

# Ameliorative Effect of *Moringa oleifera* Lam. Leaves Extract on the Sex Hormone Profile and Testicular Dysfunctions in Streptozotocin-induced Diabetic Wistar Rats

Ram Niwas Jangir, Gyan Chand Jain\*

## ABSTRACT

**Background:** Diabetes mellitus (DM) is associated with reproductive impairment. Medicinal plants and herbs are the rich source of antioxidants which may ameliorate diabetes-induced male reproductive dysfunctions and could play an important role in the management of diabetes induced male infertility. **Objectives:** The present study aimed to investigate the ameliorative effects of 70% hydroalcoholic extract of *Moringa oleifera* Lam. leaves on sex hormone alterations and testicular dysfunction in diabetic rats. **Materials and Methods:** Diabetes mellitus (DM) in male rats was induced by single intraperitoneal injection of streptozotocin (60 mg/kg b.wt.). The induced diabetic rats were treated with three different doses (100, 250, and 500 mg/kg b.wt./day, orally for 60 days) of *M. oleifera* leaves extract. The results were compared with reference antidiabetic drug glibenclamide (5 mg/kg b.wt./day) treated diabetic rats. Reproductive hormones like testosterone, FSH and LH and oxidative stress parameters in the testicular tissues were determined. Histopathological and histomorphometric changes in the testis were also investigated. **Results:** Oral administration of three doses of *M. oleifera* leaves extract or glibenclamide in diabetic rats significantly improved serum sex hormones levels and restored histopathological and histomorphometric changes as compared to diabetic control rats. Furthermore, extract treatment in diabetic rats also reduced TBARS levels and improved the levels of antioxidant markers in the testicular tissue. These results were comparable with glibenclamide. **Conclusion:** Based on these results, it may be concluded that the extract of *M. oleifera* leaves possess significant antioxidant activities as well as play a pivotal role in modulating testicular dysfunction in diabetic rats.

**Key words:** Antioxidants, Histomorphometric, *Moringa oleifera*, Streptozotocin, Testis, Testosterone.

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## INTRODUCTION

Diabetes mellitus (DM) is a prevalent chronic condition marked by high blood sugar levels and changes in carbohydrate, lipid, and protein metabolism as a result of insufficient insulin action or production, or both.<sup>[1]</sup> Diabetes mellitus is one of the fastest-growing diseases in the world today. The International Diabetes Federation claims (IDF), 451 million adults around the world were suffering from diabetes in 2017 and it is estimated that the number will reach around 693 million by 2045.<sup>[2]</sup> In recent years, DM has also been diagnosed in children and younger adults of reproductive age.<sup>[3]</sup> Impairment of reproductive functions both in men and women is one of the important complications of diabetes and up to 90% of diabetic patients suffer from reproductive dysfunctions.<sup>[4]</sup>

Several animal models and human studies have demonstrated that diabetes mellitus impairs male reproductive functions at multiple levels. Diabetes may be associated with impairment of hypothalamus-

pituitary-testicular axis resulting in decline of serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone levels. Diabetes induces morphological alteration in the testes, increase germ cell apoptosis, impairment of spermatogenesis and poor quality of sperm parameters. Furthermore, diabetes leads to erectile and ejaculatory dysfunctions and reduced libido, consequently male infertility.<sup>[5,6]</sup>

Oxidative stress is considered as the major factor for testicular impairment which consequently may lead to male hypogonadism, poor sperm parameters and infertility.<sup>[7,8]</sup> Insulin synthesized and secreted by the  $\beta$ -cells of pancreas, is thought to have a key role in the regulation of male reproductive functions. Moreover, recent studies have shown the expression of insulin in both testes and sperm. The insulin transcripts and protein have also been reported in diabetic and control rat testes with lower level of



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insulin protein in diabetic rat testes.<sup>[9,10]</sup> It is suggested that the adverse effect of diabetes may be due to abnormal insulin signaling in the brain and testes or systemic effect of insulin deficiency or both.<sup>[10,11]</sup>

Most of the currently available therapies for DM include insulin and various oral antidiabetic agents to achieve better regulation of glycemic index and associated complications. However, current therapies are associated with adverse effects and furthermore, many of these drugs do not completely ameliorate the diabetes induced male reproductive dysfunctions.<sup>[12,13]</sup> Medicinal plants and herbs are mines of large number of bioactive phytoconstituents that might serve as lead for the development of novel drugs.<sup>[14,15]</sup> Many plant extracts/bioactive components have demonstrated ameliorative effects on glycemic index, antioxidant defense markers as well as reproductive functions of male diabetic rats, suggesting the importance of phytotherapy in effective management of diabetes and associated male reproductive dysfunctions.<sup>[16,17]</sup>

