

Histological Study on the Potential Effect of Sildenafil on the Kidney and Testosterone Level in Experimentally Induced Diabetes in Male Rats

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Abstract

Background and Objectives: Diabetes mellitus is the most common cause of chronic renal disorders and end stage renal disease in developed countries. Male diabetics may also suffer from erectile and testicular dysfunction with low serum testosterone. Sildenafil has a great role in alleviating erectile dysfunction of different causes. The present study aimed at evaluating the possible effects of Sildenafil on renal changes and serum testosterone level in Alloxan induced diabetes.

Methods and Results: Thirty-six male Wistar rats were equally divided into 3 groups; control, diabetic & Sildenafil-treated. Diabetic group received single intraperitoneal injection of Alloxan (150 mg/kg). Sildenafil-treated group received oral Sildenafil (3 mg/kg/day) daily after confirmation of diabetes. Rats of each group sacrificed two and eight weeks after diabetes confirmation. In diabetic group, serum urea & creatinine and urinary albumin were increased, while cyclic guanosine monophosphate, anti-oxidants enzymes and testosterone levels were decreased. Renal corpuscles with distorted and almost complete loss of the glomerular tufts, in addition to the marked vacuolations of the tubular epithelial cells were observed. However in Sildenafil-treated group, all laboratory parameters returned back to nearly normal levels with almost normal histological architecture of renal corpuscles and most of cortical tubules.

Conclusion: Sildenafil administration in a rat model of diabetic nephropathy might have a potential renal protective effect and could restore normal serum testosterone level.

Keywords: Diabetes; Alloxan; Sildenafil; Desmin; Oxidative Stress; Testosterone

Introduction

Chronic kidney disease is increasing worldwide at an annual rate of 8%, with higher prevalence in developing countries. Diabetic Nephropathy (DN) is one of the common underlying causes [1]. In 2009, it was reported that DN represents 44% of all cases of end stage renal disease in the United States [2]. DN develops due to a complex interaction between metabolic and haemo-dynamic pathophysiological factors, which leads to renal damage [3]. Male diabetic patients show increasing incidence of erectile dysfunction (ED) that ranges between 35-75% [4]. Diabetes pathologically affects peripheral tissue innervation and vascularization, both are important for the erectile function. In addition, it causes oxidative stress (OS), which has a key role in the pathogenesis of diabetes-associated ED by acting on blood vessel endothelium, peripheral nerves and smooth muscles [5]. Additionally, Experimental diabetic animals are proved to suffer from testicular dysfunction such as low serum testosterone level and decreased fertility [4]. Nitric oxide-cyclic guanosine monophosphate (NO-cGMP) axis is essential for maintenance of renal perfusion, glomerular filtration and penile erection. Many of the biological actions of NO are mediated by cGMP which is rapidly degraded by phosphodiesterases (PDE), especially PDE-5. Decreasing bioavailability of NO in diabetes may play a crucial role in its progression [6,7]. Sildenafil, PDE-5 inhibitor, is widely used to treat ED. Its pharmacological action is due to its ability to prolong the signaling actions of NO in penile smooth muscle through raising the available cGMP pool by preventing its hydrolysis. Therefore, diabetic males suffered from ED are now treated routinely with Sildenafil [8]. Additionally, Sildenafil has several antioxidative properties. Recent data reported a potential application for Sildenafil in many experimental models of diseases rather than ED [9]. The present study was designed to determine whether sildenafil, might have protective effects on kidney and serum testosterone level in diabetic male rats.

Material and Methods

Animals

Thirty-six adult male Wistar rats were used in this study. Their body weights ranged from 150-170 g. Animal care was provided by laboratory animal house unit of Kasr Al-Ainy, Faculty of Medicine, Cairo University. The rats were treated in accordance to the guidelines approved by the Animal Use Committee of Cairo University. Rats were provided with ordinary rat chow, bred and housed in wire mesh cages at temperature ($24 \pm 1^\circ\text{C}$), with normal light-dark cycle. All animals were kept under the same environmental conditions and had free access to water and food.

Chemicals

- Alloxan was supplied as a bottle containing 25 g powder of 5,6-dioxuracil monohydrate (Oxford, Indian).
- Sildenafil Citrate was supplied as bottle containing 10 g powder (Egyptian company for chemicals and pharmaceuticals).

Experimental Design

Rats were divided into three groups, 12 rats each:

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Group I (Control group): was subdivided into two subgroups:

Subgroup Id: six rats received single intraperitoneal injection of 0.5 ml sterile normal saline.

Subgroup Is: six rats received single intraperitoneal injection of 0.5 ml sterile normal saline and after three days they received daily 0.15 ml of distilled water through gastric tube.

Group II (diabetic group): Diabetes was induced in 12 hours starved rats by single intraperitoneal injection of 0.5 ml of a freshly prepared solution of Alloxan dissolved in sterile normal saline at a dose of 150 mg/kg body weight. Seventy-two hours later, blood samples were obtained from the tail veins of the fasting rats and blood glucose level was determined to confirm induction of diabetes. Animals with blood glucose levels >250 mg/dl were considered diabetic and included in the experiment [10].

After diabetes confirmation, the rats were subdivided equally, according to the time of scarification, into two subgroups (IIa and IIb).

