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Assessment of ameliorative effects of *Moringa oleifera* Lam. on epididymal dysfunctions and fertility in streptozotocin–induced diabetic ratsRam Niwas Jangir[✉], Gyan Chand Jain

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ABSTRACT

Objective: To assess the protective effects of 70% ethanolic extract of *Moringa (M.) oleifera* leaves on fertility and epididymal dysfunctions in diabetic rats.

Methods: Thirty-six male Wistar rats were divided into six groups, each with six rats. Group I was the normal control group receiving vehicle (0.5 mL of distilled water/rat/day) orally; group II, III, IV, V and VI were injected intraperitoneally with 60 mg/kg of streptozotocin once to induce type I diabetes. Then, the diabetic rats in group II receiving vehicle (0.5 mL of distilled water /rat/day) orally; the diabetic rats in groups III, IV and V were orally treated with *M. oleifera* leaf extract at dose of 100, 250, and 500 mg/kg body weight (b.wt.)/day, respectively; the diabetic rats in group VI were administered with reference drug glibenclamide (5 mg/kg b.wt./day). The treatment lasted for 60 days. Sperm parameters (sperm count, motility and viability), fertility index, litter size, lipid peroxidation and antioxidative defense markers (*i.e.* superoxide dismutase, catalase, glutathione, and ascorbic acid) in the epididymal tissue were determined. Histopathological changes in epididymis were also evaluated.

Results: Administration of different doses of *M. oleifera* leaf extract in diabetic rats induced a remarkable dose dependent improvement in sperm parameters, fertility index, litter size, epididymal antioxidant status and also restored histopathological changes as compared to the diabetic control group. These findings were comparable with reference drug.

Conclusions: *M. oleifera* leaf extract possesses significant antioxidant activities as well as beneficial effects on epididymal dysfunctions in diabetic male rats.

KEYWORDS: Antioxidants; Epididymis; Fertility; *Moringa oleifera*; Sperm parameters; Streptozotocin

1. Introduction

Diabetes mellitus is a metabolic disease characterized by persistent

high blood glucose level. Currently, the global prevalence of diabetes mellitus is increasing rapidly and thus it is posing as an eminent threat to the public health in our society. As per the estimation by International Diabetes Federation, 536.6 million adults were suffering from diabetes globally in 2021 and the number will reach approximately 783.2 million by 2045[1]. Chronic hyperglycemia, if remains untreated, often leads to permanent impairment, dysfunction or even failure of multiple organs in the body[2]. A growing body of evidences have indicated that both type 1 and type 2 diabetes mellitus have significant correlation with damage to both male and female reproductive system[3,4].

Diabetes affects male reproductive system at multiple target sites, resulting in increasing incidence of erectile and ejaculatory

Significance

Previous studies have shown that diabetes leads to hormonal dysregulation and oxidative stress, resulting in histopathological changes in the epididymis, reduced sperm count and quality, and impaired fertility. This study revealed that administration of 70% ethanolic extract of *Moringa oleifera* leaves significantly improved histopathological conditions in epididymal tissue, enhanced oxidative defense markers, and positively affected sperm parameters in diabetic rats. Additionally, it increased both fertility index and litter size. These findings indicate that *Moringa oleifera* alleviates diabetes-induced male reproductive dysfunctions.

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dysfunctions, loss of libido, impairment of spermatogenesis, hypogonadism and infertility in men[5,6]. Numerous studies have also demonstrated an impairment of sperm count and quality, as well as marked reduction in fecundity/fertility. Studies conducted on streptozotocin (STZ)-treated diabetic rats also indicated that diabetes induced regression and atrophic changes in the epididymis and reduced in epididymal weight. Insulin replacement to these diabetic rats showed prevention of some deleterious effects, but on certain regions of epididymis only[4–7].

Reactive oxygen species (ROS) are generated by persistent hyperglycemia and the production of free radicals exceeds the defense capacity of the organism[8]. The structure and function of antioxidant enzymes are modified by glycosylation in such a manner that those are unable to catalyze the detoxification of free radicals, worsening oxidative stress in diabetic condition. Enhanced free radicals generation and oxidative stress promote lipid peroxidation resulting in cell and tissue damage in diabetic[9,10]. It is also reported that decline in antioxidant defense plays a significant role in development of male infertility[11].

Successful treatment of diabetes is a globally health issue. Currently, available treatment of diabetes including insulin and antidiabetic drugs are associated with adverse effects[12]. Herbs and medicinal plants are major sources of bioactive phytoconstituents including antioxidants which can exert synergistic or additive effects to modulate various biological pathways that lead to prevention or treatment of diabetes and associated complications, thereby providing multifaceted benefits[13]. Furthermore, many medicinal plants or bioactive phytochemicals have been reported to restore male reproductive functions in experimental diabetic animals by alleviating oxidative stress and/or improving glycemic index[14,15].

