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**Bioactive traditional plant *Cin-*
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combat against nephrotoxic effects
of aminoglycosides**

Bioactive traditional plant *Cinnamomum zeylanicum* successfully combat against nephrotoxic effects of aminoglycosides

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Abstract

Cinnamomum zeylanicum is traditionally used for the treatment of bladder and kidney problems; believed to have kidney tonic effects. The prime objective of the current study was to explore the nephroprotective effects of the plant due to its strong antioxidant properties, to provide experimental facts for their traditional use. Daily dose of 200 mg/kg of plant extract was employed alone and in combination with gentamicin for a period of twenty one days in rabbits. Biochemical kidney functioning parameters were assessed thrice throughout study period. Histopathological examination of the kidneys was performed on the last day of experimental period. Present study showed that *C. zeylanicum* significantly attenuated renal functional and histological changes associated with gentamicin as assessed by urea, creatinine, uric acid, electrolytes, urinary protein, and histopathological examination. The plant extract successfully proved to have strong nephroprotective properties, especially against aminoglycosides induced nephrotoxicities.

Introduction

Cinnamomum zeylanicum belongs to family Lauraceae, widely used as spices and food preservatives in form of oils and extracts (Yu et al., 2007). This traditional plant is extensively used for the treatment of different kidney ailment and due to the sweet bark of plant, it is known as sweet wood (Willis, 1973). The plant may have protective role in alleviating the sign and symptoms of many diseases due to oxidative stress (Ranjbar et al., 2006). The extracts of Cinnamon regularly used as food antioxidants (Mancini-Filho et al., 1998). The plant has also been used for the treatment of several diseases (Senhaji et al., 2007). The usefulness of plant extract in the treatment of gastritis, dyspepsia, disturbances in blood circulation and inflammation has been reported (Wang et al., 2009). The analgesic, antipyretic, anti-tussive and antiallergenic actions have also been

presented (Singh et al., 2008).

The essential oil obtained from the bark *C. zeylanicum* has abundant cinnamaldehyde, which have antimicrobial actions against several pathogenic bacteria and fungi (Mastura et al., 1999). Mishra and coworkers reported that the bark of *C. zeylanicum* contains some antifungal substances (Mishra et al., 2009). Most importantly, the plant possesses antioxidant activities due to its phenolic contents (Tomaino et al., 2005) evaluated by different *in vitro* assays (Lee and Shibamoto, 2002). Because of antioxidant properties the extract obtained from *C. zeylanicum* have beneficial role against free radical damage to the cell membranes (Hasani-Ranjbar et al., 2009). The plant has also been presented to stimulate antioxidant enzymes (Dhuley, 1999). Due to its antioxidant actions it possesses strong wound healing properties (Kamath et al., 2003). The



plant also proved to have insecticidal activity (Abdel Halim, 2006), with effectiveness in allergic rhinitis (Corren et al., 2008).

On the basis of above discussed text; *C. zeylanicum* comprise a number of remedial properties, particularly determined as antioxidant and free radical scavenging properties may due to strong phenolic contents (Ranjbar et al., 2006; Mancini-Filho et al., 1998; Tomaino et al., 2005; Lee and Shibamoto, 2002; Hasani-Ranjbar et al., 2009; Dhuley, 1999), which has been reported to be responsible for the amelioration of gentamicin induced renal damage (Ali and Mousa, 2001). Most importantly, the powdered bark of *C. zeylanicum* is used traditionally for the treatment of various kidney problems like kidney stones, renal colic and believed to have a tonic effect on kidney. Consequently the present work was aimed to explore the defensive effect of *C. zeylanicum* against renal disruption associated with administration of aminoglycosides.

Materials and Methods

Plant material and extraction

Three kilogram of dried bark of *C. zeylanicum* plant was purchased from local market Abbottabad Pakistan. The voucher specimen number 1024 was deposited after had identified by Umar Farooq, Professsor of Botany, Govt. College Abbottabad Pakistan. Powdered plant material was extracted with ethanol for three weeks and evaporated under reduced pressure (Bhukya et al., 2009).

