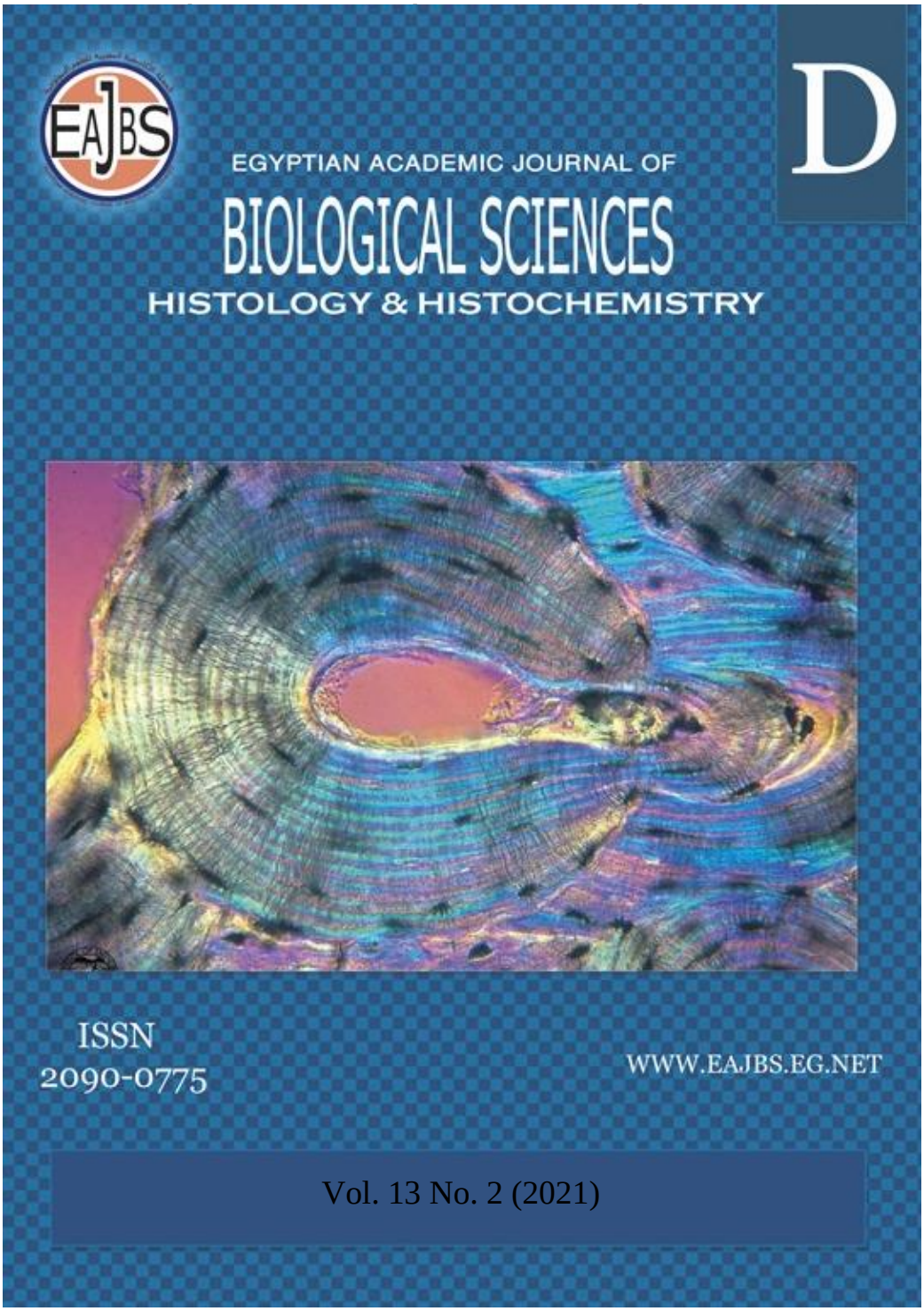

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Role of *Retama Raetam* on Gentamicin-Induced Acute Kidney Injury in Experimental Rat Model

