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Original Article	**Effect of 17-B Estradiol Supplementation on Reversion of Diabetic Renal Fibrosis**

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ABSTRACT

AIM: to examine the effects of estradiol supplementation on the attenuation and reversion of renal structural changes once they have been initiated by the diabetic milieu.

METHODS: Albino rats were randomly divided into two groups: Normal control and diabetic groups. The diabetic group was subdivided into 3 subgroups: 8-week diabetic group (D8), 16-week diabetic group (D16) and diabetic/estradiol treated group (D/E).

Blood glucose, estradiol level, body weight, kidney weight and fractional kidney weight were measured at the time of scarification. Kidney sections were stained with heamatoxylin and eosin and Sirius red stains. Renal expression of vimentin was determined by immunohistochemistry.

RESULTS: There was significant increase in blood glucose level in all diabetic groups with no significant difference between them. Plasma estradiol level was significantly decreased in the diabetic groups. Estradiol supplementation restored the plasma estradiol level to sub-physiological level and significantly attenuated the decrease in the body weight and the increase in fractional kidney weight. Furthermore, estradiol markedly decreased the structural kidney changes caused by diabetes and inhibited the increase in the amount of collagen fibers as evidenced by the decrease in the glomerulosclerosis and tubulo-interstitial fibrosis indices. Estradiol also attenuated the expression of vimentin. Estradiol had the ability to reverse the kidney as evidenced by the significant decrease in the glomerulosclerosis index as compared with that of the 8-week diabetic group.

CONCLUSION: Estradiol supplementation can reverse the structural changes caused by diabetes after it has been initiated. It can prevent and reverse the renal fibrosis.

Key Words: Diabetic nephropathy, sirius red, estradiol, vimentin.

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INTRODUCTION

Diabetic Nephropathy (DN) is one of the most frequent complications of diabetes mellitus, developing in 30–40% of patients with type 1 and type 2 diabetes mellitus *(Tuttle et al., 1991)*. It is considered as one of the main causes of end-stage renal disease (ESRD) *(Zhang et al., 2007)*.

Histologically, diabetic nephropathy is characterized by progressive accumulation of extracellular matrix (ECM) component in the glomerular mesangium and tubulo-interstitial fibrosis *(Ziyadeh, 1993; Gilbert & Cooper, 1999)*. Glomerulosclerosis and tubulo-interstitial fibrosis are prominent features of progressive diabetic nephropathy *(Bohle et al., 1991)*. Thus, attenuating ECM accumulation and/or enhancing ECM degradation is considered a prime target in the treatment of diabetic renal complications.

Myofibroblasts play a major role in the synthesis and secretion of extracellular matrix *(Essawy et al., 1997)*. Myofibroblasts are activated fibroblasts expressing a wide range of cytoskeletal proteins, in particular α-smooth-muscle actin (α-SMA) *(Gabbiani, 1992),* vimentin and desmin *(Sehmitt-Graff et al., 1*994). Also, Mesangial cell proliferation/activation, tubular damage and regeneration have been associated with the neo-expression of a wide range of cytoskeletal proteins including α -SMA and vimentin *(El Nahas et al., 1996)*.

The female sex appears to be a protective factor against the development of nondiabetic renal disease, but this protection is not as apparent in the setting of diabetes *(Silbiger & Neugarten, 2003; Metcalfe & Meldrum, 2006)*. The reduced levels of plasma estradiol and abnormal regula-

tion of estrogen receptors in the diabetic kidney might account for the loss of the female sex as a protective factor in diabetes *(Mankhey et al., 2005 & Wells et al., 2005)*.

Furthermore, several studies showed that supplementation with 17β-estradiol (E2) from the onset of diabetes protected against the development of diabetic renal injury *(Mankhey et al., 2005; Wells et al., 2005)*. In contrast, it has been reported that the incidence of glomerulosclerosis is increased in ovariectomized diabetic rats treated with estrogen *(Rosenmann et al., 1984)*. The prevention and treatment of DN in early stage and the retardation of DN development are attracting more and more attention from researchers *(Zhang et al., 2007)*.

Little is known about the ability of E2 to attenuate the progression of the disease once it has developed. This is of particular clinical importance, since the majority of patients with diabetes present with moderate or advanced renal injury.

The aim of the present study was to examine the effects of estradiol supplementation on the attenuation and reversion of renal structural changes and expression of cytoskeletal protein (vimentin) once they have been initiated by the diabetic milieu in streptozotocin (STZ)-induced diabetic rat model.

MATERIALS AND METHODS

Animal Preparation:

Thirty-two adult female albino rats (13–18) weeks old weighing (200–250g) were obtained from the Faculty of Pharmacy animal house, Mansoura University.

The animals were housed two or three to a cage at a constant temperature 18°C and humidity 45% on a 12-h light/dark cycle. They had free access to standard diet and drinking water. All the experiments were carried out according to the rules and regulations lay down by the committee on animals' experimentation of Mansoura University.

Experimental protocol:

The animals were divided randomly into 2 groups. The control group C (n= 8) received intraperitoneal injection of 0.1 mol/L of citrate buffer only while the diabetic groups (n= 24) received streptozotocin injection to induce diabetes.