*Moringa oleifera* (family-Moringaceae) is indigenous medicinal plant commonly known as Durmstick tree or Horse radish tree. *M. oleifera* leaves contains rich amount of calcium, copper, iron, phosphorus, vitamins (A, B and C,  $\alpha$ -tocopherol),  $\beta$ -carotene,  $\beta$ -sitosterol and essential amino acids.<sup>[18,19]</sup> Phytochemical studies on *M. oleifera* leaves revealed high concentration of major polyphenols such as kaempferol glycosides, quercetin-3-glycoside, rutin and chlorogenic acid.<sup>[20,21]</sup> In the indigenous system of medicine, almost every part of this plant has been used to treat a variety of ailments.<sup>[22]</sup> Decoction made with fresh flowers of *M. oleifera* and cow milk is a good herbal medicine for functional infertility and sexual weakness of both males and females.<sup>[23]</sup> Studies have also reported that *M. oleifera* enhances reproductive performance in normal animals.<sup>[24,25]</sup> The antidiabetic and antioxidant activity of *M. oleifera* leaves extract has already been reported from our laboratory,<sup>[26]</sup> and other researchers in STZ-induced diabetic rats.<sup>[27]</sup> Moreover, many studies have also shown protective effects of *M. oleifera* leaves extract against male reproductive dysfunctions in animals exposed with metals,<sup>[28,29]</sup> or other toxicants.<sup>[30,31]</sup> The present study aimed to evaluate the protective effects of hydroalcoholic extract of *Moringa oleifera* leaves on male reproductive dysfunctions especially serum levels of sex hormones, testicular histoarchitecture and oxidative stress in streptozotocin (STZ) induced diabetic male rats.

## MATERIALS AND METHODS

### Preparation of *M. oleifera* extract

The leaves of *Moringa oleifera* Lam. were collected and validated by Herbarium Incharge, Botany Department, University of Rajasthan, Jaipur (Voucher No. RUBL21056). The dried leaves of *M. oleifera* were pulverized into coarse powder. The powder of leaves (250 g) was steeped in ethanol (70%) and left to stand at room temperature for 24 hr. The mixture was extracted using a Soxhlet equipment at 60°C for 35 hr. After that, the material was filtered through filter paper. Finally, the liquid filtrate was dried in a 40°C oven to yield residue. The residue recovered from *M. oleifera* leaves was crystalline in nature, brown in colour, and yielded around 37.5 g. (15 percent of dried powder). For future use, the extract was kept at -4°C in the refrigerator.

### Animals

The animals used in the present study were colony bred, healthy, adult, Wistar albino male rats (*Rattus norvegicus*) weighing 170-205 g individually. They were kept in groups under standard laboratory conditions and provided water and adequate pallet diet (Aashirwad Food Industries, Chandigarh, India) *ad libitum*. The animals were cared for in accordance with the regulations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animal use and experimental protocols of study was approved by

the Animal Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur (India).

### Chemicals and reagents

Streptozotocin was obtained from Himedia Laboratory Limited, Mumbai, India. Glibenclamide tablets (Daonil; Aventis Pharma Ltd., India) were purchased from a local medical supply store. All of the other chemicals and reagents used were of analytical quality.

### Experimental induction of diabetes

In overnight fasting rats, diabetes mellitus was experimentally developed by intraperitoneal injecting of 0.5 ml STZ dissolved in citrate buffer (pH 4.5) at a dose of 60 mg/kg b.wt. After one week of STZ injection, diabetes was confirmed in these rats by measuring glucose levels in blood samples taken from the tail veins of overnight fasting animals using a one-touch glucometer (Johnson and Johnson, USA). The rats having blood glucose levels >250 mg/dl were considered diabetic model and used in the present study.

### Experimental groups

The experimental rats were separated into six groups, each of which had six rats. Group I: Control rats receiving 0.5 ml distilled water/rat/day, Group II: Diabetic rats receiving 0.5 ml distilled water/rat/day, Group III, IV, and V: Diabetic rats orally treated with *M. oleifera* extract at doses of 100, 250, and 500 mg/kg b.wt./day, respectively, Group VI: Diabetic rats orally treated with glibenclamide (5 mg/kg b.wt./day). All treatments were given for 60 consecutive days.

### Autopsy schedule

All the overnight-fasted animals of separate groups were autopsied under light ether anesthesia, After 24 hr of the last treatment. Blood samples were collected directly by heart puncture and centrifuged at 3000 rpm for 20 min to separate the serum, Serum samples were kept at -20°C until they were analysed. Each rat's testis were dissected, cleansed of adhering fat, washed in normal saline, and weighed separately. A small portion of the testis was cut for biochemical estimations (kept frozen at -20°C), and the rest of the testicular tissue was fixed in Bouin's fixative for histopathological analysis.

### Serum hormones

Serum samples obtained from the animals in all experimental groups at the end of final treatment (at 60 days) were used for the analysis of testosterone, LH and FSH levels through chemiluminescence (fully automatic Advia Centaur ImmunoAssay).

