

<b>Original Article</b>	<b>Structural Changes in the Ventral Lobe of the Prostate of Diabetic Rat</b> <i>Youssef Hussein</i> <i>Anatomy Department, Faculty of Medicine, Zagazig University</i>
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### ABSTRACT

**Background:** Streptozotocin has been widely used to induce type-1 diabetes in animal models.

**Aim of the Work:** This study was conducted to delineate the effects of streptozotocin (STZ)-induced diabetes on the ultrastructure of the secretory epithelium of the ventral lobe of the prostate of adult albino rats.

**Material and Methods:** Healthy thirty adult male albino rats were divided into two groups. Group I is the control and group II is the STZ-experiment group of healthy rats treated with five intraperitoneal injections STZ for 7-day intervals. The first three doses were 75 mg/kg body weight and the remaining two doses were 150 mg/kg per animal. The control group received only 0.1 ml 0.1 M citrate buffer, pH 4.4, by the intraperitoneal route. Thirty days after the detection of the diabetic status, the animals of the two groups were anaesthetized with ether inhalation and sacrificed. The ventral lobes of their prostate were removed and processed for microscopic examination.

**Results:** The histological study of the experimental group revealed a reduction in the cell height of glandular epithelium, remarkable atrophy of secretory epithelial cells with an increase in the thickness of the glandular stroma as compared with the control group. The ultrastructural distinctive features of secretory epithelial cells of the ventral lobe of the prostate of the experimental group showed ruptured microvilli, homogeneous chromatin throughout the enlarged nucleus, thickened extracellular matrix, dilated concentric cisterns of the granular endoplasmic reticulum and swollen mitochondria with disturbed cristae. The disorganized ultrastructural features of the experimental group were clear as compared with the control group.

**Conclusions:** Our results suggested adverse effects of STZ-induced diabetes on the secretory epithelium of the ventral lobe of the prostate and emphasized remarkable drastic changes in the secretory epithelial cells that consequently lead to impaired glandular function and development of prostatic pathology.

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**Key Words:** Streptozotocin, diabetes mellitus, albino rats, adult, prostate, ultrastructure, light microscopy, electron microscopy.

### INTRODUCTION

Diabetes mellitus is one of the most important diseases of the world. Besides being a medical problem, it also has social aspects because it impairs the quality of the life of the affected individuals (Mokdad, 2001).

The incidence of diabetes has been increasing rapidly during the last few years (Carvalho, et al. 2003). Diabetes is a chronic disease that affects the metabolism of carbohydrates, lipids and proteins. It is caused by a deficiency in the pancreatic secretion of insulin and /or by the inability of tissue to efficiently respond to insulin leading to hyperglycemia (Carvalho, et al. 2003).

Several diabetogenic agents are used for the induction of diabetes such as streptozotocin and alloxan. These agents lead to destruction of pancreatic beta cells. Adequate doses of these agents produce insulin deficiency in animals similar to human diabetes type I (Robbins, et al. 1989).

Diabetes causes changes in different organic systems especially the gonads and male reproductive system. Several investigators suggested that alteration of the male reproductive organs is one of the common secondary effects of diabetes, which are associated with impotence (Daubrese, et al. 1978).

Diabetes can influence the clinical manifestation of benign prostatic hyperplasia (Michel, et al. 2000; Herawi, et al. 2005). In addition, some studies have suggested that diabetes may be associated with cancer of the prostate (Will, et al. 1999; Zhou, et al. 2003; Ribeiro, et al. 2006).

The prostate is an androgen-dependent gland that plays a fundamental role in reproduction. It secretes a complex mixture of nutrients found in the seminal fluid which are essential for sperm mobility and nutrition (Costello and Franklin, 1994).

In Rodents, the prostate is formed of three pairs of lobes (ventral, lateral and dorsal) distributed around the urethra (Marker, et al. 2003). According to Price (1963), there is homology between the embryonic development of the ventral lobe of the prostate in rats and the middle prostatic lobe in humans.