Eight weeks after induction of diabetes, the diabetic animals were further subdivided into three subgroups: 8-week diabetic group D8 (n= 8) scarified immediately, 16-week diabetic group D16 (n= 8) received subcutaneous injection of 1 ml peanut oil for another 8 weeks and then scarified and diabetic/estradiol-treated group D/E (n= 8) received subcutaneous injection of estradiol every 4 days for another eight weeks and then sacrificed.

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 50 mg/kg sterptozotocin (STZ) (Sigma, St. Louis, MO, USA) in 0.1 mol/L of citrate buffer (pH 4.5) *(Liu et al., 2003)*.

Measuring blood glucose level:

Three days after STZ administration and twice per week thereafter, tail-vein blood glucose level was determined using an accutrend glucose detector (Boehringer Mannheim GmbH, Mannheim, Germany). Rats with blood glucose levels above 250 mg/dl for two consecutive weeks were considered as diabetic and were selected for the study.

Estradiol supplementation and plasma estradiol levels:

Animals in the diabetic/estradiol-treated group were subcutaneously injected with estradiol E2 (5 µg/kg in 200 µl of peanut oil; Sigma) every 4 days to mimic the cyclical nature of estrogen release dissolved in peanut oil *(Dixon & Maric, 2007)*.

At the assigned times, blood samples from tail vein were sent to laboratory. Plasma E2 levels

were measured by ELISA (Alpha Diagnostics, San Antonio, TX) according to the manufacturer's protocol.

Histological Assessment:

At the assigned times, all animals were weighted then sacrificed and their kidneys were removed, weighted. Fractional kidney weight was calculated by dividing the kidney weight by the body weight. Coronal sections from the kidney were fixed in 10% neutral formalin for 3-4 days. Kidney sections were dehydrated in ascending grades of alcohol, embedded in paraffin and sectioned at five-microns. Sections were stained with hematoxylin and eosin for histopathological assessment, Sirius red for collagen fibers *(Grimm et al., 2003)*.

Immunocytochemical staining for vimentin:

Five micron sections were placed into xylene to remove the paraffin wax, hydrated in graded ethanol and immersed into distilled water. Antigen retrieval was performed in 0.1 M citrate buffer at 90°C for 10 minutes. The sections were then incubated with 10% nonimmune goat serum for blocking of nonspecific immunostaining, followed by an overnight incubation with monoclonal mouse anti-swine vimentin (Santa Cruz Biotechnology; dilution 1:400) *(Nouwen et al., 1994)* at 4°C. The sections were washed with phosphate-buffered saline and then incubated with a biotin conjugated goat antirabbit immunoglobulin G (diluted 1:100, DAKO, Carpinteria, California) for 1 hour and last with the avidinbiotin complex (diluted 1:100, Vectastain Elite ABC kit, Vector Laboratories, Inc., Burlingame, California) for 1 hour at room temperature. A positive immunoreaction was detected after a 10-minute treatment with 3, 3'-diamino benzidine tetrahydrochloride (DAKO) and by counterstaining with Mayer's hematoxylin. Sections incubated with 10% nonimmune goat serum instead of the primary antiserum were used as negative controls.

Morphometric study:

Glomerular sclerosis and interstitial fibrosis indices were evaluated using Sirius red coloration to visualize collagen fibers. Kidney sections stained with picrosirius red observed under the light microscope, using Leica Qwin 500 image analyzer computer system. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. In each chosen field the Picrosirius red-stained area was enclosed inside the standard measuring frame and then the fibrosis area was masked by a blue binary colour to be measured.

Glomerular sclerosis index was estimated by measurement of Sirius red-stained collagen as the percentage of total glomerular surface area. For each kidney section, 50 glomeruli were systematically digitized (glomeruli were consecutively encountered by moving the microscope stage with an S-shape path) using a 400 objective and examined.

Tubulo-interstitial fibrosis index was measured by measurement of Sirius red-stained collagen as the percentage of total surface area of the renal cortex after exclusion of subcapsular cortex, glomeruli and large vessels from the analysis at magnification of ×100. A total of 25 microscopic fields were captured in a non-overlapping ''U'' direction in each kidney *(Van der Laak et al., 2000)*.

Statistics Analysis:

The data were presented as median±SD. The nonparametric data obtained were subjected to statistical analysis using Mann-Whitney test. Statistical significance was defined when $P \leq 0.05$.

RESULTS

Baseline characteristics of diabetic rats

Blood glucose level in diabetic (8 and 16 weeks) and diabetic/estradiol treated groups was highly significantly increased throughout the study period compared with the control group ($P < 0.01$). However, there was no significant difference in blood glucose level between the diabetic groups and diabetic/estradiol group ($P > 0.05$) (Table 1).

The body weight of the rats in the diabetic groups (D8: 173.5±41.9, D16: 118.75±15.3) was significantly decreased as compared with that in the control group (C: 221.25±17.88). This decrease was markedly improved after treatment with estradiol (D/E: 190.75±17.94) (Table 1).

The kidney weight was insignificantly increased with diabetes (C: 0.87 gm±0.21; D8: 0.97 gm±.34, D16: 0.98 gm±0.16), while E2 supPlementation had no effect on the kidney weight (D/E: 0.88 gm±.25).

Regarding the fractional kidney weight (kidney/body weight ratio), diabetes was associated with a significant increase in this parameter as compared with C (C: 0.4±0.1; D8: 0.61±0.3; D16: 0.84±0.18) and E2 supplementation significantly attenuated the increase in kidney/body weight ratio (D/E: 0.42±0.09) (Table 1).

