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Histological Study on The effect of Diabetes and Insulin Replacement Therapy on The prostate of Albino Rats

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Abstract:

Background: Diabetes Mellitus (DM) is a worldwide common disease and it is considered a serious public health disorder .The reproductive function is highly sensitive to changes in metabolic status, with defective reproductive capacity. **Aim of Work:** To demonstrate the histological changes in the prostate of rats with STZ induced diabetes type and to examine to what extent insulin could reverse such changes. **Materials and methods:** Twenty six male albino rats were divided into three groups. Group I: control; Group II: Diabetic was exposed to induction of diabetes by a single intraperitoneal injection of Streptozotocin (STZ) 60 mg/kg in 0.1 M citrate buffer; Group III: Diabetic then received insulin (3IU/day S.C) (for 4 weeks). The rats of each group were sacrificed after 4 weeks. The prostates were processed for electron microscopic sections and other sections stained with H&E, Masson's trichrome, immunohistochemical staining of α smooth muscle actin, Vimentin and PCNA and statistical analysis were applied. **Results:** Diabetes resulted in deterioration of normal structure of the prostatic acini and some prostatic acini showed cell proliferation with increase Masson's trichrome staining and increase immunoreactivity for α smooth muscle actin, Vimentin and PCNA. Electron microscopic examination reavealed dilatation of the cisternae of the Golgi complex, endoplasmic reticulum, and some epithelial cells showed proliferation and irregular contour of their nuclei. Insulin administration mostly reversed these changes. **Conclusion:** insulin administration greatly improved the histological architecture of the prostate gland.

Keywords: Diabetes, insulin, prostate, immunohistochemistry, electron microscope.

1- Introduction:

Diabetes mellitus (DM) is one of the most important diseases of modern society and it represents not only a medical problem but also a social one because it impairs the quality of life of affected individuals. The high mortality and negative impact of diabetes on life quality are due to the progressive impairment of multiple organ systems, caused mainly by hyperglycemia and oxidative stress **[1]**.

Diabetes is an epidemic disease characterized by the inability to produce or improperly use insulin in the human body. There are two types of diabetes: type 1 (insulin-dependent diabetes) and type 2 (non–insulin-dependent diabetes) **[2]**.

Tissue alterations caused by diabetes affect different body systems including the male reproductive system, especially the accessory sex glands and gonads. DM is a well-recognized cause of male sexual dysfunction and impairments of male fertility **[3]**.

Several investigators have conveyed the complications of DM on the structure and functions of the sexual and reproductive organs. Impotency, reduced libido, impairment of spermatogenesis, reduced sperm count and motility, decreased seminal fluid volume and decrease serum testosterone **[4]**.

The prostate gland is the largest accessory gland of the male reproductive system. It depends on different hormones such as androgens, estrogens and prolactin. The testosterone is the main hormone that is very important for the development of prostate and maintenance of its structural and functional integrity. The subtle change in the testosterone level is usually accompanied by alteration in the growth and weight of the prostate **[5]**.

Insulin is a hormone secreted by the pancreas that regulates glucose levels in the blood. Without insulin, cells cannot use the energy from glucose to carry out functions within the body. In insulin-dependent diabetes, leydig cell function and testosterone production decrease due to the absence of the stimulatory effect of insulin on these cells and an insulin-dependent decrease in FSH, which in turn reduces LH levels **[6]**.

Accordingly, this study was designed to demonstrate the histological changes induced in the prostate by induction of diabetes using STZ and also to examine to what extent insulin could reverse such changes.

2- Materials and Methods:

Drugs:

Streptozotocin (STZ): Streptozotocin (STZ) 60 mg/kg in 0.1 M citrate buffer, pH 4.5 (SIGMA Pharmaceutical Industries) applied as a single dose injection intraperitoneally.

Hormone: Insulin 3IU (Novo Nordisk, Denmark) was applied subcutaneously (s.c) daily after diabetes confirmation for four weeks.

Animals:

This study was performed on **twenty six** adult male albino rats. (Weighing 200-250 gm). The rats were bred at the Animal House of Kasr El-Aini, Faculty of Medicine, Cairo University. Every two animals were purchased and housed in a separate metal cage with mesh and wood chips for bedding. They were maintained in room temperature with a 12-hour light /dark cycle, fed with standard laboratory chow and had free access to tap water. Rats were randomly divided into 3 groups as following;

Group I: (Control) n= 6 rats. The rats were sacrificed after 4weeks. Control animals were injected with citrate buffer. (0.5 ml/day intraperitoneal) per rat.

Group II: n=10 rats. They were exposed to induction of diabetes**,** and the animals were sacrificed after 4 weeks of confirmed diabetes **[7]**.

Group III: n=10 rats. Animals were exposed to induction of diabetes then hormonal replacement therapy was given every day starting after diabetes confirmation **[8**].The rats were sacrificed after 4 weeks of confirmed diabetes and insulin therapy.

Methods:

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of Streptozotocin (STZ) 60 mg/Kg of body weight **[9]**. Dissolved in citrate buffer (pH 4.5). Estimation of serum glucose was performed after the third day of Streptozotocin (STZ) injection to confirm diabetes.

Dissection and processing of the samples:

Rats were sacrificed at the end of experimental periods by inhalation of over dose phenobarbitol **[10]**. Followed by opening the abdomen through a lower midline incision and the prostates were removed.

Specimens were then fixed immediately in formol saline 10%, dehydrated in ascending grades of ethanol and embedded in paraffin, they were processed for paraffin sections. These were cut at 5-6 µm thickness and stained.

Specimens for electron microscope were obtained, trimmed and cut into very small blocks of approximately 1 mm. The specimens were first prefixed by 2.5% glutaraldehyde freshly prepared from the stock glutaraldehyde (25 % solution BDH E/M grade) in 0.1 mol/L phosphate buffer (PH:7.4). They were kept at 4^{o}C for 2 hours **[11]**.

The methods used were:

1- H & E to demonstrate the histological changes **[12]**.
2- Masson's trichrome stain to demonstrate collagen fibers **[13]**.
3- Immunohistochemical staining for α-smooth muscle actin for smooth muscle cells, vimentin for fibroblasts and PCNA for detection of nuclear DNA **[14]**.
4- Electron microscopy to demonstrate ultrastructural changes **[11]**.

C- Morphometric Study:

Data were obtained using" Leica Qwin 500 C " image analyzer computer system Ltd. (Cambridge, England) in Histology department, Faculty of Medicine, Cairo University. Images were captured live on to the screen from sections under light microscope with video camera. The image analyzer consisted of a color video camera, Olympus microscope, colored monitor, hard disc of IBM personal computer connected to the microscope and controlled by" Leica Qwin 500 C "software . Slides were examined under the light microscope. All measurements were taken in 5 non overlapping fields for each specimen for all groups.