### Lipid peroxidation and antioxidant markers

The following parameters were measured in fresh/frozen testis samples of rats in various experimental groups: lipid peroxidation (TBARS),<sup>[32]</sup> superoxide dismutase (SOD) activity,<sup>[33]</sup> catalase (CAT) activity,<sup>[34]</sup> reduced glutathione (GSH),<sup>[35]</sup> and ascorbic acid (Vitamin C) content.<sup>[36]</sup>

### Histopathological study

Bouin's fixed testis samples were washed in distilled water to remove excess fixative, processed through a graded series of ethanol, and cleared in xylene. Then the tissue was embedded in paraffin wax and 5  $\mu$ m sections were cut using a microtome. The sections were mounted on glass slides and hematoxylin and eosin were used to counterstain. A light microscope was used to observe the slides for histopathological study. Photomicrographs of the testicular sections were taken using a Nikon digital camera attached to a light microscope.

## Histometry

### Diameter of seminiferous tubules

The size of seminiferous tubules was measured in round or nearly round tubules chosen randomly in the section. At least forty tubular profiles were measured for each animal. Measurements were taken using an ocular micrometer calibrated with stage micrometer in a light microscope. Two diameters perpendicular to each other passing through center of tubule were measured at X 100 magnification and the mean diameter was obtained.

### Quantitative determination of germ cells

Quantitative determination of germ cells in 50 round or nearly round seminiferous tubule sections per experimental group was done at the magnification of X 400 as per method reported by Leblond and Clermont (1952).<sup>[37]</sup> The germ cells (spermatogonia, preleptotene spermatocytes, pachytene spermatocytes, and round spermatids) were quantified per cross section in the VIII stage of the seminiferous tubule cycle. An ocular micrometre was used to measure the nuclear diameters of different germ cell types. A correction factor was also applied to obtain the actual numerical density of germ cells.<sup>[38]</sup>

$$\text{True Counts} = \text{Crude Counts} \times \frac{\text{Section thickness } (\mu\text{m})}{\text{Section thickness } (\mu\text{m}) + \text{Nuclear diameter } (\mu\text{m})}$$

### Diameter of Leydig cell nucleus

Using an ocular micrometre at X 1000, the diameter of 100 Leydig cell nuclei was measured on five sections for each animal, and the values were averaged and given as mean nuclear diameter of Leydig cell.

### Statistical analysis

All the data were statistically analyzed using SPSS (version 20.0) computer software (SPSS INC., Chicago, IL, USA) and represented as mean  $\pm$  standard error. All the data were statistically analyzed with One way ANOVA followed by Tukey's multiple comparison as a *post hoc* test. Values of  $P \leq 0.05$  were considered statistically significant.

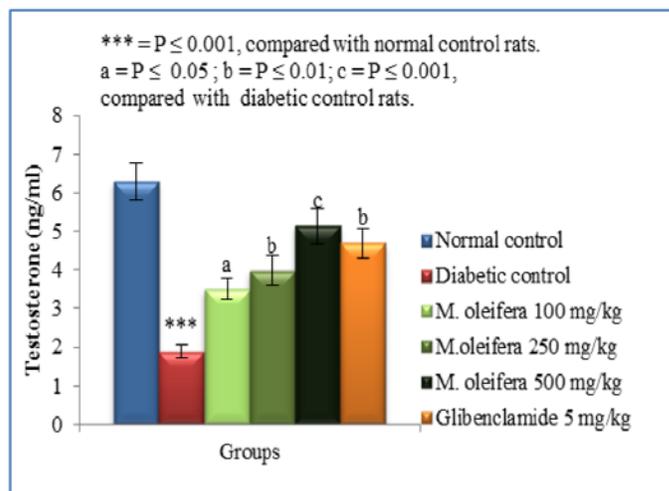
## RESULTS

### Serum sex hormone profile

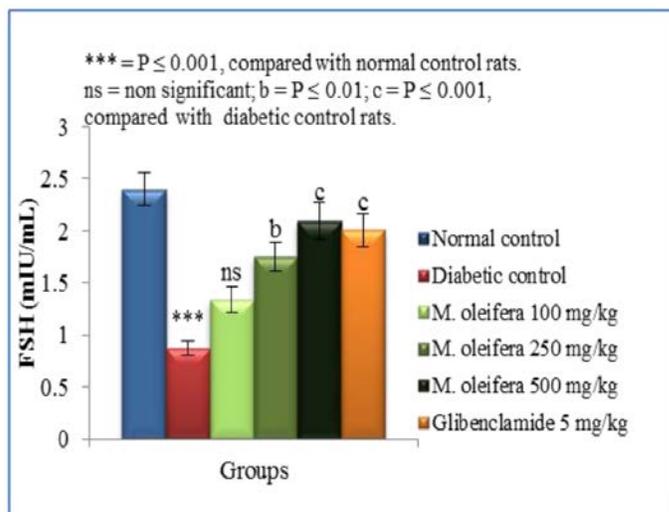
Figures 1 to 3 show changes in serum sex hormone levels in normal control, diabetic control, and experimental rats. The serum testosterone, FSH and LH levels were significantly ( $P \leq 0.001$ ) declined in diabetic control rat as compared to normal control rat. Diabetic rats treated with 250 and 500 mg/kg b.wt. doses of *M. oleifera* extract significantly ( $P \leq 0.01$  and  $P \leq 0.001$ , respectively) restored the levels of serum testosterone, LH and FSH. However, in low dose group, serum testosterone level exhibited significant increment ( $P \leq 0.05$ ) without showing any significant improvement in serum LH and FSH levels. When glibenclamide was given to diabetic rats, the serum levels of testosterone, FSH, and LH were significantly higher ( $P \leq 0.001$ ) than in diabetic control rats.

