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RESEARCH ARTICLE

Effect of Punica granatum (pomegranate) juice on the ultrastructure of the testes in the streptozetocin induced diabetes in adult albino rat

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Abstract

Diabetes mellitus is a serious metabolic disorder. Pomegranate has a variety of biological activities. The present study was designed to use a histological approach for evaluating the protective effect of pomegranate juice against streptozetocin induced diabetes in the structure of the rat testis.

MATERIAL AND METHODS: Animals were divided into six groups. The first group was control. The second group was given 1 ml pomegranate juice, while groups III to VI received 60 mg/kg/bw of streptozetocin. The third group was untreated diabetic. 0.25 ml pomegranate juice plus 0.75 ml water was given to rats in the fourth group. The fifth group was received 0.50 ml pomegranate juice plus 0.50 ml water. One ml pomegranate juice was given to rats in the six group. Treatment was given daily for 52 days. Testis specimens from all groups were examined by light, scanning and transmission electron microscopes.

RESULTS: The body weight, testis weight, the diameter of the seminiferous tubules and the epithelial height in diabetic rats were significantly reduced. Pomegranate juice increased the spermatozoa inside the seminiferous tubules. Depletion of all germinal cells were marked in untreated diabetic rats while the interstitial connective tissues were wide. Electron microscopic examination revealed vacuolations and degeneration in the spermatogenic and Sertoli cells. Basement membrane was irregular and thickened with deposition of collagen fibres. In diabetic rats treated with pomegranate juice, all the histological observations improved and dose dependant.

CONCLUSION: pomegranate juice is able to diminish the side effects of diabetes on the structure of testis.

Introduction

Diabetes mellitus is a serious metabolic disorder and a degenerative disease that harmfully affects the male reproductive function (Yanardag et al., 2005; Amaral et al., 2006). Diabetes is associated with increased generation of free radicals leading to oxidative stress and the development of atherosclerosis (Vincent et al., 2002). High levels of free radicals could lead to damage of cellular organelles and increased lipid peroxidation in the cellular membranes (Panchnadikar and Bhonde 2003).

Streptozotocin (STZ), an antibiotic with diabetogenic effects, acutely and excessively damages selective pancreatic Beta cells (Rodrigues et al., 1999). Streptozotocin-induced diabetic rats provide a model of beta-cell deterioration through glucose toxicity (Erejuwa et al., 2011). Streptozotocin enters the B cell via a glucose transporter and causes damage of DNA that induces activation of poly ADP-ribosylation. Poly ADP-ribosylation leads to depletion of cellular NAD^+ and ATP (Szkudelski, 2001).

The potential therapeutic properties of pomegranate is useful in the treatment and prevention of cancer (Lansky and Newman 2007), cardiovascular disease (Aviram et al., 2002), diabetes (Rosenblat et al., 2006), treatment of acquired

immune deficiency syndrome (Lee and Watson, 1998), in addition to its use for oral hygiene (Kim and Kim, 2002) and as an adjunct therapy to increase bioavailability of radioactive dyes during diagnostic imaging (Amorim et al., 2003).

Examples of in vivo studies of beneficial effects of pomegranate include protection of rat gastric mucosa from ethanol and aspirin toxicity (Khennouf et al., 1999; Ajaikumar et al., 2005), protection of neonatal rat brain from hypoxia (Loren et al., 2005) and prevention of male rabbit erectile tissue dysfunction (Azadzoi et al., 2005).

Pomegranate has a variety of biological activities, including potent antioxidant (Hassoun et al., 2004), antiproliferative (Seeram et al., 2005) and antiartherogenic apoptotic (Yu et al., 2005).

Pomegranate (Punica granatum L.) juice has higher antioxidant capacity than more commonly consumed fruit juices (Basu and Penugonda, 2009). Pomegranate and its derivatives have free radical scavenger and potent antioxidant activity. The most important anti-oxidant polyphenols in pomegranate juice (PJ) include the ellagitannins and anthocyanins (Gil et al., 2000). Antioxidants, in general, are compounds which dispose, scavenge, and suppress the formation of Reactive oxygen species (ROS) and lipid per-oxidation. ROS are highly reactive oxidizing agents. The overproduction of ROS stimulates DNA fragmentation and can be detrimental to sperm function, associated with peroxidative damage to the mitochondria and plasma membrane. Additionally, spermatozoa are especially susceptible to peroxidative damage, because of high concentration of polyunsaturated fatty acids and low antioxidant capacity (de Lamirande et al., 1997). Lipid peroxidation destroys the structure of lipid matrix in the membranes of spermatozoa with loss of motility and defects of membrane integrity (Sanocka and Kurpisz, 2004).

The semineferous tubules of the testis are the site of continuous spermatogenesis. In the rat testis, semineferous tubules are ensheathed by a layer of contractile cells, the peritubular myoid cells. Myoid cells are arranged to form a squamous epithelioid layer. The rapid progression of spermatozoa along the lumen of semineferous tubules toward the hilum of the testis depends upon the functional activity of myoid cells (Tripiciano et al., 1999). Sertoli cells activate the development and maintain the viability of germ cells by secreting hormonal and nutritive materials (Sawada H and Esaki, 2003). Leydig cells are essentially the only site of testosterone production in the male to form and maintain male secondary sexual structures and characteristics (Hooker, 2005).

Morphological modifications in the testicular tissue were investigated as its cellular compartments are in constant division and cell differentiation. Moreover, this tissue is of great importance for reproduction. A review of literature could not reveal any results on the histopathological effects of the use of pomegranate juice (PJ) on testis in streptozetocin induced diabetes.

The present experimental study was therefore designed to use a histological approach for evaluating the possible protective effect of PJ against streptozetocin induced diabetes in the structure of the testis of the adult albino rats using light, scanning and transmission electron microscopes.

MATERIALS AND METHODS

Sixty healthy adult male albino rats (12 weeks old) were used at the beginning of this study. The animals were housed under standard laboratory conditions (temperature 24±3°C, humidity 40–60%, a 12-h light:12-h dark cycle). A commercial pellet diet and fresh drinking water were given *ad libitum*. The handling of animals followed the rules for experimental research ethics approved by Research Ethics Committee at King Khaled University.

Forty rats were injected with 60 mg/kg/b.w of streptozotocin in 0.05 M citrate buffer (pH 5.0) intravenously via the tail vein to obtain Type-I diabetic rats (Ragbetli and Ceylan, 2010). One week after streptozotocin treatment, the diabetic rats were randomized into four groups after confirming that prandial plasma glucose concentrations were more than 160 mg/dl.

Pomegranates fruits were purchase freshly from Aseer region, K.S.A. The fruits were peeled and the seeds were squeezed with a squeezing machine. The PJ was filtered to remove any water-insoluble materials and the PJ was immediately placed in dark bottles wrapped with aluminum foil and stored at 4ºC to be used fresh daily. Pomegranate juice was given by gavage daily for 52 days. This administration period is necessary to determine the effect of PJ on sperm production because the rats need a period of 48-52 days for the exact spermatogenic cycle including spermatocytogenesis, meiosis and spermiogenesis (Bennett and Vickery, 1970).

Animals were divided into six groups. The first non diabetic group was given 1 ml distilled water orally daily. The second non diabetic group was received 1 ml PJ daily. Groups III to VI were diabetic. The third group was diabetic group and received no treatment. Only 0.25 ml PJ plus 0.75 ml distilled water was given to rats in the fourth group and named PJ-low. The fifth group was received 0.50 ml PJ plus 0.50 ml distilled water, and named PJ-middle. One ml PJ was given to rats in the six group, and named PJ-high. Distilled water and pomegranate juice were given daily for 52 days.

At the end of study, the weight of each animal was recorded. The overnight fasting rats were anesthetized with diethyl ether. Blood glucose was measured from the tail vein by using strips and the equipment One Touch Ultra 2 from Johnson and Johnson.
After weighing and sacrificing of animals, the right and left testis of each rat were separated from the body and their weight were recorded. The changes in body weights and testes weights were analyzed by two-way analysis of variance. Testes were fixed for histopathological examinations.

Chemicals
All chemicals were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA).

Light microscopy
All specimens for light microscope were cut into small pieces and fixed in a solution of 10% formaldehyde and processed for light microscopic study to get paraffin sections of 5 μm thickness. Sections were stained with Haematoxylin and Eosin (H&E) (Bancroft and Stevens, 1996). Slides were mounted using entellan and covered with coverslips prior to viewing and photography by (Nikon Eclipse E-200) light microscope.

Scanning electron microscopy
Testicular tissue were fixed with 3% glutaraldehyde in phosphate buffer for 4 h. After the samples were washed and dehydrated, then dried by the critical point in acetone method. Testis samples were mounted on an aluminium stub and then coated with gold. The prepared samples of testis were examined under the scanning electron microscope. The photographs of testis tissue were recorded using SEM 6390 LV JEOL.
Semineferous tubules were evaluated for tubular diameter and height of the tubular epithelium by using scanning electron microscope. The scale bar was 50 μm and the values for the 10 semineferous tubules for each animal were measured. These averages were used for calculating means and standard error of means and performing statistics by using prism programmed on the computer.

Transmission electron microscopy (TEM)
The testis specimens were cut into small pieces of $1mm^2$ size and fixed in 2.5% glutaraldehyde for 24 hours. Specimens were washed in 0.1 M phosphate buffer at 4°c, then post fixed in 1% osmium tetroxide at room temperature. Specimens were dehydrated in ascending grades of ethyl alcohol, then embedded in Epon resin. Semithin sections (1μm) were stained with toluidine blue in borax and examined with light microscope. Ultrathin sections (50 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate (Bancroft and Stevens, 1996). Specimens were examined and photographed with Jeol 1200 EX transmission electron microscope in the College of Medicine, King Khaled University.