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ABSTRACT

The methanol extract of the seeds of *Retama raetam* was studied for its preventive and curative effects in gentamicin-induced acute renal failure in rats. Thirty rats were divided into five different groups; each group contains six rats. Group I was served as a control group, Group II, III and IV were administered a daily dose of gentamicin (40 mg/kg body weight s.c) for 13 days. In case of group III, rats were administered with the extract at a dose of 30 mg/kg body weight on the 14th day onwards for 10 days. While, in group IV rats were treated with an oral dose of the extract equivalent to 30 mg/kg b.w. before 2hs of subcutaneous injection of gentamicin for 13 days. Group V rats were given the extract at a dose of 30 mg/kg body weight for 13 days. Serum urea and creatinine levels were measured. Moreover, histopathological as well as morphometrical investigations were performed. In the gentamicin model, the methanol extract of *R. raetam* (30 mg/kg b. wt.) reduced blood urea and serum creatinine effectively in the curative and the preventive treatment. Light microscopic examination of the renal tissues from gentamicin-treated rats revealed severe histopathological and morphometrical changes, whereas specimens obtained from extract-treated rats revealed only mild changes. These findings recommend that the methanol extract of the seeds of *R. raetam* counteract the deleterious effect of gentamicin on renal tubular function and structure.

INTRODUCTION

The kidney is a vital organ required by the body to carry out numerous main functions including the protection of homeostasis, regulation of the extracellular environment, such as detoxification, and secretion of poisonous metabolites and drugs (Ferguson *et al.*, 2008). For that reason, the kidney can be considered the most important target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific aspect in which excretion does not go easily due to toxic chemicals or drugs (Finn and Porter, 2003; Galley, 2000).

Acute kidney injury is the deterioration of the renal function over hours or days, resulting in the accumulation of toxic wastes and the loss of internal homeostasis. It can be caused by numerous etiologies (Dasari, and Tchounwou, 2014, Oh, *et al.,* 2014), and medications are a relatively common cause of kidney injury among these injuries (Rahman, *et al.,* 2012). Drug-induced nephrotoxicity is a renal dysfunction that occurs as a result of direct or indirect exposure to nephrotoxic prescribed drugs, over-the-counter products, diagnostic agents (Rahman, *et al.,* 2012), George, *et al.,* 2012. Drug-induced nephrotoxicity is an extremely common condition and is responsible for a variety of pathological effects on the kidneys (George, *et al.,* 2012). Nephrotoxicity most commonly affects the tubule interstitial compartment and manifests either acute tubular injury or acute interstitial nephritis. There is a growing incidence of drug-induced glomerular disease, including direct cellular injury and immune-mediated injury (Harrill, 2018).

Aminoglycoside antibiotics are universally used for the treatment of severe gram-negative bacterial infections. Even with their beneficial effects, aminoglycosides have significant nephrotoxic side effects (Parlakpinar *et al.,* 2003). The main widely used drug in this category is gentamicin (Reiter *et al.*, 2002). A major complication of gentamicin treatment is nephrotoxicity, accounting for 10–20% of all cases of acute renal failure according to experimental results (Erdem *et al.,* 2000). Also, about 30% of the patients treated with gentamicin for more than 7 days show some signs of nephrotoxicity that noticeably limits its use (Pedraza-Chaverri *et al.,* 2000).

Nephrotoxicity induced by gentamicin is a complex event characterized by an increase in blood urea nitrogen and serum creatinine concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure (Smetana *et al.,* 1988 and Al-Majed *et al.,* 2002). Although the pathogenesis is still not well understood, the toxicity of gentamicin in the kidney seems to relate to the generation of destructive reactive oxygen species in these cells (Reiter et al., 2002 and Al-Majed *et al.,* 2002). Reactive oxygen species have been occupied in a wide range of biological functions, but they can express both valuable and greatly toxic effects on cellular homeostasis (Mates, 2000). A large body of in vivo and in vitro evidence indicates that reactive oxygen species are important mediators of gentamicin-induced nephrotoxicity (Pedraza-Chaverri *et al.,* 2000, Kopple *et al.,* 2002, Al-Majed *et al.,* 2002 and Abdel-Naim *et al.,* 1999). It has been proposed as a causative agent of cell loss in many different pathological states as well as, in glomerular disease (Smetana *et al.,* 1988), in various models of toxic renal failure (Piotrowski *et al.,* 1996).