Moringa (M.) oleifera Lam. (family-Moringaceae) is a drought tolerant, rapidly growing, indigenous tree which is also known as Horse radish tree or Durmastick tree. *M. oleifera* possesses nutritional and medicinal properties and valuable food source for human and livestock. Almost every part of *M. oleifera* (bark, fruit, flowers, gum, leaf, root and seed) has been used to treat different diseases in the folklore medicine. *M. oleifera* leaves are reported to possess diverse pharmacological properties including analgesic, anticancer, anti-diabetic, anti-hyperlipidemic, anti-inflammatory, antioxidant, antimicrobial, antiulcer, cardioprotective, hepatoprotective, etc. *M. oleifera* leaves are rich source of vitamins (A, B, C, E, etc.), minerals (iron, calcium, copper, magnesium, phosphorus, selenium, zinc, etc.), unsaturated fatty acids and all essential amino acids. Moreover, its leaves contain several phytoconstituents including phytosterols (β -sitosterol), glycoside compounds (niazimicin, niazinin), flavonoids (isoquercetin, kaempferol), phenolics (chlorogenic acid), alkaloids, saponins and caffeoyquinic acid, etc[16,17].

Several studies have reported that leaf extract of *M. oleifera* possesses significant antidiabetic and/or antioxidant activity in STZ-induced diabetic rats[18,19]. Moreover, the aphrodisiac effect/enhance reproductive performance of *M. oleifera* leaves has also been reported

in normal rats[20], mice[21], rabbits[22] and teressa goat[23] and also ameliorate reproductive dysfunctions in stressed[24] high fructose fed[25], radiation induced[26], peroxitine induced[27], and cadmium chloride-induced[28] rats.

To our best knowledge, influence of *M. oleifera* leaf extract on fertility and epididymal dysfunctions in streptozotocin-induced diabetic rats has been reported very scanty. In this study, we investigated the influence of 70% ethanolic extract of the leaves of *M. oleifera*, on epididymal dysfunctions, fertility index and litter size in male rats with STZ-induced diabetes.

2. Materials and methods

2.1. Preparation of plant extract

M. oleifera Lam. leaves were collected in fresh condition from the adjacent areas of University of Rajasthan, Jaipur and taxonomically verified by Prof. K. P. Sharma, Department of Botany, University of Rajasthan, Jaipur (Herbarium No. RUBL21056). The leaves were washed with distilled water and dried in shade. After drying, *M. oleifera* leaves were crushed and ground into coarse powder. 200 g of powdered leaves was soaked in 70% ethanol and kept at room temperature for 24 h. The extraction was performed by subjecting the mixture to Soxhlet apparatus at 60 °C–70 °C temperature. The liquid that was extracted was subsequently filtered using a filter paper and the collected filtrate was evaporated at 40 °C in an oven to obtain residue. The residue collected from the leaves of *M. oleifera* was brown coloured, crystalline in form, and yield was found to be approximately weighing 30.5 g (15% of dried powder). The extract residue was suspended in distilled water before orally administration to experimental rats.

2.2. Animals

In the present study, total 36 healthy adult male Wistar rats (*Rattus norvegicus*) with approximate body weight of 170–210 g and age 8–10 weeks were used. The animals were kept in groups in plastic cages in a standard laboratory environment with a temperature of (25±3) °C and a 12 h–12 h light-dark cycle inside the departmental animal house (1678/Go/ReBi/S/2012/CCSEA). They were provided with water and a nutritionally adequate pellet diet (Aashirwad Food Industries, Chandigarh, India) *ad libitum*.

2.3. Reagents and chemicals

Glibenclamide tablets (Daonil; Aventis Pharma. Ltd., India) were purchased from local medical store. STZ was procured from Himedia Laboratory Limited, Mumbai, India. All other reagents and chemicals used were of analytical grade.

2.4. Experimental induction of diabetes

STZ (60 mg/kg b.wt.) dissolved in citrate buffer (pH 4.5) was intraperitoneally injected into male rats overnight fasted to induce diabetes (type-1). One week after STZ injection, diabetes induction was confirmed by measuring glucose level in the blood collected from the tail vein of fasted rats using one touch glucometer (Johnson and Johnson, USA). The rats with above 250 mg/dL blood glucose level were considered to be diabetic and selected for the current study.

