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PROTECTIVE ROLE OF *ERUCA SATIVA* EXTRACT AGAINST TESTICULAR DAMAGE IN STREPTOZOTOCIN-DIABETIC RATS

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ABSTRACT

Infertility is one of the major human health problems all over the world. *Eruca sativa* (ES, Brassicaceae) salad species are reported to have natural antioxidants like vitamin C, carotenoids and polyphenols. It exhibits antiphlogistic, diuretic, digestive, aphrodisiac and rubefacient activities. The present study evaluates the acute toxicity of ES in mice and its effect on testicular damage and fertility in male diabetic rats. Total methanolic extract of *Eruca sativa* (MEES) leaves was administered orally to mice to explore its acute toxic effect (LD_{50}). For fertility experiment, animals were rendered diabetic by single injection of streptozotocin (55 mg/kg, i.p.). Diabetic rats were treated with MEES (250 and 500 mg/kg, orally) for 8 consecutive weeks. Male fertility was evaluated by estimating genital organs weight, gonadosomatic index (GSI), epididymal sperm characters, serum testosterone, testicular thiobarbituric acid reactive substance (TBARS) and testicular glutathione (GSH) activity. Fertility parameters were further confirmed by histopathological examination of testes. The results have clearly depicted that the impact of diabetes was significantly ($p<0.01$) ameliorated by MEES administration in term of increased genital organs weight (testes 192%, epididymis 177%, seminal vesicles 378% and prostate 259%), sperm count (477%), sperm motility (248%) and sperm viability (168%). MEES also significantly increase the serum testosterone (428%), testicular GSH (250%) and decreased the testicular TBARS (37%) levels. The present study suggested that ES might have an ameliorative effect against testicular functions impaired by oxidative stress in diabetic male rats.

Keywords: Fertility, Semen Analysis, Diabetes, Testosterone, Acute Toxicity

INTRODUCTION

Infertility is one of the major health problems in life, and usually male factor is responsible for this problem **[1]**. Arab and other Islamic countries have high percentage of infertility may be due to the ancient habits and traditions, and for the reason that they refuse for medical consultation, if they have reproductive problems. Some pathogenic conditions and factors such as coronary heart diseases; diabetes mellitus; chronic liver diseases; chronic smoking; insecticides and industrial contaminants; air pollutants and, insufficient intake of vitamins have been reported to cause harmful effects on fertility **[2]**.

Diabetes mellitus greatly affects the male sexual and reproductive functions in humans as well as in animals **[3, 4]**. Decreased testosterone levels, impaired testicular function, and spermatogenic disruption has been observed in the testes of diabetic men and experimental animals, which may lead to impotence, decreased sperm motility and semen volume. It clearly means that diabetes has a significant impact on the men fertility **[5-7]**. Oxidative stress and decreased antioxidant capacity are considered to play an important role in the chronic diabetes mellitus pathogenesis **[8]**. Previously, some studies reported that stability of testicular blood barrier can be increased by antioxidants; vitamins E and C, and sperm DNA can be protected from active free radicals induced-oxidative stress **[9]**.

Eruca sativa (family, Brassicaceae) is an annual edible plant originated in the Mediterranean province, but now a day's found all around the world. It is widely consumed in many countries, e.g. Italy, Saudi Arabia etc. ES salad species are reported to have vitamins, like vitamin C, carotenoids, and polyphenols, which play an important role among natural antioxidants **[10]**. The ES leaves are most widely used in salads. Since last more than 20 centuries, a number of health-promoting or therapeutic properties have been reported for *Eruca*, such as antiphlogistic, depurative, diuretic, digestive, aphrodisiac, and rubefacient **[11, 12]**. Antidiabetic effect of ES seeds has been reported in chemically induced diabetic rats by reducing oxidative stress **[13]**. In previous studies, pharmacological activities of rockets plants have been moderately related to their strong antioxidant properties **[14, 15]**. Therefore, the present study was planned to evaluate the acute toxicity (LD_{50}) of MEES in mice, and to examine reproductive efficiency of *Eruca* leaves on male diabetic rats.