Group III (Sildenafil-treated group): Diabetes was induced and confirmed after three days as in group II. The diabetic rats received daily 0.15 ml of Sildenafil Citrate (3 mg/kg/day) dissolved in distilled water through gastric tube [11].

The rats were subdivided equally into two subgroups (IIIa and IIIb) according to the time of scarification.

Laboratory investigations

At day 0 of experiment and after 2 and 8 weeks of diabetes confirmation, blood samples were drawn from tail veins in collecting heparinized capillary tubes. Serum levels of glucose, urea and creatinine (renal function indicators), total testosterone (parameter for testicular function), cGMP and anti-oxidants enzymes; Glutathione peroxidase (GPX) and Superoxide dismutase (SOD) were assessed for all rats. In addition, 24 h-urine was collected at the same points, by putting individual rats in metabolic cages, to measure the urinary albumin. All these parameters were measured at Biochemistry Department, Faculty of Medicine, Cairo University.

Light microscopic studies

The rats of subgroups IIa and IIIa together with 3 rats from each control subgroup were sacrificed 2 weeks after diabetes confirmation while, rats of subgroups IIb and IIIb with the remaining 3 rats from each control subgroup were sacrificed after 8 weeks. The rats were anesthetized with pentobarbital (80 mg/kg, intraperitoneal) before scarification [12]. The right kidney specimens were fixed in 10% buffered formalin solution for 24 hours, dehydrated in ascending grades of ethanol and embedded in Paraffin. Serial sections of 7 µm thickness were cut and subjected to the following techniques:

- Hematoxylin and Eosin (H&E) stain [13].
- PAS stain for demonstration of parietal layer of Bowman's capsule, basal laminae and brush border of proximal and distal convoluted tubules [13]. Tissue sections were counterstained with Mayer's hematoxylin.
- Immunohistochemical stain using streptavidine-biotin peroxidase technique. The endogenous peroxidase activity was eliminated using 10% H₂O₂ for 15 minutes [14]. Sections were then incubated for one hour with primary antibody against desmin (desmin mouse monoclonal antibody) as a marker for

podocyte damage [15]. It was purchased from Dako, North America, Inc., cat number (M 0760). Tissue sections were counterstained with Mayer's hematoxylin.

Morphometric study

Using "Leica Quin 500" software image analyser computer system (Leica image system Ltd; Cambridge, England), at Histology Department, Faculty of Medicine, Cairo University.

The following parameters were measured:

- Mean number of affected Malpighian renal corpuscles (distortion, shrinkage and loss of glomerular tufts) at a magnification of × 100 in H&E stained sections.
- Mean area percent of PAS-positive reaction of brush borders and basal laminae of proximal and distal convoluted tubules, in PAS stained sections at a magnification of × 100.
- Mean optical density of PAS-positive reaction of parietal layer of Bowman's capsules × 400.
- Mean area percent of desmin positive immunoreaction in desmin-stained sections at a magnification of × 100.

These parameters were performed using image menu. The measurements were done in 10 non-overlapping randomly chosen fields for each animal.

Statistical analysis

Obtained data of the biochemical lab and morphometric results were tabulated and analyzed. Data were summarized as means and standard deviations and compared using one-way analysis-of-variance (ANOVA). P-values <0.05 was considered statistically significant. Calculations were made on SPSS software version 16 (SPSS Inc., Chicago, Illinois, USA).

Results

- No deaths were observed in rats during the experiment.
- No significant difference in laboratory, histological and immunohistochemical results in the subgroups of the control group (Id and Is). Thus, they were represented as control group (I).
- At day 0, there was non-significant difference between all studied groups in laboratory results.
- No significant difference in laboratory, histological and immunohistochemical results between subgroup IIa and IIIa.

Lab results and statistical analysis

After 2 weeks of diabetes confirmation, measurements of serum blood glucose, urea and creatinine and urinary albumin levels increased significantly in subgroup IIa (diabetic group) compared to the control ($p < 0.05$). After 8 weeks of diabetes confirmation (diabetic subgroup IIb), these parameters represented a significant increase compared to control and subgroup IIa ($p < 0.05$). Blood glucose value for subgroup IIIb (Sildenafil-treated group) showed a significant increase ($p < 0.05$) compared to control and non-significant decrease compared to subgroup IIb. Regarding serum urea and creatinine and urinary albumin values, subgroup IIIb showed a significant decrease when compared to subgroup IIb and subgroup IIIa ($p < 0.05$) and non-significant increase versus control group (Table 1).

As regards serum testosterone level in subgroup IIa, non-

Table 1: Mean \pm SD of laboratory parameters (serum blood glucose, urea, and creatinine) expressed as mg/dl, urinary albumin expressed as mg/dl, serum testosterone levels expressed as Deko gm/dl, serum cGMP expressed as pmol/ml & SOD and GPX levels expressed as u/g Hb and u/mg Hb respectively in all studied groups.