Experimental protocol

Similar weighted male rabbits were acclimatized and maintained at same diet in twelve hours cycles of dark and light after the approval from University Ethical Committee. Twenty four animals were arranged equally into four groups and were treated separately according to dosage schedule (Table I). For estimation of blood urea nitrogen (BUN), serum creatinine, creatinine clearance, serum uric acids, serum electrolytes, urinary urea, urinary creatinine, urinary protein and urinary volume; blood and urine samples were

Table I	
Different treatment groups of animals	
Group	Treatment
C	Normal saline solution 2 mL/kg/day
G	Gentamicin 80 mg/kg/day
GC-ze	Gentamicin 80 mg/kg/day and <i>C. zeylanicum</i> 200 mg/kg/day
C-ze	<i>C. zeylanicum</i> 200 mg/kg/day

collected trice throughout duration of treatment.

Biochemical assessments

BUN was assessed by following Bertholot's indophenol assay while serum creatinine was evaluated by following Jaffe reaction. Serum uric acid was estimated by chemistry analyzer with the help of reagent kits (Smith, 1985). Further, serum sodium and serum potassium was measured by flame photometric method while serum calcium by cresolphthalein complexone method (Blosser, 1985).

Histo-pathologic examination of kidney

On last day of experimental period, half of the animals in each experimental group were slaughtered for renal histopathology. Kidneys were secluded and fixed in formalin solution. Tissues were treated with ascending order of ethanol followed by xylene and fixed in wax. Blocks were cut into thin slices with help of rotatory microtome (Micros, Germany). The slides were stained with hematoxylin and eosin and studied with the help of microscope (Germany).

Statistical analysis

Results were tabulated as mean \pm SEM and all the experimental groups were compared with gentamicin treated group by using Dunnett t-test subsequent to one-way ANOVA with the help of GraphPad Prism (Version v). p value <0.05 was considered statistically significant.

Results

Mean body weight

Body weight lost significantly in group G (10.8 ± 1.1), when compared with group C ($0.2 \pm 0.9\%$). The body weight of animals treated with both gentamicin and *C. zeylanicum* were found significantly different from group G animals, exactly same as group C (Figure 1A).

Measurement of serum BUN

BUN increased significantly in group G, as 54.2 ± 2.6 mg/dL on last day of study period in comparison with group C (14.1 ± 1.1 mg/dL). Group GC-ze and C-ze were also observed significantly different when compared with group G (14.2 ± 1.2 and 13.7 ± 0.2 mg/dL vs 54.2 ± 2.6 mg/dL; Table II).

Measurement of serum creatinine

Significant increase in serum creatinine was observed in group G, as 4.0 ± 0.1 mg/dL on last day of study period in comparison with group C (0.8 ± 0.1 mg/dL). Group GC-ze and C-ze were also observed significantly different when compared with group G (0.9 ± 0.1 and 0.7 ± 0.1 mg/dL vs 4.0 ± 0.1 mg/dL; (Table II).

Table I
Biochemical kidney functioning parameters on day 0, 11 and 21 of study period

