JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2025/36436

RESEARCH ARTICLE

Effects of *Moringa oleifera* Lam. on Reproductive Organs Weight and Tissue Biochemistry in Streptozotocin-induced Diabetic Rats

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Abstract

Background: Diabetes mellitus (DM) is linked to reproductive impairments. Medicinal plants and herbs, rich in antioxidants, can potentially alleviate diabetes-induced male reproductive dysfunctions. **Aim:** To assess the ameliorative effects of 70% ethanolic extract of *Moringa oleifera* leaves (MOLE) on reproductive organ weights and biochemical markers within reproductive tissues in diabetic rats. **Methods:** Male Wistar rats were injected with a single intraperitoneal injection of streptozotocin (STZ) at a dose of 60 mg/kg to induce type I diabetes. Diabetic rats were then given oral doses of MOLE at 100, 250, and 500 mg/kg body weight per day over 60 days. The findings were compared with those of diabetic rats treated with the standard antidiabetic drug, glibenclamide (5 mg/kg body weight per day). Measurements included the absolute and relative weight of testes, epididymides, vasa deferens, seminal vesicles, and ventral prostate. Additionally, protein, glycogen, cholesterol, sialic acid, and acid and alkaline phosphatases in the testes and epididymis, along with fructose content in the seminal vesicles, were analyzed. **Results:** Oral administration of MOLE at doses of 100, 250, and 500 mg/kg body weight per day, significantly increased both the absolute and relative weight of reproductive organs and restored biochemical parameters in the reproductive tissues of diabetic rats. These effects were comparable with glibenclamide. **Conclusion:** The findings of this study suggest that MOLE significantly restored the weight of reproductive organs and improved tissue biochemical parameters in diabetic male rats.

Major Findings: The present study indicated the ameliorative effect of MOLE on diabetes-induced biochemical alterations in testicular and epididymal tissue and improved reproductive organ weight.

Keywords: Glibenclamide, *Moringa oleifera*, Reproductive Organs Weight, Streptozotocin, Tissue Biochemistry

1. Introduction

Diabetes Mellitus (DM) is a metabolic condition marked by chronically high blood sugar levels and significant disruptions in protein, fat and carbohydrate metabolism. Currently, Diabetes is the most rapidly increasing health condition globally. As per estimation by the International Diabetes Federation, 536.6 million people (10.5%) will be living with diabetes in 2021 globally, and this number will reach approximately 738.2 million (12.2%) by 2045. Alarmingly, more than half of those with diabetes are unaware they have the condition[1,2].

Numerous studies have demonstrated that diabetes negatively affects male reproductive function, contributing to male infertility. Experimental research on rodent models has shown that diabetes mellitus is significantly correlated with reduced testicular weight, atrophic and degenerative changes in the testes, decreased seminiferous tubule epithelium height, and depletion of germ cells[3-5]. Studies involving STZ-induced diabetic rats have also reported that diabetes leads to regression and atrophic changes in the epididymis, resulting in a decline in epididymal weight[6]. Additional research has shown that diabetes

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Article Received on: 01.02.2024 Revised on: 17.12.2024 Accepted on: 20.12.2024

in experimental rodents causes severe structural and secretory dysfunction, along with the reduced weight of accessory sex organs[7-9].

It is well known that hyperglycemia generates an excess of Reactive Oxygen Species (ROS) and attenuates the antioxidative defence mechanism[10]. Increasing evidence has indicated that the enhancement of oxidative stress plays a significant role in the pathogenesis of diabetic complications. Various authors have reported increased levels of Advanced Glycation End products (AGEs) and their receptors (RAGE) in the reproductive tract of both diabetic animal models and men[11,12].

Currently, available treatment of diabetes includes insulin and various oral antidiabetic drugs associated with adverse effects viz. limited efficacy, episodes of hypoglycemia, gastrointestinal intolerance and myocardial infarction etc[13,14]. Furthermore, many medicinal plants have been reported to restore male reproductive functions in experimental diabetic animals[5,15,16].