| | Group I | | Group II | | Group III | |
|---------------------|-------------------|------------------|--------------------------------|---------------------------------|-------------------------------|-------------------------------|
| | Subgroup Id | Subgroup Is | Subgroup IIa | Subgroup IIb | Subgroup IIIa | Subgroup IIIb |
| Serum blood glucose | 91.0 \pm 10.18 | 96.7 \pm 8.13 | 327 \pm 17.35* | 394 \pm 14.49* [Ⓢ] | 320 \pm 46.48* | 378.5 \pm 35.04* |
| Serum urea | 28.05 \pm 5.24 | 32.05 \pm 4.01 | 55.92 \pm 14.22* | 75.639 \pm 9.19* [Ⓢ] | 52.6 \pm 6.23* | 37.84 \pm 5.9 [Ⓢ] |
| Serum creatinine | 0.766 \pm 0.085 | 0.77 \pm 0.104 | 1.225 \pm 0.14* | 2.11 \pm 0.167* [Ⓢ] | 1.24 \pm 0.29* | 0.97 \pm 0.17 [Ⓢ] |
| Urinary albumin | 2.813 \pm 0.757 | 2.93 \pm 0.782 | 11.96 \pm 2.122* | 19.505 \pm 3.57* [Ⓢ] | 10.29 \pm 0.958* | 5.144 \pm .954 [Ⓢ] |
| Serum testosterone | 1.27 \pm 0.18 | 1.25 \pm 0.07 | 1.116 \pm 0.144 | 0.829 \pm 0.21 [Ⓢ] | 1.17 \pm 0.156 | 1.215 \pm 0.17* |
| Serum cGMP | 15.5 \pm 2.38 | 16.05 \pm 2.63 | 10.8 \pm 1.26 [Ⓢ] | 7.3 \pm 1.14 [Ⓢ] | 11.18 \pm 1.72 [Ⓢ] | 14.09 \pm 2.47 [Ⓢ] |
| Serum SOD | 920.9 \pm 13.83 | 931.4 \pm 17.9 | 782.8 \pm 27.05 [Ⓢ] | 680 \pm 40.8 [Ⓢ] | 816.3 \pm 38.6 [Ⓢ] | 890.4 \pm 57.3 [Ⓢ] |
| Serum GPX | 163.5 \pm 21.5 | 157.6 \pm 23.8 | 110.7 \pm 24.1 [Ⓢ] | 79.9 \pm 11.3 [Ⓢ] | 121.4 \pm 19.8 [Ⓢ] | 148.5 \pm 11.4 [Ⓢ] |

*Increased significantly versus control.

[Ⓢ]Increased significantly versus subgroup IIa.

[Ⓢ]Decreased significantly versus subgroup IIb and IIIa.

[Ⓢ]Decreased significantly versus control.

[Ⓢ]Decreased significantly versus subgroup IIa.

[Ⓢ]Increased significantly versus subgroup IIb.

[Ⓢ]Increased significantly versus subgroup IIb and IIIa.

significant decrease compared to control subgroups was recorded. However, after 8 weeks of diabetes confirmation, it showed the least value and expressed significant decrease compared to the control subgroups and subgroup IIa ($p < 0.05$). On the other hand, in subgroup IIIb, it recorded a significant increase when compared to subgroup IIb ($p < 0.05$) and non-significant decrease compared to the control (Table 1).

Concerning serum cGMP, SOD and GPX levels after 2 weeks of diabetes confirmation, they exhibited significant decrease in subgroup IIa compared to the control ($p < 0.05$). After 8 weeks, subgroup IIb represented a significant decrease in these parameters compared to control and subgroup IIa ($p < 0.05$). While, in subgroup IIIb they showed a significant increase when compared to subgroup IIb and subgroup IIIa ($p < 0.05$) and non-significant decrease compared to control (Table 1).

Histological and immunohistochemical results

H&E results: Sections from control group (group I) showed normal histological architecture of the kidney cortex (Figure 1a). However, features of renal glomerular and tubular lesions were shown in sections from subgroup IIa and IIIa (Figures 1b and 1c).

After 8 weeks of diabetes confirmation, sections from subgroup IIb showed progressive morphological changes than that of subgroup IIa (Figure 2a). Although, sections from Sildenafil-treated subgroup IIIb revealed almost normal histological architecture of the renal cortex (Figure 2b).

PAS stained results

Sections of the renal cortex of the control group showed normal histological structure with normal PAS reactivity (Figure 3a). While renal cortical sections of subgroups IIa, IIIa and IIb revealed atypical PAS reaction in the tubular brush borders and basal laminae (Figures 3b, 3c and 4a). This atypical reaction returned back to almost normal one in subgroup IIIb (Figure 4b).

Immunohistochemical results

Renal cortical sections of the control group revealed minor localized desmin immunoreactivity (Figures 5a and 5b). This immunoreaction became abundant in sections of subgroups IIa, IIIa and IIb (Figures