### Lipid peroxidation and antioxidant markers

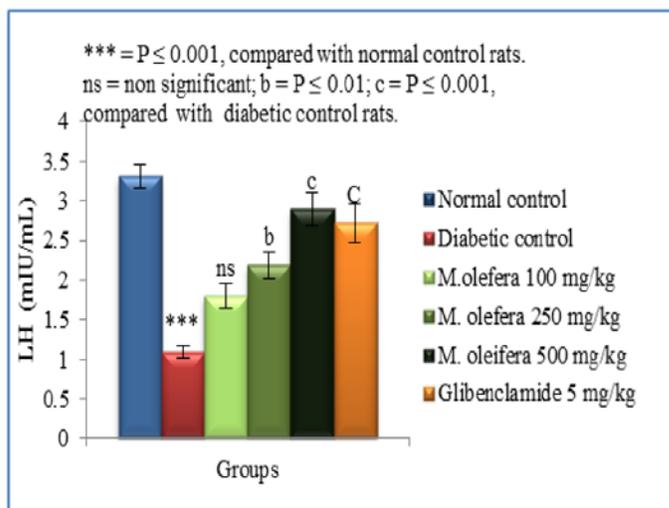
Table 1 shows the changes in lipid peroxidation (TBARS), SOD, CAT, GSH and ascorbic acid contents in testicular tissue of different experimental groups. The concentration of thiobarbituric acid reactive substances (TBARS) was used for estimation of lipid peroxidation. The TBARS concentration in the testis of diabetic rats was significantly ( $P \leq 0.001$ ) increased when compared with control rats. *M. oleifera* leaves extract treated diabetic rats demonstrated a considerably decline



**Figure 1:** Effect of *M. oleifera* extract on serum testosterone level in STZ-induced diabetic rats.



**Figure 2:** Effect of *M. oleifera* extract on serum FSH level in STZ-induced diabetic rats.



**Figure 3:** Effect of *M. oleifera* extract on serum LH in STZ-induced diabetic rats.

**Table 1: Effect of *M. oleifera* extract on lipid peroxidation and antioxidant parameters in testis of STZ-induced diabetic rats.**

Treatment	Lipid peroxidation (TBARS) (n mol MDA /mg tissue)	SOD (unit/ mg protein)	GSH (n mol/g tissue)	Ascorbic acid (mg/ g tissue)	Catalase (n mole H <sub>2</sub> O <sub>2</sub> /min/mg protein)
<b>Group I</b> Normal Control	1.64±0.10	8.60±0.30	3.14±0.22	1.46±0.10	32.53±1.45
<b>Group II</b> Diabetic Control	5.15±0.30***	4.30± 0.29***	1.25±0.10***	0.60±0.05***	15.08±1.04***
<b>Group III</b> Diabetic + Extract (100 mg/kg b.wt.)	4.05±0.25 <sup>b</sup>	5.79±0.19 <sup>a</sup>	1.79±0.11 <sup>ns</sup>	0.98±0.06 <sup>a</sup>	21.88±1.26 <sup>a</sup>
<b>Group IV</b> Diabetic + Extract (250 mg/kg b.wt.)	3.30±0.27 <sup>c</sup>	6.40±0.35 <sup>b</sup>	2.25±0.16 <sup>b</sup>	1.12±0.10 <sup>b</sup>	24.28±2.12 <sup>b</sup>
<b>Group V</b> Diabetic + Extract (500 mg/kg b.wt.)	2.25±0.13 <sup>c</sup>	7.67±0.32 <sup>c</sup>	2.80±0.20 <sup>c</sup>	1.31±0.09 <sup>c</sup>	29.56±1.32 <sup>c</sup>
<b>Group VI</b> Diabetic + Glibenclamide (5 mg/kg b.wt.)	2.37±0.18 <sup>c</sup>	7.45±0.49 <sup>c</sup>	2.65±0.18 <sup>c</sup>	1.24±0.08 <sup>c</sup>	27.33±1.94 <sup>c</sup>

**Level of significance: Values represent mean ± SEM (n=6)**

\*\*\* =  $P \leq 0.001$ , diabetic control rats compared with normal control rats

ns- non significant; a =  $P \leq 0.05$ ; b =  $P \leq 0.01$ ; c =  $P \leq 0.001$ , *M. oleifera* extract or glibenclamide treated rats compared with diabetic control rats.

of the TBARS concentration in the testicular tissue. It was moderately significant ( $P \leq 0.01$ ) for 100 mg/kg b.wt. and highly significant ( $P \leq 0.001$ ) for 250 mg/kg b.wt. as well as 500 mg/kg b.wt. dose groups as compared with diabetic control groups. When diabetic rats were given the standard antidiabetic medicine glibenclamide, the level of TBARS in the testis decreased significantly ( $P \leq 0.001$ ) when compared to diabetic rats.