RESULTS
Table 1 shows the mean ± SD of the body weight in gm, testis weight in gm, fasting blood glucose level in mg/dl, the seminiferous tubular diameter in μm and the epithelial height in μm of the different groups.
The mean of the body weight and testis weight of different groups. PJ had statistically none significant effect on body or testis weights of the none diabetic rats when compared to the control group. Group III, diabetic rats, showed a significant decrease in the mean body weight as compared to all groups. Diabetes caused statistically significant decreases in weights of testes ($P< 0.01$). Groups IV,V and Vl , diabetic rats treated by different doses of PJ, shows a significant increase in the mean body weight compared to the diabetic rats. Mild decrease in the mean body weight of group IV rats was seen when compared to the control group. Gonad/body ratio was nearly the same in all groups. Pomegranate juice decrease significantly the blood glucose level in all groups of diabetic rats.
The data of the histomorphometeric study of seminiferous tubules (STs) showed that, the Mean of diameter of STs in diabetic group was significantly lower than control group ($P< 0.05$). Also, ST diameter increased in treated diabetic groups in comparison with untreated diabetic group ($P< 0.05$).
The mean of seminiferous epithelial height was decreased in diabetic animals compared to the control group. It was observed that these values in the diabetic group were statistically significantly lower when compared with those of the PJ-treated diabetic group ($P < 0.05$) and control group. All doses of PJ provided significant increases in the diameter of ST and epithelial height when compared to the untreated diabetic group ($P < 0.001$).

Table 1: The mean ± SD of the body weight in gm, testis weight in gm, fasting blood glucose level in mg/dl, the seminiferous tubules (ST) Diameter in µm and the epithelial height in µm of the different groups.

Groups	Mean body weight ± SD	Mean testis weight ± SD	Mean fasting blood glucose ± SD	Mean ST Diameter ± SD	Mean EH ± SD
Group I control	270 ± 10.8	Rt. 2.03 ± .04 Lt. 1.94 ± .04	92 ± 3.4	288 ± 17.4	78.53 ± 3.45
Group II (PJ)	274 ± 11.2	Rt. 2.04 ± .04 Lt. 1.94 ± .04	89 ± 3.4	290 ± 17.8	78. 76 ± 3.42
Group III (diabetic)	195 ± 8.8	Rt. 1.58 ± .03 Lt. 1.51 ± .03	397 ± 12.8	170 ± 12.9	67.34 ± 3.05
Group IV (diabetic+ PJ low)	226 ± 9.4	Rt. 1.69 ± .03 Lt. 1.62 ± .03	290 ± 11.4	200 ± 13.9	70.63 ± 3.13
Group V (diabetic+PJ mid	235 ± 9.7	Rt. 1.77 ± .03 Lt. 1.69 ± .03	254 ± 11.1	228 ± 15.4	72.55 ± 3.15
Group (diabetic+PJ high	241 ± 10.1	Rt. 1.82 ± .04 Lt. 1.76 ± .04	217 ± 9.5	250 ± 17.1	74.21 ± 3.39

Light microscopy results

Examination of H and E stained sections of control testes (Group I) shows interstitial cells of Leydig prominently interspersed between the seminiferous tubules (Fig. 1). The seminiferous tubule were compacted and organized germinal cells as well as all types of cells had normal cellular attachment, five or more cell layers were seen in the epithelium of seminiferous tubule (Fig. 1). Control group revealed spermatogonia their characteristic dome shape and their oval nuclei. Primary spermatocyte appeared as large spermatogenic cell with large nucleus. Several layers of early spermatids were seen with their rounded central nuclei. Late spermatids and spermatozoa were identified by their elongated deeply stained nuclei. Sertoli cells were distributed at intervals between spermatogenic cells. Sertoli cells appear as tall cells extending from the basal membrane to the ST lumen. It is difficult to determine the outline of the cells as it is obscured by the surrounding germ cells. Both Sertoli cells and spermatogonia were seen resting on the basement membrane which was surrounded by myoid cells with flat nuclei (Fig. 1).

Examination of H and E stained sections of group II which received PJ showed the presence of normal testicular architecture and regular seminiferous tubular morphology with normal spermatogenesis. Leydig cells appeared in interstitial tissue. It was observed that PJ caused increase in spermatogenic cell density especially spermatids and spermatozoa in comparison to the control group I (Fig. 2).

The histological investigations of testicular tissue in the untreated diabetic rats (Group lll) showed alteration and distortion of both germinal epithelium and seminiferous tubules. The ST was irregular shape in its outline. The normal organization of germinal epithelium was reduced and the cells of germinal epithelium were abnormal arrangement and to some extent depletion in spermatogenic cells was seen as compared to the control group (Fig. 3). Also in diabetic rats, atrophy of the tubules was detected. In some affected tubules, the spermatogonic cells were only the major cell type seen. Some spermatids and spermatozoa could be detected in the lumen of the tubules. The multinucleated cells were seen in some of the seminiferous tubules and the number of cell layers was reduced. Sertoli cells are markedly reduced in number. There was presence of cavities and spaces within the seminiferous tubules The peritubular tissue surrounding the seminiferous tubules and interstitial cells were altered. The interstitial tissues were wide, congested with the presence of vacuoles.

The condensation of spermatogenic cells in the PJ-treated diabetic rats was observed to be increased and there was an improvement in the seminiferous tubule structure compared with the diabetic group (Figs. 4,5&6).
Examination of H and E stained sections of group IV (diabetic animals receiving low dose PJ) showed that the spermatogenic cells and sertoli cells are fewer in number and some areas lack of spermatogonia compared to the control group. Spermatids and spermatozoa could be detected in the tubules. The interstitial tissue showed exudation and congestion (Fig. 4). It was observed that a low dose of PJ caused a slight increase in spermatogenic cell density especially in spermatids in comparison to non treated diabetic group and the outline of ST is improved (Fig. 4). Middle and high doses of PJ caused marked increases in all the spermatogenic cells in comparison to the untreated diabetic group (Figs. 5&6). The interstitial tissue of group V looks more or less normal, very few small cavities were present close to the basal lamina, but the number of Sertoli cells and all other spermatogenic cells especially the spermatids and spermatozoa are increased in comparison to the diabetic group (Fig. 5). The STs of group VI were lined by several layers of spermatogenic cells. Spermatogonia and Sertoli cells were seen resting on the basement membrane followed by primary spermatocytes, early and late spermatids were nearly more or less similar to the control group (Fig. 6).
The presence of degenerating spermatogenic cells, irregularity of ST outline, cavities and germinal epithelial layers present in untreated diabetic testis were less in rats that received different doses of PJ, especially with middle and high doses (Figs. 5&6).

Scanning electron microscopy results

The outer surface of the ST is covered with single layer of flat myoid cells, whose nuclei appear as small bulges. The myoid cells appear to be arranged in a continuous monolayer. Germ cells layers can easy be seen. The STs showed the lumen filled with spermatozoa flagella.
The interstitial tissue forms a delicate lattice with well spaced cells, inbetween the STs. A distinct space was observed between the interstitial tissue and the STs (Fig. 7).
In the second group, the photograph was nearly the same like control group but the lumen is nearly completely filled with spermatozoa with long flagella (Fig. 8).
Scanning electron micrograph of testicular sections of rats of the group lll shows narrow STs with irregular outlines and wide interstitial spaces in between. The interstitial connective tissue had the amorphous material (Fig. 9). Many cavities and depletion of the germinal epithelial layers with low height are present with decrease number of spermatozoa in the lumen (Fig. 9).
Examination of sections of group IV showed more improvement with distinct STs. The layers of the spermatogenic cells are fewer in number with low in height compared to the control group but more better than the diabetic untreated group (Fig. 10). It was observed that low dose of PJ caused a slight increase in spermatogenic cell layers and improved in the irregular outline of the STs (Fig. 10). Some spermatozoa could be detected in the lumen of the tubules. Middle doses of PJ in diabetic rats resulted in marked increases in the number of layers of the spermatogenic cells and increase the lumen spermatozoa and the outline of the STs is improved in comparison to the untreated diabetic group (Fig. 11). The testes of group VI diabetic rats treated with high PJ showed a significant improvement of testicular structure. The STs were well defined. Lumen of STs was filled with spermatozoa. The STs were lined by several layers of spermatogenic cells (Fig. 12).

Electron Microscopic Results:

Electron microscopic examination of the control group (Group I) showed normal Sertoli cells with their indented irregular nuclei resting on regular basement membrane surrounded by myoid cells. Spermatogonia cell is closed to the Sertoli cell and resting also on the basement membrane (Fig. 13). The spermatid reveals that the cytoplasmic organelles are more numerous. The acrosome spread over part of nucleus. The nuclear membrane is highly electron dense under the acrosomal cap. The chromatin material is evenly distributed (Fig. 14).
Group II was shown normal spermatogenetic activity and normal seminiferous tubular structure. Sertoli cells and spermatogenic cells were rested on regular basement membrane surrounded by myoid cells with flat nuclei (Fig. 15). Large numbers of spermatozoa were present in the lumen of the STs (Fig. 16).
Electron microscopic examination of group III showed the interstitial cell of Leydig having large nucleus and vacuoles in its cytoplasm may be from dilated cisternae. Some sperms were found in the interstitial tissue escaped from damaged STs (Fig. 17). Sertoli cells were seen with atrophic nuclei and lipid droplets (Fig. 17). Some sertoli cells have been seen with vacuoles (Fig. 18). The basement membrane was irregular, thickened with deposition of collagen fibres. Myoid cells revealed irregular and atrophic nuclei (Figs. 17&18). Large vacuoles revealed in the spermatogenic cells. Other lining cells appeared vacuolated, degenerated with wide intercellular spaces.