MATERIALS AND METHODS

Plants Collection and Extract Preparation

The ES fresh leaves were purchased in October, 2013, from Al-Kharj local vegetable market, Saudi Arabia, and identified by expert taxonomist Mr. Osman Ali Elmakki. ES leaves were shade dried, coarsely pulverized, and placed in glass percolator with methanol at room temperature for 72 h (percolation method). The collected percolate was dried under reduced pressure *in vaccuo*. The obtained methanolic extract of *Eruca sativa* (MEES) was later used after suspending in the vehicle (3% v/v Tween 80 in distilled water) for further acute toxicity, and fertility studies.

Animals

Sexually mature male Wistar albino rats, approximately of same age (8-10 weeks), weighing 180-200 g, and Swiss albino mice weighing 25-30 g, were procured from the Laboratory Animal Care Unit, College of Pharmacy, Salman bin Abdulaziz University, Saudi Arabia. Mice were used for acute toxicity studies, and rats for fertility study. Animals were acclimatized for one week, at constant temperature (22 ± 2°C), humidity (55 ± 1%), and light-dark conditions (12 / 12 h light/dark ratio). Standard rodent diet was given to the animals, and drinking water *ad libitum.*

Acute Toxicity Test

Different groups of mice (n=6) were fasted overnight then treated orally with MEES at different doses (50–5000 mg/kg). Another control group received the vehicle (3% v/v Tween 80 in distilled water), and kept under the same conditions. Animals were observed for gross behavioral changes, and mortalities till 48h, and the LD_{50} were calculated **[16]**.

Justification for dose selection

MEES was nontoxic at the dose of 5000 mg/kg so, $1/20^{th}$, and $1/10^{th}$ of this dose (250 and 500 mg/kg, respectively) were selected for the study.

Induction of diabetes

Diabetes was induced in overnight-fasted Wistar albino rats by a single intraperitoneal injection of STZ (55 mg/kg) in citrate buffer (0.1 M, pH 4.5) **[17]**. Diabetic rats were confirmed by measuring the fasting blood glucose level 72 h after STZ injection. Animals with a blood glucose level above 300 mg/dl were considered diabetic, and included in the experiment.

Male Fertility Experiment

Twenty four sexually mature male Wistar albino rats (180–200 g) were randomly divided into four equal groups (n=6). The 1st group received the vehicle (2.5 mL/kg), and kept as normal control. Rats of the 2^{nd} - 4^{th} groups were rendered diabetic by a single dose of STZ (55 mg/kg, i.p.). The 2^{nd} group was kept as diabetic control, while the 3^{rd} and 4^{th} groups received MEES at doses of 250 and 500 mg/kg, respectively. Both

vehicle and MEES were administered to animals by oral intubation for 8 consecutive weeks. This administration period is necessary for completion of the spermatogenic cycle and maturation of sperms in epididymis **[18]**. At end of the experiment, blood samples were collected from orbital plexus of veins for estimation of glucose and testosterone levels.

Blood glucose and testosterone levels were estimated using commercial diagnostic kits and ELISA kits **[19]**, respectively.

One g of testicular tissue was homogenized in 9 volume of phosphate buffer and centrifuged at 12000 × *g* for 30 min at 4°C. The supernatant was collected and used for estimation of TBARS **[20]** and GSH **[21]**.

Initial and final body weights of male rats were recorded and subsequently weight changes were calculated. Rats were sacrificed under ether anesthesia and the testes, epididymis, seminal vesicles and ventral prostate were dissected out, trimmed off the attached tissues and weighed individually on electronic balance. Then, the gonadosomatic index (GSI) was calculated as: (organ weight/body weight) × 100.

The right cauda epididymidis was nicked in few sites with a scalpel blade to release the spermatozoa from the tubules on a pre-warmed slide. Two drops of warm 2.9% sodium citrate was added to semen and mixed by a cover-slip. The progressive sperm motility was evaluated within 5 min at 400× magnification from three different fields in each sample. The calculated results were finally expressed as percent motility **[22]**.

For sperm count, the left cauda epididymidis was incised and semen that oozed was quickly sucked into a red blood pipette to the 0.5 mark, and then diluted with physiological saline (0.9% NaCl) up to the 101 mark. A drop of the semen mixture was placed on the Neubauer counting chamber and viewed under the magnification of ×40. Each sample was counted two times and means value was calculated. The total numbers of sperm cells were counted and expressed as 10^6/mL **[22]**.