Group	Day 0	Day 11	Day 21
<i>Blood urea nitrogen (mg/dL)</i>			
C	13.1 ± 1.2	13.8 ± 1.0 ^a	14.1 ± 1.1 ^a
G	12.8 ± 1.1	37.8 ± 2.1	54.2 ± 2.6
GC-ze	13.7 ± 1.2	13.9 ± 1.2 ^a	14.2 ± 1.2 ^a
C-ze	13.5 ± 0.2	13.6 ± 0.2 ^a	13.7 ± 0.2 ^a
<i>Serum creatinine (mg/dL)</i>			
C	0.7 ± 0.1	0.7 ± 0.1 ^a	0.8 ± 0.1 ^a
G	0.5 ± 0.0	2.0 ± 0.1	4.0 ± 0.1
GC-ze	0.6 ± 0.0	0.7 ± 0.0 ^a	0.9 ± 0.1 ^a
C-ze	0.7 ± 0.1	0.7 ± 0.1 ^a	0.7 ± 0.1 ^a
<i>Creatinine clearance (mL/min)</i>			
C	5.7 ± 0.6	5.1 ± 0.8 ^a	5.0 ± 1.2 ^a
G	5.3 ± 0.5	2.1 ± 0.3	0.8 ± 0.1
GC-ze	5.7 ± 0.7	4.6 ± 0.5 ^a	3.8 ± 0.6 ^a
C-ze	6.3 ± 0.6	5.5 ± 0.7 ^a	5.4 ± 0.5 ^a
<i>Serum sodium (mEq/L)</i>			
C	140.5 ± 1.2	139.6 ± 0.6	140.2 ± 1.0
G	141.2 ± 0.8	140.5 ± 0.6	137.7 ± 1.1
GC-ze	141.3 ± 1.1	141.3 ± 1.1	141.2 ± 1.1 ^a
C-ze	141.2 ± 1.5	140.7 ± 1.6	140.8 ± 1.5
<i>Serum potassium (mEq/L)</i>			
C	5.3 ± 0.2	5.3 ± 0.2 ^a	5.1 ± 0.2 ^a
G	5.2 ± 0.2	4.0 ± 0.1	3.4 ± 0.2
GC-ze	5.3 ± 0.1	5.2 ± 0.1 ^a	5.1 ± 0.1 ^a
C-ze	5.4 ± 0.4	5.3 ± 0.4 ^a	5.2 ± 0.4 ^a
<i>Serum calcium (mg/dL)</i>			
C	10.1 ± 0.2	10.0 ± 0.2 ^a	9.7 ± 0.3 ^a
G	10.3 ± 0.3	8.5 ± 0.3	7.7 ± 0.2
GC-ze	10.3 ± 0.3	10.1 ± 0.2 ^a	10.0 ± 0.2 ^a
C-ze	10.3 ± 0.2	10.3 ± 0.2 ^a	10.3 ± 0.2 ^a
<i>Serum uric acid (mg/dL)</i>			
C	1.2 ± 0.1	1.4 ± 0.0 ^a	1.5 ± 0.0 ^a
G	1.1 ± 0.1	1.6 ± 0.1	2.3 ± 0.1
GC-ze	1.3 ± 0.1	1.3 ± 0.1	1.5 ± 0.1 ^a
C-ze	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.1 ^a

Mean ± SEM (n = 6); ^aconsidered significant when compared with gentamicin-treated group

Measurement of creatinine clearance

Creatinine clearance significantly decreased in group G, as 0.8 ± 0.1 mL/min on last day of study period in comparison with group C (5.0 ± 1.2 mL/min). Group GC-ze and C-ze were found to be extremely different when compared with group G (3.8 ± 0.6 and 5.4 ± 0.5 mL/min vs 0.8 ± 0.1 mL/min; Table II).

Measurement of serum sodium

No significant fall in serum sodium was observed in group G, as 137.7 ± 1.1 mEq/L on last day of study period in comparison with group C (140.2 ± 1.0 mEq/L). Further serum sodium vel of group GC-ze (141.2 ± 1.1 mEq/L) was found significantly different from group G, while C-ze (140.8 ± 1.5 mEq/L) was same as

that of group G.

Measurement of serum potassium

Significant fall in serum potassium level in group G, as 3.4 ± 0.2 mEq/L was observed on last day of study period in comparison with group C (5.1 ± 0.2 mEq/L). Further, group GC-ze and C-ze were also observed to be significantly different from group G (5.1 ± 0.1 and 5.2 ± 0.4 mEq/L vs 3.4 ± 0.2 mEq/L; Table II).

