

Nephro-protective effects of curcumin, rosemary and propolis against gentamicin induced toxicity in guinea pigs: Morphological and biochemical study

Azab El Saied Azab¹, Fathy Ahmed Fetouh², Mohamed Omer Albasha¹

¹Department of Zoology, Alegelat Faculty of Science, Zawia University, Libya

²Department of Anatomy, Faculty of Medicine, Zagazig University, Egypt

Email address:

Fghait@yahoo.com (F. A. Fetouh)

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Abstract: The kidney is a common target for toxic xenobiotics due to its capacity to extract and concentrate toxic substances by highly specialized cells and also, due to its large blood flow. Objective: The present work aimed to evaluate the effectiveness of different natural materials (curcumin, rosemary and propolis) against the histological and also biochemical alterations of gentamicin induced nephrotoxicity in guinea pigs. Materials and methods: 48 guinea pigs were used for this study and divided into 8 groups. The first 4 groups were control groups, the 5th group was the experimental and administered gentamicin at a dose of 100 mg/kg body wt for 10 days, and in the 6th, 7th, and 8th groups, gentamicin was co-administered with curcumin, rosemary, and propolis at the doses of 200 mg, 220 mg, and 100 mg/kg body wt respectively. The animals were sacrificed and the kidneys were dissected and specimens were obtained. The specimens were processed for light microscopic examinations. Blood samples were obtained for assessment of urea, creatinine and uric acid levels. Results: In gentamicin treated animals, there were structural changes. The proximal convoluted tubules showed degenerated epithelial lining with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. The afferent arteriole showed thickening in its wall and degeneration of endothelial lining with extensive perivascular infiltration of inflammatory cells. Massive interstitial hemorrhage was seen. Also, the serum urea, creatinine, and uric acid were elevated. Co-administration of curcumin, rosemary, and propolis significantly improved the structural changes in the kidney and the blood urea, creatinine and uric acid were significantly declined. Conclusion: It can be concluded that, the gentamicin has adverse effects on the kidney. Different natural materials as curcumin, rosemary, and propolis were able to protect the kidney against these effects. So, the patients should be advised to take one of these materials while they are treated by gentamicin.

Keywords: Nephrotoxicity, Histology, Biochemical, Gentamicin, Curcumin, Rosemary, Propolis

1. Introduction

The kidney is a common target for toxic xenobiotics due to its capacity to extract and concentrate toxic substances by highly specialized cells and also, due to its large blood flow (about 21% of cardiac output) [1,2]. Aminoglycoside antibiotics have long been used in antibacterial therapy. Gentamicin is an aminoglycoside antibiotic derived from *micomonospora purpurea*. It is effective against most of the life threatening Gram negative bacterial infection [3]. The gentamicin still constitutes the only effective therapeutic alternative against microorganisms, pseudomonas, proteus

and serratia that are insensitive to other antibiotics [4]. It has been estimated, that up to 30 % of patients treated with aminoglycosides for more than 7 days show some signs of nephrotoxicity [5,6]. In addition, gentamicin nephrotoxicity accounts for 10-15% of all cases of acute renal failure [7] and nearly 10-25% of human patients treated with gentamicin exhibit increased blood urea nitrogen concentration subsequent to a reduction of glomerular filtration rate [3,8]. This gentamicin induced renal injury is related to increase the generation of superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney [4,9,10]. Several natural products have been used to protect the toxicities induced by drugs. Herbs