A viability (percentage of live spermatozoa) study was also done using stain (eosin and nigrosin). Thin smears of stained semen were prepared and observed under a light microscope at ×400 magnification. Viable sperm remained colorless while non-viable sperm stained red. For the percentage viability, the stained and the unstained sperm cells were counted and mean value for each was recorded. The slides were stained with eosin–nigrosin (5 slides/ rat) and observed under a light microscope at 400× magnifications to determine the percentage of morphologically abnormal spermatozoa. Sperm cells (total 300 cells) were examined on each slide (1500 cells for

each rat), for the head, tail and total abnormality rates of spermatozoa.

One testis from each rat was fixed in freshly prepared formalin solution (10%). The fixed specimens were then processed, cleared in xylene and embedded in paraffin. Sections were cut at 4–6 μm thickness and stained with Hematoxylen and Eosin (H&E) for histological examination.

Statistical Analysis

Data are expressed as means ± SEM. Statistical analysis was done by using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test for comparisons in different treatment groups. $P<0.05$ was considered to be statistically significant. Statistical analysis was performed using SPSS program (version 8) software package (SPSS_ Inc., USA).

RESULTS

No mortalities observed with oral administration of MEES during 48h of observation even at the highest dose (5000 mg/kg). The tested extract did not produce any symptom of acute toxicity and none of the mice exhibit hyperactivity, convulsions, sedation, hypothermia, and respiratory distress. Accordingly, it suggested that oral LD_{50} of MEES is higher than 5 g/kg b.wt.

The mean level of glucose in STZ-induced diabetic rats (546.6 ± 17.47 mg/dL) was significantly higher at the time of sacrifice than normal control group (115.67 ± 12.35 mg/dL). Administration of MEES at doses of 250 and 500 mg/kg to rats for 8 weeks reduced the severity of STZ-induced hyperglycemia **(Table 1)** in a dose dependent manner (351.33 ± 16.83 and 318.00 ± 12.86 mg/dL, respectively). The results presented in Table 1 showed that STZ significantly decreased the serum testosterone level (2.42 ± 0.16 ng/mL) as compared to normal control group (10.22 ± 0.15 ng/mL). Serum testosterone level of diabetic animals treated with MEES at doses of 250 and 500 mg/kg for 8 consecutive weeks showed significant increase (8.96 ± 0.24 and 10.36 ± 1.37, respectively) when compared with that of the diabetic controls.

The results presented in **Table 1** showed that oxidative stress induced by STZ significantly increased the testicular TBARS and decreased the testicular GSH level. The testicular TBARS was significantly declined while the testicular GSH activity was significantly elevated following MEES medication of diabetic rats at doses of 250 and 500 mg/kg for 8 weeks as compared with those of diabetic control rats.

Effect of oral administration of MEES to rats for 8 weeks at levels of 250 and 500 mg/kg on body and genital organs weights are recorded in **Table 2**. The baseline weight of the rats at the beginning of the study was similar in all groups. At the end of the study period (8 weeks), all rats except

diabetic controls increased in their final body weight but the maximum increase was found in normal control animals (16.67%). Diabetic control rats showed the highest decrease in their final body weight (-4.99%). While, diabetic rats treated with MEES at levels of 250 and 500 mg/ kg showed improvement in their final weights (6.33 and 10.91%, respectively). The absolute weights and GSI of the testes, epididymis, ventral prostate and seminal vesicles were reduced dramatically in diabetic control rats as compared to the normal control group. However, statistically significant improvement in the absolute weights and GSI were noted in MEES treated groups as compared to the diabetic control rats.