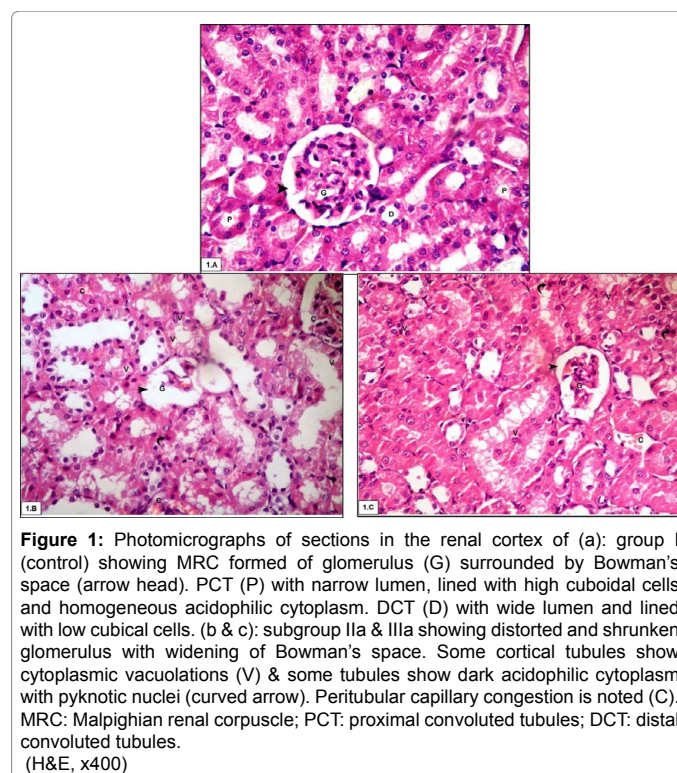


Figure 1: Photomicrographs of sections in the renal cortex of (a): group I (control) showing MRC formed of glomerulus (G) surrounded by Bowman's space (arrow head). PCT (P) with narrow lumen, lined with high cuboidal cells and homogeneous acidophilic cytoplasm. DCT (D) with wide lumen and lined with low cubical cells. (b & c): subgroup IIa & IIIa showing distorted and shrunken glomerulus with widening of Bowman's space. Some cortical tubules show cytoplasmic vacuolations (V) & some tubules show dark acidophilic cytoplasm with pyknotic nuclei (curved arrow). Peritubular capillary congestion is noted (C). MRC: Malpighian renal corpuscle; PCT: proximal convoluted tubules; DCT: distal convoluted tubules. (H&E, x400)

6a, 6b, 6c, 7a, 7b & 7c). On the other hand, the immunoreaction was apparently reduced in subgroup IIIb (Figures 6d & 7d).

Morphometric results

Mean numbers of affected renal corpuscles, mean optical density of PAS-positive material of parietal layer of Bowman's capsules and mean area percent of positive desmin immunoreaction showed a significant increase in subgroups IIa and IIIa compared to control ($p < 0.05$). While, after 8 weeks (subgroup IIb), they represented a significant increase compared to control and subgroup IIa ($p < 0.05$). In subgroup IIIb, there was significant decrease in these three parameters when compared to subgroups IIb and IIIa ($p < 0.05$) (Table 2).

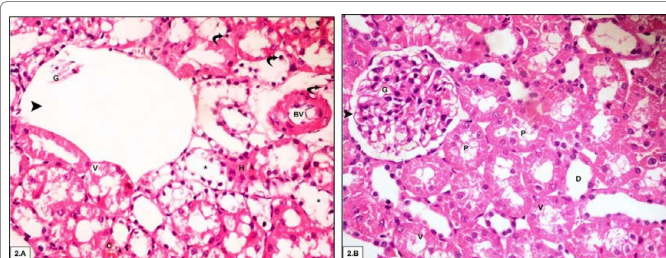


Figure 2: Photomicrographs of sections in the renal cortex of (a): subgroup IIb showing MRCs with distorted and almost complete loss of glomerular capillaries (G) with widening of Bowman's space (arrow head). Cortical tubules show loss of normal architecture (*), marked cytoplasmic vacuolations (V), dark acidophilic cytoplasm with pyknotic nuclei (curved arrows). Acidophilic hyaline material (H) in-between tubules is seen. Peritubular capillary congestion (C) & blood vessel (BV) with thickened walls and narrow lumen are noted. (b): subgroup IIIb showing apparently normal MRCs formed of glomerulus surrounded by Bowman's space, normal PCT (P) & DCT (D) except for the presence of few areas of cytoplasmic vacuolations. (H&E, x400)

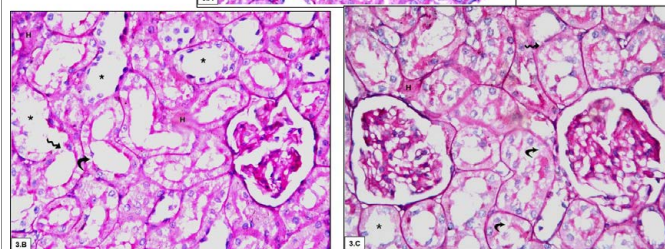
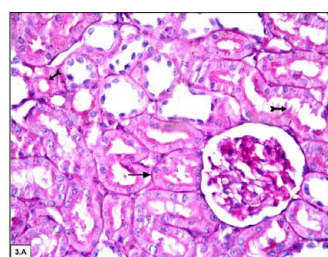


Figure 3: Photomicrographs of sections in the renal cortex of (a): group I (control) showing multiple cortical tubules with preserved brush borders (bifid arrows) and basal laminae (arrow). (b & c): subgroups IIa & IIIa showing partial (curved arrows) or complete loss (*) of the brush borders of most of the tubules. Interrupted basal laminae around cortical tubules are seen (wavy arrow). Note hyaline material (H) in between tubules. (PAS, x400)