There are a number of approaches in the literature to attenuate gentamicin nephrotoxicity including the use of Polyascorbic acid (Swan, *et al.,* 1992), melatonin (Ozbek *et al.,* 2000), vitamin E, superoxide dismutase (Pedraza-Chaverri *et al.,* 2000), ginkgo biloba extract (Maldonado *et al.,* 2003), diallyl disulfide (Pedraza-Chaverrý *et al.,* 2003), taurine (Aysen, *et al.,* 2003), and garlic extract (Perla, *et al.,* 2003).

Retama raetam is an indigenous plant belonging to the Fabaceae and is common in the north and east Mediterranean region (Boulos, 1999; El Bahri *et al.,* 1999 and Mittler *et al.,* 2001). It has been found that *Retama raetam* possess a significant hypoglycemic effect in both normal and strptozotocin diabetic rats (Maghrani *et al.,* 2003), exhibited a significant diuretic effect in normal rats (Maghrani, *et al.,* 2005 a), and

glycosuria inhibition of renal glucose reabsorption (Maghrani *et al.*, 2005 b).

Therefore, this experimental study was designed to investigate the possible protective and curative effects of *Retama raetam* on Acute kidney injury induced by gentamicin in a rat model.

MATERIALS AND METHODS

Chemicals:

Garamycin:

Scientific Name: Gentamicin 80 mg, **Type:** Ampoules, **Producing Company:** Schering/Memphis, **Molecular Formula:** $C_{21}H_{43}N_5O_7$ and **Molecular Weight:** 477.6

Garamycin (Manufactured by Memphis Co. for Pharm. & Chem. Ind. Cairo- A.R.E. under the authority of Schering-Plough Corporation / U.S.A.) as aqueous solution each ml of Garamycin injection contains the gentamicin sulfate, equivalent to 40 mg. Urea estimation kit and creatinine were used for this study.

Extraction and Isolation:

The powder of the dried *Retama raetam* was defatted with methyl chloride (CH_3Cl) and extracted with methanol-water [CH_3OH: H2O (7:3)] at room temperature. The extract was filtered, evaporated under reduced pressure, and lyophilized (200 g). At the time of use, the extract was suspended in distilled water at the desired concentrations (30 mg/kg b. wt.). The purpose of using this medicinal plant was its high constituent of flavonoids. Methyl alcohol extract was used due to its boiling point being very low, easily evaporated, and low in cost. Methyl extracts are more common in use than ethyl extracts. Methyl alcohol is toxic, but in the present study the dried part of the employed plant was extracted and all methyl alcohol was evaporated. The chemical constituents of *Retama raetam* aqueous alcoholic extract include genistein 8-C-glucoside, orobol 8-C-glucoside, apigenin 8-C-glucoside orobol, genistein, and apigenin.

Animals:

In this investigation, 30 healthy adult Sprague-Dawley rats (Experimental Animal House, National Research Centre, Egypt) weighing between 120 and 150 g were used. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (25±3 ◦C) during the experimental period. All experimental procedures were conducted in accordance with the guide to the care and use of laboratory animals. The rats were provided rat chow and water *ad libitum*.

Experimental Design:

Rats were divided into five groups, six rats each one. Group I was served as control. Group II was injected with Gentamicin for 13 days (40 mg/kg body wt., s.c) (Akria, et al., 1994). Group III was studied as the curative activity of the extract. In this group, rats were treated with the extract (30 mg/kg body wt., orally) from the 14th day onwards, for 10 days. Group IV was studied as the preventive activity of the extract of *Retama*. This group was treated with extract (30 mg/kg body wt., orally) from the 1st day onwards, along with gentamicin (40 mg/kg body wt., s.c.) daily for 13 days. Group V rats were administered with the extract (30 mg/kg body wt., orally) for 13 days. At the end of work, blood was withdrawn on the 14th day in the 1st, 2nd, 4th and 5th groups and the 3rd group blood was withdrawn on the 24th day to assess renal functions.

