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Effect Of *Moringa Oleifera* Extract On Some Sperm Parameters And Testis In Diabetic Rats

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Abstract This study aims to demonstrate the effectiveness and ability of the methanolic extract of Moringa oleifera (*M. oleifera*) leaves to improve some sperm parameters and testis in diabetic rats. Twenty healthy adult male Wistar rats divided equally into 4 groups of (n = 5) were used in this investigation. First group represents the negative control, while second group is the positive control, whereas the following two groups represent the treated groups of *M. oleifera* leaves with a concentration of 300mg/kg and 600mg/kg for 4 weeks. At the end of the experiment, the rats were anesthetized and sperm collected from epididymis to calculate their numbers and abnormalities in them by light microscope. Meanwhile, the testis was extracted for histological study. The totals of treatment with the extract in the *M. oleifera* groups showed a significant decrease in the level of fasting sugar (P <.00). The number of sperm was significant increased (P <.01) whereas, significant decrease in the number of abnormal sperm (P <.00). The histological study showed improvement in the process of sperm production. From this study we conclude the ability of the *M. oleifera* leaves extract to improve some sperm parameters and histology of testis in diabetic rats.
Keywords: *Moringa oleifera*, spermatogenesis, sperm count, abnormal sperm.

تأثير مستخلص أوراق المورنجا اوليفيرا *Moringa Oleifera* على بعض خصائص الحيوانات المنوية والخصية على الفئران المصابة بالسكري

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الملخص تهدف هذه الدراسة الى إظهار فاعلية وقدرة المستخلص الميثانولي لاوراق المورنجا اوليفيرا *Moringa Oleifera* في تحسين بعض خصائص الحيوانات المنوية والخصية على الفئران المصابة بالسكري. في هذا البحث تم استخدام 20 فأر ذكر بالغ وسليم من نوع Wistar rats حيث قسمت الفئران الى اربع مجموعات (N=5). المجموعة الأولى تمثل المجموعة الضابطة السليمة، بينما الثانية تمثل المجموعة الضابطة الموجبة اما المجموعتان الاخريان تمثلان المجموعتين اللاتان تم تجريعهما بمستخلص أوراق المورنجا بتركيز 300 مجم/ كجم و 600 مجم/كجم لمدة اربع أسابيع. في نهاية التجربة تم تخدير الفئران وجمع الحيوانات المنوية من البربخ لحساب اعدادها والتشوهات الموجودة بها بواسطة المجهر الضوئي كما تم استخراج الخصية واجراء الدراسة النسيجية عليها. أظهرت النتائج ان الحيوانات المعاملة بمستخلص المورنجا حدث بها انخفاض معنوي في مستوى السكر الصائم (P< .00) و زيادة في اعداد الحيوانات المنوية (P< .01) و انخفاض في عدد الحيوانات المنوية غير الطبيعية بشكل ملحوظ (P< .00) في حين أظهرت الدراسة النسيجية تحسن في عملية انتاج الحيوانات المنوية. من هذه الدراسة نستنتج قدرة المستخلص على تحسين خصائص الحيوانات المنوية والانسجة في الخصية لدى الفئران المصابة بالسكري.

الكلمات المفتاحية: المورنجا اوليفيرا، تكوين الحيوانات المنوية، عدد الحيوانات المنوية، الحيوانات المنوية غير الطبيعية.

1-Introduction

Diabetes mellitus (DM) portrays a metabolic issue of various etiologies described by constant hyperglycemia with the unsettling influences of carbohydrate, fat, and protein digestion coming about because of imperfections in insulin secretion, insulin activity, or both. Diabetes is rapidly rising as a significant general wellbeing challenge and requests extraordinary consideration towards its administration [1][2][3] [4]. Hyperglycemia coming about because of uncontrolled glucose regulation is generally perceived as the causal connection among diabetes and diabetic complications. Brief scenes of hyperglycemia cause tissue harm by mechanisms including continual acute changes in cellular metabolism. Nevertheless, exposure to high glucose also causes cumulative changes in long-lived macromolecules, which continue despite restoration of euglycemia. A lot of information accentuate four key metabolic pathways as being significant supporters of hyperglycemia-actuated cell damage: (1) increased polyol pathway flux; (2) increased advanced

glycation end product (AGE) formation; (3) activation of protein kinase C (PKC) isoforms and (4) increased hexosamine pathway flux[5]. Diabetes has been proved to adverse effect male reproductive function and its effects can be seen in expanded commonness of infertility. About 90% of diabetics experience change in sexual function, including a decrease in libido, impotence and infertility. Moreover a reduction in semen volume, sperm counts, motility, and abnormal sperm morphology [6][7][8][9].