Despite the known harmful effects of diabetes on the secretory epithelium of accessory sex glands, there is a lack of detailed information about the involvement of cell organelles participating in the glandular secretory process. Thus, the aim of the present study was to determine the possible effects of streptozotocin-induced diabetes on the histology and ultrastructure of the secretory epithelium of the ventral lobe of the prostate of albino rat.

## MATERIAL AND METHODS

Thirty adult male rats aged 3 months were divided into two groups, control and experimental. Both groups received balanced Purina chow ad libitum in the form of pellets granules. The control group received only 0.1 ml 0.1 M citrate buffer, pH 4.4, by intraperitoneal route. The experimental group was injected with streptozotocin as the diabetogenic agent (Sigma Chemical Company) administered in 0.1 M citrate buffer, pH 4.4, as vehicle. Each animal received five intraperitoneal injections with 7-day intervals. The first three doses were 75 mg/kg body weight and the remaining two doses were 150 mg/kg per animal according to Cagnon et al. (2000).

The diabetic status of the animals was confirmed 24 hours later by estimation of plasma glucose concentration from tail vein blood sample using one touch horizon ACE glucometer. Induction of

diabetes was also identified by using multistix 10-SG reagents strips (Bayer) to determine the approximate variation of glucose (mg/dl) in urine. Two Multisix 10-SG tests were performed on all animals before the first streptozotocin injection for quantitative analysis of glucose as a control standard.

Thirty days after the identification of the diabetic status, the animals of the two groups were anaesthetized with ether inhalation and sacrificed.

For light microscopic examination; The specimens were carefully dissected, then removed and fixed in 10% formol saline, dehydrated, cleared and embedded in paraffin wax. The sections were cut and stained with hematoxylin and eosin.

For electron microscopy, The specimens were immediately removed and fixed in 3% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 for a period of 3 h, and post-fixed in 1% osmium tetroxide in the same buffer for 2 hr. The samples were dehydrated in ascending grades of alcohol and embedded in resin. Ultrathin sections were cut and stained with toluidine blue and prepared for light microscopy for the selection of the specific areas to be examined by transmission electron microscope (Watson, 1958). This work was done at Faculty of Science, Ain Shams University.

The food, water intake, body weight and prostate weight of the animals were measured throughout the experiment. The variations in food (g/day), water intake (ml/day), body weight (g/day) and prostate weight (g/day) were submitted to analysis. The data were analyzed statistically by Student's t-test.

## RESULTS

**Blood glucose level and Urine analysis:** The control animals had no glucose in the urine (0 mg/dl). The experimental group had an average glucose level of 1050 mg/dl in the urine. The glucose levels in the blood were checked every other day. The normal blood glucose level in rats is 70- 125 mg/dl, but the rats with fasting blood glucose level 140 mg/dl or more were considered diabetic according to Igarashi et al. (2000).

**Nutritional assessment and body weight:** In the control group, means and standard deviations of

daily food, water intake, body weight and prostate weight were obtained. Means and standard deviations of daily food ( $9.90 \pm 0.64$ ), water intake ( $24.33 \pm 1.58$ ), body weight ( $250.9 \pm 9.6$ ) and prostate weight ( $0.7 \pm 0.10$ ) were significantly higher in the diabetic group (Table). Despite the higher food intake, the diabetic group showed a remarkable loss of body weight.

**Table 1:** Assessment of nutrition and body weight (Means  $\pm$  standard deviations).

	Control	Diabetic	P value
<b>Food (gm/day)</b>	6.75 $\pm$ 0.3	9.90 $\pm$ 0.64	P<0.05
<b>Water (ml/day)</b>	5.31 $\pm$ 0.11	24.33 $\pm$ 1.58	P<0.05
<b>Body weight (gm)</b>	311.6 $\pm$ 6.7	250.9 $\pm$ 9.6	P<0.05
<b>Prostate weight (gm)</b>	0.4 $\pm$ 0.06	0.7 $\pm$ 0.10	P<0.05

