

<b>Original Article</b>	<b>Can Curcumin Prevent Gentamicin-Induced Nephrotoxicity in Rats? A Histological Study</b> <i>Ashraf M. F. Kamel</i> <i>Histology Department, Faculty of Medicine, Cairo University</i>
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### ABSTRACT

**Background:** Free oxygen radicals play an important role in the pathogenesis of gentamicin (GEN) nephrotoxicity and curcumin (CMN) has a strong antioxidant activity.

**Aim of the Work:** The possible protective role of CMN on GEN-induced nephrotoxicity was investigated in albino rats by histological and morphometric methods.

**Materials and Methods:** Thirty-six adult male albino rats were randomly divided into six equal groups. Group I: Control group received intraperitoneal (i.p.) injection of 0.9% saline, Group II received 1 ml corn oil orally for 21 days, Group III received 200 mg/kg CMN dissolved in corn oil orally for 21 days. Group IV received 100 mg/kg GEN dissolved in 0.9% saline i.p. for seven days from day 14, Group V received corn oil for 21 days and GEN for seven days from day 14 and lastly Group VI received CMN for 21 days and GEN from day 14 for seven days. At the end of the experiment, animals were weighed, sacrificed and the kidneys were dissected out, then processed for light and electron microscopic studies. Morphometric and statistical analysis were also performed.

**Results:** Treatment with GEN led to significant limitation of weight gain, hyaline degeneration and nuclear pyknosis in the renal proximal tubules, shrinking of glomeruli and weak PAS reaction. Ultrastructurally, the proximal tubules exhibited loss of microvilli, thickened tubular basement membrane, condensed nuclei, rarefied cytoplasm with dark bodies and reduced number of mitochondria. Podocytes displayed fusion of secondary foot processes and thickening of the glomerular basement membrane with focal loss of its trilamellar structure. Concomitant administration of CMN with GEN revealed an observable protection against these changes.

**Conclusion:** CMN showed a protective effect against nephrotoxicity induced by GEN in albino rats.

**Key Words:** Gentamicin, curcumin, nephrotoxicity, oxidative stress, antioxidant, histology, ultrastructure.

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### INTRODUCTION

Gentamicin (GEN) is an aminoglycoside class of bactericidal antibiotics that is very effective in treating life-threatening gram-negative infections (Bentley *et al.*, 2010). However, nephrotoxicity is the major side effect of aminoglycosides accounting for 10–15% of all cases of acute renal failure (Shifow *et al.*, 2000). Previous studies have also shown that 30% of the patients treated with gentamicin for more than 7 days show some signs of nephrotoxicity (Appel, 1990). In fact, renal function is usually closely monitored in the in-patient setting, during therapy. Despite the introduction of newer and less toxic antibiotics, gentamicin continues to serve a useful clinical

role in the treatment of serious enterococcal, mycobacterial and Gram-negative infections, due to its high efficacy, low cost, and the low levels of bacterial resistance (Pedraza-Chaverri *et al.*, 2003).

It has been shown that the specificity of GEN renal toxicity is related to its selective preferential accumulation in the renal proximal convoluted tubules and in lysosomes (Nagai & Takano, 2004). It has been reported that GEN-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule that leads to loss of its brush border integrity (Pedraza-Chaverri *et al.*, 2003).

Nephrotoxicity induced by GEN is a complex phenomenon leading to the development of an array of morphological and functional alterations, which include glomerular lesions that interfere with glomerular hemodynamics and proximal tubule injury ranging from alterations in tubular transport to necrosis of tubular epithelium followed by deterioration of renal functions and then acute renal failure (*Stojiljkovic et al., 2008-a*).

However, the mechanism of GEN-induced nephrotoxicity is not completely known. *Balakumar et al. (2010)* reported that GEN induced nephrotoxicity by specifically inhibiting protein synthesis in renal proximal tubular cells resulting in acute tubular necrosis, followed by acute renal failure. Moreover, GEN has been shown to increase the generations of reactive oxygen species (ROS) like superoxide anions ( $O_2^-$ ), hydroxyl radicals (OH) and hydrogen peroxides ( $H_2O_2$ ), as well as reactive nitrogen species (RNS) in the renal cortex that eventually lead to renal structural and functional deterioration (*Wang et al., 2006; Martinez-Salgado et al., 2007; Balakumar et al. 2008*). Generally, ROS have the capacity to cause cell death by necrosis or apoptosis (*Klamt et al., 2009*).

The oxidative damage had been supported by the results of administration of several compounds with antioxidant properties and/or ROS scavengers which were able to ameliorate the severity of GEN-induced renal damage (*Erdem et al., 2000; Maldonado et al., 2003; Pedraza-Chaverri et al., 2003; Parlakpinar et al., 2005; Ali et al., 2005; Balakumar et al., 2010*).

*Parlakpinar et al. (2005)* believed that because of nephrotoxicity, the safety of aminoglycosides was lagging behind that of other wide-spectrum antibiotics. Accordingly, the value and utility of gentamicin in clinical practice would be greatly increased if some means could be found to protect the kidney against GEN-induced damage.

The levels of antioxidants synthesized in the body cannot be manipulated by simple means. On the other hand, the levels of antioxidant vitamins can be increased easily by dietary means or supplementation (*Gutteridge & Halliwell, 2000*). Trends on applying nutri-

tional antioxidants in diseases related to oxidative stress have gained immense interest in recent years. Plant products are known to exert their protective effects by scavenging free radicals and modulating the antioxidant defense system.

Curcumin (CMN) is a major yellow pigment and the active constituent in the ground rhizome turmeric of *Curcuma longa* Linn. It is widely used as a spice, food preservative and herbal medicine in different countries (*Okada et al., 2001; Joe et al., 2004*). It has a broad spectrum of biological activities such as anti-inflammatory, anti-neoplastic, antimutagenic and antioxidant activities with no reported side effects (*Naik et al., 2004; Jagetia, 2007*).

Curcumin acts as a scavenger of free radicals and is considered to be an effective antioxidant against oxidative tissue damage. It can significantly inhibit the generation of ROS both in vitro and in vivo (*Biswas et al., 2005; Okada et al., 2001*). More specifically, curcumin significantly decreases the levels of free oxygen radicals and this protective effect of CMN is attributed to its free radical scavenging activity in addition to its ability to induce detoxification enzymes (*Manikandana et al., 2004*).

Thus, the aim of the present study was to investigate any possible protective effect of CMN supplementation on GEN-induced nephrotoxicity in albino rats by light and electron microscopic techniques.

## MATERIALS AND METHODS

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### Drugs and Chemicals

Gentamicin sulfate, available commercially as Epigent<sup>®</sup> (80 mg/2 ml ampoules), was obtained from the Egyptian International Pharmaceutical Industries Co. (EIPICO, 10<sup>th</sup> of Ramadan City, Egypt).

Curcumin (diferuloylmethane) [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] was obtained from Sigma chemical company (St Louis, MO, USA).

All other chemicals used were of good quality and highest analytical grade.

## Animals

This study was carried out on thirty-six adult male albino rats weighing 200–220 g. The rats were obtained from the Animal House of the Faculty of Medicine, Cairo University. Animals were housed in clean stainless steel cages under standard conditions of humidity, temperature and fed with standard diet and allowed water ad libitum. All animals were treated in accordance with the international guidelines for the care and use of laboratory animals.

