Original Article

Ameliorative Effect of *Moringa oleifera* Fruit Extract on Reproductive Parameters in Diabetic-induced Male Rats

Amira Kamalrudin[1], Malina Jasamai[2], Mahanem Mat Noor[1*]

ABSTRACT
Background: Diabetes mellitus negatively impact the male reproductive parameters. The ability of *Moringa oleifera* fruit to improve the reproductive parameters in diabetic-induced male has yet to be documented. **Aim:** To investigate the anti-hyperglycaemic potential of *M. oleifera* fruit aqueous extract and its effect on reproductive parameters in diabetic-induced male rats. **Materials and Methods:** A total of 20 male Sprague Dawley rats were divided into four groups; normal control (without diabetic induction), negative control (diabetes without treatment), positive control (diabetes with metformin) and treatment group (diabetes with 500 mg/kg *M. oleifera* fruit aqueous extract). Treatments were given *via* oral gavage for 21 consecutive days. Analysis of fasting blood glucose level, sperm quality, testicular histology and relative testis weight were performed. **Results:** The administration of *M. oleifera* fruit aqueous extract exhibited a significant reduction in fasting blood glucose level and a pronounced increase in sperm quality (sperm count, motility, viability and morphology) compared with the negative control group. *M. oleifera* fruit extract restored the histology of the testes as they were seen to be packed with sperms and showed an organised spermatogenesis. The relative testis weight showed no significant difference between all groups. **Conclusion:** The results obtained suggested that *M. oleifera* fruit aqueous extract possessed an anti-hyperglycaemic property and improved the reproductive parameters in diabetic-induced rats.
Key words: *Moringa oleifera* fruit, Male reproductive, Diabetes mellitus, Sperm quality, Fasting blood glucose level.

Amira Kamalrudin[1], Malina Jasamai[2], Mahanem Mat Noor[1*]

[1]*School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, MALAYSIA.*
[2]*Drug and Herbal Research Centre, Faculty of Pharmacy, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, MALAYSIA.*

Correspondence

Dr. Mahanem Mat Noor

School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, MALAYSIA.

Phone no : +60192633785

E-mail: mahanem@ukm.edu.my

INTRODUCTION

Diabetes mellitus is a sign of impairment of glucose homeostasis in the body characterised by a progressive deterioration of insulin function and/or lack of insulin production. The global prevalence of diabetes among men aged 18 years and above has been increasing in the past years, from 11.6% in 2006[1] to 15.2% in 2011[2] and 17.5% in 2015.[3] This trend is expected to keep increasing at an alarming rate. Thus, it becomes a great concern as men are in their active reproductive age and it might affect their reproductive functions. A total of 48.5 million couples which made up 15% of the global population have been affected by infertility.[4] Of all the infertility cases reported, 30–50% of them were due to male factors alone.[5]

Male infertility is a common side effect of diabetes mellitus. Avicenna, father of the early modern medicine, has specifically stated in his book, The Canon of Medicine, that the collapsed of the sexual function is one of the main complication of diabetes mellitus.[6] Oxidative stress has been established as a crucial implication of diabetes mellitus. Increased oxidative stress in diabetic patients is a result of chronic hyperglycemic state that lead to protein oxidation and glycation.[7] A total of 30-80% of sperm damage is due to the

significant increase of reactive oxygen species (ROS).[8] ROS damages the plasma membrane of the sperm which negatively affects the sperm motility and its ability to fertilise the oocyte. Besides, diabetes mellitus was found to induce the loss of sperm motility *via* the mitochondrial DNA damage associated with the activation of mitochondrial ROS generation. Numerous reports have demonstrated a higher sperm DNA fragmentation in diabetic men compared to normal control.[9] In addition, Shrilatha[10] found out that the streptozotocin-induced diabetic rats manifested a significant increase of oxidative stress in the testis. The onset of diabetes mellitus is also known to alter the production of reproductive hormones by interrupting the hypothalamus-pituitary-testicular axis. The expression of insulin has been detected in the hypothalamus and pituitary gland.[11] The absence of insulin signalling in the hypothalamus of knock-out mice inhibits the development of Leydig cells, thus disrupts the biosynthesis of testosterone by the Leydig cells.[12]