As shown in **Table 3**, the sperm count was significantly reduced in diabetic control rats (12.4×10^6/mL) compared to normal controls (74.6×10^6/mL). The epididymal spermatozoa of MEES-treated rats at doses of 250 and 500 mg/ kg exhibited gradual improvement in sperm count in a dose-dependent manner (47.5 and 59.2×10^6/mL, respectively). In control diabetic rats, the percentage of sperm motility was drastically reduced (26.5%) while the percentage of sperm abnormality was significantly increased (46.6%) comparatively to normal controls (88.4% and 10.3%, respectively). The most common abnormalities seen in the examined seminal smears of diabetic rats were headless sperms, bent tail and coiled tail (Figure 1). In comparison with the diabetic control rats, MEES at doses of 250 and 500 mg/kg, significantly elevated the percentage of sperm motility (58.9 and 65.8%, respectively) and reduced the percentage of sperm abnormality (13.8 and 12.9%, respectively). There was a significant decrease in the percentage of sperm viability of diabetic control rats (52.7%) compared to normal controls (91.4%). Both doses of MEES improved sperm viability of diabetic rats into 80.3 and 88.5%, respectively.

Histopathology of testes of the normal control rats showed normal basement membrane with normal round or oval seminiferous tubules in addition to normal germ cells at various stages covering complete spermatogenic cycle **(Figure 2-A)**. Testis of the diabetic control rats showed severe degenerative changes in most seminiferous tubules which became atrophied. Other tubules were completely free from spermatozoa. Widespread immature germinal cells were present in the seminiferous tubular lumen of the diabetic control group **(Figure 2-B)**. MEES at doses of 250 and 500 mg/kg for 8 consecutive weeks restored these changes towards normality **(Figure 2-C and 2-D)**.

DISCUSSION

In the current study, no acute toxicity and mortality was observed in mice after oral administration of MEES at doses up to 5000 mg/kg during 48 h of observation. Accordingly, oral LD_{50} of the MEES was found to be higher than 5000 mg/kg. Therefore, it can be said that ES plant is highly safe since substances with LD_{50} higher than 50 mg/kg are non-toxic **[23]**. The non-toxic nature of ES as observed in acute toxicity study in mice is well supported in rats following medication for 8 weeks in male fertility study. Treatments with 250 and 500 mg/kg of MEES were well tolerated by all the animals, as there were no toxic effects or mortalities observed throughout the experiment.

Several reports showed that reproductive functions are noticeably exaggerated by diabetes mellitus which can lead to reduced fertility **[24]**. The STZ-induced diabetes is most commonly used model in the literature, relating changes in the male reproductive tract as part of the disease **[25]**. In target organs of diabetic patients, hyperglycemia may leads to structural and functional changes **[26]**. In present study, diabetic rats found to have significantly increased mean blood glucose level. The increased level of reducing sugars that result due to the oxidative stress of hyperglycemia can easily react with lipids and proteins to produce reactive oxygen species (ROS) which leads to the development of diabetic complications **[27]**. In STZ-diabetic rats, ROS impair Leydig cell function and decreases testosterone level **[28]**.

The main reason for the male infertility is oxidative stress, and an increased level of seminal ROS was reported in a large number of infertile men **[29]**. Plasma membranes of spermatozoa are highly susceptible to ROS-induced damage, due to high content of polyunsaturated fatty acid **[30, 31]**. The present investigation showed that STZ-induced hyperglycemia lead to an increased oxidative stress in testes, which was manifested by an increase in TBARS level and decrease in antioxidant GSH. These observations corroborate with the previous findings showing an increase in testis TBARS level and decrease in the testicular antioxidant activity of GSH in the STZ-diabetic rats **[32]**. Our study demonstrated that oral administration of MEES to STZ- hyperglycemic rats significantly neutralized these effects due to its antioxidant effect as evidenced by significant increase of GSH and reduced TBARS levels in their testicular homogenate. The present study results depicted that MEES treatment results in reduced oxidative stress and may have a therapeutic role in ROS mediated pathogenic conditions. Accordingly, the

effect of MEES on the semen analysis may be depend on the plant nature that has several antioxidant constituents including glucosinolates, flavonoids and carotenoids **[14]**.