are generally considered safe and proved to be effective against various human ailments and their medicinal uses have been gradually increasing in developed countries [11]. Natural antioxidants strengthen the endogenous antioxidants defenses and restore the optimal balance by neutralizing reactive species [12]. Curcumin as one of the naturally occurring dietary substances has been used since ancient times for promoting human health [13]. Curcumin is a major yellow pigments in rhizomes of *curcuma longa* linn which is used widely as a spice and coloring agent in several foods [13-15]. It represents a class of anti-inflammatory and anti-oxidant reported to be a potent inhibitor of ROS formation [14,16]. Rosemary (*Rosmarinus Officinalis*) is a herb commonly used as spice and flavoring agent in food processing. It is composed of dried leaves and flowers constitutes a particularly interesting source of biologically active phytochemicals as it contains a variety of phenolic compounds including carnosol, carnosic acid, rosmanol, 7-methyl-epirosemanol, isorosmanol, rosmadial and caffeic acid [12,17]. Rosemary has anti-inflammatory action [18]. Propolis is resinous natural product collected from cracks in the bark of trees and leaf buds which are enriched with the salivary enzymes of honey bees. It has gained popularity and was used extensively in healthy drinks and foods to improve well-being and prevent diseases such as inflammation, heart diseases, diabetes and even cancer [19-21]. It has more than 180 compounds including flavonoids, phenolic acids and its esters [20,21]. Melatonin and caffeic acid phenethyl ester (CAPE) are compounds of honey bee propolis, that were recently found to be potent free radical scavengers and antioxidants [22]. Propolis possesses several biological properties such as anti-inflammatory, antioxidant, anticancer, antibiotic and antifungal activities [19-21]. Most of the previous literatures studied the protective effects of one antioxidant substance on the biochemical nephrotoxicity of the gentamicin but not much of them which studied the morphological alterations. Also, to our knowledge, the evidence reporting the protective effect of rosemary and propolis against gentamicin induced nephrotoxicity are very few.

2. Aim of the Work

The present work aimed to evaluate effectiveness of different natural materials (curcumin, rosemary and propolis) against the histological and biochemical alterations of gentamicin induced nephrotoxicity in guinea pigs.

3. Materials and Methods

3.1. Chemicals

Gentamicin and curcumin were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). Rosemary was purchased as dried rosemary leaves from a herbal store in Zagazig city, Egypt. Aqueous rosemary extract was prepared according to the method of Amin and Hamza [23]. Briefly,

ten gm of dried plants was slowly boiled in 100 ml of distilled water and heated for 30 minutes. The extracts were then filtered and directly administered orally by gavage to the animals. The given dose was 220 mg/kg b. wt. Crude propolis was obtained from honey bee colonies in the Faculty of Agriculture, Zagazig University, Egypt. Aqueous propolis extract was prepared according to the method of El-khayat et al. [24]. Briefly, propolis was kept dry and freeze-dried (-40 °C) until used. Propolis samples were mixed with distilled water, heated gently and filtered through Whatman no:1 filter paper. Propolis was freshly prepared and administered to animals orally by gavage at a dose of 100 mg/kg b. wt. Gentamicin was injected intraperitoneally at the dose of 100 mg/kg b. wt for ten successive days on the bases that the nephrotoxicity occurs with a wide range of gentamicin doses (20-180 mg/kg b. wt, as found by Elfarra et al. [25], Kumar et al. [26], Ali et al. [27] and Cuzzocrea et al. [9]. The choice of the doses of curcumin, rosemary and propolis were based on the results of the previous studies, where the antioxidant effects of these agents were confirmed. Curcumin was given orally at a dose of 200 mg/kg b. wt by gavage [28,29]. Rosemary was given at a dose of 220 mg/kg b. wt orally by gavage [30,31] and the propolis was given at a dose of 100 mg/kg b. wt orally by gavage [24].

3.2. Animals

48 adult male guinea pigs (*Cavia porcellus*) weighting 480-530 gm were used for this study. The animals were obtained from animal house unit in the Faculty of Veterinary Medicine, Zagazig University, Egypt. The animals were housed in a room under standard conditions of ventilation, temperature (25 ± 2°C), humidity (60-70%) and light/dark condition (12/12). The animals were provided with tap water *ad libitum* and fed with the standard commercial chow. The animal procedures were performed in accordance with Guide Lines for Ethical Conduct in the Care and Use of Animals.

3.3. Experimental Design

After one week of acclimation, the animals were randomized and divided into 8 groups (6 guinea pigs for each) as follow:

Group 1 (control group): The animals received intraperitoneal injection of saline (0.5 ml/day for 10 days).

Group 2 (curcumin only): The animals received curcumin (200 mg/kg b. wt /day) orally by gavage for 10 days.

Group 3 (rosemary only): The animals received rosemary (220 mg/kg b. wt /day) orally by gavage for 10 days.

Group 4 (propolis only): The animals received propolis (100 mg/kg b. wt /day) orally by gavage for 10 days.

Group 5 (gentamicin treated group): The animals received intraperitoneal injection of gentamicin only (100 mg/kg b. wt /day) for 10 days.

Group 6 (gentamicin/curcumin co-administered): The animals received intraperitoneal injection of gentamicin (100 mg/kg b. wt /day) concurrently with curcumin (200

mg/kg b. wt /day) orally for 10 days.