Numerous anti-diabetic drugs are available in the market such as metformin and glibenclamide but those drugs do not completely address the reproductive

problems commonly faced by diabetic men. Unlike modern synthetic drugs, herbs provide holistic treatment as it can tackle multiple diseases simultaneously. Herbs play a significant role in the discovery and development of medicinal drugs in which 25% of modern medicines were derived from natural plants.[13]

Few herbs have been documented as highly potential anti-diabetic agents. One of such plant is *Lunasia amara.* It has been documented that *L. amara* extract inihibits the carbohydrate-metabolizing enzyme in the intestine in an *in vitro* study, hence forbidding the glucose uptake into the blood stream.[14] Nevertheless, the most effective anti-hyperglycemic dose of *L. amara* (500 mg/kg) was found to be toxic to the reproductive parameters in diabetic-induced rats as it significantly damages the sperm quality.[15] The diabetic-induced sperm damage may be worsen by the anti-diabetic mechanism of *L. amara.*

Therefore, there is an urgent need to study the potential of medicinal herbs in regulating diabetes mellitus and male infertility comprehensively in order to treat both of the pathological conditions without rising any negative side effects. Throughout the years, traditional herbs have been widely used to treat diabetes and infertility in many communities around the world.[16] One of them is *Moringa oleifera* Lam. which is a native plant in subtropical and tropical countries.[17] *M. oleifera* is also known as the Drumstick tree because of the long slender shape of its fruit. Recent studies have reported that *M. oleifera* fruit extract is capable of significantly lowering the blood glucose level in streptozotocin-induced rats.[18-19] In addition, *M. oleifera* seeds extract has also been discovered to improve sperm quality in non-diabetic experimental animals.[20-21]

Many works reported the pro-fertility effects of *M. oleifera* fruit among normal rats however, reports on the effect of *M. oleifera* fruit on reproductive parameters among diabetic models are scarce. Therefore, this study aimed to investigate the anti-hyperglycaemic effect of *M. oleifera* fruit aqueous extract and its ability to improve reproductive parameters in diabetic-induced male rats.

MATERIALS AND METHODS

Chemicals

All the reagents were procured from Sigma-Aldrich Inc. (USA), unless stated otherwise. Commercial assay kit for testosterone was purchased from Cayman Chemical Company (USA).

Experimental animals

A total of 20 male Sprague Dawley rats (300–500 g) at age between 10–12 weeks were provided by the Animal House, Universiti Kebangsaan Malaysia (UKM), Malaysia. The rats were acclimatized for three days prior to the experiment and were kept at 28°C under 12h light/dark cycle. The rats received standard pellet diet and water *ad libitum*. Animal maintenance was strictly performed according to the guidelines approved by the Animal Ethics Committee of Faculty of Medicine, Universiti Kebangsaan Malaysia, Malaysia (FST/2017/MAHANEM/29-MARCH/833-MARCH-2017-FEB.-2019).

Preparation of *M. oleifera* fruit aqueous extract

Fresh fruits of *M. oleifera* were collected from Tasek Gelugor, Penang, Malaysia. The fruits were authenticated by Mr. Damanhuri, a botanist from the Herbarium of Faculty of Science and Technology, UKM, Malaysia. Voucher specimen (40344) was deposited in the aforementioned Herbarium. The *M. oleifera* fruits were weighed, washed and dried at 50°C for 6 h. A total of 450 g dried powdered fruits were extracted with distilled water at 80°C for three hours using reflux apparatus. The ratio of the dried powdered fruits to the distilled water was 1:10. The extract

was filtered and freeze-dried for five days to yield a total of 68.57 g yellow powdered extract.

Induction of experimental diabetes

The rats were fasted for 16h prior to the diabetic induction. Diabetes was induced by a single intravenous injection of streptozotocin (STZ) at a dose of 50 mg/kg body weight, dissolved in 0.1 M citrate buffer at pH 4.5. Rats with fasting blood glucose (FBG) level elevated above 13 mmol/l were considered diabetic and were used in the experiment.[22]

Experimental design

The animals were randomly divided into four groups with five rats in each group; normal control (without diabetic induction), negative control (diabetes without treatment), positive control (diabetes with metformin) and treatment group (diabetes with 500 mg/kg of *M. oleifera* fruit aqueous extract). Treatments were given for 21 consecutive days *via* oral gavage after five days of STZ injection. At the end of the treatment, FBG level was determined and the rats were euthanised through a single intravenous injection of 130 mg/kg sodium pentobarbital. Finally, sperms were collected and reproductive organs were dissected out, weighed and preserved.