The following parameters were measured:

1) Measurement of the epithelial height in H&E stained sections using the interactive measuring menu. This was done at a magnification of x400 for all animals of all groups.

2) Measurement of the mean area % of collagen fibers: It was done on Masson's trichrome stained sections. Detection of the areas of collagen (occupied by the blue color) stained sections were observed at x 10 objective lens. Grey image is for optical density.

3) Measurement of the mean area % occupied by strong brown cytoplasmic staining in α-SMA, Vimentin and PCNA immune-stained sections. This was done by masking reaction with the binary colour to specify areas of brown reaction to be detected then measured at X 40 objective lens.

4) Area % was measured in relation to a fixed measuring frame with an area (7381.1) μm^2.

D- Statistical study [15].

Quantitative data were summarized as means and standard deviations and Comparison between study groups was made using one analysis of variance (ANOVA) followed by post hoc Tukey test. P values <0.05 were considered statistically significant and <0.01 highly significant. Calculations were made on SPSS software (version 16).

3- Results:

1-Hematoxylin and Eosin stained prostatic sections (plate1):

Histological examination of rat prostatic sections in **the control group (GI)** revealed that the prostate consists of branched tubulo-acinar glands embedded in a fibro muscular stroma. The prostate gland showed multiple acini with infolded mucosa lined by a simple columnar epithelium **(GI)**. The sections in **groupII (diabetic group)** revealed the acini were separated by widely spaced connective tissue with dilated congested blood vessels. This was associated with loss of infolding of glandular mucosa of the most acini and thinning of the lining epithelium. Some prostatic acini showed cell proliferation. The sections in **groupIII (treated group)** showed the prostatic acini have well formed lining epithelium and the mucosal folds with minimal stroma in between.

2- Masson's Trichrome stained prostatic sections (plate2):

Histological examination of rat prostatic sections in **the control group (GI)** revealed the stroma of the prostate had a mixture of collagen fibers and smooth muscle fibers. The sections of **groupII (diabetic group)** showed widened and thick stroma with increase of collagen fibers when compared to the control group. While the sections of **groupIII (treated group)** revealed decreased collagen fibers in-between the acini and thinning of the septa between the acini when compared to the diabetic group.

3-Immuno-Histochemical Results:(α-SMA staining)(plate3):

Histological examination of **the control group (GI)** revealed positive actin immuno-staining in the cytoplasm of the smooth muscles of the stroma. The sections of **groupII** exhibited abundant positive immunoreactivity when compared to that of the control group. The sections in **groupIII** showed scanty positive actin immuno-staining similarly to that in the control group.

4-Immuno-Histochemical Results:(Vimentin staining)(plate4):

Histological examination of **the control group (GI)** revealed few cells with positive immunoreactivity in the stroma. The sections of **groupII** showed many cells with positive imumunoreactivity for vimentin staining in the stroma. The sections in **groupIII** revealed few cells with positive immunoreaction as compared to groupII.

5-Immuno-Histochemical Results:(PCNA staining)(plate5):

Histological examination of the **control group (GI)** revealed few cells with positive nuclear immune reaction for PCNA in the epithelial cells of the prostatic acini. The sections of **groupII** expressed numerous cells exhibiting positive nuclear immune reaction for PCNA in the acinar cells. While the sections of **groupIII** revealed few cells with

positive nuclear immune reaction in the epithelium of the prostatic acini.

6- Ultrathin Sections Results (plate 6):

Electron microscopic examination of rat prostatic sections **in the control group (GI)** showed the prostatic epithelium formed of tall columnar cells with basally located oval-shaped nuclei. In the cytoplasm well-developed Golgi complex and the granular endoplasmic reticulum were formed of numerous flattened and parallel reticulum cisterns. Secretory vesicles with different electron density were observed in the apical cytoplasm and the microvilli covered the surface of the cell facing the lumen and thinning of the glandular stroma between the acini were observed.

Electron microscopic examination of the sections of **groupII** showed morphological alterations four weeks after confirmation of the diabetic state. There was atrophy of the secretory epithelium characterized by a general reduction of the cytoplasm and low cuboidal cells with basally located nuclei. Some acini exhibited proliferation of the epithelial cells with different sizes and irregular contour of the nuclei arranged in various layers of the epithelium. The cisterns of the Golgi complex were dilated and those of the granular endoplasmic reticulum were distributed in a concentric manner throughout the cytoplasm and decreased of apical secretory vesicles. While the sections of **groupIII** revealed the ultrastuctural features were similar to those verified in the control group. The epithelial cells revealed basal oval nuclei, well-developed Golgi complex, supranuclear secretory vesicles and microvilli on the cell surface and flattened, parallel reticulum cisterns of the rough endoplasmic reticulum were verified.

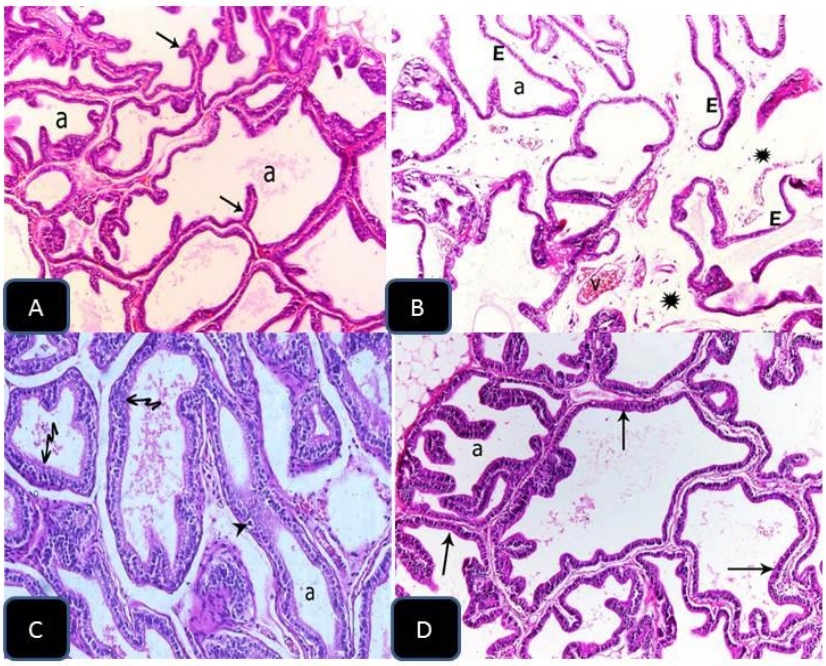

Plate (1): Photomicrograph of the prostate of **A (control group):** showing multiple acini with infolded mucosa (a) lined by a simple columnar epithelium (thin arrow). **B (diabetic group):** showing wide spaces between acini (star) with dilated congested blood vessels (V) in addition to loss of infolded glandular mucosa (a) and thinning of the secretory epithelium observed in most of the acini (E).**C (diabetic group)**: showing loss of infolded glandular mucosa of the acini (a) with proliferation of the secretory epithelium in some acini (wavy arrows). **D (treated group):** showing restoration of the lining simple columnar epithelium (thin arrows) with mucosal folds of the acini (a). **(H&E: A, B, C, & D x100).**