**Histological study:**

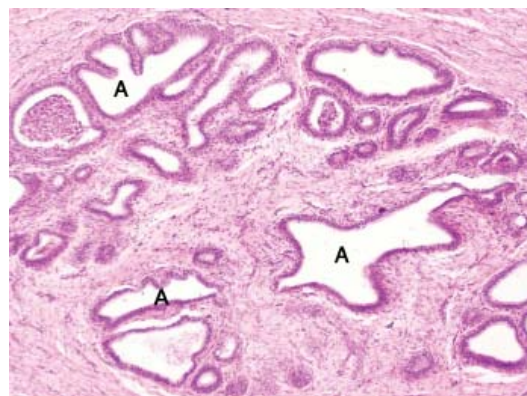
- **A- Light microscopy:** Control group: The prostate showed acini of different size with a folded mucosa (Figs. 1,2). The secretory epithelium was columnar resting on the basement membrane and apical pale acidophilic cytoplasm. The oval-shaped nuclei were located in the basal region. In the stroma, thin and packed smooth muscle layers were visible around the acini (Fig. 2).

**Experimental group:** They showed remarkable atrophy of secretory epithelial cells and the nuclei were occupying most of the cytoplasmic region. The cells were cuboidal in shape with a significant decrease of folding (Figs. 3,4). The glandular stroma presented marked thickening of the extracellular matrix with hypertrophied smooth muscle cells (Fig. 5).

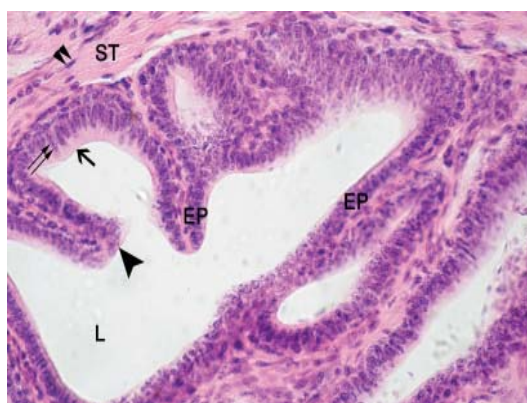
- **B- Electron microscopy:** Control group: They showed columnar cells resting on a clearly visible and intact basal lamina (Figs. 6,8). The nucleus was oval in shape, containing condensed chromatin in the peripheral region and the nuclear envelope appeared with smooth outline and its pores were evident (Fig. 6). The epithelial cell lining the acini showed apical microvilli, rounded secretory vesicles and numerous mitochondria in the cytoplasm. There were concentric parallel cisterns of the granular endoplasmic reticulum in the perinuclear region of the cell (Figs. 6,7). Intact microvilli were seen lining the lumen of the acinus, some of which

were cut longitudinally while some others cut transversely (Fig. 7).

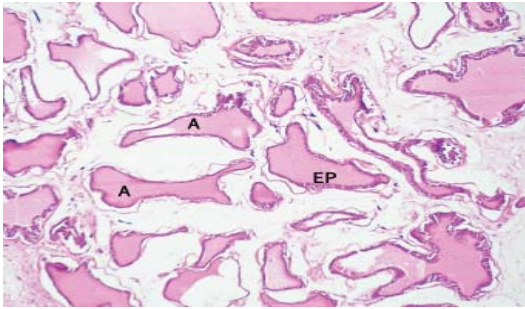
**Experimental group:** There were atrophy of the secretory epithelial cells resting on the disturbed basal lamina (Fig. 9). The basally located nucleus with clearly visible irregular nuclear membrane occupied most of the cell. The chromatin was disturbed in a homogeneous manner throughout the nucleus (Figs 9,10). The microvilli on the cell surface were ruptured (Figs. 9,11). The cytoplasm showed dilated concentric cisterns of the granular endoplasmic reticulum and cytoplasmic residual bodies. The mitochondria became swollen with disturbed cristae (Fig. 10). Most of the secretory vacuoles were electron lucent (immature cell) in the apical region (Fig. 11). The extracellular matrix in the stroma was remarkably thickened and showed irregular smooth muscle cells and collagen fibres. Blood vessels and fibroblasts were observed in the interstitial tissue (Fig. 12).