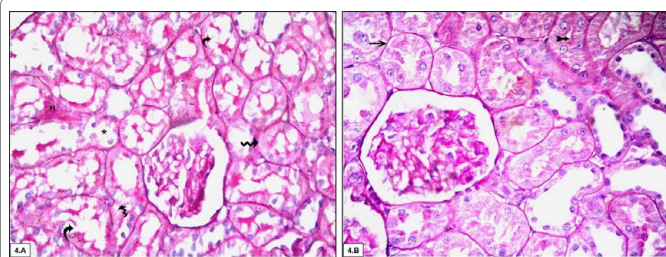


Figure 4: Photomicrographs of sections in the renal cortex of (a): subgroup IIb showing many tubules with partial (curved arrows) or complete loss (*) of the brush borders & Interrupted basal laminae (wavy arrows). Note hyaline material (H) in between tubules. (b): subgroup IIIb showing multiple cortical tubules with almost preserved brush borders (bifid arrow). Preserved basal laminae are seen around most of cortical tubules (arrow). (PAS, x400)

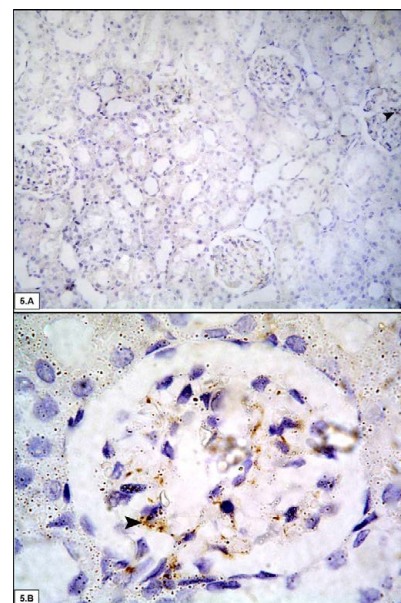


Figure 5: Photomicrographs of sections in the renal cortex of the control group (I), show minimal glomerular positive immunoreaction in the podocytes' cytoplasm and processes (arrowhead). (Immunohistochemical stain for desmin) (5.a desmin x 200) & (5.b desmin x 1000)

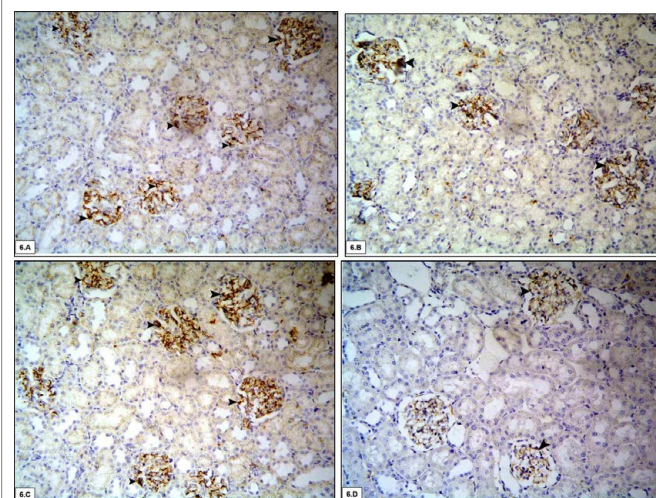


Figure 6: Photomicrographs of sections in the renal cortex from (a) subgroup IIa, (b) subgroup IIIa, (c) subgroup IIb & (d) subgroup IIIb. (a), (b) & (c) show widespread positive desmin immunoreaction in podocytes (arrowheads). However, photomicrograph (d) shows apparent diminution in the positive desmin immuno-reaction in the podocytes. (Immunohistochemical stain for desmin x 200)

Mean numbers of affected renal corpuscles in subgroup IIIb showed significant increase versus control. However, mean optical density of PAS-positive material of parietal layer of Bowman's capsules and mean area percent of positive desmin immunoreaction recorded non-significant increase compared to the control group. Regarding the mean area percent of PAS positive material a significant decrease in all the experimental subgroups compared to control ($p < 0.05$) was observed. After 8 weeks, subgroup IIb (diabetic group) represented a significant decrease compared to subgroup IIa ($p < 0.05$). Subgroup IIIb

Table 2: Mean \pm SD of morphometric parameters in all studied group.

| Parameters | Group I | Group II | | Group III | |
|---|-------------------|--------------------------------|--------------------------------|--------------------------------|------------------------------------|
| | | Subgroup IIa | Subgroup IIb | Subgroup IIIa | Subgroup IIIb |
| No of affected corpuscles | 3.8 \pm 0.63 | 15.4 \pm 2.22 [*] | 24.6 \pm 4.427 [°] | 14.7 \pm 2.668 [*] | 8.1 \pm 0.93 [°] |
| Optical density of PAS +ve material of parietal layer | 97.59 \pm 18.58 | 136.57 \pm 14.9 [*] | 179.3 \pm 15.8 [°] | 130.57 \pm 11.2 [*] | 102.7 \pm 33.98 [°] |
| Area % of desmin | 0.204 \pm 0.03 | 4.123 \pm 0.837 [*] | 6.73 \pm 1.07 [°] | 3.0 \pm 0.47 [*] | 0.696 \pm 0.04 [°] |
| Area % of PAS +ve material | 39.18 \pm 2.016 | 19.129 \pm 2.35 ^β | 16.18 \pm 3.51 ^{β°} | 20.079 \pm 2.15 ^β | 28.9 \pm 2.176 ^{β&} |

*Increased significantly (p<0.05) versus control.

