Protective Effects of Pomegranate Peel Extract on the Diabetic – Induced Damage in Rat Testis

Original Article

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ABSTRACT

Introduction: Diabetes mellitus (DM) is known to cause a state of general oxidative stress. The decreased fertility rates have been attributed to testicular inflammation, oxidative stress and apoptosis in male diabetic patients. Pomegranate peel extract (PPE), known for its high content of bioactive constituents, posses anti-inflammatory, antioxidant, antidiabetic properties.
Aim of the Work: To investigate the potential benefits of PPE against testicular injury-induced by diabetes.
Material and Methods: Forty adult male albino rats were randomly allocated to four groups: control group, PPE group (500 mg/kg/day orally for 8 weeks), diabetic group (received single intraperitoneal injection of 60 mg/kg STZ) and diabetic treated with PPE group. At the end of the experiment, the testes were dissected out and processed for light microscopy, immunohistochemical stainning and transmission electron microscope. Serum testosterone hormone were investigated.
Results: Examination of diabetic group revealed a significant reduced serum testosterone level, severely damaged testis with thickened tunica albuginea, thickening and discontinuity of the tubular basement membrane, shrunken tubules with occluded lumen, depleted germ cells, markedly lost elongated spermatids, congestion and significantly increased caspase-3 immunopositive cells area percent. TEM results revealed shrunken nuclei, ballooned mitochondria, cytoplasmic vacuolations and wide intercellular spaces. Diabetic treated with PPE group showed restoration of normal arrangement of the seminiferous tubules with lumen full with mature elongated spermatids and narrow interstitial spaces in-between.
Conclusion: DM induced damaging effect on the testis, increased the expression of caspas-3 apoptotic marker, fibrosis and lowered the testosterone level. However, PPE administration counteracted most of these changes so it could be used as adjuvant therapy for DM.

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Key Words: Diabetes mellitus; pomegranate peel extract; testis, TEM.

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INTRODUCTION

Diabetes mellitus (DM) is a group of common chronic endocrine metabolic illnesses associated with hyperglycemia. It is predicted that 366 million people will be diabetic by the year 2030[1].

Countries of Middle East and North Africa have the highest prevalence of diabetes until 2040 (from 35.4 million in 2015 to 72.1 million in 2040)[2].

DM is classified into two broad types, type 1 due to absolute deficiency of insulin secretion and type 2 due to lack of tissue response to insulin[3]. DM is believed to be as a major cause of morbidity and mortality among individuals in developing countries as well as developed countries. DM is strongly linked to male infertility, particularly to adolescents[4].

DM is known to be associated with generalised oxidative stress as hyperglycemia promotes glucose auto-oxidation leading to free radicals production. Hyperglycemia had been implicated in several functional and histopathological alterations in many organs of diabetic cases, in addition, oxidative stress secondary to DM obviously affected the testicular structure[5].

The high proportion of polyunsaturated fatty acids (PUFA) in mammalian testicular tissue and sperm cells, decreased oxygen tension and lack of antioxidant defence mechanisms explain the increased susceptibility to membrane lipid peroxidation and free radical damage leading to affections of sperm motility and fertility. Furthermore, the decreased fertility rates have been attributed to testicular inflammation, oxidative stress and apoptosis in male diabetic patients[6].

Nowadays, there is an urgent need for different complementary and alternative therapies especially for subjects with chronic disorders, such as DM. It is well established that medicinal plants are the most preferred among the various complementary and alternative therapeutic options[7].

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There is a mention of pomegranate (Punica granatum L; “seeded apple”) in the Torah, Bible, and Al-Quran as gifts and heavenly fruits of God conferring good luck, wealth, and power of fertility[8].

Pomegranate is one of the members of Punicaceae family. It is considered as one of the oldest edible fruits which is broadly planted in Mediterranean regions including Egypt, Iran, Iraq and India. It is also grown in the USA, China, Japan and Russia[9].

It is well known that pomegranate posses anti-inflammatory, antioxidant, antidiabetic properties as well as antidiarrheal, antitumor and antiproliferative effects[10].

All Pomegranate parts (fruits with its juice and peel, flowers and leaves, and roots), have been efficiently used as a traditional treatment of various diseases. Recently, Pomegranate is a well-known functional food providing several health-promoting benefits[11].

Pomegranate juice as well as pomegranate peel extract (PPE) contain high content of bioactive constituents such as flavonols, anthocyanidins, tannins, phenolic acids, gallic acid, and ellagic acid that exhibit antioxidant effects and inhibit oxidative stress[12].

It has been reported that PPE exerted more efficient antioxidant activities than pomegranate juice[13].

Therefore, this study aimed to investigate the potential benefits of PPE against testicular injury-induced by diabetes.

MATERIAL AND METHODS

Experimental animals

Forty adult male albino rats (150 and 250 g) were housed at the animal house of Bilharzial Research Unit, Faculty of Medicine, Ain Shams University. Animals were manipulated in accordance with guidelines approved by the animal Committee of Ain Shams University. The rats were freely supplied with diet and water access with appropriate environmental conditions at a temperature of 23 0C.

Induction of diabetes

The rats of diabetic groups received a single intraperitoneal injection of streptozotocin (STZ) (60 mg/kg dissolved in 0.1 M cold citrate buffer) after twelve hours of fasting. The animals were supplied with 5% glucose solution to resist the hyperglycemia which resulted from STZ. After three days, rats with serum glucose levels more than 250 mg/dL was considered to be diabetic. STZ was purchased from Sigma Company, St. Louis, Mo, USA.

Preparation of PPE

Pomegranate peels were separated from fresh fruit which were brought from the local markets then the peel were cut into small pieces. The pieces were dried in shade for 10 days before grounding. The dried peel were ground into fine powder. The powder of pomegranate peel were separately extracted in water (1:10 water/volume). PPE supplied at a dose of 500 mg/kg orally.

Experimental design

The rats were randomly divided into 4 groups (n=10):

Control group: received citrate buffer intraperitoneal single injection.

PPE group: rats received PPE 500 mg/kg orally in aqueous solution once per day).

Diabetic group: rats subjected to induction of diabetes.

Diabetic treated with PPE group: rats were subjected to induction of diabetes and received PPE as PPE group for eight weeks.

At the end of the experiment (8 weeks) blood samples were obtained from tail vein and testosterone hormone level was estimated. Rats from all groups were anaesthetized using ether inhalation then they were sacrificed.

For light microscopy, the abdominal wall was opened and the testes were extracted from each rat. The right testes were fixed in Bouin’s solution for 48 hours, then sliced and processed for paraffin embedding. Sections of 5 µm thickness were stained with hematoxylin and eosin (H&E), PAS and Masson trichrome’s stain[14] for their histopathological examination.

Immunohistochemical staining

Testicular sections were prepared and mounted on positively charged slides and stained by caspase-3 which is an indicator for apoptosis and counterstained with haematoxylin[15].

Transmission Electron Microscopic (TEM) Study

The left testes were cut in small pieces of 1mm2 size and fixed in 2.5% glutaraldehyde for 24 hours. Specimens were washed by 0.1 M phosphate buffer at 4 °C then postfixed in 1% osmium tetroxide. Dehydration of the specimens by ascending grades of ethanol and epoxy resin embedding were achieved. Finally, uranyl acetate and lead citrate staining for the ultrathin sections were carried out. Specimens were studied with (Jeol-Jem 1010 Japan) transmission electron microscope in Faculty of Science, Azhar University.

Morphometric Study

Area percent for collagen fibres stained by Masson trichrome’s stain and area percent for caspase-3 immunopositive cells were measured by image analyser computer system Leica Qwin 500 (England) in five different randomly selected non overlapping fields per animal in each group at objective X 400.

Statistical analysis

The results were expressed as mean±SD. One way anova test followed by Post-Hoc (Bonferoni) were used to analyze the data and to compare between groups using SPSS software (SPSS Inc., Chicago, Illinois, USA). The differences were considered statistically significant if *P-value* was < 0.05.

RESULTS

Biochemical results

Diabetic group showed a significant reduction in the mean serum testosterone hormone level as compared to the control group. PPE administration caused a significant increase ($p < 0.05$) in the level of testosterone as compared to the diabetic group (Table 1).

Light microscopic results

Hematoxylin and Eosin stained sections

H&E-stained sections of the control group showed closely packed seminiferous tubules with interstitial spaces contains Leydig cells. The lumina of the tubules were full of mature elongated spermatids (Figure 1a). The seminiferous tubules were lined with germinal epithelium which was composed of tall Sertoli cells with their characteristic pale nuclei with finely dispersed chromatin and well developed nucleolus, and spermatogonia resting on the basement membrane. The spermatocytes appeared with dark nuclei with coarse chromatin granules. The round immature spermatids with their round central nuclei were arranged into two to three layers with elongated spermatids in-between (Figure 1b). The sections of PPE group showed similar results to the control group.