Plasma estradiol level

Plasma estradiol level was highly significantly decreased in the diabetic groups as compared with the control group (C: 117.88±13.05 pg/ml; D8: 23.56±4.42pg/ml; D16: 14.75±6.78 pg/ml), while E2 supplementation significantly increased plasma estradiol level (D/E: 35.31±4.58pg/ml). However, this level was still significantly low as compared with the control level (Table 1).

Histological examination:

- **Control group:**

Haematoxylin and eosin-stained sections of the control group revealed the normal histological structure of the kidney (Fig. 1). Sirius red stain revealed fine collagen fibers inside the glomeruli and in the glomerular basement membrane. Delicate collagen fibers were observed surrounding the renal tubules and blood vessels in the cortex (Fig. 2). The glomeulosclerosis index and tubulointerstitial fibosis index were 14.99±11.59 and 8.69±6.13 respectively (Table 2).

Positive vimentin immunoreactivity was detected in the glomerular epithelial and endothelial cells (Fig. 3). Minimal vimentin immunoreactivity was detected in the peritubular interstitium (Fig. 4).

- **Eight-week diabetic group:**

Diabetes for 8 weeks was associated with glomerular injury. Several glomeruli showed mesangial widening with hypercellularity (mesangial expansion). Tubulo-interstitial lesions were characterized by infiltration of mononuclear cells and tubular dilatation with tubular cells vacuolation (Fig. 5).

There was an overall increase in the intensity of Sirius red staining in kidney sections of 8-week diabetic rats. Coarse collagen fibers could be detected around and inside the glomeruli. Also, there was increase in the amount of Sirius red-stained collagen fibers in tubulo-interstitial area around theproximal and distal convoluted tubules and collecting ducts (Fig. 6). Diabetes for 8 weeks caused significant increase in the glomerulosclerosis index (23.94±13.51) and non-significant increase in the tubulo-interstial fibosis index (13.43±4.58) (Table 2).

In kidneys from 8-week diabetic rats, there was an increase in the glomerular cells vimentin immunostain (Fig. 7). There was an increase in positive vimentin expression in the peritubular cells (Fig. 8) and tubular cells expression of vimentin was also noted (Fig. 7).

- **Sixteen-week diabetic group:**

Diabetes for 16 week caused progressive damage of the kidney as there was marked mesengial expansion and tubular dilatation and tubular cells vacuolations (Fig. 9). By Sirius red stain, the collagen fibers were markedly increased (Fig. 10). The glomerulosclerosis index (43.52±16.35) was significantly increased, but tubulo-interstial fibrosis index (16.87±9.67) was nonsignificantly increased as compared with the 8-week diabetic group (Table. 2). Vimentin positive stain distribution was similar to 8-week diabetic groups (Figs. 11, 12).

- **Estradiol-treated group:**

In estradiol-treated group, some glomerular abnormality has been detected in the form of mesengial expansion in some glomeruli together with also tubular vacuolation (Fig. 13). Fine Sirius red-stained collagen fibers could be detected inside and around the glomeruli and around the ducts and tubules (Fig. 14). Interstitial fibrosis was significantly reduced by estradiol therapy as compared with that of untreated 16-week diabetic rats. The glomeulosclerosis and tubulointerstitial fibrosis indices were 17.37±7.79 and 10.32±5.11. The glomeulosclerosis index was significantly decreased as compared with that of the 8-week diabetic group. However; the tubulo-

interstitial fibrosis index was nonsignificantly decreased as compared with that of the 8-week diabetic group (Table 2).

The changes in vimentin immunostain expression were considerably reduced by estradiol treatment. Only few glomerular and interstiatial positive vimentin reaction could be detected (Figs. 15, 16).

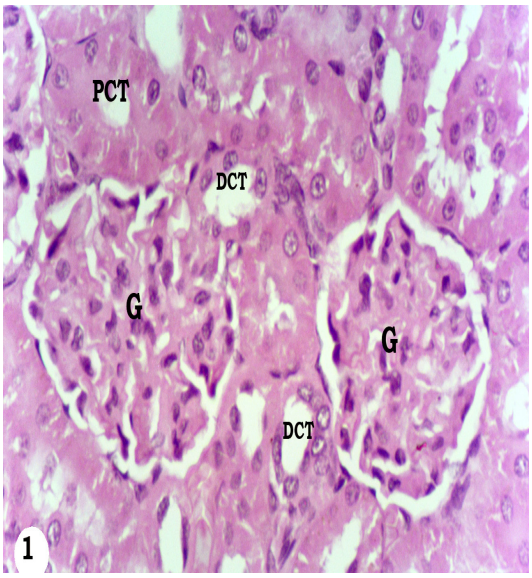

Fig. 1: A photomicrograph of a section of the rat kidney of the control group showing glomeruli (G), Proximal convoluted tubules (PCT) and distal convoluted tubules (DCT). Hx.&E.; X400