Anti-hyperglycemic study

Blood was drew out from the tail vein of 16-h fasted rats. The fasting blood glucose (FBG) level was determined prior to the treatment and at the end of the treatment using Accu-Chek® Performa glucometer (Roche Diabetes Care GmbH, Manheim, German).

Sperm quality analysis (sperm count, motility, viability and morphology)

Cauda epididymis was excised and suspended in pre-warmed 15 mL Biggers-Whitten-Whittingham (BWW) medium (94.5 mM NaCl, 4.8 mM KCl, 1.7 mM $CaCl_2$, 1.2 mM $KHPO_4$, 1.2 mM $MgPO_4.7H_2O$, 25.1 mM $NaHCO_3$, 5.5 mM glucose, 0.25 mM sodium pyruvate, 21.6 mM sodium lactate, 10 mM penicillin-streptomycin, pH 7.4) supplemented with bovine serum albumin (BSA). The epididymal tissue was carefully minced in the medium to allow the sperms to disperse into the medium. Then, the sperm suspension was incubated in 5% carbon dioxide incubator (37°C, 30 min) to capacitate the sperms. The assessments of sperm count, motility, viability and morphology were carried out according to the World Health Organization (2010) laboratory manual.[23]

Determination of relative testis weight

Rats were sacrificed and the testes were dissected out, washed with 0.9% sodium chloride and weighed. The testes of each rat were measured individually. The average value of both testes obtained from each rat was taken as one observation. The relative testes weight was calculated based on the following equation:[24]

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight (g)}} \times 100$$

Histological evaluation of testis

Testes were fixed overnight in Bouin's solution. Next, the testes were dehydrated in a series of graded alcohol and embedded in paraffin wax. The epididmal tissues were then sectioned at 5 μm, stained with haematoxylin and eosin (H and E) and mounted with DPX. The stained sections were examined qualitatively. The mean diameter of round seminiferous tubules was measured under a light microscope (Digital

Microscope Carl Zeiss, USA) with the help of image analyser (iSolution Lite, IMT i-Solution Inc., USA).[25]

Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics version 22 software (SPSS Inc., USA). Data were analysed by one-way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. Correlations were assessed using Pearson's correlation coefficient. P value < 0.05 was considered statistically significant. Results were expressed as mean ± standard error mean (SEM).

RESULTS

Fasting blood glucose level

The effects of *M. oleifera* fruit extract on FBG level are displayed in Table 1. *M. oleifera* extract at 500 mg/kg significantly ($p < 0.05$) produced an anti-hyperglycemic effect in STZ-induced rats (11.1 ± 0.81 mmol/L) compared with negative control (23.7 ± 0.89 mmol/L). *M. oleifera* fruit extract exhibited the glucose-lowering effect similar to that of metformin where FBG level was reduced by 43.94% (in *M. oleifera*) and 45.90% (in metformin) after the 21-day treatment.

Sperm quality (sperm count, motility, viability and morphology)

The effects of *M. oleifera* fruit extract on reproductive parameters are displayed in Table 1. The negative control group revealed a marked reduction ($p < 0.05$) in sperm counts which is $(2.80 ± 0.58) × 10^6$ than the normal control, $(88.80 ± 5.24) × 10^6$. Whereas, oral administration of *M. oleifera* fruit extract significantly ($p < 0.05$) increased the sperm count and the percentage of viable sperms compared with negative control group. In addition, a strong negative correlation was observed between FBG level and sperm count in *M. oleifera*-treated group ($r = -0.83$; $p < 0.05$). Sperm motility was restored in the *M. oleifera*-treated rats in which the percentage of progressive (PR) sperms increased while the percentage of non-progressive (NP) and immotile (IM) sperms decreased significantly ($p < 0.05$), in contrast to the negative control group. Moreover, a pronounced increase ($p < 0.05$) in the percentage of viable sperms was observed in *M. oleifera*–treated group (69.17 ± 2.34 %) compared