However, sections of diabetic group showed severely damaged testes. The elongated spermatids were markedly lost. Many seminiferous tubules appeared shrunken with occluded lumen containing multinucleated giant cells. There were separation of the spermatogonia and Sertoli cells from the basement membrane. Many vacuolar spaces replaced the depleted cells. The interstitial spaces were wide and oedematous with dilated congested blood vessels (Figure 2a). Other tubules showed discontinuity of the basement membrane with wide lumen and marked depletion of the germ cells (Figure 2b). Sertoli appeared with karyolitic nuclei and lost their cytoplasmic extensions. The round spermatids were degenerated with pyknotic nuclei. The interstitial spaces contained degenerated Leydig cells, acidophilic hyaline material and dilated congested blood vessels (Figure 2c).

On the other hand, sections of the diabetic treated with PPE group showed preservation of normal arrangement of the seminiferous tubules with lumen full of mature elongated spermatids and narrow interstitial spaces in-between (Figure 3a). Most of the seminiferous tubules showed different layers of the germinal epithelium and its normal cellular components of Sertoli cells, spermatogonia, spermatocytes, rounded spermatids and elongated spermatids (Figure 3b).

Masson's trichrome stained sections

The control group showed thin tunica albuginea surrounding the testicular tissue without detectable collagen fibers between the seminiferous tubules (Figure 4a). The diabetic group revealed thick tunica albuginea and thickened congested blood vessels in-between. There were increased collagen fibers in interstitial spaces (Figure 4b). The diabetic treated with PPE group showed tunica albuginea with thickness similar to the control (Figure 4c). Statistical analysis revealed a significant decrease ($p < 0.05$) in area percent of collagen fibers of the diabetic treated with PPE group as compared to the diabetic group (Table 2).

PAS stained sections

The control group showed PAS positive reaction in the basement membrane, round spermatids and elongated spermatids. The interstitial tissue showed PAS positive reaction in Leydig cells. (Figure 5a). The diabetic group revealed strong positive reaction in thickened basement membrane of the seminiferous tubules and the thickened interstitial blood vessels (Figure 5b). The diabetic treated with PPE group showed PAS positive reaction in the basement membrane, round spermatids and elongated spermatids similar to the control group (Figure 5c).

Caspase-3 stained sections

Sections of the control group revealed negative immunoreactivity in the cytoplasm of spermatogenic cells and the interstitial cells of Leydig (Figure 6a). The diabetic group showed strong positive immunoreactivity in the cytoplasm of spermatogenic cells and Leydig cells (Figure 6b), while the diabetic treated with PPE group showed negative caspase-3 immunoreactivity in most cells with few positive immunoreactive cells (Figure 6c). Statistical analysis revealed a significant decrease ($p < 0.05$) in area percent of caspase-3 immunopositive cells of the diabetic treated with PPE group as compared to the diabetic group (Table 2).

TEM results

The control group revealed normal Sertoli cell appeared with indented triangular nucleus having fine granular homogenous chromatin with well-developed nucleolus and its dense cytoplasm contained mitochondria. Both Sertoli and spermatogonia cells were lying on the basement membrane. The spermatocytes showed large nearly rounded nuclei with evenly distributed granular chromatin and their cytoplasm contained mitochondria and endoplasmic reticulum (Figure 7a). The middle pieces of elongated spermatids appeared with peripheral mitochondria and central microtubules while the heads of elongated spermatids contained elongated dense nuclei (Figure 7b).

The diabetic group revealed extremely disturbed germinal epithelium with wide intercellular spaces and many cytoplasmic vacuolations. Sertoli cells appeared with electron dense nucleus and its cytoplasm showed ballooned mitochondria with destructed cristae, lipofuscin granules and electron dense granules. Sertoli cells and spermatogonia were lying on thickened basement membrane with irregular dilated myoid cell nucleus, (Figure 8a). The deformed spermatocytes contained dense heterochromatic nuclei and

granular cytoplasm showing dilated rough endoplasmic reticulum and ill-defined organelles (Figure 8b). The round spermatids showed many vacuoles. The spermatogenic cells revealed shrunken nuclei and numerous cytoplasmic vacuolations (Figure 8c). The elongated spermatids were markedly degenerated with detached mitochondrial sheath and apparently decreased in their number (Figure 8d).

The diabetic treated with PPE group showed Sertoli cells with finely intended triangular nuclei. Sertoli cells and spermatogonia were resting on apparently normal thickness basement membrane with regular myoid cell nucleus. The primary spermatocytes revealed nuclei with regularly arranged chromatin (Figure 9a). The middle pieces of elongated spermatids showed peripheral mitochondria and central microtubules while the heads of elongated spermatids appeared with its acrosomal system (Figure 9b).

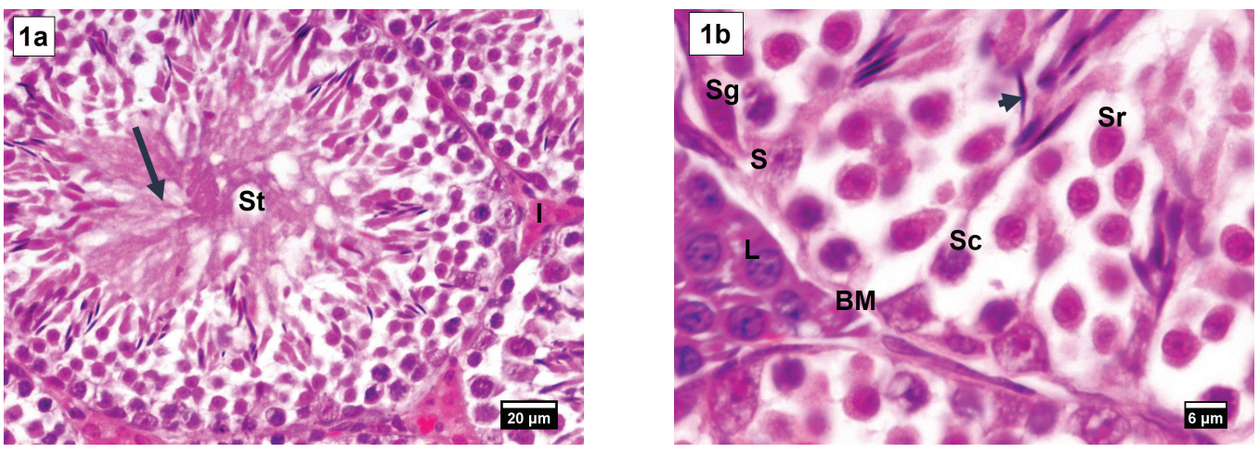

Fig. 1: Photomicrographs of sections in the testis of the control group showing: a) the seminiferous tubules (St) with narrow interstitial spaces (I) in between and lumen filled with mature elongated spermatids (arrow) [H & E; X 400]. b) the well-arranged germinal epithelium with Sertoli cells (S) and spermatogonia (Sg) resting on basement membrane (BM), spermatocytes (Sc), round immature spermatids (Sr), mature elongated spermatids (arrowhead) and Leydig cells (L) [H & E; X 1000].