Moringa oleifera commonly known as the drumstick tree, is recognized for its exceptional nutritional profile, containing over 90 essential chemical compounds. The leaves of *M. oleifera* are rich in vitamins (A, B, C, E), minerals (such as calcium, copper, magnesium, iron, phosphorus, selenium, and zinc), and proteins, including all essential amino acids. Phytochemical analysis of *M. oleifera* leaves are rich source of bioactive secondary metabolites, such as flavonoids (isoquercitrin, kaempferol), phenolic acids (chlorogenic acid), phytosterols (β-sitosterol), glycosides (niazimicin, niazinin), alkaloids, saponins, and caffeoylquinic acid, which possess both nutritional and pharmaceutical properties[17,18]. Numerous scientific studies have highlighted the antidiabetic and antioxidant activity of *M. oleifera* leaves in diabetic animal models[19,20]. Furthermore, *M. oleifera* has been reported to have aphrodisiac effects in healthy animals[21,22] and to alleviate male reproductive dysfunctions caused by exposure to metals or other toxic substances[23-25]. Previously we have reported ameliorative effects on testicular dysfunctions in STZ-induced diabetic rats[26].

This study sought to examine the effects of a 70% ethanolic extract of *Moringa oleifera* leaves on both the absolute and relative weights of reproductive organs, along with the concentrations of protein, glycogen, cholesterol, sialic acid, and activity of acid and alkaline phosphatases in the testes and epididymal tissues of STZ-induced diabetic rats.

2. Materials and Methods

2.1 Preparation of *Moringa oleifera* Leaves Extract

Leaves of the plant were gathered and authenticated by the herbarium curator at the Department of Botany, University of Rajasthan, Jaipur (RUBL21056). After collecting, the leaves were washed with distilled water and air-dried in the shade at room temperature. Once dried, they were ground into a coarse powder. A 250g sample of this powdered material was suspended in 70% ethanol and kept at room temperature for 24 hours. Extraction was then carried out using a Soxhlet apparatus at a temperature of 60°C–70°C. The extract was filtered, and the filtrate was evaporated in an oven at 40°C to yield a residue, which was stored in an airtight container in a refrigerator until use.

2.2 Experimental Animals

Male Wistar rats (*Rattus norvegicus*), weighing between 170-210 g, were used in this study. The animals were housed in groups in standard plastic cages under controlled laboratory conditions, maintaining a temperature of 25±3 °C and a 12-hour light-dark cycle in the department's animal facility. They were provided with water and a standard pellet diet *ad libitum*. Ethical approval for the study was obtained from the Institutional Animal Ethical Committee.

2.3 Experimental Induction of Diabetes

Type 1 Diabetes Mellitus (DM) was induced in male rats that had been fasted overnight by administering an intraperitoneal injection of streptozotocin (sourced from Himedia, India), dissolved in citrate buffer (pH 4.5), at a dose of 60 mg per kg of body weight. One-week post-injection, the onset of diabetes was confirmed by measuring fasting blood glucose levels from blood, collected from the tail vein of the treated rats. Blood glucose levels were measured using a OneTouch glucometer (Johnson and Johnson, USA). Rats with blood glucose levels exceeding 250 mg/dl were identified as diabetic and included in the study.

2.4 Experimental Design

The control and diabetic rats were distributed into 6 separate groups, each containing 6 animals, and were treated as follows:

Group I (Normal Control): Rats were given 0.5 ml of distilled water per rat per day orally for 60 days.

Group II (Diabetic Control): Rats were treated with 0.5 ml of distilled water per rat per day for 60 days.

Groups III, IV, and V (Diabetic + Extract): Rats received varying doses of MOLE (100, 250, and 500 mg/kg body weight respectively, suspended in 0.5 ml of distilled water per rat per day, orally for 60 days.