with negative control (6.67 ± 0.91 %) and positive control (11.44 ± 1.44 %). The percentages of sperms with normal morphology were similar in normal control (33.76 ± 1.98 %) and *M. oleifera*–treated group (30.00 ± 2.36 %). These values were significantly ($p < 0.05$) higher than that of the negative control (10.00 ± 0.63 %). Even though metformin significantly ($p < 0.05$) improved the percentage of sperms with normal morphology compared with the negative control group, the effect was not as good as recorded by the *M. oleifera*-treated group. The *M. oleifera*-treated group displayed normal sperm morphology with intact hooked head, midpiece and tail, similar to that of normal control group. On the contrary, the negative control group revealed various abnormal sperm morphologies such as detached head and tail, bent midpiece, short tail and coiled tail (Figure 1).

Relative testes weight

No significant changes were observed in the relative testes weight between all groups after 21 days of treatment (Table 1).

Testicular histology

Histological testis section of normal control group (Figure 2A) shows that the seminiferous tubules are in normal shape with Leydig cells in between them. The tubule is lined with many layers of spermatogenic cells representing different stages of development; spermatogonia, spermatocyte and spermatids, while the lumen is highly packed with spermatozoa. Sertoli cells are seen at interval in between the spermatogenic cells. On the other hand, negative control group (Figure 2B) shows abnormal shape of seminiferous tubules with no interstitial space in between. The stratification of spermatogenic cells seems to be mixed and cannot be distinguished. Furthermore, the lumen of the tubule is devoid of spermatozoa. Meanwhile, the positive control group (Figure 2C) shows the presence of vacuoles in the seminiferous tubules. The tubules were round in shape yet contained depleted spermatogenic cells and a very few spermatozoa in the lumen. The scarcity of spermatozoa in the lumen suggested an impaired spermatogenesis. Interestingly, histological examination of the testicular tissue of *M. oleifera*-treated group (Figure 2D) did not exhibit any microscopic lesion and disorganisation of the tubular elements. The lumen is tremendously packed with spermatozoa and the tubules returned to its round shape with adequate interstitial space in
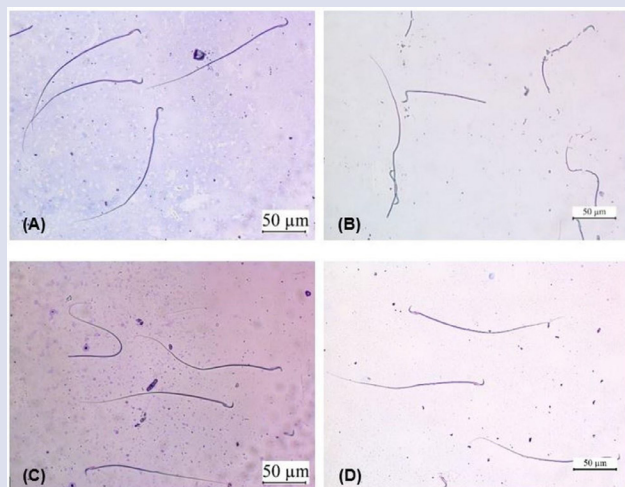
Figure 1: Photomicrograph of sperms of (A) normal control, (B) negative control, (C) positive control and (D) *M. oleifera*-treated group. Giemsa staining. Magnification: 400×. The scale bar shown in the figure represents 50 μm.
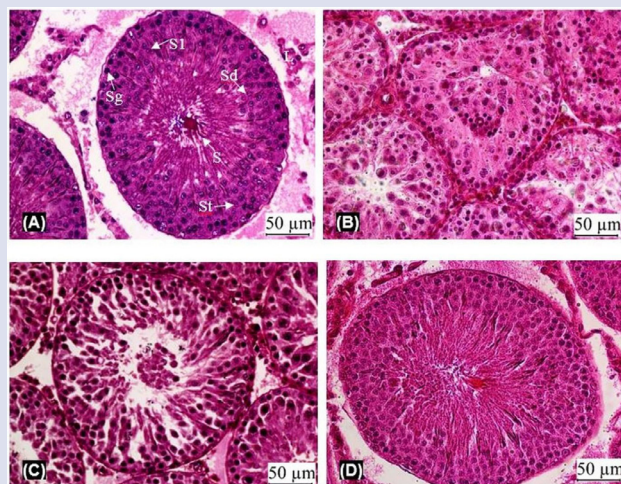
Figure 2: Photomicrograph of histological section of testis in normal control group (A), negative control group (B), positive control group (C) and *M. oleifera*-treated group (D). Spermatogonia (Sg), primary spermatocytes (S1), spermatids (Sd) and spermatozoa (S) in normal control group. Magnification 400×. H&E staining. The scale bar shown in the figure represents 50 μm.