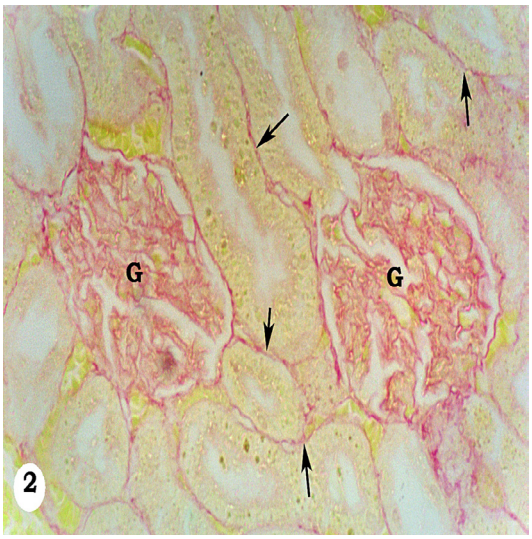

Fig. 2: A photomicrograph of a section of the rat kidney of the control group showing red stained collagen fibers inside the glomeruli (G) and delicate collagen fibers surrounding the tubules (arrows). Sirius red; X400

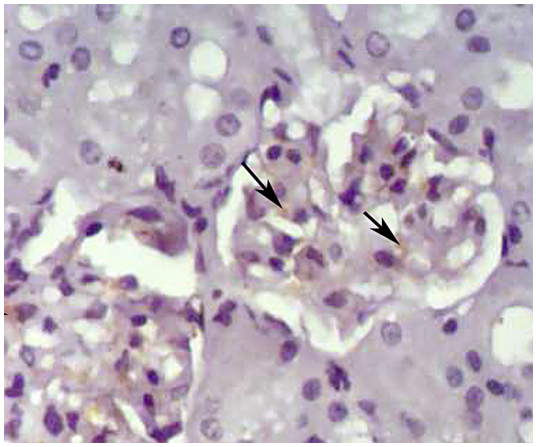

Fig. 3: A photomicrograph of a section of the rat kidney of the control group showing vimentin positive reaction (arrows) inside the glomeruli. Vimentin immunoperoxidase stain; X400

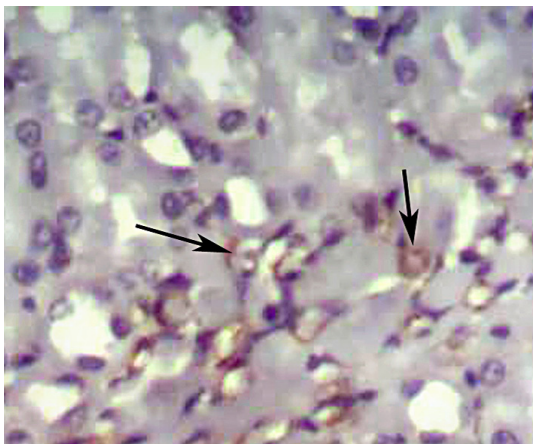

Fig. 4: A photomicrograph of a section of the rat kidney of the control group showing vimentin positive reaction (arrows) in the peritubular interstitium. Vimentin immunoperoxidase stain; X400

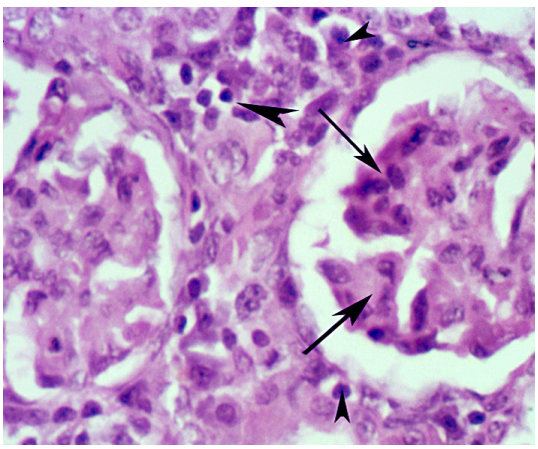

Fig. 5: A photomicrograph of a section of the rat kidney of 8 weeks diabetic group showing glomerular expansion (black arrows), mononuclear cells infiltration (arrowheads), tubular dilatation (asterisk) and tubular cells vacuolations (red arrows). Hx. & E.; X400

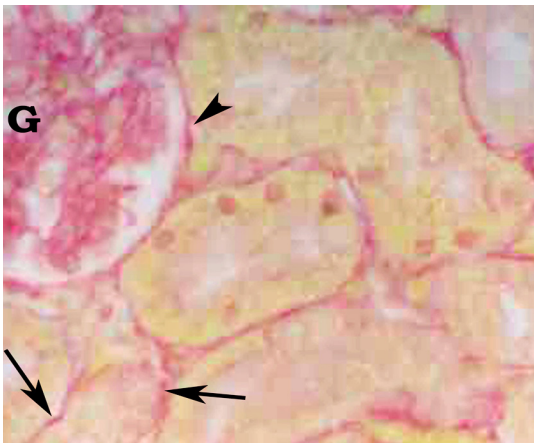

Fig. 6: A photomicrograph of a section of the rat kidney of 8 weeks diabetic group showing coarse collagen fibers around (arrowheads) and inside the glomeruli (G) and coarse collagen fibers around the tubules (arrows). Sirius red; X400