Group 7 (gentamicin/rosemary co-administered): The animals received intraperitoneal injection of gentamicin (100 mg/kg b. wt /day) concurrently with rosemary (220 mg/kg b. wt /day) orally for 10 days.

Group 8 (gentamicin/propolis co-administered): The animals received intraperitoneal injection of gentamicin (100 mg/kg b. wt /day) concurrently with propolis (100 mg/kg b. wt /day) orally for 10 days.

At the end of the experimentation and 24 hours after the last dose, the animals were sacrificed by injecting ketamine (intraperitoneal) under general anesthesia, then rapidly dissected and subjected to the following examinations:

3.3.1. Histological Examination

The kidneys were exposed by mid line incision and then rapidly dissected from the surrounding structures. Kidney specimens were obtained and fixed in buffered 10 % formaldehyde solution for 24 hours and processed for paraffin sections of 5 micron thickness. The sections were stained with Hematoxylin and Eosin and examined under binocular light microscope (Zeiss)[32].

3.3.2. Biochemical Analysis

Blood samples were drawn by cardiac puncture and centrifuged at 3000 rpm for 15 minutes to harvest the serum with which the renal function assessment were analyzed. The levels of urea, creatinine, and uric acid were assessed in the sera of control and experimental animals [33,34].

Statistical analysis

The values were presented as means \pm SD of different groups. Differences between the mean values were estimated using one way ANOVA. The results were considered statistically significant when $p < 0.05$.

4. Results

Histologically, by light microscopic examination, the kidney appeared with normal structures in normal control and positive control animals (curcumin, rosemary, and propolis administered). The glomerulus appeared normal with intact Bowman's capsule and Bowman's space. The proximal convoluted tubules appeared in cross and longitudinal sections with intact limiting basement membrane and their lumens showed intact brush borders. The distal convoluted tubules showed wide lumens with low cuboidal lining cells. An afferent arteriole appeared near the glomerulus (Fig. 1).

In gentamicin treated animals, the proximal convoluted tubules showed degenerated epithelial lining with disruption of their brush borders and presence of epithelial debris inside their lumens. The renal corpuscle appeared with degeneration of the glomerulus and disrupted Bowman's capsule. The afferent arteriole appeared near the glomerulus with thickening in its wall and degeneration of endothelial lining. Extensive perivascular infiltration of inflammatory cells and massive interstitial hemorrhage were seen (Fig. 2)

In animals co-administered with gentamicin and

curcumin, most of the proximal convoluted tubules appeared with normal epithelial lining and regained their brush borders. Some tubules still have diminished brush borders. The glomerulus appeared normal with intact Bowman's capsule (Fig. 3).

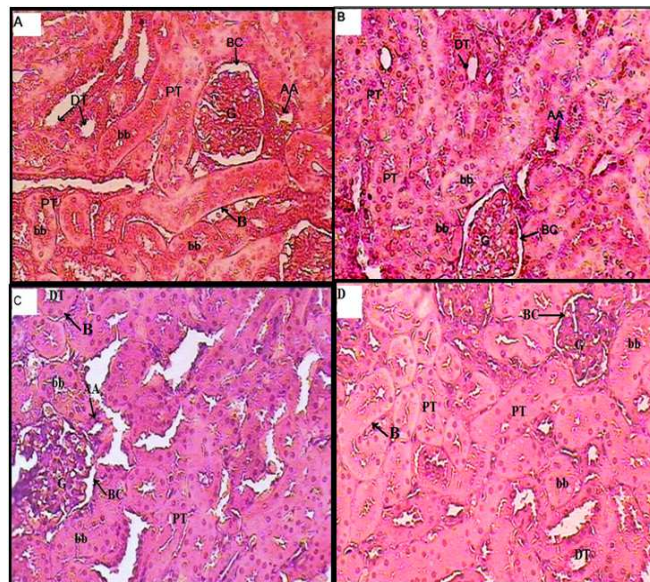


Fig 1. Light micrograph of sections in the kidney of control guinea pigs; A: negative control (administered saline), B: positive control (administered curcumin only), C: positive control (administered rosemary only), D: positive control (administered propolis only). Glomerulus (G), Bowman's capsule (BC), proximal convoluted tubules (PT), basement membrane (B) brush border (bb), distal convoluted tubules (DT), afferent arteriole (AA) (Haematoxylin & Eosin $\times 400$)

