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A light microscopic investigation of the renoprotective effects of α-lipoic acid and α-tocopherol in an experimental diabetic rat model

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ABSTRACT

We investigated the effects of α-lipoic acid (AL) and α-tocopherol (AT) on renal histopathology in a streptozotocin (STZ) induced diabetic rat model. Adult male rats were divided into six groups: group 1, saline only; group 2, AL only; group 3, AT only; group 4, STZ only; group 5, STZ + AL; group 6 STZ + AT. Experimental diabetes was induced by STZ. AL and AT were administered for 15 days. Kidney sections were examined using a light microscope after hematoxylin and eosin (H & E), periodic acid-Schiff (PAS) and caspase-3 staining. Histological damage to glomeruli, tubule epithelial cells and basement membrane was observed in group 4. Administration of AT and AL reduced renal injury in the diabetic rats. Group 5 exhibited a greater curative effect on diabetic rats than group 6. AT and AL may be useful for preventing diabetic renal damage.

KEYWORDS

α-lipoic acid; α-tocopherol; diabetes; kidney; rat; streptozotocin

Diabetes mellitus (DM) affects carbohydrate, lipid and protein metabolism and is characterized by high levels of fasting and postprandial plasma glucose. The global prevalence of DM is increasing (Satman 2011). DM has an estimated worldwide prevalence of 387 million in 2014; this number is expected to increase to 592 million by 2035 according to the International Diabetes Federation (Donath 2014). Hyperlipidemia, inflammation and altered antioxidant profiles are common complications of DM (Das et al. 2012).

DM is characterized by hyperglycemia caused by insulin insufficiency (Crawford and Cotran 1999). Various experimental and clinical observations suggest that hyperglycemia increases directly or indirectly the formation of free radicals, which causes oxidative stress. Increased oxidative stress and protein glycosylation play important roles in the development of diabetic complications such as diabetic nephropathy (DN), angiopathy, retinopathy and neuropathy (Agardh et al. 2002; Liptakova et al. 2002).

Streptozotocin (STZ) is used to induce experimental DM. STZ is a narrow spectrum antibiotic with oncolytic, oncogenic and diabetogenic properties (Herr et al. 1960). STZ causes DM by destroying β cells in the pancreas. The degree and duration of hyperglycemia depends on the duration and dose of administration and on the experimental animal involved (Tozzo et al. 1997; Imaeda et al. 2002). A single dose of 50 mg/kg is sufficient to induce DM in dogs, rats and mice (Carney et al. 1979; Büyükdevrim 1989; Kim et al. 2001). The clinical symptoms, and biochemical, morphological and ultrastructural findings for STZ induced DM in experimental animals are similar to those of humans with DM. Therefore, STZ induced DM models are used extensively to study DM and its pathogenesis (Hirose et al. 1982; Fujihara et al. 1992; Gaber et al. 1994).

Characteristics of kidney sections from STZ diabetic rats include thickened glomerulus and tubule basement membranes, increased mesangial matrix cells, podocyte hypertrophy and structural disturbances, hyperplasia of parietal leaf cells, vacuolization and atrophy of tubule cells, and fibrosis (Melin et al. 1997).

STZ produces oxidants that selectively destroy the islets of Langerhans β-cells (Lenzen 2008). High concentrations of reactive oxygen species (ROS) can cause tissue damage directly (Chapple 2006). ROS also play a role in apoptosis (Pourova et al. 2010). STZ causes production of both nitric oxide (NO) and reactive oxygen radicals, OH^- and ROO^- (Ohkuwa et al. 1995). NOS inhibitors and antioxidants reduce the hyperglycemia caused by DM (Tanak et al. 1995; Haluzik et al. 1998; Vardı et al. 2005). Organisms possess enzymatic and nonenzymatic antioxidant defense systems to protect against the effects of ROS. Exogenous antioxidants also can be used to protect against the effects

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of free radicals (Reed et al. 1953; Shay et al. 2009). α-Lipoic acid (AL) is used to treat DM, because it increases consumption of glucose by the cells (Eguíluz Lumbreras et al. 2010). AL and its reduced form, dihydrolipoic acid, reduce oxidative stress by scavenging free radicals in both membranous and aqueous domains (Packer et al. 1995; Packer 1998). AL also may be effective for both preventing and treating oxidative stress under conditions of ischemia-reperfusion injury (Romero et al. 1992; Suzuki et al. 1992), DM (Henriksen et al. 1997; Ziegler et al. 1999), HIV infection (Suzuki et al. 1992; Fuchs et al. 1993) and neurodegenerative diseases (Packer et al. 1997).

α-Tocopherol (AT), a form of vitamin E, is a fat soluble vitamin that can be located inside or outside the cell, or in the cell membrane. AT suppresses or eliminates ROS. AT is an important antioxidant for preserving membrane integrity (Rodrigo et al. 2007). AT supplementation may have beneficial effects on metabolic control of DM due to its antioxidant activity, which affects lipid oxidation, protein glycation and insulin sensitivity. AT exhibits beneficial effects on nuclear factor kappa B (NF-κB) associated inflammatory response, pre-fibrosis markers and oxidative stress in diabetic mice (Shin et al. 2016). AT controls low-density lipoprotein (LDL) oxidation and its cytotoxic effects on rat mesangial cells in vitro (Saborio et al. 1999).

