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A Study on Root Extract of *Boerhaavia diffusa* as Nehroprotective Agent against Drug Induced Nephrotoxicity and Comparison with Vitamin-C and Vitamin-E.

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ABSTRACT

A study on root extract of *Boerhaavia diffusa* as nehroprotective agent against drug induced nephrotoxicity and comparison with vitamin-C and vitamin-E. Wistar-albino male rats weighing 125–150gms, are utilized for the present study.Blood samples were collected with cardiac puncture for biochemical investigations like blood urea, uric acid, creatinine, serum Na, K, Ca, determination. One way ANOVA and Bonferroni Multiple Comparison. Hyaline cast formation is observed in PCT with atrophic glomeruli effecting half of the cortical region when administered to rats with 80mg/kg b.w. administration of gentamycin. *Boerhaavia diffusa* 800mg/kg.bw and 200mg/kg.bw+250mg/kg.bw of Vit-C and Vit-E rejuvenated necrotic cells of kidney . Gentamycin must be given in the lowest effective therapeutic doses in patients with normal kidney function along with punarnava or vitamins.

Keywords: gentamycin, glomeruli, lymphatic infiltration, proximal convoluted tubules, punarnava, Vitamin-C, Vitamin-E.

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INTRODUCTION

Drug-induced nephrotoxicity is an important cause of renal failure. Aminoglycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity. Acidic phospholipids, broadly distributed in the plasma membranes in various tissues, were considered to be the binding site of aminoglycosides in brush-border membrane of proximal tubular cells [1]. Hydroxyl radicals play a role in the pathogenesis of gentamycin nephrotoxicity, gentamycin can induce suppression of Na(+)-K(+)-ATPase activity and DNA synthesis in rats proximal tubules leading to renal injury; this injury may be relevant to reactive oxygen metabolites generated by gentamycin. Renal cortical mitochondria is the source of reactive oxygen metabolites, which induces renal injury [2].

Pharmacological studies have demonstrated that *Boerhaavia diffusa Linn.,* exhibits a wide range of properties such as diuretic [3]; nephrotic syndrome[4]; antiurolithiatic [5] antioxidant and antidiabetic activity.

Antioxidants are molecules, which interact with free radicals and terminate the chain reaction before vital molecules are damaged. They donate an electron to stabilize a free radical. Antioxidants have long been known to reduce the free radical mediated oxidative stress caused by elements and compounds in the environment [6]. Although there are several enzymes systems within the body that scavenge free radicals, the principal micronutrient (vitamin) antioxidants are vitamins E, beta-carotene, and vitamin C.

Vitamin C (ascorbic acid, ascorbate) represents the major water-soluble antioxidant in plasma and can also act as an antioxidant by reacting with free radicals. Vitamin E includes a group of lipid soluble compounds, tocopherol and tocotrienols that act as antioxidants defending the organism against oxidative stress. Ascorbic acid (Vitamin C) has been studied extensively in modulating lead intoxication. Ascorbic acid is known to have number of beneficial effects against lead toxicity. It acts mainly as an antioxidant molecule and its beneficial effects could be attributed to its ability to complex with lead [7].

Vitamin E (α-tocopherols) are multifaceted antioxidants, that scavenge oxygen free radicals, lipid peroxides and singlet oxygen [8]. They act as membrane stabilizers by their positive influence on membrane lipid organization. Vitamin C (ascorbic acid) is a good free radical scavenger and is known to represent the first line of antioxidant defense in the living cell [9,10]. It reacts with activated oxygen more readily than any other aqueous component and protects critical macromolecules from oxidative damage.

Since Gentamycin-induced nephrotoxicity has very important clinical consequences, different potentially therapeutic approaches to prevent or attenuate it have been proposed. Accordingly, this study is aimed at determining the possible protective effects of Vitamin-C and Vitamin-E against gentamycin-associated acute kidney injury and to compare the nephroprotective effect of *Boerhaavia diffusa* Linn., to that of Vitamins in Gentamycin nephrotoxicity.

SUBJECTS AND METHODS

- Wistar rats from animal house, MIMS medical college.
- NaCl–NaOH.
- Gentamycin 20ml ampules from apollo pharmacy, MIMS hospital , Vizianagaram, Andhra pradesh, given intraperitoneally.
- *Boerhaavia diffusa* aqueous root extract powder along with Sodium CMC administered orally.
- Vitamin-C in water and Vitamin-E 1:6 dilution in sesame oil given orally.