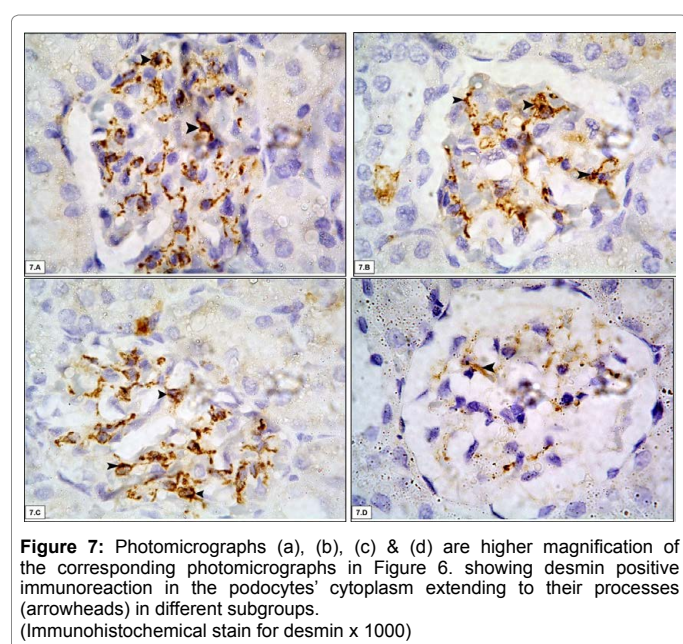
[°]Increased significantly versus subgroup IIa.

[°]Decreased significantly versus subgroups IIb and IIIa.

^βDecreased significantly versus control.

[°]Decreased significantly versus subgroup IIa.

[&]Increased significantly versus subgroups IIb & IIIa.



showed statistically significant increase compared to subgroups IIb and IIIa (p<0.05) (Table 2).

Discussion

One of the major complications and causes of mortalities associated with Diabetes Mellitus (DM) is diabetic nephropathy [16].

DM is considered as one of the major risk factors for ED where it is associated with an increase in superoxide formation in the vasculature that decreases the bioavailability of NO. This is followed by impairment of NO-dependent smooth muscle relaxation and therefore the normal erectile response [17]. In addition, it was reported that diabetic male rats shows decreased fertility with low serum testosterone level [4].

In the present study, Alloxan was chosen to induce diabetes in Wistar rats as it could induce renal histological and functional changes similar to those occurring in human diabetic nephropathy [18,19].

Diabetes was induced in rats of group II and III of the current work (blood glucose level >250 mg/dl) 3 days after Alloxan administration. This could be explained as Alloxan produces oxygen free radicals that destroys pancreatic β -cells and causes severe hypoinsulinaemia and hyperglycemia (type I diabetes) [10].

In the present study, two weeks following confirmation of diabetes (subgroup IIa), it could be assumed that the renal function was deteriorated. This assumption was based on the significant increase in the mean values of serum urea and creatinine when compared to the control group. This increase, indicates the severity of the clinical renal damage and destruction of functioning nephrons associated with DN [3,20]. Additionally, there was significant increase in the mean value of urinary albumin when compared to the control group as previously detected [21]. Such findings could be explained by increased OS and reduced antioxidative ability in diabetes that results into renal tubular injury and leads to gradual loss of renal function [21]. Further explanation came from what was stated in another study [7]. They reported that DN is characterized by OS, podocyte damage and glomerulosclerosis that occur due to increased PDE-5 activity associated with diabetes. Increase in PDE-5 level exacerbates NO-cGMP pathway dysfunction by decreasing cGMP level [22]. They clarified that the reduction in NO activity associated with diabetes may be due to defect in NO synthesis or NO quenching through the production of superoxide radicals. This explanation could be supported in the current work by the significant decrease in the mean value of cGMP in diabetic subgroup IIa compared to control group.

Another support to the assumption of renal function deterioration in this study came from the abundant desmin positive immunoreactivity within the cytoplasm and the processes of the podocytes of subgroup IIa. That was furtherly supported by the significant increase in its mean area percent versus the control group. This observation is in line with an earlier study [23], which recorded that desmin glomerular expression is significantly higher in podocytes of untreated diabetic rats, indicating podocytes damage. This finding could be explained based on that podocytes are essential components of the renal glomerular filtration barrier that are injured at the early stages of glomerular damage [24]. Podocytes reacts to injury, including DN, by altered expression of intermediate filaments and strong expression of desmin. So, desmin could be considered as a sensitive marker for their injury [25-28].

Further enforcement for the deterioration of the renal functions was achieved by the renal histological appearance in subgroup IIa. The renal glomeruli were distorted and shrunken. This finding was supported by significant increase in mean number of affected renal corpuscles when compared to control. This is concomitant with what was reported in a previous study [29]. It could be explained by the disturbance in glucose metabolism and generation of reactive oxygen species (ROS) accompanying persistent and chronic hyperglycemia in DM. The ROS triggers oxidative stress (OS), enhances lipid peroxidation, DNA damage and protein degradation and exhausts the antioxidative defense systems [30,31]. This explanation was enforced

by the significant decrease in the serum level of the antioxidants (SOD and GPX) versus the control group.