Group VI (Diabetic + Standard Drug): Rats were administered 5 mg/kg body weight of Glibenclamide (standard drug) orally for 60 days.

The following day, after the final treatment, all overnight-fasted animals from the various groups were weighed and then euthanized under mild ether anesthesia.

2.5 Organ Weight

The reproductive organs, such as the "testes, epididymides, vas deferens, seminal vesicles, and ventral prostate", were carefully dissected, cleaned of any attached fat and blood clots, and weighed individually using a digital electronic balance. Half of the tissues from each organ were preserved in Bouin's fluid for histopathological examination, while the remaining tissues were stored at -20°C for biochemical analysis.

2.6 Tissue Biochemistry

Frozen/fresh tissue samples of testis and epididymis were used for quantitative estimations of total protein[27], glycogen[28], sialic acid[29], total cholesterol[30], acid phosphatase and alkaline phosphatase (Diagnostic kit, Accurex Biomedical Pvt. Ltd. Mumbai, India) and Seminal vesicle tissue used for fructose analysis[31].

2.7 Data Analysis

All data were analyzed statistically using SPSS 20.0. The results were presented as mean ± SEM and tested for variance. Statistical analysis was performed using one-way ANOVA, followed by Tukey's post hoc test. Differences in means were considered significant at $p < 0.05$.

3. Results

3.1 Reproductive Organs Weight

Changes in the absolute and relative weight of reproductive organs of the various experimental groups are presented in Table 1. The absolute weight have been expressed as mg±SEM and relative weight as mg/100 g b.wt.±SEM.

Diabetic control rats exhibited a highly significant decrease ($P \leq 0.001$) in the absolute weights of the testes, epididymides, vas deferens, seminal vesicles, and ventral prostate compared to normal control rats. Administering MOLE at a dose of 100 mg/kg body weight to diabetic rats led to a significant increase ($P \leq 0.01$) in the absolute weights of these organs. Furthermore, treatment with MOLE at doses of 250 mg/kg and 500 mg/kg body weight, as well as with glibenclamide, produced a highly significant increase ($P \leq 0.001$) in the absolute weights of these reproductive organs relative to diabetic control rats.

In diabetic control rats, the relative weights of the testes, epididymides, vas deferens, and ventral prostate were significantly lower ($P \leq 0.01$) than those in normal control group I rats. Administration of MOLE at doses of 250 and 500 mg/kg body weight per day resulted in a notable increase in the relative weight of the testes and epididymides ($P \leq 0.05$ and $P \leq 0.01$, respectively) compared to diabetic control rats. However, rats treated with the lower dose of MOLE exhibited no significant changes in the relative weights of these organs. Diabetic rats treated with glibenclamide showed a slight but notable increase ($P \leq 0.05$) in the relative weights of the testes and epididymides compared to the diabetic control group.

Administration of MOLE at a dose of 500 mg/kg body weight led to a remarkable increase ($P \leq 0.01$) in the relative weight of the vas deferens compared to diabetic control rats, while the low and medium doses showed no significant effect. Diabetic rats treated with glibenclamide also demonstrated a notable increase ($P \leq 0.05$) in the relative weight of the vas deferens compared to untreated diabetic controls.

In diabetic rats, treatment with MOLE at doses of 250 and 500 mg/kg body weight significantly increased

the relative weight of the ventral prostate (P≤0.05 and P≤0.01, respectively) compared to untreated diabetic control rats, while the low dose showed no significant effect. Likewise, diabetic rats treated with glibenclamide exhibited a remarkable increase (P≤0.01) in the relative weight of the ventral prostate compared to the untreated diabetic control rats.

In STZ-induced diabetic rats, the relative weight of the seminal vesicle was significantly reduced (P ≤ 0.001) compared to normal controls. Treatment with MOLE at doses of 250 and 500 mg/kg resulted in a notable elevation in the relative weight of the seminal vesicles (P ≤ 0.05 and P ≤ 0.001, respectively), while the lower dose of 100 mg/kg had no significant effect compared to diabetic control rats. Diabetic rats administered glibenclamide exhibited a notable increase (P ≤ 0.01) in the relative weight of the seminal vesicles compared to the diabetic control group.