The experiments done were approved by the institutional animal ethics committee and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India.. CPCSEA No. 753/03/C/CPCSEA.

Wistar male rats weighing 125–150 g, are utilized for the present study. Experiments were performed with the permission of the institutional ethics committee. In the present study, male Wistar rats were used and are grouped as follows:

Group I: 10 albino rats with 1ml saline for 10 days and are sacrificed on the 11th day.

Group-II: 6 albino rats with 80mg/kg. bw of gentamycin for 10 days.

Group-III: A- 6 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with BD (punarnava) 800mg/kg.bw. By the end of 1 wks all the 6 rats were sacrificed to see the changes.

B- 6 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with BD (punarnava) 800mg/kg.bw. By the end of 4 wks all the 6 rats were sacrificed to see the changes.

C- 6 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with BD (punarnava) 800mg/kg.bw. By the end of 8 wks all the 6 rats were sacrificed to see the changes.

Group IV: A- 6 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with Vit-C 200mg/ kg .bw and Vit-E 250 mg/kg.bw orally for 1 wk and sacrificed by the end of the 1 week.

B- 6 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with Vit-C 200mg/ kg .bw and Vit-E 250 mg/kg.bw orally for 4wks and sacrificed by the end of the 4^{th} week.

C-6 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with Vit-C 200mg/ kg .bw and Vit-E 250 mg/kg.bw orally for 8wks and sacrificed by the end of the 8^{th} week.

All rats were kept under observation for 1 week prior to the experiments to permit the animals to adjust to the environment. All animals were fed standard rat chow and were provided tap water to drink ad libitum.

They were housed in a facility with 12–12 h light–dark cycle that is maintained at 25°C. All animals were weighed before the injections. The animals were anaesthetised with ether inhalation. Blood was drawn from retro-orbital plexus for biochemical investigations.

Histopathological examination

Processing of isolated kidneys

Bilateral paraumbilical vertical incisions were made. Right and left kidneys were removed quickly, weighed and preserved in 10% formalin. Anterior half of Kidneys from all groups were fixed in 10% neutral buffered formalin and processed for histological procedure. The isolated kidneys were cut into small pieces of 5mm thickness and preserved in formalin (10% solution). The kidney tissues were washed in running water for about 3 hrs. This was followed by dehydration with alcohol of increasing strength: 70%-1hr, 80%-1hr, 90%-1hr, Absolute alcohol-I-1hr, Absolute alcohol-II-1hr. The tissues are cleared in Xylene-I and Xylene-II for 1 hr each. The tissues were then processed in liquid paraffin i.e., wax-I-1hr and wax-II-1 hr . Liquid paraffin wax was poured into Leukart (L)-shaped blocks. The kidney tissues were embedded in liquid paraffin and were allowed to cool.

Staining

The blocks were sectioned using rotary microtome to get sections of 4-5 µ thickness. The sections were dried completely before staining. Sections were stained with Haematoxylin and Eosin, Masson's trichrome, and Periodic Acid Schiff and are examined under light microscope at 100 x and 400 x magnification.

Statistical analysis of the results obtained was done by using ANOVA and Bonferroni multiple Comparison by using SPSS software.

The experiments done were approved by the institutional animal ethics committee and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. CPCSEA No. 753/03/C/CPCSEA.

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RESULTS

Biochemical parameters

Administration of GM produced significant increase ($p<0.01$) in serum urea level when compared to normal group, indicating nephrotoxicity. Administration of BD 800 mg/kg.bw for 8 wks (G-III)and 200mg/kg.b.w of Vit-C and 250mg/kg.bw of Vit-E(G-IV) for 4 weeks from the 11th day on GM 80mg /kg.b.w for 10days(G-II) produced significant decrease in serum urea level which are nearly similar to that of control group (G-I). Increase in the serum urea level induced by gentamycin was reversed on treatment with both , the aqueous extracts of BD and vitamins.

