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Original Article

Effect of Estradiol Supplementation on Induced Diabetic Changes in the Vagina of Albino Rat: An Experimental Immunohistochemical Study

Rania N. Sherif and Dalia M. Saleh

Anatomy and Embryology Department, Faculty of Medicine, Mansoura University

ABSTRACT

Background: Estrogen hormone and receptors (ER) play an important role in maintaining vaginal health. Therefore, their disruption may adversely affect vaginal structure and function. Limited studies are available investigating the effects of diabetic complications on ER expression and distribution in the vaginal wall.

Aim of the Work: This work aimed to study the effects of diabetes-induced changes on the vaginal structure, the expression of ERα as well as to determine whether the supplementation of estradiol can ameliorate these changes or not.

Material and Methods: Thirty female albino rats were divided into 3 equal groups, 10 rats each. The first was the control received the vehicle only, the second was the diabetics, received a single intraperitoneal injection of alloxan (150mg/kg), and the third was diabetic/estradiol treated. Eight week-diabetic animals were injected subcutaneously with estradiol 20µg/kg/day dissolved in peanut oil for 8 weeks. By the age of 16 weeks, the animals were sacrificed, blood samples were collected to estimate the serum estradiol level and the vagina was removed and processed for paraffin sections at 5µm thick. For routine histopathological assessment H and E was used. Masson's trichrome used for collagen fibers and estrogen immunoperoxidase stains for ERα.

Results: Diabetic rats showed highly significant decline in the serum estradiol level (19.6 ± 8.4 pcg/ml) compared to the controls (126.6 ± 7.6 pcg/ml). Histopathological examination revealed thinning of the vaginal epithelial layers, increase in the collagen deposition in the submucosa, marked atrophy in the muscularis layer and decrease in ERα immunostaining. Treatment of diabetic animals with estradiol for eight weeks led to its increase to a sub-physiological level (35.1 ± 5.7pcg/ml) and marked hypertrophy of the muscularis layer and re-stratification of the vaginal epithelium. Moreover, there was marked reduction in the nuclear and cytoplasmic ERα immunostaining in the epithelium and increase in its expression in the stroma of the lamina propria and in the muscularis layer as compared to the control group.

Conclusion: Diabetes-induced structural changes in the vagina may be a consequence of decreased levels of estrogen. The increase in the estradiol level, even at a sub-physiologic level, can ameliorate these atrophic effects.

Key Words: lumbar epidural space, anatomy, morphometry, CT scan, Egyptians.

Corresponding Author: Dr. Dalia Saleh, Anatomy Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt, e- mail: meteor_381@hotmail.com, Mobile: 0106723381.

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by disrupted glucose homeostasis with harmful effects on many organs such as the kidneys, eyes, nervous system and heart. It can lead to increased rate of mortality when untreated *(Wild et al., 2004)*. Diabetic women have a higher prevalence rate of sexual dysfunction in comparison to non-diabetic females. Most of the symptoms are consistent with autonomic neuropathy and decrease in sexual arousal such as loss of libido, diminished clitoral sensitivity, vaginal discomfort and dryness, which may be related to vaginal atrophy *(Wincze et al., 1993)*.

The vaginal epithelium has important functions in vaginal lubrication by production of mucin

glycoproteins, which is thought to be regulated by estrogens. It is suggested that both type I *(Enzlin et al., 2002)* and type II *(Erol et al., 2002)* diabetes impede the normal stratification of the vaginal epithelium that protects the vaginal wall from abrasions and trauma during coitus and decreases the risk of infections *(Gorodeski, 2007)*.

Estrogen is known to regulate diverse physiological processes in the body and the female reproductive tract is its main target. Previous studies have demonstrated that estrogen deprivation by ovariectomy resulted in marked reductions in vaginal lubrication *(Min et al., 2003)*, blood flow and ERα expression *(Kim et al., 2004)*, and epithelial thickness *(Pessina et al., 2006)*, and that estradiol treatment restored these structural and physiological parameters to control levels.

Estrogen action is primarily mediated via binding to specific intracellular receptors in target cells *(Gronemeyer, 1992)*. Two types of ER have been discovered to date: ERα and ERβ *(Kuiper et al., 1996; Mosselman et al., 1996)*. These molecules are members of a superfamily of nuclear-transcription factors with highly homologous DNA binding and ligand binding domains *(Pettersson et al., 1997)*. The two receptors bind 17 β-estradiol with high affinity and specificity *(Kuiper et al., 1997)*.

Little is known regarding the mechanism of diabetes-induced vaginal atrophy and the existence of conflicting data regarding estradiol actions and the possibility that it might be related to glucose homeostasis and insulin resistance have put estradiol replacement therapy under intense investigation *(Barros et al., 2006)*. Thus, this study was designed to investigate the effects of diabetes on the structure of the vagina and on the expression of ER-α and the mechanism by which these changes occur. It was also aimed to determine whether estrogen replacement therapy could ameliorate these changes.

MATERIALS AND METHODS

Animal Preparation: Thirty adult female albino rats (8–10 weeks old) weighing (200–250 g) were obtained from the Faculty of Pharmacy animal house (Mansoura University). The animals were divided randomly into 3 groups. Group 1; control (n=10) received vehicle only; Group 2; diabetic (n=10) and Group 3; diabetic/estradiol treated (n=10). All animals were housed in cages with softwood granules as bedding. They had free access to standard diet and drinking water.

Induction of Diabetes: Animals subjected to induction of diabetes were allowed to fast for 12 hours prior to the experiment and rendered diabetic by a single dose of intraperitoneal injection of alloxan tetrahydrate (Sigma, St. Louis, MO, USA) 150 mg/kg and then were kept in the fasting state for another 12 hours *(Vogel, 2002)*. After 18 hours of injection of alloxan, diabetes was confirmed by testing blood sugar. Rats with blood glucose levels above 200 mg/dl for two consecutive weeks were considered as diabetic and were selected for the study. Eight weeks from induction of diabetes, the diabetic animals were further divided into two treatment groups: diabetic (n=10) received vehicle only and diabetic with estradiol supplementation (n=10) received estradiol for another eight weeks.