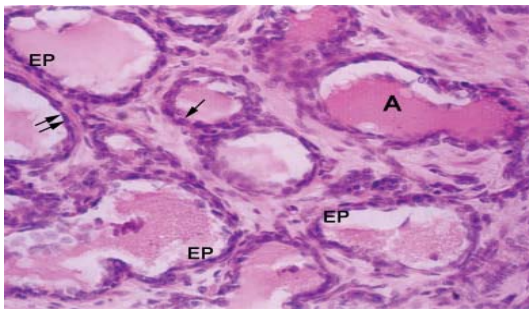
**Fig. 1:** A photomicrograph of the ventral lobe of the prostate of a control rat showing prostatic acini (A) with different size and shape. (H & E; X100)



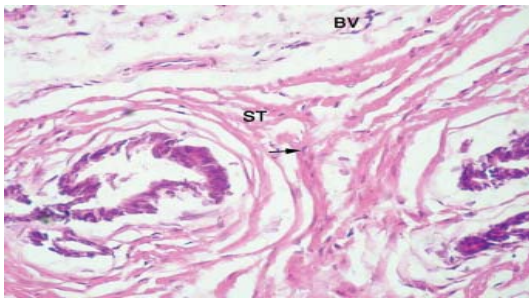
**Fig. 2:** A photomicrograph of the ventral lobe of the prostate of a control rat showing simple epithelial cells (EP) of columnar type (double arrows) with basally located nuclei. Mucosal folding (arrow head) are seen in the lumen (L). A light zona (arrow) is observed in the supranuclear region. Notice that the stroma (ST) is formed of closely packed smooth muscle cells (double arrow heads). (H & E; X 400)



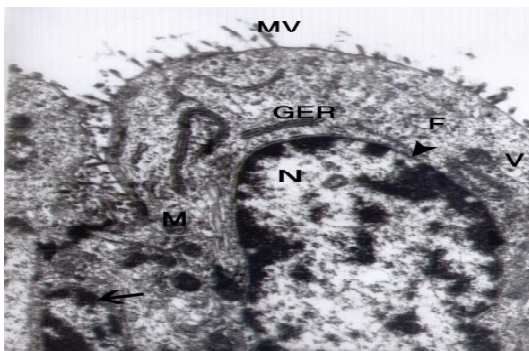
**Fig. 3:** A photomicrograph of the ventral lobe of the prostate of an experimental rat showing prostatic acini (A) lined by epithelial cells (EP). (H & E; X100)



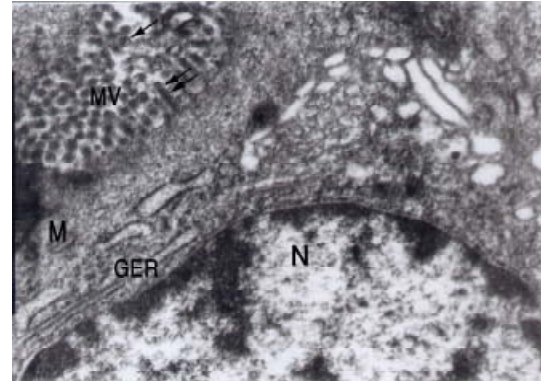
**Fig. 4:** A photomicrograph of the ventral lobe of the prostate of an experimental rat showing prostatic acini (A). They are lined by cuboidal (arrow) and flat (double arrows) epithelial cells (EP). (H & E; X200)



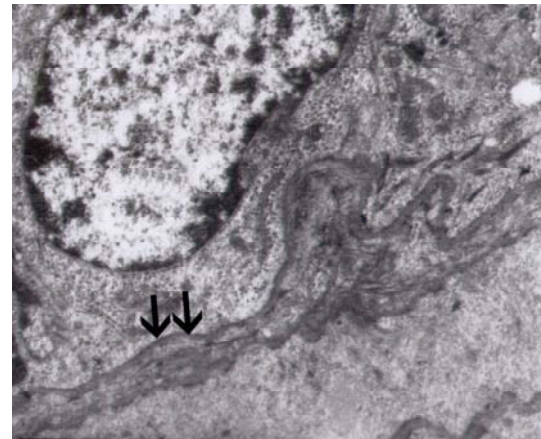
**Fig. 5:** A photomicrograph of the ventral lobe of the prostate of an experimental rat showing the stroma (ST) with marked thickening of the extracellular matrix. Notice that the stroma is formed of hypertrophied smooth muscle cells (arrow) and blood vessels (BV). (H & E; X400)