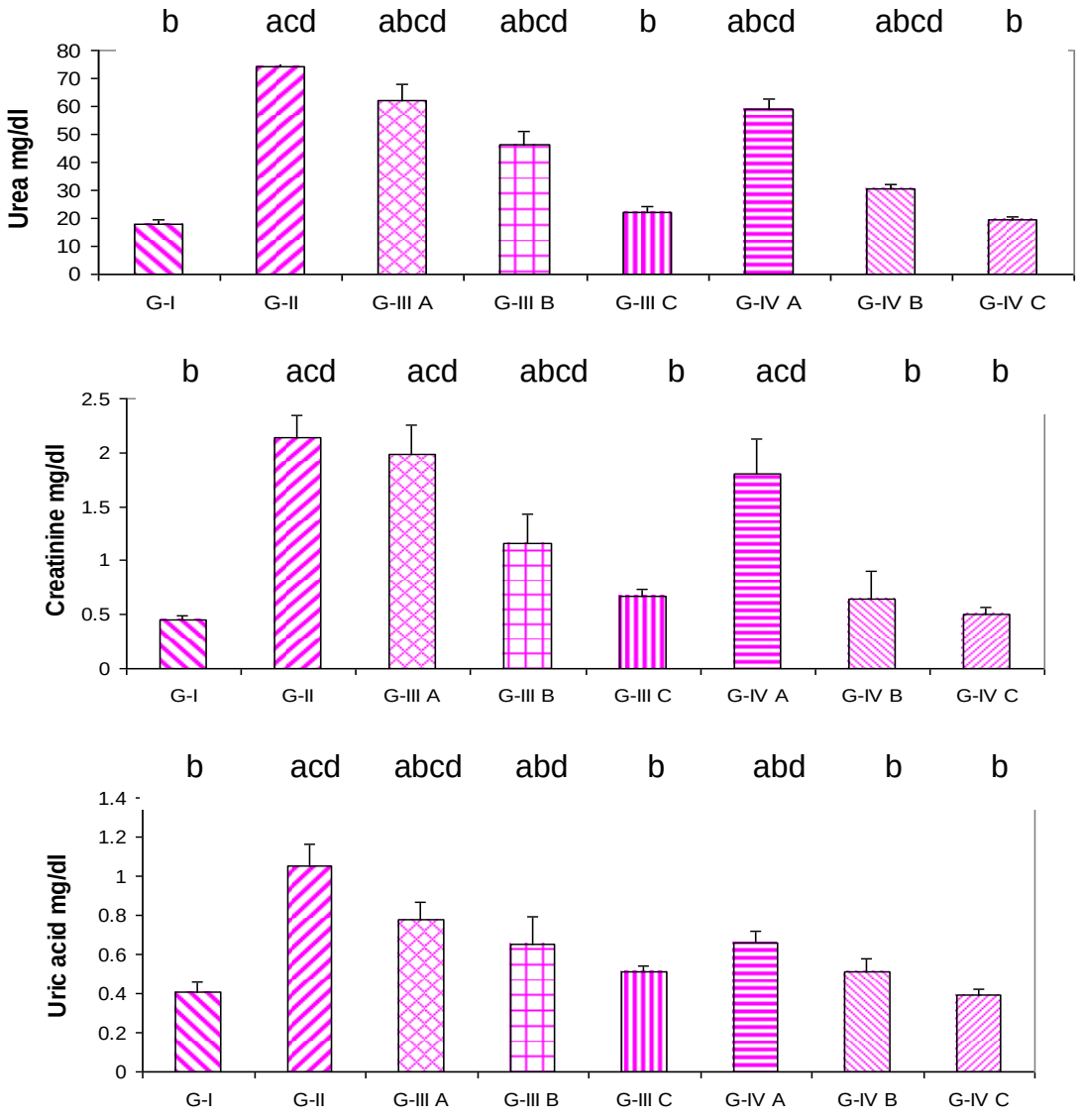

Figure 1: Urea, Creatinine and Uric acid levels in the following groups. Mean+SD (n=6 except control group n=10)

Group I- saline control for 10 days
Group-II- 80mg/kg. b.wt of GM for 10 days
Group-III – A)80mg/kg. b.wt of GM for 10 days + from 11th day BD 800mg/kg. b.wt for 1 week ,B) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day BD 800mg/kg. b.w for 4 wks ,C) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day BD 800mg/kg. b.w for 8 wks.
Group-IV-- A)80mg/kg. b.wt of GM for 10 days + from 11th day 200mg/kg.bw Vit-C and 250mg/kg. b.wt Vit-E for 1 week ,B) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day 200mg/kg.bw Vit-C and 250mg/kg. b.wt Vit-E for 4 wks ,C) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day 200mg/kg.bw Vit-C and 250mg/kg. b.w Vit-E for 8 wks.
a is significantly different from control group (G-I)