## Treatment and experimental design:

Rats were divided into six equal groups (6 rats each). Groups I, II and III were used as control while groups IV, V and VI were experimental groups. Animals were treated according to the following:

- **Group I – Control Group:** Received intraperitoneal (i.p.) injection of 0.9% saline for 7 days, at the same volume as GEN-treated rats.
- **Group II – Corn oil-treated group:** Received daily 1 ml corn oil orally by gastric gavage for 21 days.
- **Group III – CMN treated group:** Received CMN (200 mg/kg body weight [bw] /day) dissolved in corn oil and administered to animals orally by gastric gavage for 21 days (Chuang et al., 2000).
- **Group IV – GEN-treated group:** Received GEN in 0.9% saline at a dose of 100 mg/kg bw /day i.p. for 7 days. (Karadeniz et al., 2008).
- **Group V – GEN and corn oil (CMN vehicle) treated group:** Received GEN (100 mg/kg bw /day) i.p. for 7 days and 1 ml corn oil daily orally by gastric gavage for 14 days before GEN and then continued concomitantly with GEN treatment (i.e. for 21 days).
- **Group VI – GEN and CMN-treated group:** Received GEN (100 mg/kg bw /day) i.p. for 7 days and CMN (200 mg/kg bw /day) orally by gastric gavage for 14 days before GEN and then continued concomitantly with GEN treatment (i.e. for 21 days).

All animals were carefully observed for any abnormal appearance or behavior during the treatment. Twenty-four hours after the administration of the last dose (on the 22<sup>nd</sup> day), rats were weighed then sacrificed by decapitation under light isoflurane anesthesia. The kidneys were dissected out and small specimens were selected for Electron Microscopy (E.M.) while the rest of the organ specimens were immediately fixed in 10% formol saline. Paraffin sections of 5µm thickness were prepared and stained with hematoxylin and eosin (Hx. & E.) and PAS reaction (Bancroft & Gamble, 2002). For E.M., the chosen kidney specimens were trimmed into 1 mm<sup>3</sup> pieces, fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.4) and post-fixed in 1% osmium tetroxide. Ultrathin sections (60–70 nm) were mounted on copper grids and contrast-stained with uranyl acetate and lead citrate (Hayat, 2000). Transmission electron microscopic (T.E.M.) analysis was carried out by a JEM-1400A transmission electron microscope (JEOL, Tokyo, Japan) operated at 80 kV at the Faculty of Agriculture Research Park, Cairo University.

## Body weight analysis:

The body weights of all rats were recorded at the beginning of the experiment and just before sacrifice. The percentage of change in body weight for each animal was calculated by the following formula (Ali et al., 2005):

$$\frac{\text{Final body weight} - \text{initial body weight}}{\text{Initial body weight}} \times 100$$

## Quantitative morphometric analysis:

The measurements were obtained using Leica Qwin 500 image analyzer computer system (England) connected to the light microscope for the following parameters:

**1. Glomerular morphometry:** In ten Hx. and E.-stained sections, 10 renal corpuscles from the renal cortex of each rat were chosen randomly at a magnification of x100. This allowed analysis of 100 glomeruli per rat. The area of the renal corpuscle and the area of its glomerulus were measured, using the interactive measurement menu of the image analysis software. The area of the urinary space was calculated by subtracting

the area of the glomerulus from the area of the renal corpuscle (Stojiljkovic et al., 2008-a).

**2. The optical densities of PAS stained sections:** this was measured in ten fields per specimen at a magnification of x100.

#### Statistical Analysis:

The areas of glomeruli, renal corpuscles and urinary spaces as well as the optical densities of the PAS reaction in the different groups were presented as mean and standard deviation ( $\pm$ SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey HSD test to compare variables among the different groups. A value of  $P < 0.05$  was considered significant. Data was tabulated and represented graphically.

## RESULTS

The body weights as well as the histological and morphological results were similar in

the control groups (I, II & III) and thus were considered as one group (control group). Similarly, both the GEN group and GEN plus CMN vehicle groups (groups IV & V) had the same features and accordingly they were also regarded as one group (GEN-treated group).

No deaths or remarkable signs of external toxicity were observed in the groups of rats given GEN either alone or in combination with CMN or corn oil. However, decreased food intake was noticed in rats which received GEN alone and GEN plus corn oil (groups IV & V).

#### Body weight:

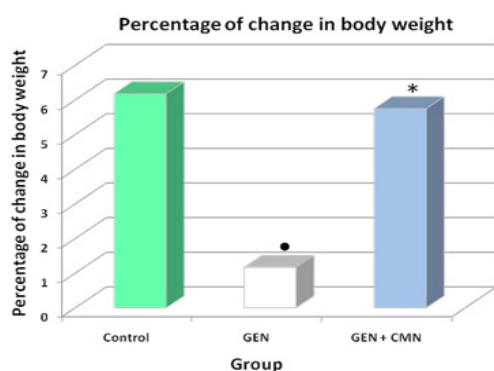
GEN administration significantly decreased ( $P < 0.05$ ) the percentage of change in body weight (between the initial and final body weights) as compared to the control group. Concomitant administration of CMN with GEN significantly increased the percentage of change in body weight ( $P < 0.05$ ) as compared to the GEN-treated group. (Table 1; Histogram 1).

**Table 1:** Mean values  $\pm$ SD of initial and final body weights of rats in the different groups and the percentage of change in body weights

Group	Initial body weight (g) (mean $\pm$ SD)	Final body weight (g) (mean $\pm$ SD)	Change (%) (mean $\pm$ SD)
Control group	212.83 $\pm$ 5.53	225.94 $\pm$ 3.99	6.18 $\pm$ 1.48
GEN group	207.5 $\pm$ 7.18*	209.92 $\pm$ 7.42*	1.17 $\pm$ 0.72*
GEN+CMN group	210.17 $\pm$ 6.46*	222.25 $\pm$ 7.52*	5.75 $\pm$ 1.33*

\* Significant as compared to control group ( $P < 0.05$ )

\* Significant as compared to GEN-treated group ( $P < 0.05$ )



**Histogram 1:** Comparison between the mean values of the percentage of change in body weights in the different groups

● Significant as compared to control group ( $P < 0.05$ )

\* Significant as compared to GEN-treated group ( $P < 0.05$ )

#### I. Histological results:

Light microscopic examination of Hx.&E.-stained sections of the kidneys of control rats showed the renal corpuscles, proximal and distal convoluted tubules in the cortex. Each renal corpuscle consisted of a glomerular capillary tuft surrounded by Bowman's capsule. The capsule consisted of a parietal layer of simple squamous epithelium and a visceral layer enveloping the capillaries of the glomerulus. Between the two layers of the capsule, there was the Bowman's (urinary) space. The proximal convoluted tubules had narrow lumen and were lined by simple cuboidal epithelium. The cells had acidophilic cytoplasm and large rounded nuclei. The distal tubules

had wider lumen and less acidophilic cytoplasm than the proximal tubules (Fig. 1). The medulla showed thin descending limbs of the loops of Henle were regular, rounded and lined by simple squamous epithelium whose nuclei protruded slightly into the lumen. The collecting tubules and thick ascending limb of loop of Henle were wider and less regular in shape and were lined by simple cuboidal epithelium (Fig. 2). A strong positive PAS reaction was seen in the apical and basal membranes of the proximal and distal tubule cells and brush border in proximal tubules. Distal tubule cells were more lightly stained and had wider lumen than the proximal tubules. The basement membrane of the parietal layer of the Bowman capsules and glomerular tufts showed a strong positive PAS reaction (Fig. 3).