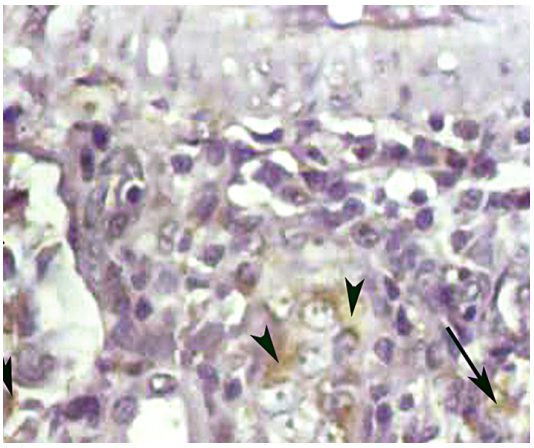

Fig. 7: A photomicrograph of a section of the rat kidney of 8 weeks diabetic group showing increased vimentin positive reaction (arrows) inside the glomeruli and vimentin positive reaction in some tubular cells (arrowheads). Vimentin immunoperoxidase stain; X400

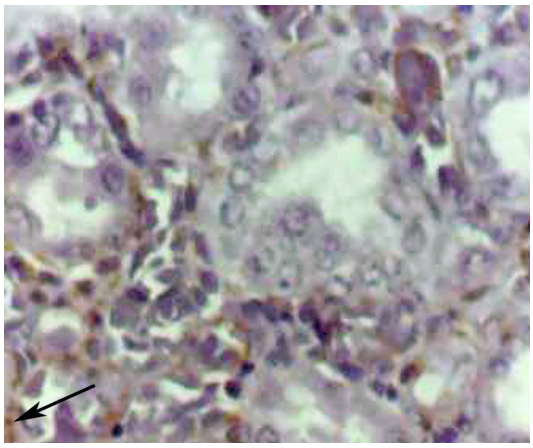

Fig. 8: A photomicrograph of a section of the rat kidney of 8 weeks diabetic group showing vimentin positive reaction (arrows) around the tubules. Vimentin immunoperoxidase stain; X400

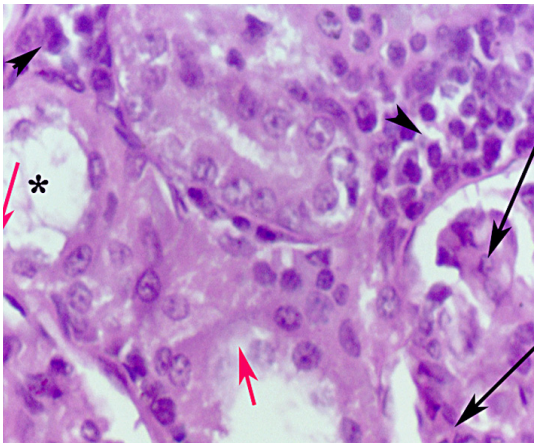

Fig. 9: A photomicrograph of a section of the rat kidney of 16 weeks diabetic group showing glomerular expansion (black arrows), mononuclear cells infiltration (arrow heads), tubular dilatation (asterisk) and tubular cells vacuolations (red arrows). Hx. & E.; X400

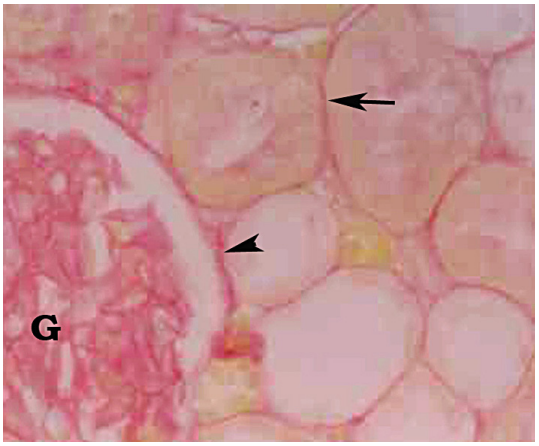

Fig. 10: A photomicrograph of a section of the rat kidney of 16 weeks diabetic group showing coarse collagen fibers around (arrowheads) and inside the glomeruli (G) and coarse collagen fibers around the tubules (arrows). Sirius red; X400

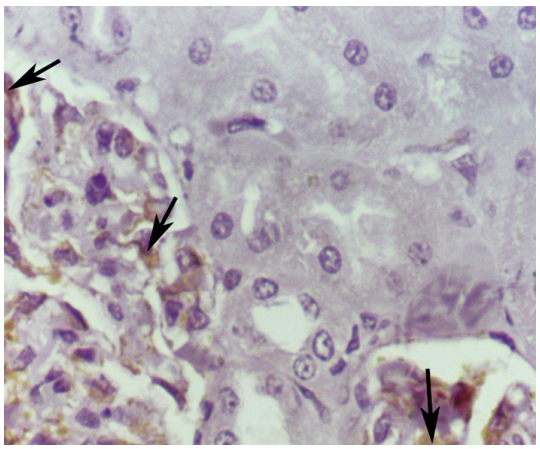

Fig. 11: A photomicrograph of a section of the rat kidney of 16 weeks diabetic group showing increased vimentin positive reaction (arrows) inside the glomeruli. Vimentin immunoperoxidase stain; X400