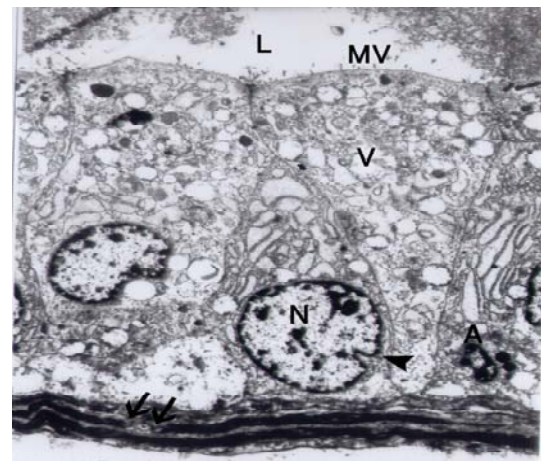
**Fig. 6:** An electronmicrograph of the epithelial cell of the ventral lobe of the prostate of a control rat: The cell is simple columnar epithelium with an oval nucleus (N). The nucleus (N) appears with peripheral heterochromatin and smooth nuclear envelope (arrow head). Mitochondria (M), parallel cisterns of the granular endoplasmic reticulum (GER), apical microvilli (MV), free ribosome (F) and secretory vacuoles (V) are seen. Desmosomal junction (arrow) is seen between the adjacent cells. (X7,500)



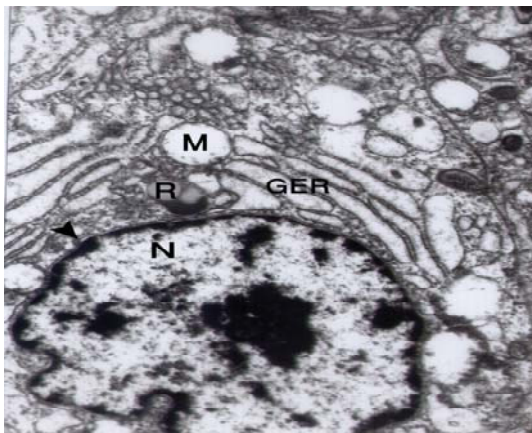
**Fig. 7:** An electronmicrograph of the epithelial cell of the ventral lobe of the prostate of a control rat showing the apical region. Microvilli (MV) line the lumen of the acinus. Some of which are cut longitudinally (double arrows) while some other cut transversely (arrow). Notice the parallel cisterns of the granular endoplasmic reticulum (GER), mitochondria (M) and nucleus (N). (X10,000)



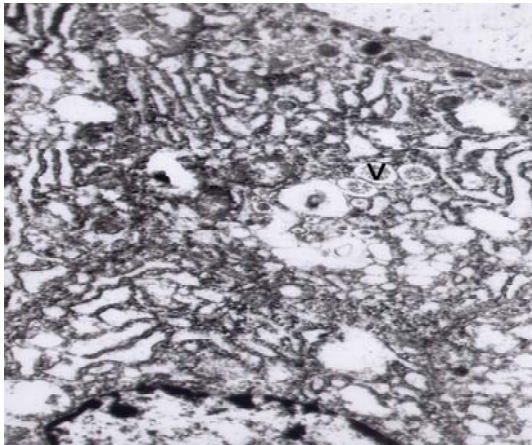
**Fig. 8:** An electronmicrograph of the ventral lobe of the prostate of a control rat showing the basal region. The epithelial cell can be seen resting on the clearly visible basal lamina (double arrows). (X 4,000)