Estradiol supplementation: Animals in the diabetic/estradiol-treated group were subcutaneously injected with 0.002% estradiol (1 ml/kg/day, Sigma-Aldrich Corporation, St Louis, Missouri, USA) 20 μg estradiol dissolved in 1 ml peanut oil, whereas non-estradiol supplemented animals were injected with peanut oil only *(Liu et al., 2004)*.

Monitoring of Blood Glucose Level: Blood glucose levels were monitored every two weeks using an accutrend glucose detector (Boehringer Mannheim GmbH, Mannheim, Germany). Rats with blood glucose levels ≥ 200 mg/dl for two consecutive weeks were considered diabetic and were allowed to survive for 16 weeks.

Measurement of plasma estradiol level: At the end of the experiment, the animals were anesthetized with intraperitoneal injection of sodium pentobarbitone (40 mg/kg), weighed and blood samples were collected (via direct cardiac puncture) and were sent to the laboratory for measurement of plasma estradiol levels by ELISA (Alpha Diagnostics, San Antonio, TX) according to the manufacturer's protocol.

Assessment of estrus cycle phase: Smears of vaginal cells were prepared from all animals before being sacrificed. A cotton swab moistened in 0.9% saline was inserted into the vagina, manipulated

in a circular fashion and then smeared onto a glass slide. Samples were fixed with 70% alcohol and stained with hematoxylin and eosin. The phase of the estrous cycle was determined from the morphological characteristics of the vaginal mucosal cells *(Marcondes et al., 2002)*.

Histological Assessment: After 16 weeks, all animals in the proesterus phase, to minimize hormonal related variations, were sacrificed by an over dose of ether inhalation and their vagina were denuded from the skin and removed en bloc. The vagina was opened with a longitudinal incision and the distal end was notched for proper orientation. Tissues were then immersed in 10% neutral buffered formalin for 3-4 days. After fixation, the mid-section of each vagina was dehydrated in ascending grades of alcohol, embedded in paraffin and sectioned at five-microns. Tissue sections were deparaffinized, rehydrated in graded alcohol solutions (100, 95, 70%), and stained with hematoxylin and eosin for histopathological assessment, Masson's trichrome for collagen fibers and estrogen immunoperoxidase stains for ERα *(Cushman et al., 2009)*.

Statistics Analysis: Data were expressed as means ± SD. Differences between groups were determined using independent sample student t-test after testing for normal distribution. Significant differences were attributed with $P \leq 0.05$.

RESULTS

Alloxan induced hyperglycemia in rats: Administration of alloxan resulted in significant elevations (≥ 3 fold) in blood glucose level after one week of induction of diabetes that was sustained throughout the duration of the study. On average, the mean blood glucose level was 87 ± 9.6 mg/dl in the control, 248.4 ± 20.1 mg/dl in the diabetic group, and 231.8 ± 24.7 mg/dl in the diabetic/estradiol group. This was accompanied by a significant decrease in body weight in the diabetic group although they were on normal diet, the average body weight was 249.6 ± 8.1 g in the control, 231.2 ± 7.8 g in the diabetic, and 242.6 ± 11.5 g in diabetic/estradiol treated (Table 1).

Diabetes causes decrease in the estradiol level in the blood: The average serum estradiol level in the control rats was 126.6 ± 7.6 pcg/ml. Administration of alloxan resulted in highly significant decrease in the serum estradiol level to 19.6 ± 8.4 pcg/ml in the diabetic animals. Estradiol supplementation for 8 weeks to the diabetic rats resulted in an increase in the serum estradiol level to 35.1 ± 5.7 pcg/ml which was highly significant below the level of that of the control group (Table 1).

Diabetes induces atrophy of the vagina: Cross sections of the vaginal wall in the control group showed superficial cornification of the squamous epithelium which was composed mainly of squamous cells. There were no leukocytes infiltration in the epithelium or in the lumen, and mitotic figures were rare (Figs. 1, 2). Masson's trichrome-stained sections showed normal mucosal layer, normal distribution of smooth muscle and connective tissue, and normal microvasculature with prominent blood vessel channels (Figs. 7, 8).

Vaginal tissue cross-sections from diabetic rats tended to have epithelium that was more uniformly thin with fewer layers of cells in comparison to that of the control. The surface epithelium was

Table 1: Blood glucose, body weight, and plasma estradiol concentration of control, diabetic and diabetic/estradiol treated rats. Values are presented as means ± SD.

	Blood Glucose (mg/dl)	Body Weight (g)	Estradiol (pg/ml)
Control	87.0 ± 9.6	249.6 ± 8.1	126.6 ± 7.6
Diabetic	248.4 ± 20.1	231.2 ± 7.8	19.6 ± 8.4
P (vs control)	P ≤ 0.001**	P ≤ 0.05*	P ≤ 0.001**
Diabetic/Estradiol	231.8 ± 24.7	242.6 ± 11.5	35.1 ± 5.7
P (vs control)	P ≤ 0.001**	P ≥ 0.05	P ≤ 0.001**
P1 (vs diabetic)	P ≥ 0.05	P ≥ 0.05	P ≤ 0.05*

* The mean difference is significant (P ≤ 0.05)

** The mean difference is highly significant (P ≤ 0.001)

covered by a layer of mucinous cells which started to shed in the lumen (Figs. 3, 4).

The submucosal layer and the diabetes-induced vaginal fibrosis were examined by Masson's trichrome stain. Micrographs of the vagina showed that collagen fiber density appeared considerably enhanced in the diabetic animals. This was more obvious in the muscularis layer which was consistently thin with less well-developed bundles and was infiltrated by collagenous fibers (Figs. 9, 10).

Estradiol reverses vaginal atrophy in diabetic rats: In the estradiol-treated group, vaginal epithelium was a target for estrogenic activity resulting in an increase in the thickness of the vaginal epithelium which was formed of multi-layer of columnar epithelial cells filled with vacuoles. In the superficial layers, epithelial cells were hypertrophic forming a squamous layer on the surface (Figs. 5, 6). Submucosal tissue thickness was increased with an increase in the blood supply. The muscularis layer consisted of large, well-defined bundles of smooth muscle (Figs. 5, 11, 12).

