[c]	77.45 ± 0.90[c]
EEDK 750 mg / kgBW	74.35 ± 3.38[d]	73.37 ± 1.58[d]	82.83 ± 2.31[d]
P* value	**0.001**	**0.001**	**0.001**

4. Conclusion

The conclusions that can be drawn in this study are as follows. Moringa leaf ethanol extract contains phytochemicals in the form of alkaloids, tannins, saponins, triterpenoids, and polyphenols. The ethanol extract of Moringa leaves significantly decreased blood sugar levels in line with the increasing dose of Moringa leaf extract given (P value = 0.001). The ethanol extract of Moringa leaves showed a protective effect on testicular tissue through a significant increase in density, motility, and viability of epididymal semen in line with the increasing dose of ethanol extract of Moringa leaves given (P value = 0.001).

5. References

1. Abd Eldaim, M. A., Shaban Abd Elrasoul, A. dan Abd Elaziz, S. A. (2017) "An aqueous extract from Moringa oleifera leaves ameliorates hepatotoxicity in alloxan-induced diabetic rats," Biochemistry and Cell Biology, 95(4), hal. 524–530. doi: 10.1139/bcb-2016-0256
2. Abu, A. H., Ahemen, T. dan Ikpechukwu, P. (2013) "the Testicular Morphometry and Sperm Quality of Rabbit Bucks Fed," Agro Search, 13(1), hal. 49– 56.
3. Akunna, G. G. et al. (2012) "Ameliorative Effect of Moringa oleifera (drumstick) Leaf Extracts on Chromium-Induced Testicular Toxicity in Rat Testes," World J Life Sci. and Medical Research, 2(2), hal. 20–26. Tersedia pada: http://www.rrpjournals.com/.
4. Fatoumata, B. et al. (2020) "Antidiabetic properties of Moringa oleifera: A review of the literature," Journal of Diabetes and Endocrinology, 11(1), hal.18–29.doi: 10.5897/jde2019. 0136.
5. Fedail, J. S. et al. (2016) "Gum arabic improves semen quality and oxidative stress capacity in alloxan induced diabetes rats," Asian Pacific Journal of Reproduction, 5(5), hal. 434–441.doi: 10.1016/j.apjr.2016. 07.014.
6. Meldawati, (2011). Pengaruh Ekstrak Buah Morinda Citrifolia Linn Terhadap Kualitas, Kuantitas Sperma Dan Kadar Malondialdehyde Testis Tikus Wistar Diabetes Mellitus. "Tesis". Medan.
7. Olayaki, L. A. et al. (2015) "Methanolic extract of Moringa oleifera leaves improves glucose tolerance, glycogen synthesis and lipid metabolism in alloxan-induced diabetic rats," Journal of Basic and Clinical Physiology and Pharmacology, 26(6), hal. 583–593. doi: 10.1515/jbcpp-2014-0129.
8. Radiansah, R. (2013). Ekstrak Daun Kelor (Moringa oleivera) sebagai alternatif untuk menurunkan kadar gula darah pada mencit. Jurnal Akademia Kimia. 2(2) : 1-5. Palu
9. Sadoughi, D., Edalatmanesh, M. A. dan Rahbarian, R. (2019) "Protective effect of curcumin on quality parameters of sperm and testicular tissue alterations in alloxan-induced diabetic rats as animal model," Indonesian Biomedical Journal, 11(3), hal. 240–246. doi: 10.18585/inabj.v11i3.733.
10. Samsulrizal, N. et al. (2011) "Effect of Ficus deltoidea leaves extracts on sperm quality, LDH-C 4 activity and testosterone level in

alloxan-induced male diabetic rats," 2011 IEEE Colloquium on Humanities, Science and Engineering, CHUSER 2011, (October), hal. 888–891. doi: 10.1109/CHUSER.2011.6163864.

11. Syarifuddin, N. A. et al. (2017) "Improving libido and sperm quality of bali bulls by supplementation of Moringa oleifera leaves," Media Peternakan, 40(2), hal. 88–93. doi: 10.5398/medpet.2017.40.2.88.
12. Vargas-Sánchez, K., Garay-Jaramillo, E. dan González-Reyes, R. E. (2019) "Effects of moringa oleifera on glycaemia and insulin levels: A review of animal and human studies," Nutrients, 11(12), hal. 1–19. doi: 10.3390/nu11122907.