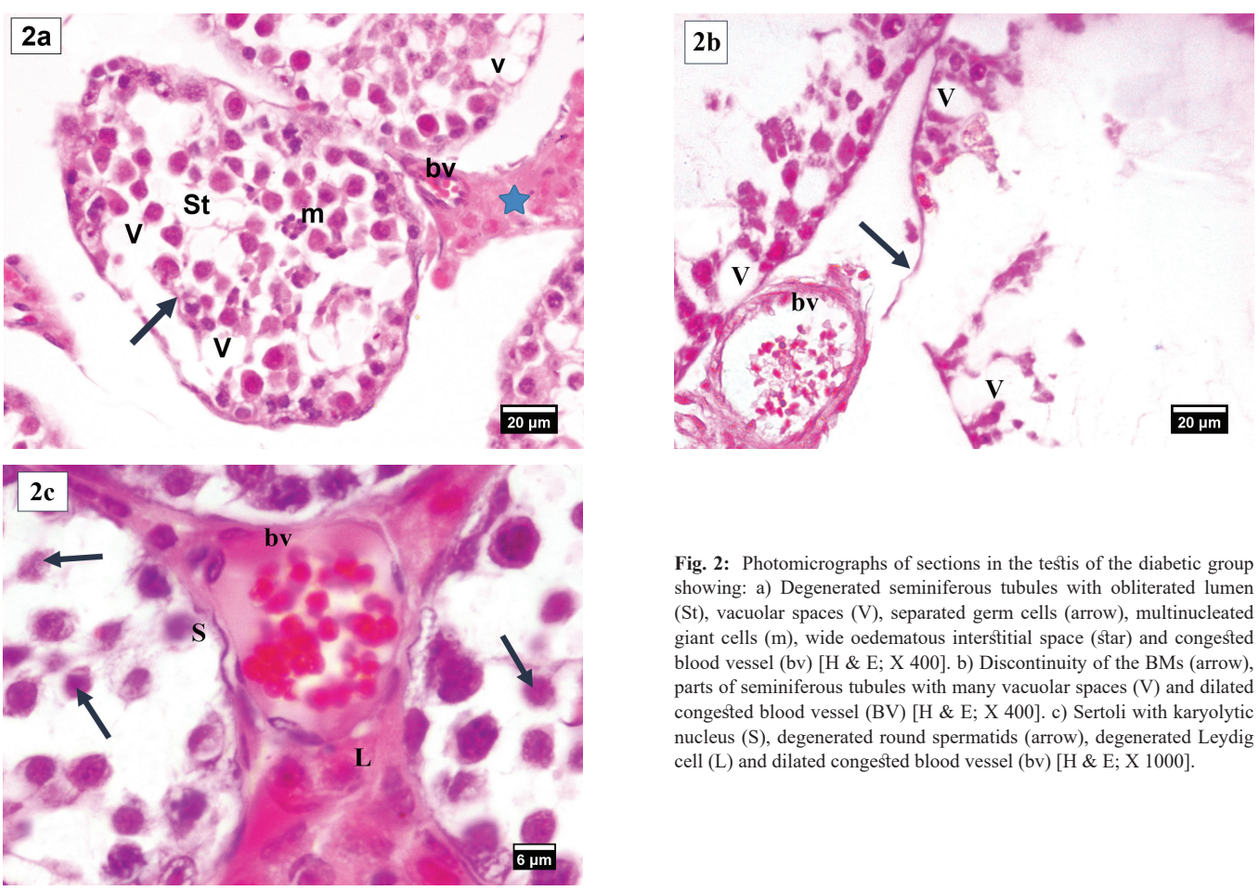

Fig. 2: Photomicrographs of sections in the testis of the diabetic group showing: a) Degenerated seminiferous tubules with obliterated lumen (St), vacuolar spaces (V), separated germ cells (arrow), multinucleated giant cells (m), wide oedematous interstitial space (star) and congested blood vessel (bv) [H & E; X 400]. b) Discontinuity of the BMs (arrow), parts of seminiferous tubules with many vacuolar spaces (V) and dilated congested blood vessel (BV) [H & E; X 400]. c) Sertoli with karyolytic nucleus (S), degenerated round spermatids (arrow), degenerated Leydig cell (L) and dilated congested blood vessel (bv) [H & E; X 1000].

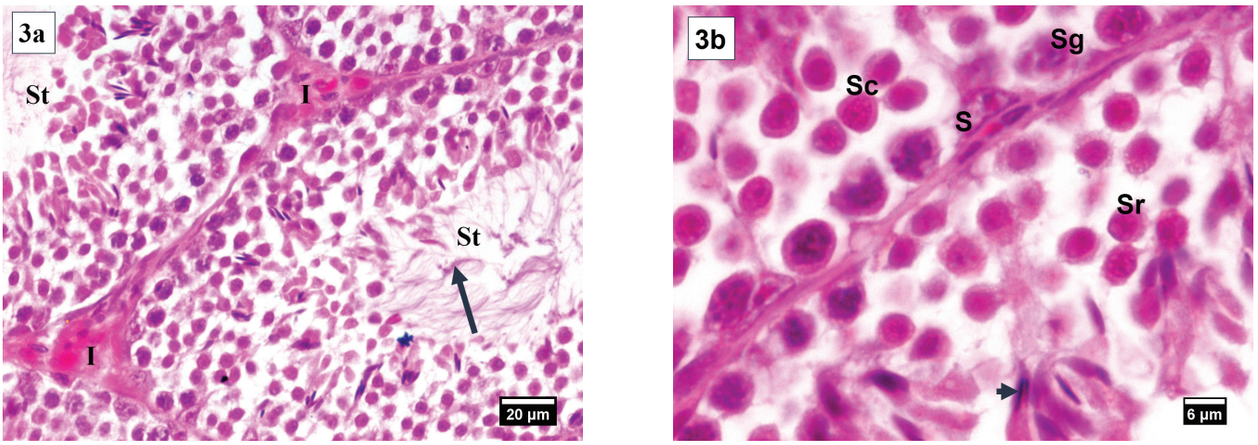

Fig. 3: Photomicrographs of sections in the testis of the diabetic treated with PPE group showing: a) Preservation of normal arrangement of the seminiferous tubules (St) with lumen full with mature elongated spermatids (arrow) and narrow interstitial spaces (I) [H & E; X 400]. b) The lining germinal epithelium, Sertoli cells (S), spermatogonia (Sg), spermatocytes (Sc), round spermatids (Sr) and mature spermatids (arrowhead) [H & E; X 1000].

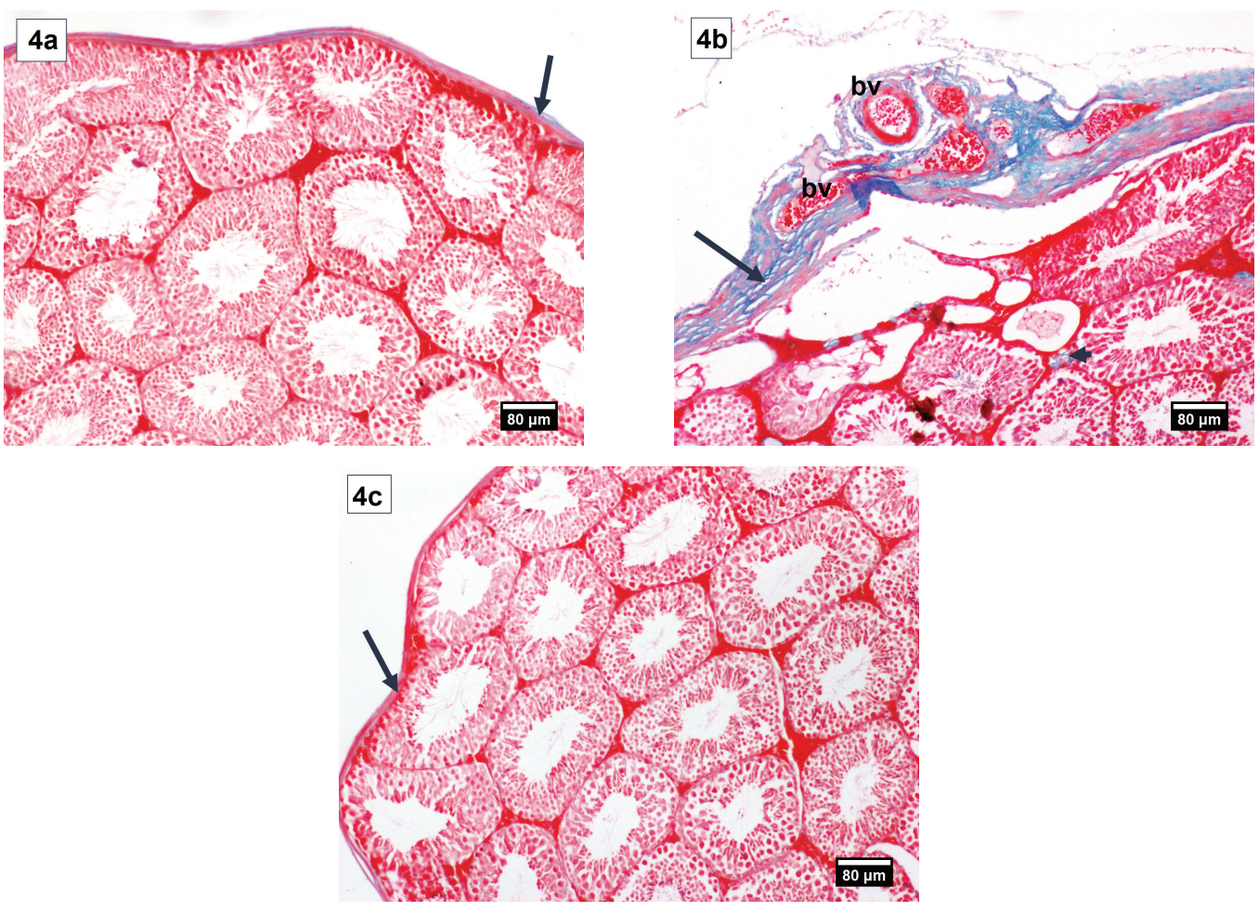

Fig. 4: Photomicrographs of sections in the testis from different groups. a) Control group showing thin tunica albuginea surrounding the testicular tissue (arrow). b) Diabetic group showing thick tunica albuginea (arrow), thickened congested blood vessels (bv) and increased collagen fibers in interstitial spaces (arrow head). c) Diabetic treated with PPE group showing thin tunica albuginea (arrow). Masson trichrome stain; X 100