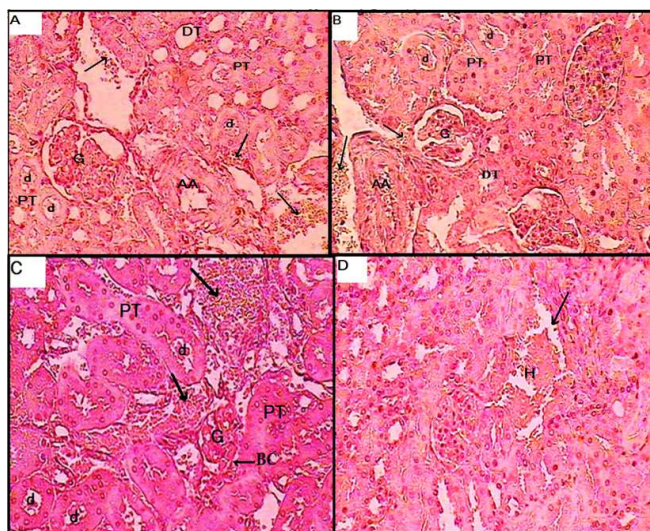


Fig 2. Light micrograph of sections in the kidney of gentamicin treated guinea pigs. A&B: The proximal convoluted tubules (PT) show degenerated epithelial lining with epithelial debris (d) inside the lumens. The distal convoluted tubules (DT) appear normal. The glomerulus (G) is degenerated. The afferent arteriole (AA) shows thickening in the wall and degeneration of endothelial lining with perivascular infiltration of inflammatory cells (arrows). C: Shows degenerated glomerulus with disrupted Bowman's capsule (BC). Epithelial debris (d) appears inside lumens of the proximal convoluted tubules (PT). Extensive inflammatory cell infiltrations appear in the interstitial tissues (arrows). D: Congested intertubular capillary (arrow) is seen with massive hemorrhage (H). (Haematoxylin & Eosin $\times 400$)

In animals co-administered with gentamicin and rosemary, the proximal convoluted tubules appeared with normal epithelial lining and regained their brush borders. The glomerulus appeared more or less normal with intact Bowman's capsule (Fig. 4).

In animals co-administered with gentamicin and propolis, most of the proximal convoluted tubules appeared with normal epithelial lining with their brush borders, but some tubules appeared regenerating with disrupted brush borders. The glomerulus appeared normal with intact Bowman's capsule (Fig. 5)

Biochemically, the levels of serum urea, creatinine and uric acid increased in the gentamicin treated group in comparison with control group with significant differences. The values decreased in the co-administered gentamicin and curcumin, gentamicin and rosemary and gentamicin and propolis with significant differences (Table 1 and Figs. 6,7,8)..

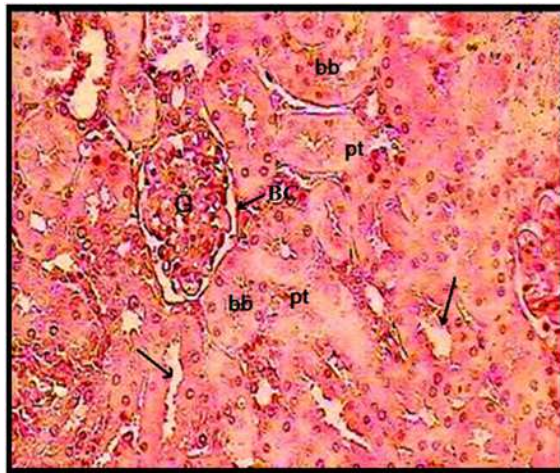


Fig 3. Light micrograph of a section in the kidney of gentamicin treated guinea pigs co-administered curcumin. Most of the proximal convoluted tubules (PT) appear with normal epithelial lining and regained their brush borders (bb). Some tubules still have diminished brush borders (arrows). The glomerulus (G) appears normal with intact Bowman's capsule (BC). (Haematoxylin & Eosin $\times 400$)



Fig 4. Light micrograph of a section in the kidney of gentamicin treated guinea pigs co-administered rosemary. The proximal convoluted tubules (PT) appear with normal epithelial lining and regained their brush borders (bb). The glomerulus (G) appears more or less normal with intact Bowman's capsule (BC). (Haematoxylin & Eosin $\times 400$)