M. oleifera is the most boundless species having a place with the Moringaceae family , which have extra 13 types of trees and bushes initially spread in a several Asian nations, for example, India, Pakistan, Bangladesh, Afghanistan and Sri Lanka[10]. *M. oleifera* leaves have been portrayed to contain an attractive wholesome equalization, containing nutrients, minerals, amino acids, and fatty acids . Also, these leaves are accounted for to contain different types of antioxidant compounds, for example, ascorbic corrosive, flavonoids, phenolics, and carotenoids . As indicated by a several authors, different preparations of *M. oleifera* leaves are utilized for their antiinflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, antineoplastic, antipyretic, antiulcer, cardioprotectant, and hepatoprotectant activities[11].

2-Materials and Methods

Twenty healthy adult male rats weighing 200-300 g were obtained from animal house of Misurata University. All rats were housed in gang cages under standard laboratory conditions (12 h light–dark cycle) and fed a standard commercial laboratory-pelleted diet . Food and water were provided *ad libitum*.

2.1-Extract Preparation

The leaves of *M. oleifera* were collected, identified and authenticated at Department of Botany, Sebha University Herbarium. The collected leaves were air dried under shade for 15 days and pulverized. The pulverized leaves were extracted by maceration with 80 % methanol (1:10 w/v) for 24 h at room temperature in an orbital shaker. The *M. oleifera* methanolic leaves extract solution was centrifuged and evaporated by oven at 40 ^{0}C. The extract was weighed, percent yield calculated, and stored in tight container protected from light at 4 ^{0}C until used.[12][13][14].

2.2- Phytochemical analysis of *M. oleifera* leaf extracts

The percentage extraction yield of *M. oleifera* leaves was determined by Akinyeye *et. al.*[15]. The content of total phenolic compounds was determined using Folin-Ciocalteu assay[12]. Whereas, the total flavonoids content in the extracts was determined by aluminum colorimetric assay with some modifications [16].

2.3- Induction of diabetes

Experimental diabetes was induced in over-night fasted rats by a single intraperitoneal dose of Streptozotocin (STZ) (45 mg/kg)[17] dissolved in acetate buffer (pH 4.5). One week post-STZ injection, the blood glucose level was measured in rats deprived of food and water over- night using reagent strips (On-Call Plus) with a drop of blood obtained by lateral tail-vein puncture. Animals were considered diabetic if blood glucose levels were higher than 200 mg/dl.

2.4- Experimental design

Twenty adult male rats were divided into five groups (n = 5) as follows:

Group I: Healthy rats receiving the equivalent volume of the vehicle (distilled water) daily by oral gavage for 4 weeks (negative control).

Group II: Diabetic rats receiving the equivalent volume of the vehicle (distilled water) daily by oral gavage for 4 weeks (positive control).

Group III: Diabetic rats treated with *M. oleifera* extract (300 mg/kg) by oral gavage for 4 weeks.

Group IV: Diabetic rats treated with *M. oleifera* extract (600 mg/kg) by oral gavage for 4 weeks.

2.5- Semen and histopathological study sampling

At the end of the experimental period, the rats were anesthetized with halothane and sacrificed. Semen was collected the cauda epididymis and was diluted with normal saline 0.9% [18] for measurement of sperm count and abnormality morphology[19].

testis fixed in modified Davidson fluid (MDF) for 48 h then briefly washed in tap water before being transferred to 10% neutral buffered formalin (NBF) for storage prior to trimming and processing for histological investigation[20].

2.6- Statistical analysis

Data obtained from laboratory test were entered, edited and analyzed using the statistical software SPSS® version 20. Results represent as the mean ± standard deviation. One-way analysis of variance (ANOVA) with the least significant difference(LSD) test was conducted to determine the significant differences between groups. A value of $P<0.05$ was considered statistically significant.

3- Results

3.1- Phytochemical analysis of *M. oleifera* leaf extracts

The percentage extraction yield of *M. oleifera* leaves was 24.6% . The contents of total phenol and total flavonoid are shown in(Table 1)

(Table 1): *M. oleifera* content of total phenol and total flavonoid.

