

Acacia senegal Inhibits Diabetic Nephropathy Ultrastructure Alteration and Renal Biomarkers Changes in Rats

Acacia senegal Inhibe la Alteración de la Ultraestructura de la Nefropatía Diabética y los Cambios en los Biomarcadores Renales en Rata

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SUMMARY: Diabetic nephropathy (DN) is the most common complication of diabetes. Several studies have been done in a trial to protect against this problem at the ultrastructure level. This study investigates the protective effect of oral administration of *Acacia senegal* (AS) against the development of DN. Sixty male albino rats were randomly divided into six groups: control, *Acacia senegal* control, Diabetic untreated, diabetic insulin-treated, Diabetic AS treated, and Diabetic insulin and AS combined treated groups. Plasma glucose, HbA1c, serum Albumin, creatinine, urine creatinine was measured using specific kits. Determinations of creatinine clearance and blood pressure were done. The renal tissues of both kidneys were prepared to investigate under both light (LM) and electron microscope (EM). Ultrastructure examination of renal rats tissue of diabetic untreated rats showed the destruction of the glomerular basement membrane and endothelial cells together with hemorrhage in glomerular capsules (Bowman's capsules). On the other side, both LM and EM revealed improving the endothelial cells and the other glomerular capsules structures, especially with the combined treated group, which confirmed the improvement of the biochemical investigation in the study. In conclusion, from the present study, using the oral AS together with SC insulin could be protected against the development of DN.

KEY WORDS: Diabetic nephropathy; *Acacia senegal*; Gum Arabic; Ultrastructure electron microscope.

INTRODUCTION

Diabetic nephropathy is a progressive chronic kidney function deterioration occurring in patients, particularly with uncontrolled diabetes mellitus. It is considered the leading cause of chronic kidney disease (CKD) and end-stage renal disease (ESRD) globally, with more projection in developed countries. (Zimmet, 2001). At least 25 % of patients with type I and 30 % with type II DM eventually develop diabetic nephropathy (DN) that may progress to end-stage renal disease (ESRD) (Kruzel-Davila *et al.*, 2016). The problem of DN becomes more apparent, especially in type I DM, as it manifests early in life, so ESRD may develop earlier, producing an additional burden for patients and health services (Pálsson & Patel, 2014). They claimed that micro- and macro-vascular complications are the primary pathophysiological core for diabetic nephropathy. Hyperglycemia stimulates the extra-generation of

inflammatory biomarkers, and reactive oxygen species (ROS) represents a common mechanism underlying the vascular injury observed in diabetes. Elevated levels of vascular cell adhesion molecules such as soluble vascular cell adhesion protein 1, soluble intercellular adhesion molecule-1, E-selectin, and P-selectin represent a common mechanism underlying vascular injury via increasing the interaction between the leukocytes, platelets, and endothelium that help the trans-endothelial migration of leukocytes. Diabetes mellitus induces these vascular cell adhesion molecules, which are recognized biomarkers of diabetes-induced vascular injury. In addition, dyslipidemia is regarded as a risk factor for vascular damage and the progression of hypertension.

Damage of the glomeruli is usually accompanied by

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progressive hypoproteinemia with subsequent generalized edema in diabetes. The progressive reduction of the glomerular filtration rate (GFR) could lead to end-stage renal disease after several years from the development of diabetes (An *et al.*, 2015).

Pathophysiology of diabetic nephropathy begins with different degrees of disturbances in the functions of the nephrons. Initially, as diabetes is a macrovascular and microvascular disorder, it causes vasoconstriction in the vascular entity of the glomeruli followed by severe damage of the glomeruli, renal tubules, and other renal vasculature. The status of diabetic nephropathy may be monitored by measuring two values: the amount of protein in the urine (proteinuria) and serum creatinine. The amount of proteinuria reflects the degree of damage to any still-functioning glomeruli. The serum creatinine value can be used to calculate GFR, which reflects the percentage of the glomeruli that are no longer filtering the blood (An *et al.*, 2015).

Gum Arabic (AS) is a polysaccharide water-soluble dietary fiber obtained from the dried gummy exudates from the stems and branches of *Acacia senegal* (Ahmed, 2020). It is considered one of the US Foods and Drug Administration (Smolinske, 1992). In Middle Eastern countries, AS is widely used to treat patients with chronic kidney disease and end-stage renal disease. It was found to increase fecal nitrogen excretion to decrease the production of free oxygen radicals (An *et al.*, 2015). It modestly counteracts renal injury following acute gentamicin nephrotoxicity in rats (Ali *et al.*, 2003). In healthy mice, AS treatment has been shown to increase creatinine clearance and renal ADH excretion, and intestinal and renal excretion of Mg^{2+} and Ca^{2+} . It has also been shown to lower plasma concentration of 1,25 (OH)₂ D₃ and used for its overdose toxicity (Nasir *et al.*, 2012). Several studies have shown the favorable effects of AS on blood glucose concentration and renal function. The studies reported AS has multiple antioxidants, anti-lipidemic, and anti-inflammatory effects. AS has been shown to decrease blood pressure, to decrease plasma cholesterol concentrations in rats, and to display antimicrobial activity and has been shown to reduce blood pressure (Nasir *et al.*, 2012). It decreases plasma cholesterol concentrations in rats and displays highly intestinal antimicrobial activity.