Estrogen receptor immunostaining: Sections from the control rats immunostained with a specific antibody to ERα demonstrated a low to moderate level of ER expression in the various laminae of the vaginal wall (Figs. 13, 14). ERα localization was mostly nuclear with minimal cytoplasmic staining. On the basis of immunostaining of ERα, three zones were identified in the epithelium of control animals: (1) a basal zone in which the nuclei demonstrated low to moderate staining, (2) an intermediate, or parabasal, zone in which the nuclei were minimally immunoreactive, and (3) a superficial, or juxtaluminal, zone in which the nuclei were totally unstained. The cytoplasm of the epithelial cells was minimally stained throughout all the three zones.

After induction of diabetes, ERα immunostaining was decreased in the vaginal epithelium and was rarely detected in the stroma (Figs. 15, 16). Estradiol supplementation in diabetic animals markedly reduced nuclear and cytoplasmic ER immunostaining in the epithelium. On the other hand, ERα immunostaining was detected in the stroma of the lamina propria and in the muscularis layer (Figs. 17, 18).

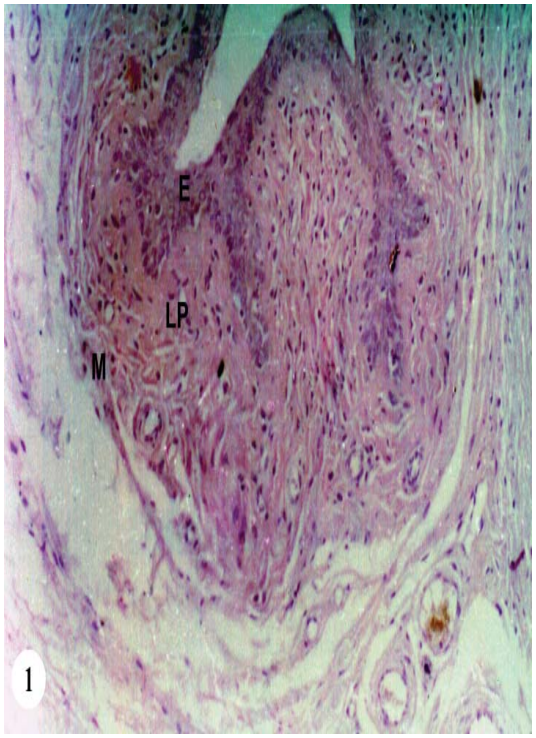

Fig. 1: A photomicrograph of a section of the rat vagina of the control group showing epithelial layer (E), lamina propria (LP) and muscularis layer (M). Hx. & E.; X100

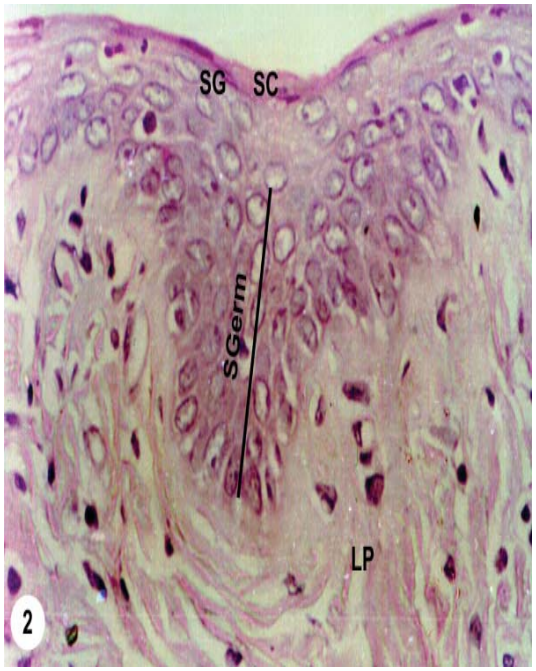

Fig. 2: High magnification of a section of the rat vagina of the control group showing the multicellular epithelial layer which is formed of columnar cells forming stratum germinative (SGerm), squamous cells in the top layer forming a startum granulosum (SG) and is covered by an amprphous eisonophilic band forming stratum cornium (SC). There is no leukocyte infiltration in the lamina propria (LP). Hx. & E.; X400

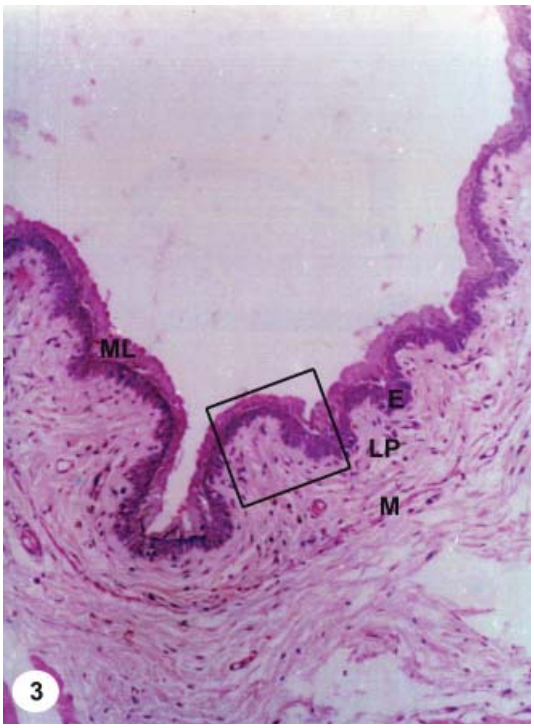

Fig. 3: A photomicrograph of a section of the rat vagina of the diabetic group showing decrease in the height of the epithelium (E) which becomes more basophilic and seen covered by a mucinous layer of cells (ML). There is attenuation of the lamina propria (LP) and marked atrophy of the mascularis layer (M). Hx. & E.; X100