Cortical renal sections stained with Hx.&E. from GEN-treated rats showed small sized glomeruli with narrowing of the Bowman's space. The proximal tubules displayed hyaline degeneration and dark nuclei of the tubular cells while the distal tubules showed hyaline casts more than the proximal tubules. Some blood vessels appeared congested (Figs. 4, 5). The medulla showed vacuolar degeneration, cell desquamation and hyaline casts in the collecting tubules as well as interstitial congestion (Fig. 6). The PAS reaction was weak in the apical membrane but was moderate in the basal membrane of the proximal and distal convoluted tubular cells. A moderate reaction in the distorted glomeruli was also observed (Fig. 7).

On the other hand, the renal sections stained with Hx.&E. from rats treated with both GEN and CMN showed that apart from mild congestion of blood vessels and slightly reduced size of renal corpuscles, there was a nearly similar picture to that of the control of the glomerular capillary tufts, proximal and distal convoluted tubules as well as the loops of Henle and collecting tubules (Figs. 8, 9). A strong positive PAS reaction was seen in the apical and basal membranes of the proximal and distal tubule cells as well as in the glomerular tufts comparable to the control (Fig. 10).

Electron microscopic examination of ultrathin sections of the kidneys of control rats showed that the proximal convoluted tubular

cell displayed numerous tall apical microvilli with many pinocytotic vesicles of various sizes and lysosomes in the cytoplasm immediately beneath the microvilli. The nucleus was rounded and displayed extended chromatin and prominent nucleolus. The tubular basement membrane was thin and the basal cell membrane exhibited basal infoldings containing numerous elongated mitochondria arranged at right angles to the basement membrane and exhibiting regular cristae and intact membranes (Fig. 11). A glomerular capillary loop inside the renal corpuscle was seen lined by a thin layer of fenestrated endothelial cytoplasm and contained red blood corpuscles. The nuclei of several podocytes were seen, with their primary processes giving rise to numerous secondary foot processes resting on the glomerular basement membrane. Higher magnification, clarified the three components of the glomerular barrier. The fenestrated capillary endothelium was closely applied to the luminal surface of the glomerular basement membrane; on the opposite side are podocyte's secondary foot processes, separated by filtration slits of uniform width. The central dark and wide lamina densa of the glomerular basement membrane could be seen bordered on each side by a pale and narrower lamina rara (Fig. 12).

The ultrastructure of the kidneys of GEN-treated rats showed distortion and partial loss of the apical microvilli of the proximal convoluted tubules as well as reduction of the pinocytotic vesicles in the apical cytoplasm. The nuclei were irregular with increased quantity of condensed chromatin. The tubular basement membrane was moderately thickened and exhibited disappearance of the basal infoldings. The mitochondria were apparently diminished in number and moved away from the basal membrane, changing from elliptical to become almost circular in outline. The cytoplasm was electron dense in some regions (curved arrows), rarefied in other regions and exhibited a number of dark bodies (Figs. 13, 14). The secondary foot processes of podocytes related to some glomerular capillary loops displayed focal fusion and the glomerular basement membrane exhibited foci of thickening as well as partial loss of its trilamellar structure in some areas (Fig. 15).

Ultrathin kidney sections from rats treated with both GEN and CMN revealed



nearly similar appearance as the control of the proximal convoluted tubular cell with intact apical microvilli, euchromatic nucleus and thin tubular basement membrane displaying basal infoldings containing numerous elongated mitochondria (Fig. 16). Moreover, the secondary foot processes of the podocytes and the glomerular basement membrane had a comparable appearance to that of the control apart from some thickening of the central lamina densa and slight narrowing of lamina rara (Fig. 17).

**II. Morphometric and statistical results:**

**1. Glomerular morphometry:** The mean area in  $\mu\text{m}^2$  ( $\pm\text{SD}$ ) of the glomeruli, renal corpuscles and urinary spaces in the control

groups were 8021 ( $\pm 2496$ ), 11041 ( $\pm 2841$ ) and 3019 ( $\pm 611$ ) respectively (Table 2; Histogram 2).

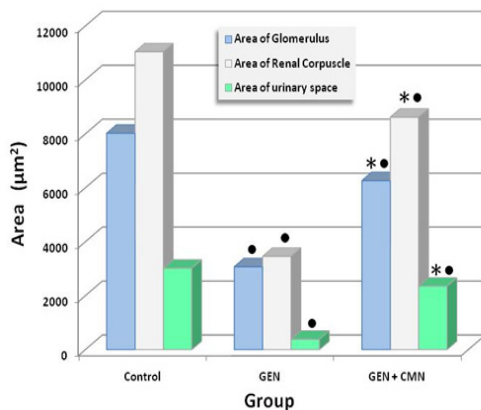
GEN administration significantly decreased ( $P < 0.05$ ) the mean area in  $\mu\text{m}^2$  ( $\pm\text{SD}$ ) of the glomeruli, renal corpuscles and urinary spaces to become 3073 ( $\pm 827$ ), 3457 ( $\pm 957$ ) and 385 ( $\pm 161$ ) respectively compared to the control (Table 2; Histogram 2).

Concomitant administration of CMN with GEN significantly increased ( $P < 0.05$ ) the mean area  $\mu\text{m}^2$  ( $\pm\text{SD}$ ) of the glomeruli, renal corpuscles and urinary spaces to become 6266 ( $\pm 1671$ ), 8619 ( $\pm 2054$ ) and 2352 ( $\pm 696$ ) respectively compared to the GEN-treated group (Table 2; Histogram 2). Meanwhile, these figures were still significantly lower ( $P < 0.05$ ) than the control group.

**Table 2:** Mean values  $\pm$ SD of areas of glomeruli, areas of renal corpuscles and areas of the urinary spaces in the different groups.

	Area of Glomerulus ( $\mu\text{m}^2$ ) (mean $\pm$ SD)	Area of Renal Corpuscle ( $\mu\text{m}^2$ ) (mean $\pm$ SD)	Area of urinary space ( $\mu\text{m}^2$ ) (mean $\pm$ SD)
Control group	8021 $\pm$ 2496	11041 $\pm$ 2841	3019 $\pm$ 611
GEN group	3073 $\pm$ 827*	3457 $\pm$ 957*	385 $\pm$ 161*
GEN + CMN group	6266 $\pm$ 1671**	8619 $\pm$ 2054**	2352 $\pm$ 696**

• Significant as compared to control group ( $P < 0.05$ ).  
 \* Significant as compared to GEN-treated group ( $P < 0.05$ ).



**Histogram 2:** Comparison between the mean values of areas of glomeruli, areas of renal corpuscles and areas of the urinary spaces in the different groups.

• Significant as compared to control group ( $P < 0.05$ )  
 \* Significant as compared to GEN-treated group ( $P < 0.05$ )

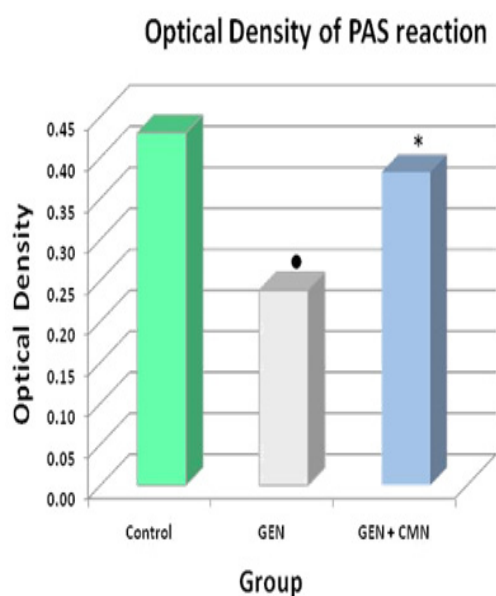
**2. The optical densities of PAS stained sections:** The mean optical density ( $\pm$ SD) of PAS reaction in the control groups was 0.43 ( $\pm$ 0.06). GEN administration significantly decreased ( $P < 0.05$ ) the optical density to 0.24 $\pm$

(0.04) compared to the control. On the other hand, concomitant administration of CMN with GEN significantly increased ( $P < 0.05$ ) the optical density to 0.38 ( $\pm$ 0.04) compared to the GEN treated group (Table 3; Histogram 3).