Testosterone in humans or androstenedione in animals are synthesized in the Leydig cells. It has been reported that testosterone initiate and maintain spermatogenesis in seminiferous tubules **[33]**. Navarro-Casado *et al*., **[25]** reported decreased serum testosterone levels in STZ-treated diabetic rats when compared with normal controls rats. This report concurs with our results in which diabetic control rats exhibited a significant decrease in serum level of testosterone. This decrease might be due to testicular oxidative stress induced by STZ-hyperglycemia. Testicular oxidative stress causes reduction in testicular testosterone production, either as a result of injury on the Leydig cells or on endocrine structures, such as the anterior pituitary **[34]**. MEES at doses of 250 and 500 mg/kg to diabetic rats improved the serum level of testosterone toward the normal control value. The improvement of testosterone level by MEES may be adduced to stimulation of the hormone synthesis by the Leydig cells **[35]**.

Body weight measurement provides knowledge on the general health of animals, and it can play an important in elucidation of reproductive effects **[36]**. In this study, the final body weight of diabetic control rats was severely reduced after 8 weeks of STZ injection. In diabetic rats, tissue proteins breakdown may be one reason for the decreased body weight **[37]**. The final body weights of diabetic rats that medicated with MEES at 250 and 500 mg/kg were not altered indicating that MEES protected the animals against the deleterious effect of STZ on body weight. Creasy **[38]** has reported that weights of testes and accessory sex organs are important that can be used in evaluation of negative effect on male reproduction. Additionally, our results demonstrated that in diabetic control rats, a significant reduction in testes weight and the accessory reproductive glands was observed as compared to normal control rats. This result indicated that STZ-induced hyperglycemia caused structural alteration in the reproductive organs of male rats. Reductions of body, testicular and accessory sex organs weights in diabetic control animals, observed in this study, have already been reported in hyperglycemic animals **[39, 40]** and can be associated with hormonal and metabolic changes observed in diabetic animals and human beings. It has also been demonstrated that maintenance of the weights of the accessory reproductive glands depends on testosterone level **[41]**. Accordingly, the significant increase in the reproductive organ weights of diabetic rats

that medicated with both doses of MEES in this study may be attributed to the improvement in testosterone levels. In addition, Zitzmann **[42]** have demonstrated that physiologic concentration of testosterone play an important role in spermatogenesis, so a significant increase of testosterone levels following MEES treatment could increase the number and function of somatic and germinal cells of testis followed by testis weight improvement.

Diabetes may leads to insufficient production of spermatozoids by causing gonadal dysfunction and decreased testosterone production **[43]**. Moreover, sperm motility and count are considered as the important factors that affect male fertility **[44]**. In this investigation, the reduction in epididymal sperm motility and count of the diabetic control rats could be connected to the reduction in the serum testosterone level. We corroborate with Hassan et al., **[45]** and Scarano et al., **[40]** who reported epididymal oligospermia and reduced blood testosterone level in streptozotocin-induced hyperglycemia in rats. Gong & Han **[46]** confirmed this explanation as they stated that decreased epididymal sperm motility and count suggested a restriction of testosterone to the epididymis. In addition, high levels of ROS adversely affect normal sperm motility and viability as a result of polyunsaturated fatty acids lipid peroxidation in the head and mid-piece **[47]**. Similarly, germinal sperm cells are highly sensitive and easily compromised by high levels of ROS **[48]**.

Dosing of MEES (250 and 500 mg/kg) to STZ-diabetic rats for 8 weeks has been found to reduce their testicular ROS and restore normal sperm motility and count. The increased sperm count and motility noted in MEES-treated groups may be attributed to the improvement in testosterone levels. In another explanation, Ganong **[49]** mentioned that seminal vesicle secretes fructose, phosphorylcholine, ergothioneine and prostaglandins that are responsible for enhancing motility of sperm. Accordingly, the improvement weight and histological structure of seminal vesicle is responsible for the increased sperm motility observed in MEES-treated groups. The deleterious effect of diabetes on sperm viability and abnormalities corroborates with the previous studies **[25, 50]**. The percentages of sperm viability were significantly improved while those of sperm abnormalities were significantly reduced in diabetic groups medicated with MEES (250 and 500 mg/kg) compared to the diabetic control rats. This result may suggest that MEES could have an effect on spermiogenesis process and it could favor normal sperm production. Additionally,

testosterone deficiency (like that observed in our diabetic control rats) produces immature sperm from the Sertoli cells by early sloughing of spermatids **[51]**. However, the most common abnormality of the epididymal sperm observed was detached heads and coiled tails, in the present study. The improvement in percentages of sperm abnormality and viability, in the present study, coincided with the increased testosterone level in the serum of MEES-treated rats.