In comparison to control rats, diabetic control rats had a significant ( $P \leq 0.001$ ) decrease in SOD and CAT activity, as well as GSH and ascorbic acid concentrations in the testicular tissue. Administration of extract at 100, 250 and 500 mg/kg b.wt. doses caused a significant dose dependent ( $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$  respectively) increase in the SOD and CAT activities as well as ascorbic acid level in the testis as compared with diabetic control rats. However, a significant ( $P \leq 0.01$  and  $P \leq 0.001$  respectively) rise of GSH concentration was recorded only in 250 and 500 mg/kg b.wt. dose groups. In comparison to diabetic control rats, diabetic rats treated with glibenclamide showed a highly significant ( $P \leq 0.001$ ) increase in CAT and SOD activity, as well as GSH and ascorbic acid concentrations.

## Histomorphometric analysis

### Germ cells counts

The mean testicular germ cells population count per seminiferous tubule cross sections has been depicted in Table 2. Untreated diabetic control rats had a significant ( $P \leq 0.001$ ) decrease in the population of various germ cell types. Oral administration of *M. oleifera* extract at 250 and 500 mg/kg b.wt. doses to diabetic rats induced a significant ( $P \leq 0.01$  and  $P \leq 0.001$  respectively) increase in the mean number of spermatogonia in seminiferous tubules as compared to diabetic control rats. However, diabetic rats given a low dose of the extract (100 mg/kg b.wt.) had a non-significant rise in the number of spermatogonia. When diabetic rats were treated with *M. oleifera* extract at three different doses (100, 250 and 500 mg/kg b.wt.) a statistically significant ( $P \leq 0.05$ ,  $P \leq 0.01$  and  $P \leq 0.001$ , respectively) dose dependent increase in the population of preleptotene and pachytene spermatocyte was observed as compared with diabetic control rats. Diabetic rats given the medium (250 mg/kg b.wt) and high

doses (500 mg/kg b.wt) of *M. oleifera* extract had a substantial ( $P \leq 0.001$ ) increase in the population of round spermatids, while the diabetic rats treated with low dose of the extract showed slightly significant ( $P \leq 0.05$ ) elevation as compared to diabetic control rats. Glibenclamide treated diabetic rats showed a significant ( $P \leq 0.001$ ) elevation in the quantity of spermatogonia, preleptotene and pachytene spermatocytes and round spermatids when compared to diabetic control rats.

### Diameter of seminiferous tubules and Leydig cell nucleus

Table 2 shows the changes in mean seminiferous tubule diameter and Leydig cell nuclear diameter in the normal control and experimental groups. Streptozotocin induced a significantly substantial ( $P \leq 0.001$ ) decrease in the diameters of the seminiferous tubules and Leydig cell nuclei in rats. When diabetic rats were given three different doses of *M. oleifera* extract (100, 250 and 500 mg/kg b.wt.) it caused a dose dependent significant ( $P \leq 0.05$ ,  $P \leq 0.01$ ,  $P \leq 0.001$ , respectively) increase in the mean seminiferous tubule and Leydig cell nuclear diameters as compared with untreated diabetic control rats. Glibenclamide treated diabetic rats showed a highly significant ( $P \leq 0.001$ ) increase in both Leydig cell nuclear diameter and seminiferous tubular diameter when compared to diabetic control rats.