Although several studies had been done almost over the decades in a trial to save the kidneys from developed nephropathy in diabetes, none of them was satisfactory and demonstrated adequate protection of the kidneys from diabetic renal insults. So, this study aimed to evaluate the effects of highly purified AS powder to alleviate the deteriorating impact of diabetes on the renal tissue at the level of the electron microscopic ultrastructure and light

microscopic pathological changes in a rat model of diabetic nephropathy. Our target is a trial to get a natural product for helping in the control of diabetes mellitus and preventing the development of its renal complications.

MATERIAL AND METHOD

Animals and experimental design. This study followed a randomized-controlled animal experimental design. It is a prospective study and was conducted following the ethical guidelines of the Helsinki Declaration update on 64th WMA General Assembly, Fortaleza, Brazil, October 2013 for investigations of laboratory animals. Also, it was approved by the ethical committee approval (No. REC # 2013-02-07) of the College of Medicine, King Khalid University, Abha, Saudi Arabia. Sixty male Sprague-Dawley rats were used in this study and were gained from the animal house of King Khalid University; weighed 150 and 200 g and were fed on a standard rat chow with free water access: 12:12-h light/dark cycle. The animals were randomly divided into the following groups (n=10 each); Control non-treated group (C) where rats were injected intraperitoneally (i.p.) once with citrate buffer only (0.1 M, pH 4.5), control AS treated (CA) group, where rats were injected i.p. with the buffer as C group and received AS in a dose 2020 g/kg body weight freshly prepared in 1 ml of distilled water daily through an oral gavage Diabetic group (D1): DM was induced in rats by a single i.p. injection of streptozotocin (STZ) in a dose of 65 mg/kg (El Karib *et al.*, 2016); diabetic + insulin (DI) group, where DM was induced in rats as D group and was received mixtard insulin Humulin 70/30 (70 % human insulin isophane suspension and 30 % human soluble insulin injection [rDNA origin]). In a dose of 0.75 IU/100g body weight in 0.75 ml volume subcutaneously (SC) once daily starting 48 h after induction of diabetes (El Karib *et al.*, 2016); diabetic + AS (DA) group where DM was induced in rats as DM group and was received the same dose of AS as in CA group starting 48 h after induction of diabetes (An *et al.*, 2015) (R 9); diabetic + insulin + AS (DIA) group, where DM was induced in rats as DM group and was received both insulin and AS with the same doses and time as in groups DI and DA, respectively. The groups C, CA, D, and DA daily received SC injection of 0.75 mL saline while C, D, and DI groups daily received 1 mL of water by oral gavage. DM was verified on the 5th day after STZ injection by measuring blood glucose by rat tail blood sampling. Those rats were with the non-fasting blood glucose level of ≥ 20 mmol/L were considered diabetic (Kedziora-Kornatowska *et al.*, 1998). Although the failure rate for the development of diabetes was expected to be 17 % and the death rate was 30 %, dead and failed rats were excluded from our study

groups from the start. All diabetic rats have received a single dose of 20 ml of glucose 5 % through oral gavage immediately after induction of diabetes to prevent severe hypoglycemia that may occur.

Collection of urine: At the end of the experiment (the end of 12th week); 24 hrs urine was collected for each rat which was kept in a metabolic cage for 24 hours. The urine was collected in a container containing 0.1 N HCL to prevent bacterial growth. The collected samples were used for the estimation of urinary creatinine concentration and urinary proteins over 24 hrs.

Measurement of blood pressure. The systolic blood pressure measurement was done 48hrs after induction of diabetes in a fixed day weekly and at the end of the experimental protocol (12 weeks) in conscious rats using tail-cuff plethysmography pre-warmed for 10 min in a thermostatically controlled restrainer (XBP1000; Kent Scientific, Torrington, CT). The mean of at least three separate recordings on three occasions was obtained.

Blood and tissue samples. At the end of the 12th week, after measuring the blood pressure, retro-orbital blood samples (3 ml each) were obtained under anesthesia with sodium thiopental (Bio-chem, Austria; 40 mg/kg i.p.) after overnight fasting [39]. Samples were divided into two parts; the first part was used to prepare the serum was separated and used for the determination of albumin and creatinine levels. The other part of the blood was added to ethylenediamine-tetra-acetate acid (EDTA) and centrifuged at 1,000 rpm for 15 min. Plasma was separated and stored at -80 °C for assay of the total plasma glucose and HbA1c. Then, the animals were killed by decapitation. Both kidneys were dissected out, cut into small pieces to prepare light (Right kidneys) and electron microscopic examination (left kidneys).

Determination of plasma glucose and HbA1c % levels. Plasma glucose was determined using the glucose oxidase method (Roche) (Trinder, 1969). HbA1c % was determined according to the chemical separation and colorimetric method based on carbohydrates' phenol sulfuric acid reaction (Nayak & Pattabiraman, 1981).