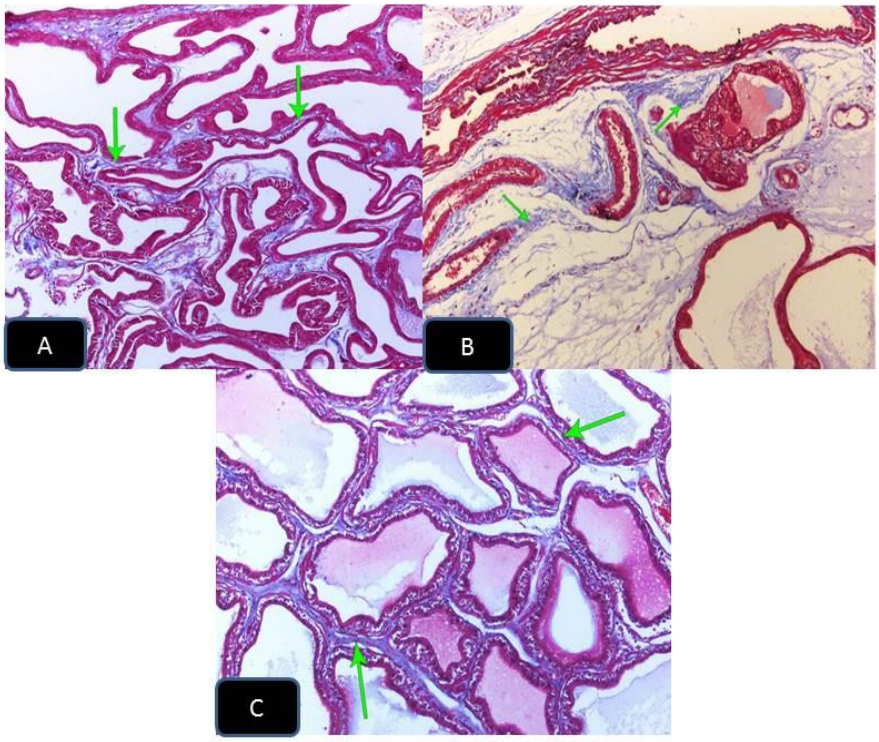

Plate (2): Photomicrograph of the prostate stained with Masson's Trichrome **A (control group):** showing the collagen fibers in the prostatic stroma (green arrows). **B (diabetic group)**: showing increase of collagen fibers in the stroma between the widely separated acini (green arrows). **C (treated group):** showing moderate decreased of collagen fibers between the acini (green arrows) and thinning of the septa between the acini. **(Masson's Trichrome: A, B, & C x100).**

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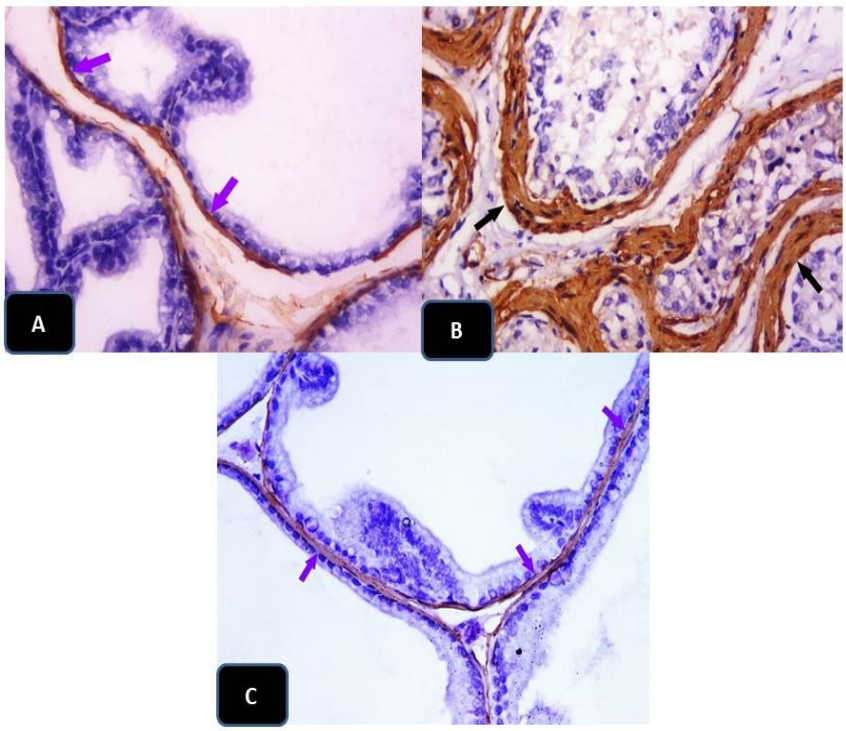


Plate (3): Photomicrograph of the prostate stained with α-SMA **A (control group):** showing positive immuno reaction for actin immuno-staining detected in the stroma (thick arrows). **B (diabetic group):** showing abundant positive immunoreactivity for α smooth muscle actin in the stroma (thick arrows). **C (treated group):** showing scanty positive actin immunoreaction in the smooth muscles of the prostatic stroma (violet arrows). **(α-SMA immunostaining: A, B, & C x400).**