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b is significantly different from gentamycin group(G-II)
c is significantly different from BD treated (G- IIIC)
d is significantly different from Vitamins group(G-IVC) (P<0.05)

The serum urea level in BD 800 for 8 weeks treated group and vitamins for 8 weeks treated group did not produce significant difference when compared to the control group. All the groups produced significant difference when compared to gentamycin group p<0.001. BD 800 for four and eight weeks treated group and vitamins for 4 and 8 weeks treated group produced significant difference when compared to gentamycin group (p<0.001).The serum uric acid level in BD 800 for 8 weeks treated group and vitamins for 4 and 8 weeks treated group did not produced significant difference when compared to control group, indicating that the values are similar to each other. All the groups produced significant difference when compared to gentamycin group p<0.001 (Figure:1).

BD 800 for 8 weeks treated group could not produce significant difference when compared to gentamycin group but vitamins for 8 weeks treated group produced significant difference in serum sodium levels p < 0.001. BD 800 for 8 weeks treated group and vitamins for 8 weeks treated group produced significant difference in Serum Potassium level when compared to gentamycin group p<0.001. Gentamycin produced significant change in calcium levels when compared to control group(p<0.001). (Figure:2).

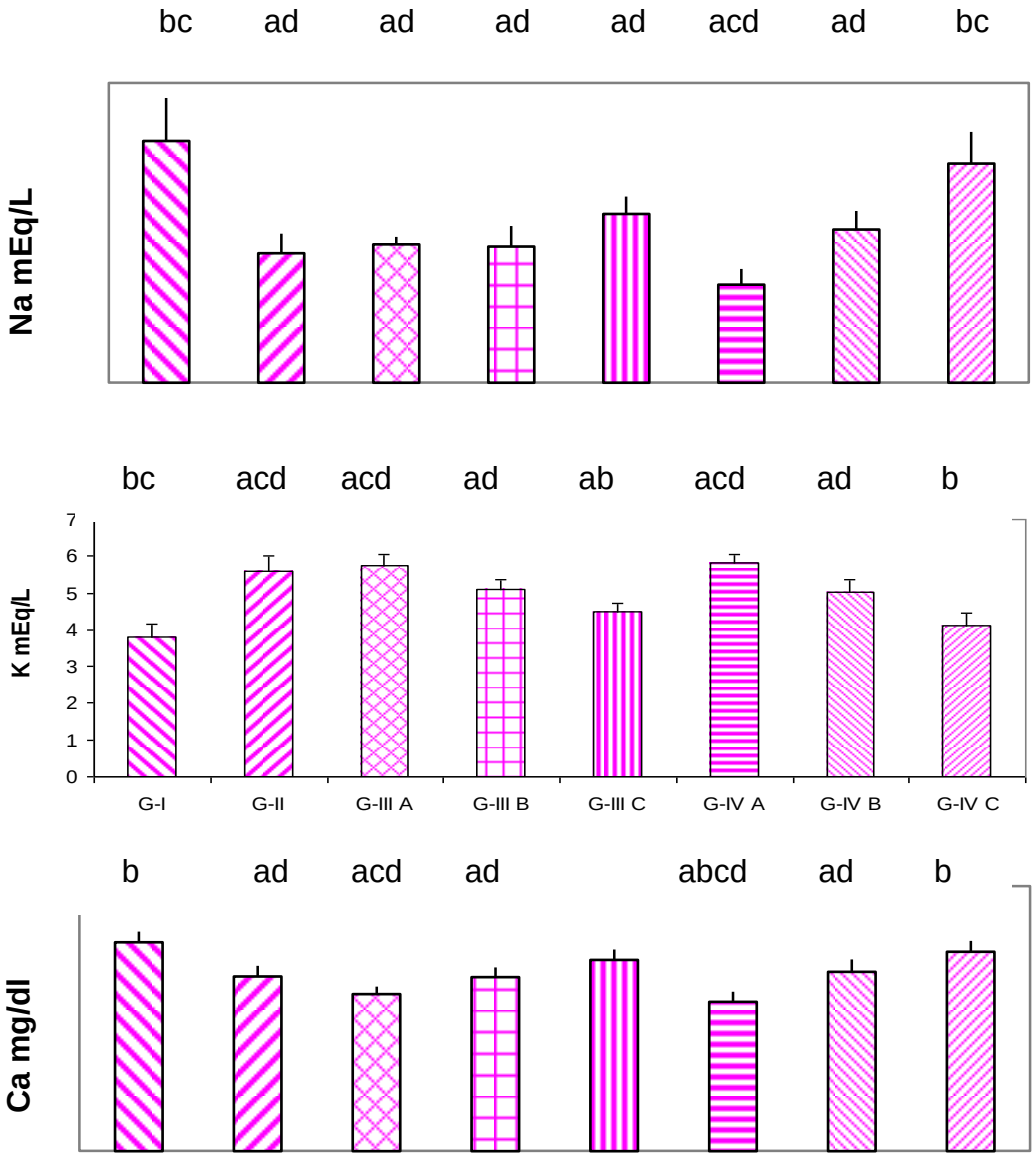

Figure 2: Sodium,Potassium and Calcium levels in the following groups. Mean+SD (n=6 except control group n=10)

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Group I- saline control for 10 days
Group-II- 80mg/kg. b.wt of GM for 10 days
Group-III – A)80mg/kg. b.wt of GM for 10 days + from 11th day BD 800mg/kg. b.wt for 1 week ,B) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day BD 800mg/kg. b.w for 4 wks ,C) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day BD 800mg/kg. b.w for 8 wks.
Group-IV-- A)80mg/kg. b.wt of GM for 10 days + from 11th day 200mg/kg.bw Vit-C and 250mg/kg. b.wt Vit-E for 1 week ,B) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day 200mg/kg.bw Vit-C and 250mg/kg. b.wt Vit-E for 4 wks ,C) 80mg/kg. b.wt of gentamycin for 10 days + from 11th day 200mg/kg.bw Vit-C and 250mg/kg. b.w Vit-E for 8 wks.