Our histopathological findings support the biochemical results. In control rats testicular tissue, the compacted seminiferous tubules, organized germinal cells and all types of cells found to be normal. In diabetic control group, the normal germinal epithelium organization was reduced and cells had abnormal cellular attachment, in addition, spermatogenic cells depletion was also seen. Widespread immature germinal cells were present in the seminiferous tubular lumen of the diabetic control group. This shows that spermatogenesis was not completed and was impaired **[52]**. These alterations may be due to oxidative properties of STZ. Our study showed that MEES treatment (250 and 500 mg/ kg) protected testicular seminiferous tubules against STZ toxicity. It reduced the histological damage by reducing sloughing of the germinal cells and increasing spermatogenesis. The protective effect of MEES treatment may be due to its protection of cellular membranes against oxidative damage.

CONCLUSION

In conclusion, the present study showed that STZ induced - diabetes results in deterioration in testosterone and semen quality and many histological changes in the testis. Oral administration of MEES for 8 weeks has a protective effect against testicular damage induced by STZ-hyperglycemia. We think that this protective effect of MEES may be due to its antioxidant property. Therefore, the present study suggests that MEES or ES leaves intake as a salad may be helpful for diabetic patients to minimize the reproductive deterioration.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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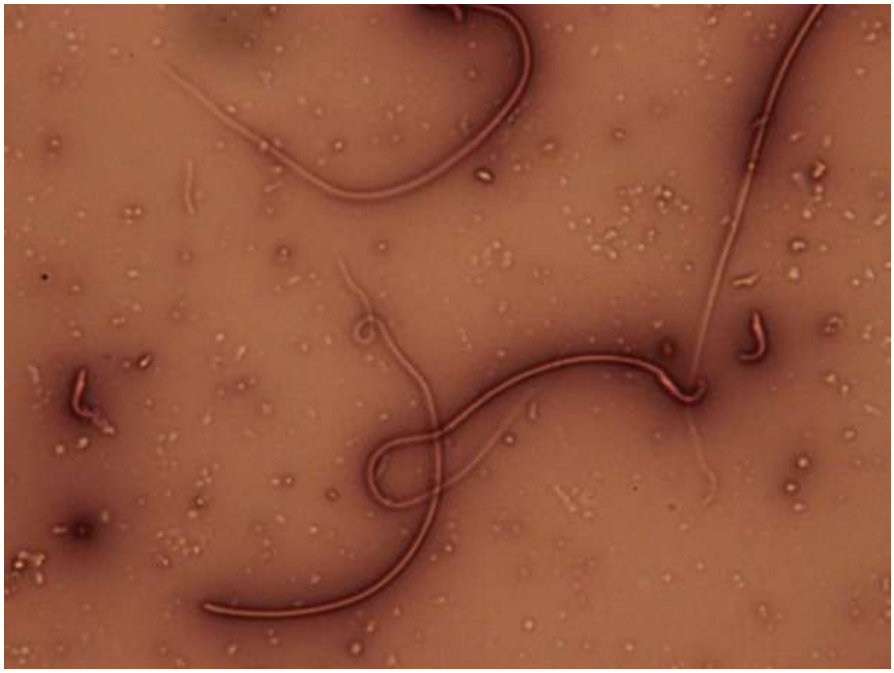

Figure 1: Showing Headless Sperms (A) Bent Tail (B) and Coiled Tail (C) Obtained From Rats Normal Sperm Morphology was Observed in Diabetic Control Rats. (Eosin-Nigrosin, X 400)