Low AT concentrations are observed in patients with type 2 diabetes mellitus (DM2) and these levels are even lower when complications of the disease occur (Pazdro and Burgess 2010). Hsu et al. (2002) reported a positive effect of AT on oxidation of LDL-c in patients with DM2 who received supplemental 1200 IU AT/day for 3 months. These investigators found low plasma AT concentration together with increased lipid oxidation products in the kidney tissue, which suggest the presence of oxidative stress as observed in experimental models (Gotoda et al. 1995) and in patients with progressive renal diseases (Richard et al. 1991; Rajbala et al. 1997). The use of vitamins can reduce complications such as nephropathy, angiopathy, neuropathy and embryopathy if DM has already developed (Halliwell 2002; Montonen et al. 2004; Saldeen and Saldeen 2005).

Hyperglycemia induced oxidative stress has been implicated in the development and progression of DN (Tan et al. 2007). Increased intracellular glucose stimulates local production of oxygen free radicals, which causes oxidative stress (Forbes et al. 2003; Koo et al. 2002). Other factors known to be increased with DM, such as angiotensin II (Ang II), increase formation of oxygen radicals and cause oxidative tissue damage (Ishii et al. 2001; Privratsky et al. 2003). Intrarenal renin-angiotensin system (RAS) activation and high glucose may act together to increase tubule apoptosis in diabetes independent of systemic hypertension (Brezniceanu et al. 2008). ROS demonstrate the association between renin-angiotensin system (RAS) activation and renal cell apoptosis in DM (Bhatti et al. 2005; Brezniceanu et al. 2008).

ROS induce apoptosis in mesangial cells, tubule cells and podocytes (Brezniceanu et al. 2008; Ha and Lee 2000; Winiarska et al. 2009). Caspases, especially caspases-3 and -9, play important roles in high glucose induced apoptosis of tubule epithelial cells (Allen et al. 2003). Detection of activated caspases in situ is a marker for apoptosis. Caspases belong to the cysteine protease family and are found as inactive precursor proteins in the cells of many mammals. Active caspase-3, which is cleaved in situ, is an executioner caspase that participates in morphological changes characteristic of apoptosis, which can be identified by histological staining (Kang et al. 2001).

We investigated histological changes in kidney tissue in STZ induced diabetic rats and determined the effects of AL and AT treatment using histological and immunohistochemical methods.

Material and methods

Animals

We used 60 200–300 g male Wistar albino rats obtained from the Mersin University Faculty of Medicine Experimental Animal Breeding and Research Center, Turkey. Our study was approved by the local Experimental Animal Ethics Committee (2016/39). The rats were housed in cages at 21 °C and 55–60% humidity with a 12 h light:12 h dark cycle (lights on 08:00–20:00). Cages were cleaned daily and rats had access to standard pellet chow and water *ad libitum.*

Experimental design

The rats were divided randomly into six groups of 10: three control groups (groups 1–3) and three treatment groups (groups 4–6). DM was induced by intraperitoneal (i.p.) injection of a single dose of 40 mg/kg STZ in freshly prepared 0.1 M citrate buffer, pH 4.5. DM groups were injected i.p. once with either 300 µl citrate buffer or streptozotocin solution. After 3 days, blood samples were collected from the tail vein. Blood glucose levels were measured using a glucometer (Accu-Check; Roche, Selangor, Malaysia) and values ≥ 300 mg/dl were considered diabetic.

Rats were assigned randomly to six groups: three control (groups 1–3) and three treatment (groups 4–6). The groups were constituted as follows: group 1, 10 mg/kg/day saline only (i.p); group 2, 100 mg/kg/day AL for 15 days (i.p); group 3, 40 mg/kg/day AT for 15 days (i.p); group 4, 10 mg/kg/day STZ only (i.p); group 5, STZ +

100 mg/kg/day AL for 15 days (i.p); group 6, STZ + 40 mg/kg/day AT for 15 days (i.p).

Histopathology

Kidney tissue samples were fixed in neutral buffered 10% formalin for 48 h. Samples were dehydrated through an ascending alcohol series, cleared with xylene and embedded in paraffin. Sections were cut at 5 µm using a microtome, rehydrated, stained with hematoxylin and eosin (H & E) (Cardiff et al. 2014) and mounted on slides.

For H & E staining, kidney damage was scored based on inflammatory cell infiltration and glomerular collapse or congestion. Glomerular damage, i.e., capillary collapse and narrowing or absence of Bowman's space, was scored as: 0, absent; 1, damage to < 25% of glomeruli; 2, damage to 25–50% of glomeruli; 3, damage to > 50% of glomeruli. Inflammation and congestion were scored as 0, absent; 1, mild; 2, moderate; 3, severe for a maximum score = 9.

We also used periodic acid-Schiff (PAS) staining (Tunçdemir 2006) to identify tubule epithelial cell microvilli, basal membrane and tissue carbohydrates such as glycogen.

Immunohistochemistry

Caspase-3 activity was measured as an indicator of kidney tissue apoptosis. Sections were mounted on polylysine coated slides for immunohistochemical staining. Sections were rehydrated through 100, 95 and 70% alcohol. For antigen retrieval, sections were heat-treated (when needed) with citrate buffer, pH 7.6, (Thermo Scientific, Fremont, CA) and placed in a pressure cooker (Retriever 2100) (Aptum, Southampton, UK) for 15 min. After cooling for 20 min at room temperature, sections were washed with phosphate-buffered saline (PBS). Sections were immersed in 3% hydrogen peroxide for 10 min to inhibit endogenous peroxidase activity, then washed with PBS. Sections were incubated with protein-V blocking reagent (Thermo Scientific) for 5 min. Sections were incubated for 2 h with primary rat polyclonal caspase-3 antibody (NeoMarkers, Fremont, CA) diluted 1:300, then rinsed in PBS and incubated with biotinylated goat anti-polyvalent antibody for 10 min and streptavidin peroxidase for 10 min at room temperature. A polyvalent HRP kit (Thermo Scientific) was used according to the manufacturer's instructions. Staining was completed with chromogen (AEC; Thermo Scientific) + substrate buffer (AEC) (Thermo Scientific) for 15 min. Sections were rinsed with PBS and distilled water, then counterstained with Mayer's hematoxylin for 1 min. The sections were rinsed first in tap water, then in distilled water, dehydrated rapidly through an ascending alcohol series and held for 1 min in the last absolute ethanol before clearing with xylene and mounted with large volume vision mount (Thermo Scientific).