Serum and urine creatinine, serum creatinine and Albumin (mg/dl) level\|s and Creatinine Clearance calculation (ml/min). Jaffe's procedure determined serum and urine creatinine levels, where creatinine reacts with picrate ion in an alkaline medium to yield an orange red creatinine picrate complex. The rate of complex formation is measured spectrophotometrically at 490-510 nm and is proportional to the creatinine concentration (Spencer, 1986).

Serum albumin was measured by electro-immune diffusion using anti-rat albumin antiserum (Nordic). Creatinine clearance was calculated using the specific equation.

Total Urine Proteins (mg/24 h): This method is based on the binding of Coomassie brilliant blue to protein (Sedmak & Grossberg, 1977). The protonated form of Coomassie blue is a pale orange-red color, whereas the unprotonated form is blue. When proteins bind to Coomassie blue in acid solution, their positive charges suppress the protonation, resulting in blue color. The binding dye to the protein causes a shift in the absorption maximum of the dye from 465 to 595 nm and it is the increase in absorbance at 595 nm that is monitored.

Histological examination. Right kidneys were excised, the renal cortex was separated, cut into small pieces as the left kidneys but; were immediately fixed in 10 % formal saline for 24 hours. Paraffin blocks were prepared, and 5 mm thick sections were subjected to Hematoxylin and eosin (H&E) stain to elucidate the structural changes by light microscope.

Transmission electron microscopy examination (TEM). As the renal glomeruli are present mainly in the cortex of the nephrons, we chose to evaluate the histopathological and ultrastructure changes as consequences of diabetic nephropathy and the effect of AS on the cortical part of the kidney. After blood sampling, the animals were killed using a lethal dose of thiopental sodium injection (75 mg/kg i.p.), as mentioned above. Left kidneys were excised, the renal cortex was separated, cut into small pieces, and immediately fixed in 2.5 % glutaraldehyde for 24 hours; then washed with phosphate buffer (0.1 M, PH 7.4). Post-fixation was made in 1 % osmium tetroxide buffered to PH 7.4 with 0.1 M phosphate buffer at 4 °C for 1-2 hours. The samples were washed in phosphate buffer to remove excess fixative, dehydrated through ascending grades of ethanol followed by clearing in propylene oxide. The specimens were embedded in Araldite 502 to form gelatin capsules. Polymerization was obtained by placing the capsules at 60 °C. Semi-thin sections (~1 mm thick) were stained with toluidine blue for orientation and observation. Ultra-thin sections (100 nm) were prepared using ultra-microtome and picked up on uncoated copper grids. Following double staining with uranyl acetate and lead citrate, three-to-five random micrographs for each section were examined and photographed using a JEM-1011-JEOL transmission electron microscope, Japan, at 80 kV.

Statistical analysis. Data analysis was performed using SPSS version 22 (IBM) (SPSS Inc., Chicago, IL). Data were expressed as mean \pm SEM. Nonparametric tests were performed to test for significance. Kruskal-Wallis test was used to compare between multiple groups, and the Man-

Whitney U test was applied for the comparison between two groups. The post hoc Scheffe's test was applied to identify the source of statistical significance. P-Values < 0.05 were considered statistically significant.

RESULTS

Rate tail systolic blood pressure changes. No significant changes were observed in all experimental groups of rats when compared with the control groups.

Plasma glucose (mg/dl) and HbA1c. Blood glucose HbA1c levels showed insignificant changes in both control and control + AS groups. On the other hand, the diabetic untreated group showed significant increases in blood glucose and HbA1c levels compared with the control groups (Fig. 1A, B). Insulin treatment to the DM+Ins group significantly reduced both blood glucose HbA1c compared with the DM untreated group. While the diabetic AS treated group showed insignificant changes compared with the DM untreated group, they demonstrated a significantly high level of both Blood glucose HbA1c levels. In the combined treated group,

a significant reduction in both diabetic parameters appeared compared with DM and DM+AS groups (Fig. 1A, B).

Serum Creatinine (mg/dl). At the end of the experimental protocol, serum creatinine levels were significantly higher in DM, DM+Ins, and DM+AS groups than in control levels. On the other hand, the diabetic combined treated group (DM+Ins+AS group) demonstrated a significant reduction in serum creatinine level compared with additional diabetic untreated or diabetic insulin or AS treated groups individually (Fig. 1C).

Creatinine clearance (ml/min). Creatinine clearance (ml/min) was calculated (at the end of the study (week 12). The diabetic combined treated group (DM+Ins+AS) did not show any significant differences in creatinine clearance when compared with both control groups (Fig. 1D). However, DM untreated, DM+Ins, and DM+AS groups had significantly lower creatinine clearance when compared to the control groups (Fig. 1D). Administration of concomitant insulin and AS to diabetic rats in the DM+Ins+AS group resulted in a significant improvement in creatinine clearance when compared to diabetic rats treated with insulin only (DM+Ins group) (P = 0.023) (Fig. 1D).