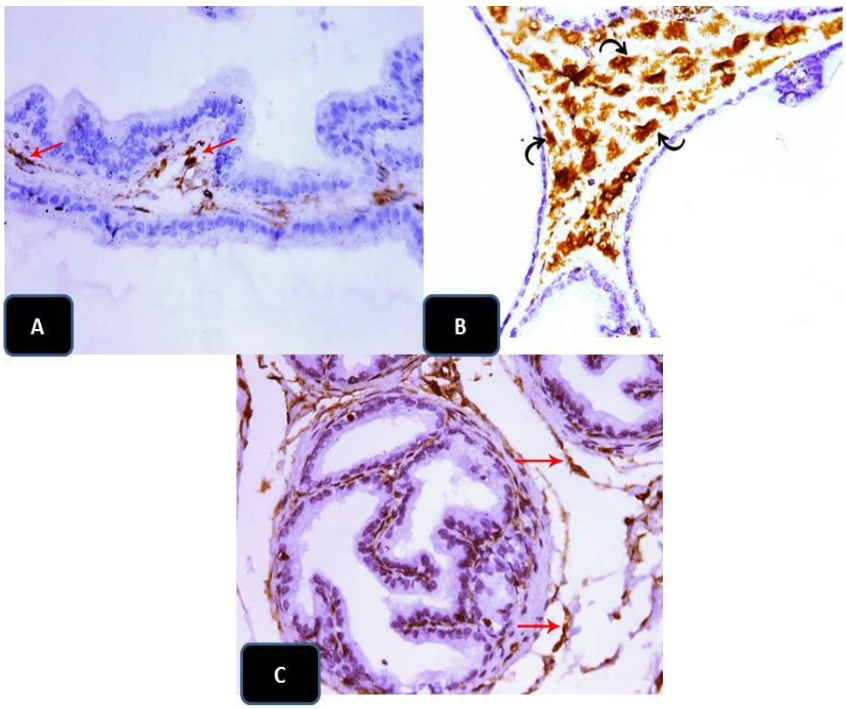


Plate (4): Photomicrograph of the prostate stained with Vimentin **A (control group):** showing few cells with positive immuno reaction for vimentin-staining within the stroma (red arrows). **B (diabetic group)**: showing many cells with positive immunoreactivity for vimentin staining in the stroma (curved arrows). **C (treated group):** showing few cells with positive immunoreaction for vimentin staining in the stroma (red arrows). (**Vimentin immunostaining: A, B, & C x400).**

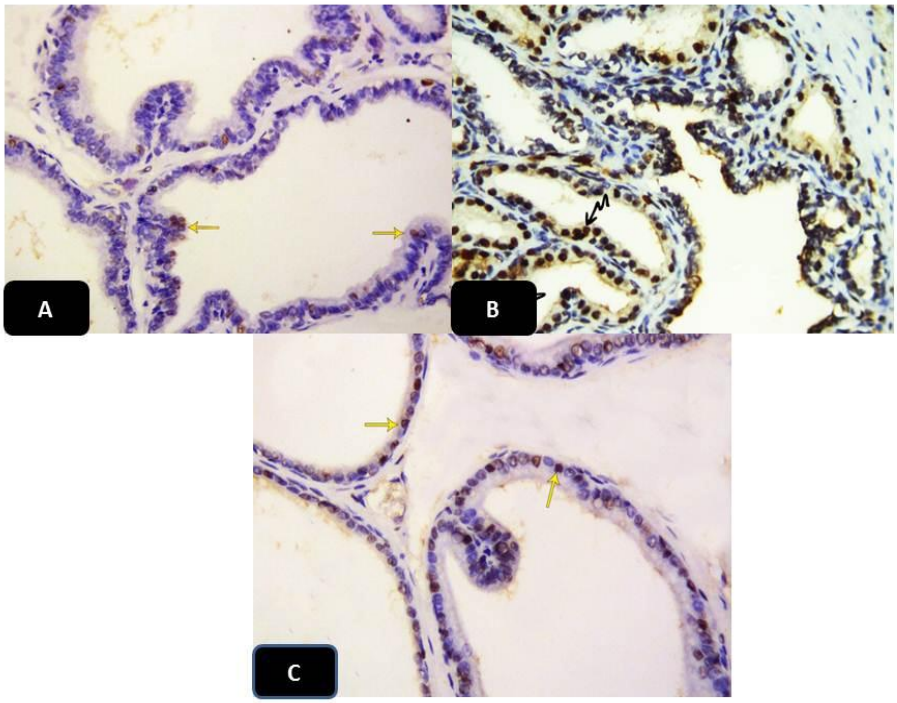


Plate (5): Photomicrograph of the prostate stained with PCNA **A (control group):** showing few cells exhibiting positive nuclear immunostainig for PCNA in the acinar epithelium (yellow arrows). **B (diabetic group):** showing numerous cells exhibiting positive nuclear immune reaction for PCNA in the acinar epithelial cells (wavy arrows). **C (treated group):** showing few epithelial cells exhibiting positive nuclear reaction (yellow arrows). **(PCNA immunostaining : A, B, & C x400).**