Apoptosis of cells of the glomerulus and proximal and distal tubules was scored semiquantitatively. Apoptosis scores and the percentage of activated caspase-3 immunoreactivity in sections/animal were determined (Table 1). Glomerulus and tubule caspase-3 immunoreactivity was scored as: 0, absent; 1, damage to < 25% of glomeruli and < 25% of tubules injured; 2, damage to 25–50% of glomeruli and 25–50% of tubules injured; and 3, damage to > 50% of glomeruli and > 50% of tubules injured for a maximum score = 9. All sections were examined by an histologist blinded to the identity of the groups using a light microscope (Eclipse Ni-U) and camera (DS-Fi3) (Nikon Instruments Inc., Melville, NY) and analyzed with an Image Analysis System (NIS-Elements Documentation 5.02; Nikon Corp., Tokyo, Japan).

Statistical analysis

Data are reported as median and minimum –maximum values. The Kruskal-Wallis test was used for group comparisons and pair-wise comparisons were performed using the Conover method. Values for $p \leq 0.05$ were considered statistically significant.

Table 1. Histological damage scores

Group	H & E total damage score	Caspase-3 immunoreactivity total H-score	Proximal tubule caspase-3 timmunoreactivity	Distal tubule caspase-3 timmunoreactivity
1	0 (0–0)	1 (0–1)[a]	0 (0–1)[a]	0 (0–1)[a]
2	0 (0–1)	1 (0–1)[a]	0 (0–1)[a]	0.5 (0–1)[a]
3	0 (0–2)	1 (0–2)[a]	0.5 (0–1)[a]	0.5 (0–1)[a]
4	1 (0–3)	4.5 (3–5)[b]	1 (1–2)[b]	3 (2–3)[b]
5	0 (0–1)	2 (1–3)[c]	1 (0–1)[a,c]	1 (1–2)[c]
6	0 (0–2)	3 (2–4)[b]	1 (1–2)[b,c]	2 (1–2)[c]
p	0.100	< 0.001	0.013	< 0.001

Data are medians and minimum-maximum values. Differences between groups with different superscript letters are statistically significant. Differences between groups with the same superscript letters are not statistically significant. Group 1, saline group; group 2, AL only; group 3, AT only; group 4, STZ only; group 5, STZ + AL; group 6, STZ + AT.

Results

Glomerulus and tubule structures in group 1 were histologically normal; no congestion, glomerular collapse or inflammation and rare and slight tubule caspase-3 immunoreactivity was observed. The sections stained with PAS exhibited normal glomerulus and tubule basement membranes (Figure 1). Groups 2 and 3 generally exhibited normal histology; however, slight tubule damage and inflammation were observed occasionally in these groups. Varying degrees of glomerular collapse, congestion, and narrowing and local occlusion of Bowman's spaces were observed in sections from these groups. Tubule caspase-3 immunoreactivity was increased in groups 2 and 3 compared to group 1, but the difference was not statistically significant (Figures 2, 3).

Sections from group 4 exhibited glomerular collapse with narrowing and occlusion of Bowman's space. Various degrees of inflammation and congestion also were found in sections from group 4. We found accumulation of eosinophilic material in the lumen of some tubules and vacuolization in podocytes and tubule epithelial cell cytoplasm. Variable caspase-3 immunoreactivity was observed in most renal tubules, especially distal tubules.

PAS stained sections showed that many glomerulus and tubule basement membranes were thickened by DM. Tubule damage was accompanied by cell swelling and intracytoplasmic vacuolization in group 4 (Figure 4). Administration of AL (group 5) or AT (group 6) reduced glomerulus collapse, congestion, inflammation and tubule caspase-3 immunoreactivity compared to group 4. The accumulation of eosinophilic material in the tubule lumen and vacuolization also were decreased in groups 5 and 6 compared to group 4. Damage was decreased significantly in group 5 and tubule caspase-3 immunoreactivity was decreased significantly compared to group 6 (Table 1).