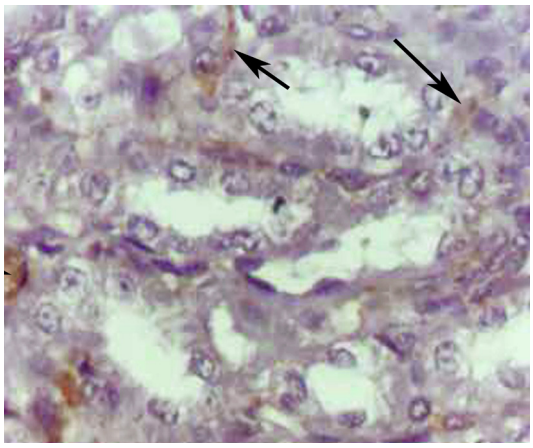

Fig. 12: A photomicrograph of a section of the rat kidney of 16 weeks diabetic group showing increased vimentin positive reaction (arrows) around the tubules. Vimentin immunoperoxidase stain; X400

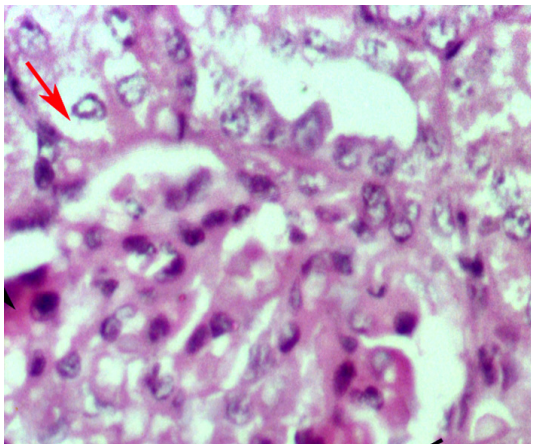

Fig. 13: A photomicrograph of a section of the rat kidney of estradiol treated group showing little glomerular expansion (black arrows), tubular dilatation (asterisk) and tubular cells vacuolations (red arrows). Hx. & E.; X400

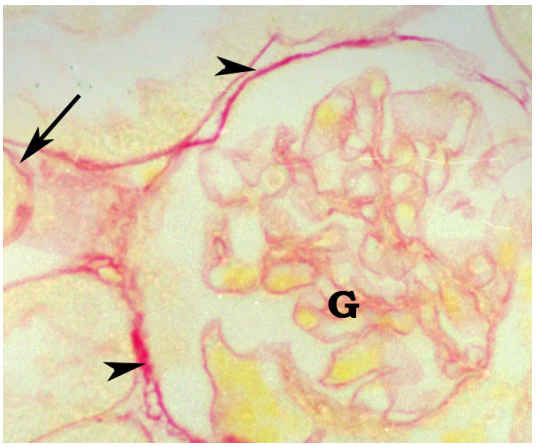

Fig. 14: A photomicrograph of a section of the rat kidney of estradiol treated group showing fine collagen fibers around (arrowheads) and inside the glomeruli (G) and fine collagen fibers around the tubules (arrows). Sirius red; X400

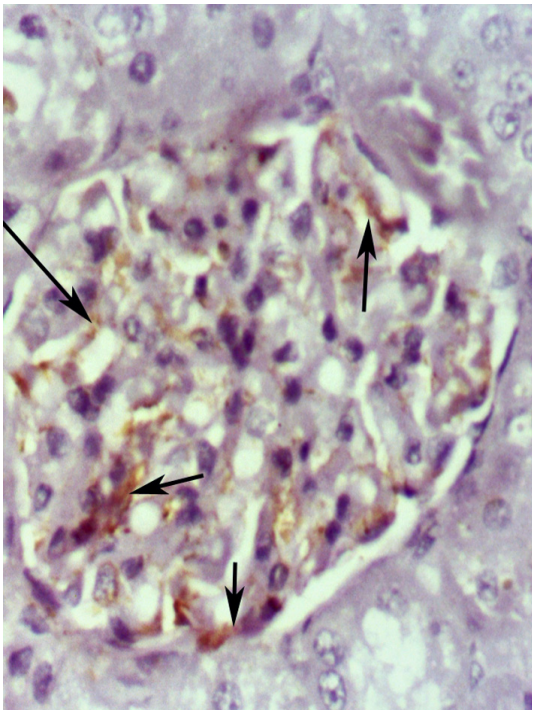

Fig. 15: A photomicrograph of a section of the rat kidney of estradiol treated group showing increased vimentin positive reaction (arrows) inside the glomeruli. Vimentin immunoperoxidase stain; X400

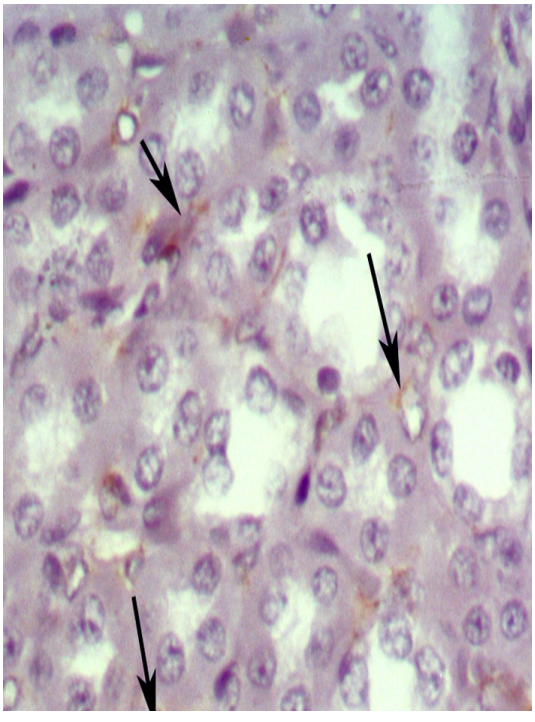

Fig. 16: A photomicrograph of a section of the rat kidney of estradiol treated group showing little vimentin positive reaction (arrows) around the tubules. Vimentin immunoperoxidase stain; X400

Table 1: Body weight, kidney weight, blood glucose level and blood estradiol level in different groups.