a is significantly different from control group (G-I)
b is significantly different from gentamycin group(G-II)
c is significantly different from BD treated (G- IIIC)
d is significantly different from Vitamins group(G-IVC), ($P<0.05$)

The parameters of GM group are statistically significant when compared to control group. Parameters of BD group and Vitamin group are statistically significant when compares to GM group $P<0.01$.

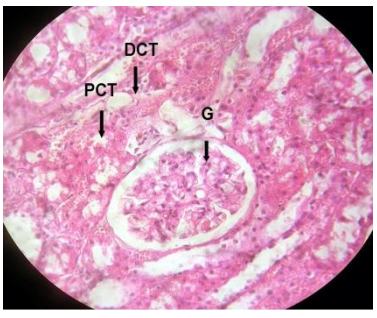
Histopathological observations

Group-I: Male albino rats with intake of normal saline showed normal architecture of renal glomeruli with intact bowmans capsule. H & E stained sections of the cortex of the kidney in control group showed presence of the glomeruli with blood capillaries surrounded by capsular space and Bowman's capsule. The PCT were lined by pyramidal cells, with acidophilic cytoplasm and rounded, basally located basophilic nuclei and having a free striated brush border. The distal convoluted (DCT) were lined by cubical epithelium, with light acidophilic cytoplasm and rounded, centrally situated basophilic nuclei. The lumina of the DCT were wider than that of PCT(Figure:3)

Group-II: The most severely affected were the proximal convoluted tubules (PCT) showing dilatations (Figure:4). PAS stained sections of the cortex of the kidney in gentamycin treated group showed faint PAS positive reaction. Lymphocytic infiltration has increased and 100% of the animals showed necrotic changes and lymphatic infiltration.(Figure:5)

Group-III: The renal cortex of the rats given with 800mg/ kg.bw of BD showed similar histological structure as that of control group . PAS stained sections of the cortex of the kidney showed PAS positive reaction in the brush borders of the PCT and basement membrane of renal tubules and glomerular capillaries (Figure:6). There is decreased degenerative and necrotic changes in PCT and glomerulus . PAS stained sections of the cortex of the kidney in this group were nearly similar to that of the control group. There was no difference when compared to control group. Hyaline cast disappeared and by the end of eight weeks, 80% of glomeruli have regained their normal structure and enclosed by continuous bowman's capsule showing continuity in the Bowman's membrane and regeneration of tubular epithelium (Figure:7)