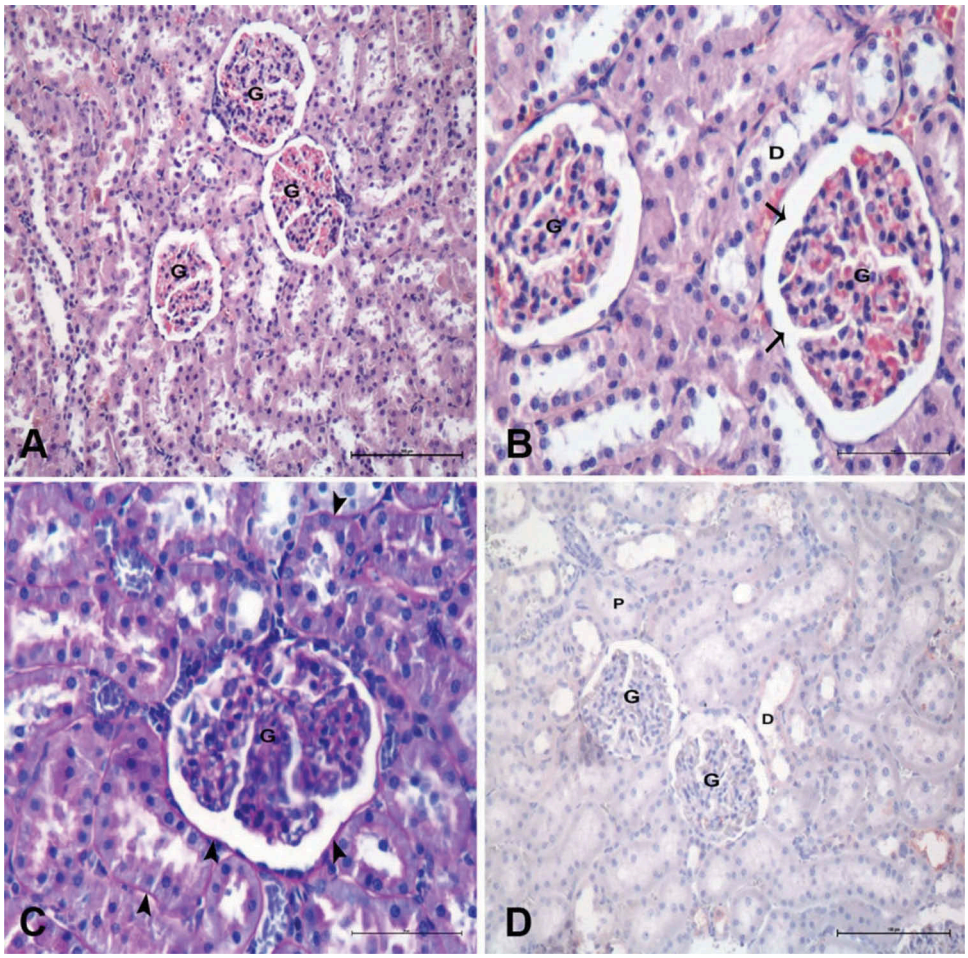

Figure 1. Group 1 sections. Glomerulus, tubules and basement membranes appeared normal. A, B) H & E. Scale bars = 100 (A), 50 µm (B). C) PAS. Scale bar = 50 µm. D) Caspase-3. Scale bar = 100 µm. G, glomerulus; D, distal tubule; P, proximal tubule; arrow, Bowman's space; arrowhead, glomerular and tubule basement membranes.

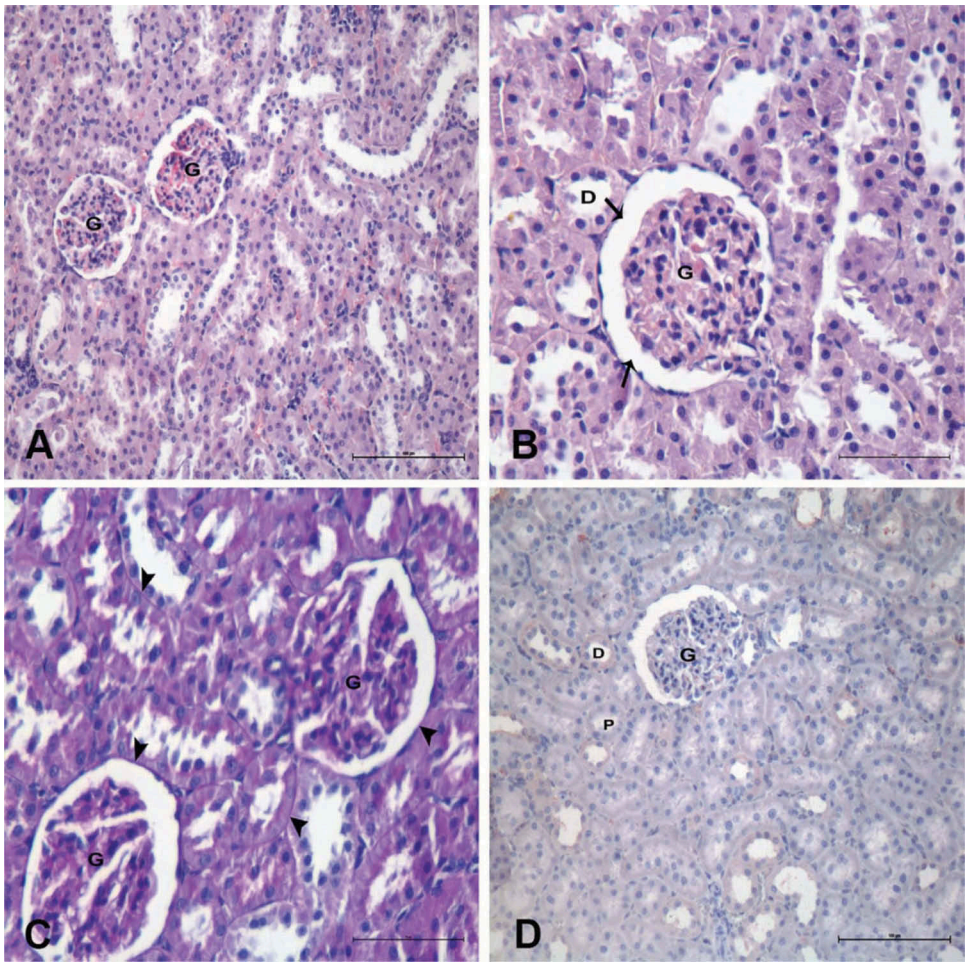

Figure 2. Group 3 sections. AT treated sections exhibited generally normal histology. Minimal and infrequent tubule and glomerular damage was detected. A, B) H & E. Scale bars = 100 µm (A), 50 µm (B). C) PAS. Scale bar = 50 µm. D) Caspase-3. Scale bar = 100 µm. G, glomerulus; D, distal tubule; P, proximal tubule; arrow, Bowman's space; arrowhead, glomerular and tubule basement membranes.