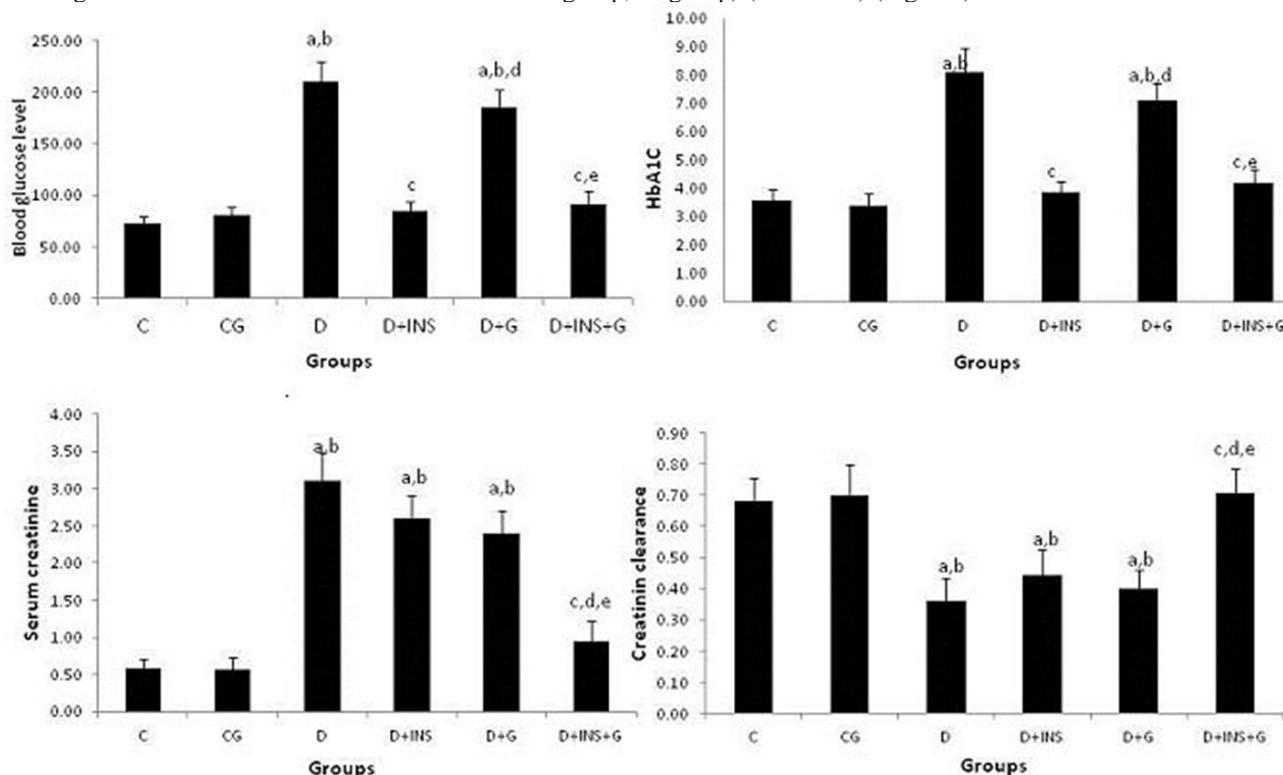


Fig. 1. Blood glucose (mg/dl), HbA1c, serum creatinine (mg/dl), and creatinine clearance in all experimental groups of rats (n=10), week 12. Values are expressed as Mean \pm SE. Values were considered significantly different at P < 0.05. A: significantly different when compared to control group (C). B: significantly different compared to control+AS (C+AS). C: significantly different compared to diabetic non-treated rats (DM). D: significantly different when compared to diabetic rats treated with insulin (DM+Ins). E: significantly different when compared to diabetic rats treated with AS (DM+AS). DM+Ins+AS: diabetic rats treated with concomitant insulin and AS.

24 hours total urine proteins (L/24hrs) and urine creatinine mg/dl.

Twenty-four hours total urine proteins (mg/24hrs) and creatinine mg/dl in the C+AS group showed insignificant changes in total urine proteins and creatinine mg/dl when compared with the control group (P=0.943) (Fig. 2A). While the DM, DM+Ins, and DM+AS groups revealed significantly higher total urine protein and creatinine mg/dl levels than the control group (P = 0.006, P=0.000, and P = 0.001, respectively) (Fig. 2B). Twenty-four hours of urine protein and creatinine mg/dl in DM+Ins+AS was insignificant changed when compared with the control group (P=0.595) but was significantly lowered (P = 0.000) (46.3 %) compared with the DM+Ins group (Fig. 2B).

Light microscope. Histological examination of renal tissue in C or C+AS groups showed normal renal tissue structures (Fig. 3A, B). At the end of the 12th week after induction of diabetes, the diabetic rats' showed thickened basement membrane of the glomeruli along with changes in the density of mesenchyme, atrophy, and complete degeneration of glomerular capillaries with focal hemorrhage and tubular necrosis of both proximal and distal tubular epithelial cells (Fig. 3C). Histological examination of renal tissue in insulin-treated Diabetic group (DM+Ins) revealed partial damage of glomerular capillaries, intact epithelial cells with dilated and some injury to both proximal and distal tubules (Fig. 3D). Treatment with AS alone in the DM+AS group showed hypertrophy and partial damage of glomerular epithelial cells with the destruction of glomerular capillaries and less damaged proximal and distal tubular epithelial cells (Fig. 3E). Maximum improvement was seen in the histological

examination. The concomitant group (DM+I+AS) showed almost the regular structured glomerular capsular epithelium and glomerular capillaries endothelium, proximal and distal tubular epithelium (Fig. 3E).