**Table 3:** Mean values  $\pm$ SD of optical densities of the PAS reaction in the different groups.

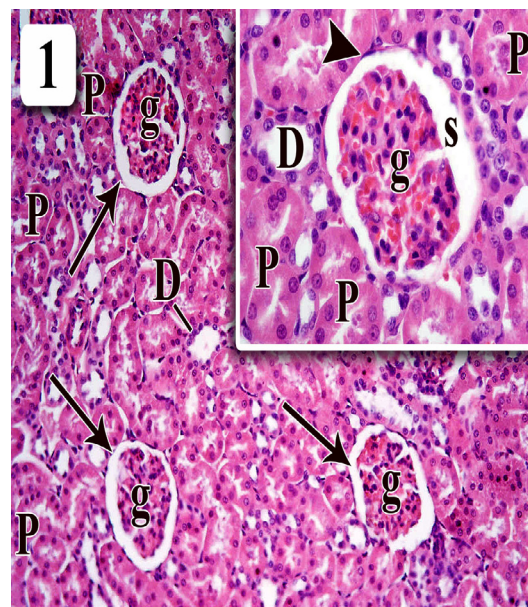
	Control group (mean $\pm$ SD)	GEN group (mean $\pm$ SD)	GEN + CMN group (mean $\pm$ SD)
Optical Density of PAS reaction	0.43 $\pm$ 0.06	0.24 $\pm$ 0.04*	0.38 $\pm$ 0.04*

• Significant as compared to control group ( $P < 0.05$ ).  
\* Significant as compared to GEN-treated group ( $P < 0.05$ ).



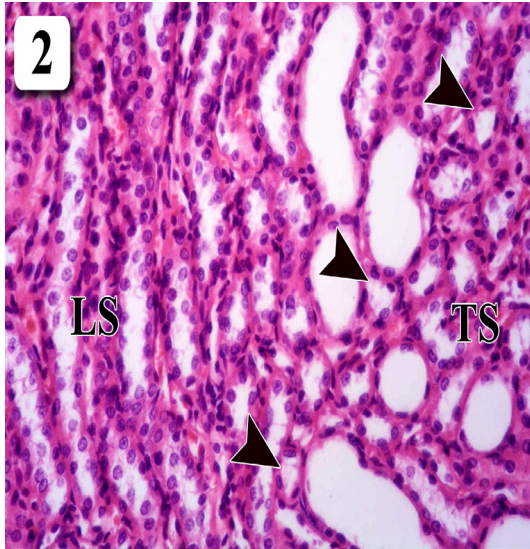
**Histogram 3:** Comparison between the mean values of the optical densities of the PAS reaction in the different groups

• Significant as compared to control group ( $P < 0.05$ )  
\*Significant as compared to GEN-treated group ( $P < 0.05$ )

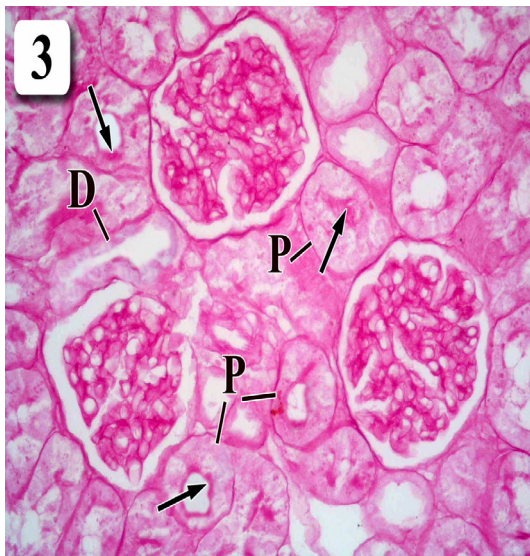


**Fig. 1:** A photomicrograph of a section in the renal cortex of a control rat showing the renal corpuscles (arrows) consisting of a glomerular capillary tuft (g) surrounded by Bowman's capsule. Note the proximal (P) and distal convoluted tubules (D). Inset: higher magnification of renal cortex showing glomerular capillary tuft (g) surrounded by Bowman's capsule with the simple squamous epithelium of its parietal layer (arrowhead) and Bowman's space (s). The proximal convoluted tubules (P) show narrow lumen, and are lined by simple cuboidal epithelium with acidophilic cytoplasm and large rounded nuclei. The distal tubules (D) have wider lumen and less acidophilic cytoplasm.  
Hx.&E.; x200; inset: x400

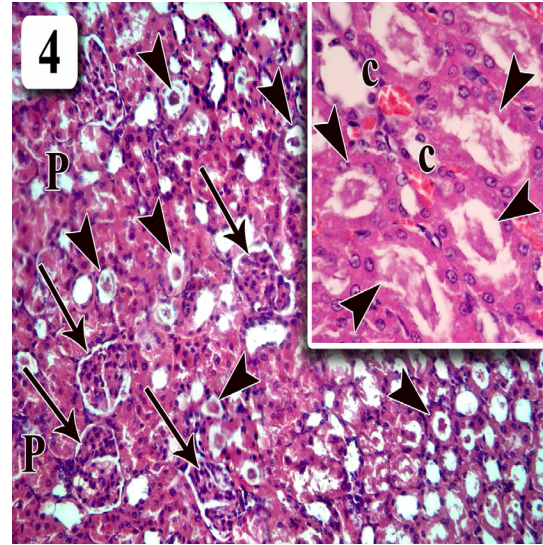




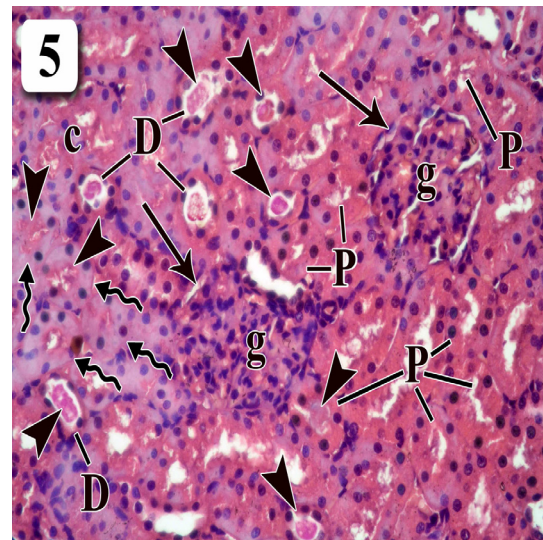
**Fig. 2:** A photomicrograph of a section in the renal medulla of a control rat showing regular and rounded thin descending limbs of the loops of Henle (arrowheads) lined by simple squamous epithelium whose nuclei protrude slightly into the lumen. Collecting tubules and thick ascending limbs of loops of Henle cut in transverse (TS) and longitudinal (LS) sections are wider and less regular in shape and are lined by simple cuboidal epithelium.  
Hx.&E.; x400