Table 1: Effects of *Moringa oleifera* fruit extract on FBG level, sperm quality, seminiferous tubules diameter and relative testes weight in diabetic-induced male rats.

Parameters	Normal control	Negative control (Diabetes)	Positive control (Diabetes + metformin)	Diabetes + *M. oleifera* treatment
FBG level				
Before treatment (mmol/l)	4.4 ± 0.10*	19.9 ± 0.98	24.4 ± 0.81	19.8 ± 0.62
After treatment (mmol/l)	4.9 ± 0.20*	23.7 ± 0.89	13.2 ± 0.81*	11.1 ± 0.81*
Sperm count (×10^6)	88.80 ± 5.24*	2.80 ± 0.58	14.40 ± 1.86	76.60 ± 3.47*
Sperm motility				
Progressive (%)	40.03 ± 1.90*	6.67 ± 0.18	6.38 ± 0.59	58.44 ± 2.43*
Non-progressive (%)	14.43 ± 1.15*	0.00 ± 0.20	5.06 ± 1.17*	14.24 ± 1.28*
Immotile (%)	25.54 ± 1.48*	53.33 ± 1.83	48.56 ± 2.21	11.81 ± 1.15*
Sperm viability (%)	54.46 ± 4.66*	6.67 ± 0.91	11.44 ± 1.44	69.17 ± 2.34*
Normal sperm morphology (%)	33.76 ± 1.98*	10.00 ± 0.63	21.36 ± 0.99*	30.00 ± 2.36*
Seminiferous tubule diameter (μm)	257.07 ± 1.27*	106.72 ± 0.43	144.34 ± 2.03	233.65 ± 2.95*
Relative testis weight (g/100g)	0.32 ± 0.05	0.29 ± 0.08	0.29 ± 0.10	0.30 ± 0.03

*$p < 0.05$ compared to negative control

between them. *M. oleifera*-treated group shows the histological structure of testis similar to the normal control group in which the presence of different stages of spermatogenic cells and the highly packed spermatozoa in the lumen indicating normal spermatogenesis. The quantitative analysis of the testes revealed a significant ($p < 0.05$) increase in the diameter of the seminiferous tubules of *M. oleifera*-treated group compared to the negative control group (Table 1). Additionally, the seminiferous tubules diameter showed a strong inverse correlation with the FBG level in *M. oleifera*-treated group (r = -0.89; p < 0.05).

DISCUSSION

Diabetes mellitus causes disruption of sperms quality, disturbance in hypothalamus-pituitary-gonadal axis and erectile dysfunction.[26] One of the underlying mechanisms of infertility in diabetic subjects is due to the increase of oxidative stress in light of the fact that it is one of the main contributing factors that leads to various diabetic complications.[9] This hypothesis was further supported by Shrilatha[10] who found out that there was a significant increase of oxidative stress in the testes of diabetic-induced rats after 6 weeks of STZ injection. STZ damages the pancreatic β-cells and consequently inhibits insulin production.[22] Insufficient insulin production prevents the uptake of glucose into the body cells, thus causes a striking rise of blood glucose level.[27]

M. oleifera pods and seeds are known to be rich in antioxidants.[28] The *M. oleifera* fruit aqueous extract displayed a higher antioxidant activity compared to ethanolic extract. Phytochemical screening of *M. oleifera* fruit aqueous extract revealed the presence of alkaloids, glycosides, flavonoids, triterpenes and tannins.[29] Hence, the reason to why aqueous extraction was applied in the present study. This present study showed that the *M. oleifera* fruit aqueous extract is able to significantly reduce the FBG level similar to that of metformin. Related finding has been reported by Gupta *et al.*[19] where *M. oleifera* fruit methanol extract was discovered to possess an anti-hyperglycaemic effect without causing hypoglycaemic in non-diabetic rats. *M. oleifera* fruit inhibits the enzymatic activity of α-amylase and α-glucosidase in the intestine which hinders the breakdown of complex carbohydrate into simple sugar to be absorbed into the blood vessels, thus preventing hyperglycaemic state.[18] The anti-hyperglycaemic effect of *M. oleifera* fruit might also be due to the presence of bioactive compounds such as niazinin, niazirin A, and 4-[(6-deoxy-α-l-mannopyranosyl) oxy]-benzaldehyde.[30] Meanwhile, positive control group demonstrated a decreased FBG level due to the role of metformin