2.5. Experimental design

The control and diabetic rats were distributed into six separate groups, each with six animals and were treated as follows-Group I : Normal control rats receiving vehicle (0.5 mL of distilled water/rat/day) orally for 60 days. Group II : Diabetic rats receiving vehicle (0.5 mL of distilled water /rat/day) orally for 60 days. Group III, IV and V : Diabetic rats receiving *M. oleifera* extract with a dose of 100, 250 and 500 mg/kg bwt./day respectively orally for 60 days. Group VI : Diabetic rats receiving reference drug glibenclamide (5 mg/kg bwt./day) orally for 60 days. Based on the LD₅₀ of *M. oleifera* leaf extract and findings from previous antidiabetic studies, different doses were selected for this study[29–31].

2.6. Autopsy schedule

After 24 h of the last treatment, all the overnight fasted rats of different groups were weighed and scarified under mild ether anesthesia. The epididymis of each animal was dissected out, cleaned off from adherent fat and washed with normal saline and weighed on a digital electronic balance. A part of epididymis was cut for biochemical estimations analysis and rest parts of the epididymis tissues were fixed in Bouin's fixative for histopathological studies.

2.7. Sperm parameters

2.7.1. Sperm motility and density

The sperm motility and density were determined by mincing the cauda epididymis (100 mg) in 2.0 mL normal saline (0.9% NaCl, 37 °C) and the suspension was filtered using a nylon mesh to separate the tissue from the sperm. The numbers of spermatozoa were counted by Neubauer's counting chamber of hemocytometer under an Olympus light microscope (×100). The sperm count was expressed as million/mL of suspension. Sperm motility was measured by counting both motile and immotile spermatozoa under the light microscope at 100× magnification. Sperm motility was expressed as percentage (%) [32].

2.7.2. Sperm viability

To assess sperm viability, one drop of 10% nigrosin and 1%

Eosin-Y aqueous solution was deposited in a microcentrifuge tube. A drop of sperm sample was added to it and mixed properly. The mixture was placed on a glass slide to observe under the Olympus light microscope (×400). The red stained (dead) and without stained (alive) sperm were estimated by counting at least 200 cells [33].

2.8. Fertility and litters size

To assess the reproductive performance of the experimental and control rats, mating test was conducted during the last 5 days of the treatment period. Male rats were cohabited overnight with normal cycling (untreated) proestrous or estrous females in 1:2 ratios to facilitate mating. The presence of copulatory plug and sperms in the vaginal smears, in the next morning, were regarded as confirmation of mating and this was considered to be the first day of pregnancy. The mated females (sperm positive) were separated until parturition. The number of live pups (litters) delivered on the day of parturition were recorded. The fertility was calculated as the total number of females with sperm positivity divided by total number of females multiplied by 100.

2.9. Epithelial cell height

The mean height of 100 cauda epithelial cells was measured from 5 sections of each animal using ocular micrometer at ×400 and mean values were calculated.

2.10. Lipid peroxidation and antioxidant defense system markers

Fresh/frozen epididymis tissue samples of rats of different experimental groups were used for estimation of following parameters: Lipid peroxidation (TBARS) [34], superoxide dismutase (SOD) [35], reduced glutathione (GSH) [36], ascorbic acid [37] and catalase [38].

2.11. Histopathological study

To perform histopathological studies, Bouin's fixed epididymis tissues were washed in distilled water, dehydrated in a graded ethanol series and cleared in xylene. Subsequently, the tissues were embedded in paraffin. 5 µm thick tissue sections were cut using microtome, sections were mounted on glass slides and counter stained with eosin and hematoxylin. The sections were observed under light microscope to detect histopathological effects. Photomicrographs of the histopathological slides were captured by a Nikon digital camera mounted on a light microscope.

2.12. Statistical analysis

SPSS 20.0 (SPSS INC., Chicago, IL, USA) was used to analyze all statistical data. All the data were analyzed with one-way

ANOVA variance followed by Tukey's as a *post hoc* test. The data of experiments were presented as mean±standard deviation (mean±SD). Differences of means were considered significant at $P<0.05$.

2.13. Ethics statement

The animals were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the experimental protocol was approved by the departmental Animal Ethical Committee.

3. Results

3.1. Sperm parameters

Changes in sperm parameters (count, motility and viability) in different experimental groups and normal controls were presented in Figure 1. A highly significant ($P<0.001$) decline of the sperm count, sperm motility and sperm viability was observed in cauda epididymis of STZ-induced diabetic rats when compared with normal control rats. Treatment of diabetic rats with three different doses (100, 250 and 500 mg/kg b.wt.) of *M. oleifera* extract exhibited a significant ($P<0.05$, $P<0.01$ and $P<0.001$, respectively) dose dependent increase in both sperm count and sperm motility as compared with diabetic control rats. The number of viable sperms was also improved significantly ($P<0.01$ and $P<0.001$) in the diabetic rats treated *M. oleifera* extract (250 and 500 mg/kg b.wt., respectively) as compared with diabetic control rats. However, in the low dose group of *M. oleifera* extract (100 mg/kg b.wt.), no significant increase in the percentage of viable sperm was observed. Diabetic rats treated with glibenclamide also exhibited significant ($P<0.001$) elevation in the sperm count, sperm motility and sperm viability as compared to diabetic control rats.