3.2 Tissue Biochemistry

Table 2 presents the results of biochemical parameter analyses across various tissue samples in the different experimental groups.

In the untreated diabetic rats, protein content in both the testis and epididymis was significantly reduced (P≤0.001). However, treatment with MOLE at doses of 100, 250, and 500 mg/kg body weight led to a significant increase in protein levels in both organs (P≤0.05, P≤0.01, and P≤0.001, respectively). Diabetic rats administered glibenclamide also demonstrated a significant rise (P≤0.001) in protein contents in the testis and epididymis compared to diabetic controls.

Streptozotocin treatment caused a highly remarkable decrease (P≤0.001) in glycogen concentration in both the testis and epididymis compared to normal controls. However, administration of medium (P≤0.01) and high doses (P≤0.001) of MOLE significantly elevated

Table 1. Effect of MOLE on reproductive organ weight in STZ-induced diabetic rats

Treatment	Testes		Epididymides		Vas deferens		Seminal vesicles		Ventral prostate	
	A (mg)	R (mg/100g b.wt.)	A (mg)	R (mg/100g b.wt.)	A (mg)	R (mg/100g b.wt.)	A (mg)	R (mg/100g b.wt.)	A (mg)	R (mg/100g b.wt.)
Group I Normal Control	2856.00± 68.08	1223.52± 21.74	1226.67± 31.77	525.50± 10.44	367.50± 10.68	157.40± 3.45	1174.17± 13.88	503.59± 9.45	626.00± 18.40	267.44± 5.74
Group II Diabetic Control	1593.00± 65.70***	1053.36± 40.77**	677.33± 38.24***	448.34± 21.17**	197.00± 13.57***	129.83± 6.92**	593.83± 12.86***	393.33± 11.83***	350.33± 14.45***	231.40± 7.64**
Group III Diabetic+Extract (100mg/kg)	2100.33± 64.79 ^{b}	1102.76± 25.64^{ns}	891.17± 23.20 ^{b}	467.96± 8.32 ^{ns}	262.17± 10.90 ^{b}	137.54± 4.42 ^{ns}	776.00± 23.12 ^{b}	407.42± 9.02 ^{ns}	446.33± 13.63 ^{b}	234.51± 6.73 ^{ns}
Group IV Diabetic+Extract (250mg/kg)	2359.00± 80.25 ^{c}	1197.88± 16.64^{a}	988.50± 35.74 ^{c}	501.88± 8.43 ^{a}	290.67± 11.25 ^{c}	147.56± 3.13 ^{ns}	860.00± 32.38 ^{c}	436.62± 8.97 ^{a}	506.67± 19.86 ^{c}	257.21± 5.46 ^{a}
Group V Diabetic+Extract (500mg/kg)	2512.33± 94.24 ^{c}	1212.84± 30.27 ^{b}	1065.67± 33.39 ^{c}	514.68± 9.68 ^{b}	318.33± 11.54 ^{c}	153.73± 3.75 ^{b}	961.33± 30.92 ^{c}	464.48± 11.72 ^{c}	548.67± 12.94 ^{c}	265.19± 4.28 ^{b}
Group VI Diabetic+ Glibenclamide (5mg/kg)	2422.17± 109.04 ^{c}	1195.95± 30.24 ^{a}	1024.00± 42.49 ^{c}	505.50± 11.24 ^{a}	303.67± 14.84 ^{c}	149.77± 4.67 ^{a}	916.00± 33.57 ^{c}	452.49± 8.54 ^{b}	530.50± 19.58 ^{c}	261.99± 4.53 ^{b}