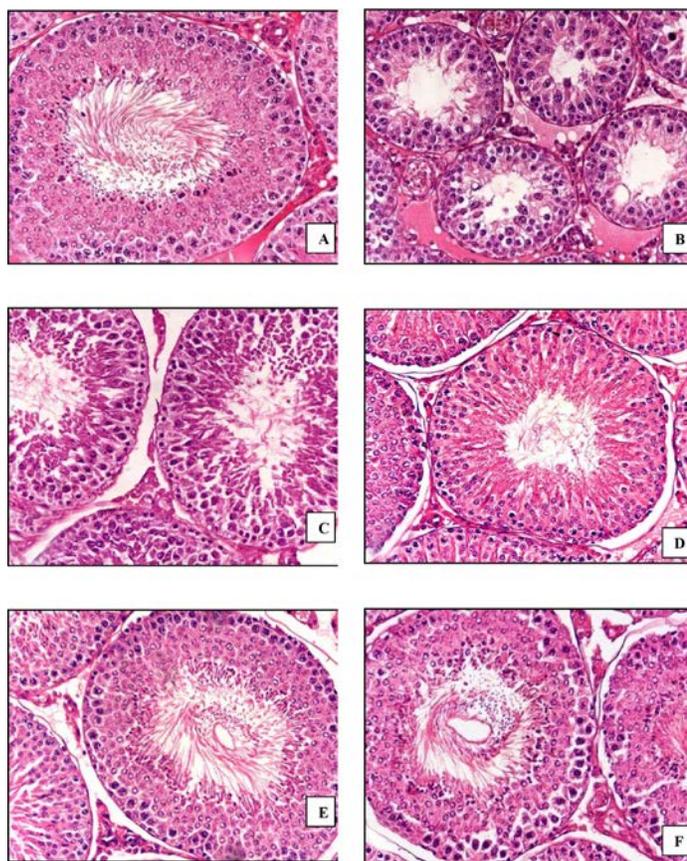
### Histology

Figure 4 depicts histomorphological pictures of the testis of control and different experimental groups. Testis of normal control rat exhibited normal testicular architecture, seminiferous tubular morphology, interstitial structure and spermatogenesis (Figure 4A). Histopathological examination of the testis of STZ-induced diabetic rat showed marked necrotic and atrophic changes as evidenced by reduction of germinal epithelium height, vacuolization of epithelium in many areas, depletion and derangement of spermatogenic cells. The Sertoli cells were reduced in number and exhibited degenerative changes. The interstitial spaces were widened with scattered Leydig cells showing degenerative and atrophic changes (Figure 4B; Table 2). Diabetic rat treated with low dose

**Table 2: Effect of *M. oleifera* extract on histomorphometric indices of seminiferous tubule and Leydig cell nuclear diameter of STZ-induced diabetic rats**

Treatment	Spermatogonia	Preleptotene spermatocyte	Pachytene Spermatocyte	Round spermatid	Seminiferous tubular diameter (µ m)	Leydig cell nuclear diameter (µ m)
Group I Normal Control	6.33±0.25	20.17±1.08	23.67±1.52	64.87±2.37	272.67±8.32	6.67±0.33
Group II Diabetic Control	3.83±0.31***	11.50±0.76***	13.83±1.25***	28.00±2.06***	168.50±9.75***	3.50±0.26***
Group III Diabetic + Extract (100 mg/kg b.wt.)	5.20±0.19 <sup>ns</sup>	15.85±0.85 <sup>a</sup>	19.37±1.07 <sup>a</sup>	40.03±2.45 <sup>a</sup>	210.17±10.24 <sup>a</sup>	4.85±0.25 <sup>a</sup>
Group IV Diabetic + Extract (250 mg/kg b.wt.)	5.65±0.27 <sup>b</sup>	17.35±0.89 <sup>b</sup>	21.50±1.42 <sup>b</sup>	47.40±2.75 <sup>c</sup>	230.67±10.70 <sup>b</sup>	5.45±0.30 <sup>b</sup>
Group V Diabetic + Extract (500 mg/kg b.wt.)	6.00±0.43 <sup>c</sup>	19.20±1.04 <sup>c</sup>	22.60±1.17 <sup>c</sup>	58.23±3.02 <sup>c</sup>	261.17±9.31 <sup>c</sup>	6.33±0.28 <sup>c</sup>
Group VI Diabetic + Glibenclamide(5 mg/kg b.wt.)	5.85±0.35 <sup>c</sup>	17.85±1.01 <sup>c</sup>	22.00±1.15 <sup>c</sup>	56.40±3.29 <sup>c</sup>	248.50±8.69 <sup>c</sup>	6.20±0.37 <sup>c</sup>

Level of significance: Values represent mean ± SEM (n=6)\*\*\* = P ≤ 0.001, diabetic control rats compared with 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 diabetic control rats



**Figure 4:** Photomicrograph of testis (A) Normal control rats exhibiting normal histoarchitecture. (B) Diabetic control rats exhibiting severe degenerative, atrophic changes and impairment of spermatogenesis. (C) *M. oleifera* extract (100mg/kg b.wt.) treated diabetic rat showing mild improvement of seminiferous tubular histomorphology. (D) *M. oleifera* extract (250mg/kg b.wt.) treated diabetic rat showing moderate restoration of histomorphology of seminiferous tubules and interstitial tissue. (E) *M. oleifera* extract (500mg/kg b.wt.) treated diabetic rat exhibiting near normal histoarchitecture. (F) Glibenclamide treated diabetic rat displaying significant restoration of histoarchitecture. (H&E x200).

of *M. oleifera* extract (100 mg/kg b.wt.) exhibited mild prevention of degenerative and atrophic changes in seminiferous tubules as evidenced by slight improvement of various germ cell populations, presence of few sperm in the tubular lumen and slight edema of interstitial cells.

Exfoliation and derangement of spermatogenic cells was still present in the seminiferous tubules (Figure 4C; Table 2). Administration of medium dose of *M. oleifera* extract (250 mg/kg b.wt.) in diabetic rats moderately improved the histoarchitecture of the testis as indicated by increased population of spermatogenic cells (spermatogonia, preleptotene spermatocyte, pachytene spermatocyte and spermatids) with displaying characteristic arrangement. Leydig cell nuclear diameter was also increased. Presence of good number of spermatozoa in the lumen of seminiferous tubules was also recorded. (Figure 4D; Table 2). The testis of diabetic rats receiving *M. oleifera* extract at high dose (500 mg/kg b.wt.) level exhibited near normal histological structure of most of the seminiferous tubules, as evidenced by a considerable increase in the quantity of spermatogenic cells and their characteristic tiered organisation. The diameter of the seminiferous tubules increased, and the lumen was filled with a significant number of spermatozoa. The Leydig cells also exhibited almost normal morphology (Figure 4E; Table 2). The seminiferous tubules of diabetic rats treated with glibenclamide had a near-normal appearance, with all the developmental stages of spermatogenic cells and a good amount of sperms in the lumen. Leydig cell shape and their nuclear diameter were also improved (Figure 4F; Table 2).