Electron Microscope. A transmission electron micrograph (TEM) in C or C+AS groups a normal glomerulus showed standard glomerular capsule (Bowman's capsule) structure with normal parietal cell, epithelial cells, basement membranes with foot processes, mesangial and endothelial cells (Fig. 4A). TEM Examination of the glomerulus in diabetic animal group showed glomerular capsule with damaged parietal cells, vacuolar degenerated epithelial cells with irregular nucleus, thin damaged basement membranes with focal fusion foot processes and the glomerular capillaries were opened together with damaged endothelial lining (Fig. 4B). The glomerulus in the diabetic + insulin group examined by the TEM showed glomerular capsule with partial destruction of the parietal cells, degenerated epithelial cells, thickened basement membranes with focal fusion foot processes, damaged organelles in the urinary space, and swollen endothelial cells within glomerular capillaries (Fig. 3C). TEM of a person with diabetes + AS group showed the glomerulus less degenerated epithelial cells, immune deposition in the mesangial matrix, damaged endothelial cells, and thickened basement membranes with focal hump-like deposit. On the other hand, the DM+AS+Ins group examined by TEM revealed almost standard glomerular structure with normal basement membranes, partially healthy foot processes, normal endothelial and epithelial cells with few vacuoles with scanty focal hump-like deposit and swollen endothelial cells (Fig. 3E).

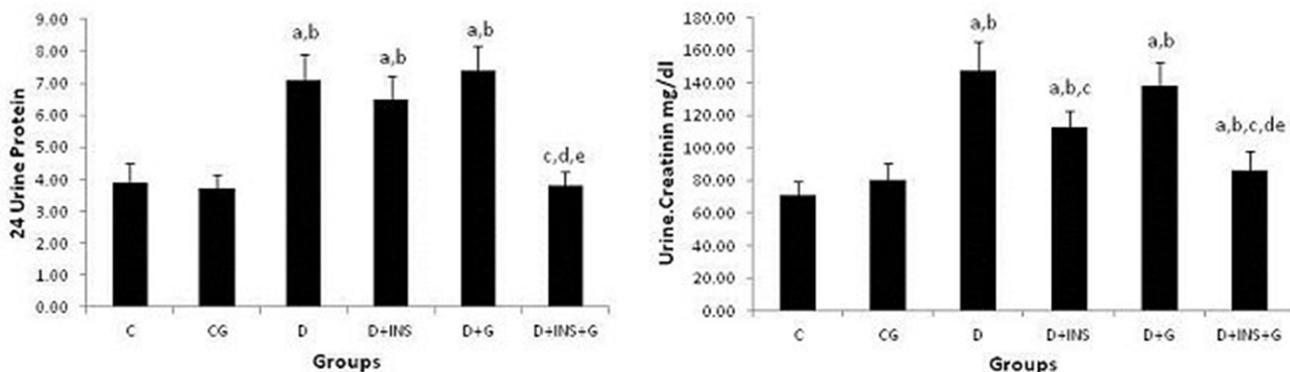


Fig. 2. 24 hrs total urine proteins (L/24hrs) and urine creatinine (mg/dl) concentration in all experimental groups of rats. Values are expressed as Mean ± SE for 10 rats/group. Values were considered significantly different at P< 0.05. A: significantly different when compared to control group(C). B: significantly different compared to control+AS (C+AS), C: significantly different compared to diabetic non-treated rats (DM). D: significantly different when compared to diabetic rats treated with insulin (DM+Ins), E: significantly different when compared to diabetic rats received AS (DM+AS). DM+Ins+AS: diabetic rats received concomitant insulin and AS.