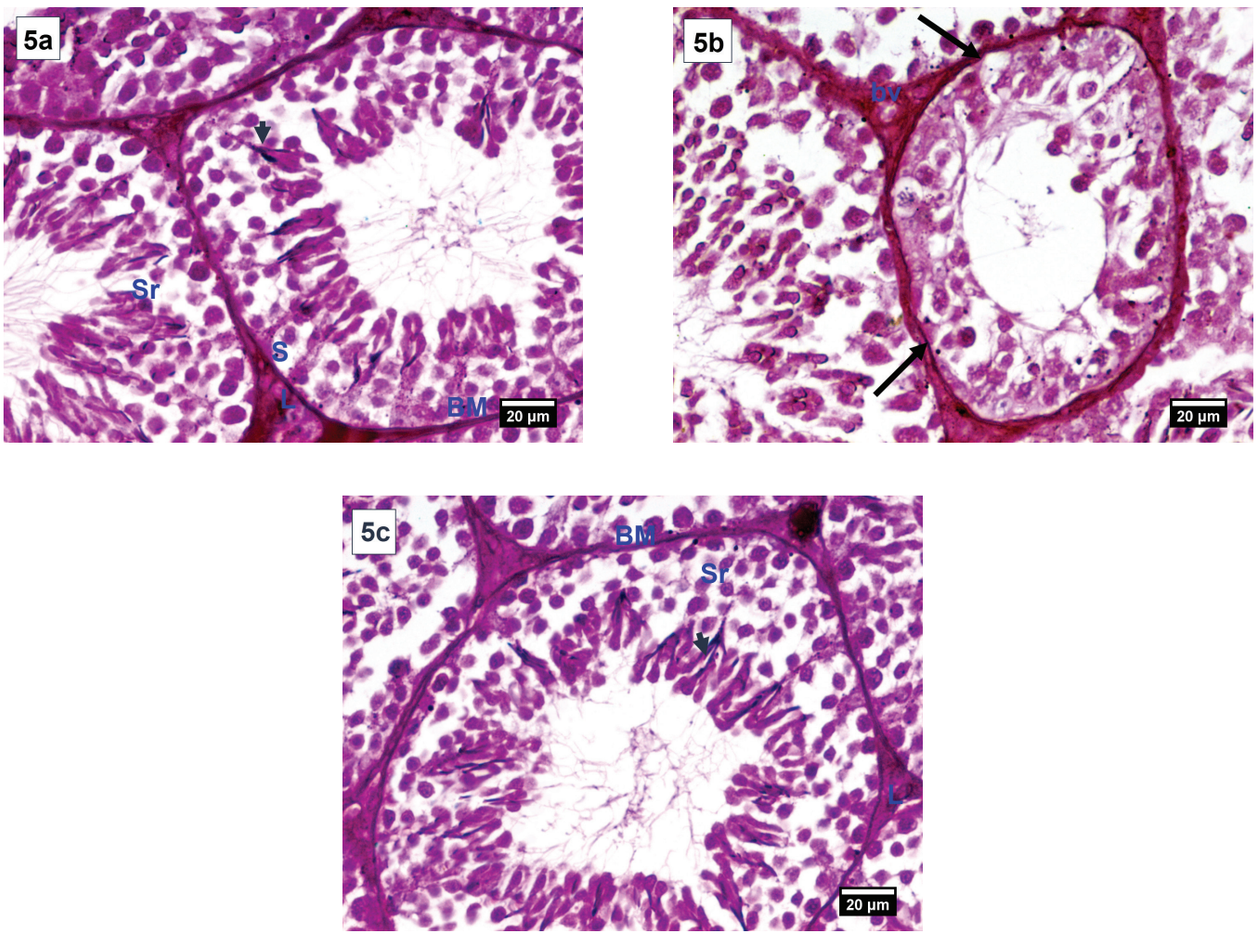

Fig. 5: Photomicrographs of sections in the testis from different groups. a) Control group showing seminiferous tubules with PAS positive reaction in thin basement membrane (BM), round spermatids (Sr), elongated spermatids (arrowhead) and Leydig cells (L). b) Diabetic group showing strong positive reaction thickened basement membrane of the seminiferous tubules (arrow) and the thickened interstitial blood vessels (bv). c) Diabetic treated with PPE group showing diabetic treated with PPE group showing PAS positive reaction in thin basement membrane (BM), round spermatids (Sr), elongated spermatids (arrowhead) and Leydig cells (L). PAS; X 400

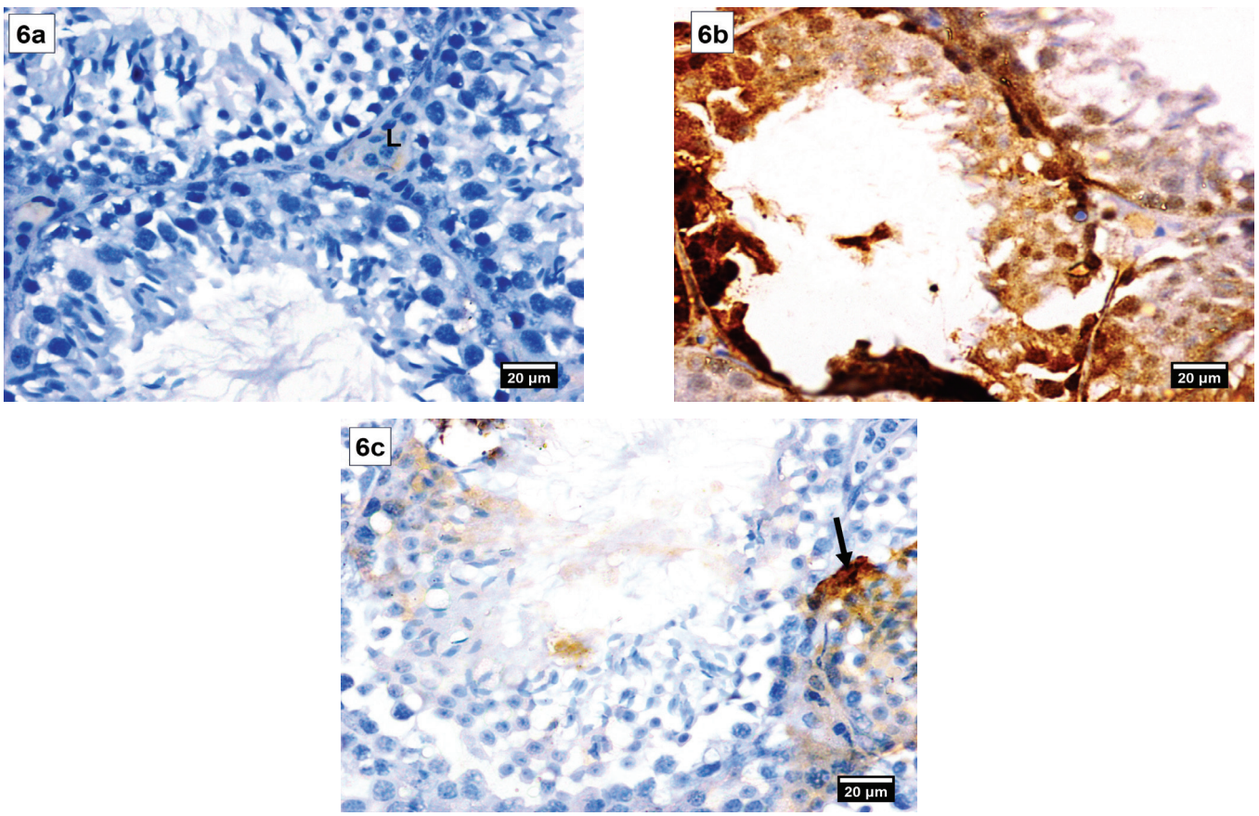

Fig. 6: Immunohistochemical staining for the demonstration of caspase 3 in the testis from different groups. a) Control group showing negative immunoreactivity in cytoplasm of spermatogenic cells and interstitial cells of Leydig (L). b) Diabetic group showing strong positive immunoreactivity in the cytoplasm of spermatogenic cells and Leydig cells. c) Diabetic treated with PPE group showing few positive immunoreactive cells (arrow). Caspase 3; X 400