Disintegration of tubular cells, vacuolization of sertoli cells and spermatogonia cells were seen in most of STs. Ealy spermatids appeared in the second layer (Fig. 18).
Spermatids were often maloriented to each other and to the tubular lumen. Some early spermatids and spermatids with acrosomal caps had lipid droplets and amorphous cytoplasmic materials in the lumen (Fig. 19). Disorganization of maturation, malorientation of spermatids and hypospermatogenesis were marked in untreated diabetic rats.
Electron microscopic examination of group IV showed Sertoli cells with large indented nuclei and prominent nucleoli. Spermatogonia cells were normal in shaped without vacuoles. The cells were seen resting on less irregular and less thickened basement membrane (Fig.20) compared to the diabetic group.
Examination of group V revealed mild irregular and thin basement membrane with flat regular myoid cells. Sertoli and spermatogenic cells more or less normal with lipid droplets and resting on the basement membrane (Fig. 21).
Electron microscopic examination of group VI showed thin regular basement membrane and normal Sertoli and spermatogenic cells (Fig. 22). The basement membrane and the cells are more improved with the treatment of diabetic rats with high PJ. Many spermatids with acrosomal cap covering the anterior half of the nuclei were seen in the lumen of the seminiferous tubules (Fig. 23).

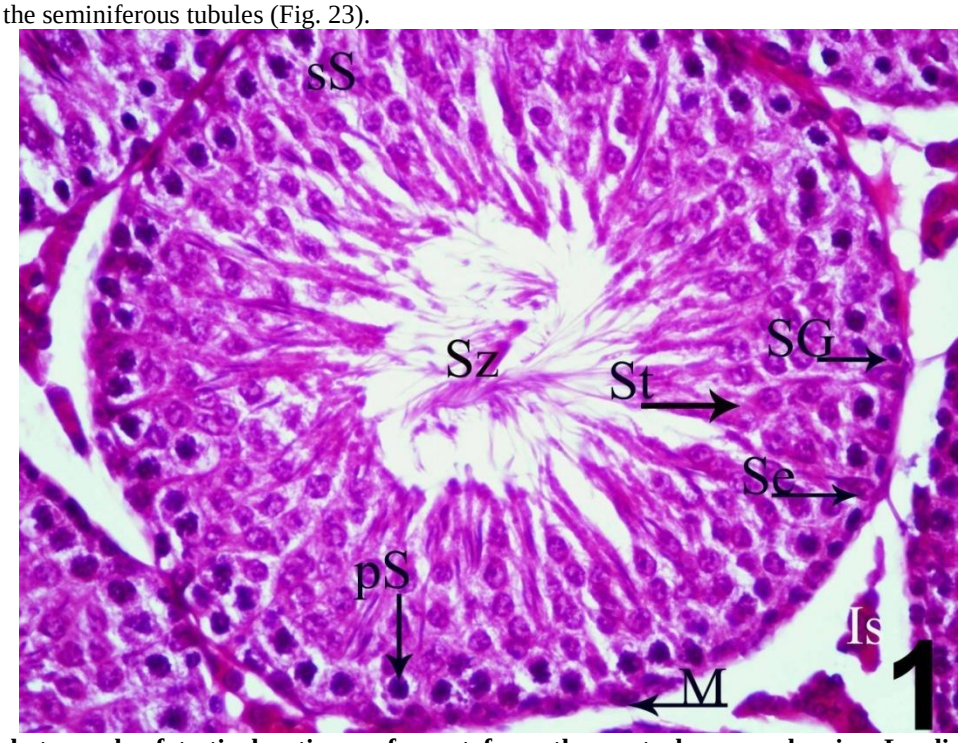

Fig. 1:- A photograph of testicular tissue of a rat from the control group showing Leydig cells in the interstitial tissues (Is), the myoid cells (M), spermatogonia (SG), Sertoli cells (Se), primary spermatocytes (pS), secondary spermatocyte (sS) and elongated spermatids (St). (Hx&E X 400)

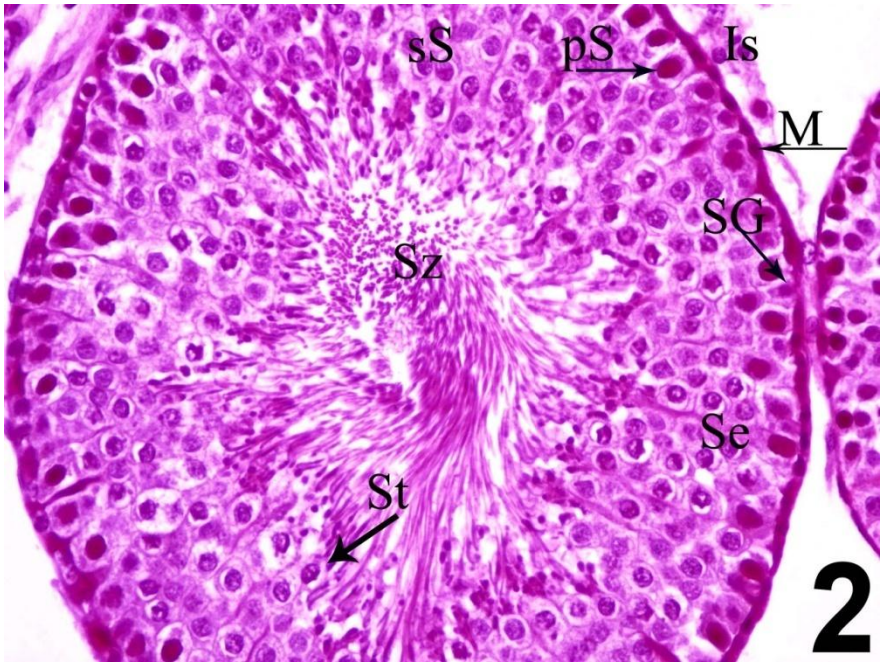

Fig. 2:- A photograph of testicular tissue of a rat from the PJ treated group showing normal testicular architecture and regular seminiferous tubular morphology with normal spermatogenesis, the myoid cells (M), spermatogonia (SG), sertoli cells (Se), primary spermatocytes (pS), secondary spermatocyte (sS), and elongated spermatids (St). Leydig cells appeared in interstitial tissue (Is). (Hx&E X 400)

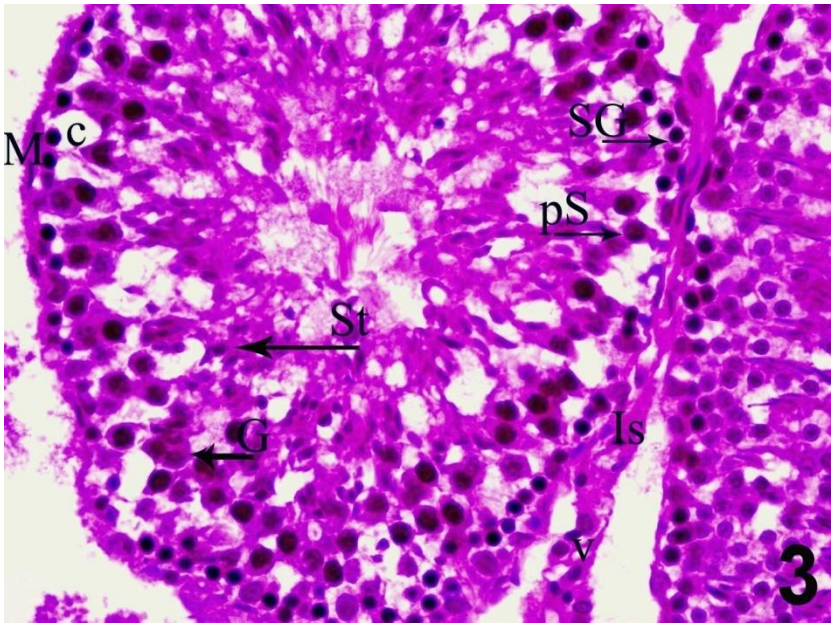

Fig. 3:- A photograph of part of seminiferous tubule of diabetic rat showing depletion of germ cells. The seminiferous tubules have irregular shape and the germinal epithelium is disorganized with many cavities (c). Giant cells with two nuclei (G) are seen.Myoid cells (M), spermatogonia (SG), primary spermatocytes (pS) and spermatids (St) are present. The interstitial tissue (Is) is widened and vacuolated. (Hx&E X 400)