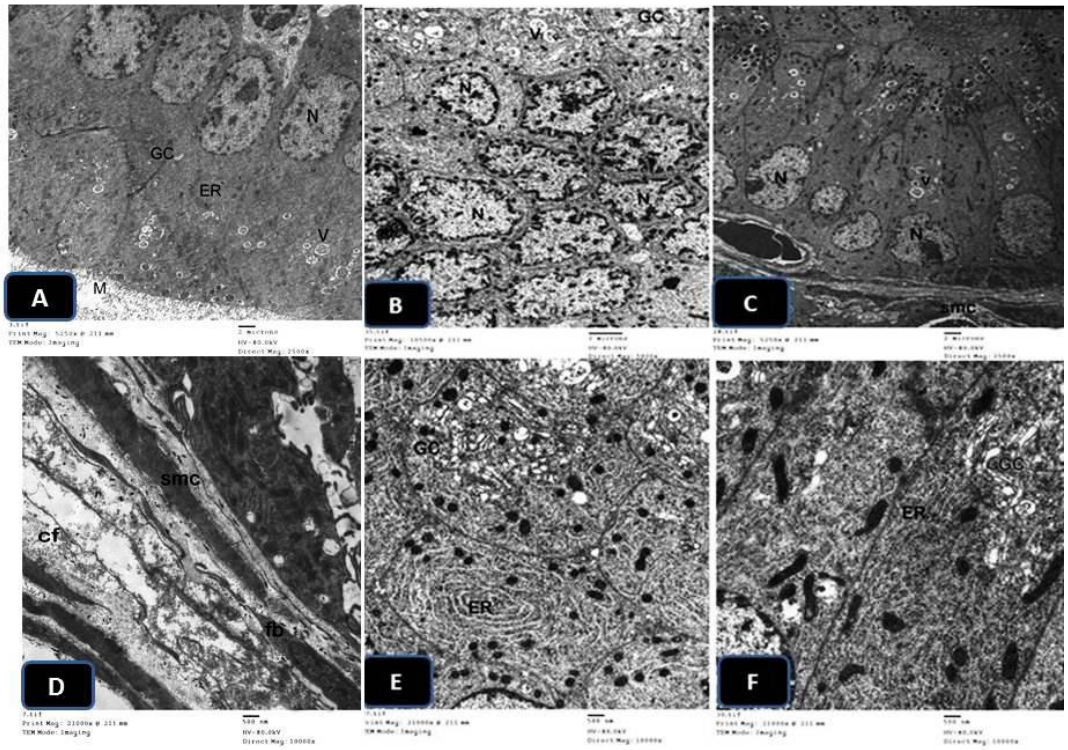


Plate (6): TEM micrograph of the prostate of **A (control group):** showing tall columnar cells with a basal oval nuclei (N) with clearly visible nucleolus (N), well-developed Golgi complex (GC). Flattened and parallel reticulum cisterns (ER), supranuclear secretory vesicles (v) can be seen. Microvilli line the cell surface facing the lumen (M). **B (diabetic group):** showing proliferation of the epithelial cells with irregular contour of the nuclei (N), distention of the cisterns of the Golgi complex (GC) and decreased of apical secretory vesicles (V). **C (treated group):** showing basal oval nuclei (N), secretory vesicles (V) in the apical region of cytoplasm and thin stroma that exhibited normal appearance of smooth muscle cells (smc). **(A, B, & C x 2500)**. **D (control group):** showing thin stroma with collagen fibers (cf) and fibroblast (fb) seen among smooth muscle cells (smc). **E (diabetic group):** showing dilated Golgi cisternae (GC) with dilated rough endoplasmic reticulum cisternae arranged in concentric manner (ER). **F (treated group):** showing well-developed Golgi complex (GC). Flattened and parallel cisterns of the granular endoplasmic reticulum (ER). (**D, E, & F x 1000)**.

Morphometric Results:

1. **Epithelial heights in the studied groups of the experiment (Table 1)**

   -There was significant decrease in mean epithelium heights in groupII compared to control group.

   -No significant difference was detected between control group and groupIII.

2. **The mean area percent of collagen fibers in the studied groups of the experiment (Table 2)**

   -There was significant increase in mean area percent of collagen fibers in group II, compared to control group and groupIII.

   -No significant difference was detected between control group and groupIII.

3. **The mean area % of α-Smooth Muscle Actin in the studied groups of the experiment (Table 3)**

   -There was significant increase in mean area percent of α-Smooth Muscle Actin in groupII, compared to control group and groupIII.

   -No significant difference was detected between control group and groupIII.

4. **The mean area % of Vimentin in the studied groups of the experiment (Table 4)**

   -There was significant increase in mean area percent of Vimentin in groupII, compared to control group and groupIII.

   -No significant difference was detected between control group and groupIII.

5. **The mean area % of PCNA in the studied groups of the experiment (Table 5)**

   -There was significant increase in mean area percent of PCNA in groupII, compared to control group and groupIII.

   -No significant difference was detected between control group and groupIII.

Table 1: Mean Epithelial heights ± SD in the studied groups after four weeks of diabetes confirmation.

	Control group		**Diabetic group**		**Treated group**	
Mean Epithelial heights	16.99	± 7.32	10.13	± 1.98	15.35	± 4.13

Table 2: Mean area % of Collagen ± SD in the studied groups after four weeks of diabetes confirmation.

	Control group		**Diabetic group**		**Treated group**	
Mean area % of collagen	1.06	± 0.06	3.38	± 0.78	1.14	± 0.59

Table 3: Mean area % of α-Smooth Muscle Actin immunopositivity ± SD in the studied groups after four weeks of diabetes confirmation.

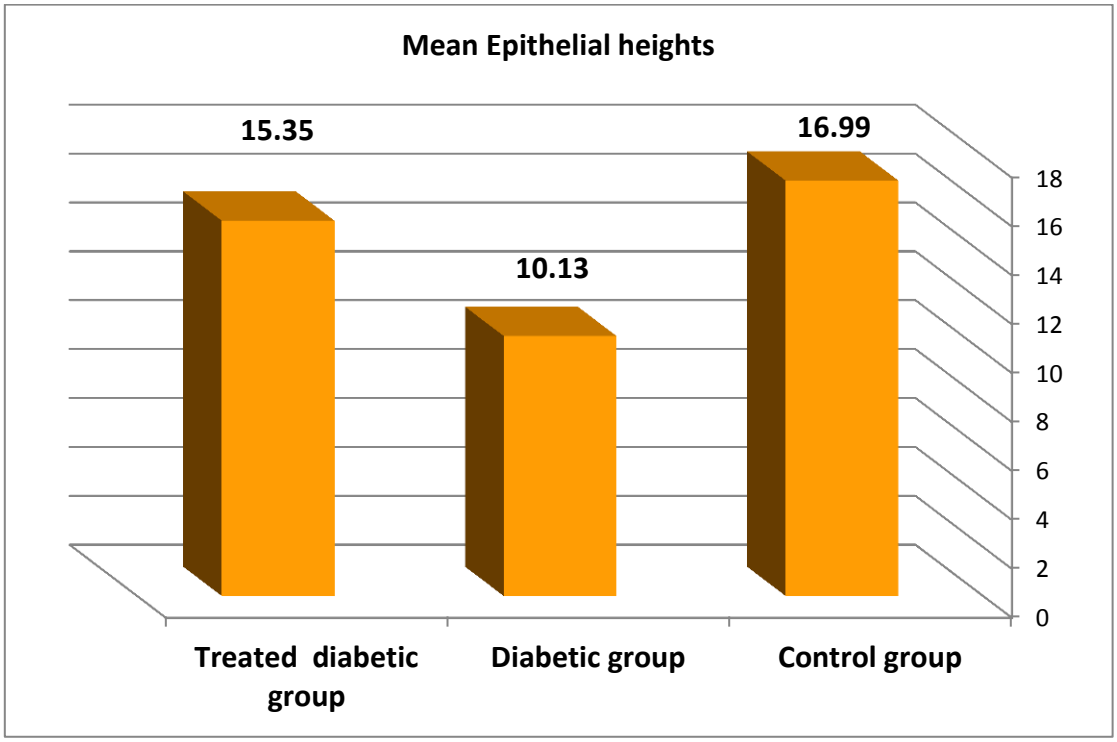
	Control group		**Diabetic group**		**Treated group**	
Mean area % of α-Smooth Muscle Actin	4.08	± 0.36	6.84	± 2.29	4.27	± 0.69