In addition, some cortical tubular cells showed cytoplasmic vacuolations and some showed dark acidophilic cytoplasm with pyknotic nuclei. The cytoplasmic vacuolations which was also detected in another study [15], could be attributed to increase fluid uptake as a result of altered permeability of the cell membrane [29,32]. The cell membrane damage could be referred to OS as reported formerly [29]. Another explanation was provided by an earlier study [33] that referred cytoplasmic vacuolations to lactate accumulation in the tubules of the kidney. This results in increased osmotic pressure with subsequent water influx. However, appearance of cells with increased acidophillia and pyknotic nuclei could be due to loss of cytoplasmic RNA (which binds the blue dye; Hematoxylin) and increased binding of denatured cytoplasmic proteins to Eosin [34].

Furthermore, the PAS-stained sections of subgroup IIa in the current study revealed partial or complete loss of the brush borders in most of the tubules with interruption of the basal laminae of some parts of the renal tubules. This finding was supported by significant decrease in mean area percent of PAS positive material in this subgroup compared to the control group. These results are concomitant with the formerly reported marked reduction in PAS positive materials in the brush borders and the basement membranes of some tubules in the Alloxanated diabetic rats. This finding could be attributed to diminution of glycogen content by the damaging effect of diabetes on the cytoplasmic organelles and the associated enzymes responsible for glycogen metabolism [29]. An earlier study [35] postulated the decrease in mucopolysaccharide content in the kidneys of Alloxanated diabetic rats to glycogenolysis. Moreover, complete or partial loss of the brush borders of most of the tubules could be explained based on that the brush border membranes (BBM) are prime targets of ROS. These ROS are released during pathogenesis of diabetes. In addition, they may increase the rigidity of the diabetic BBM which is an important finding in pathogenesis of DN [36].

The OS resulting from diabetic hyperglycemia may lead to extracellular matrix (ECM) deposition. This could be explained by that ROS, cytokines and growth factors are synthesized by the inflammatory cells at the site of injury. They do not only exacerbate the renal damage but also lead to stimulation of ECM proteins and poorly ordered matrix deposition. This results into consequent basement membrane thickening [3,21], which might explain the hyalinization detected in-between cortical tubules in subgroup IIa. In addition, it could support the significant increase in the mean optical density of PAS positive material of the parietal layer of Bowman's capsules in this subgroup compared to control group.

Peritubular congestion detected in subgroup IIa of the current study might be a consequence of hyperglycemia-activated protein kinase-C in the endothelium that increases the production of adhesion molecules. These adhesive molecules bring more leukocytes in the blood vessels creating more congestion and disruption of the endothelial functions [37].

In the current work, 8 weeks following confirmation of diabetes (subgroup IIb), there was aggravation of the histological lesions of the MRCs and the cortical tubules. It could be supported by the significant increase in the mean number of the affected MRCs and mean density of PAS positive material in the parietal layer in this subgroup compared to subgroup IIa. Additionally, there was significant decrease in the mean

area percent of PAS immune-positive materials in subgroup IIb versus subgroup IIa. This exacerbation of the renal histological lesions could be assumed to be due to intensification of diabetic OS that was supported by the significant decrease in the mean values of antioxidants (SOD and GPX) in this subgroup versus subgroup IIa.

The aggravation of the renal histological lesions could explain the worsening of the renal function detected in subgroup IIb. As there was significant increase in the mean values of serum urea and creatinine and urinary albumin and significant decrease in the mean value of cGMP, in subgroup IIb versus subgroup IIa. In addition, there was more abundant desmin positive immunoreactivity within the cytoplasm and processes of the podocytes of subgroup IIb and significant increase in its mean area percent compared to subgroup IIa.

The cortical blood vessels of subgroup IIb appeared with thickened walls and narrow lumens. This finding is similar to that stated previously where it could be referred to increased angiotensin II due to diabetes. The increase of angiotensin II stimulates proliferation of smooth muscle and mesangial cells. They added that this vascular hypertrophy is a progressive complication that may lead to hypertension and nephropathy [19].

In subgroup IIb of the present study, the testosterone level exhibited significant decrease when compared to the control and subgroup IIa. This marked reduction of serum testosterone level could be explained as diabetes induces OS in testicular tissue and inhibits androgenesis by Leydig cells [38]. OS restricts transfer of cholesterol to mitochondria and reduces the expression of steroidogenic acute regulatory protein in Leydig cells [39].

Examination of cortical sections from Sildenafil-treated rats, 2 weeks after confirmation of diabetes (subgroup IIIa), revealed histological changes similar to that of subgroup IIa. There were some shrunken glomeruli, vacuolations of cytoplasm of some tubular cells and dark acidophilic cytoplasm with pyknotic nuclei in the others. Many tubules showed interrupted basal laminae and either partial or complete loss of the brush borders. In addition, there were hyaline material in between the tubules and peritubular capillary congestion. This similarity was enforced by the non-significant decrease in the mean number of the affected MRCs and the mean density of PAS positive materials in the parietal layer of this subgroup versus subgroup IIa. Additionally, there was non-significant increase in the mean area present of the PAS positive materials when compared to subgroup IIa.

Such similarity between the histological changes of subgroup IIa and IIIa could support the absence of functional improvement of the kidneys detected by the non-significant decrease in the mean values of serum urea and creatinine and urinary albumin in subgroup IIIa versus subgroup IIa.