"Values are presented as mean ± SEM (n=6), Levels of significance: ** = P ≤ 0.01; *** = P ≤ 0.001, for comparisons between diabetic control rats and normal control rats. Ns = not significant; a = P ≤ 0.05; b = P ≤ 0.01; c = P ≤ 0.001, for comparisons between MOLE or glibenclamide-treated rats and diabetic control rats. Abbreviations; A-Absolute; R-relative"

Table 2. Effect of MOLE on tissue biochemical parameters in STZ-induced diabetic rats

Treatment	Protein (mg/gm)		Glycogen (mg/gm)		Cholesterol (mg/gm)		Sialic acid (mg/gm)		Acid phosphatase (IU/g tissue)		Alkaline phosphatase (IU/g tissue)		Fructose (mg/gm)
	T	E	T	E	T	E	T	E	T	E	T	E	SV
Group I Normal Control	202.96 ±6.83	218.50 ±8.70	3.54 ±0.13	3.34 ±0.19	6.44 ±0.33	6.03 ±0.23	4.95 ±0.18	5.60 ±0.40	2.94 ±0.15	2.80 ±0.14	31.83 ±1.61	27.30 ±1.81	5.85 ±0.26
Group II Diabetic Control	139.17 ±5.53***	156.33 ±5.92***	2.45 ±0.15***	2.10 ±0.13***	9.28 ±0.30***	8.65 ±0.23***	3.05 ±0.20***	3.22 ±0.16***	2.08 ±0.13**	1.92 ±0.11**	16.26 ±1.02***	13.32 ±.94***	3.85 ±0.27***
Group III Diabetic+ Extract (100mg/kg)	166.50 ±4.53[a]	188.67 ±6.02[a]	2.90 ±0.19[ns]	2.43 ±0.11[ns]	8.01 ±0.23[a]	7.49 ±0.29[ns]	3.69 ±0.26[ns]	4.08 ±0.15[a]	2.41 ±0.16[ns]	2.15 ±0.12[ns]	21.01 ±1.74[ns]	17.77 ±1.48[ns]	4.60 ±0.21[ns]
Group IV Diabetic+ Extract (250mg/kg)	178.33 ±6.82[b]	196.67 ±8.63[b]	3.17 ±0.14[b]	2.87 ±0.15[b]	7.59 ±0.28[b]	7.07 ±0.32[b]	4.12 ±0.18[b]	4.42 ±0.22[b]	2.70 ±0.13[a]	2.53 ±0.14[a]	24.66 ±1.25[b]	21.15 ±1.20[b]	5.18 ±0.24[b]
Group V Diabetic+ Extract (500mg/kg)	189.17 ±6.18[c]	206.83 ±8.28[c]	3.36 ±0.13[c]	3.11 ±0.15[c]	6.83 ±0.21[c]	6.38 ±0.29[c]	4.65 ±0.20[c]	5.17 ±0.16[c]	2.88 ±0.14[b]	2.74 ±0.16[b]	28.11 ±1.50[c]	24.08 ±1.57[c]	5.55 ±0.29[c]
Group VI Diabetic+ Glibenclamide (5mg/kg)	186.33 ±7.35[c]	208.83 ±6.61[c]	3.26 ±0.10[b]	3.12 ±0.12[c]	7.15 ±0.28[c]	6.56 ±0.32[c]	4.45 ±0.18[c]	5.02 ±0.24[c]	2.82 ±0.09[b]	2.68 ±0.15[b]	27.25 ±1.33[c]	23.46 ±1.68[c]	5.40 ±0.24[b]

"Values are presented as mean ± SEM (n=6), Levels of significance: ** = P ≤ 0.01; *** = P ≤ 0.001, for comparisons between diabetic control rats and normal control rats. Ns = not significant; a = P ≤ 0.05; b = P ≤ 0.01; c = P ≤ 0.001, for comparisons between MOLE or glibenclamide-treated rats and diabetic control rats. Abbreviations; T-Testes; E-Epididymis; SV-Seminal Vesicle"

glycogen levels in these organs, while the low-dose group did not show a significant increase. Diabetic rats treated with glibenclamide exhibited a remarkable increase in glycogen concentration in both the testis (P≤0.01) and epididymis (P≤0.001) compared to diabetic controls.