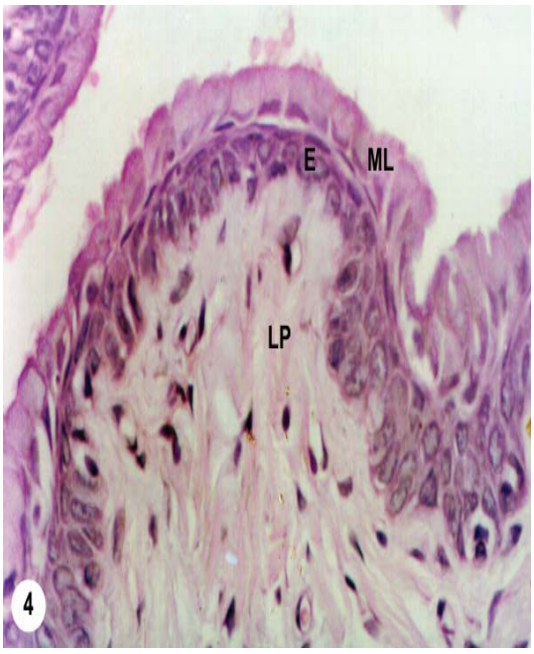

Fig. 4: High magnification of the square in the Fig. 3 of the diabetic group showing decrease in the thickness of the epithelium (E). Note the mucinous layer (ML) which starts to shed from the underlying epithelial layer. There is no leukocyte infiltration in the lamina propria (LP). Hx. & E.; X400

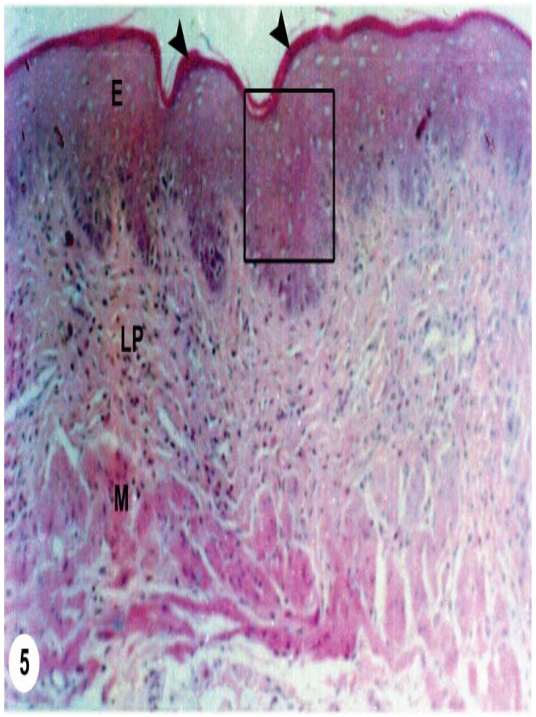

Fig. 5: A photomicrograph of a section of the rat vagina of the diabetic/estradiol treated group showing increase in the thickness of the epithelium (E) which is covered by a cornified eosinophilic layer (arrowheads). The lamina propria (LP) is as thick as the control and the muscularis layer (M) becomes hypertrophied and is formed of well developed bundles. Hx. & E.; X100

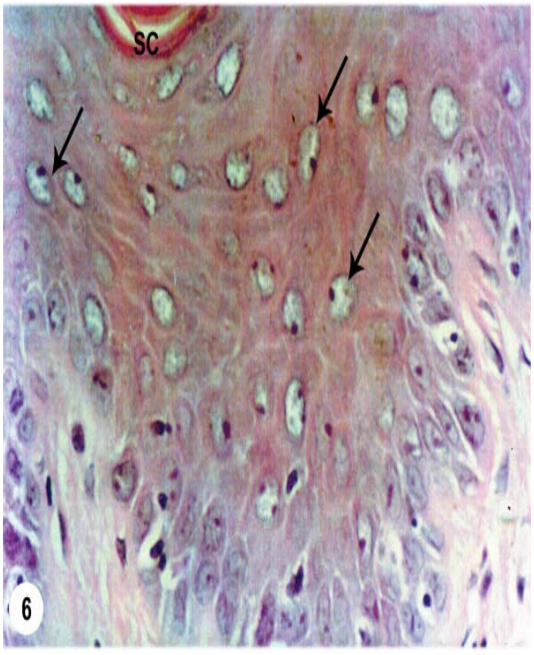

Fig. 6: High magnification of the square in the Fig. 5 of the diabetic/estradiol treated group showing increase in the thickness of the epithelium, which is formed of cells filled with vacuoles and eccentric nuclei (arrows) and covered by a stratum cornium (SC). Hx. & E.; X400

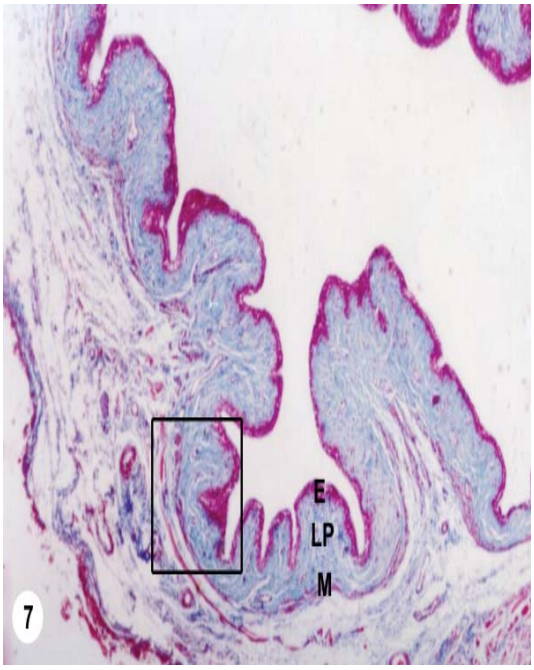

Fig. 7: A photomicrograph of a section of the rat vagina of the control group showing the epithelial layer (E), lamina propria (LP) and muscularis layer (M). Masson's trichrome; X40