Renal Functions:

Blood Urea:

Urea concentration in the blood was estimated by the enzymatic method using a Urease enzyme kit. Absorbance was read from a UV-240 vis spectrophotometer (Shimadzu Corporation, Japan).

Serum Creatinine:

Creatinine level in serum was estimated by the alkaline picrate method, using a creatinine kit.

Absorbance was read from a UV-240 vis spectrophotometer (Shimadzu Corporation, Japan).

Histological Examination:

For light microscopic evaluation, portions of each kidney were fixed in a 10% neutral phosphate-buffered formalin solution. Following dehydration in an ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5 μm were stained with hematoxylin & eosin (H & E). Five coded slides from each group were examined. Light microscopy was used to evaluate tubular necrosis which was graded as follows: mild (+): only single-cell necrosis and slight degenerative changes, moderate (++): tubular necrosis at different foci throughout the cortex and severe (+++): extensive and marked tubular necrosis throughout the cortex.

Statistical Analyses:

The results were expressed in the terms of mean ± S.E. and the statistical significance of differences between means was estimated by ANOVA using SPSS software for Windows (Version 9.05). Results were considered statistically significant at $P < 0.05$.

RESULTS

Biochemical Results:

Gentamicin administration for thirteen days resulted in a significant elevation in serum creatinine and urea as compared with the control one. On the other hand, elevations in the serum creatinine and blood urea were significantly at $P < 0.05$ attenuated by the extract of *Retama*. Pre or post-treatments indicate diminution in Gentamicin-induced nephrotoxicity at $P < 0.05$. The extract of *Retama* treatment alone did not change the renal function tests when compared to control values (Table 1).

Table 1: Effect of Gentamicin and its combination with Retama on creatinine and urea

Groups / Parameters	Creatinine (mg dl−1)	Urea (mg dl−1)
Control	0.56 ± 0.09	44 ± 2.8
Gentamicin alone	1.37 ± 0.19*	110 ± 5.6*
Pre-treatment with Retama + Gentamicin	0.82 ± 0.17**	77 ± 3.0**
Post treatment with Retama and Gentamicin	0.77 ± 0.18**	79 ± 2.9**
Retama alone	0.58 ± 0.19	42 ± 1.78

Data are means ± S.E of six rats for each group.

* Significant difference as compared to controls at $P < 0.05$

** Significant difference as compared to rats receiving gentamicin alone at $P < 0.05$.

Acute Toxicity Studies:

The methanol extracts of *Retama* (seeds), when orally administered in the dose range of 30 mg/kg body wt. did not produce any significant changes in the autonomic or behavioral responses, including death during the observation period.

Histopathological Results:

The tissue of the kidney is divided into an outer cortex and an inner medulla. The functional unit of the kidney consists of two major components, the renal corpuscle and the renal tubule. The renal corpuscles lie in the cortex and each is formed of two structures, Bowman's capsule and the glomerulus. Bowman's capsule is formed of the doubled-walled epithelium; the external layer forms the outer limit of the renal corpuscle and is called the parietal layer. The internal layer envelops the glomerulus and is called the visceral layer. The spaces between the two layers of the Bowman's capsule are known as urinary spaces. The glomerulus is a tightly coiled network of anatomizing capillaries.

The renal tubules extend from Bowman’s capsules to its junction with a collecting duct and include the

proximal convoluted tubule and the distal convoluted tubule. Sections of the proximal and distal convoluted tubules are shown in the cortex. The proximal convoluted tubule is the largest and its lumen is narrow. It is lined with simple cuboidal epithelium. The distal convoluted tubules are lined with simple cuboidal epithelium. The cytoplasm of these cells appears somewhat less acidophilic than in the proximal convoluted tubules, and in cross-sections the nuclei appear numerous as the number of cells is far more than that of the proximal convoluted tubules. The lumina of the distal convoluted tubules are larger than those of the proximal convoluted tubules (Fig. 1.A).