Group-IV: Treatment with Vitamin E and Vitamin C in gentamycin induced rats for 4 weeks showed normal glomerular structure and regeneration of tubular epithelium though moderate tubular changes were observed(Figure:8). Strong PAS positive reaction is observed in the basement membrane and brush border of proximal convoluted tubules (Figure:9). Deposition of collagen fibers is observed with Masson's Trichrome indicating regeneration of Bowman's capsule (Figure:10)



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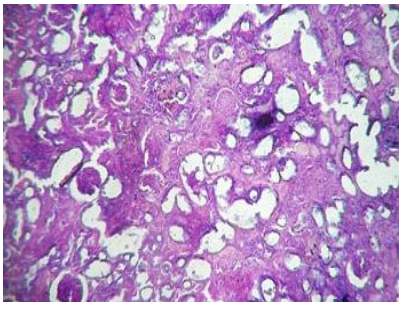
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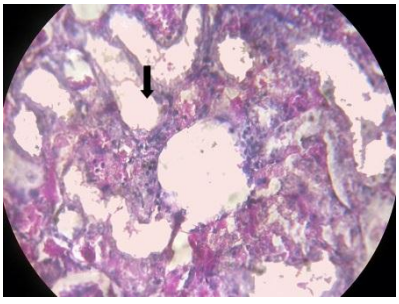
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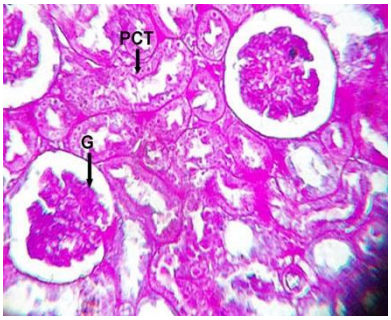
Figure 3: Histology of the renal cortex in controls showing normal glomerulus(G) and Proximal Convoluted Tubules (PCT), Distal convuluted tubule(DCT), magnification X400. H&E Stain

Figure 4: Histology of the renal cortex in 80 mg/kg.bw of GM induced rats showing dilated proximal convoluted tubules, magnification X400. PAS Stain

Figure 5:Histology of the renal cortex in 80 mg/kg.bw of GM induced rats showing dilated proximal convoluted tubules, magnification X400. PAS Stain



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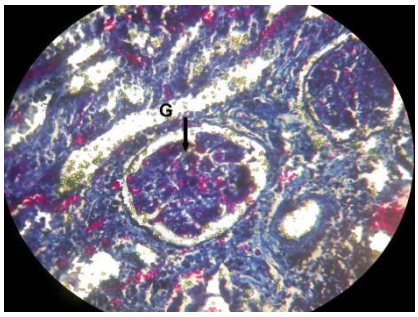

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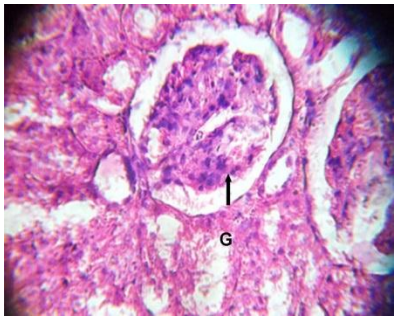

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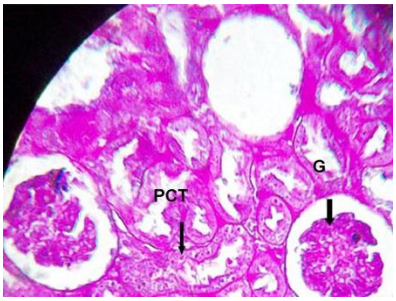

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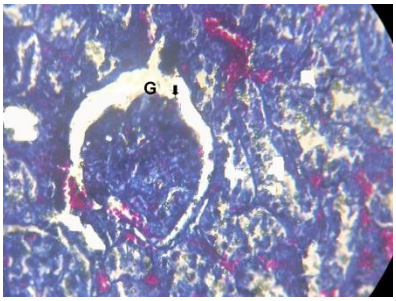

Figure: 10

Figure 6: Histology of the renal cortex in 80 mg/kg.bw of GM induced rats +800mg/kg.bw of BD showing normal cytoarchitecture, magnification X400. PAS Stain

Figure 7: Histology of the renal cortex in 80 mg/kg.bw of GM induced rats +800mg/kg.bw of BD showing normal cytoarchitecture, magnification X400. MT Stain