	Body Weight	Kidney weight	Kidney Fraction weight	Blood glucose level	Blood Estradiol level
Control	221.25±17.88	0.87±.21	0.4±.1	84.74±10.18	117.88±13.05
8-week Diabetes	173.5±41.91*	0.97±.34	0.61±.3*	217.75±9.67**	23.56±4.42**
16-week Diabetes	118.75±15.3**, #	0.98±.16	0.84±.18**	219.5±16.37**	14.75±6.78**, #
Estradiol-treated group	190.75±17.94#, ##	0.88±.25	0.42±.09#, ##	228.25±5.2**	35.31±4.58# ,# #

* Significant VS control.
# Significant VS 8 weeks Diabetes.
** Highly Significant VS control.
# # Highly significant VS 16 weeks Diabetes.

Table 2: Glomerulosclerosis and tubulo-interstitial fibrosis indices in different groups.

	Glomerulosclerosis index	Tubulo-interstitial fibrosis index
Control	14.99±11.59	8.69±.6.13
8-week Diabetes	23.94±13.51*	13.43± 4.58
16-week Diabetes	43.52±16.35 **, #	16.87± 9.67*
Estradiol-treated group	17.37±7.79 #, ++	10.32± 5.11 +

* Significant VS control.
+ Significant VS 16 weeks Diabetes.
** Highly Significant VS control.
++ Highly significant VS 16 weeks Diabetes.
# Significant VS 8 weeks Diabetes.

DISCUSSION

The pathophysiology of diabetic nephropathy is characterized by a progressive loss of renal function and deposition of extracellular matrix (ECM) leading to widespread tissue fibrosis.

In the present study, diabetes for 8 weeks was associated with degenerative changes in the kidney as glomerular expansion, tubular dilatation and tubular cells vacuolations, mononuclear cell infiltration and significant increase of the amount collagen fibers in the glomeruli and tubular interstitium. This is in agreement with several previous studies *(Jackle-Meyer et al., 1995; Ma et al., 2004; Mankhey et al., 2007)*. Longstanding diabetes for 16 weeks was associated with progressive damage of the kidney in the form of progressive glomerulosclerosis and tubulo-interstitial fibrosis, with mononuclear cells infiltration. It has been reported that glomerular sclerosis and tubulo-interstitial fibrosis correlate with disease progression *(Gilbert & Cooper, 1999)*. Myofibroblasts in the glomeruli and interstitium are involved in the pathogenesis of glomerulosclerosis and tubulo-interstitial fibrosis *(El Nahas et al., 1996)*. Activation of those myofibroblasts could be attributed to local activation of growth factors and cytokines, including angiotensin, endothelin and TGF-β in response to hyperglycemia *(Wolf & Ziyadeh, 1999; Chen et al., 2003)*.

Activated myofibroblasts express a wide range of cytoskeletal proteins, as α-SMA and vimentin *(Gabbiani, 1992; Sehmitt-Graff et al., 1994)*. There is accumulating evidence, as has been suggested by *Menè et al. (1989)* that the activated mesangial cell develops myofibroblast-like features, i.e., characteristics of both smooth muscle cells and fibroblasts *(Gown, 1990)* in in vivo glomerular injury.

In the present study, the diabetic groups showed different grades of mesangial staining of vimentin. There was a positive correlation between mesangial vimentin expression and glomerular sclerosis. In contrast, *Yonemoto et al. (2006)* found positive correlation between mesangial sclerosis and mesangial α-SMA expression, but not vimentin. This could be explained by the instability of the vimentin that makes the filamentous vimentin dis-assembled into smaller units *(Nagasawa et al., 1997)*.

In the current study, diabetes caused decrease in circulating estradiol levels. This is in agreement with previous studies that found lower circulating levels of estradiol in diabetic women *(Resnick & Howard, 2002)*. Examining the effects of E2 supplementation in a diabetic animal model with intact ovaries would produce more valuable information regarding the potential use of E2 supplementation in diabetic women.

In the present study, E2 supplementation restored the estradiol plasma level to sub-physiological level. This is of a great benefit as it has been proved that low doses of estrogen do not affect urine albumin excretion (*Garg et al., 1994)*, while high doses of estrogen lead to development of macroalbuminuria in women with type 1 diabetes *(Ahmed et al., 2005)*. This suggests that the dosage of treatment plays a critical role in the overall effect of sex hormones on target organs.

It has been reported that diabetic nephropathy is associated with increased synthesis of ECM and decrease in ECM degradation by MMPs *(Mason & Wahab, 2003; Lee & Ha, 2005)*. Estradiol regulates renal ECM protein expression in vitro *(Silbiger et al., 1999; Potier et al., 2002; Dubey et al., 2003)* and in vivo *(Blush et al., 2004; Chin et al., 2005)*. *Mankhey et al. (2007)* reported that supplementation with E2 from the onset of diabetes decreases ECM synthesis and increases ECM degradation, thus having a dual renoprotective role relating to ECM metabolism.