PAS stained sections showed that AL and AT protected against glomerulus and tubule basement membrane thickening and other damage caused by DM (Figures 5, 6).

Discussion

STZ damages pancreatic β cells, which causes DM (Kim et al 2001); therefore, we used STZ to create a DM model. DN is a common cause of end stage renal failure (Wolf and Ziyadeh 1999). DN occurs with variable severity in the STZ DM model (Tunçdemir 2006). DM causes characteristic changes in the kidney including glomerulus and tubule epithelium hypertrophy, glomerulus and tubule basal membrane thickening, glomerular mesangial intracellular matrix accumulation, tubule damage and fibrosis (Cortes et al. 1987; Tunçdemir 1998; Sanai et al. 2000). DM induced cellular hypertrophy is particularly prominent in glomerular and tubule-interstitial areas, and in areas of proximal tubules and distal tubules (Ziyadeh and Goldfarb 1991). Accumulation of glycogen in renal tubules causes cell damage or death, but this can be corrected by insulin therapy (Melin et al. 1997; Bamri-Ezzine et al. 2003). We observed cellular swelling, intracytoplasmic vacuolization and glycogen accumulation in tubule epithelial cell and podocyte cytoplasm in group 4. Glomerular collapse, narrowing of Bowman's space, variable levels of inflammation, congestion and accumulation of eosinophilic material in interstitial tissue were found in group 4. Our findings are consistent with earlier reports (Cam et al. 2003; Vardı et al. 2005, 2006; Sargın 2009).

Tubule apoptosis, atrophy and dilation occur as a result of kidney damage (Yoo et al. 2000). Apoptosis is initiated by ischemia, exogenous toxins or endogenous stimuli. Apoptosis has been reported to participate in the development of renal damage in both experimental models and clinical findings of glomerulonephritis, acute and chronic renal failure

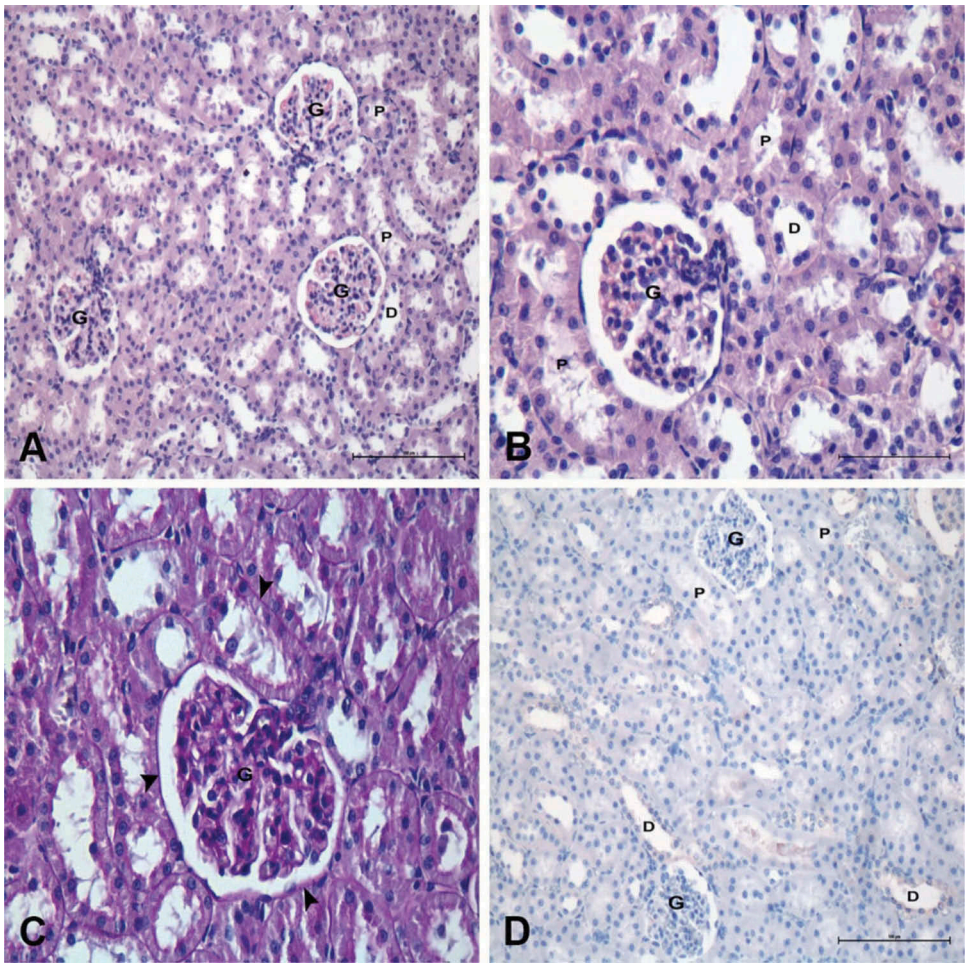

Figure 3. Group 2 sections exhibited generally normal histology. Infrequent and minimal tubule and glomerular damage were detected. A, B) H & E. Scale bars = 100 µm (A), 50 µm (B). C) PAS. Scale bar = 50 µm. D) Caspase-3. Scale bar = 100 µm. G, glomerulus; D, distal tubule; P, proximal tubule; arrow, Bowman's space; arrowhead, glomerular and tubule basement membranes.

and DN. It has reported that apoptosis is increased in the subtotal nephrectomy animal model of chronic renal failure, which is correlated with the progression of renal fibrosis (Graham et al. 1998). Apoptosis also is associated with fibrosis in kidney tissue (Ortiz 2000).