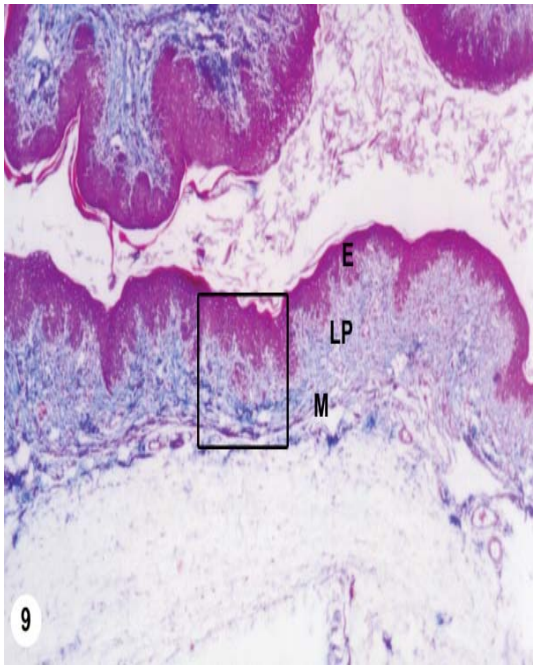

Fig. 9: A photomicrograph of a section of the rat vagina of the diabetic group showing the decrease in the folding of the epithelial layer (E), increase in the deposition of the collagenous fibers in the lamina propria (LP) and marked atrophy of the mascularis layer (M). Masson's trichrome; X40

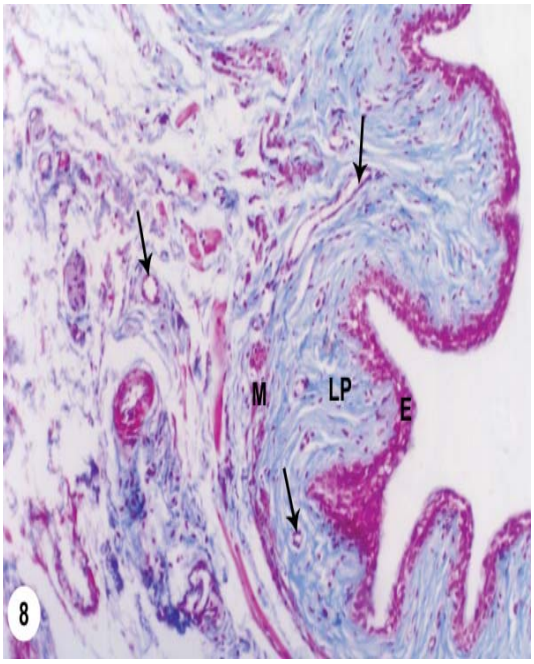

Fig. 8: High magnification of the square in Fig. 7 of the control group showing epithelial layer (E), lamina propria (LP) and muscularis layer (M). Note the rich blood vessels within the stroma and connective tissue (arrows). Masson's trichrome; X100

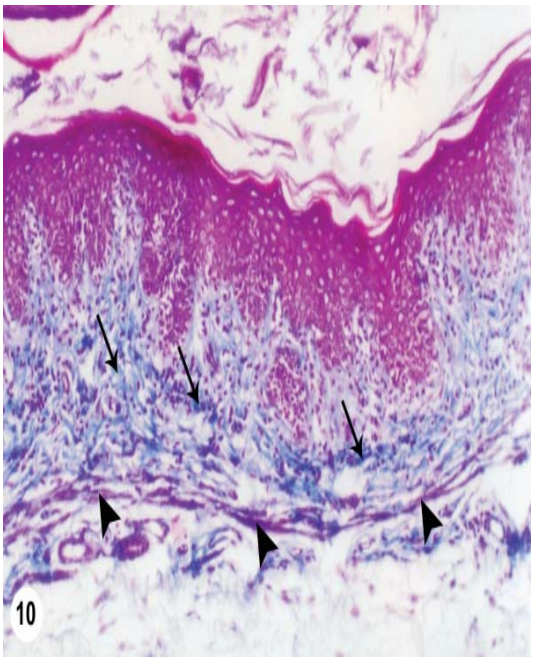

Fig. 10: High magnification of the square in Fig. 9 of the diabetic group showing enhancement of the connective tissue content, which became more thick and irregular (arrows). Note the atrophy of the mascularis layer (arrowheads) and its infiltration by the connective tissue bundles. Masson's trichrome; X100

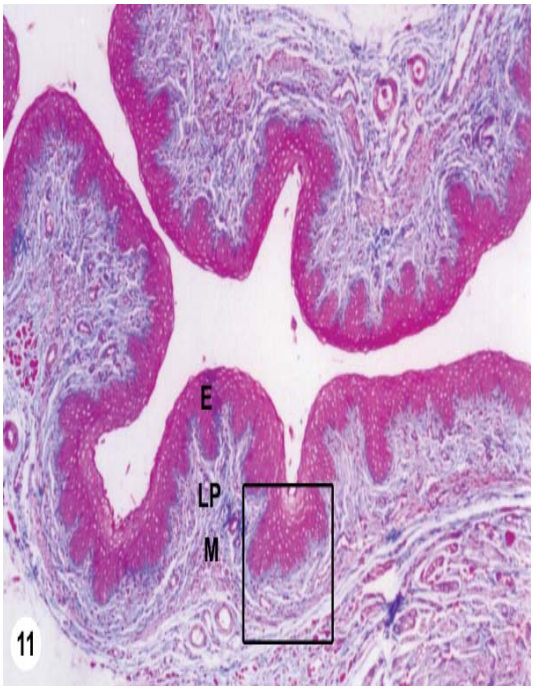

Fig. 11: A photomicrograph of a section of the rat vagina of the diabetic/estradiol treated group showing increase in the thickness of the epithelium (E), the lamina propria (LP) and the well developed muscularis layer (M). Masson's trichrome; X40

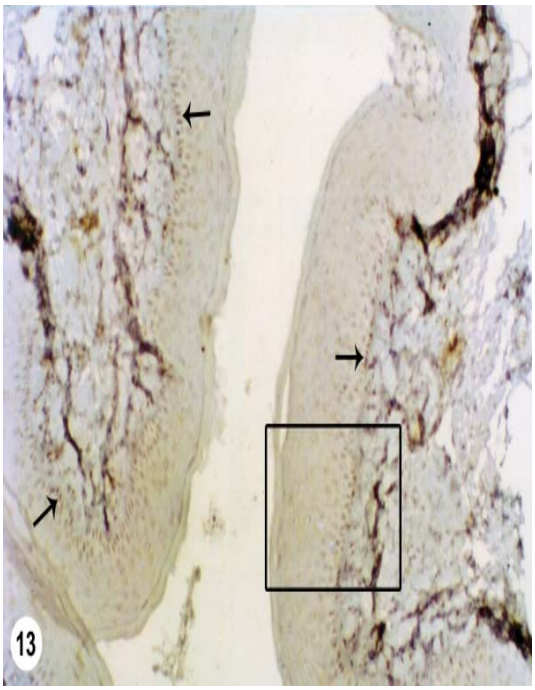

Fig. 13: A photomicrograph of a section of the rat vagina of the control group showing ERα positive cells (arrows) in the epithelial layer especially in the basal layer. ERα immunoperoxidase stain counter stained with Hx.; X100