The medulla is formed of a large number of collecting tubules and the ascending and descending limbs of Henle's loops. The collecting tubule is large in size, lined with simple cuboidal epithelium and displays a wide lumen. The loops of Henle are lined with simple squamous epithelium; the nuclei of its cells appear protruded into the lumina of such tubules (Fig. 1.B).

Light microscopy of the kidney sections from rats after daily treatment with subcutaneous doses of gentamicin equivalent to 40 mg/kg body wt. for 13 consecutive days demonstrated severe histological alterations. Hemorrhage, as well as lymphocyte infiltration in the interstitial tissue of the cortex, were demonstrated. The renal corpuscles showed different stages of glomerular degeneration and necrosis. These degenerative changes appeared in the form of hypercellularity, extensive, partial, or complete glomerular degeneration. The renal corpuscles also showed congestion and wide urinary spaces. Some proximal convoluted tubules revealed complete loss of their epithelial lining while others showed partial desquamation or necrosis of their lining cells. The nuclei of these necrotic cells exhibited signs of pyknosis and karyolysis (Figs. 1. C, D).

In Rats of the preventive group that orally given the extract of *Retama* at a dose equivalent to 30 mg/kg body wt. from the 1st day onward, along with gentamicin (40 mg/kg body wt., s.c.) daily for 13 days, microscopic examination of kidney section indicated the appearance of structure nearly to the control one (Fig. 2. A). Few renal and corpuscles and tubules showed mild degeneration (Fig. 2. B).

Histopathological investigation of the kidney forms the curative group that was treated with the extract (30 mg/kg body wt., orally) from the 14th day onward, for 10 days demonstrated that the structure of the kidney appeared more or less like normal (Figs.2.C). Sections of few renal and corpuscles and tubules showed moderate degeneration (Figs.2.D). On the other hand, there were not any microscopical differences between the control and only the extract of *Retama*. -treated groups (Fig. 2. E).

Morphological Changes In Kidney Tissue:

The morphological changes in the kidney were graded and results were scored. The kidneys of the control group showed normal kidney parenchyma. Gentamicin-treated rats showed more extensive and marked tubular necrosis (+++). In the gentamicin + the extract of *Retama*-treated rats light tubular changes was observed. In some rats' mild marked tubular necrosis was found (+). In case the curative group gentamicin + the extract of *Retama*-treated rats' dense tubular changes was observed. In some rats marked tubular necrosis was seen (++). The extract of *Retama* apparently reduced kidney tissue damage. No microscopical differences between the control and only the extract of the Retama-treated group (Table 2).

Table 2: Histological damage grading in the kidney of a different group of rats

Groups	Tubular necrosis grade			
Control	-	-	-	-
Gentamicin alone	-	-	-	+++
Pre-treatment with Retam + GEN	-	+	-	-
Post treatment with retam and GEN	-	-	++	-
Retama alone	-	-	-	-

(+): mild tubular necrosis grade
(++) moderate tubular necrosis grade
(+++): severe tubular necrosis grade