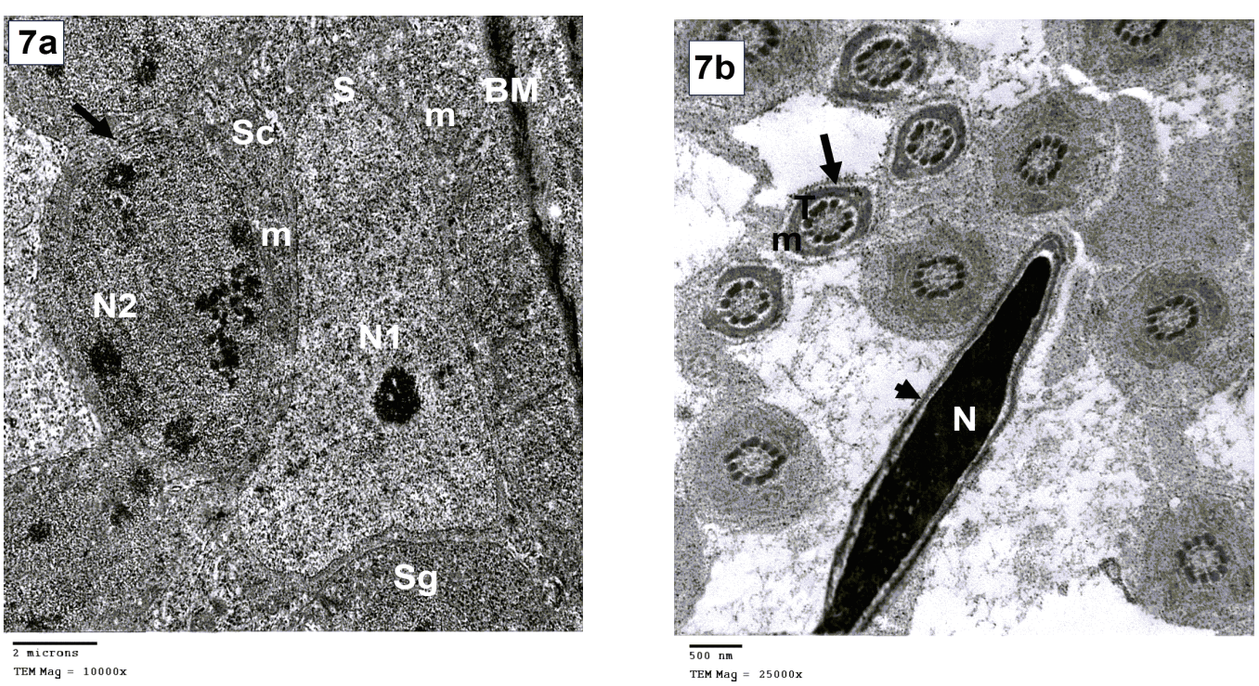

Fig. 7: Electron photomicrographs of sections of the testis of the control group showing: a) Sertoli cell (S) having indented triangular nucleus (N1), part of spermatogonia cell (Sg) lying on basement membrane (BM), primary spermatocyte (Sc) with large nearly rounded nucleus (N2), smooth endoplasmic reticulum (arrow) and mitochondria (m). × 10000 b) Cross-section at the middle pieces of elongated spermatids (arrows) having peripheral mitochondria (m) and central microtubules (T) and head of elongated spermatids (arrow head) with its elongated dense nucleus (N). × 25000

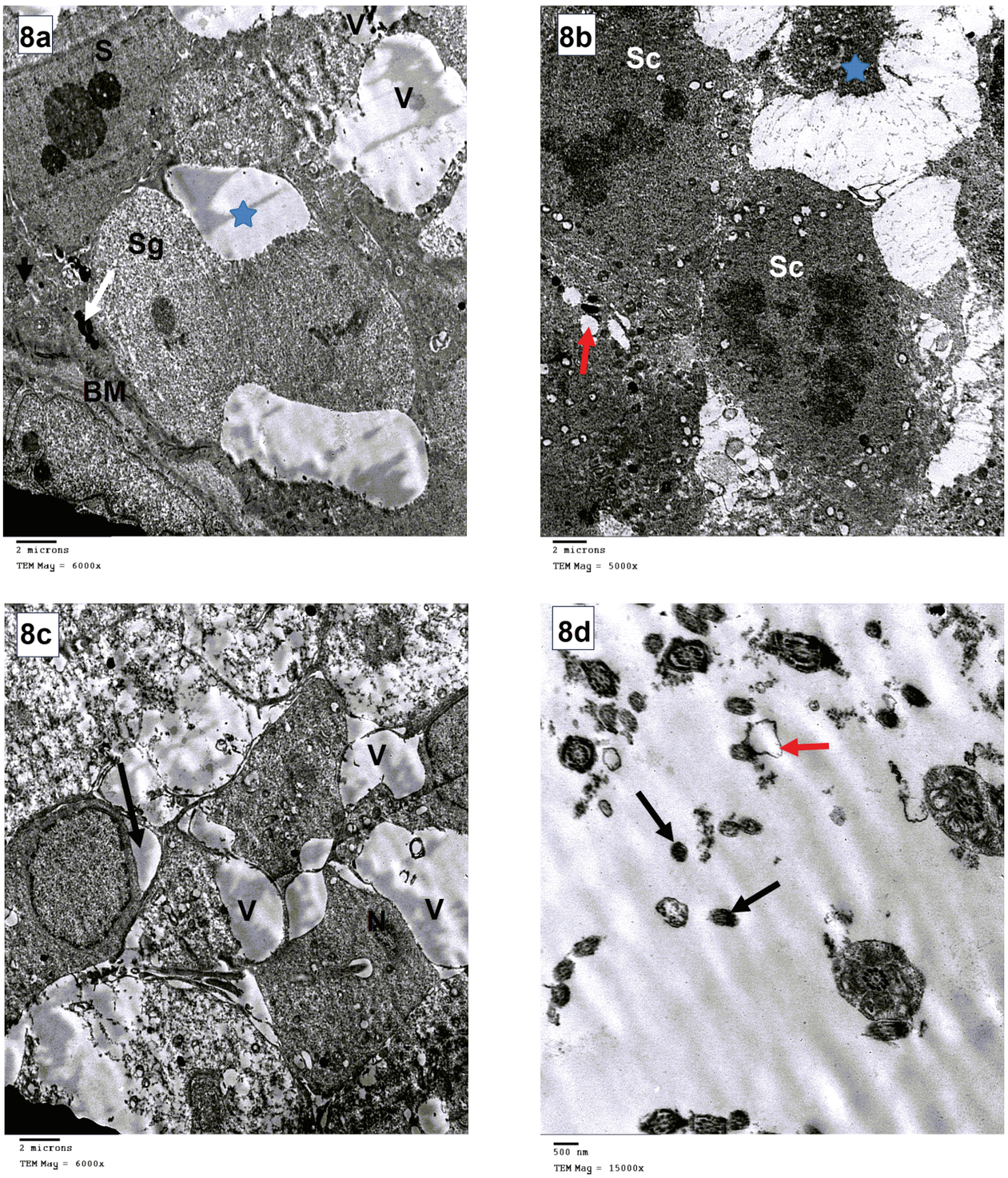

Fig. 8: Electron photomicrographs of sections of the testis of the diabetic group showing: a) Thickened basement membrane (BM), irregular myoid cell nucleus (arrowhead), wide intercellular spaces (star), cytoplasmic vacuolations (V), Sertoli cell (S) appearing with electron dense nucleus, ballooned mitochondria (m), lipofuscin granules (arrowhead) and numerous electron dense granules (arrow) and spermatogonia (Sg). × 6000 b) Deformed spermatocyte (Sc) with dense heterochromatic nucleus, dilated rough endoplasmic reticulum (red arrow) and degenerated germ cell (star). × 5000 c) Round spermatids with vacuoles (arrow). Notice the shrunken nucleus (N) and many cytoplasmic vacuolations (V) × 6000. d) Markedly degenerated elongated spermatids (arrow), detached mitochondrial sheath (red arrow) and marked apparent decrease in their number. × 15000