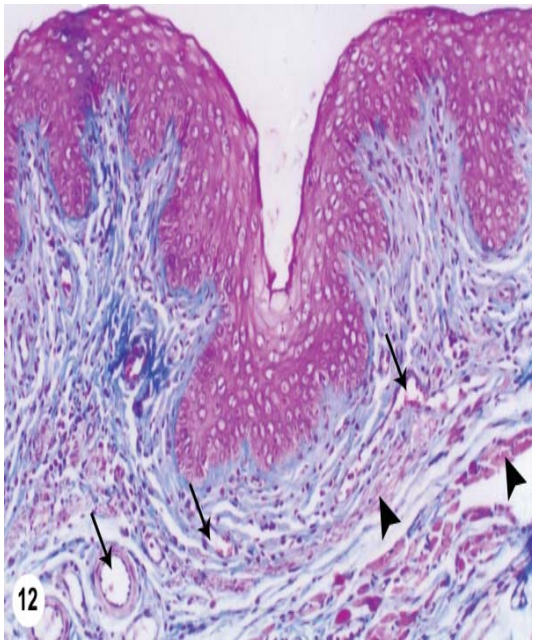

Fig. 12: High magnification of the square in Fig. 11 of the diabetic/estradiol treated showing the hypertrophied muscularis layer (arrowheads) which is formed of well developed bundles. Note the rich blood supply (arrows). Masson's trichrome; X100

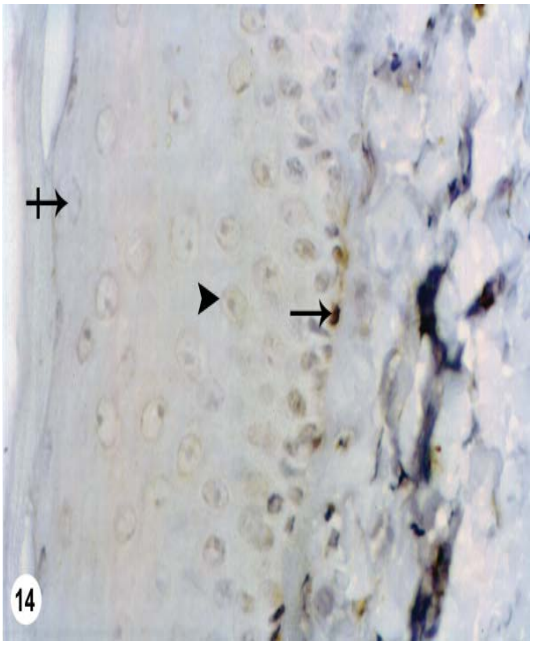

Fig. 14: High magnification of the rectangle in Fig. 13 of the control group showing ERα positive cells (arrow) in the epithelial layer especially in the basal layer, week positive cells are seen in the intermediate layers and the reaction is mainly nuclear (arrowhead). Note the negatively stained cells in the most superficial layers (crossed arrow). ERα immunoperoxidase stain counter stained with Hx.; X400

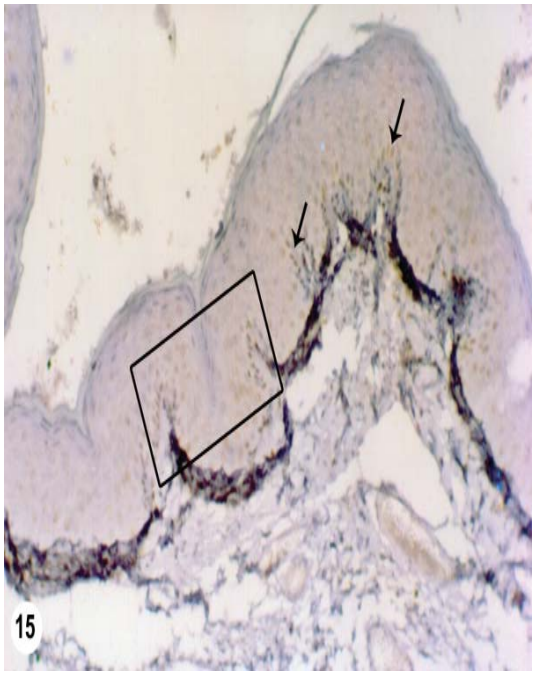

Fig. 15: A photomicrograph of a section of the rat vagina of the diabetic group showing few ERα positive cells (arrows) in the epithelial layer. ERα immunoperoxidase stain counter stained with Hx.; X100

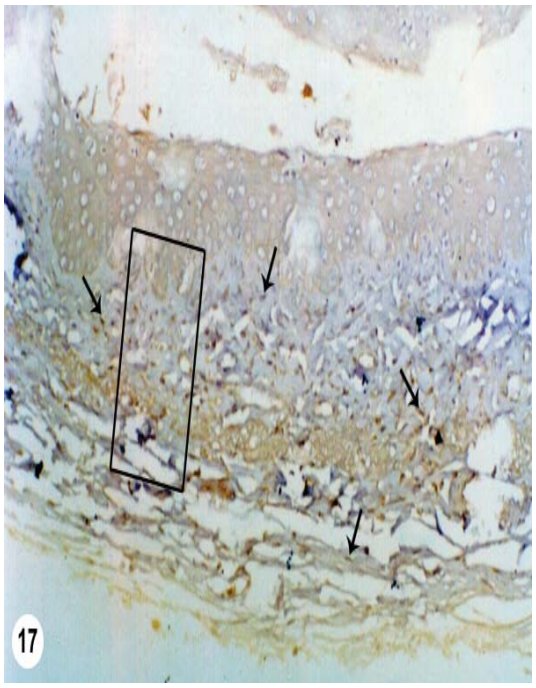

Fig. 17: A photomicrograph of a section of the rat vagina of the diabetic/estradiol treated group showing ERα positive cells (arrows) in the stroma of the lamina propria and in the muscularis layer. ERα immunoperoxidase stain counter stained with Hx.; X100