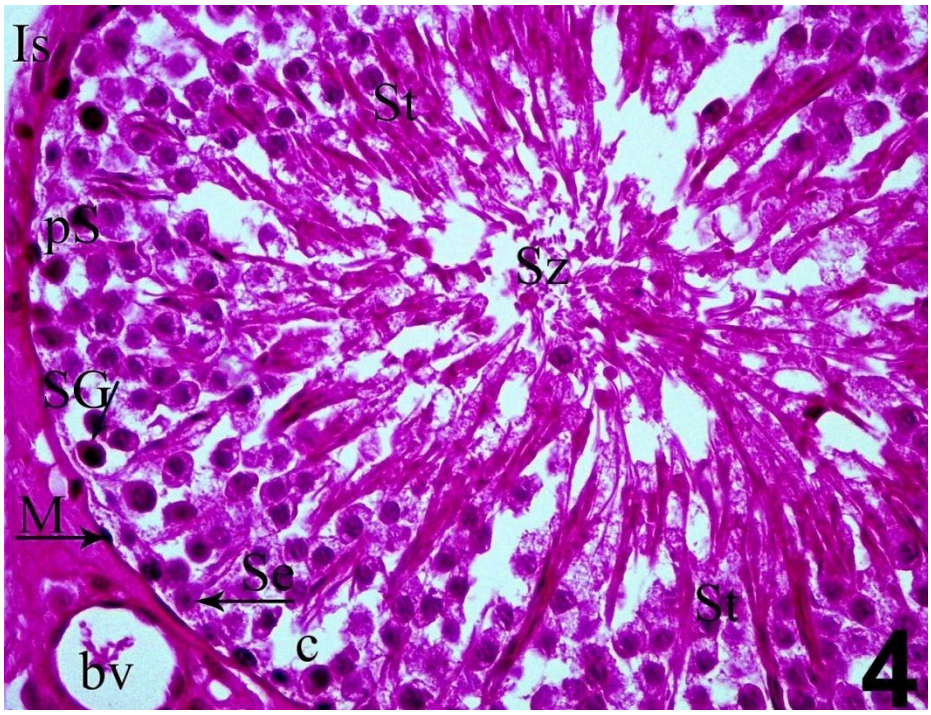

Fig.4:- A photograph of testicular tissue of a rat from the low PJ treated diabetic rat group showing an improved testicular arrangement, seminiferous epithelium with spermatogonia (SG), primary spermatocytes (pS), Sertoli cells (Se) spermatids (St) and spermatozoa (Sz) in the lumen. Notice the presence of cavities (C) and degenerated cells. The interstitial tissues (Is) is congested, vacuolated (v) with wide blood vessel(bv). (Hx&E X400)

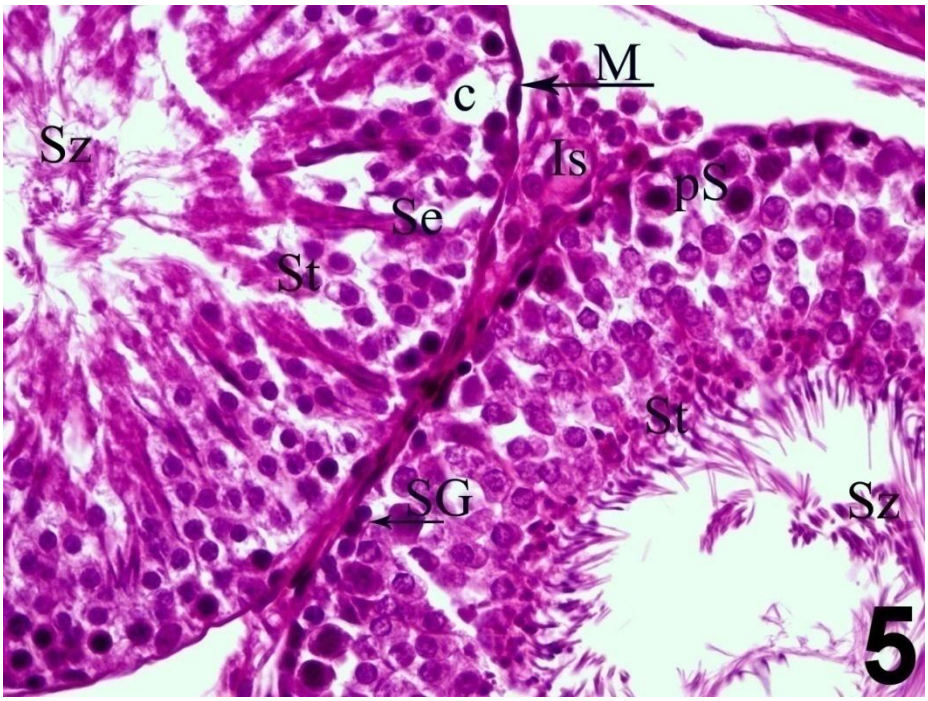

Fig. 5:- A photograph of testicular tissue of a rat from the middle PJ treated diabetic rat group showing the myoid cells (M), Sertoli cells (Se), spermatogonia (SG), primary spermatocytes (pS), spermatids (St) and spermatozoa (Sz) in the lumen. Some cavities (c) appear close to the basal lamina. The interstitial tissue (Is) with its cells looks more or less normal (Hx&E X 400)

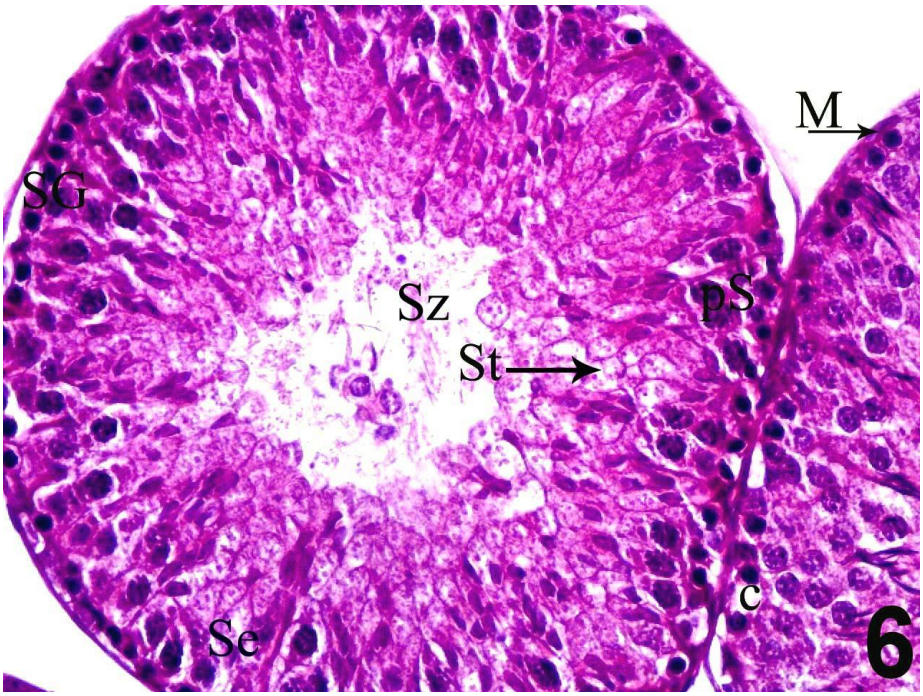

Fig. 6:- A photograph of testicular tissue of a rat from the high PJ treated diabetic rat showing the myoid cells (M), spermatogonia (SG), primary spermatocytes (pS), spermatids (St) and spermatozoa (Sz) in the lumen. Some cavities (c) appear close to the basal lamina. The pathological changes are reduced. (Hx&E X400)

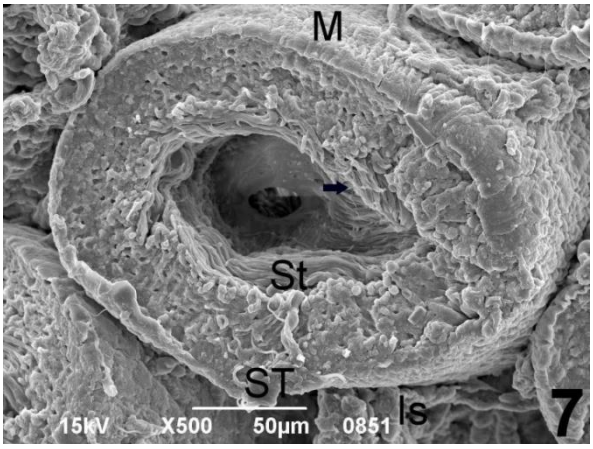

Fig. 7:- Scanning electron micrograph of testicular sections of rats of the control group showing: seminiferous tubule (ST) with flat myoid cells (M) and interstitial spaces (Is) in between. Notice the presence of spermatids (St) and spermatozoa with long flagellae (arrow) in its lumen (bar = 50 µm).

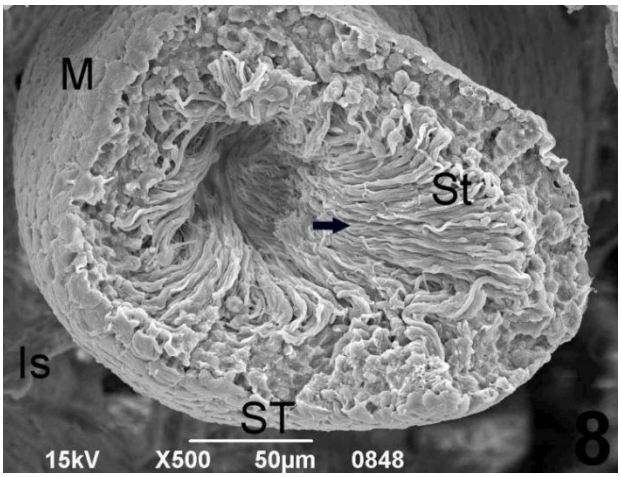

Fig. 8:- Scanning electron micrograph of testicular sections of rats of the PJ treated group showing: seminiferous tubule (ST) with flat myoid cells (M) and interstitial spaces (Is) in between. Notice that its lumen is completely occupied by spermatids (St) and spermatozoa with long flagellae (arrow) (bar = 50 µm).

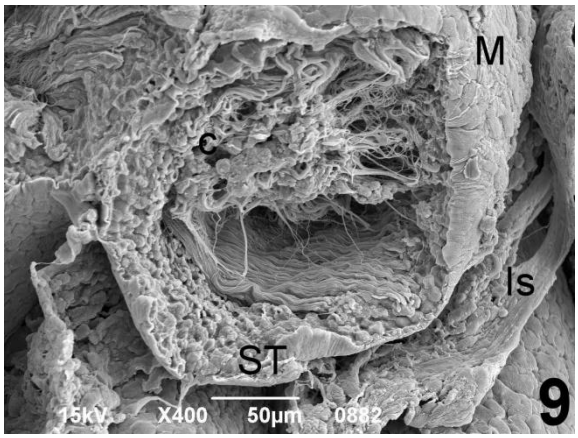

Fig. 9:- Scanning electron micrograph of testicular sections of rats of the untreated diabetic group showing: seminiferous tubule (ST) with flat myoid cells (M) and wide interstitial tissues (Is) and spaces. Notice the irregularity of the ST outline and the presence of cavities with the depletion of the germinal epithelial layers (bar = 50 µm).