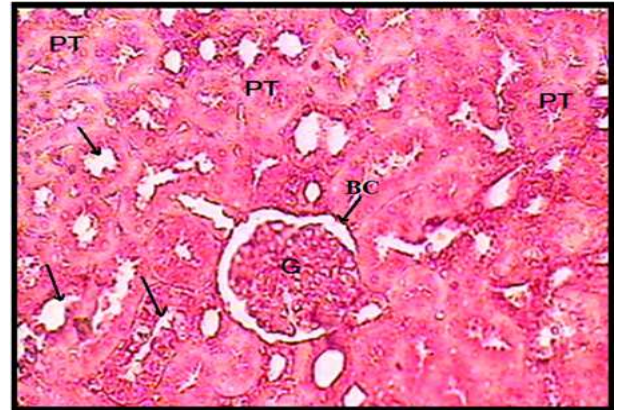


Fig 5. Light micrograph of a section in the kidney of gentamicin treated guinea pigs co-administered propolis. Most of the proximal convoluted tubules (PT) appear with normal epithelial lining with their brush borders, but some tubules appear regenerating with disrupted brush borders (arrows). The glomerulus (G) appears normal with intact Bowman's capsule (BC). (Haematoxylin & Eosin $\times 400$)

Table 1. Effect of curcumin, propolis and rosemary on serum urea, creatinine, and uric acid in the kidney of gentamicin treated male guinea pigs (*Cavia porcellus*) in different groups.

Group	Urea (mg/dl) Mean \pm SD	Creatinine (mg/dl) Mean \pm SD	Uric acid(mg/dl) Mean \pm SD
Control	21.2 \pm 2.9	0.525 \pm 0.019	1.317 \pm 0.248
Curcumin	16.3 \pm 1.03	0.503 \pm 0.014	1.883 \pm 0.117
Propolis	16.2 \pm 1.17	0.302 \pm 0.016	1.45 \pm 0.105
Rosemary	15.7 \pm 0.75	0.31 \pm 0.013	1.183 \pm 0.075
Gentamicin	54.2 \pm 1.2*	1.358 \pm 0.308*	3.283 \pm 0.279*
Gentamicin + Curcumin	29.8 \pm 3.2**	0.618 \pm 0.025**	2.117 \pm 0.116**
Gentamicin+ Propolis	27.8 \pm 1.2**	0.36 \pm 0.014**	1.983 \pm 0.014**
Gentamicin+ Rosemary	24.5 \pm 1.9**	0.448 \pm 0.036**	1.85 \pm 0.187**

* $p < 0.05$ when compared to control group, ** $p < 0.05$ when compared to gentamicin treated group, all data are mean of 6 animals

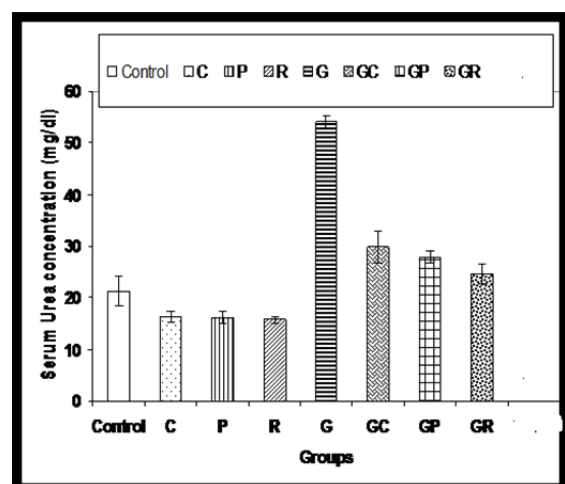


Fig 6. The serum urea level in different animals groups. The level is the highest in gentamicin treated group (G) in comparison with control groups; normal control, curcumin treated (c), propolis treated (p), and rosemary treated (R). The level shows declining in co-administered gentamicin and curcumin (GC), gentamicin and propolis (GP) and gentamicin and rosemary (GR).