## DISCUSSION

It is well known that male reproductive functions are regulated by hypothalamus-pituitary-testicular (HPT) axis. In the present study, a significant reduction in the serum levels of FSH, LH and testosterone was recorded in STZ-induced diabetic rats. These outcomes are in agreement with the findings of several researchers who also reported significant decrease in the concentration of FSH, LH and testosterone in STZ-induced diabetic rats<sup>[39-41]</sup> as well as in diabetic patients.<sup>[42,43]</sup> The observed reduction in serum testosterone, LH and FSH levels in diabetic rats suggest that diabetes perturbs FSH and LH hormone synthesis and secretion from anterior pituitary gland and consequently disturbs the functions of Leydig and Sertoli cells leading to impairment of steroidogenesis and spermatogenesis in the testes. The observed decline in the FSH and LH hormones in diabetic rats may be correlated with insulin deficiency, since many researchers have shown a strong correlation between insulin level and the pituitary biosynthesis and secretion of FSH and LH hormones.<sup>[11,44,45]</sup>

The finding of the current study revealed that treatment of diabetic rats with *M. oleifera* extract/glibenclamide resulted in considerable improvement of serum testosterone, LH and FSH levels. These results are consistent with Ebong *et al.*, (2014)<sup>[46]</sup> who also found a substantial increase in the levels of testosterone, LH and FSH in STZ-induced

diabetic rats treated with 80% ethanolic extract of *M. oleifera* leaves. Similar ameliorative effects of glibenclamide on testosterone, LH and FSH levels have also been observed in STZ-induced diabetic rats.<sup>[12,41]</sup> The observed ameliorative effects on serum testosterone, LH and FSH in *M. oleifera* extract treated diabetic rats could be due to restorative effects of the phytoconstituents present in the extract on pancreas, as well as on the synthesis and secretion of insulin, resulting in resumption of pituitary as well as Leydig and Sertoli cells functions in the testes.

The results of the present investigation demonstrated a highly significant elevation in TBARS level along with a marked decline in the antioxidant defense parameters (SOD, CAT, GSH and ascorbic acid) in testicular tissue of STZ-induced diabetic control rats. The results of this investigation are in accordance with many earlier findings where similar decline of antioxidant defense markers along with elevation of TBARS levels were recorded in the testis of diabetic animals.<sup>[47-51]</sup> It is well established that oxidative stress play central role in development of reproductive complications leading to infertility.<sup>[52,53]</sup> Thus the observed decline in the antioxidant molecules could result from their inactivation by excessive generation of reactive oxygen species (ROS) or by glycation of the enzymes. In addition to this, the observed increase of oxidative stress in the testis might also be due to reduced testosterone level.<sup>[54]</sup>

Treatment of diabetic rats with *M. oleifera* extract/glibenclamide increased the activities of enzymatic antioxidants (SOD and CAT) as well as nonenzymatic antioxidants (GSH and ascorbic acid), while lowering TBARS levels in the testes. The observed improvement in these parameters could be due to presence of potent antioxidant phytochemicals in the extract of this plant.<sup>[26,55]</sup> Many researches have established the antioxidant properties of polyphenolic substances such as quercetin,<sup>[56]</sup> rutin,<sup>[57]</sup> naringenin,<sup>[58]</sup> beta carotene,<sup>[59]</sup> in the testis/sperms of diabetic animals. Furthermore, various research have revealed that *Moringa oleifera* leaf extract has antioxidative or ameliorative actions against male reproductive dysfunctions in animals exposed to metals<sup>[28,29]</sup> or other toxicants.<sup>[30-31]</sup>

Histopathological examinations of the reproductive organs, particularly the testis, are indicated as the most sensitive end point in evaluation of male reproductive toxicity.<sup>[60]</sup> Histological observation of the testis of the diabetic control rat showed significant atrophic and degenerative changes in the testis. The epithelium of seminiferous tubules exhibited marked degeneration and disorganization of germ cells. The mean diameter of seminiferous tubules and the number of different types of germ cells along with spermatozoa were significantly decreased. The interstitial spaces have been expanded, and there are a few atrophic Leydig cells with tiny nuclei in the interstitial spaces. These findings are consistent with previous research that found similar atrophic and degenerative alterations and impairment of spermatogenesis was observed in the testis of diabetic rats.<sup>[5,61-64]</sup>