Figure 8: Histology of the renal cortex in 80 mg/kg.bw of GM induced rats +200mg/kg.bw of Vit-C+250mg/kg.bw of Vit-E showing normal cytoarchitecture, magnification X100. PAS Stain

Figure 9: Histology of the renal cortex in 80 mg/kg.bw of GM induced rats +200 and 250mg/kg.bw of Vit-C+ Vit-E showing normal cytoarchitecture, magnification X400. PAS Stain

Figure 10: Histology of the renal cortex in 80 mg/kg.bw of GM induced rats +200 and 250mg/kg.bw of Vit-C+ Vit-E showing normal cytoarchitecture, magnification X400. MT Stain

DISCUSSION

In a study, Kidney section of normal rats showed normal renal architecture , Kidney section of acetaminophen induced control rats with showing glomerular degeneration, tubular brush border loss, tubular dilatation, necrosis of epithelium and interstitial oedema. Kidney section in *Boerhaavia diffusa Linn.,* (200 mg/kg)-treated rats showing mild tubular damage. (d) Kidney section extract of *Boerhaavia diffusa Linn.,* (400 mg/kg)-treated rats showing almost normal renal architecture [11]**.** After intramuscular administration for up to 10 days, gentamycin (80 mg/kg/body Wt) alone caused significant reduction in GFR, glomerular changes and secondary tubular casts evident by significant increase in serum BUN and decreased creatinine clearance [12- 15] . Pre-treatment of rats with vitamin E gave rise to increased changes in nephrotoxicity on day 10, between the groups receiving concurrent gentamycin ± vitamin E and those receiving pre-treatment

with vitamin E ± gentamycin [16,17]). This was particularly marked by significant changes in BUN concentration.

Sodium, potassium, calcium and phosphorus [18] explicitly showed the electrolyte abnormalities upon treatment of rats with gentamycin (80 mg/kg/body Wt). Relevant effects of vitamin E therapy prior to gentamycin administration in the studies that the activity of Na/K pumps in cell membrane have been regulated suggest, a striking association between oxidative pathways, hyperhomocystinemia, suppression or decrease in glomerular synthesis of thromboxane B2, O2-, MDA, H2O2, antidiuretic potential of vitamin E[19] and scavenging of free radicals due to turnover of glutathione and vitamin C [20].

Histopathologic results also showed minimal changes in renal tissue, indicating the influence of vitamin E pretreatment against gentamycin-induced nephrotoxicity. The present biochemical and histological results supported each other strongly. In a study the author provided evidence that pre-treatment of vitamin E can prevent both the functional and histological renal changes induced by gentamycin in rats [21]. In the present study also there is regeneration in the tubular epithelium of PCT which is in harmony with the above authors.

Experimental gentamycin nephrotoxicity has been investigated in various animal models so far, and several approaches considering different mechanisms have been attempted to reduce the nephrotoxicity of gentamycin and related aminoglycosides [22]. Among these, the most consistent effect has been observed with the use of antioxidant agents such as vitamin C and vitamin E [21]. All groups in a study were treated during 8 consecutive days. Quantitative evaluation of gentamycin(G)-induced structural alterations and degree of functional alterations of kidney were performed by histopathological, morphometrical and biochemical analyses in order to determine potential beneficial effects of vitamin C co-administration with gentamycin. In G-group the proximal convoluted tubules showed cytoplasm vacuolation with dark inclusions in the epithelial cells and coagulation-type necrosis, while in GVC-group necrosis was not observed. The glomerular basement membrane was significantly thickened ($p<0.05$) in G-group animals than in other groups. Nuclear optical density of the tubular epithelial cells in GVC-group was significantly higher ($p<0.05$) compared to G-group. Blood urea and serum creatinine concentration were significantly elevated, while potassium concentration was lowered in G-group compared to other groups ($p<0.01$ for each).Concomitant administration of gentamycin and vitamin C resulted in a significant reduction of morphological and functional kidney alterations [23].

In the present study there is regeneration of tubular epithelium with administration of BD 800mg/kg.bw for 8 weeks in 80mg/kg.bw of gentamycin induced renal damage rats and also in rats treated with Vitamin C 200mg/kg.bw + 250mg/kg.bw of Vitamin E for 8 weeks in gentamycin induced nephrotoxicity .

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