3.2. Fertility index

The STZ-treated diabetic control rats recorded a markedly reduced fertility (41.66%) as compared to normal control rats (91.7%).

Administration of different doses of *M. oleifera* extract (100, 250 and 500 mg/kg b.wt.) in diabetic rats induced a remarkable dose dependent improvement in the fertility (58.3%, 75.0%, 83.3% respectively). Diabetic rats treated with glibenclamide also revealed an improvement in fertility index as compared to diabetic control rats (83.3% vs. 41.7% respectively) (Figure 2).

3.3. Litter size

In comparison to normal rats, a significant decline in the mean litter size was observed in STZ-induced diabetic rats. However, when the diabetic rats were treated with 250 and 500 mg/kg b.wt. doses of *M. oleifera* leaf extract, a significant ($P<0.01$ and $P<0.001$, respectively) improvement in the mean litter size was observed as compared with diabetic control rats. While, in low dose (100 mg/kg b.wt.) group, a nonsignificant increase in the mean litter size was recorded. Diabetic rats treated with glibenclamide also exhibited a highly significant ($P<0.001$) increase the mean litter size as compared to diabetic control rats (Figure 3).

3.4. Cauda epithelial cell height

Changes in cauda epithelial cell height in normal control and different experimental groups are depicted in Figure 4. In comparison to normal control rats, the STZ-induced diabetic rats denoted a highly significant ($P<0.001$) reduction in the cauda epithelial cell height as compared to normal control rats. When diabetic rats treated with *M. oleifera* extract treatment at 250 and 500 mg/kg b.wt. doses demonstrated a significant ($P<0.01$ and $P<0.001$ respectively) elevation in the epithelial cell height as compared with diabetic control rats. While treatment of the diabetic rats with low dose (100 mg/kg b.wt.) of the extract showed non-significant increase. Treatment of diabetic rats with glibenclamide also showed a significant ($P<0.001$) increase in epithelial cell height as compared with the diabetic control rats.

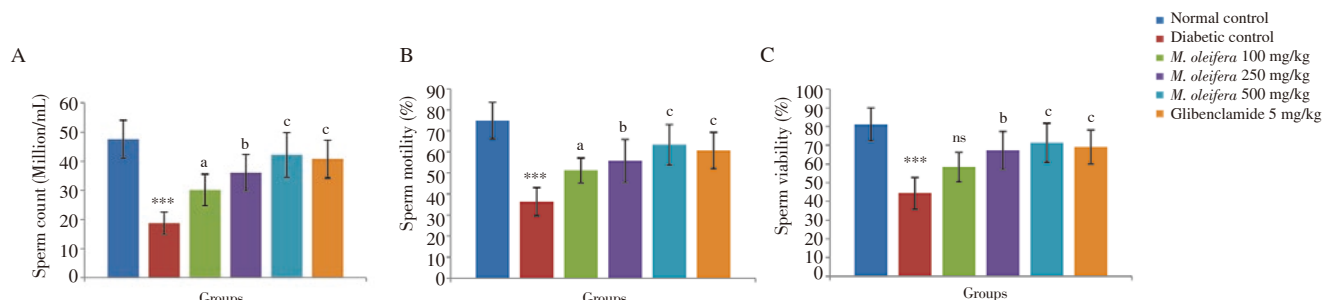


Figure 1. Effects of *Moringa (M.) oleifera* leaf extract on sperm parameters (A: sperm count, B: sperm motility, C: sperm viability) in streptozotocin (STZ)-induced diabetic rats. Values are presented as mean±SD and all the data are statistically analyzed with one-way ANOVA. *** $P<0.001$: the diabetic control rats compared with the normal control rats; ns: non significant, ^a $P<0.05$; ^b $P<0.01$; ^c $P<0.001$: *M. oleifera* extract or glibenclamide treated rats compared with the diabetic control rats.

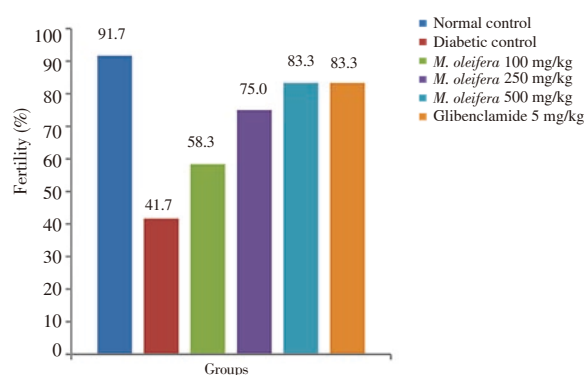


Figure 2. Effects of *M. oleifera* on fertility in STZ induced-diabetic rats.