**Fig. 3:** A photomicrograph of a section in the renal cortex of a control rat showing a strong positive PAS reaction seen in the apical and basal membranes of the proximal (P) and distal (D) tubule cells. Arrows indicate the brush border in proximal tubules. Distal tubule cells (D) are more lightly stained and have wider lumen than proximal tubules. The basement membrane of the parietal layer of the Bowman capsules and glomerular tufts also exhibit a strong positive PAS reaction.  
P.A.S.; x400

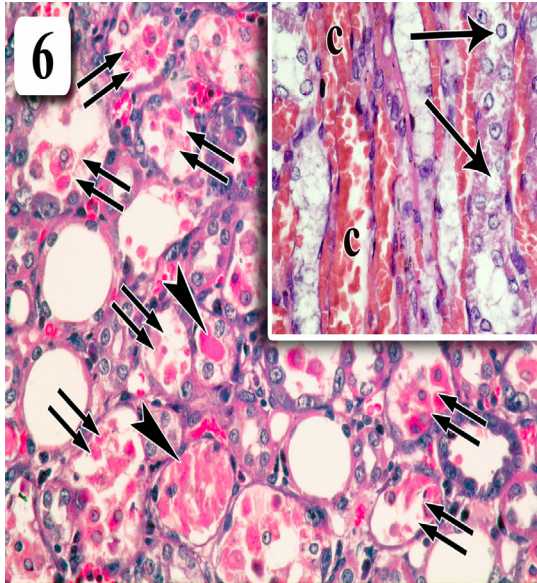


**Fig. 4:** A photomicrograph of a section in the renal cortex of a GEN-treated rat showing the renal corpuscles (arrows) appearing small in size with narrowing of the urinary space and hyaline casts (arrowheads) in the distal convoluted tubules (D) rather than in the proximal tubules (P). Inset: higher magnification of renal cortex showing hyaline casts (arrowheads) in the distal convoluted tubules as well as congestion of blood vessels (c).  
Hx. & E.; x200; inset: x400

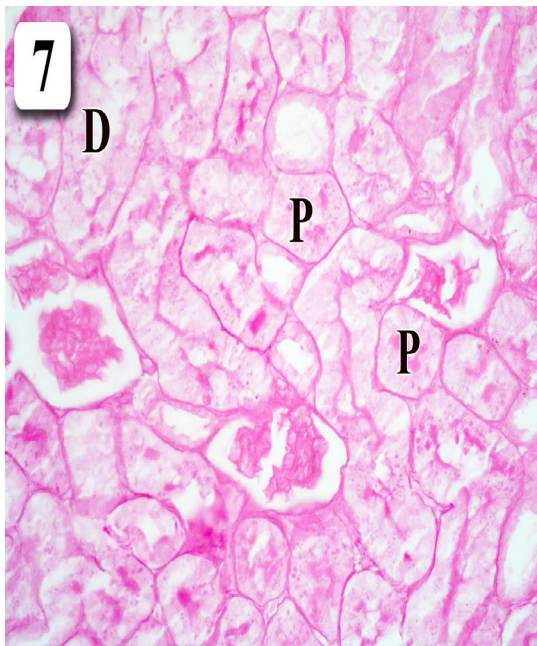


**Fig. 5:** A photomicrograph of a section in the renal cortex of a GEN-treated rat showing more affection of the renal corpuscles (arrows) appearing small in size with obliteration of the Bowman's space and presence of hyaline casts (arrowheads) in the distal convoluted tubules (D) more than in the proximal convoluted tubules (P). The proximal convoluted tubules (P) exhibit hyaline degeneration (curved arrows) and dark nuclei. Note presence of congested blood vessels (c).  
Hx. & E.; x400

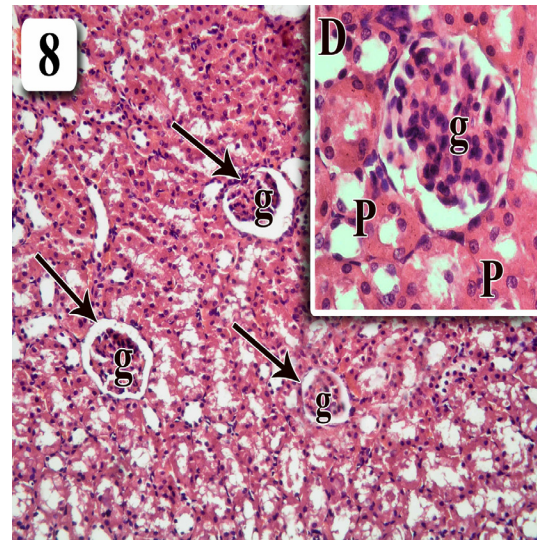




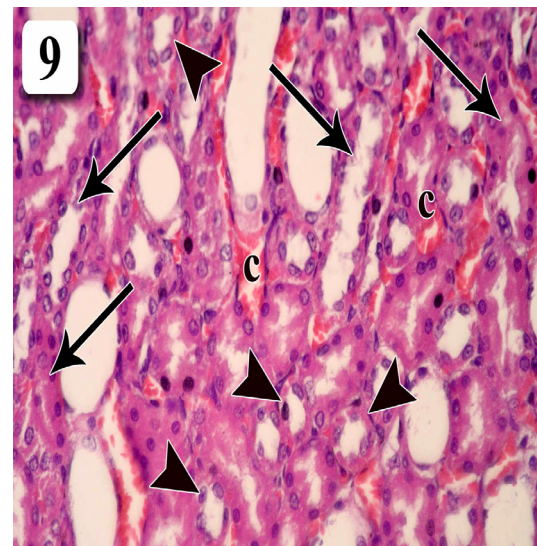
**Fig. 6:** A photomicrograph of a section in the renal medulla of a GEN-treated rat showing cell desquamation (double arrows) and hyaline casts (arrowheads) in the collecting tubules. Inset: longitudinal section of the medulla showing vacuolar degeneration (arrows) in the collecting tubules and interstitial congestion (c).  
Hx. & E.; x400; Inset: x400



**Fig. 7:** A photomicrograph of a section in the renal cortex of a GEN-treated rat showing a weak positive PAS reaction was seen in the apical membrane, but was moderate in the basal membrane of the proximal (P) and distal (D) tubule cells. A moderate reaction in the distorted glomeruli is also observed.  
P.A.S.; x400

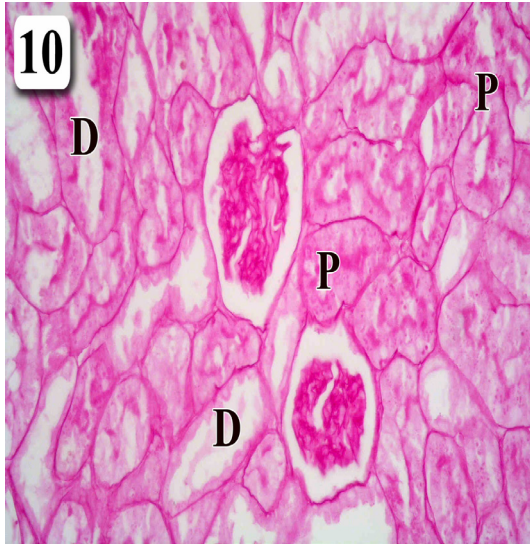


**Fig. 8:** A photomicrograph of a section in the renal cortex of a rat treated with both GEN and CMN showing slightly reduced size of renal corpuscles (arrows). Otherwise, the glomerular capillary tufts (g), the proximal and distal convoluted tubules present a nearly similar picture to that of the control. Inset: higher magnification of renal cortex showing that the renal corpuscle with the Bowman's capsule and glomerular capillary tuft (g) as well as the proximal (P) and distal convoluted tubules (D) have a similar appearance to that of the control.  
Hx. & E.; x200; Inset: x400