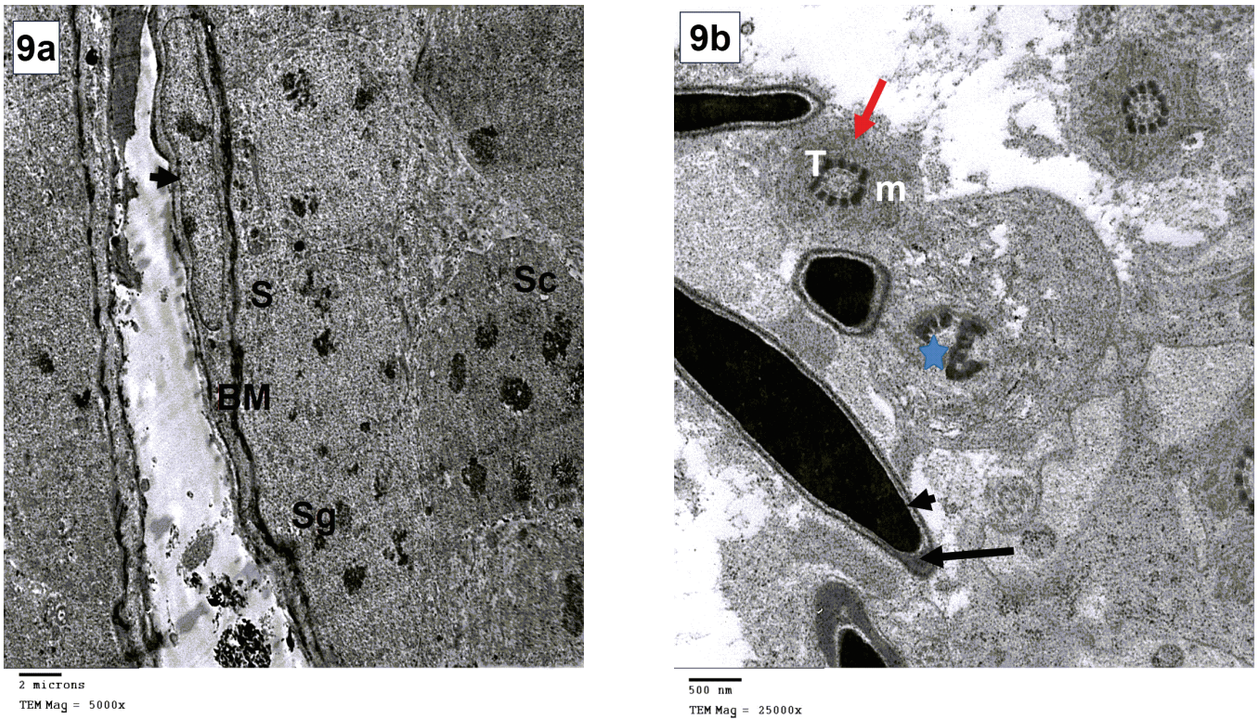

Fig. 9: Electron photomicrographs of sections of the testis of the diabetic treated with PPE group showing: a) Myoid cell nucleus (arrowhead), Sertoli cell with finely intended triangular nucleus (S) and spermatogonia (Sg) resting on basement membrane (BM), primary spermatocyte having nucleus with regularly arranged chromatin (Sc). × 5000 b) The middle pieces of elongated spermatids (red arrow) having peripheral mitochondria (m) and central microtubules (T) and heads of elongated spermatids (arrow head) with its acrosomal system (arrow). Notice one distorted middle piece (star). × 25000

Table 1: Serum testosterone levels

Parameter	Control	PPE	Diabetic	Diabetic +PPE
Mean serum testosterone (ng/ml)	3.7 ±0.4	3.8±0.3	1.9 ±0.3[a,b]	3.1 ±0.6[a,b,c]

Data are expressed as mean ± SD. $p > 0.05$: no significant difference, $p < 0.05$: significant difference.
a = significantly different from control group, b = significantly different from PPE, c = significantly different from Diabetic group.

Table 2: Area percent of collagen fibers and caspase-3 immunoreaction

Parameter	Control	PPE	Diabetic	Diabetic +PPE
Area percent of collagen fibers	4.1±0.92	4.3±0.73	12.1±1.67[a,b]	6.32±0.92[a,b,c]
Mean area percent of caspase-3 immunoreaction	0.12 ± 0.01	0.14 ± 0.03	6.11 ± 0.73[a,b]	1.7 ± 0.45[a,b,c]

Data are expressed as mean ± SD. $p > 0.05$: no significant difference, $p < 0.05$: significant difference.
a = significantly different from control group, b = significantly different from PPE, c = significantly different from Diabetic group.

DISCUSSION

Induction of diabetes resulted in dysfunction of the reproductive system in male rats. Diabetes is considered one of the major risk factors for male subfertility or infertility. Meanwhile, in *vivo* studies proved that diabetes induces alterations in testicular and germ cells in adult rats[16].

The present study revealed low testosterone levels in diabetic group. This could be attributed to the high estradiol concentrations induced by the action of aromatase enzyme during testosterone metabolism that leads to reduction of luteinizing hormone secretion from the pituitary gland and subsequent reduction of plasma testosterone level in diabetic rats[17].

Moreover, hyperglycemia-induced Leydig cells dysfunction leads to suppression in testosterone production[18]. The DM-induced inflammation is proved by excess production of the inflammatory cytokines in diabetics. This overexpression of inflammatory cytokines inhibits testosterone synthesis and impairs spermatogenesis[19].

In the present study, the testes of the diabetic group showed thickened tunica albuginea and basement membrane of the seminiferous tubules as detected by PAS stain, and confirmed by TEM results.

The observed thickened tunica albuginea and tubular basement membrane could be assigned to high collagen content that resulted from fibroblast dysfunction or

glycation of collagen fibrils. This thickening in tunica albuginea and basement membrane hinders the fair vascularity of testicular cells, therefore raising the probability for their atrophy and damage[16].

Diabetes leads to increase of the thickness of the basement membrane due to impairment of the turnover of basement membrane proteins, as these proteins were glycosylated and causes the decrease in the amount of producing sperm[20]. Moreover, marked increase of PAS expression in diabetes is due to glycogen accumulation inside the cells[21].

In the present study, there was marked loss of the spermatozoa. The other germinal cells were few and arranged discretely. Moreover, Leydig cells were markedly reduced in the interstitial tissues which showed congested blood vessels, oedema, and eosinophilic exudate. Similar results found by Abd Elsamie *et al*.[22].

These deleterious changes observed in the diabetic group could be related to the hyperglycemia-mediated increased cellular oxidative damage due to excess reactive oxygen species (ROS) production and reduction of the antioxidant defence system. High ROS levels were responsible for increasing the apoptosis and atrophy of the tubular epithelium and Leydig cells leading to testicular damage and subsequently disrupted sperm count and function[23].

Also the TEM results of the present study showed wide intercellular spaces and many cytoplasmic vacuolations of most of the cells with destructive changes in the mitochondria and endoplasmic reticulum of Sertoli cells and irregular myoid nuclei.

The interactions between Sertoli, myoid, and Leydig cells are important for control of spermatogenic process in the testis[24]. The ultrastructural changes of Sertoli and spermatogenic cells induced by diabetes are due to the fluctuations in pituitary gonadotropins and these changes influence the normal spermatogenesis in rats[16].

In the present study, the diabetic group showed giant multinucleated cells. The appearance of these cells can be attributed to inability of the primary spermatocytes to complete the division to produce haploid sperm cells[25]. These cells were considered as a cellular self-destructive behaviour resulting in formation of non–functional pathogenic cells[26].

In this study, sections of diabetic rats showed a marked elevation in the expression of the caspase-3. TEM results also showed spermatogenic cells with shrunken nuclei and numerous cytoplasmic vacuolations. Furthermore, the elongated spermatids were markedly degenerated with detached mitochondrial sheath. This could be attributed to the increased testicular cell apoptosis associated with DM[27].

The increased apoptosis of Leydig cells resulted in reduced androgen biosynthesis and low serum testosterone levels which negatively affected spermatogenesis and caused decreased sperm output and fertility. Moreover, the inflammatory signs as congestion, oedema, and haemorrhage is caused by oxidative stress and apoptosis[28].

Prolonged hyperglycemia is a key factor for the oxidative stress associated with DM due to the auto-oxidation of glucose and glycosylation of protein which causes overproduction of free radicals. These free radicals lead to the oxidative stress of several tissues such as testis[29].

DM increased lipid peroxidation products such as MDA, and depleted antioxidant defence mechanism including GSH content, SOD activity and HO-1level in testicular tissues[30].

Among all germ cells, spermatozoa are considered the most affected by oxidative stress due to their high PUFA content which is concerned with the fluidity of their membranes needed for sperm maturation, spermatogenesis, sperm capacitation, acrosome reaction and gamete fusion. In this regard, lipid peroxidation of spermatozoa causes disruption of the lipid matrix of their membranes leading to loss of intracellular ATP with subsequent axonal injury, reduced sperm viability, and altered mid-piece morphology and could inhibit spermatogenesis in severe cases[31].

Testicular tissue is characterized by the high proliferating rate of all its cells but the most differentiating cell is the sperm cell. Thus, sperms consume high glucose amount for their metabolism and their function. DM with its consequent oxidative damage, apoptosis and inflammation results in testicular dysfunction with disrupted steroidogenesis and spermatogenesis[32].

Previous experiments have concluded that oxidative stress and inflammation formed the main core for testicular dysfunction in DM[33,34].

Moreover, testicular interstitial fibrosis associated with DM alters the testicular spermatogenic environment leading to reduction of testosterone secretion and spermatogenesis disruption with the resultant male infertility[35].