The present study showed that estradiol supplementation after 8 weeks of onset of diabetes is renoprotective as it attenuated the structural changes associated with diabetes, attenuated the progression of glomerulosclerosis and tubulointerstitial fibrosis and attenuated the increase in the amount of collagen fibers and reduced vimentin expression.

Dixon and Maric (2007) observed that supplementation with E2 for 8-week following the 9-week period of diabetes downregulates ECM protein and TGF-β protein expression in diabetic nephropathy model. Estradiol and its metabolites inhibit TGF-β protein expression *(Neugarten et al., 2000; Matsuda et al., 2001)*, increase the activity of ECM-degrading enzymes, matrix metalloproteinases *(Potier et al., 2001)*, reverse type IV collagen gene transcription and protein synthesis and suppress mesangial cell type I collagen gene transcription and protein synthesis *(Neugarten et al., 2000; Zdunek et al., 2001)*. These actions shift the balance of matrix metabolism away from matrix accumulation.

It has been found that in type II diabetic patients, replacement with E2 reduced proteinuria and improved creatinin clearance *(Szekacs et al., 2000)*. These findings indicate that E2 may prevent and/or reverse the decline in renal function associated with diabetic nephropathy.

No known study in the literature commented on the effect of E2 on reversion of the fibrosis in the diabetic nephropathy. In the present study, the glomerulosclerosis and tubulo-interstitial fibrosis indices after E2 supplementation were lower than those of the 8-week diabetic group. It might indicate that E2 plays a role in prevention as well as reversion of the kidney fibrosis. This is confirmed by the lower expression of vimentin in the glomeruli and tubulointerstitium. This could be due to the effect of E2 in increasing the expression and activity of both MMP-2 and MMP-9 and reducing TIMP protein expression which not only reduce ECM synthesis but also increase ECM degradation *(Mankhey et al., 2007)*. The decrease in glomeruloscelosis index was significant. However,

the decrease in tubulo-interstitial fibrosis index was insignificant as compared with those of the 8-week diabetic group. This might indicate that E2 has different mechanism of action on the mesangial cells and interstitial myofibroblasts.

In conclusion, sex hormones play an important role in the pathophysiology of diabetic renal disease. The supplementation with E2 to restore the levels of estradiol even to sub-physiological level, could reverse the renal fibrosis in diabetic nephropathy. Comparing the level of expression of MMP-2 and MMP-9 needs further investigations. Further studies should be done to determine the minimal dose of estradiol that can protect the kidney with minimal side effects and the therapeutic effect of other estrogen receptors stimulating drugs on diabetic nephropathy.

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دراسة تأثير اعطاء - بيتا استراديول على انعكاس التليف الكلوى السكري

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ملخص البحث

الهدف من البحث: دراسة تأثير اعطاء ١٧-بيتا استراديول على تخفيف وانعكاس التغيرات الهيكلية الكلوية بعد حدوثها نتيجة لمرض السكري.

طريقة البحث: تم تقسيم الفئران البيضاء عشوائيا الى مجموعتين: مجموعة ضابطة و مجموعة مصابة بداء السكرى. ثم تم تقسيم المجموعة المصابة بالسكرى الى ثلاث مجموعات فرعية: مجموعة مصابة بالسكرى لمدة ٨ أسابيع و مجموعة مصابة بالسكرى لمدة ١٦أسبوع و مجموعة مصابة بالسكرى و تعالج بالأستراديو.

وفى وقت التضحية تم قياس نسبة الجلوكوز ومستوى استراديول في الدم، ووزن الجسم ووزن الكلية الكلى والنسبى. ثم تم صباغة مقاطع من الكلية بصبغات الهيماتوكسلين والايوسين وسيريوس الحمراء كما تم تحديد التعبيرالكلوى للفيمنتين بواسطة الصبغة الهيستوكيميائية المناعية.

نتيجة البحث: كانت هناك زيادة ذات دلالة احصائية في مستوى السكر في الدم فى المجموعات الفرعية المصابة بالسكري مع عدم وجود فرق كبير بينهم. وانخفض مستوى الاستراديول بشكل ملحوظ في البلازما فى المجموعات المصابة بالسكري. و قد نجح اعطاء الاستراديو فى استعادة مستوى الاستراديول فى البلازما إلى مستوى دون المستوى الفسيولوجي كما تسبب فى تراجع ملموس فى معدل الانخفاض في وزن الجسم والزيادة في الوزن النسبى للكلية. وعلاوة على ذلك، نجح الاستراديول فى خفض التغييرات الهيكلية فى الكلية الناتجة عن مرض السكري بشكل ملحوظ ، وحال دون الزيادة في كمية ألياف الكولاجين بدليل الانخفاض في مؤشرات التليف فى الكبيبات و الفضاء حول الأنابيب. كما أضعف الاستراديول أيضا التعبير عن الفيمنتين. و قد تمكن الأستراديول من عكس التليف الكلوى بدليل الانخفاض ذو الدلالة الأحصائية فى مؤشر التليف فى الكبيبات بالمقارنة مع المؤشر الخاص بالمجموعة المصابة بالسكرى لمدة٨ أسابيع.

الخلاصة: يمكن للاستراديول أن يعكس التغيرات الهيكلية فى الكلية الناتجة عن مرض السكري بعد حدوثها كما يمكنه منع وعكس التليف الكلوي.