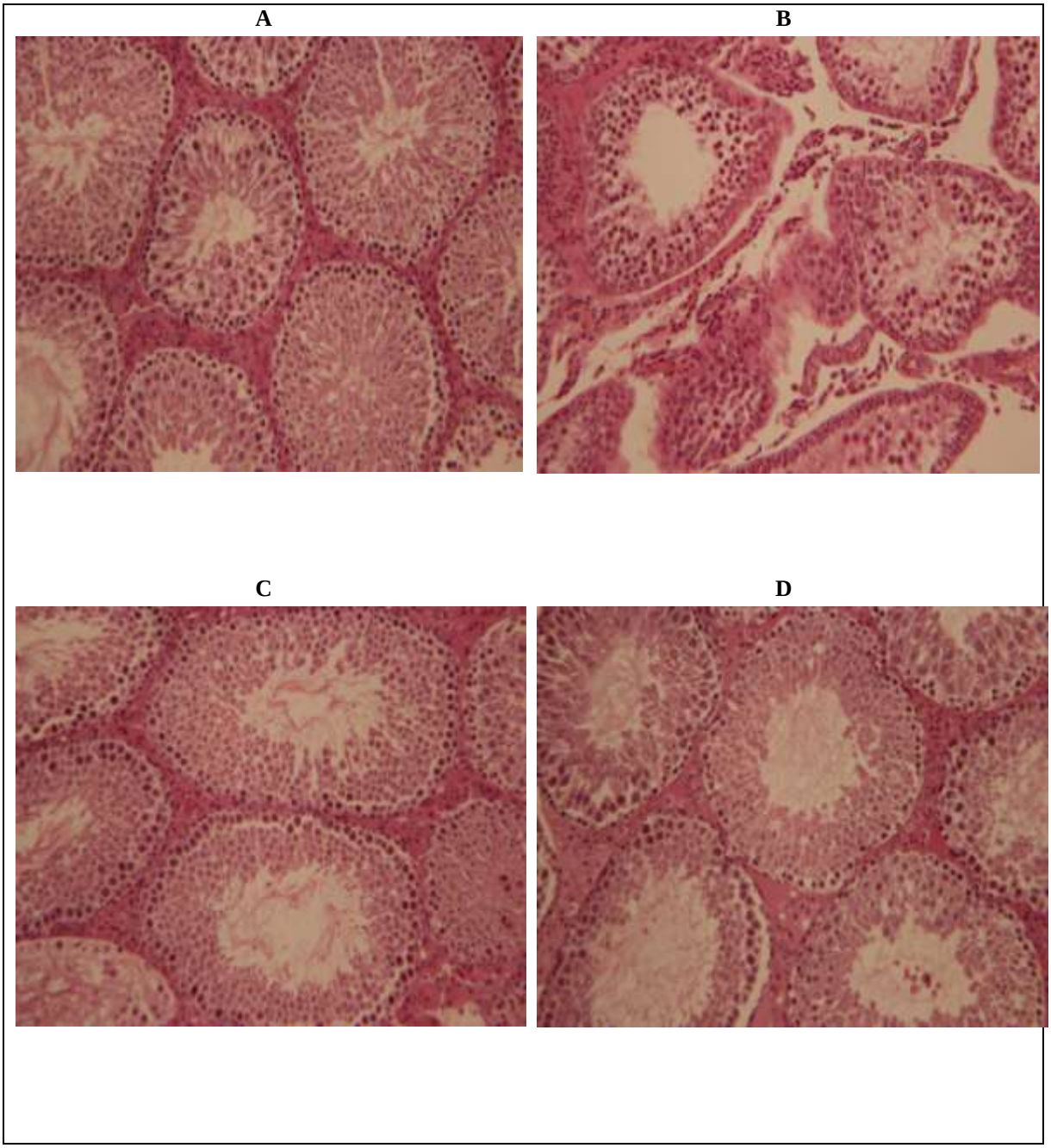

Figure 2: Photomicrograph of Testicular Histology of the (A) Control Rats Showing Normal Seminiferous Tubules and Normal Structure of Germinal Epithelium. (B) Diabetic Control Rats, Showing Atrophic and Degenerated Seminiferous Tubules Associated with Incomplete Spermatogenesis and Sloughing of Degenerated Germ Cells. (C & D) Diabetic Rats Treated with MEES at Doses of 250 and 500 mg/ kg, Respectively Restored the Degenerative Changes in the Seminiferous Tubules Towards Normality. (H & E, X 200)

Table 1: Effect of Oral Administration of MEES for 8 Weeks on Blood Levels of Glucose and Testosterone and Testicular Levels of TBARS and GSH of Diabetic Male Rats