Model summary of phenol					
Model	Coeffi-cients	Std Error	Sig	R^2	*M. oleifera* phenol mg/g GAE
Const-ant	0.008	0.003	0.10	0.999	71.33
x	0.009	0.001	0.00		
Model summary of flavonoid					
Model	Coeffi-cients	Std Error	Sig	R^2	*M. oleifera* flavonoid mg/g QE
Const-ant	0.004	0.02	0.04	0.99	90.25
x	0.007	0.001	0.00		

3.2- Effect of *M. oleifera* on Blood Glucose Level

glucose level in diabetic groups treated with *M. oleifera* decreased significantly compared with the positive control group (Table 2) The results showed that the treatment with *M. oleifera* significant decrease in the level of glucose over a period of 4 weeks, (P <.010, P <.004, P <.009, P <. 000)

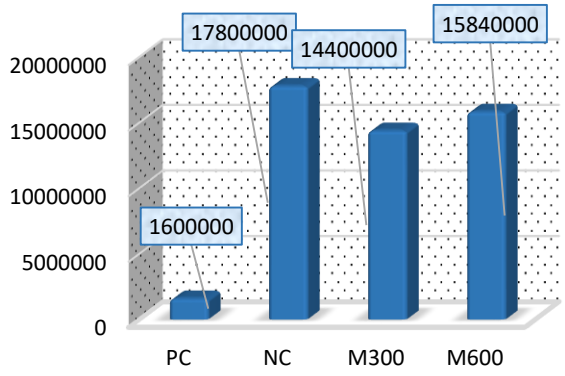
(Table 2): Effect of *M. oleifera* on Blood Glucose Level.

	1st week	2nd week	3rd week	4th week
PC	62±4.91	70±4.06	65.25±5.87	68.75±2.86
NC	535.7±47.2	451.7±25,06	412.50±34.9	424.50±29.7
M300	421.4±89.01	273.6±73.1	194.60± 82.9	84.80±21.7
M600	347.6±66.6	259.6±75.3	207.60±90	130.60±22.3

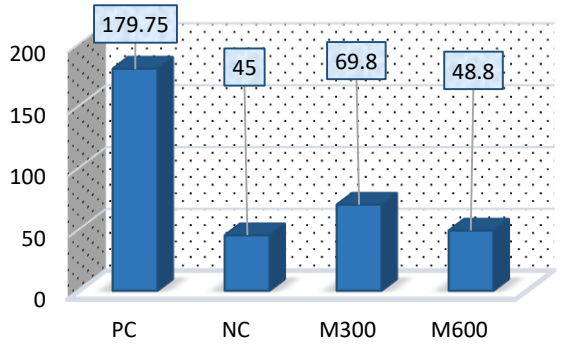
positive control **(PC)**, negative control (**NC**), *M. oleifera* 300mg/kg (**M300)**, *M. oleifera* 600mg/gk. (**M600**).

3.3- Effect of *M. oleifera* on epididymal sperm count and abnormality morphology

Epididymal sperm result are shown in Fig 1 and Fig 2. There was a significant increase in sperm count ($p<0.01$) in diabetic groups treated with *M. oleifera* compared with the positive control group whereas, a significant decrease was observed in sperm abnormality morphology (P <.000) in diabetic groups treated with *M. oleifera* compared with the positive control group.



(Figure 1) Effect of *M. oleifera* on sperm count: positive control **(PC)**, negative controL (**NC**), *M. oleifera* 300mg/kg (**M300)**, *M. oleifera* 600mg/gk (**M600**).



(Figure 2) Effect of *M. oleifera* on abnormality morphology: positive control **(PC)**, negative controL (**NC**), *M. oleifera* 300mg/kg (**M300)**, *M. oleifera* 600mg/gk (**M600**).

3.4- Histopathological study

The histological photomicrographs of testicular sections of normal, diabetic and treated diabetic rats were illustrated in Fig. 3

Histological sections of negative control group (Fig 3, A) testis showed that each seminiferous tubules has a normal spermatogenic cell line formed of a number of spermatogonia, spermatocytes, spermatids and spermatozoa arranged around central lumen and alternating with the sertoli cells. Whereas, testicular sections of positive control (Fig 3, B) group rats show loss of germ cells, abnormality of germinative epithelium, degenerated and atrophic seminiferous tubules, sertoli and Leydig cells, severe destruction of spermatids and spermatozoa, with sparse to missing germ cells.

Treatment with *M. oleifera* revealing a markedly enhancement of testis abnormalities, as illustrated in (Fig 3, C and D). showing sperm with a normal spermatogenic cell line, seminiferous tubules, sertoli and Leydig cells

4- Discussion

Oxidative stress is the main cause of diabetes complications. It mainly originates from high blood glucose levels, where the reactive oxygen species and the free radicals production exceeds the defense capacity of the organism and disrupt the cellular reduction–oxidation balance. Therefore, glycemic control and increased antioxidant defense are necessary to reduce the body damage in diabetic conditions [21]. For many years, *M. oleifera* was used in the traditional treatment of diabetes and infertility[22].

Oral administration of *M. oleifera* for 4 weeks decreased fasting glucose levels compared with positive control. Previous studies have demonstrated that *M. oleifera* reduces hypoglycemia by minimizing gluconeogenesis and supporting the regeneration of pancreatic b cells in rat[23][24].