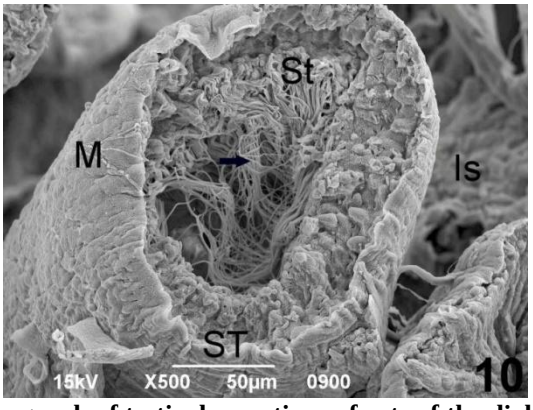

Fig. 10:- Scanning electron micrograph of testicular sections of rats of the diabetic group treated with low PJ showing: seminiferous tubule (ST) with flat myoid cells (M) and wide interstitial tissues (Is) and spaces. Notice the irregularity of the ST outline, the presnce of cavities and the depletion of the germinal epithelial layers is improved and many spermatids (St) are present with their flagellae (arrow) in the lumen (bar = 50 µm).

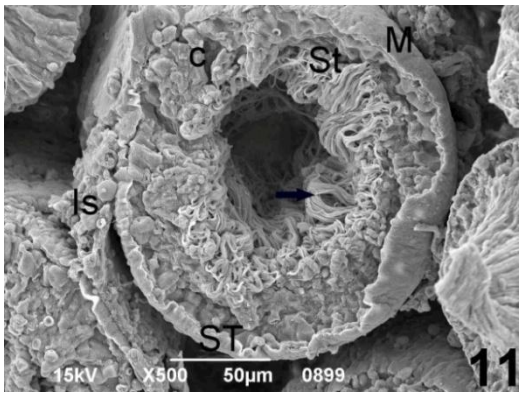

Fig. 11:- Scanning electron micrograph of testicular sections of rats of the diabetic group treated with middle PJ showing: seminiferous tubule (ST) with flat myoid cells (M) and wide interstitial tissues (Is) and spaces. Notice the spermatids (St) and spermatozoa present with their flagellae (arrow) in the lumen and layers of germinal epithelium and cavities (c) (bar = 50 µm).

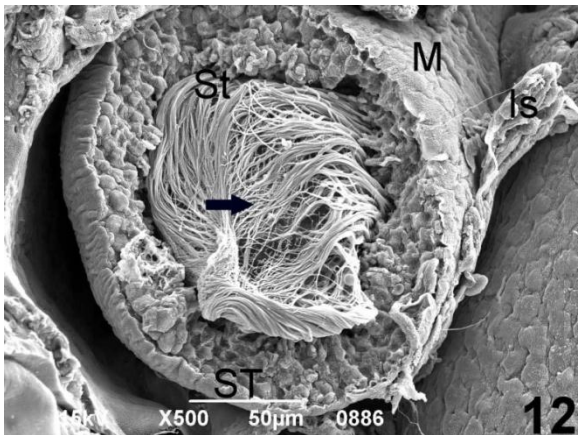

Fig. 12:- Scanning electron micrograph of testicular sections of rats of the diabetic group treated with high PJ showing: seminiferous tubule (ST) with flat myoid cells (M) and wide interstitial tissues (Is) and spaces. Notice the large amount of spermatids (St) and spermatozoa with their flagellae (arrow) present in the lumen and many layers of germinal epithelium (bar = 50 µm).

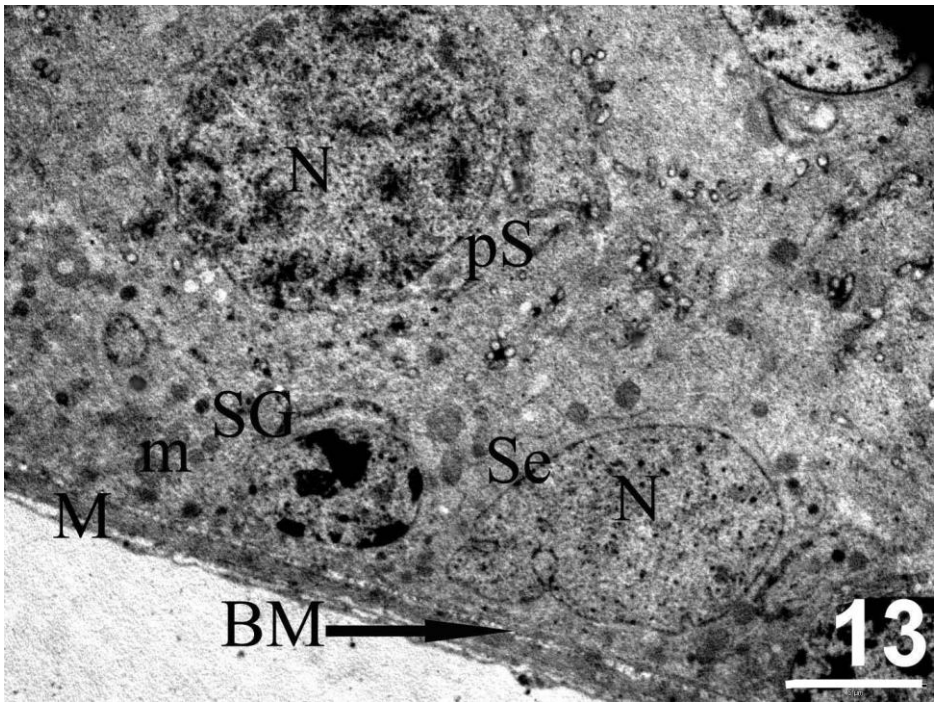

Fig. 13:- Transmission electron micrograph of testicular sections of rats of the control group showing: Sertoli cell (Se) with its idented nucleus (N), spermatogonia (SG) with mitochondria (m) closed to regular basement membrane (BM) and myoid cell (M). Notice the presence of primary spermatocyte cell (pS) with its large nucleus (N). (bar = 5 µm X 4000)

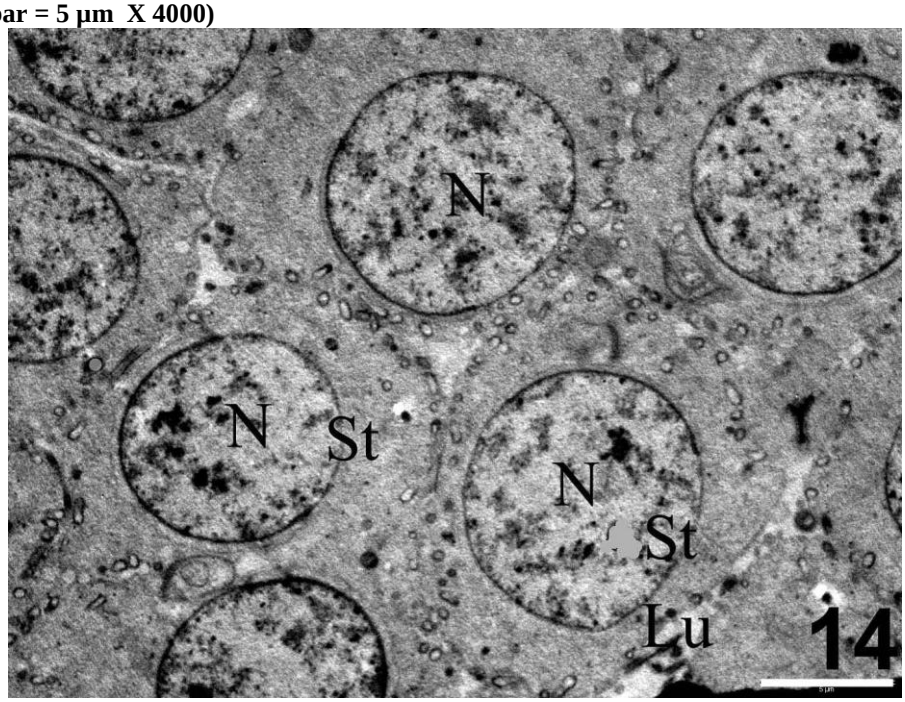

Fig. 14:- Transmission electron micrograph of testicular sections of rats of the control group showing: cap phase spermatids (St) with its large rounded nucleus (N) close to the lumen (Lu). The acrosome spread over part of nucleus. The nuclear membrane is highly electron dense under the acrosomal cap. (bar = 5 µm X 4000)