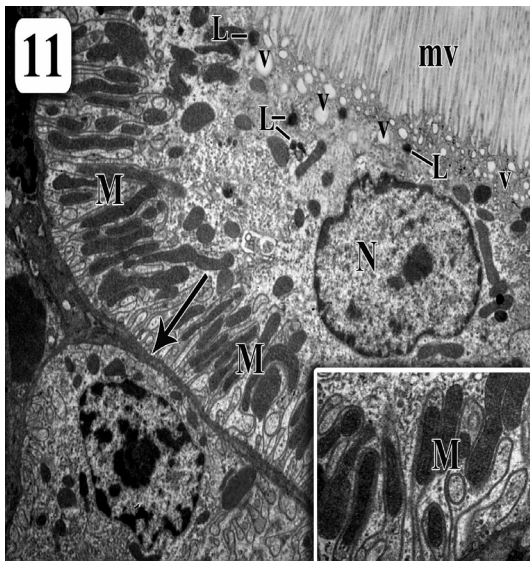


**Fig. 9:** A photomicrograph of a section in the renal medulla of a rat treated with both GEN and CMN showing that apart from mild congestion of blood vessels (c) there is a comparable picture to that of the control regarding the thin descending limbs of the loops of Henle (arrowheads), the collecting tubules and the thick ascending limbs of the loops of Henle (arrows).  
Hx. & E.; x400

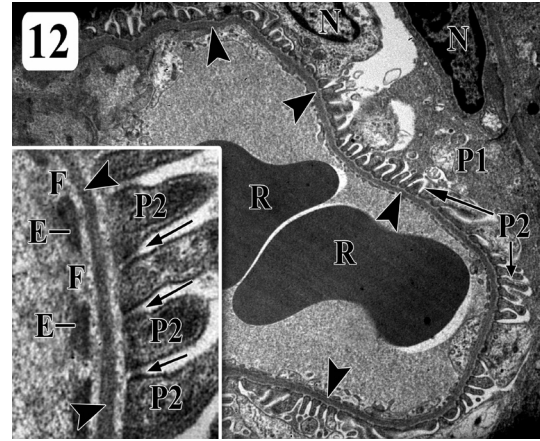




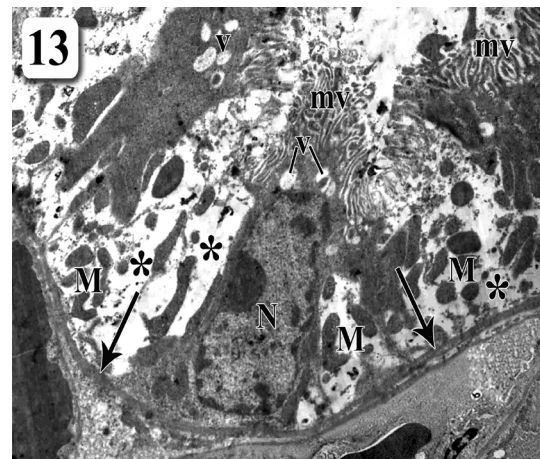
**Fig. 10:** A photomicrograph of a section in the renal cortex of a rat treated with both GEN and CMN showing a strong positive PAS reaction seen in the apical and basal membranes of the proximal (P) and distal (D) tubule cells as well as in the glomerular tufts. P.A.S.; x400



**Fig. 11:** Electron micrograph of the kidney of a control rat showing a proximal convoluted tubular cell displaying numerous tall apical microvilli (mv) with many pinocytotic vesicles (V) of various sizes as well as lysosomes (L) in the cytoplasm immediately beneath the microvilli. The nucleus (N) is rounded with extended chromatin and prominent nucleolus. The tubular basement membrane (arrow) is thin and the basal cell membrane exhibits basal infoldings containing numerous elongated mitochondria (M) arranged at right angles to the basement membrane. **Inset:** shows higher magnification of mitochondria (M) exhibiting regular cristae and intact membranes in the basal membrane infoldings. X6,000; inset: x12,000

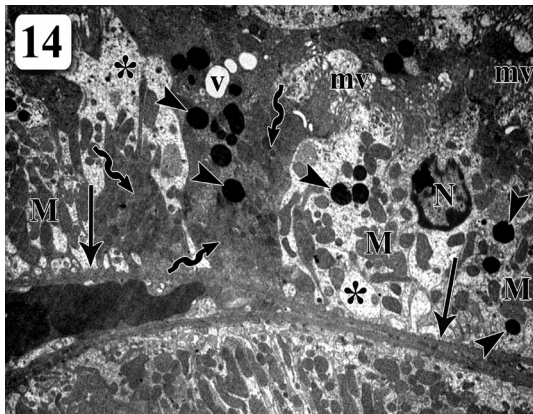


**Fig. 12:** Electron micrograph of the kidney of a control rat showing a glomerular capillary loop inside a renal corpuscle lined by a thin layer of fenestrated endothelial cytoplasm and containing red blood corpuscles (R). The nuclei of several podocytes (N) are seen, with their primary processes (P1) giving rise to numerous secondary foot processes (P2) that rest on the glomerular basement membrane (arrowheads). **Inset:** a higher magnification of the glomerular barrier showing the fenestrated (F) capillary endothelium (E) on the luminal surface of the glomerular basement membrane (arrowheads) exhibiting a dark wide central lamina densa bordered on each side by a narrow pale lamina rara. On the opposite side are podocyte's secondary foot processes (P2), separated by filtration slits of uniform width (arrows). X10,000; inset: x40,000

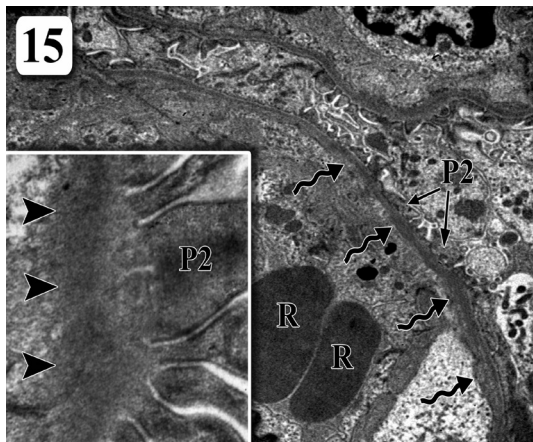


**Fig. 13:** Electron micrograph of the kidney of a GEN-treated rat showing a proximal convoluted tubular cell displaying distortion and partial loss of apical microvilli (mv) as well as reduction of the pinocytotic vesicles (V) in the apical cytoplasm. The nucleus (N) is irregular with increased quantity of condensed chromatin. The tubular basement membrane (arrow) is moderately thickened and exhibits disappearance of the basal infoldings. The mitochondria (M) are apparently diminished in number and moved away from the basal membrane, changing from elliptical to almost circular outline. Wide cytoplasmic rarefied spaces (\*) dispersing the organelles are seen. X6,000