Increased oxidative stress and glycosylation end products contribute to the pathogenesis of chronic complications of DM (Liptakova et al. 2002). The organism possesses enzymatic and nonenzymatic antioxidant defense systems to counteract these harmful radicals. Exogenous antioxidants also can be used to combat the effects of free radicals (Reed et al. 1953; Ohkuwa et al. 1995). Antioxidants and nitric oxide synthase (NOS) inhibitors are helpful for treatment of DM by reducing hyperglycemia caused by STZ (Reed et al. 1953; Liptakova et al. 2002; Vardı et al. 2005, 2006). The NF-kB and mitogen-activated protein (MAP) kinase signaling pathways are activated by the oxidative stress caused by DM. NF-kB pathway; endothelin-1 (ET-1) is regulated by increased thrombomodulin and proinflammatory cytokines such as interleukin-1α (IL-1α), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) (Neumann 1999; Ahmed 2005).

Because it increases cellular glucose utilization, AL therapy also has been used for treating DM (Eguíluz Lumbreras et al. 2010). Oxidative stress occurs in DM; the main targets of ROS are membranes of mitochondria and endoplasmic reticulum, and lipids in plasma. ROS have been detected in mitochondria and endothelial cells. It has been suggested that albuminuria that accompanies DM may contribute to development of endothelial dysfunction. Mitochondrial ROS also contribute to the formation of glomerular basement membrane thickening, mesangial matrix expansion and tubule dysfunction (Memişoğulları 2005; Altan et al. 2006).

Maritim et al. (2003) reported that AL reduces lipid oxidation by eliminating free radicals. Melhem et al. (2002) prevented glomerular injury during the early

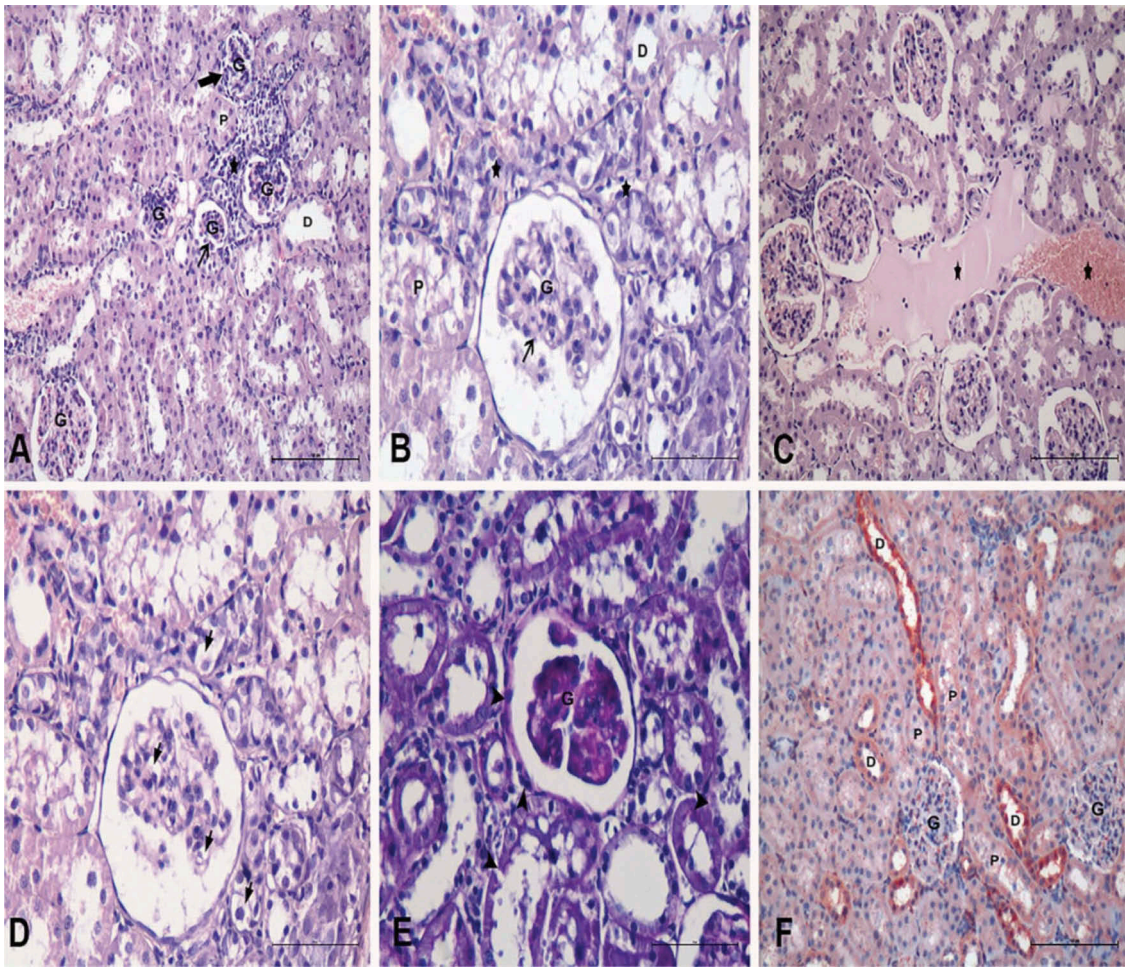

Figure 4. Group 4 sections. A, B) Glomerular collapse (thin arrows), narrowing of Bowman's space (thick arrow), varying levels of inflammation (asterisk) and congestion were common. H & E. Scale bars = 100 µm (A), 50 µm (B). C, D) Asterisk, congestion and accumulation of eosinophilic material; arrow, vacuolization in podocytes and tubule epithelial cell cytoplasm. H & E. Scale bars = 100 µm (C), 50 µm (D). E) Glomerular basement membrane thickening and tubule basement membrane damage were observed. Arrowhead, thickened glomerular and damaged tubule basement membranes. PAS. Scale bar = 50 µm. F) Caspase-3 immunoreactivity was observed at different density in most renal tubules, especially distal tubules (red-brown stained regions). Scale bar = 100 µm. G, glomerulus; D, distal tubule; P, proximal tubule.

period of DM by daily application of AL in an STZ group not treated with insulin; they attributed the effects to both its glycemic control and antioxidant effect.