Measurement of serum calcium

Significant fall in serum calcium was observed in group G, as 7.7 ± 0.2 mg/dL on last day of study period in comparison with group C (9.7 ± 0.3 mg/dL). However, group GC-ze and C-ze were found significantly

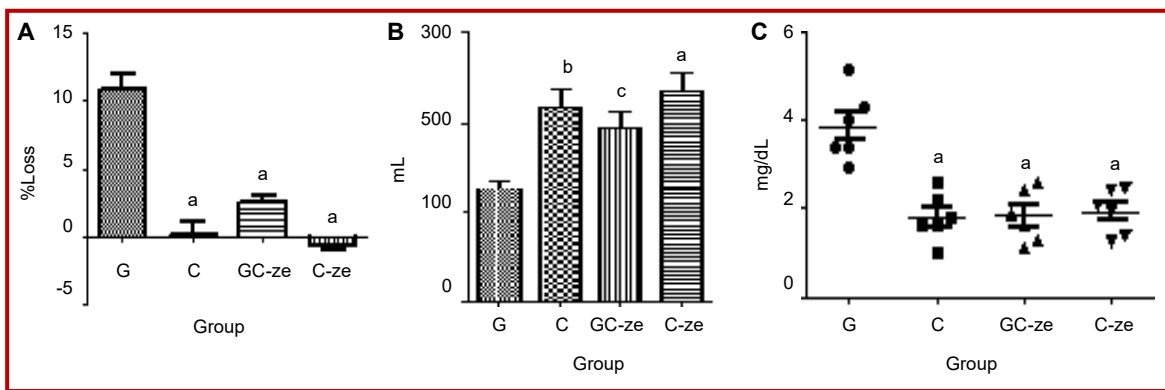


Figure 1: Changes in body weight, urinary volume and urinary protein excretion in various groups. (A) Percent loss in mean body weight in group G animals (gentamicin treated) significantly different from control group, extract treated group and animals treated with both gentamicin and extract. (B) Urinary volume measured on day 21st presented; Group G significantly different from group GC-ze, very significantly different from group C, and extremely significantly different from group C-ze. (C) Urinary excretion of protein on day 21st significantly different from group C, and other treated groups

different from group G (10.0 ± 0.2 and 10.3 ± 0.2 mg/dL vs 7.7 ± 0.2 mg/dL; Table II).

Measurement of urinary volume

Significant decrease in urinary volume of group G, as 126.0 ± 9.1 mL was observed in comparison with group C (217.0 ± 19.8 mL). Group GC-ze (195.0 ± 17.9 mL) and C-ze (236.0 ± 19.8 mL) were also found significantly different from group G (Figure 1B).

Measurement of urinary protein excretion

Significant rise in the excretion of protein was noted in group G animals as 3.9 ± 0.3 mg/dL on last day of study period in comparison with group C animals as 1.8 ± 0.2 mg/dL. Urinary protein excretion of group GC-ze and C-ze were found significantly different from group G animals (Figure 1C).

Measurement of serum uric acid

Significant increase in group G, as 2.3 ± 0.1 mg/dL was observed on last day of study period in comparison

with group C (1.5 ± 0.0 mg/dL). Group GC-ze and C-ze were found significantly different when compared with group G (1.5 ± 0.1 and 1.4 ± 0.1 mg/dL vs 2.3 ± 0.1 mg/dL; Table II).

Histopathologic examination of the kidney

Group C animals presented normal tubules with no hydropic abnormalities. Glomeruli appeared normal with no confirmed identification of necrosis; renal medulla presenting normal distal tubules with a little hazy appearance as shown in Figure 2A-B. However, group G animals presented necrosis of proximal tubules with disrupts cellular structure. Additionally atropic glomeruli with hydropic abnormalities and a number of ruptured tubules were also found; renal medulla showed loss of cellular pattern with large number of ruptured tubules as shown in Figure 3A-B. Further, group GC-ze and C-ze animals presented normal structures, with no common abnormalities, except few ruptured tubules in case of some animals of group GC-ze; renal medulla of both groups presenting normal