Parameters	Normal control	Diabetic control	Diabetic + MEES	
			250 mg/ kg	500 mg/ kg
Glucose (mg/ dL)	115.67±12.35	546.60±17.47[a]	351.33±16.83[b]	318.00±12.86[b]
Testosterone (ng/ mL)	10.22±0.15	2.42±0.16[a]	8.96±0.24[b]	10.36±1.37[b]
TBARS (nmol of MDA formed/min/g Tissue)	1.43±0.13	6.33±0.25[a]	2.49±0.20[b]	2.34±0.26[b]
GSH (µmoles/ g Tissue)	0.20±0.01	0.06±0.01[a]	0.13±0.01[b]	0.15±0.02[b]

MEES=Methanolic Extract of *Eruca sativa;* The data are presented as means ± SEM, (n = 6); [a] Significant difference as compared with normal control group (P < 0.05); [b] Significant difference as compared with diabetic control group (P < 0.05)

Table 2: Effect of Oral Administration of MEES for 8 Weeks on the Body and Genital Organs Weight and GSI of Diabetic Male Rats

Parameters	Normal control	Diabetic control	Diabetic + MEES	
			250 mg/ kg	500 mg/ kg
Body weights (g)				
Initial	187.7±7.62	188.3±6.59	187.9±6.54	189.7±6.83
Final	219.0±8.01	178.9±6.95[a]	199.8±5.96[b]	210.4±8.68[b]
Body weight change (%)	16.67	-4.99	6.33	10.91
Absolute organs weights (g)				
Testes	2.47±0.16	0.84±0.03[a]	1.54±0.11[b]	1.61±0.12[b]
Epididymides	0.80±0.07	0.31±0.02[a]	0.51±0.04[b]	0.55±0.03[b]
Prostate	0.52±0.04	0.17±0.01[a]	0.32±0.02[b]	0.44±0.03[b]
Seminal vesicles	1.47±0.09	0.32±0.02[a]	1.09±0.07[b]	1.21±0.10[b]
GSI (per body wt, %)				
Testes	1.13±0.08	0.52±0.04[a]	0.88±0.06[b]	0.88±0.05[b]
Epididymides	0.36±0.03	0.19±0.01[a]	0.29±0.02[b]	0.31±0.02[b]
Prostate	0.24±0.02	0.11±0.01[a]	0.18±0.01[b]	0.24±0.02[b]
Seminal vesicles	0.67±0.05	0.20±0.01[a]	0.62±0.04[b]	0.66±0.04[b]

MEES=Methanolic Extract of *Eruca sativa;* The data are presented as means ± SEM, (n = 6); [a] Significant difference as compared with normal control group (P < 0.05); [b] Significant difference as compared with diabetic control group (P < 0.05)

Table 3: Effect of Oral Administration of MEES for 8 Weeks on the Epididymal Sperm Characters of Diabetic Male Rats

Parameters	Normal control	Diabetic control	Diabetic + MEES	
			250 mg/ kg	500 mg/ kg
Count (x10^6/ mL)	74.6±3.11	12.4±0.86[a]	47.5±1.88[b]	59.2±2.65[b]
Progressive motility (%)	88.4±3.86	26.5±0.96[a]	58.9±2.85[b]	65.8±3.50[b]
Sperm morphology (%)				
Normal	89.7±3.25	53.4±2.18[a]	86.2±3.60[b]	87.1±3.31[b]
Abnormal head	2.4±0.15	18.5±0.93[a]	3.6±0.21[b]	3.5±0.20[b]
Abnormal tail	7.3±0.44	25.7±0.94[a]	8.7±0.47[b]	8.2±0.42[b]
Other abnormalities	0.6±0.05	2.4±0.17[a]	1.5±0.11[b]	1.2±0.08[b]
Total abnormalities	10.3±0.68	46.6±1.85[a]	13.8±0.84[b]	12.9±0.85[b]
Sperm viability (%)	91.4±3.85	52.7±2.23[a]	80.3±3.57[b]	88.5±3.50[b]

MEES=Methanolic Extract of *Eruca sativa;* The data are presented as means ± SEM, (n = 6); [a] Significant difference as compared with normal control group (P < 0.05); [b] Significant difference as compared with diabetic control group (P < 0.05)