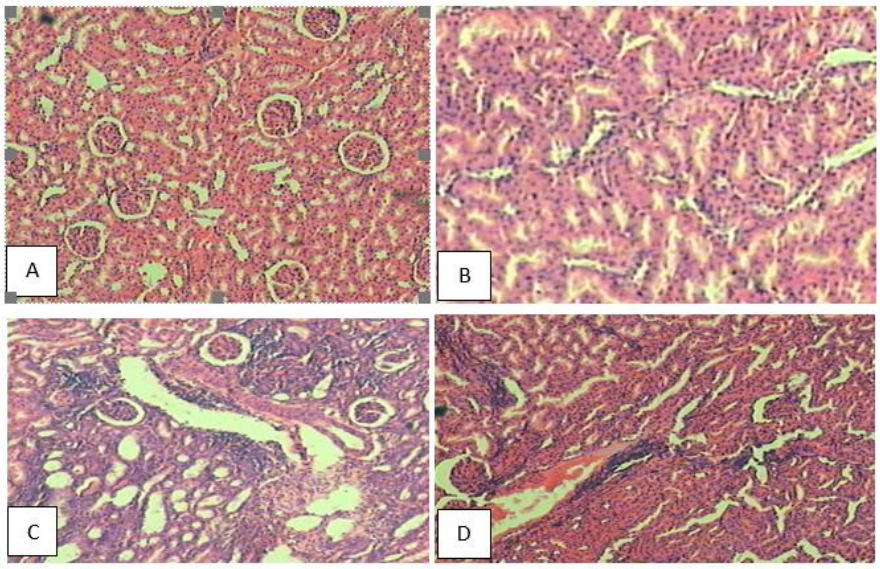

Fig. 1: Sections of kidney of rat shows (A) control group cortex, (B) control group medulla, (C) cortex of gentamicin treated group, and (D) (H & E stain, x100).

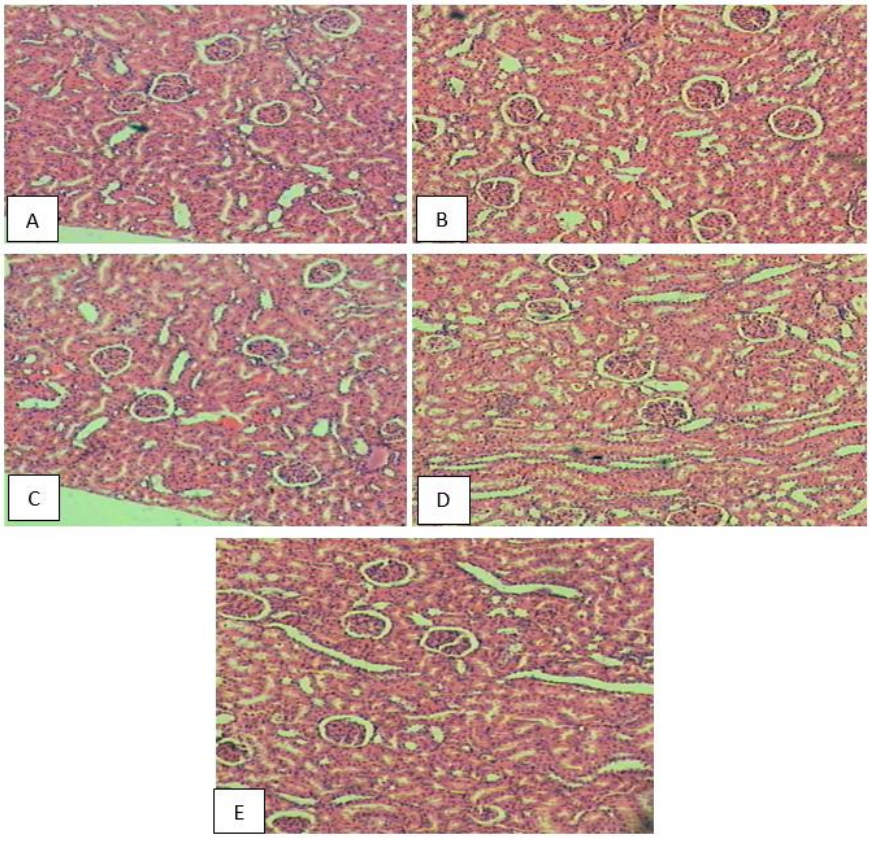

Fig. 2: Sections of kidney show (A, B) cortex from curative group (C, D) cortex from treated group, (E) extract alone (H & E stain, x100).

DISCUSSION

As the clinical use of aminoglycosides may be limited by the progress of nephrotoxicity, it is important to be aware of those hazard factors associated with a greater rate of renal damage. The beginning of decreasing renal function caused by aminoglycosides usually occurs after 1 week's treatment (Walker and Duggin, 1988 and Solgaard *et al.,* 2000).