In the present study, the diabetic treated with PPE group showed histological structure comparable to the control, regular arrangement of the tunica albuginea and seminiferous tubules at both light microscope and TEM results, and recovery of the testosterone level. The spermatozoa cells were more abundant in the lumen of the seminiferous tubules. Leydig cells were apparently increased in the interstitial spaces. Moreover, the caspase-3 sections of the diabetic treated with PPE group revealed significant reduction of the immunopositive cells as compared to the diabetic group

Dietary intake of antioxidants can suppress the oxidation of the cellular constituents so inhibit oxidative stress. Thus, our diet should be enriched with these antioxidants to prevent various chronic diseases[36].

Pomegranate juice and PPE were demonstrated to expose more efficient antioxidant activity than frequently consumed fruit juices, such as grape, cranberry, grapefruit or orange juice[37].

The antioxidant activities of pomegranate are attributed to its rich constituents of vitamin C, vitamin E, polyphenols, tannic acid, and bioflavonoids (catechins and other complex flavonoids)[38]. It is stated that bioactive compounds of pomegranate extract improved the natural testicular antioxidant defences production that caused increased sperm count and testosterone level[39].

It is well established that pomegranate possesses anti-hyperglycaemic activity due to enhancement of insulin secretion or due to the potentiation of insulin action. Furthermore, pomegranate can help the improvement of pancreatic B-cell function leading to increased insulin secretion[40].

Moreover, pomegranate healing activity owed to its role as a free radical scavenger protected pancreatic β-cells from damage by neutralizing the free radical effect[41].

Furthermore, pomegranate peel were shown to have the highest antioxidant levels as compared to the peel, pulp and seed of 28 different fruit kinds[42]. Qu *et al*.[43] also concluded that the pomegranate peel is considered a rich source of natural antioxidants and its antioxidant activity exceeded that of the seeds and pulp.

The pomegranate peel possesses effective antitumor, antioxidant, antiviral and anti-inflammatory activities[44]. Previous researches have concluded that punicalagin, a polyphenol of pomegranate, exerted high powerful antioxidant potency[45]. Pomegranate peels were demonstrated to have antioxidant and anti-inflammatory activities in several animal species and several tissues[46].

Furthermore, Valadares *et al*.[47] proved that pomegranate could protect DNA and inhibit chromosomal destruction in mice. PPE inhibits leukocyte apoptosis in rats[48].

CONCLUSIONS

DM led to structural and ultrastructural changed on the testis and lowered the serum testosterone level and increased fibrosis of the testicular tissue. Also, DM elevated the expression of caspase-3. PPE administration returned the serum testosterone level near to the normal, markedly reduced the fibrosis and the other inflammatory signs and reduced the expression of caspase-3. Thus, PPE could be considered as a natural, safe adjuvant therapy in diabetic patient to protect the testes from the harmful effect of diabetes.

CONFLICT OF INTERSTS

There are no conflicts of interest.

REFERENCES

1. Shaw JE, Sicree RA and Zimmet PZ: Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract. (2010) 87:4–14.
2. Osati S, Homayounfar R and Hajifaraji M: Metabolic effects of vitamin D supplementation in vitamin D deficient patients (a double-blind clinical trial). Diabetes Metab Syndr. (2016) 10(1):S7–10.
3. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. (1998) 15(7):539-53.
4. Maresch CC, Stute DC, Alves MG, Oliveira PF, de Kretser DM, Linn T. Diabetes-induced hyperglycemia impairs male reproductive function: a systematic review. Hum Reprod Update. (2018) 24(1): 86-105.
5. Abdella EAB, Al Waleed H, El Hassan F, Asim I, Salwa KM and Samia, A: Investigate Evaluation of Oxidative Stress and Lipid Profile in STZ-Induced Rats Treated with Antioxidant Vitamin. Pharmacology & Pharmacy (2014) 5: 272-279.
6. Rashid K and Sil PC: Curcumin ameliorates testicular damage in diabetic rats by suppressing cellular stress-mediated mitochondria and endoplasmic reticulum-dependent apoptotic death. Biochim Biophys Acta. (2015) 1852(1):70-82.
7. Wanchai A and Phrompayak D: Use of complementary and alternative medicine among Thai patients with type 2 diabetes mellitus. J Integr Med. (2016) 14(4): 297–305.
8. Jurenka J: Therapeutic applications of pomegranate (Punica granatum L.): a review. Altern Med Rev. (2008) 13:128.
9. Matthaeus B and Ozcan M: Pomegranate plant (Punica granatum L.) composition, antioxidant activity, therapeutic effect and antimicrobial activity. Journal of Applied Pharmaceutical Science (2016) 21:160-167.
10. Abdel Moneim AE, Othman MS, Mohmoud SM and El-Deib KM: Pomegranate peel attenuates aluminum-induced hepatorenal toxicity. Toxicol Mech Methods. (2013) 23(8):624-633.
11. Melgarejo-Sánchez P, Núñez-Gómez D, Martínez-Nicolás JJ, Hernández F, Legua P and Melgarejo P: Pomegranate variety and pomegranate plant part, relevance from bioactive point of view: a review. Bioresour. Bioprocess. (2021) 8:2.
12. Kandylis P and Kokkinomagoulos E: Food Applications and Potential Health Benefits of Pomegranate and its Derivatives. Foods. (2020) 9(2):122.
13. Yan L, Zhou X, Shi L, Shalimu D, Ma C and Liu Y: Phenolic profiles and antioxidant activities of six Chinese pomegranate (Punica granatum L.) cultivars. International journal of food properties (2017) 20 (1):S94-107.

14. Suvarna SK, Layton C and Bancroft JD: Immunohistochemical techniques. In: Bancroft's Theory and Practice of Histological Techniques, 7th ed., Churchill Livingstone Elsevier, London. 2013; pp: 387-418.

15. Buchwalow IB and Böcker W: Working with Antibodies. In: Immunohistochemistry: basics and methods. Springer, Heidelberg, (2010) pp: 31–39.

16. Kianifard D, Sadrkhanlou RA and Hasanzadeh S: The ultrastructural changes of the sertoli and leydig cells following streptozotocin induced diabetes. Iran J Bas Med Sci (2011) 15: 623-635.

17. Hasan MM, El-Shal AS, Mackawy AMH, Ibrahim EM, Abdelghany EMMA, Saeed AA and El-Gendy J: Ameliorative effect of combined low dose of pioglitazone and omega-3 on spermatogenesis and steroidogenesis in diabetic rats. J Cell Biochem. (2020)121:1524–40.

18. Heeba GH and Hamza AA: Rosuvastatin ameliorates diabetesinduced reproductive damage via suppression of oxidative stress, inflammatory and apoptotic pathways in male rats. Life Sci. (2015)141:13–9.

19. Coştur P, Filiz S, Gonca S, Çulha M, Gülecen T, Solakoğlu S, Canberk Y and Çalışkan E: Expression of inducible nitric oxide synthase (iNOS) in the azoospermic human testis. Andrologia. (2012) 44(1):654–60.

20. Guneli E, Tugyan K, Ozturk H, Gumustekin M, Cilaker S and Uysal N: Effect of melatonin on testicular damage in streptozotocin-induced diabetes rats. Eur Surg Res. (2008) 40:354-360.

21. Ravikumar SS, Menaka TR, Vasupradha G, Dhivya K, Dinakaran J and Saranya V: Cytological intracellular glycogen evaluation using PAS and PAS-D stains to correlate plasma glucose in diabetics. Indian J Dent Res. (2019) 30(5):703-707.

22. Abd Elsamie MS, Ali MHM, Mahmoud OM and Kamel EM: Influence of each hesperidin and insulin on diabetes-induced testicular alterations in adult albino rats. Eur J Anat. (2021)25 (2): 197-210.

23. Alves MG, Martins AD, Cavaco JE, Socorro S and Oliveira PF: Diabetes, insulin-mediated glucose metabolism and Sertoli/ blood-testis barrier function. Tissue Barriers. (2013) 1:e23992.

24. Skinner MK: Cell–cell interactions in the testis. Endocrinol Rev. (1991)12: 45–77.

25. Rotter V, Schwartz D, Almon E, Goldfinger N, Kapon A, Meshorer A, Donehower LA and Levine A.J: Mice with reduced levels of p53 protein exhibit the testicular giant-cell degenerative syndrome. Proc.Natl.Acad. Sci.U.S.A.(1993) 90(19):9075-9079.

26. Hess RA and Nakai M: Histopathology of the male reproductive system induced by the fungicide benomyl. Histol. Histopathol. (2000) 15(1):207-224.