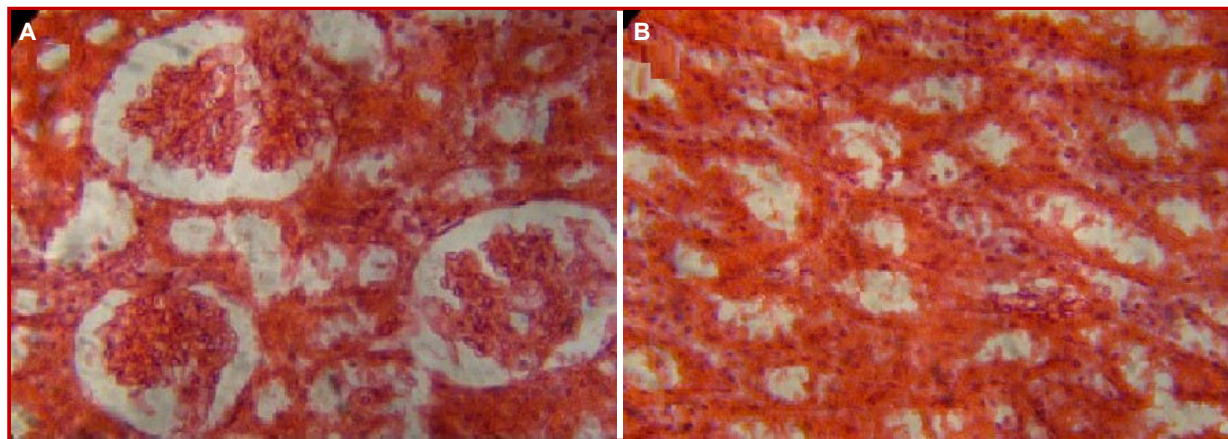


Figure 2: Microphotographs of Group C (control); (A) renal cortex showed normal glomeruli and normal structures and (B) renal medulla presenting normal distal tubules with a little hazy appearance

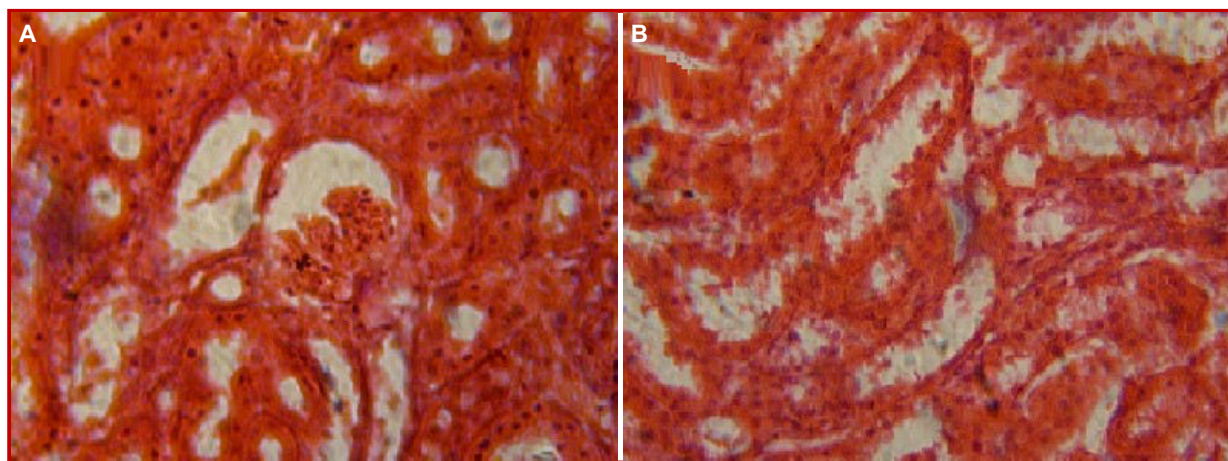


Figure 3: Microphotographs of Group G (Gentamicin treated); (A) renal cortex presenting glomerular atrophy with hydropic changes, hyaline filled lumina, necrosis and loss of cellular pattern and (B) renal medulla presenting loss of cellular pattern with a number of ruptured and dilated collecting tubules

tubules with no proper abnormality except some ruptured tubules in group C-ze, as shown in Figure 4A-B, 5A-B.