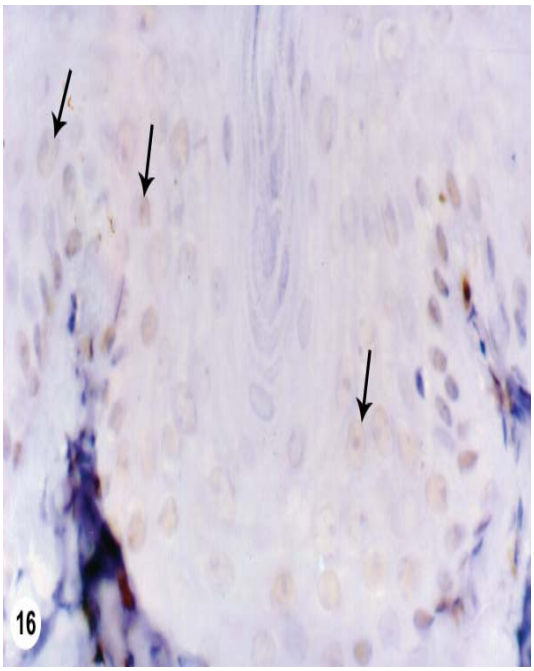

Fig. 16: High magnification of the rectangle in Fig. 15 of the diabetic group showing decrease in the expression of ERα in the epithelial layer with few ERα positive cells (arrows). The reaction was faint in the cytoplasm and negative in the nucleus. ERα immunoperoxidase stain counter stained with Hx.; X400

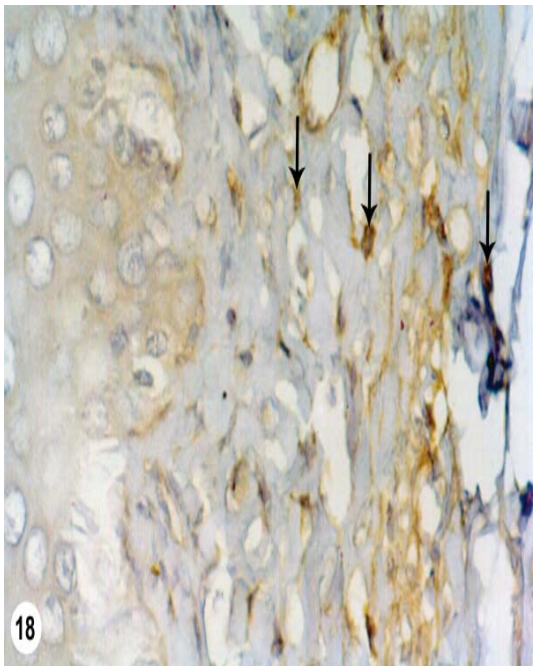

Fig. 18: High magnification of the rectangle in Fig. 17 of the diabetic/estradiol treated group showing positive ERα within the stroma of the lamina propria (arrows) while the epithelial cells are negatively stained. ERα immunoperoxidase stain counter stained with Hx.; X400

DISCUSSION

This study showed that the diabetic state produced a decrease in the mean plasma estradiol level and marked changes in the vaginal tissue structure which was characterized by thinning of the epithelium, increase in collagen deposition and atrophy of the muscularis layer. The immunostaining of ERα in the epithelium of the vagina was decreased comparable to that of the control group. These changes resembled those observed by other authors in type 1 and type 2 diabetic animals *(Cushman et al., 2009)* and also in the ovariectomized animals *(La Marca et al., 1999; Kim et al., 2004; Pessina et al., 2006)*.

Thinning of the epithelium with loss of its outer zones, lack of mitotic figures, and atrophy of the muscularis layer in the vagina of diabetic animals suggest a possible disruption in the cell proliferation and/or growth. Diabetes has been shown to disrupt cell growth and proliferation and to induce apoptosis in the heart *(Cai et al., 2002)*, retina *(Martin et al., 2004)*, pancreas *(Garris & Garris, 2005)*, kidney *(Menini et al., 2007)*, and spinal cord *(Gao & Gao, 2007)*.

The changes in the architecture of the vaginal connective tissue including deposition of dense, compact and less uniform collagen fiber and marked atrophy of the muscularis layer observed in this study were also reported by *Kim et al. (2006)*. Similar diabetes-induced changes in elastic fiber networks were well documented in the tissue of the corpora cavernosa of male patients where elastic fibers appeared shortened or absent in the tunica albuginea, ultimately contributing to erectile dysfunction *(Akkus et al., 1997)* and also in the clitoral cavernous smooth muscle in alloxan-induced type 1 diabetic rabbits *(Park et al., 2002)*. Such marked degeneration changes in the connective tissue are likely to alter the tonicity of the vaginal wall and modify vaginal compliance.

The metabolic disturbance produced by the lack of insulin and by hyperglycemia has been shown to cause a decrease in the serum levels of FSH and LH in different animal models. This was accompanied by loss of sensitivity of ovarian cells to these hormones and alteration in their capacity to synthesize ovarian reproductive hormones, namely, estrogens from follicular cells and progesterone from luteal cells *(Ballester et al., 2004)*.

Diabetic state and lack of insulin production also inhibit aromatase activity which is an insulin-dependent enzyme essential for the conversion of 4-androstenedione and testosterone to estrone and estradiol causing a decrease in estrogen biosynthesis and an increase in testosterone level *(Yoon et al., 2005)*. The alteration of the estrogen and testosterone levels can cause an imbalance in the hypothalamic–hypophyseal–gonodal axis. The exogenous administration of these hormones, and not insulin, are able to restore the normal stability of the hormonal parameters.