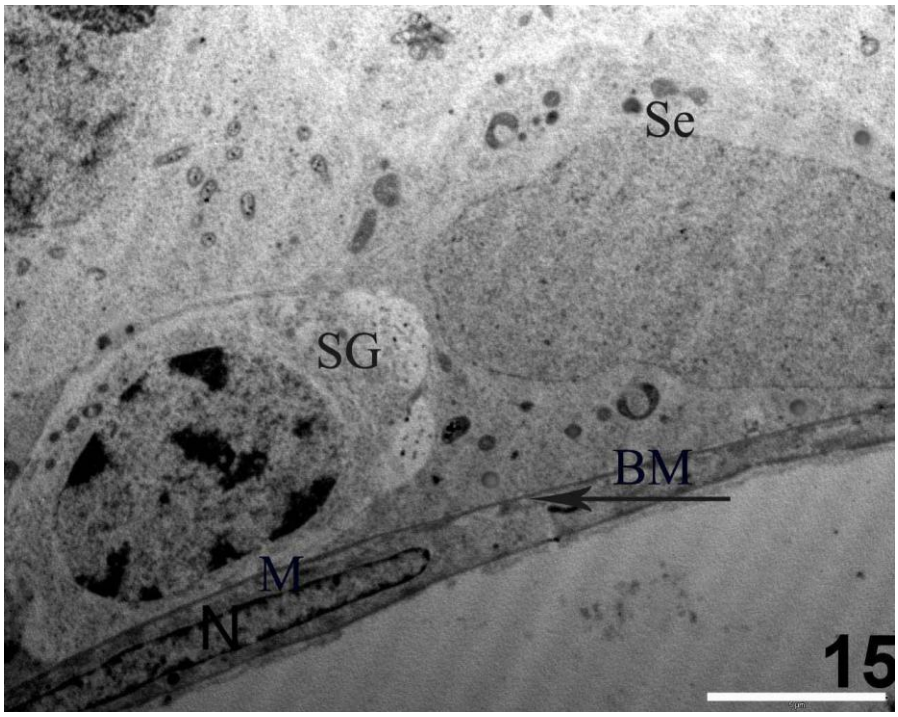

Fig. 15:- Transmission electron micrograph of testicular sections of rats of the pj treated group showing: spermatogonia (SG), and Sertoli cell (Se) resting on the regular basement membrane (BM) with flat myoid cell (M) nucleus (N). (bar = 5 µm X 5000)

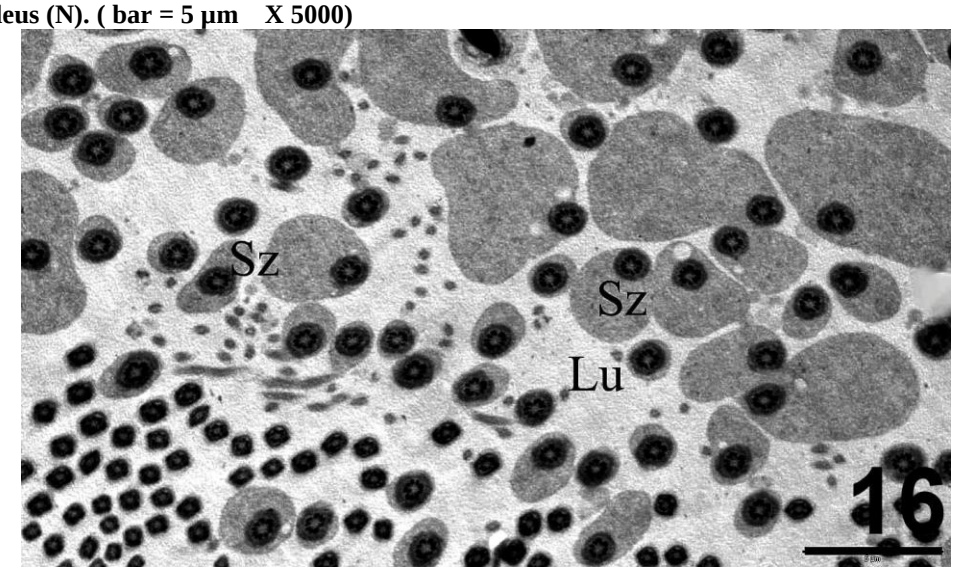

Fig. 16:- Transmission electron micrograph of testicular sections of rats of the pj treated group showing: large numbers of spermatozoa (Sz) in the lumen. (bar = 5 µm X 4000)

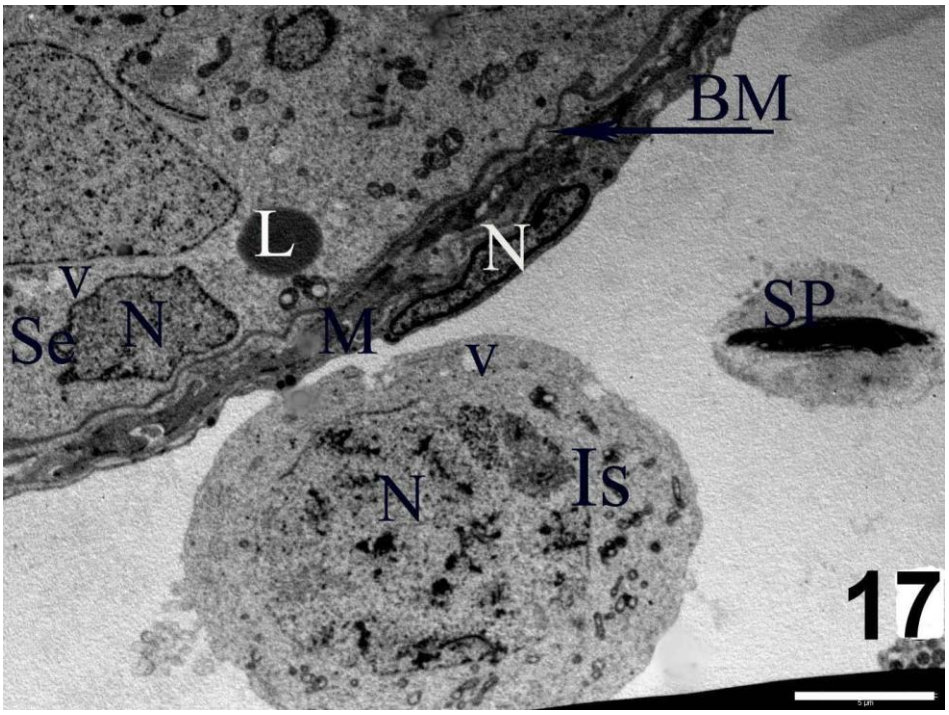

Fig. 17:- Transmission electron micrograph of testicular sections of rats of the diabetic group non treated showing: irregular basement membrane (BM), atrophy of sertoli cell (Se) nucleus (N) with the presence of vacuoles (v), lipid droplets (L), flat myoid cells (M) with irregular nucleus (N) and large interstitial cell (Is) of Leydig having large euchromatic nucleus (N) and vacuoles (v). Notice the presence of sperm (SP) outside the seminiferous tubule. (bar = 5 µm X 4000)

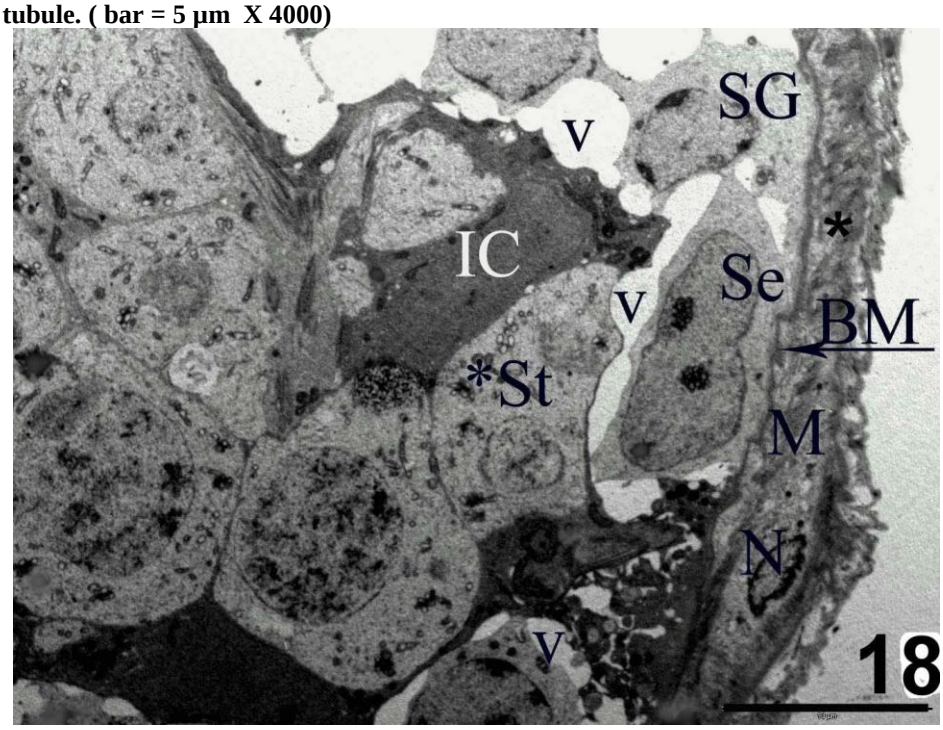

Fig. 18:- Transmission electron micrograph of testicular sections of rats of the diabetic group non treated showing: marked irregular and thickened basement membrane (BM), spermatogonia (SG) with vacuole (v), atrophy of Sertoli cell (Se) nucleus (N) with the presence of vacuole (v) also and primary spermatocyte (pS). Notice the nuclear atrophy (N) of myoid cell and the deposition of collagen fibres (*). (bar = 10 µm X 3000)