The observed degenerative and atrophic alterations in the diabetic rats' testes could be related to impairment of HPG axis resulting in lowered levels of the testosterone, LH and FSH as a consequence of insulin insufficiency. LH is thought to promote Leydig cells proliferation through a mechanism that involves insulin and insulin like growth factor (IGF-1) signalling.<sup>[65]</sup> Thus, hypoinsulinemia may cause a decrease in the number of Leydig cells and their metabolic activities in STZ-induced diabetic rats. These factors play a role in the decrease in testosterone level. The present study's findings of lower nuclear diameter and atrophy in Leydig cells also corroborate this theory. Adequate testosterone level in testis is required for maintenance of normal morphology of seminiferous tubules and spermatogenesis.<sup>[66]</sup> The depletion of germ cell populations in the testis of diabetic rat also supports diminished availability of testosterone. Degeneration and atrophy of Sertoli cells also affects normal spermatogenic function, as Sertoli cells provide biochemical

and structural support for developing sperm cells and also secrete many growth factors and transport proteins.<sup>[67]</sup>

Furthermore, under condition of hyperglycemia and insulin shortage, testicular cells may undergo metabolic adaptations and utilise alternate substrate as a source of energy, which induce oxidative stress.<sup>[3]</sup> Overproduction of free radicals and ROS causes attenuation of endogenous antioxidant enzymes, decline of testicular functions and thus lead to germ cells necrosis, apoptosis and cell death because the membrane of testicular germ cells is rich in polyunsaturated fatty acids and is highly susceptible to lipid peroxidation and oxidative stress. Several investigations have also reported increased apoptotic germ cell death in the testes of STZ-induced diabetic rats and mice.<sup>[63,68-70]</sup> According to recent research, mitochondria play a key role in germ cell apoptosis. ROS triggers chain reactions that activating mitochondria to release cytochrome c with caspase activation, resulting in induction of apoptosis.<sup>[7,71]</sup>

Treatment of diabetic rats with *M. oleifera* extract/glibenclamide significantly restored the testicular histoarchitecture. The seminiferous tubules were large showing active spermatogenesis. Leydig cells also showed near normal morphology. These ameliorative effects might be due to restoration of insulin and testosterone levels and attenuation of oxidative stress by virtue of individual or synergetic action of the phytoconstituents present in the extract. The findings of the present study are in accordance with earlier studies where *M. oleifera* leaves/flower extract has shown significant improvement of testicular histoarchitecture and functions in diabetic rats.<sup>[46,55,72,73]</sup> Diabetic rats receiving glibenclamide treatment also showed significant restoration of testicular histomorphology. These results are also in like with earlier findings in diabetic rats.<sup>[41,74,75]</sup>

## CONCLUSION

In conclusion, the outcomes of the present study provide clear evidence that the phytoconstituents present in the hydroalcoholic (70%) extract of *M. oleifera* leaves possess significant antioxidant activities as well as play a pivotal role in modulating testicular lesions and in improvement of serum sex hormone levels in diabetic male rats. Needless to say, further studies are needed to isolate and characterize the bioactive principle(s) which are responsible for such activity.

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## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

## ABBREVIATIONS

**b.wt.:** Bodyweigh; **DM:** Diabetes Mellitus; **STZ:** Streptozotocin; **ROS:** Reactive Oxygen Species; **FSH:** Follicle Stimulating Hormone; **LH:** luteinizing Hormone; **GSH:** Glutathione; **SOD:** SuperOxide Dismutase; **CAT:** Catalase; **TBARS:** ThioBarbituric Acid Reactive Substances.

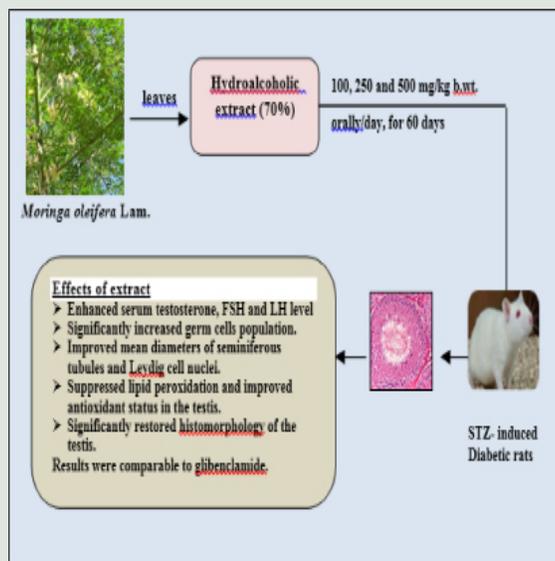
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## GRAPHICAL ABSTRACT



## SUMMARY

- Protective effects of hydroalcoholic extract of *Moringa oleifera* Lam. leaves on testicular tissue of streptozotocin induced diabetic rats were investigated.
- Leaves extract treatment in diabetic rat considerably increased serum testosterone, FSH and LH levels.
- Leaves extract treatment in diabetic rat considerably improved the reduced levels of SOD, CAT, GSH and ascorbic acid as well as significantly declined lipid peroxidation.
- Leaves extract treatment in diabetic rats remarkably improved the germ cells population, diameter of seminiferous tubules and nuclear diameter of Leydig cells.
- Leaves extract treatment in diabetic rats also significantly restored histomorphology of testis.
- The results were comparable with standard antidiabetic drug glibenclamide.

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