Estrogen receptors belong to the steroid–thyroid hormone nuclear receptor supergene family *(Nilsson et al., 2001)*. While ER-β is much more widely distributed throughout the body, ER-α is mainly expressed in the uterus, vagina, ovaries, oviduct, pituitary and mammary glands, which are classical sites of estradiol actions *(Hall & McDonnell, 1999)*. Moreover, ER-α mediates protective anti-inflammatory effects in vascular *(Darblade et al., 2002; Ardelt et al., 2005)* and nonvascular tissues *(Vegeto et al., 2003; Ghisletti et al., 2005)*. In this study, ER-β levels were not determined since ER-α has been shown to be the predominant subtype expressed in the rat vagina *(Mowa & Iwanaga, 2000)*.

ER-α immunostaining was reduced throughout the vaginal wall in the diabetic animals. Previous laboratory studies have indicated that diabetes disrupts estrogen signaling in a variety of tissues. Estrogen receptor binding and ERα levels have been shown to be reduced in the pituitary of diabetic animals resulting in affection of sexual receptivity and reproduction *(Coirini et al., 1980)*. Also, nuclear retention of estradiol-bound ER has been shown to be of shorter duration in both the pituitary and uterus of diabetic animals *(Weisenberg et al., 1983)*.

Another mechanism that could explain the pathological findings in this study is the state of oxidative stress which is a well-known consequence of diabetes. This oxidative state may differentially regulates the expression of ER-α and ER-β causing dysregulation of estrogen action within the vagina *(Tamir et al., 2002)*.

The results of the current study revealed that estradiol supplementation has increased the proliferation of the epithelium and restored the muscularis layer as compared with the control. Also, estradiol supplementation, at sub-physiological levels, has up-regulated ER-α immunostaining in the stroma and muscularis layers of the diabetic rats but not in the epithelium in comparison to the non-treated diabetics. This is in accordance with *Cushman et al. (2009)* who demonstrated that estradiol supplementation in diabetic animals restored most of the diabetes-induced changes in the vagina despite of the persistence of hyperglycemia.

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دراسة تأثير الاستراديول على التغييراتِ الناتجة عن مرض البول السُكّرى على مهبلِ الجرذ الأبيض: دراسة تجريبية هستوكميائية مناعية

رانيا شريف، داليا صالح

قسم التشريح و الأجنة ـ كلية الطب ـ جامعة المنصورة

ملخص البحث

يلعب كل من هورمون الاستروجين ومُستقبِلاته دورَ هام في الإبْقاء على الصحةِ المهبليةِ، وقد تؤدى عرقلتِهم إلى تأثير سلبى على تركيب ووظيفةِ المهبل. ولا توجد دِراسات كافية عن تأثير مرض البول السكرى على توزيعِ مستقبلات هورمون الاستروجين في الجدار المهبلي. ويهدف هذه البحث إلى دراسة التغييراتِ الناجمة عن مرض البول السكّرىَ على التركيبِ الهستولوجى للمهبل و على توزيع مستقبلات هورمون الاستروجين به ولبحث ما إذا كانت المعالجةِ بالاستراديول يُمْكِنُ أَنْ تؤدى الى تحسّينَ هذه التغييراتِ.

تم تقسيم الجرذان البيضاء بشكل عشوائي إلى ثلاث مجموعاتِ وتكونت كل مجموعة من عشرة جرذان. المجموعة الأولى (المجموعة الضابطة) تلقت مذيب الدواء فقط. المجموعة الثانية (المصابون بالسُكر) تلقت حقنة وحيدة بتجويف البطن من عقار الألوكسان بجرعة مقدارها ١٥٠ مللجرام/كجم. المجموعة الثالثة (المصابون بالسكر والمعالجون بالاستراديول) وفيها استخدمت الجرذان المصابة بالسكر لمدة ثمانية أسابيع وحقنت تحت الجلد بالاستراديول المذاب فى زيت الفول السودانى بجرعة ٢٠ ميكروجرام/كجم وذلك لمدة ثمانية أسابيعِ أخرى.

وقد تم التضحية بالحيوانات بعد ستة عشر أسبوعاً منذ بداية التجربةِ، وجُمِعتْ عينات الدمّ من الجرذان لتَحديد نسبة مستوى الاستراديول بها. كما تم تجهيز قطاعات من المهبل وصباغتها بالهيماتوكسيلين والايوسين لفحص التغيرات الهستوباثولوجية بها وبصبغة ماسون ترايكروم لتقييمِ محتوى الكولاجينِ بها وبالصبغة المناعية لمستقبلات هرمون الاستروجين (ألفا).

وأظهرت الدراسة أن مستوى الاستراديول فى دم الجرذان المصابة بمرض السُكّرُ قد قل بصورة كبيرة وذات دلالة إحصائية وذلك مقارنة بالمجموعة الضابطة. كما أظهر الفحص الهستولوجى ضمور فى جدار المهبل وفى الطبقاتِ الطلائيةِ المهبليةِ، مع زيادة في ألياف الكولاجينَ فى الغشاء تحت المخاطي وضمور ملحوظ فى طبقة العضلات الملساء ونقص فى مستقبلات هرمون الاستروجين (ألفا). وبعد معالجة الحيواناتِ المصابة بمرض السُكّر بالاستراديول لمدة ثمانية أسابيعِ فقد أدى ذلك إلى تضخّم ملحوظ فى طبقة العضلات الملساء مع إعادة تصفيف فى طبقات الغشاء الطلائى. علاوة على ذلك، كان هناك انخفاض ملحوظُ في الصبغة المناعية النووية والسيتوبلازمية لمستقبلات هرمون الاستروجين (ألفا) داخل الغشاء الطلائى، كما كان هناك زيادةِ في تعبير هذه المستقبلات داخل النسيج الاساسى للطبقة تحت الغشاء المخاطى وطبقة العضلات الملساء مُقارنة بالمجموعة الضابطة.

ويخلص هذا البحث إلى أنّ التغييرات الهيكلية بالمهبل والناجمة عن مرض السُكّر قَدْ نتجت جزئياً بسبب نقص مستوى الاستروجين بالدم وأن زيادته بالدم ولو إلى النسبة تحت الفسيولوجية يُمْكِنُ أَنْ تحسن من مظاهر الضمور فى المهبل.