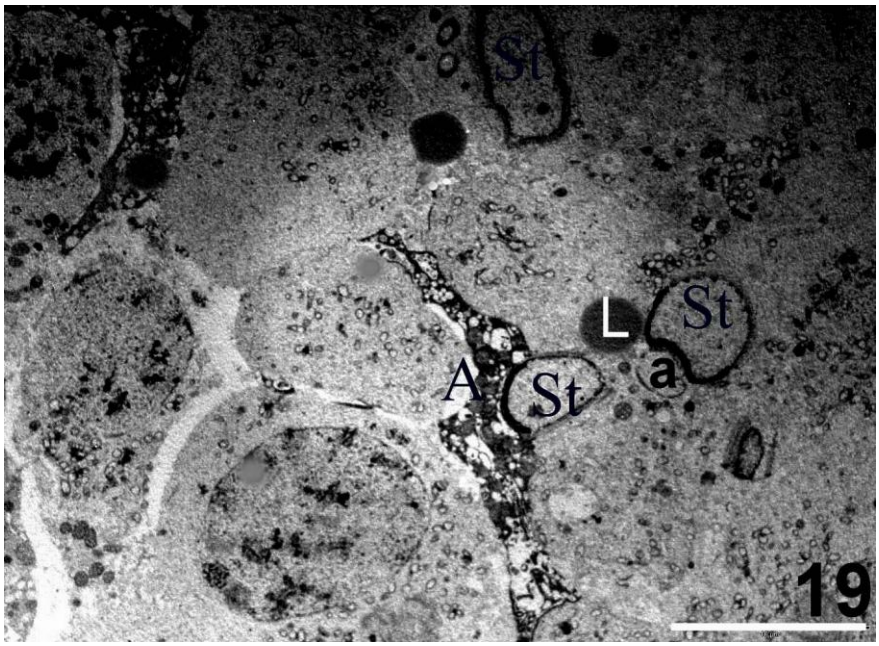

Fig. 19:- Transmission electron micrograph of testicular sections of rats of the diabetic group non treated showing: spermatids (St) in cap phase close to the lumen, acrosomsl vesicle (a), lipid droplet (L). Notice the presence of amorphous cytoplasmic materials (A) in the lumen. (bar = 10 µm X 3000)

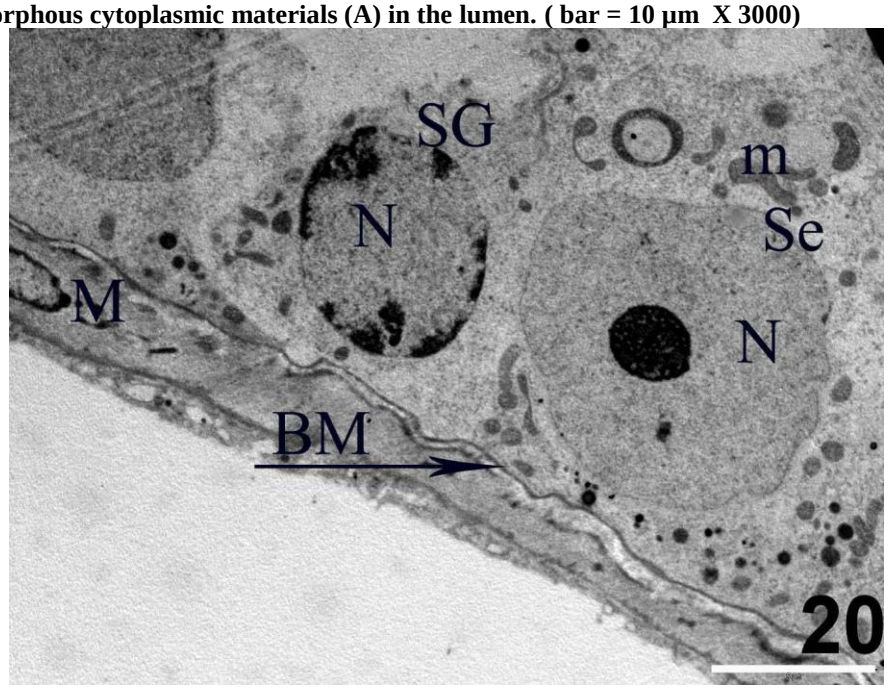

Fig. 20:- Transmission electron micrograph of testicular sections of rats of the diabetic group treated with low PJ showing: irregular basement membrane (BM), flat myoid cell (M), spermatogonia (SG) and sertoli cell (Se) with indented nucleus (N) and condensed nucleolus (n) and the presence of mitochondria (m). (bar = 5 µm X 5000)

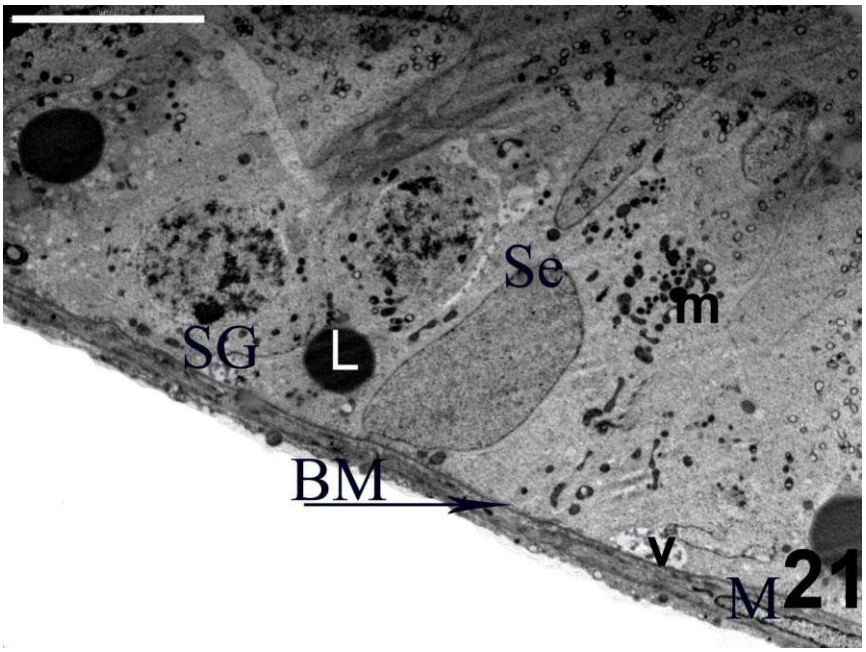

Fig. 21:- Transmission electron micrograph of testicular sections of rats of the diabetic group treated with middle PJ showing: mild irregular basement membrane (BM), spermatogonia (SG) and sertoli cell (Se). Notice the presence of mitochondria (m), lipid droplets (L) and vacuole (v). (bar = 10 µm X 3000)

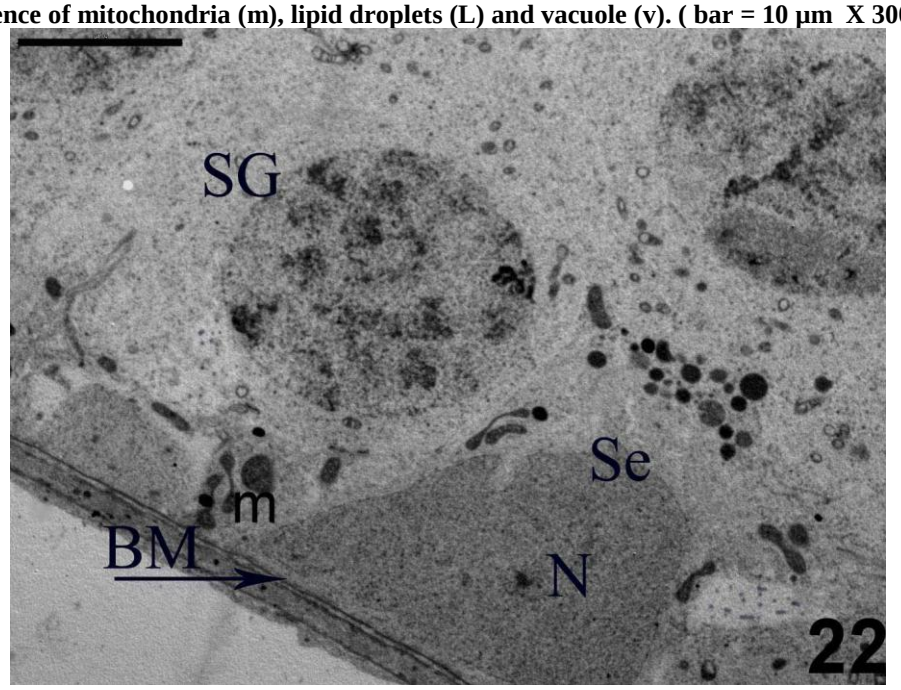

Fig. 22:- Transmission electron micrograph of testicular sections of rats of the diabetic group treated with high PJ showing: sertoli cell (Se) with indented nucleus (N) and mitochondria (m) resting on regular basement membrane (BM) and the presence spermatogonia (SG). (bar = 5 µm X 5000)

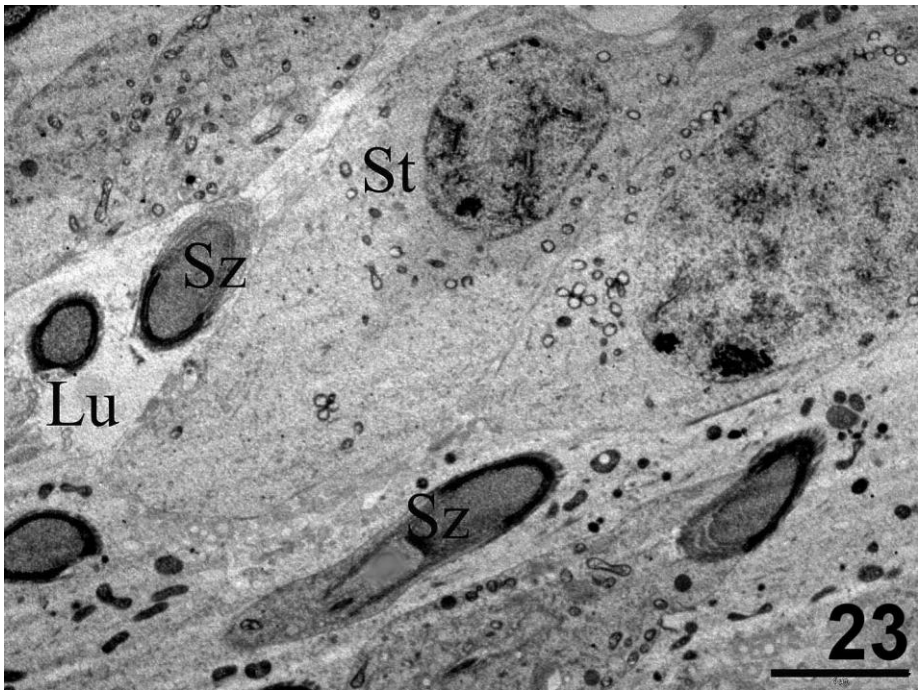

Fig. 23:- Transmission electron micrograph of testicular sections of rats of the diabetic group treated with high PJ showing spermatids (St) and many spermatozoa (Sz) with acrosomal cap covering the anterior half of the nuclei close to the lumen (Lu). (bar = 5 µm X 4000)

DISCUSSION

Diabetes mellitus is a chronic disease affecting many tissues and systems of the body. The incidence of diabetes is increasing and this will result in a 50% increase over the next ten years. The increasing incidence of diabetes may result in the decline in male fertility (Bener et al., 2009) as it is manifested by spermatogenic alteration (Shrilatha and Muralidhara, 2007).