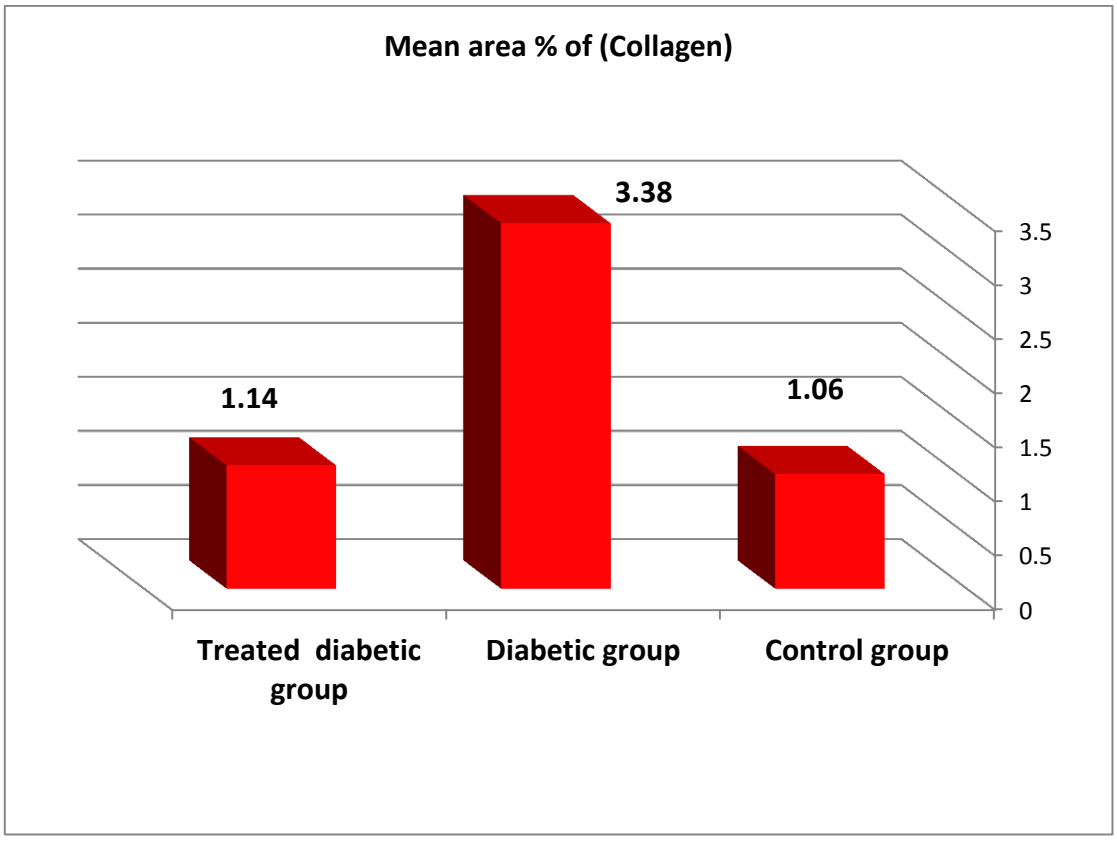
Table 4: Mean area % of Vimentin immunopositivity ± SD in the studied groups after four weeks of diabetes confirmation.

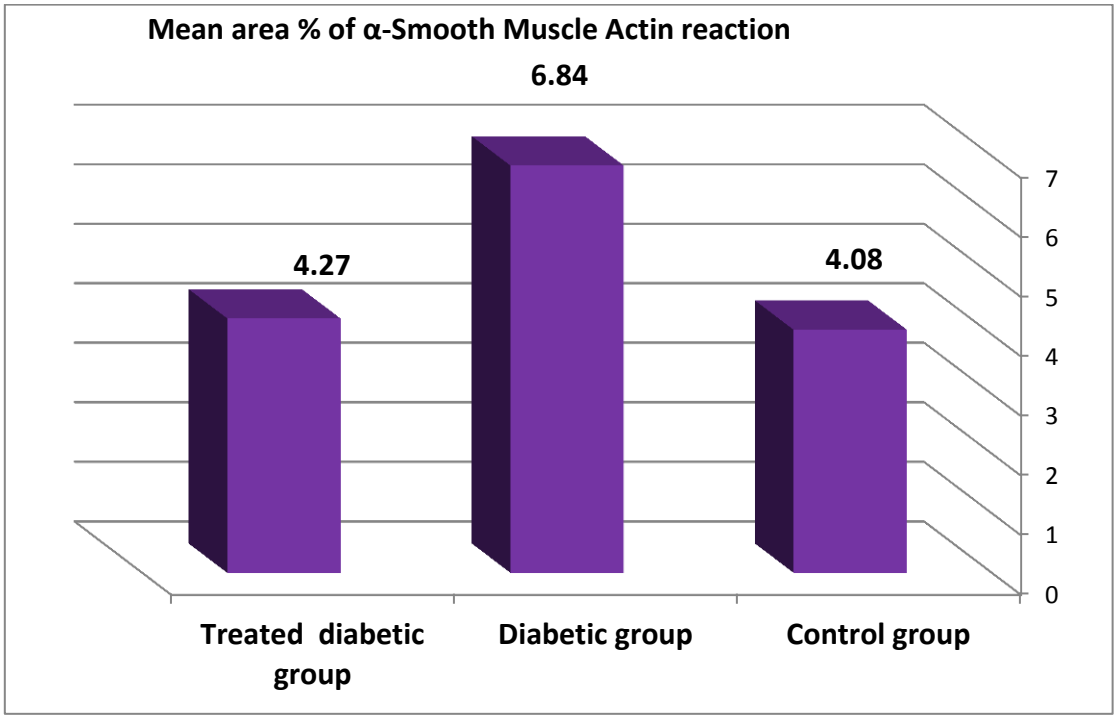
	Control group		**Diabetic group**		**Treated group**	
Mean area % of Vimentin	1..48	± 0.47	5.53	± 1.41	1.74	± 0.37