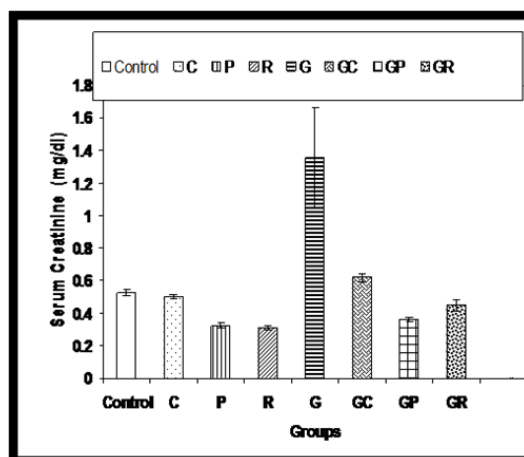


Fig 7. The serum creatinine level in different animals groups. The level is the highest in gentamicin treated group (G) in comparison with control groups; normal control, curcumin treated (C), propolis treated (p), and rosemary treated (R). The level shows declining in co-administered gentamicin and curcumin (GC), gentamicin and propolis (GP) and gentamicin and rosemary (GR).

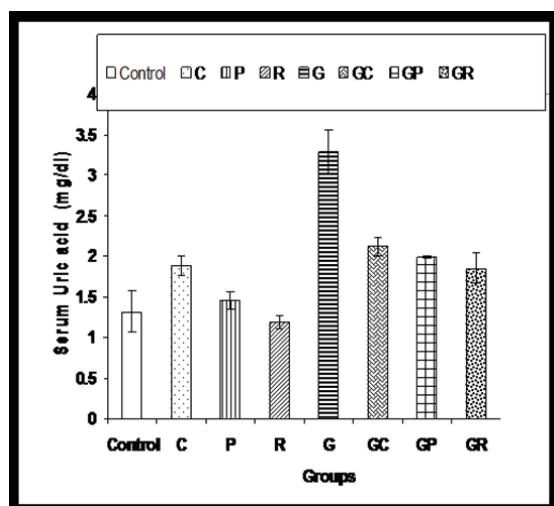


Fig 8. The serum uric acid level in different animals groups. The level is the highest in gentamicin treated group (G) in comparison with control groups; normal control, curcumin treated (C), propolis treated (p), and rosemary treated (R). The level shows declining in co-administered gentamicin and curcumin (GC), gentamicin and propolis (GP) and gentamicin and rosemary (GR).

5. Discussion

In the present study, the gentamicin had adverse effects on the kidney structure mainly on the proximal convoluted tubules, renal corpuscle, vessels, and interstitial tissues. The proximal tubules showed degeneration of the epithelial lining with disruption of their brush borders and presence of epithelial debris in the lumen. The renal corpuscles showed degeneration in the glomerulus and disrupted Bowman's capsule. The vessels showed thickening of the wall and degeneration of the endothelial lining. The interstitial tissues showed perivascular infiltration of inflammatory cells and interstitial massive hemorrhage. This is in agreement with many authors who reported the toxicity of gentamicin on the

kidney. Padmini and Kumar [35] observed epithelial cell degeneration and granular deposits in tubular lumens with evidence of tubular epithelial cell desquamation and lymphocytic infiltration around the proximal convoluted tubules. Manikandan et al. [36] observed damaged glomerular structure, tubular necrosis, thickening of capillary walls and atrophy of glomerular tuft. Lakshmi and Sudhakar [37] observed epithelial desquamation in proximal tubules, tubular necrosis, epithelial oedema and glomerular hypertrophy. Souza et al. [38] observed apoptosis, intracellular oedema, basement membrane interruption, glomerular narrowing of the Bowman's capsule and acute tubular necrosis. Ali and Bashir [39] and Elfarra et al. [25] observed severe and complete necrosis of the proximal tubules throughout the cortex. Nale et al. [40] observed degenerative changes in glomeruli with hypercellularity and also atrophy of the glomeruli. Few areas showed interstitial hemorrhage and infiltration of mononuclear cells. Kang et al. [41] observed diffuse cellular necrosis in the proximal tubules. In addition, the lumens of the tubules were filled with degenerated and desquamated cells, massive hyaline cast and severe inflammatory cell infiltrations. The finding of the present study supported by the previous literatures indicated that the gentamicin has affinity to the proximal tubules without distal tubules affection. On the other hand, Nale et al. [40] observed tubular changes like diffuse tubular swelling and loss of tubular epithelium which were prominent at many foci in both proximal and distal tubules. Lakshmi et al. [42] observed epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells. Some literatures recorded slight effects on the distal tubules. Ullah et al. [43] observed loss of the cellular pattern with the presence of necrosis mostly in the proximal tubule in rabbit kidney treated with gentamicin. Alarifi et al. [44] found that the tubular damage is more prominent in proximal tubules than distal tubules. This predominant toxicity in the proximal tubules is caused by taking up the aminoglycosides into the epithelial cells of the renal proximal tubules and stay for a long time which lead to nephrotoxicity [45]. Also, the accumulation of gentamicin in proximal renal tubules leads to brush border network damage [46]. The proximal convoluted tubules are the primary sites for re-absorption and active transport. This leads to a higher concentration of gentamicin in the epithelial lining of these tubules and suggests that the gentamicin toxicity is related to its accumulation in the proximal tubules [44,47]. Also, the tubular necrosis were observed without any changes in glomerular structure [9].