Discussion

In the present work we investigated the offset property of *C. zeylanicum* against the nephrotoxic side effects of aminoglycosides due to their antioxidant potentials. These nephrotoxic effects are dependent on dose and its duration of treatment, especially when the drug is used at higher doses (Bennett et al., 1999). Gentamicin as the most important member of aminoglycoside group, used at different doses (40/60/80 mg/kg/day) to produce nephrotoxic effects, followed in the present work at 80 mg/kg/day to generate renal toxicity (Gilbert et al., 1989).

Significant increase in creatinine, BUN and uric acid

with a decrease in creatinine clearance was observed in the present exploration in group G animals in comparison with control group animals. Which is in agreement with previous reported findings as, significant fall in creatinine clearance with rise in serum creatinine and BUN was produced after treatment with gentamicin (Moghaddam et al., 2010). Further, animals treated with combine therapy of extract and gentamicin proved safe from nephrotoxicity like group C-ze animals, confirmed the nephroprotectant properties of *C. zeylanicum*.

Body weight progressively decreased in group G animals when compared with group C animals. Further, animals treated with both gentamicin and *C. zeylanicum* were found with no significant loss in their body weight. Gentamicin treated animals were found with fall in urinary volume and rise in excretion of protein, significantly dissimilar to that of group C animals. Further, urinary volume and urinary protein excretion of animals treated with combine administra-

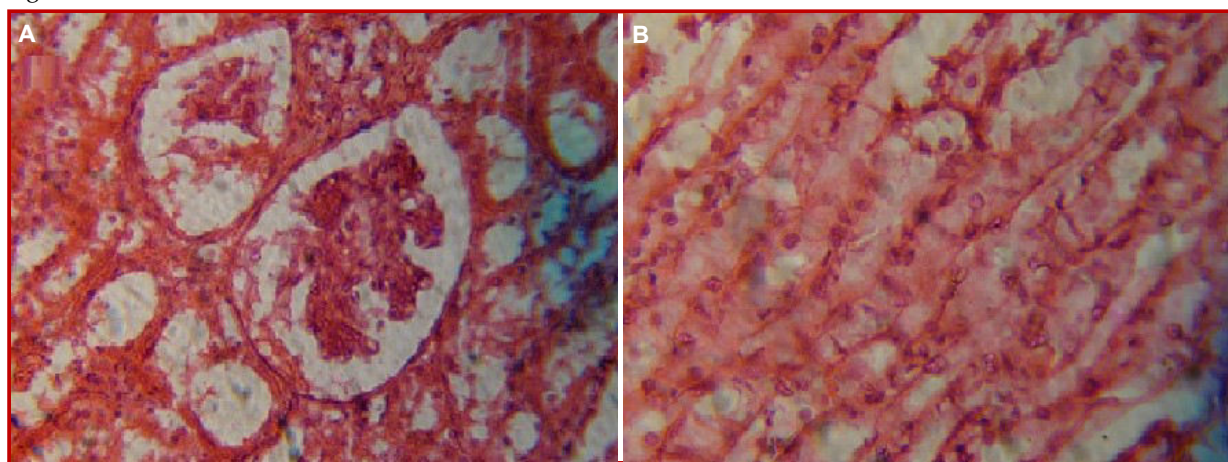


Figure 4: Microphotographs of Group GC-ze (Gentamicin and *C. zeylanicum* treated); (A) renal cortex presenting normal structures with some ruptured tubular cells and (B) renal medulla presenting normal tubules with no proper evidence of abnormality