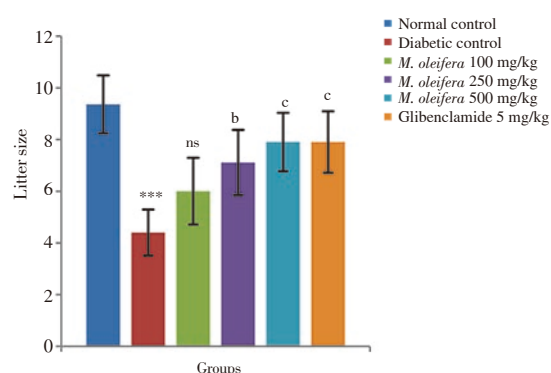


Figure 3. Effects of *M. oleifera* on litter size in STZ-induced diabetic rats. Values are presented as mean±SD and all the data are statistically analyzed with one-way ANOVA. *** $P<0.001$: the diabetic control rats compared with the normal control rats; ns: non significant, ^b $P<0.01$, ^c $P<0.001$: *M. oleifera* extract or glibenclamide treated rats compared with the diabetic control rats.

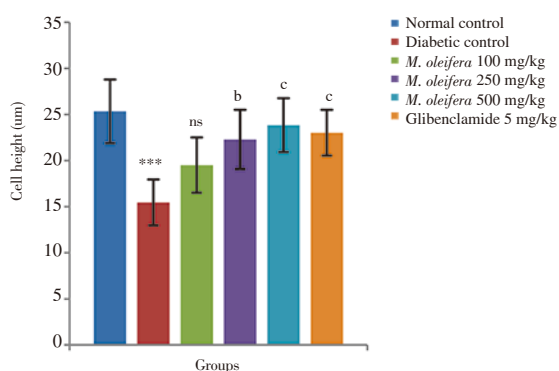


Figure 4. Effects of *M. oleifera* on epididymal cell height in STZ-induced diabetic rats. Values are presented as mean±SD and all the data statistically analyzed with one-way ANOVA. *** $P<0.001$: the diabetic control rats compared with the normal control rats. ns: non significant, ^b $P<0.01$, ^c $P<0.001$: *M. oleifera* extract or glibenclamide treated rats compared with the diabetic control rats.

3.5. Lipid peroxidation and antioxidant defense markers

Changes observed in the levels of lipid peroxidation (TBARS) and

antioxidant defense markers in epididymis of normal control and different experimental groups are shown in Table 1. STZ-induced diabetic rats exhibited a significant ($P<0.001$) elevation in TBARS concentration concomitant with significant decline ($P<0.001$) in the activities of catalase and SOD as well as concentration of ascorbic acid and GSH in epididymal tissue as compared with normal control rats.

Treatment of diabetic rats with a low dose (100 mg/kg b.wt.) of *M. oleifera* extract induced a slightly significant ($P<0.05$) decline of the TBARS concentration concomitant with significant ($P<0.05$) elevation in the activity of catalase and levels of GSH and ascorbic acid in epididymal tissue as compared with diabetic control rats. However, the rise of SOD activity was non-significant. Treatment of diabetic rats with medium dose (250 mg/kg b.wt.) of extract caused a markedly significant ($P<0.001$) decline in the TBARS level with concomitant significant ($P<0.01$) increase of catalase and SOD activities as well as concentration of GSH and highly significant ($P<0.001$) rise of ascorbic acid levels in the epididymis as compared with diabetic control rats. Diabetic rats treated with high dose of extract (500 mg/kg b.wt.) or glibenclamide caused a significant ($P<0.001$) decline in the TBARS level with concomitant significant increase ($P<0.001$) in the activities of catalase and SOD as well as concentration of ascorbic acid and GSH in the epididymis as compared with diabetic control rats.