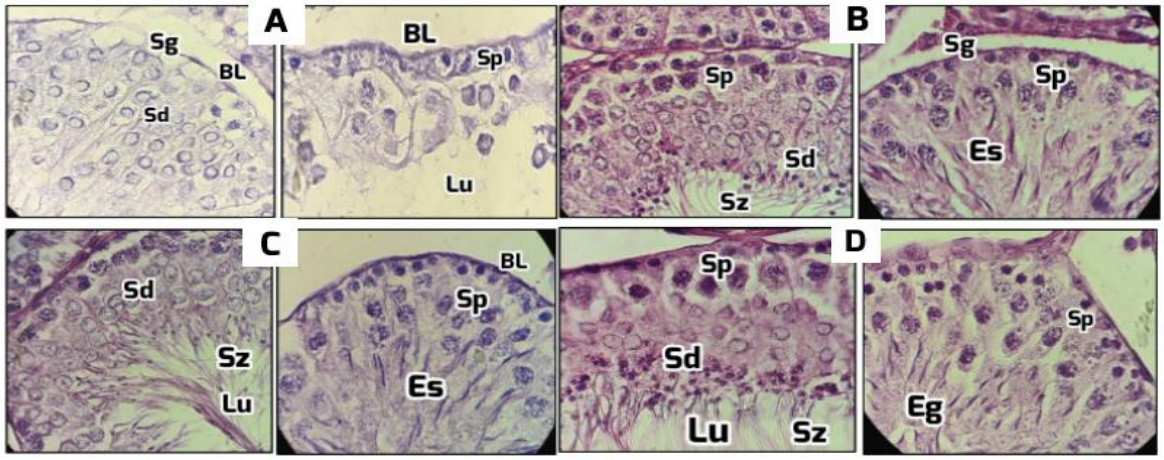
Phenols and flavonoids in *M. oleifera* inhibit the activity of α-glucosidase, pancreatic α-amylase, and intestinal sucrose, contributing to antihyperglycemic properties[25][26]. Chlorogenic acid is a compound found in *M. oleifera* that reportedly enhances insulin activity by triggering the AMP-activated protein kinase (AMPK) pathway[24]. Quercetin can protect insulin-producing pancreatic β cells from STZ- induced oxidative stress and apoptosis in rats[27] and an apical inhibitor of GLUT2 [28]. It has also been shown to activate adenosine monophosphate-activated protein kinase (AMPK), to increase glucose uptake through stimulation of GLUT4 in skeletal muscle, and to decrease the production of glucose through downregulation of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase in liver[29]. So, the enhancement in the fasting blood sugar illustrated in the present investigation might be

attributed to the presence of such phytochemicals in *M. oleifera*.

The impairment of gonadal antioxidant potential of the male reproduction system causes testicular disturbance and failure in sperm production. Furthermore, disorders in the endocrine control of spermatogenesis have also been observed in diabetic patients, with reduced levels of essential hormones, such as FSH, LH and testosterone [21]. In the present experiment, disturbances in spermatogenesis, sperm count and sperm abnormal morphology in rats have been improved with *M. oleifera* application which modulates oxidative stress damage. Moreover, the preliminary phytochemical examination of the leaves extract of *M. oleifera* revealed the existence of alkaloids, phenols, , and flavonoids. It was reported that phytochemical existing in *M. oleifera* leaves reign fertility potentiating properties, and may increase the level of testosterone in the body[30], as noticed in this study (result not show) . Moreover, alkaloids and flavonoids alter the androgen levels[31]. As a result the improvement in the sexual function illustrated in the present investigation might be attributed to the presence of such phytochemicals in *M. oleifera*.

5- CONCLUSION

The present results suggest that the *M. oleifera* leaf extracts administration improve sperm parameters and histology of testis in diabetic adult male rat which lead to improvement in the process of sperm production. It also support to the usage of



(Fig. 3) Histological photomicrographs of testicular sections (x100): negative control **(A)**, positive control **(B)**, *M. oleifera* 300mg/kg **(C)**, *M. oleifera* 600mg/gk. **(D)**. spermatogonia (Sg), spermatocytes (Sp), spermatids (Sd), elongated spermatids (Es), basal lamina (BL), lumen (Lu), spermatozoa (Sz). All tissues are fixed in MDF-NBF 10% fixative and haematoxylin–eosin stained.

M. oleifera as a sexual function enhancing medicine. Thus, this study may prove to be an effective and safe alternative remedy in reproductive disorders. However, more studies are needed concerning the possible underlying mechanisms of action of *M. oleifera* leaf extracts on spermatogenesis.

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