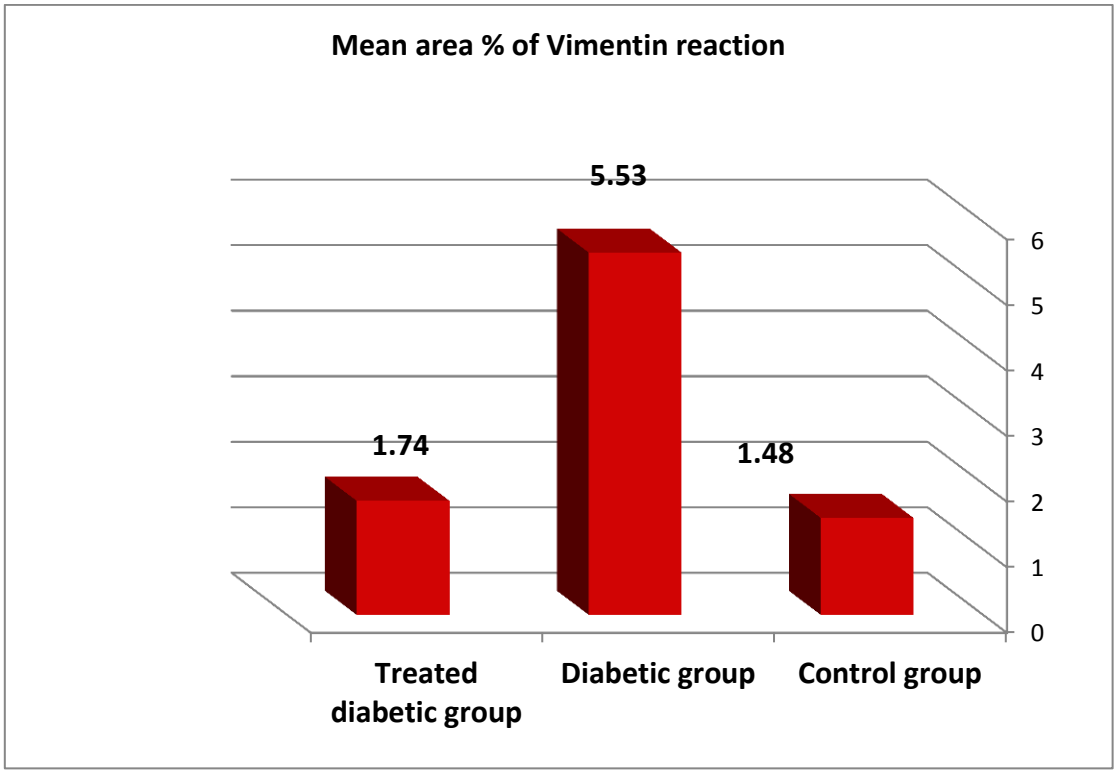
Table 5: Mean area % of PCNA immunopositivity ± SD in the studied groups after four weeks of diabetes confirmation.

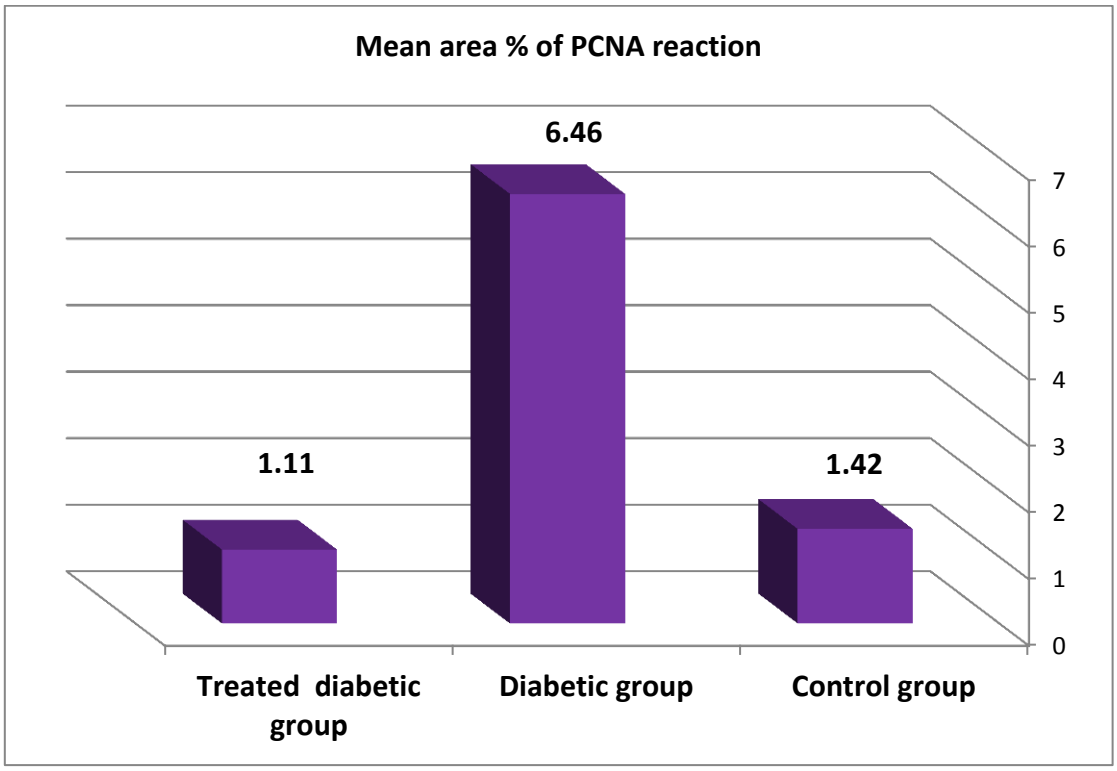
	Control group		**Diabetic group**		**Treated group**	
Mean area % of PCNA	1.42	± 0.64	6.46	± 3.38	1.11	± 1.00


Mean Epithelial heights
15.35
10.13
16.99
18
16
14
12
10
8
6
4
2
0
Treated diabetic group
Diabetic group
Control group


Mean area % of (Collagen)
1.14
3.38
1.06
3.5
3
2.5
2
1.5
1
0.5
0
Treated diabetic group
Diabetic group
Control group


Mean area % of α-Smooth Muscle Actin reaction
6.84
4.27
4.08
7
6
5
4
3
2
1
0
Treated diabetic group
Diabetic group
Control group


Mean area % of Vimentin reaction
5.53
1.74
1.48
6
5
4
3
2
1
0
Treated diabetic group
Diabetic group
Control group



4- Discussion:

Diabetes mellitus is one of the oxidative stress (OS) conditions in which free radicals are increased and anti-oxidant mechanisms are inhibited. Effect of DM on male reproductive function can be explained, through the impact of OS, caused by the inequality between reactive oxygen species (ROS) production and antioxidant defense mechanisms **[16]**.

This study was performed to demonstrate the histological changes induced in the prostate after streptozotocin-induced diabetes on the prostate gland in adult rats and the role of insulin replacement therapy in ameliorating the diabetic effect on the prostate.

In the present study, examining the control group of the prostate in H&E stained sections revealed normal histological architecture of the prostate. The prostate consists of branched tubulo-acinar glands embedded in a fibro muscular stroma. The prostatic acini with infolded mucosa was lined by a simple to pseudostratified columnar epithelium.

At the level of the electron microscope, examination of the control group revealed that the epithelial cells were high cuboidal with basally located oval-shaped nuclei. In the cytoplasm, the Golgi complex was developed and formed by numerous flattened and parallel reticulum cisterns

located in the perinuclear and basal region of the cell. Rounded secretory vacuoles containing secretion were observed in the apical cytoplasm. Microvilli covered the surface of the cell facing the lumen. The glandular stroma was thin and consisted of smooth muscle fibers, collagen fibers and fibroblast.