Cholesterol levels in both the testis and epididymis were significantly elevated (P≤0.001) in STZ-induced diabetic rats compared to normal controls. MOLE treatment at doses of 250 and 500 mg/kg remarkably reduced cholesterol levels in both organs (P≤0.01 and P≤0.001, respectively), while the low-dose group showed a slight reduction (P≤0.05) in testicular cholesterol without significant changes in epididymal cholesterol. Glibenclamide treatment significantly restored cholesterol levels in both the testis and epididymis (P≤0.001) compared to diabetic controls.

In diabetic control rats, there was a remarkable reduction (P≤0.001) in sialic acid levels in both the testis and epididymis compared to normal controls. Administration of MOLE at doses of 250 and 500 mg/kg resulted in a notable increase in sialic acid levels in both organs (P≤0.01 and P≤0.001, respectively) compared to the diabetic controls, while the 100 mg/kg dose led to a significant increase (P≤0.05) only in the epididymis. Diabetic rats treated with glibenclamide also showed a significant elevation (P≤0.001) in sialic acid concentration in both the testis and epididymis relative to the diabetic controls.

In diabetic control rats, acid phosphatase activity in both the testis and epididymis was notably reduced (P≤0.01) compared to normal controls. The administration of MOLE at doses of 250 and 500 mg/kg led to a notable increase in acid phosphatase activity

(P≤0.05 and P≤0.01, respectively) in both organs, while the low-dose group showed no significant change. Treatment with glibenclamide also significantly elevated acid phosphatase activity (P≤0.01) in both the testis and epididymis compared to diabetic controls.

A significant decrease (P≤0.001) in alkaline phosphatase activity was observed in both the testis and epididymis of diabetic control rats compared to normal controls. Treatment with MOLE at 250 and 500 mg/kg doses significantly increased alkaline phosphatase activity (P≤0.01 and P≤0.001, respectively) in both organs, while no significant change was observed in the low dose group. Glibenclamide treatment also caused a highly significant increase (P≤0.001) in alkaline phosphatase activity in both the testis and epididymis compared to diabetic controls.

Streptozotocin administration led to a highly significant reduction (P≤0.001) in seminal vesicle fructose concentration compared to normal controls. MOLE treatment at medium (P≤0.01) and high doses (P≤0.001) significantly elevated the fructose concentration in diabetic rats, Meanwhile, the low-dose group exhibited no significant increase. Diabetic rats treated with glibenclamide also exhibited a remarkable increase (P≤0.01) in seminal vesicle fructose content compared to diabetic controls.

4. Discussion

Measuring the weight of testicular and accessory sex organs is a critical endpoint for evaluating the antispermatogenic and antiandrogenic effects of test compounds[32]. In this study, streptozotocin-induced diabetic control rats showed a significant decrease in the relative weight of the testes, epididymides, vas deferens, seminal vesicles, and ventral prostate gland. These findings align with previous research that observed similar reductions in the relative or absolute weight of these organs in experimental-induced diabetic rats[33-36]. The observed weight reduction in these organs is likely due to decreased serum testosterone levels in diabetic conditions, as testosterone is essential for maintaining the structural and functional integrity of the testis and accessory sex organs[35]. Insulin deficiency in diabetes reduces serum testosterone levels by inhibiting LH and FSH production[37,38].

The weight and size of the testes are typically maintained by fluid secretion from Sertoli cells and the mass of mature spermatogenic cells[39]. In diabetic rats, a decrease in the absolute and/or relative weight of the testes may stem from the loss of spermatids, spermatozoa, and Sertoli cells, as well as reduced steroid biosynthesis by Leydig cells[40]. The observed reduction in epididymal weight may be linked to decreased levels of androgens and spermatozoa in the epididymal lumen. The lower weight of the vas deferens might be due to a reduced number of spermatozoa and degenerative changes in the duct, Lack of androgens can also be held responsible for this decline in vas deferens weight[41].