27. Jiang X, Chen J, Zhang C, Zhang Z, Tan Y, Feng W, Skibba M, Xin Y and Cai L: The protective effect of FGF21 on diabetes-induced male germ cell apoptosis is associated with upregulated testicular AKT and AMPK/Sirt1/PGC-1α signaling. Endocrinology. (2015) 156(3): 1156-1170.

28. Ballester J, Muñoz MC, Domínguez J, Rigau T, Guinovart JJ and Rodríguez-Gil JE: Insulin-dependent diabetes affects testicular function by FSH- and LH-linked mechanisms. J Androl. (2004) 25(5): 706-719.

29. Bhattacharya S, Manna P, Gachhui R and Sil PC: D-Saccharic acid 1,4-lactone protects diabetic rat kidney by ameliorating hyperglycemia-mediated oxidative stress and renal inflammatory cytokines via NF-κB and PKC signaling, Toxicol. Appl. Pharmacol. (2013) 267 (1): 16–29.

30. Mohasseb M, Ebied S, Yehia MA and Hussein N: Testicular oxidative damage and role of combined antioxidant supplementation in experimental diabetic rats. J Physiol Biochem. (2011) 67:185–94.

31. Turk G, Sonmez M, Aydin M, Yuce A, Gur S, Yuksel M, Aksu EH and Aksoy, H: Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin. Nutr. (2008) 27: 289-296.

32. Ding GL, Liu Y, Liu ME, Pan JX, Guo MX, Sheng JZ and Huang HF: The effects of diabetes on male fertility and epigenetic regulation during spermatogenesis. Asian J Androl. (2015) 17:948–53.

33. Ghosh S, Chowdhury S, Das AK and Sil PC: Taurine ameliorates oxidative stress induced inflammation and ER stress mediated testicular damage in STZ-induced diabetic Wistar rats. Food Chem Toxicol. (2019) 124: 64-80.

34. Khosravi Z, Sedaghat R, Baluchnejadmojarad T and Roghani M: Diosgenin ameliorates testicular damage in streptozotocin-diabetic rats through attenuation of apoptosis, oxidative stress, and inflammation. Int Immunopharmacol. (2019) 70: 37-46.

35. Aksglaede L and Juul A: Testicular function and fertility in men with Klinefelter syndrome: A review. Eur J Endocrinol. (2013) 168: R67-R76.

36. Rouhi SZ, Moklesur R, Asmah R, Saad A and Fauziah O: The effect of pomegranate fresh juice versus pomegranate seed powder on metabolic indices, lipid profile, inflammatory biomarkers, and the histopathology of pancreatic islets of Langerhans in streptozotocin-nicotinamide induced type 2 diabetic Sprague–Dawley rats. Complementary and Alternative Medicine. (2017) 17:156-69.

37. Tapias V, Cannon J and Greenamyre J: Pomegranate juice exacerbates oxidative stress and nigrostriatal degeneration in Parkinson's disease. Neurobiol. Aging. (2014) 35(5):1162-76.

38. Ahmed MA, El Morsy EM and Ahmed AA: Pomegranate extract protects against cerebral ischemia/reperfusion injury and preserves brain DNA integrity in rats. Life Sci. (2014) 21; 110:61–69.

39. Atilgan D, Parlaktas B, Uluocak N, Gencten Y, Erdemir F, Ozyurt H, Erkorkmaz U and Aslan H: Pomegranate (Punica granatum) juice reduces oxidative injury and improves sperm concentration in a rat model of testicular torsion-detorsion. Exp Ther Med. (2014) 8:478–82

40. Papaccio G, Pisanti FA, Latronico MV, Ammendola E and Galdieri M: Multiple low-dose and single highdose treatments with streptozotocin do not generate nitric oxide. J. Cell Biochem. (2017) 77: 82–91.

41. Tzulker R, Glazer I, Bar-Ilan I, Holl D, Aviram M and Amir R: Antioxidant activity, polyphenol content and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. J Agric Food Chem. (2007) 55:9559–9570.

42. Li Y, Guo C, Yang J, Wei J, Xu J. and Cheng S: Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Fd. Chem. (2006) 96: 254-260.

43. Qu W, Pan Z and Ma, H: Extraction modeling and activities of antioxidants from pomegranate marc. J. Fd. Eng. (2010) 99: 16-23.

44. Prakash CV and Prakash I: Bioactive chemical constituents from pomegranate (Punica granatum) juice, seed and peel–A review. Int J Res Chem Environ. (2011) 1:1-18.

45. Seeram N, Lee R, Hardy M, Heber D. Rapid large scale purification of ellagitannins from pomegranate husk, a by-product of the commercial juice industry. Sep Purif Technol. 2005; 41:49–55.

46. Bakeer MR, El-Attrouny MM and Abdelatty AM: Effect of dietary pomegranate peel (Punica granatum L.) and Aloe vera gel (Aloe barbadensis miller) supplementation on testicular antioxidant biomarkers and spermatogenesis enzymes in mature V-Line rabbit bucks. J Anim Physiol Anim Nutr (Berl). (2021)105(1):175-182.

47. Valadares MC, Pereira ERT, Benfica PL and Paula JR: Assessment of mutagenic and antimutagenic effects of Punica granatum in mice. Brazilian Journal of Pharmaceutical Sciences (2010) 46(1): 121-7.

48. Toklu HZ, Sehirli O, Ozyurt H, Mayadağli AA, Ekşioğlu-Demiralp E, Cetinel S, Sahin H, Yeğen BC, Ulusoylu Dumlu M, Gökmen V and Sener G: Punica granatum peel extract protects against ionizing radiation-induced enteritis and leukocyte apoptosis in rats. J Radiat Res. (2009) 50:345–53.

الملخص العربى

التأثيرات الوقائية لمستخلص قشر الرمان على خصية الجرذان من الأضرار الناتجة عن مرض السكري

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الخلفية: من المعروف أن داء السكري يسبب حالة من الإجهاد التأكسدي العام، ويُعزى انخفاض معدلات الخصوبة إلى التهاب الخصية والإجهاد التأكسدي وموت الخلايا المبرمج لدى مرضى السكري من الذكور. هذا ويتميز مستخلص قشر الرمان، المعروف بمحتواه العالي من المكونات النشطة بيولوجيًا، بخصائص مضادة للالتهابات، ومضادة للأكسدة، ومضادة لمرض السكر.

الهدف من الدراسة: التحقيق في الفوائد المحتملة لمستخلص قشر الرمان ضد إصابة الخصية التي يسببها مرض السكري.

المواد والطرق: تم تقسيم أربعين فأرًا بالغًا من ذكور الجرذان البيضاء بشكل عشوائي لأربع مجموعات: المجموعة الضابطة، مجموعة مستخلص قشر الرمان (٥٠٠ مجم / كجم / اليوم عن طريق الفم لمدة ٨ أسابيع)، مجموعة مرض السكري (تم تلقي حقنة واحدة داخل الصفاق من الستربتوزوتوسين ٦٠ مجم / كجم) ومجموعة مرض السكري الذين عولجوا بمستخلص قشر الرمان. وفي نهاية التجربة، تم تشريح الخصيتين ومعالجتهما من أجل الفحص المجهري الضوئي والإلكتروني ومن أجل الصبغة الكيميائية المناعية، كما تم فحص هرمون التستوستيرون في الدم.

النتائج: أظهر فحص مجموعة مرض السكري انخفاضًا ملحوظًا في مستوى هرمون التستوستيرون في الدم، وتلفًا شديدًا في الخصية مع سماكة الغلالة البيضاء، وسماكة وانقطاع الغشاء القاعدي لأنابيب الخصيه، كما تقلصت العديد من الأنابيب مع انغلاق تجويفها، وفقدان لكثيرمن الخلايا المبطنه لأنابيب الخصيه، وفقدان واضح للحيوانات المنوية، واحتقان، وزيادة مساحة الخلايا المناعية المصبوغه بمضاد caspase-٣ بشكل ملحوظ. وأظهرت نتائج الفحص بالمجهر الإلكتروني تقلص النوى، وتضخم الميتوكوندريا، والفراغات السيتوبلازمية، والمساحات الواسعة بين الخلايا. بينما أظهرت مجموعة مرضى السكري الذين عولجوا بمستخلص قشر الرمان استعادة الترتيب الطبيعي للأنابيب المنوية مع تجويف ممتلئ بالحيوانات المنوية الناضجة ومسافات خلالية ضيقة بين أنابيب الخصيه.

الخلاصة: يسبب داء السكري تأثيرًا ضارًا على الخصية ويزيد من التعبير عن علامة موت الخلايا المبرمج المصبوغه ب caspase-٣ ويخفض مستوى هرمون التستوستيرون ولكن مستخلص قشر الرمان منعت معظم هذه التغييرات بحيث يمكن استخدامها كعلاج مساعد لمرض السكري.