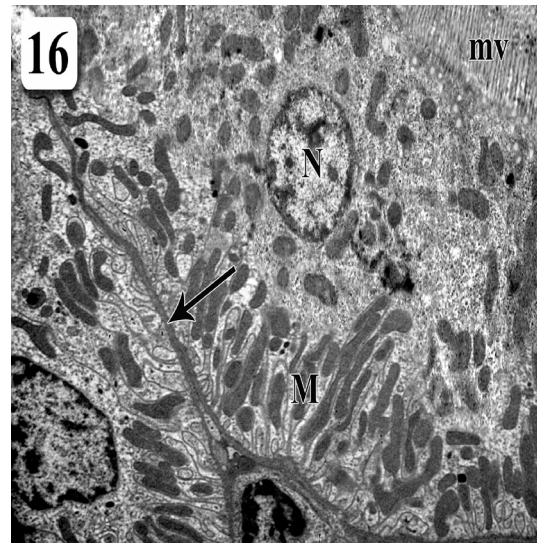




**Fig. 14:** Electron micrograph of the kidney of a GEN-treated rat showing a proximal convoluted tubular cell displaying distortion and diminution of the apical microvilli (mv) as well as reduction of the pinocytotic vesicles (V) in the cytoplasm beneath the microvilli. The nucleus (N) is irregular with more condensed chromatin. The tubular basement membrane (arrow) is thickened with reduced basal infoldings. The mitochondria (M) are apparently diminished in number and moved away from the basal membrane, changing from elliptical to almost circular outline. The cytoplasm is electron dense in some regions (curved arrows), rarefied (\*) in other regions and exhibits a number of dark bodies (arrowheads). X6,000



**Fig. 15:** Electron micrograph of the kidney of a GEN-treated rat showing focal fusion of secondary foot processes (P2) of podocytes related to a glomerular capillary loop inside a renal corpuscle. The glomerular basement membrane exhibits patchy foci of thickening and partial loss of its trilamellar structure in focal areas (curved arrows). Red blood corpuscles (R) are seen inside the capillary loop. Inset: focal fusion of secondary foot processes (P2) and glomerular basement membrane exhibiting foci of thickening and partial loss of its trilamellar structure (arrowhead). X10,000; inset: x40,000



**Fig. 16:** Electron micrograph of the kidney of a rat treated with both GEN and CMN showing nearly similar appearance as the control of proximal convoluted tubular cell with intact apical microvilli (mv), euchromatic nucleus (N) and thin tubular basement membrane (arrow) displaying basal infoldings containing numerous elongated mitochondria (M). X6,000



**Fig. 17:** Electron micrograph of the kidney of a rat treated with both GEN and CMN showing a picture almost similar to the control of the glomerular basement membrane (arrowheads) and the secondary foot processes (P2) of the podocytes related to a glomerular capillary loop enclosing red blood corpuscles (R). Inset: a higher magnification of the secondary foot processes (P2) and glomerular basement membrane (arrowheads) revealing a comparable appearance to the control apart from some thickening of lamina densa and narrowing of lamina rara. X10,000; inset: x40,000



## DISCUSSION

Gentamicin is an aminoglycoside widely used in clinical practice for the effective treatment of gram-negative infections for many years, but its renal toxicity is well-known and limits its clinical usefulness to some extent. The nephrotoxic potential of GEN complicates the patients' conditions, prolongs the hospital stay and increases the medical costs and most seriously may progress to renal failure (Sande & Mandell, 1990). Therefore, identification of a therapeutic approach to protect against gentamicin-induced renal damage would have very important clinical consequences for the safer use of this drug.

In the current study, GEN was administered to albino rats in order to induce nephrotoxicity. Out of all laboratory animals, the rat was employed in this study since it was considered an excellent model of acute renal failure to test compounds, extracts or drugs which could be used to prevent side effects of gentamicin in humans (Ali, 1995). In the literature, several dosage schemes have been reported for gentamicin administration in rats. However, the dose of GEN used in the present study (100 mg/kg), was chosen because it has been reported to induce marked nephrotoxicity (Parlakpinar et al., 2005) and was regarded as suprathreshold (Stojiljkovic et al., 2008-a).

It was observed in this study, that all the rats which received GEN had experienced a decrease in food intake as compared to the rats of the other groups. Loss of appetite was previously documented with GEN treatment (Erdem et al., 2000) and might be partly responsible for the significant retardation in weight gain in the GEN-treated rats in the present study as compared to the control. This effect on body weight had also been reported in previous studies (Murakami et al., 1998; Erdem et al., 2000). Moreover, the limited weight gain might be ascribed to renal tubular injury, with the subsequent inability of the tubular cells to reabsorb water leading to dehydration and failure to gain weight to the same extent as control rats.

In the current study, light microscopic examination of renal sections of rats treated with GEN revealed hyaline degeneration and

dark nuclei in the proximal tubules, shrinking of glomeruli and significantly decreased PAS reaction. Hyaline casts were demonstrated in the distal and collecting tubules. Moreover, desquamated cells were observed inside the lumen of the collecting tubules.

Ultrastructurally, the proximal tubules exhibited several changes including partial loss of microvilli and thickened basement membranes. The cytoplasm was rarefied in some regions and electron dense in other areas with reduced and distorted mitochondria in addition to the presence of dark bodies. The nuclei displayed increased condensation of chromatin. However, no ultrastructural changes could be seen in the distal tubules.

The rarefaction may be due to cytoplasmic edema. This is in accordance with Gilbert et al. (1978) who described marked cell edema, cytoplasmic disorganization and mitochondrial disruption.

On the other hand, the dark bodies observed in the present study probably represented lysosomes which might have increased in size in an attempt to deal with the intracellular edema and debris. These dark bodies were in contrast to the myelin-like structures or the myeloid bodies previously described by Mingeot-Leclercq and Tulkens (1999). The authors believed that these myeloid bodies were induced by the accumulation of GEN in the proximal tubules with enlargement and subsequent rupture of lysosomes and progressive deposition of phospholipids and proteins in a concentric lamellar manner.

Moreover, the dark nuclei in Hx. & E. sections and the E.M. finding of increased condensation of nuclear chromatin together with areas of dark cytoplasm in the proximal tubular cells noticed in this study could denote apoptosis. This is also supported by Servais et al. (2006) who believed that GEN accumulated in the lysosomes of the renal proximal tubular cells and caused apoptosis at clinically relevant doses.

Furthermore, the podocytes displayed localized fusion of secondary foot processes and thickening of the glomerular basement

membrane with focal loss of its trilamellar structure. The tubular basement membrane affection in the current study is consistent with *Stojiljkovic et al. (2008-a, b)* who demonstrated in their two studies, significant increase in the thickness of the glomerular basement membrane.

The histological changes in the proximal convoluted tubules induced by GEN, in the present study, were also observed by several previous studies (*Pedraza-Chaverri et al., 2000; Cuzzocrea et al., 2002; Karahan et al., 2005; Stojiljkovic et al., 2008-a*). All these structural changes confirm that the accumulation of GEN in the proximal tubule cells was associated with nephrotoxicity (*Nagai & Takano, 2004*).

In the current study, morphometry and statistical analysis showed a statistically significant decrease in the mean areas occupied by the glomeruli, renal corpuscles and urinary spaces compared to the control. This is consistent with the findings of previous researches that revealed reduced size of glomeruli (*Said, 2010; Sayed-Ahmed & Nagi, 2007*). On the other hand, *Stojiljkovic et al. (2008-b)*, *Yaman and Balikci (2010)* reported glomerular hypertrophy, while *Abdel-Raheem et al. (2010)* described the presence of atrophy in many glomeruli and hypertrophy in few others.

Experimental data suggested that both vascular (glomerular) and tubular targets were involved in drug-induced nephrotoxicity (*Rodriguez-Barbero et al., 2000*). In all the experimental models of nephrotoxicity developed so far, there were several processes that seem to make a major contribution to the reduction in glomerular filtration rate; a characteristic of toxic renal failure. Among these, the most important was a reduction in renal blood flow secondary to an increased renal vascular and tubular necrosis with the subsequent tubular obstruction and fluid leak (*Fent et al., 1988*).