Marked changes were detected on examining prostatic sections of the diabetic group (II) (four weeks post diabetes) where it revealed that the acini were separated by wide connective tissue septa and demonstrated a loss of their mucosal folds associated with thinning of their lining epithelium and dilated congested blood vessels, this can explained by the dependence of the prostate gland on different hormones such as androgens, estrogens and prolactin.

The testosterone is the main hormone that is very important for the development of prostate and maintenance of its structural and functional integrity. The subtle change in the testosterone level is usually accompanied by alteration in structure and function of the prostate **[17]**.

In the current work, examining the prostate of diabetic group (II) exhibited thick fibromuscular stroma of the prostate due to significant increase in collagen fibers compared to group (I).

Another supportive study revealed that diabetes led to a marked decrease in testosterone levels and decrease in the prostate epithelial height and increase the thickness of the fibromuscular stroma by increasing smooth muscles and collagen fibers **[18]**.

Our findings agree with those of other investigators who observed atrophied prostatic secretory epithelial cells, stromal hypertrophy. These can be explained by stromal remodeling, which appears to be another common prostatic response to diabetes. This involves morphological changes in smooth muscle cells as well as increase of collagen fibers **[19]**. At least in part, these changes are similar to those caused by castration and can be interpreted as the result of decrease in testosterone serum levels. According to changes in collagen and smooth muscles have been related to remodeling tissue that follows androgenic decrease **[20]**.

In the present work EM examination of the diabetic group (II) revealed the prostatic acini presented less infolded mucosa than that observed in the control group. Atrophied secretory epithelium with cuboidal cells was verified together with thickening of the elements of the extracellular matrix. The collagen fibers were distributed among the smooth muscles and thick smooth muscle cells were found.

Similar findings were also observed it was reported that prostatic damage that may occur due to hyperglycemia may be eminent from these microvascular changes that occur due to the excessive release of ROS during oxidative stress leading to inflammation of the prostate **[21]**.

Our current study showed a positive expression in the cytoplasm of the smooth muscles in diabetic groups. The mean area % of α smooth muscle actin immunostaining was significantly increased in group (II) compared with control group .

Group (II) stained with vimentin exhibited increase in cytoplasmic staining of many fibroblasts cells of the stroma that was confirmed by positive immunoreactivity and morphometric results.

An important finding noted in the present study was the presence of cell proliferation by demonstrating PCNA in group (II). Some nuclei of the control epithelium exhibited positive immune-reactivity. In the diabetic group (II) numerous cells showed positive nuclear reaction. This is assured by morphometric studies in the form of significant increase in the mean area % in the diabetic group (II) when compared to the control group. This was followed by decrease in the rate of cell proliferation in group (III) to reach a rate similar to that of the control group.

This agrees with a previous study that suggested that diabetic individuals had a high incidence of prostatitis, and this inflammation could stimulate the incidence of other forms of prostatic pathology. The immune system is damaged in diabetic individuals, and it could explain the high incidence of prostatitis in diabetics **[22]**. The inflammation process may induce carcinogenesis through morphological and genetic damage in the cells, and it also can create a microenvironment rich in cytokines and growth factors increasing cell proliferation **[23]**.

Similar findings were also reported that the aggressive release of oxygen radicals from macrophages and neutrophils during acute or chronic inflammation may lead to compensatory cellular proliferation, which further exacerbates oxidative stress, and in such conditions, hyperplastic proliferation may occur in the prostate tissue **[24]**. Several lines of evidence suggested that ROS plays a vital role in the regulation of important cellular processes such as proliferation. Excessive production of ROS or inadequacy in a normal cell's anti-oxidant defense system (eg, anti-oxidant enzymes) can cause pathological conditions associated with aberrant growth and neoplastic transformation **[25]**.

Another study presented opposing findings to the results in the present study stated that the diabetes led to a significant

reduction in androgen levels together with an eight-fold reduction in cell proliferation levels in the acinar epithelium. The positive correlation between testosterone levels and epithelial proliferation as androgens are the major growth factor for prostate cells. Under normal physiological conditions, the proliferation in the acinar epithelium is indirectly controlled via androgenic stimulation of stromal cells, which secrete growth factors such as FGF-7, FGF-10 and IGF-I **[26]**.

In contrast to our finding, it was reported that both testosterone and insulin promote prostatic cell proliferation and change in the level of the hormone lead to the destabilization of cellular equilibrium and modulation of the insulin-receptor signaling in the prostate gland resulted in reduced epithelial cell proliferation in the prostate gland **[27]**.

Our findings partially disagree with another study who reported that the hyperglycemia down regulates androgen receptor (AR) through NF-κB activation, and that this inhibitory effect is further increased by TNFα. However, they found that down regulation of (AR) induced by diabetes is associated with significant tumor growth retardation **[28]**.

This finding demonstrated in the current work by light microscope examination of the diabetic group (II) were documented by EM examination revealed intraepithelial neoplasia (PIN) identified in some acini, characterized by proliferation of the epithelial cells with irregular contour of the nuclei. Dilated rough endoplasmic reticulum and Golgi complex cisternae were observed in the cellular cytoplasmic. Occasional vesicles containing secretion of low electron density material was verified in the apical region of the cell and discontinuous of the microvilli which covered the cellular surface.

The findings of the present study were concomitant with those reported that in a diabetic state there was atrophy of the secretory epithelium, dilated cisterns of Golgi complex, rough endoplasmic reticulum distributed in a concentric manner, rupture of the microvilli on the cell surface with some prostatic acini showing intraepithelial neoplasia (PIN) and thickening of the extracellular matrix elements **[29]**.

It is worth notice that, the results of the present study showed that the experimental group that received insulin showed an improvement in histological feature and immunohistochemical results in the form of restoration of the lining simple columnar epithelium with appearance of mucosal folds in all acini within normal stroma in between the acini with decreased cell proliferation in the prostatic acini.

In the present work EM examination of the treated group (III) exhibited the total epithelial cell volume was greater than that observed in the diabetic groups, well-developed Golgi complex, flattened cisterns of the Golgi complex, supranuclear secretory vesicles, microvilli on the cell surface with flattened and parallel cistern of the rough endoplasmic reticulum were verified and the prostatic stroma with collagen, fibroblasts and smooth muscle cells were similar to that of the control group.

These findings were in agreement with those of other investigators, who demonstrated insulin treatment maintained testosterone levels and preserved at least partly the cell morphology and collagen fiber organization of the prostatic stroma **[30]**.

5- Conclusion:

It could be concluded that the reproductive dysfunction is one of the common secondary effects of diabetes. It may affect male reproductive functions at multiple levels including variation in accessory sex glands, reduced testosterone level. Insulin replacement therapy in diabetes normalizes hormone levels of LH and testosterone and greatly improves the histological architecture of the prostate gland in diabetic rats.

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