Many authors explained the mechanisms of gentamicin induced nephrotoxicity. The gentamicin administration is suggested to increase renal cortical lipid peroxidation, nitric oxide generation and mitochondria hydrogen peroxide production and all these molecules induce cellular injury and necrosis by several mechanisms, including peroxidation, protein denaturation and DNA damage [10,48]. Also, the gentamicin induces renal tubular damage via energy depletion in renal tubular cells beside inducing of oxidative

stress [49]. In addition, It induces cellular injury and necrosis by reducing the efficiency of antioxidant enzymes in the kidney such as superoxide dismutase, catalase, glutathione peroxidase and glutathione [50]. Gentamicin binds to the phospholipids of the cell membrane of the renal tubules and enters inside the cells, then it binds to subcellular organelles, alters the mitochondrial respiration and small amount may be taken up by lysosomes [51]. Moreover both, lysosomes and mitochondria have been shown to send death signals through the activation of specific stress sensors. Also, lysosomes membrane rupture and release of acid hydrolases contribute to apoptosis and necrosis of proximal tubular cells [52]. In the present study, co-administration of curcumin in animals treated by gentamicin ameliorated the structural changes of the kidney. This is in agreement with Manikandan et al. [36] who found that curcumin affords curative role against nephrotoxicity induced gentamicin exposure and reduces gentamicin induced renal injury and this is supported by Biswas et al. [53] who found that curcumin has anti-inflammatory and antioxidant properties with a potent ability to inhibit reactive oxygen species formation.

In the present study, co-administration of rosemary to animals treated with gentamicin regained the structural changes to normal. This is in agreement with Tavafi and Ahmadvand [54] who found that co-treatment of gentamicin and rosmarinic acid significantly decreased the tubular necrosis. Also, the rosemary aqueous extract alleviates the nephrotoxicity induced by CCL4 in albino rats [31]. The protective effect of rosemary can be explained that rosemary extract has a high scavenging capacity of different types of reactive oxygen and nitrogen species, mostly free radicals, as thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals [55]. Also, the rosemary aqueous extract alleviates the toxicity induced by lead on the kidney through stimulation of endogenous antioxidant defense system [56]. In addition, rosemary has anti inflammatory action [18].

In the present study, co-administration of propolis to gentamicin treated animals improves the structural changes induced by gentamicin. This is in agreement with Osman and Tantaway [57] who observed that oral administration of propolis extract to rabbit significantly protected against histopathological alterations induced by gentamicin. Atta et al. [58] found that propolis ameliorated the renal alterations induced by gentamicin administration as indicated by maintenance of the activity of antioxidant enzymes. Park and Kahng [59] found that propolis extract had profound anti-inflammatory effects on both chronic and acute inflammations.

Functionally, the levels of serum urea, creatinine, and uric acid increased in gentamicin treated group in comparison with control groups. This is in agreement with Moghaddam et al. [60], Soliman et al. [61] and Sivachandran and Hariharan [62] who found that the gentamicin nephrotoxicity is functionally characterized by increase in serum creatinine, urea, and blood urea nitrogen.

Co-administration of curcumin, rosemary, and propolis ameliorated the renal function. This is in agreement with Salem et al. [63] who found that co-administration of propolis with gentamicin decreased the rise in blood urea and serum creatinine. This effect is probably due to the antioxidant protective effect of propolis which could have accumulated in the cells of the proximal convoluted tubules of the kidney where propolis was reported to be collected and secreted [64]. Also, co-treatment of gentamicin and rosmarinic acid significantly decreased serum creatinine and urea [54].

6. Conclusion

It can be concluded that, the gentamicin has adverse effects on the kidney. Different natural materials as curcumin, rosemary, and propolis were able to protect against these effects. So, the patients should be advised to take one of these material while they are treated by gentamicin.

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