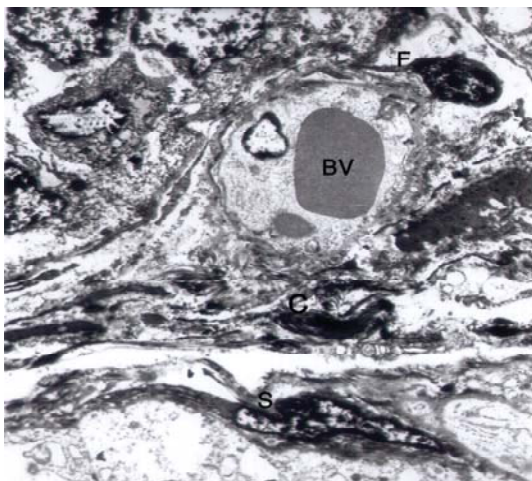
**Fig. 9:** An electronmicrograph of the ventral lobe of the prostate of an experimental rat showing atrophied epithelial cell rested on the disturbed basal lamina (double arrows). The nuclear envelope (arrow head) shows irregular outline with discontinuity of the microvilli (MV) in the lumen (L) of the acinus. Secretory vacuoles (V) are seen in the apical region of the cytoplasm. (X 4,000)



**Fig. 10:** An electronmicrograph of the epithelial cell of the ventral lobe of the prostate of an experimental rat showing the apical region. The nucleus (N) with the clearly visible irregular nuclear membrane (arrow head) is seen. The cytoplasm shows dilatation of the cisterns of the granular endoplasmic reticulum (GER), swollen mitochondrial (M) with disturbed cristae. Cytoplasmic residual (R) bodies (end products of lysosomal action) are seen. (X7,500)



**Fig. 11:** An electronmicrograph of the epithelial cell of the ventral lobe of the prostate of an experimental rat showing the apical region. Most of the secretory vacuoles (V) are electron lucent. (X7, 500)



**Fig. 12:** An electronmicrograph of the ventral lobe of the prostate of an experimental rat showing the basal region. Notice the collagen fibres (C), smooth muscle cell (S) with irregular outline, blood vessels (BV) and fibroblast (F). (X4000)

## DISCUSSION

The present study demonstrated a high glucose levels in the urine of diabetic animals. Glycosuria is one of the determining factors in the identification of diabetes type I. It has observed in animals after they had been submitted to any of diabetogenic drugs and also in spontaneously diabetic animals (Hunt and Bailey, 1961; Ader, et al. 1998).

From the results of this work, the body weight of diabetic animals was reduced even after ingestion of high amounts of ration and water. An imbalance in food intake and poor utilization of food has been reported for both diabetic human and experimentally diabetic animals (Seethalakshmi, et al. 1987).

The histological study showed a marked atrophy of the glandular secretory epithelium with reduction of the cytoplasm in diabetic animals when compared to the controls. Particularly important among these changes was stromal tissue hypertrophy. This coincides with that reported by Cagnon et al. (2000); Carvalho et al. (2003).

Several experiments have been carried out to analyze accessory sex glands, including the prostate, under androgen deprivation. These experiments showed a disorganized morphology of the secretory epithelium and the stroma with an increase in smooth muscle cells, collagen and elastic fibres in the ventral lobe of the prostate of castrated rats (De Carvalho and Line, 1996; Kiess and Gallaher, 1998).

De Carvalho and Line (1996); De Carvalho et al. (1997) suggested that the morphology of the secretory epithelium and the stroma were important for the structural maintenance of the integrity of the secretory epithelium. The stroma-epithelium interaction is known to be essential for the homeostasis of accessory sex organs. Thus, interruption of this equilibrium, such as observed with androgen depletion, leads to histological and biochemical disorders (Okuda, et al. 1991; Chan, et al. 2003). On this basis, it is suggested that diabetes may have effect on the morphology of the prostate.

The prostatic stroma has been considered as the principal compartment in glandular functioning due to its role in the maintenance of prostate homeostasis and its morphophysiological invol-

vement in diseases such as benign prostatic hyperplasia and cancer (Zhang, *et al.* 2003; Adley and Yang, 2006; De Marzo, *et al.* 2006). Sund *et al.* (1983) and Isaacs and Coffey (1989) have associated benign prostatic hyperplasia and cancer with androgen deprivation in both humans and rodents.