3.6. Histology

Histopathological studies of epididymis of normal control rats revealed large tubules with wide lumen. Tubules were lined with ciliated pseudostratified columnar epithelium and distinct by fibromuscular layer. The tubular lumen was full of spermatozoa (Figure 5A). Histopathological studies of epididymis of diabetic control rat showed a considerable reduction in the size of tubules and epithelium cell height and increase in intertubular stroma. The epithelial lining of tubules in diabetic rats showed degenerative changes and loss of stereocilia. Most of tubules were devoid of spermatozoa (Figure 5B). Diabetic rats treated with *M. oleifera* extract (100 mg/kg b.wt.) showed slightly increased tubular size and epithelial cell height, slight decrease in intertubular connective tissue, presence of degenerating sperm in lumen of the tubules was also observed (Figure 5C). Administration of medium dose (250 mg/kg b.wt.) of *M. oleifera* extract in diabetic rats exhibited moderate prevention of degenerative changes and improved tubule size, epithelial cell height. An adequate number of spermatozoa were also present in the lumen of tubules (Figure 5D). The cauda epididymis of diabetic rats receiving high dose (500 mg/kg b.wt.) of *M. oleifera* extract exhibited near normal histoarchitecture as evidenced by large tubule size, increase height of epithelial cell lining, presence of long stereocilia, decrease of intertubular stroma and good quantity of spermatozoa in the lumen of tubules (Figure 5E). Treatment of

Table 1. Effect of *M. oleifera* leaf extract on lipid peroxidation and antioxidant parameters in epididymis of streptozotocin-induced diabetic rats.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Lipid peroxidation (TBARS) (n mol MDA mg-tissue)	1.75±0.36	5.71±0.79 ^{***}	4.50±0.58 ^a	3.59±0.68 ^c	2.41±0.44 ^c	2.55±0.53 ^c
SOD	8.28±1.01	3.76±0.57 ^{***}	5.04±0.62 ^{ns}	5.78±1.03 ^b	7.37±0.48 ^c	7.15±0.87 ^c
Catalase	37.22±4.72	17.52±3.80 ^{***}	28.82±4.86 ^a	31.47±7.08 ^b	34.93±6.07 ^c	32.92±5.17 ^c
GSH	3.40±0.38	1.33±0.25 ^{***}	2.10±0.28 ^a	2.45±0.58 ^b	3.12±0.48 ^c	3.01±0.55 ^c
Ascorbic acid	1.60±0.27	0.71±0.11 ^{***}	1.15±0.14 ^a	1.28±0.27 ^c	1.45±0.33 ^c	1.40±0.25 ^c

Values are presented as mean±SD and all the data are statistically analyzed with one-way ANOVA. ^{***} $P<0.001$: the diabetic control rats compared with the normal control rats. ns: non significant, ^a $P<0.05$, ^b $P<0.01$, ^c $P<0.001$: *M. oleifera* extract or glibenclamide treated rats compared with the diabetic control rats. SOD: superoxide dismutase, GSH: reduced glutathione, TBARS: thiobarbituric acid reactive substance, MDA: malondialdehyde. Group I is the normal control group receiving vehicle (0.5 mL of distilled water/rat/day) orally; group II, III, IV, V and VI are injected intraperitoneally with 60 mg/kg of streptozotocin once to induce type I diabetes, and then respectively receive vehicle (0.5 mL of distilled water /rat/day), *M. oleifera* leaf extract at dose of 100, 250, and 500 mg/kg body weight (b.wt.)/day, and reference drug glibenclamide (5 mg/kg b.wt./day) for 60 days.