The weight of the seminal vesicle and prostate glands are used in hormonal bioassay of androgen activity[42,43]. A marked reduction in the relative weight of accessory reproductive glands in diabetic rats suggests glandular tissue atrophy, decreased secretory function, and an inadequate androgen supply[44].

Treatment of diabetic rats with MOLE leads to a significant improvement in the absolute and/or relative weights of the testes and other accessory sex organs. This effect may result from enhanced glycemic control and redox balance, which help restore spermatogenic and steroidogenic functions. These results are also supported by many studies which have reported significant improvement in reproductive organ weight in diabetic[45] and normal rats[46] as well as in mice[47] treated with *Moringa oleifera* leaves extract. Diabetic rats administrated with glibenclamide also showed an ameliorative effect on the testes and accessory reproductive organ weight. These results are also compatible with earlier findings[33,48,49].

Biochemical analysis of marker parameters offers insight into the structural and functional condition of organs. In the present investigation, STZ-induced diabetic control rats exhibited a remarkable reduction in testicular protein content compared to control rats. These results are consistent with previous research that reported a similar decrease in testicular protein content in diabetic rats[50,51]. This reduction in testicular protein levels may be due to decreased testosterone levels and testicular secretory activity, disruptions in protein synthesis or metabolism, or a reduced number of germ cells and spermatozoa in the testes[52,53]. The total protein concentration in epididymis was also reduced significantly in diabetic control rats. The decline of the protein concentration in epididymis

suggests an under-supply of androgens, loss of spermatozoa and secretary activity of epithelium. The principal cells of the epididymal epithelium synthesize and secrete various types of proteins under the influence of androgens that ensure the maturation of spermatozoa[54,55]. The reduction in epididymal protein concentration may impact the internal environment of the epididymis, thereby affecting sperm maturation. Treatment of diabetic rats with MOLE/glibenclamide showed significant improvement in the total protein concentration of testosterone level, spermatogenesis and secretary functions of these organs. These results are compatible with Shah and Khan, (2014) who also reported significant improvement in the concentrations of testicular total proteins in diabetic rats treated with glibenclamide[50].

In the present study, the concentration of glycogen in diabetic control rats was significantly reduced in both testis and epididymis. These results agree with earlier studies in which a similar decline of glycogen content was reported in the testis of diabetic rats[56,57]. The reduced glycogen contents in the testis and epididymis were restored in diabetic rats following MOLE/ glibenclamide treatment. This might be due to the restoration of insulin secretion and glucose homeostasis in the blood. These findings are consistent with previous studies where significant improvement of glycogen content in the testis of diabetic rats has been observed after treatment of plant extracts possessing antidiabetic activity or glibenclamide[56,57].

Cholesterol serves as an obligatory precursor for steroid biosynthesis in gonads and adrenal cortex 58,59. In the present study, a significant increase in cholesterol content was observed in both the testes and epididymis of diabetic control rats. When compared, these observations align with previous findings that reported a similar increase in testicular cholesterol content in diabetic rats[33,60,61]. The augmented cholesterol content could be due to impairment of androgen biosynthesis due to reduced LH secretion by insulin deficiency in the diabetic state. Furthermore, reduced levels of testosterone and impairment of steroidogenic enzyme activity in Leydig cells have already been reported in diabetic rats[35,56].

The increased cholesterol levels found in the epididymis of diabetic rats may be associated with disturbances in cholesterol metabolism or transport. Additionally, reduced utilization of cholesterol by spermatozoa could be a contributing factor, as this corresponds with the reduced sperm count seen in the present study. Treatment of diabetic rats with MOLE or glibenclamide effectively inhibited cholesterol buildup in both the testis and epididymis, likely due to the restoration of steroidogenesis and spermatogenic activity facilitated by the phytoconstituents in the extract. Previous studies have similarly reported the beneficial effects of plant extracts and glibenclamide on testicular cholesterol levels in diabetic rats[33,60,61].