STZ is an alkylating agent actively transported into pancreatic β cells via the Glut-2 glucose transporter. It reacts at many sites in DNA but in particular at the ring nitrogen and exocyclic oxygen atoms of the DNA bases (Cardinal et al., 2001). STZ affected β cells of islets of Langerhan's by release of toxic radicals leading to β cell death (Raza et al., 2004) that caused insufficient production of insulin and consequently, the elevation of blood glucose level. The morphological alterations observed in the testes of STZ-induced diabetic rats are not caused by a direct effect of the drug, but rather by diabetes (Ballester et al., 2004).

Rats received 60 mg/kg/body weight of STZ became diabetic manifested by increase in the mean blood glucose level and decrease in the mean body weight which were statistically significant as compared to the control group. These findings are similar to those of some researchers (Hassan and Abdel Moneium, 2001; Mallick et al., 2007). The body weight of diabetic rats significantly reduced during the course of diabetes. The reduction of body weight can be due to breakdown of tissue proteins in diabetic rats (Yanardag et al., 2005) . In the present study reduction of body weight was less in PJ treated diabetic rats with a dose of 0.25, 0.5, 1ml /kg of body weight daily for 52 days.

Rats testicular weight was reduced in diabetic group compared to control group (Group l) or PJ group (Group ll). This reduction of gonadal weight was accompanied with general body weight loss. Testicular weight was improved in all PJ treated diabetic rats (Groups lV, V, Vl). Diabetic rats testicular weight is decreased around 20 percent compared to healthy rats (Hassan and Abdel Moneium, 2001; Navarro-Casado et al., 2010). Yanardag et al, (2005) reported that the testicular weight increase in short time and decrease in long time diabetic rats. The comparison of gonad/body weight ratio between experimental groups indicated that, the loss of weight of gonad in diabetic groups was dependant to body weight changes.

In the testicular tissue, mean of the STs diameter are used to assess histopathologic damage (Uguralp et al., 2004). In the present study, the diameter of the STs in diabetic rats was strongly reduced. Decrease of the diameter of STs was reported in recent studies (Guneli et al., 2008; Kianifard et al., 2011). It was accompanied also with decrease in the height of germinal epithelium. Germ cell depletion, decrease in cellular population and depress the activity of spermatogenesis in the untreated diabetic rats were reported also (Kianifard et al., 2011).

These histological observations in STs, indicates depressed cellular activity of spermatogenic cells and the STs became atrophied during the course of diabetes. Decrease of the diameter of STs explains in part the apparent

increase in the interstitial areas. The decreased diameter of the STs in diabetic rats may be due to low gonadotrophins and testosterone (Ballester et al., 2004). STs atrophy and the decrease in spermatogenic cells were morphological indicators of spermatogenesis failure (Cameron et al., 1985). Administration of PJ to the diabetic rats ameliorated these histopathological deficits by providing structural protection against the impairment of STs.

The results on light microscopic level revealed histological structure of the ST of control rat in agreement with that described by Fawcett (1993).

Group II treated with PJ shows marked increase in spermatids and spermatozoa in the lumen. PJ increased spermatogenic cell density, sperm concentration, sperm motility and decreased abnormal sperm in male rats (Turk et al., 2008).

Examination of the diabetic rats testis specimens by light microscope showed that the STs were severely affected. Many cavities and spaces appeared in the ST leading to decrease the number of Sertoli cells and spermatogonic cells. This finding indicates that the conversion of spermatogonic cells to primary spermatocytes is reduced in diabetic conditions. These changes in cellular conversion lead to reduction in spermatid production in most of the tubules. The abnormal spermatogenesis occurred with diabetes coincided with the results of other researchers (Cameron et al., 1985; Altay et al., 2003; Ricci et al., 2009). Whereas Scarano et al,(2006) did not find any alterations in the testicular structure, possibly due to the reason that they used very short duration of diabetic conditions before terminating the experiments. ST in the untreated diabetic group showed the presence of giant multinucleated cells. These cells may resulted from the inability of the primary spermatocytes to undergo meiotic division (Rotter et al., 1993). The presence of these cells were considered to be the end result of germ cell degeneration (Hess and Nakai, 2000).

The increase in interstitial connective tissue and amorphous material was seen in testes of diabetic rats in the present study. Other study also reported the increase in interstitial area in testis of diabetic rats (Hassan and Abdel Moneium, 2001).

Testicular tissue damage can primarily be accounted for by decreased glucose utilization due to lack of insulin in STZ-induced diabetic rat (Mallick et al., 2007). STZ-induced diabetes resulted in significant reduction of the GSH-Px antioxidant enzymes activities in the testis. while a mild dose of PJ caused a slight increase in spermatogenic cell density especially spermatozoa, both middle and high doses of PJ provided marked increases in all the spermatogenic cells ranging from spermatogonia, spermatocytes, spermatids to spermatozoa when compared to the untreated diabetic group. Additionally PJ provided an increase in the diameter of ST and germinal cell layer thickness. In this study, improvements observed in spermatogenic cell may be attributed to prevention of excessive generation of free radicals, produced by spermatozoa themselves by means of the antioxidant property of PJ.

Rats of the groups which were treated with PJ revealed a significant decrease in the mean blood glucose level as compared to the diabetic rats receiving no treatment. The hypoglycemic effect of PJ had been documented (Jurenka, 2008). Several reports have demonstrated that PJ possesses a lot of antioxidative activity (Jurenka, 2008). In diabetic rats treated with PJ all the histological observations seemed to be improved and dose dependant as comparison with untreated diabetic rats. These findings specify that PJ can diminish the side effects of diabetes on the structure of testis. Effect of PJ may be through the lowering of the elevated blood glucose levels and as antioxidant.

The Electron microscopic picture of the control ST are in agreement with that reported by Kuehnel (2003). EM examination of untreated diabetic rats revealed vacuolations and degeneration in the spermatogenic and Sertoli cells. Interstitial cells of Leydig showed vacuoles may be from dilated cisternae. Diabetes caused oxidative stress stimulating macrophages and other inflammatory cells to secrete cytokines. Cytokines might lead to inhibit Leydig cells and affecting spermatogenesis (Konrad et al., 1998). Cameron and his colleagues (1985) did not find either diminution or change on both light and electron microscope of Leydig cells of the diabetic rats.

Dilatation of the intercellular spaces of ST cells were present in untreated diabetic rats. Acute stress can prompt intercellular space dilation (Farré et al., 2007). The basement membrane structure is considered to ensure fluid regulation from the inter-tubular medium toward the lumen of the STs (Yamamoto et al., 1987). The basement membrane of STs of diabetic rats revealed irregularity, increase in thickness and deposition of collagen fibres. This thickening appears to be caused by increased collagen content. Seminiferous tubular walls were found to be thickened in diabetic man (Cameron et al., 1985). Thickness of the basement membrane may be due to impairment of the turnover of basement membrane proteins (Nicholls and Mandel, 1989). Changes in the STs basement membrane could impair spermatogenesis (Skinner, 1991).

Sertoli cells send numerous fine cytoplasmic processes which envelope the associated germ cells. Sertoli cells revealed vacuolated cytoplasm and nuclear atrophy as reported previously (Cameron et al., 1985; Murray et al., 1981). Structural degeneration of Sertoli cell is the cause of germ cell sloughing and spermatid malorientation (Cameron et al., 1985). Sertoli cells are responsible for the control of testis development (Renato de France et al., 1995).

Tarleton et al,(1990) showed testicular histological changes which included disorganization of maturation, malorientation of spermatids and hypospermatogenesis in diabetic animals. The plasma membrane of sperms contains a high amount of unsaturated fatty acids. Therefore, it is particularly susceptible to peroxidative damage.

Although little information is available on the effect of PJ on diabetes but from our study it was found that PJ had a more positive and potent effect on the testes of diabetic rats. Improvement of testicular tissue observed in the present work after administration of PJ may suggest that the antioxidant properties of PJ can mop up free radicals produced by streptozotocin

The present results confirmed that STZ-induced diabetes mellitus caused testicular pathology in male rats as has been reported by others (Guneli et al., 2008). Also it revealed for the first time that administration of PJ to diabetic rats attenuated the testicular pathology and spermatogenic disruption in the testis. In the present study, we have elaborated the protective effects of PJ against STZ-diabetes-induced damages of the testis structure of male rats.

In conclusion, this research demonstrated that diabetes caused damage in the testicular structures and PJ administration improves these structural deficits by providing protection against the impairment of seminiferous tubules and the loss of spermatogenic cell series.

Disclosure:

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