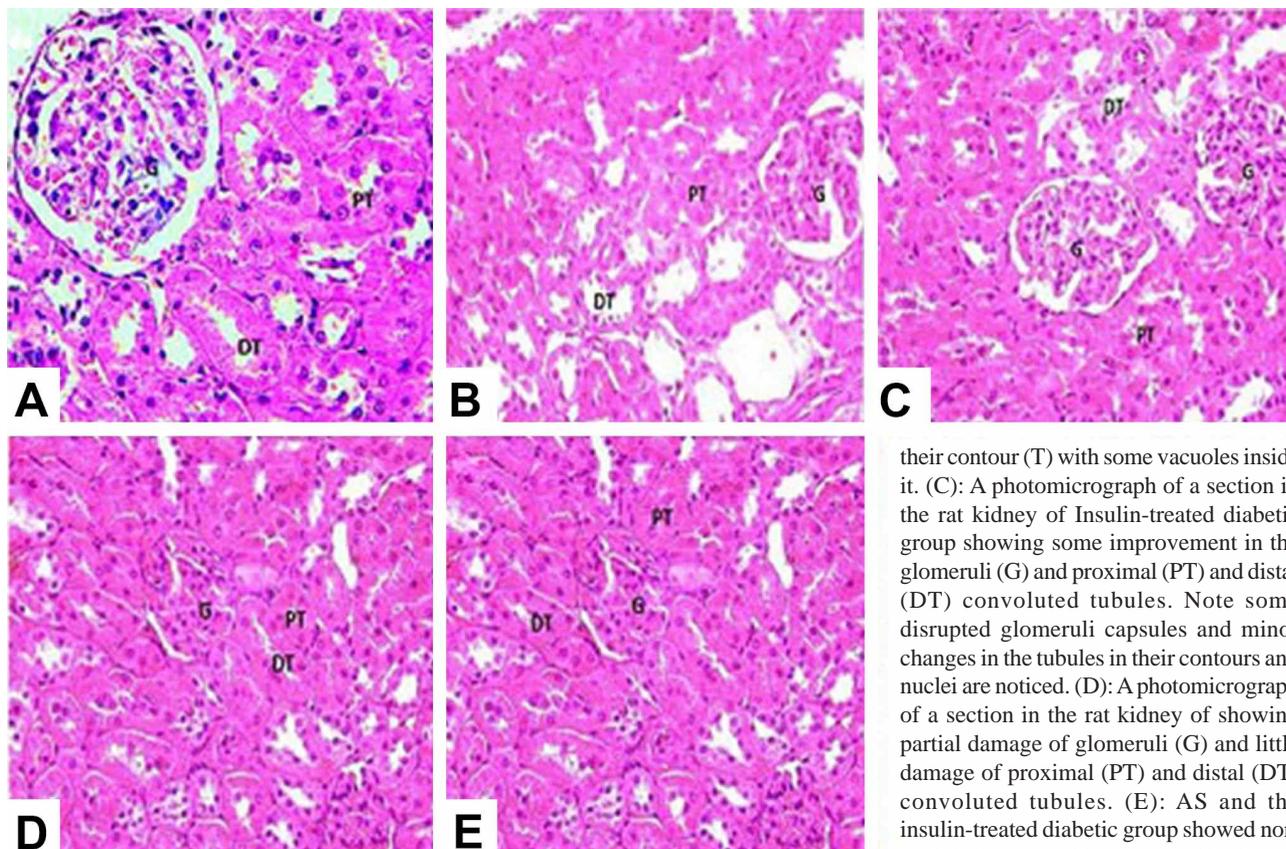


Fig. 3. Hematoxylin and eosin micrographs of the renal glomeruli in the different rats groups. (A): A photomicrograph of a section in the rat kidney of the control group showing normal cellular structure with intact glomeruli and regular tubular contour. There were no signs of necrosis or cellular damage. The renal glomeruli (G) with a tuft of capillaries and normal glomeruli capsules are seen. Notice the contour of the proximal (PT) and distal (DT) convoluted tubules is intact and regular with intact nuclei. (B): The microscopic hematoxylin and eosin histology slides of kidney of the diabetic group showing atrophied glomeruli (G) with widened urinary space and degenerated tubules are seen with disturbed

their contour (T) with some vacuoles inside it. (C): A photomicrograph of a section in the rat kidney of Insulin-treated diabetic group showing some improvement in the glomeruli (G) and proximal (PT) and distal (DT) convoluted tubules. Note some disrupted glomeruli capsules and minor changes in the tubules in their contours and nuclei are noticed. (D): A photomicrograph of a section in the rat kidney of showing partial damage of glomeruli (G) and little damage of proximal (PT) and distal (DT) convoluted tubules. (E): AS and the insulin-treated diabetic group showed normal kidney architecture with intact epithelial cells and tubules. (D): A photomicrograph of a section in the rat kidney of AS and insulin-treated diabetic group showing marked improvement in the glomeruli (G) with its glomeruli capsule and proximal (PT) and distal (DT) convoluted tubules contours and intact nuclei (H&E; X 400).

DISCUSSION

The primary aim of this study was to investigate the potential protective effect of oral administration of *Acacia senegal* in the water against diabetic nephropathy development in rats regarding the ultra-structure electron microscopic and histopathological changes. Approval of the occurrence of nephropathy in diabetic rats was confirmed by the presence of albuminuria accompanied by the reduction of the serum albumin level in this group (Hauser & Josephson, 2013; Afkarian *et al.*, 2016). Also, the elevation of the serum creatinine and its clearance with the reduction in glomerular filtration rate is inconsistent with the development of diabetic nephropathy (Qi *et al.*, 2017). The damage is exerted on all kidney compartments includes the glomerulus, the renal tubules, the renal vasculature, and the

interstitium (Qi *et al.*, 2017). Renal fibrosis is the final common pathway of DN. This fibrosis is a product of multiple mechanisms, including renal hemodynamic changes, abnormal glucose metabolism associated with oxidative stress, inflammatory reaction, and an overactive renin-angiotensin-aldosterone system (Calle & Hotter, 2020). In the present study, the histopathological changes were verified in renal injuries by diabetes. Thickening of the basement membrane of the capillary endothelium was observed together with destruction and hemorrhage. In the glomerular capsule, some nephrons have been seen by LM (Fig. 3A). This is in agreement with the previous studies of Alicic *et al.* (2017) and Lin *et al.* (2018). A transmission electron micrograph (TEM) of non-treated diabetic rats