as an established anti-diabetic drug that is capable to inhibit gluconeogenesis which in turn reduce glucose production by the liver.[31]

The results obtained also showed that the administration of *M. oleifera* fruit extract significantly enhanced the reproductive parameters of diabetic-induced male rats in addition to its anti-hyperglycemic effect. The improvement of sperm quality might be due to the antioxidant activity of flavonoids which strengthen the sperm antioxidant defence system, thereby preventing lipid peroxidation reaction on the sperm membrane.[21,32] The improvement in both the histology of testes and the diameter of the seminiferous tubules might also be due to the reduction of oxidative stress in the testes and the pronounced elevation of testosterone which is crucial for the regulation of spermatogenesis. Sharma and Paliwal[33] reported that the presence of antioxidants, especially saponins, in *M. oleifera* fruit extract reduce the oxidative stress in the testis which in turn increase the production of testosterone.

In contrast, administration of metformin into the diabetic-induced rats did not significantly improve the sperm quality and testosterone level compared to negative control. Experimental studies suggested that metformin inhibits the *in vitro* proliferation of Leydig cells, thus reducing the production of testosterone. Meanwhile, the treatment of metformin in the *in vivo* setting was reported to be associated with a significant decrease in the size of the testis and the population of the Sertoli cells that affects the spermatogenesis efficiency.[34]

CONCLUSION

M. oleifera fruit aqueous extract was able to ameliorate the reproductive parameters in diabetic-induced rats. The daily administration of 500 mg/kg *M. oleifera* fruit aqueous extract for 21 days reduced the diabetic-associated reproductive problems which could be due to the antioxidant and androgen-increasing properties of *M. oleifera* fruit extract. The current data provide an evidence on the efficacy of *M. oleifera* fruit aqueous extract as a complementary agent to improve male reproductive parameters specifically in diabetic subjects. However, detailed mechanism on how the reproductive parameters in diabetic-induced rats were improved is yet to be elucidated.

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CONFLICT OF INTEREST
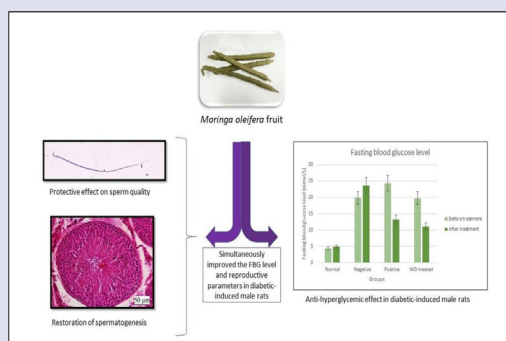
The authors declare no conflict of interest.

ABBREVIATIONS

STZ: Streptozotocin; **FBG:** Fasting blood glucose; **H and E:** Hematoxylin and Eosin; **MO:** *Moringa oleifera.*

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GRAPHICAL ABSTRACT

Moringa oleifera fruit

Protective effect on sperm quality

Simultaneously improved the FBG level and reproductive parameters in diabetic-induced male rats

Fasting blood glucose level

■ Before treatment
■ After treatment

Anti-hyperglycemic effect in diabetic-induced male rats

Restoration of spermatogenesis

SUMMARY

- The induction of experimental diabetes by streptozotocin injection increased the fasting blood glucose level and thus impaired the reproductive function of male rats. To the best of the authors' knowledge, there was no report on the ability of *M. oleifera* fruit extract in recovering the specifically diabetic-derived male reproductive problems while manifesting its anti-hyperglycemic effect. This study provided evidence that the administration of *M. oleifera* fruit aqueous extract at a dose of 500 mg/kg restored both fasting blood glucose level and reproductive parameters simultaneously in diabetic-induced male rats.

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