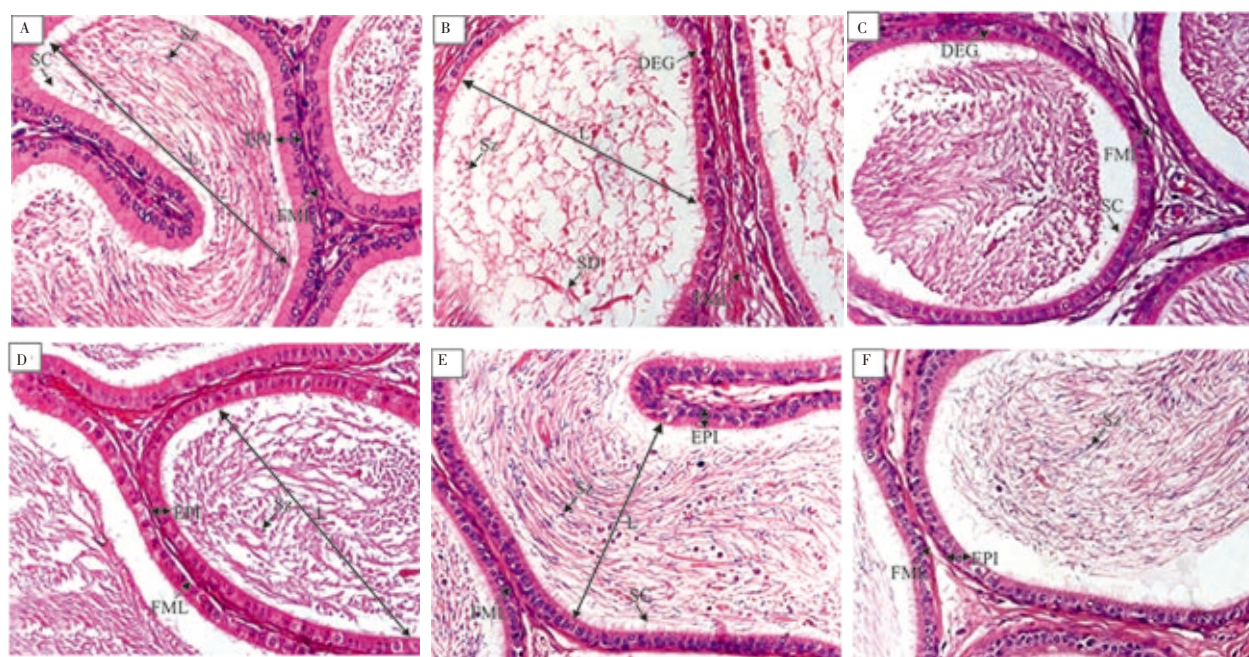


Figure 5. Photomicrograph of cauda epididymis of (A) normal control rats showing normal histoarchitecture including large tubules with wide lumen (L) having spermatozoa, tubules lined with ciliated (SC) pseudostratified columnar epithelium (EPI) and distinct by fibromuscular layer (FML). (B) Diabetic control rats showing shrinkage of tubules, thick fibromuscular layer (FML), sperm debris (SD) in the tubular lumen, loss of spermatozoa as well as degenerative and atrophic changes (DEG). (C) *M. oleifera* extract (100 mg/kg b.wt.) treated diabetic rat showing slight decrease in fibromuscular layer (FML), and mild prevention of degenerative and atrophic changes (DEG). (D) *M. oleifera* extract (250 mg/kg b.wt.) treated diabetic rat showing moderate improvement of histoarchitecture including increased lumen of tubule (L) and epithelial cell height (EPI). (E) *M. oleifera* extract (500 mg/kg b.wt.) treated diabetic rat depicting near normal appearance of histoarchitecture as evidenced by large lumen of tubule (L), increased epithelial cell height (EPI), presence of long stereocilia (SC) and good quantity of spermatozoa (Sz). (F) Glibenclamide treated diabetic rat showing marked restoration of histomorphology depicted by increased epithelial cell height (EPI) and good quantity of spermatozoa (Sz) (H & E staining; magnification: ×200).

diabetic rats with glibenclamide also showed significant recovery of the degenerative effects as evidenced by significant improvement of epithelial cell height and numbers of spermatozoa in the lumen and reduction in intertubular stroma (Figure 5F).

4. Discussion

In the present investigations, a highly significant decrease in

cauda epididymal sperm count, motility and viability of diabetic rats was recorded when compared with normal control rats. These findings are consistent with earlier report where similar decrease in sperm count, motility, and viability was recorded in STZ-induced diabetic rats/mice [39,40] and also in diabetic subject [41]. The observed alteration in sperm parameters may be correlated with insulin deficiency, low testosterone levels and increased with oxidative stress and lipid peroxidation.

There is inverse relationship between the lipid peroxidation and

motility of spermatozoa[42]. Plasma membrane of spermatozoa have a higher amount of polyunsaturated fatty acids (PUFA) and their cytoplasm is comprised of low concentration of antioxidants rendering them more susceptible to ROS-induced damages[43,44]. Increased lipid peroxidation in male reproductive tract is correlated with deleterious changes in spermatozoa morphology, motility, concentration and membrane integrity and ultimately causes infertility[45,46]. Increased formation of advanced glycation end products (AGEs) during diabetic state in man has been suggested to be correlated with poor sperm qualities[47,48].

The result of present study showed that sperm count, viability and motility in diabetic rats, significantly improved after treatment with leaves extract from *M. oleifera* in a dose dependent manner. The observed ameliorative effect of these extracts of sperm parameters might be due to presence of large number of bioactive phytoconstituents (minerals, vitamins and polyphenols and flavonoids) which have improved glycemic index, antioxidant status and restored the levels of sex hormones. These results are consistent with earlier studies where similar amelioration of blood glucose level and improvement of male reproductive functions was observed in diabetic rats[49], cryptorchid rats[50], normal rats[51], hydroxyurea intoxicated rats[52], radiation induced rats[26], peroxitine induced rats[27] and cadmium chloride induced rats[28] after treatment with *M. oleifera* leaf/seed extract.