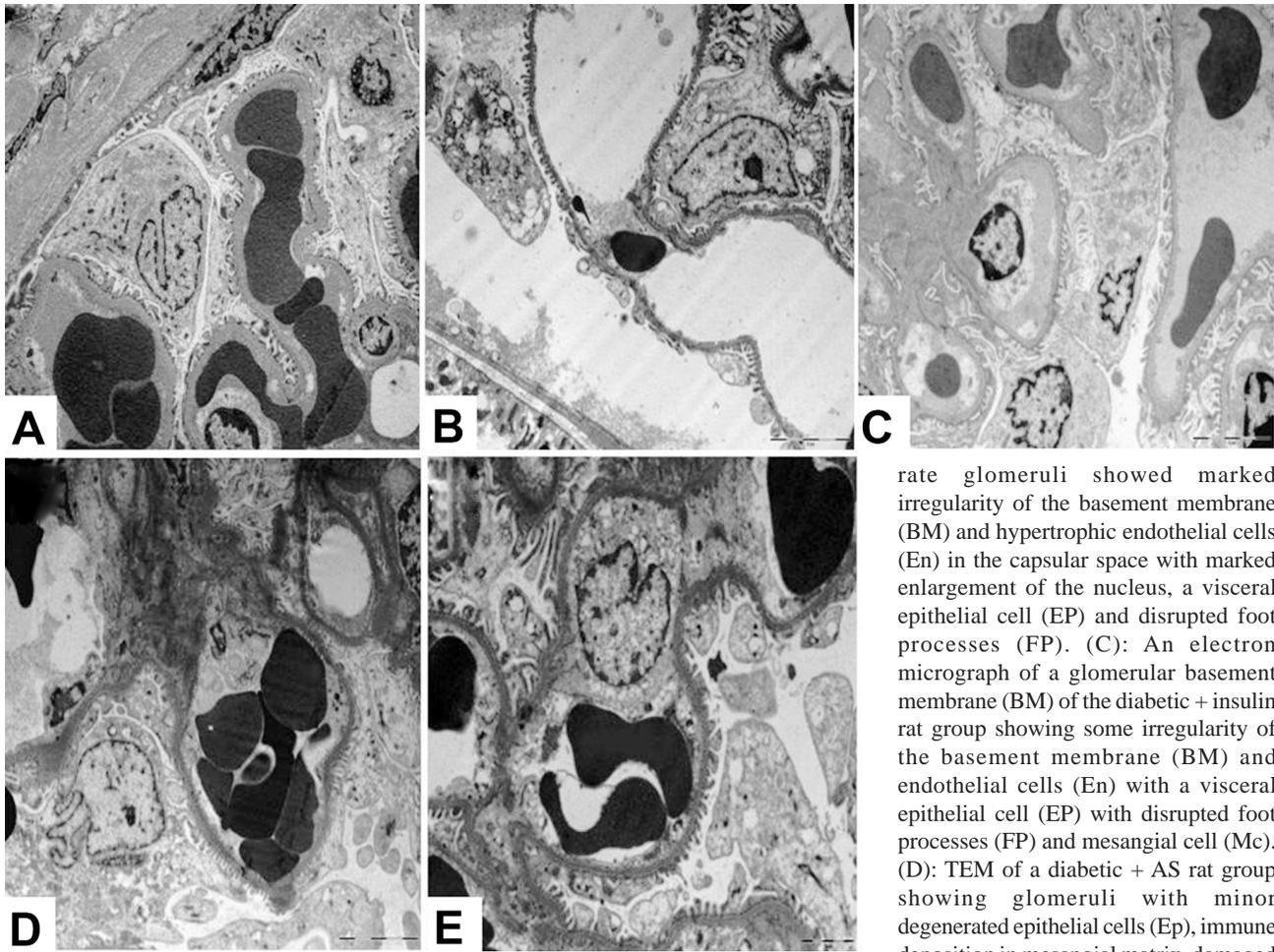


Fig. 4. Electron micrographs of the renal glomeruli in the different rats groups. (A): An electron micrograph of the kidney glomerulus of the control kidney showing its basement membrane (BM), endothelial cell (En), a visceral epithelial cell (EP) with foot processes (FP), and mesangial cell (MC). (B): TEM of the diabetic

rate glomeruli showed marked irregularity of the basement membrane (BM) and hypertrophic endothelial cells (En) in the capsular space with marked enlargement of the nucleus, a visceral epithelial cell (EP) and disrupted foot processes (FP). (C): An electron micrograph of a glomerular basement membrane (BM) of the diabetic + insulin rat group showing some irregularity of the basement membrane (BM) and endothelial cells (En) with a visceral epithelial cell (EP) with disrupted foot processes (FP) and mesangial cell (Mc). (D): TEM of a diabetic + AS rat group showing glomeruli with minor degenerated epithelial cells (Ep), immune deposition in mesangial matrix, damaged endothelial cell (En), and thin basement membranes (BM) with disrupted food processes (FP), and focal hump-like deposit. (E): TEM of a diabetic + AS + insulin rat group in which glomeruli showed normal basement membranes (BM), normal foot processes (FP), normal endothelial cell (Ec) with few vacuoles (V).