Özkan et al. (2005) reported that AL protects against early diabetic glomerular damage better than high doses of vitamins C and E, and that the insulin signaling pathway that increases glucose transport can be activated by high concentrations of AL (Özkan et al. 2005; Yaworsky et al. 2000). Consistent with the literature, we found that AL treatment prevented the deleterious effects of DM.

AT can be used to treat degenerative changes caused by DM (Devaraj and Jialal 2000a, 2000b; Wu et al. 2007). AT therapy, especially at high doses, clearly is beneficial for LDL oxidation, isoprostanes and decreased inflammatory markers including C-reactive protein, pro-inflammatory cytokines and PAI-1 levels. Therefore, it appears that AT therapy could be an additional therapy for diabetes (Boaz et al. 2000). Haliga et al. (2017) reported changes characteristic of DN in DM animals including increased glomerular size, active mesangial cells, PAS stained mesangial deposits, thickened basal capillary membranes and capillary lumina filled with erythrocytes. AT supplementation reduced the histopathology of DN (Kutlubay et al. 2007; Haliga et al. 2017).

Meruva et al. (2016) investigated the effect of AT on the antioxidant defense system in STZ induced diabetic rats. They observed degenerative effects including altered Bowman's capsules, focal necrosis of renal tubules, altered cytoplasm resolution and vacuolar modifications. Following AT treatment, the renal parenchyma exhibited regenerative changes in the distal tubules and Bowman's

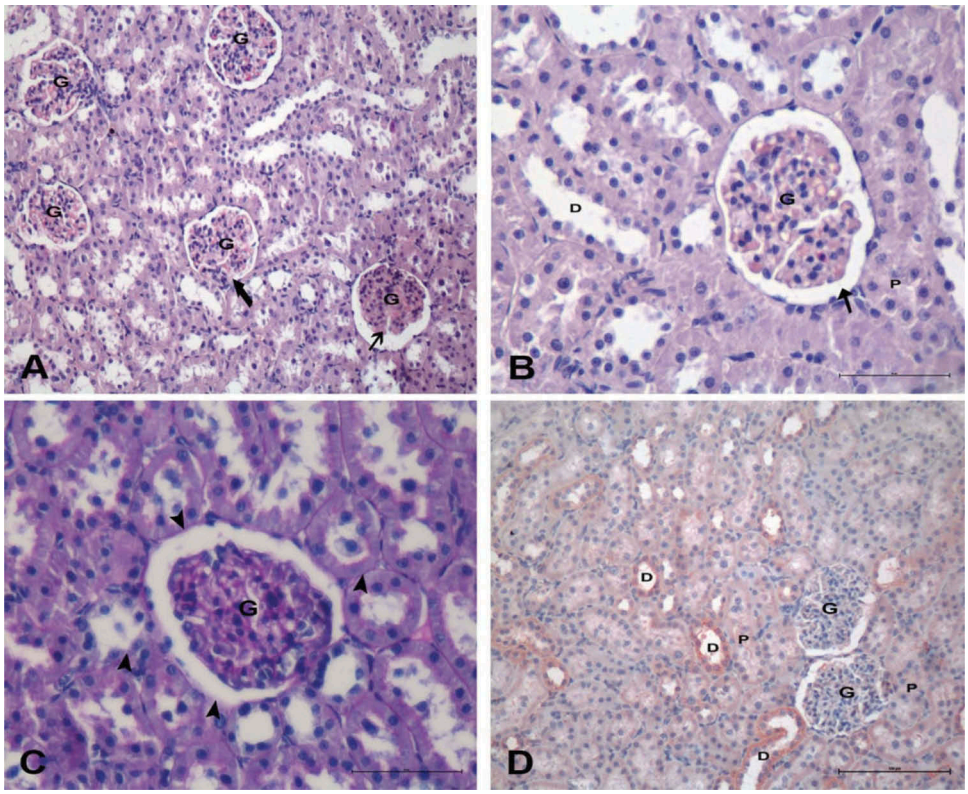

Figure 5. Group 6 sections. Tubule damage and caspase-3 immunoreactivity was decreased in group 6 compared to group 4. A) Thin arrows, glomerular collapse; thick arrow, narrowing of Bowman's space. Scale bar = 100 µm. B) Thin arrow, normal Bowman's space. H & E. Scale bar = 50 µm. C) Arrowhead, normal glomerular and tubular basement membranes. PAS. Scale bar = 50 µm. D) Occasional red-brown stained regions indicate caspase-3 immunostaining. Scale bar = 100 µm. G, glomerulus; D, distal tubule; P, proximal tubule.

capsule (Meruva et al. 2016). Consistent with the literature, we found that AT treatment prevented the deleterious renal effects of DM.