This absence of the renal functional improvement could be enforced by the abundant desmin positive immunoreaction and the non-significant decrease in its mean area present in subgroup IIIa versus subgroup IIa.

This non-improvement state of subgroup IIIa compared to subgroup IIa might be explained by the progressive effect of the OS of diabetes that was supported by the non-significant increase in the mean values of antioxidants when compared to subgroup IIa. Another explanation could be the longer time that might be needed for Sildenafil to compensate for the renal injury.

Sildenafil-treated subgroup IIb of the current study, 8 weeks

after diabetes confirmation, demonstrated almost normal histological architecture of renal corpuscles and most of the tubules. This finding was enforced by the significant decrease in the mean number of the affected renal corpuscles when compared to subgroup IIb. It could be explained by the antioxidant properties of Sildenafil [9,40]. This explanation was supported in the current study by the significant increase in the mean values of the antioxidants (SOD and GPX) in subgroup IIIb versus subgroup IIb. In addition, they showed non-significant decrease compared to control group. The OS improvement effect of Sildenafil was suggested to occur through increase in cGMP that in turn inhibits NADPH oxidase expression and activity, thereby reducing superoxide and H₂O₂ production [31,41]. This suggestion was confirmed in the current study by significant increase in cGMP in Sildenafil-treated subgroup IIIb versus diabetic subgroup IIb and its non-significant decrease versus control group. Sildenafil could elevate cGMP through two alternative ways. The first way occurs through inhibiting PDE-5 enzyme that degrades cGMP. The second one depends on the ability of Sildenafil to induce the expression of mRNA of endothelial nitric oxide synthase (eNOS). This, in turn, increases NO; the main activator of soluble guanylyl cyclase enzyme that synthesizes cGMP [42].

Additionally, there was preservation of the brush borders and basal laminae of most of the cortical tubules in subgroup IIIb in the present work. This was confirmed by the significant increase in the mean area percent of PAS positive material when compared to subgroup IIb. The increase of glycogen deposition in basement membranes and brush borders of renal tubules is a sign of glycogenesis [29]. Moreover, subgroup IIIb recorded significant decrease in the mean optical density of PAS positive material of parietal layer of Bowman's capsules when compared to subgroup IIb. This finding could be explained by the ability of Sildenafil to affect the balance between Matrix metalloproteinases (MMPs) and their inhibitors by inhibiting the activity of the kinases, including mitogen-activated protein kinases (MAPK) and MAPK kinase (MAPKK). Thus, Sildenafil has the potentiality to prevent the action of mitogenic stimuli that could activate glomerular or tubulointerstitial cell proliferation [6].

In subgroup IIIb of the current study, Sildenafil could restore the renal function detected by the significant decrease in the mean values of serum urea and creatinine versus subgroup IIb and its non-significant increase versus the control group. Furthermore, the mean value of urinary albumin in the same subgroup revealed significant decrease compared to subgroup IIb and non-significant increase versus control group, as reported in a prior study [3]. This might be based on the ability of Sildenafil to increase NO/cGMP activity that results into renal vasodilation. Thus, Sildenafil may restore glomerular filtration [43].

Further support to the ability of Sildenafil to reestablish the renal function in the current study came from restoration of podocytes and blood renal barrier functions. That was spotted through diminished desmin positive immunoreactivity in podocytes' cytoplasm. Moreover, there was significant decrease in the mean area percent of desmin positive reaction in subgroup IIIb compared to subgroup IIb and its non-significant increase versus control group. This finding is in line with what was reported previously [7].

Subgroup IIIb of the current study revealed significant increase in the mean testosterone level compared to subgroup IIb and its non-significant decrease versus the control group. The restored testosterone level could be explained by Sildenafil cytoprotective antioxidant effect that has been clearly introduced in an earlier study [44]. Another postulated mechanism by which Sildenafil could

increase steroidogenesis is through its direct effect on Leydig cell steroidogenesis. PDE-5 inhibition is involved in androgen biosynthesis stimulation through activation of NO-cGMP signaling pathway in Leydig cells [45-49].

Noteworthy is that the mean values of blood sugar level in Sildenafil-treated subgroups (IIIa and IIIb) showed significant increase than that of the control group and non-significant decrease versus subgroups (IIa and IIb) respectively. This finding might demonstrate that Sildenafil couldn't improve diabetes type I. This assumption was also stated in a previous study which reported that Sildenafil has no influence on either streptozotocin-induced diabetes type I or non-insulin dependent diabetes type II, in rats [3]. However, in an earlier study Sildenafil is found to be able to reduce hyperglycemia and insulin resistance in female rats with diabetes type II [50].

In the present study, it could be concluded that diabetes type I resulted in pronounced oxidative stress that affected renal function and structure and reduced testosterone level. Sildenafil administration for 8 weeks proved to have a potential renal protective capacity at both morphological and functional levels. This effect might be related to the antioxidant properties, radical scavenging effect and selective inhibition of PDE-5 resulting in intra-cellular cGMP accumulation. That in turn, resulted in elevation of NO level which reduced oxidative injury in diabetes. Thus, Sildenafil might have a potential novel in restoration of kidney function and structure in diabetic male patient especially those with low testosterone level.

Accordingly, it is recommended to use Sildenafil in patients with ED, especially those with diabetic nephropathy and low testosterone level.

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