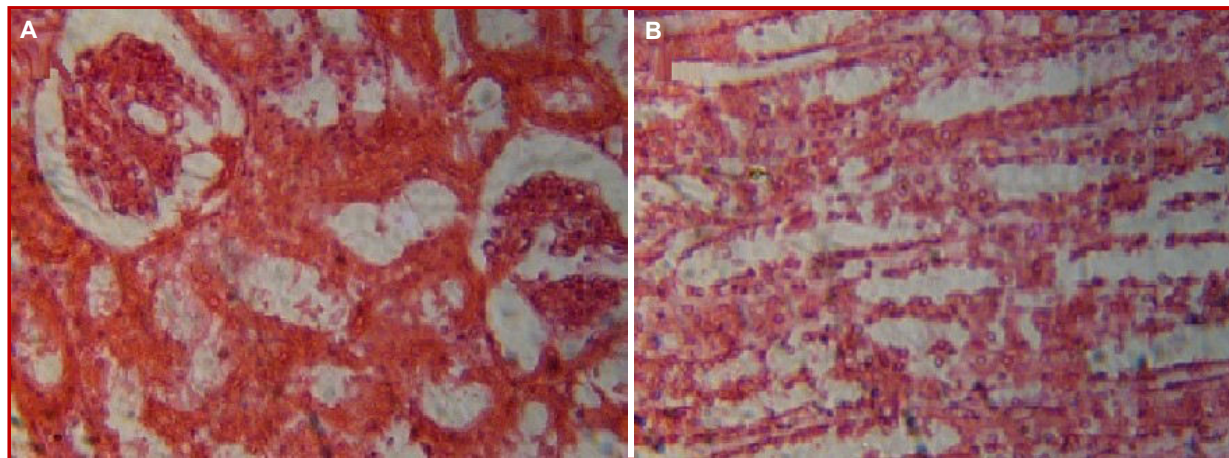


Figure 5: Microphotographs of Group C-ze (*C. zeylanicum* treated); (A) renal cortex presenting no common abnormality and (B) renal medulla presenting few ruptured tubules

tion of gentamicin and *C. zeylanicum* were found significantly different from gentamicin treated animals. Sodium excretion has been reported to improve with the reduction in re-absorption of potassium by toxic gentamicin treatment (Derakhshanfar et al., 2007). Gentamicin has also been reported to produce hypokalemia at large doses as in present study for group G animals, opposite in case of animals treated with combine gentamicin and *C. zeylanicum* (Bennett et al., 1999). *C. zeylanicum* treated animals alone and in combination with gentamicin significantly protect from reduction in serum potassium different from group G animals (Asif et al., 2012). Serum calcium decreased significantly in group G animals in the present work, significantly different from group C and all other treated groups as reported previously (Lambie, 1991). However, in opposition; gentamicin has been presented with no sound effect on serum calcium (Lambie, 1991). Further, in the present work serum sodium was found nearly same in all groups including gentamicin treated group. However, altered serum sodium has been presented previously (Bennett et al., 1999).

Increase in serum creatinine was reported to be linked with necrosis of tubules (Solez, 1983), may because of accumulation of debris in the tubules as like in the present findings necrosis and increase in creatinine are side by side in gentamicin treated animals. In opposition it has been presented that necrosis and other functional parameters are independent to each other (Luft et al., 1977).

The increase excretion of urinary excretion in group G animals in the present study may due to renal casts and hyaline in the tubules, responsible for blocking of tubules causes renal toxicity (Solez, 1983). In addition kidney histology presented group G with necrosis and hydropic abnormalities as reported previously (Bennett et al., 1999). No proper abnormality like hydropic

abnormalities, cast cells or necrosis were identified in group C animals and animals treated with both gentamicin and *C. zeylanicum*, nor in animals treated with *C. zeylanicum* alone, confirmed the nephroprotectant properties of *C. zeylanicum*.

Conclusion

C. zeylanicum successfully attenuated renal structural and functional derangements associated with aminoglycosides assessed by histological and biochemical parameters, may due to its strong antioxidant potentials.

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Conflict of Interest

Authors declare no conflict of interest

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