The prevention of reproductive dysfunction observed in glibenclamide treated diabetic rats in present study also may be supported by the finding of other researchers[53,54]. Among the diabetic control rats, a significant decline in mean litter size and fertility (%) was observed in our current study. Similar to our findings, many studies have also reported a significant reduction of fertility/fecundity in experimental diabetic animals[55,56]. This decline of fertility in diabetic rats might be due to defect of sperm maturation, diminished sperm count, motility and increased spermatozoa abnormalities[57]. Altered sex behaviour or loss of libido could also be an important cause of diminished fertility[56,58].

In present study, the observed decline of mean litter size in diabetic control rats in comparison with the control rats could be due to pre-implantation or post-implantation losses. It is well known that diabetes causes excessive generation of free radicals and increased lipid peroxidation which cause sperm nuclear and mitochondrial DNA damage[59]. Furthermore, low fertilization rates, defective pre-implantation embryonic development and increased rates of miscarriage are associated with DNA damage in the male germ cells[60].

The fertility rate and mean litter size of the diabetic rats improved significantly when treated with *M. oleifera* extract/glibenclamide. This might be due to improved testosterone level, sperm parameters (sperm count, viability and motility), restoration of antioxidant status and glycemic control. These results are compatible with other previous studies; similar improvement of fertility rate and litter size have been recorded in diabetic rats treated with extract of

antidiabetic plants[55,61].

The results of the study also showed a very significant increase in TBARS level along with a significant reduction in antioxidant parameters (ascorbic acid, catalase, GSH and SOD) in epididymal tissues. The results of the present study are in accordance with numerous previous reports that showed similar decline of antioxidant defense markers along with increase of TBARS level in epididymis of diabetic animals[53,56,62]. The high level of lipid peroxidation marker TBARS in the diabetic control rats is a reflection of insufficiently of antioxidant defense in combating ROS mediated oxidative stress. Furthermore, the epididymis has low antioxidant profile[43]. Oxidative stress has been shown to play a central role in the pathophysiology of diabetes and its reproductive complications leading to infertility[42,44].

Treatment of diabetic rats with *M. oleifera* extract/glibenclamide, significantly increased the activities of antioxidant enzymes (catalase and SOD) and the levels of nonenzymatic antioxidants (ascorbic acid and GSH) concomitant with decline of TBARS level in epididymis. The antioxidant phytochemicals that are present in the extract of the plant might be responsible for the observed improvement in those parameters[4,63].

The epididymis is a highly specialized organ that plays a vital role in sperm maturation, transporatation, storage and protection of spermatozoa[64,65]. The cauda epididymis of STZ-induced diabetic rats also showed atrophic and degenerative changes including loosening of epithelium, massive vacuolization, damage of basement membrane and almost absence of spermatozoa in tubular lumen and hyperplasia of intertubular stroma. The height of epithelial cells lining of the tubules was reduced. These results are consistent with previous studies that reported similar degenerative and atrophic changes in epididymis of diabetic rats[66,67]. It is well known that testosterone plays a pivotal role in maintenance of structural and functional integrity of the epididymis[65]. Therefore, these degenerative and atrophic changes in epididymal histoarchitecture may be associated with lower supply of androgen to the organ. Furthermore, the depletion of antioxidant defense markers due to hyperglycemia in response to diminished insulin level could cause disruption of structural and functional integrity of epididymis in diabetic rats.

Diabetic rats treated with *M. oleifera* leaf extract/glibenclamide demonstrated a significant dose dependent restoration of cauda epididymal histoarchitectue as evidenced by large tubule lined with columnar type cells bearing long stereocilia, lumen contains good number of spermatozoa and intertubular stroma was reduced. The observed ameliorative changes might be due to increase in serum testosterone, insulin levels and reduction of hyperglycemia induced oxidative stress. Parallel to present study, other researchers have also reported preventive effects of medicinal plants possessing antidiabetic and antioxidant activities[49,67].

However, the current study has some limitations. The study was exclusively conducted on induced diabetic rats, extrapolating these

findings to naturally occurring diabetes in human subjects requires further investigation and additional research is necessary to isolate and identify the bioactive phytoconstituent(s) contributing to these activities.

In conclusion, the results of present study suggest that the 70% ethanolic extract of the leaves of *M. oleifera* plant possesses significant antioxidant activities and ameliorates diabetes-induced epididymal dysfunctions and infertility which justify the traditional use of plant as aphrodisiac and for management of diabetes. Needless to say, further studies are needed to isolate and characterize the bioactive phytoconstituent(s) that are involved in these activities.

Conflict of interest statement

The authors declare no conflicts of interest.

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Authors' contributions

Prof. Gyan Chand Jain conceived and designed the study and Dr. Ram Niwas Jangir conducted the experiments, collected data, performed statistical analysis, and wrote the manuscript. Prof. Gyan Chand Jain critically reviewed and edited the manuscript. Both authors contributed to the interpretation of results and approved the final version for submission.

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