Due to the treatment with some antioxidants protects the gentamicin-induced renal injury, in the present study we focused on the effect of pre-treatment with *Retama* + gentamicin and post-treatment with *Retama* and gentamicin on the renal damage and injury induced by gentamicin using both biochemical determinations and the morphology of the kidney using light microscopy.

Results of this study confirmed that gentamicin at a dose of 40 mg/kg/day produces nephrotoxicity as evident by the elevation of serum creatinine and urea. On the other hand, urea begins to rise only after a marked renal parenchymal injury occurs (Erdem *et al.,* 2000). In the present study, an increase in serum Cr and BUN levels induced by creatinine and urea was significantly blocked by *Retama*. The success of *Retama* in reducing creatinine and urea concentrations could be attributed to its antioxidant properties because it has been found that ROS may be involved in the impairment of GFR (Pedraza-Chaverri *et al.,* 2000).

General mechanisms that cause nephrotoxicity include changes in glomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy (Zager, 1997; Schnellmann and Kelly, 1999; Schetz *et al.,* 2005; Ferguson *et al.,* 2008).

Kidneys can keep a constant filtration rate as well as maintain the displacement of urine through regulation of blood flow in afferent and efferent arteries for adjustments or maintenance of intraglomerular pressure.

Circulation of prostaglandin is used for the expansion of afferent arteries (Naughton, 2008). Anti-prostaglandin drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) or drugs having anti-angiotensin activity for the prevention of blood pressure elevation including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers have been shown to induce nephrotoxicity in the glomerulus (Olyaei *et al.,* 1999; School Werth *et al.,* 2001; Palmer, 2002).

Because renal tubules, especially proximal tubule cells, are exposed to drugs in the process of concentration and reabsorption through the glomerulus, they are influenced greatly by drug toxicity (Perazella, 2005). Cytotoxicity occurs due to the damaged mitochondria in tubules, the disturbed tubular transport system, and the increase in oxidative stress by free radical generation (Zager, 1997; Markowitz and Perazella, 2005). The cytotoxicity-inducing drugs include aminoglycoside antibiotics, antifungal agents such as amphotericin B, antiretroviral drugs such as adefovir, anticancer drugs such as cisplatin and foscarnet (Markowitz *et al.,* 2003; Prezella, 2005; Markowitz and Perazella, 2005).

Nephrotoxic drugs often induce inflammation in the glomerulus, proximal tubules, and surrounding cellular matrix, and then fiberize the kidney tissue. Inflammation that disturbs normal kidney functions and induces toxicity includes glomerulonephritis, acute and chronic interstitial nephritis. Glomerulonephritis has been shown to be closely related to proteinuria (Prezella, 2005). Acute interstitial nephritis, a type of drug-

induced immune response, is induced by NSAIDs and antibiotic drugs such as rifampicin (Rossert, 2001). Chronic interstitial nephritis occurs frequently by long-term use of calcineurin inhibitors, lithium, some anticancer drugs, or analgesics (Perneger *et al.*, 1994; Fored *et al.*, 2001; Isnard Bagnis, *et al.*, 2004; Rodriguez-Iturbe and Garcia Garcia, 2010). In the case of chronic interstitial nephritis, early detection is especially important because it is difficult to diagnose until most of the functionality of the kidney is destroyed.

These findings correlated well with the renal histological examination which revealed that more extensive and marked tubular necrosis in the gentamicin-treated kidney. Similar changes were also reported by (Kumar *et al.*, 2000) and others (Al-Majed *et al.*, 2002) demonstrating structural changes in renal tissue of gentamicin-treated animals and its protection by various agents. Administration of Team reversed kidney damage with especially a marked reduction in tubular damage induced by gentamicin.

Conclusions

Drug-induced nephrotoxicity is closely associated with acute renal injury. However, traditional nephrotoxicity assays such as measurement of the concentration of serum urea and creatinine or morphological and histological levels that provide useful information to diagnose nephrotoxicity earlier and more selectively in case of using *Retama* pre-treatment or post-treatment gentamicin

Ethical Approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed. We respected the welfare of animals and excluded situations when animals were in pain.

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