We found that AL exhibited a more curative effect for diabetic rats in group 5 than for group 6. AL is a free radical scavenger that is absorbed from the diet, transported into cells and reduced to dihydrolipoic acid (DHLA), which exhibits even greater antioxidant activity than lipoic acid (Biewenga et al. 1997). The AL/DHLA oxidation-reduction couplet possesses a greater redox potential than AT, vitamin C, coenzyme Q and glutathione (Biewenga et al. 1997; Podda and Grundmann-kollmann 2001). AL/DHLA can regenerate reduced forms of vitamin E, vitamin C, coenzyme Q and glutathione, thereby maintaining the endogenous reduced state, which counteracts oxidative stress (Biewenga et al. 1997; Packer et al. 1997). Biewenga et al. (1997) reported several useful properties of AL: antioxidant effects, metal chelation, ROS scavenging, regeneration of endogenous antioxidants and repair of oxidatively damaged biomolecules.

Bamri-Ezzine et al. (2003) reported that apoptosis participates in the development of DN. Clear cells that accumulated glycogen were identified in renal tissues of rats with STZ induced DM. Clear cells were concentrated in the thick ascending limbs and distal tubules. The clear cells exhibited features of apoptosis. these investigators also identified active caspase-3 in clear cell nuclei using immunoelectron microscopy. It appeared that epithelial cells in thick ascending limbs and distal tubules developed DN in response to hyperglycemia and apoptosis. These investigators suggested that apoptosis plays a significant role in renal epithelial cell loss during DM.

Although the kidney exhibits little mitosis under normal conditions, it can regenerate itself if injured or partially ablated. Acute tubule necrosis may occur due to ischemia or exposure to reagents that are toxic to epithelial cells. Damaged tubules are repaired after injury following a burst of cell proliferation (Kitamura et al. 2005). These investigators isolated highly proliferative renal cells from the S3 segment of proximal tubule in adult rat kidney. Studies using immunostaining of human tissue have shown that cells of the proximal tubule are multipotent progenitor cells with self-renewal properties (Bruno et al. 2009; Kim et al. 2011; Ward et al. 2011). Hishikawa et al.

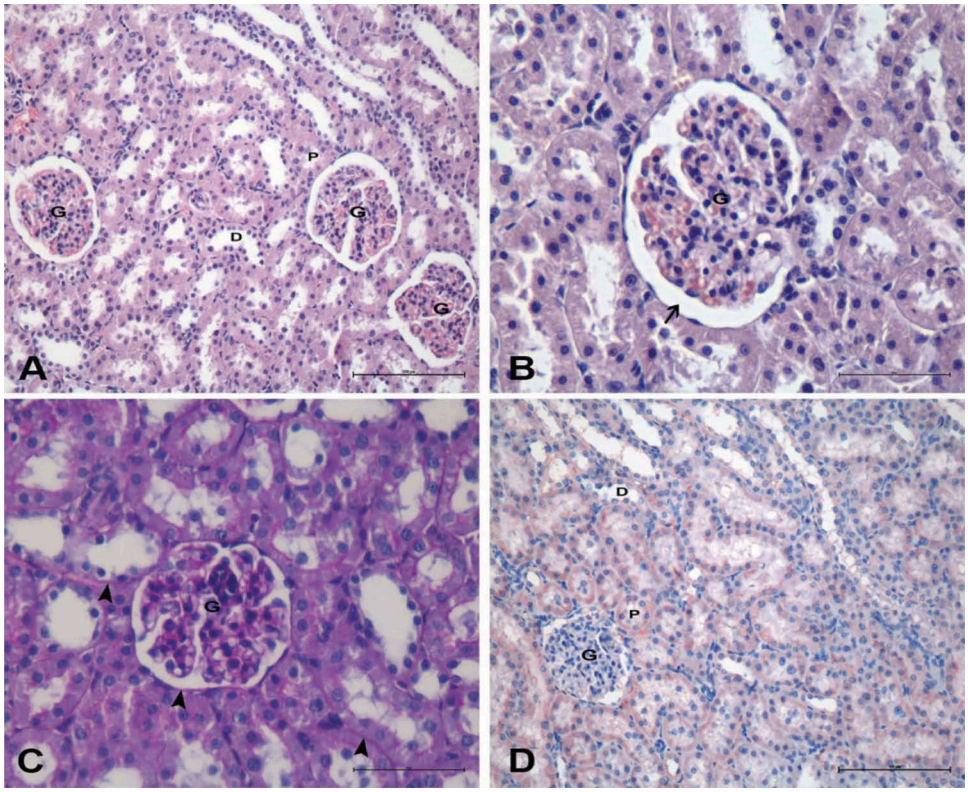

Figure 6. Group 5 sections. Tubule damage and caspase-3 immunoreactivity were decreased significantly in group 5 compared to groups 4 and 6. A, B) Histology generally was normal. Arrow, normal Bowman's space. H & E. Scale bars = 100 µm (A), 50 µm (B). C) Arrowhead, normal glomerular and tubule basement membranes. PAS. Scale bar = 50 µm. D) Significantly decreased caspase-3 immunoreactivity compared to groups 4 and 6. Scale bar = 100 µm. G, glomerulus; D, distal tubule; P, proximal tubule.

(2015) reported that a progenitor cell is required for organ homeostasis and repair, because progenitor cells continuously replace lost cells following damage. Therefore, the literature supports the self-renewal properties of proximal tubules and may account for the decreased caspase-3 immunoreactivity in proximal tubules that we observed. We found less caspase-3 positive immunoreactivity in the proximal tubule than the distal tubule and attribute this to the self-regenerative capacity of the proximal tubules.

The damaging effects of DM decreased significantly in groups 5 and 6, and tubule caspase-3 positive immunoreactivity also decreased. AL exhibited an ameliorative effect on diabetic rats and reduced kidney damage. AT treatment also prevented the deleterious renal effects of DM, but AL treatment was more effective than AT treatment. Also, it appears that proximal tubule cells have a greater potential for self-renewal than distal tubule cells.

Declaration of interest

The authors declare no conflict of interest.

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