Sialic acid is essential for preserving the structural stability of the sperm membrane and promoting sperm maturation[62]. Its level in the testis and epididymis are associated with androgen concentrations[63]. This study recorded a significant reduction in sialic acid levels in both the testis and epididymis of diabetic control rats, which may be attributed to testosterone deficiency in the diabetic state. These results align with previous findings that also reported a significant decline in sialic acid concentration in the testis[64] and epididymis[7] of diabetic rats. Treatment with MOLE or glibenclamide significantly increased sialic acid levels in both the testis and epididymis, likely through enhanced serum sex hormone levels and reduced oxidative stress.

A marked reduction in acid phosphatase (ACP) activity was also observed in the testis and epididymis of diabetic control rats compared to normal controls. The decreased ACP activity in the testis may reflect testicular degeneration, likely due to reduced steroidogenesis and heightened oxidative stress, which may be correlated with decreased gonadotropin secretion in the diabetic state (caused by insulin deficiency). The reduced ACP activity in the epididymis could be linked to a lower number of spermatozoa and their decreased activity. These findings are consistent with several studies that have reported a decline in ACP activity in the testes of diabetic rats[65,66], although some studies have reported an increase in ACP activity[67], potentially due to differing treatment durations. Treatment with MOLE or glibenclamide significantly restored ACP activity in both the testis and epididymis, likely due to improved steroidogenic and gametogenic functions facilitated by the phytoconstituents in these extracts.

In the present study, diabetic control rats showed a significant decline in the activity of alkaline phosphatase (ALP) in both testis and epididymis as compared to

normal control rats. These findings are consistent with Atta *et al.*,[65] who also observed a significant decline in testicular ALP activity in diabetic rats. However, other workers have reported an increase in ALP activity in diabetic rats[66,68]. This discrepancy in results might be due to the different duration of the experiment. The reduced alkaline phosphatase activity in the epididymis of diabetic control rats may result from a degenerative alteration in the epididymal epithelium and reduced spermatozoa count in the lumen. The reduced alkaline phosphatase activity level in both testis and epididymis was significantly improved following MOLE/glibenclamide treatment. This might be due to improved sex hormone secretion, improved spermatogenic activity and amelioration of antioxidant status and insulin secretion[65].

Fructose is secreted from the seminal vesicle and other sex accessory glands. Fructose synthesis and secretion by seminal vesicle depends upon the secretion of testosterone from the Leydig cells of the testis[51,69-71]. The fructose concentration in seminal vesicles was significantly reduced in diabetic control rats. These findings are consistent with previous reports that have demonstrated a notable decrease in seminal vesicle fructose content in diabetic rats[48,56,66,72]. Treatment with MOLE or glibenclamide in diabetic rats significantly enhanced fructose levels in the seminal vesicle. This observed increase in fructose content in seminal vesicles might be correlated with improved testosterone levels, spermatogenic activity and increased insulin secretion by the bioactive phytoconstituents present in the extracts. These results are further supported by the findings of other researchers who have also reported restoration of fructose levels in the seminal vesicles of diabetic rats after treatment with glibenclamide plant extracts having antidiabetic activity[33,56,66,72].

5. Conclusion

The findings from the present study indicated the ameliorative effect of MOLE on diabetes-induced biochemical alterations in testicular and epididymal tissue and improved reproductive organ weight. The possible mechanisms of this action might be due to their antidiabetic and antioxidative effects.

6. Acknowledgement

The authors express their gratitude to the Head of the Department of Zoology at the University of Rajasthan, Jaipur, for providing the essential facilities.

7. Author Contribution

Dr. Ram Niwas Jangir: The author has conducted the experimental work, 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. All authors have read and agreed to the published version of the manuscript.

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