showed damaging parietal cells of glomerular capsule with vacuolar degenerated o the epithelial cells, thickened basement membranes with focal fusion foot processes (Fig. 4A). The constriction of the efferent arterioles and dilation of afferent arterioles was observed, resulting in glomerular capillary hyperfiltration that gradually changes to hypofiltration over time (Schlöndorff & Banas, 2009). In diabetic nephropathy, the kidney functions progressively decline, and the glomerular basement membrane (GBM) becomes more permeable and has less filtration efficiency (Wang *et al.*, 2018). An increase in the mesangial cells and the mesangial matrix has been observed, which invaded the glomerular capillaries and produced deposits called Kimmelstiel-Wilson nodules. The mesangial cells and matrix can progressively expand and consume the glomerulus completely, shutting off the filtration not seen in our study.

Either insulin or *Acacia senegal* treatment individually partially improved the chemical biomarkers of nephropathy, including proteinuria, serum albumin, serum creatinine, and its clearance, indicating a limited improvement of GFR. However, histopathological examination by LM confirmed this improvement as the basement membrane of the glomerular capsules integrity was more cleared with minor hemorrhage and hypertrophy. In addition, partial damaging of glomerular capillaries has been seen. The TEM of these individually treated groups showed glomerular capsule with partial, degenerated epithelial cells and small patches of immune deposition in the mesangial matrix. The thickened basement membrane with focal fusion foot processes, less damaged organelles, and still endothelial cells are swollen with bleeding has been observed.

Furthermore, our data that point to the reduction of blood sugar, HbA1c, serum creatinine, and improvement of creatinine clearance by simultaneous treatment with either insulin or *Acacia senegal* are in agreement with previous studies that demonstrated the beneficial effects of *Acacia senegal* in diabetes (Figs. 1, 2, 3 and 4) (Mohammed *et al.*, 2020). This might be accountable for the observed improvement of the renal ultrastructure. Combined treatment with insulin plus *Acacia senegal* significantly improved these ultrastructure changes where LM showed; basement membrane appeared normal, normal foot processes, and endothelial cells. The epithelial cells were seen with few vacuoles, and their nuclei appeared usually. These observations in TEM images supported our hypothesis that concurrent treatment with *Acacia senegal* and insulin improved the structure and the renal function biomarkers in diabetic nephropathy rats (Fig. 4). This agrees with the previous study that demonstrated the beneficial effects of insulin in diabetic nephropathy (Nasir, 2013), which more improved by adding *Acacia senegal* administration (Figs. 1, 2, 3 and 4). The significant reduction in the levels of renal markers upon treatment with *Acacia senegal* together with insulin might account for the observed improvement of the kidney ultrastructure (Figs. 3 and 4).

In summary, our data demonstrate that DM-induced nephropathy ultrastructural alterations associated with hyperglycemia, proteinuria, and hypoalbuminemia were improved by combined treatment with insulin and *Acacia senegal*. Further studies are in need in humans as a potential line adjuvant to use *Acacia senegal* with insulin for renal protection against the development of diabetic nephropathy.

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RESUMEN: La nefropatía diabética (ND) es la complicación más común de la diabetes. Se han realizado varios estudios de ensayo para abordar esta dificultad a nivel de ultraestructura. Este estudio investiga el efecto protector de la administración oral de *Acacia senegal* (AS) contra el desarrollo de la ND. Se dividieron sesenta ratas albinas machos aleatoriamente en seis grupos: control, control de *Acacia senegal*, diabéticos no tratados, diabéticos tratados con insulina, diabéticos tratados con AS y grupos tratados con compuesto de insulina diabética + AS. Se midieron utilizando kits específicos, glucosa plasmática, HbA1c, albúmina sérica, creatinina en sangre y en orina. Se registraron la creatinina y la presión arterial. Los tejidos renales de ambos riñones se prepararon para investigar tanto con microscopio óptico (MO) como electrónico (ME). El examen de la ultraestructura del tejido renal de ratas diabéticas no tratadas mostró la destrucción de la membrana basal glomerular y las células endoteliales junto con hemorragia en las cápsulas glomerulares (cápsulas de Bowman). Por otro lado, tanto MO como ME revelaron una mejora de las células endoteliales y las estructuras capsulares glomerulares, en el grupo tratado con el compuesto, lo que confirmó la mejora de la investigación bioquímica. En conclusión, el uso de AS oral en combinación con insulina podría proteger contra el desarrollo de ND.

PALABRAS CLAVE: Nefropatía diabética; *Acacia senegal*; Goma arábiga; Microscopio electrónico de ultraestructura.

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