In this study, the structural changes observed in diabetic animals are similar to those observed in castrated animals (De Carvalho, *et al.* 1996,1997; Vilamaior, *et al.* 2000). These morphological changes observed in the stroma could be an attempt to maintain the integrity of the epithelium and consequently of the secretory process. Therefore, it may be concluded that diabetes lead to effective remodeling of the glandular stroma similar to the effect of castration.

The present study showed changes in the secretory epithelium of the diabetic animals. These changes mainly affected the organelles involved in the secretory process, in addition to the extracellular matrix. Drastic structural changes in the organelles have been reported in the ventral lobe of the prostate in diabetic mice (Cagnon, *et al.* 2000; Carvalho, *et al.* 2003; Ribeiro, *et al.* 2006). Vilamaior *et al.* (2000) indicated that relevant structural changes of cellular organelles occurred in the accessory sex glands involved in the secretory process in castrated animals, as has been observed in experimental diabetes. Kubo *et al.* (1998) also demonstrated that castration induced degeneration of biological membranes in the accessory sex glands, as characterized by dilatation of Golgi complex and atrophy of the cisterns of granular endoplasmic reticulum, leading to a faulty secretory mechanism.

The present findings showed atrophy of the cells. At low magnification, these cells had enlarged nuclei and decrease of mucosal folds. These data are compatible with the results of Wang *et al.* (2008) who stated that the enlarged nuclei with visible nucleoli and slight infolding luminal surface in cells with partial atrophy are commonly present in prostate cancer. The results of this work showed thickness and hypertrophy of the extracellular matrix. Cunha *et al.* (2003); Ribeiro *et al.* (2006) said that modification of the stroma cells and hypertrophy of the extracellular matrix were

the first step in the development of prostate cancer. Thus, it may be assumed that diabetes causes impairment of the reproductive process and might lead to premalignant lesions.

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## التغيرات التركيبية في الفص البطني لبروستاتا الفأر المصاب بداء البول السكري

يوسف حسين

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### ملخص البحث

كان هدف الدراسة توضيح التغيرات التركيبية في الشريان الأورطي المساعد في الفئران المسنة ومقارنة تأثير اعطاء هورمون الأستروجن يهدف هذا البحث الى تحديد تأثير (STZ) Streptozotocin المسبب لمرض البول السكري على التركيب الدقيق للخلايا الظهارية المفرزة في الفص البطني لبروستاتا الفئران البالغة. وقد استخدم في هذا البحث ثلاثون من الفئران الذكور البالغين حيث تم تقسيمهم إلى مجموعتين: المجموعة الأولى هي الضابطة أما المجموعة الثانية فهي المجموعة التجريبية. تم إستحداث مرض البول السكري بحقن خمسة جرعات من (STZ) داخل التجويف البريتوني بفاصل أسبوع بين كل جرعة. أول ثلاث جرعات كانت ٧٥ مجم/كجم أما الجرعة الرابعة والخامسة كانت ١٥٠مجم/كجم من وزن الجسم. تم إستئصال الفص البطني لغدة البروستاتا بعد ثلاثين يوم من ظهور السكر في الدم ثم أعدت العينات للفحص بواسطة الميكروسكوب الضوئي والإلكتروني. بعد فحص الأنسجة المكونة للفص البطني في المجموعة التجريبية لهذا العمل وجد الآتي:

- ١- تغير شكل الخلية من المستطيلة إلى المكعبة.
- ٢- ضمور ملحوظ في الخلايا الظهارية المفرزة لسائل البروستاتا.
- ٣- زيادة في سمك النسيج الأساسي للخلايا.
- ٤- تمزق في الخمائل الدقيقة وتضخم نوى الخلايا وإتساع الحوض المركزي لشبكة البلازما الداخلية المحيطة وإنفتاح الميتوكوندريا.

وقد اظهرت نتائج هذا البحث تغييرات قاطعة في شكل وتركيب الخلايا الظهارية المفرزة في الفص البطني لغدة البروستاتا والتي من الممكن أن تؤدي إلى إعاقة الوظيفة ونشوء عطب بالبروستاتا.