The glomerular dimensions and its contractile state seemed to be a major determinant of renal hemodynamic regulation and filtration (*Mene et al., 1989*). *Rodriguez-Barbero et al. (2000)* considered glomerular

contraction as one of the major effects of toxicants on glomerular structures. Glomerular contraction was expressed as a decrease of the cross-sectional glomerular area in comparison to the initial size or to the control (*Rodriguez-Barbero et al., 2000*). *Stojiljkovic et al. (2008-a)* reported that when GEN was administered to experimental animals, there was a marked fall in the renal blood flow and glomerular filtration rate with a rise in renal vascular resistance, which occurred without permanent glomerular structural changes and also without a direct relationship with tubular damage.

It has been proposed that oxidative stress and ROS played a considerable role in GEN-induced nephrotoxicity since it was found that  $O_2^-$ ,  $H_2O_2$  and  $OH$  increase with GEN treatment (*Balakumar et al., 2010*). Therefore, drugs or plant products having direct or indirect antioxidant properties might have the potential to prevent gentamicin-induced nephrotoxicity.

In this respect, various antioxidant agents that scavenge or interfere with ROS production have been demonstrated to prevent successfully GEN-induced renal damage such as polyaspartic acid (*Gilbert et al., 1989*), L-arginine (*Can et al., 2000*), melatonin (*Shifow et al., 2000*), carvedilol (*Kumar et al., 2000*) and some medicinal plants such as arabic gum (*Al-Majed et al., 2002*).

The present study showed significant improvement in weight gain in rats when CMN was simultaneously given with GEN which reflected a general protective effect of CMN against GEN-induced nephrotoxicity. In the same context, the tubular and glomerular alterations induced by GEN treatment were alleviated by concomitant supplementation with CMN. This was evident by the light and E.M. appearance of the renal cortex and medulla which displayed a nearly similar picture to that of the control apart from presence of mild congestion of blood vessels. Morphometry and statistical analysis showed a statistically significant increase in the mean areas of glomeruli, renal corpuscles and urinary spaces as compared to the GEN-treated group, but were still significantly lower than the control group.

Furthermore, the PAS reaction significantly increased in the membranes of proximal and distal tubules as well as in the glomerular tufts as compared to the GEN-treated group.

This is in accordance with two biochemical studies reported that CMN prevented GEN-induced renal toxicity (Ali *et al.*, 2005; Farombi & Ekor, 2006).

The mechanism by which curcumin exerted its reno-protective action is not certain. However, it is well established that CMN has a strong antioxidant action (Biswas *et al.*, 2005; Jagetia, 2007) which may have neutralized the ROS and oxidative stress documented in GEN-induced nephrotoxicity. Moreover, CMN treatment was able to ameliorate the nephrotoxicity of other drugs that might have predisposed to the production of free radicals such as adriamycin and cisplatin (Venkatesan *et al.*, 2000; Antunes *et al.*, 2001). Meanwhile, regarding the safety aspects, CMN at the dose used in this study, has not exerted any histological damage in the kidneys, thus confirming its possible beneficial effect.

In conclusion, this study showed that the histological changes of GEN-induced nephrotoxicity were prevented by the co-administration of CMN. Gentamicin should be carefully used clinically to prevent the occurrence nephrotoxicity especially in high-risk patients. At the same time, the renal function should be carefully monitored during therapy. Moreover, curcumin should be further investigated clinically to explore its efficacy to protect the kidneys against GEN-induced nephrotoxicity by virtue of its strong antioxidant property.

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## هل بإمكان الكركم أن يقي الكلى من التسمم المستحدث بواسطة الجنتاميسين في الجرذان؟ دراسة هستولوجية

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### ملخص البحث

تلعب شوارد الأكسجين الحرة دوراً هاماً في النشوء المرضي لتسمم الكلى الناتج من الجنتاميسين وفي نفس الوقت يتميز الكركم بفعالية عالية كمانع للتأكسد.

استهدف البحث الحالي دراسة الدور الوقائي المحتمل للكركم على تسمم الكلى المستحدث بواسطة الجنتاميسين في الجرذان البيضاء باستعمال طرق هستولوجية وقياسية مورفولوجية.

وقد أجريت هذه الدراسة على ست وثلاثين من ذكور الجرذان البيضاء البالغة تم تقسيمها الى ست مجموعات اشتملت كل منها على ستة جرذان: المجموعة الأولى (المجموعة الضابطة) حققت داخل التجويف البريتوني بـ ٠,٩٪ محلول ملح والمجموعة الثانية أعطيت ١ مللتر من زيت الذرة عن طريق الفم والمجموعة الثالثة أعطيت كركم 002 مجم/كجم مذاب في زيت الذرة عن طريق الفم لمدة 12 يوماً والمجموعة الرابعة حققت داخل التجويف البريتوني بالجنتاميسين ١٠٠ مجم/كجم مذاب في ٠,٩٪ محلول ملح لمدة ٧ أيام والمجموعة الخامسة أعطيت كل من الجنتاميسين بالحقن داخل التجويف البريتوني لمدة ٧ أيام وزيت الذرة عن طريق الفم لمدة ١٤ يوماً قبل الجنتاميسين و٧ أيام معه والمجموعة السادسة أعطيت كل من الجنتاميسين بالحقن داخل التجويف البريتوني لمدة ٧ أيام والكركم مذاب في زيت الذرة عن طريق الفم لمدة ١٤ يوماً قبل الجنتاميسين و٧ أيام معه.

تم تشريح كل المجموعات بعد مرور ٢٤ ساعة من آخر جرعة للجنتاميسين ثم استخرجت الكليتان من كل جرد وجهزت العينات لصبغها بالهيماتوكسيلين والأيوسين وتفاعل حمض البريوديك للفحص بالمجهر الضوئي ثم جهزت شرائح دقيقة للفحص بالمجهر الإلكتروني وكذلك تمت دراسة العينات بجهاز التحليل الضوئي للصور واستخلاص دلالاتها إحصائياً.

وقد أظهرت النتائج أن إعطاء الجنتاميسين أدى إلى نقص ذا دلالة احصائية في الوزن لدى الجرذان وكذلك تنكس زجاجي وزيادة كثافة الأنوية بخلايا الأنبيبات القريبة بالكلى وانكماش بالكبيبات وتفاعل ضعيف لحمض البريوديك. وأظهر التركيب الدقيق وجود تدهم في الخملات الدقيقة بالحافة القمية لخلايا الأنبيبات القريبة بالكلى وزيادة سمك الغشاء القاعدي وزيادة كثافة الأنوية مع تداخل بالساييتوبلازم وظهور أجسام داكنة ونقص بالحبيبات الخيطية. كما ظهر وجود التحام بؤري بين الزوائد القمية الثانوية للخلايا القمية وزيادة سمك الغشاء القاعدي الكبّي مع فقدانه للتركيب الثلاثي الطبقات في بؤر متفرقة. وقد أدى استعمال حمض الكركم مع الجنتاميسين الى حماية ملحوظة ضد هذه التغيرات.

الخلاصة: تبين أن للكركم دور وقائي بالكلى ضد التسمم المستحدث بواسطة الجنتاميسين في الجرذان البيضاء. ولذلك ينصح ايضاً بعمل دراسات سريرية من أجل تأكيد فاعلية استعمال الكركم للوقاية من الآثار السلبية للجنتاميسين على الكلى في البشر.