

## An Experimental Study of Biochemical and Histopathological Study on Gentamycin Induced Renal Failure in Albino Rat And The Effectiveness Of Punarnava (BOERHAEVIA Diffusa) On Reversal Of Renal Damage

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**Abstract:** 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. 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. By using one way ANOVA the results are significant at .001. Hyaline cast formation is observed in PCT with atrophic glomeruli effecting half of the cortical region when rats treated with 80mg/kg b.w. administration of Punarnava 400mg and 800mg/kg.bw rejuvenated necrotic cells of kidney. Gentamicin must be given in the lowest effective therapeutic doses in patients with normal kidney function along with punarnava.

**Key words:** gentamicin, glomeruli, lymphatic infiltration, proximal convoluted tubules, punarnava

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### I. 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. Hydroxyl radicals play a role in the pathogenesis of gentamicin nephrotoxicity, gentamicin 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 gentamicin. Renal cortical mitochondria is the source of reactive oxygen metabolites, which induces renal injury (Nephrol Dial Transplant. 1994;9 Suppl 4:135-40[1]).

Pharmacological studies have demonstrated that *B. diffusa* exhibits a wide range of properties such as diuretic [Gaitonde BB, et al 1974[2]; nephrotic syndrome [Singh RH et al 1972[3]; antiurolithiatic [Pareta SK et al 2011[4]; antioxidant and antidiabetic activity. Due to the combination of diuretic, antioxidants and anti-inflammatory activities *B. diffusa* is regarded as therapeutically highly efficacious for the treatment of inflammatory renal diseases and common clinical problems such as nephrotic syndrome, oedema, and ascites. Boerhavia Diffusa has been reported to be useful in the treatment of elephantiasis, night blindness, corneal ulcers and nephritic syndrome (Mishra J, et al 1980[5], Singh RH, et al 1972[3]).

The protection offered by the *B.diffusa* aqueous extract could have been due to the presence of any of the active principles contained in it. Literature has shown *B. diffusa* contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. Flavonoids and other antioxidant constituent of medicinal plants have been reported to inhibit xenobiotic induced nephrotoxicity in experimental animal models due to their potent anti-oxidant effects [Devipriya S et al 1999[6]. Very few studies of histopathology were reported in literature with the effectiveness of punarnava (*Boerhaevia diffusa*) on gentamicin induced renal failure in Albino rats and to estimate the damage and revival of renal tissue. So, the present study is taken up to record the anti-nephrotoxic effects of punarnava.

### II. Materials And Methods

Wistar - albino 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 albino rats were used and are grouped as follows:

\*group I- 6 albino rats with normal saline for 10 days and are sacrificed on the 11<sup>th</sup> day.

\*(group-II- 6 albino rats with 400mg/kg. b.wt of punarnava(pure extract obtained in capsules from himalaya product-each capsules containing 250mg of extract of pure punarnava powder). for 10 days and are sacrificed on the 11<sup>th</sup> day.

\*group-III-10 albinorats with 80mg/kg. b.wt of gentamycin for 10 days. On 11<sup>th</sup> day 6 animals are sacrificed and remaining 4 are left without giving any treatment to see whether there is self regeneration.

\*group-IV-10 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with punarnava 400mg/kg. b.wt for 3weeks by the end of third wk two rats were sacrificed to see the regenerative changes. By the end of six weeks another two rats were sacrificed to see the changes.by the end of 9 wks all the 6 rats were sacrificed to see the changes.

\*group-V- 10 albino rats with 80mg/kg. b.wt of gentamycin for 10 days and from 11th day treated with with punarnava 800mg/kg. b.wt for 3weeks by the end of 3<sup>rd</sup> wk two rats were sacrificed to see the regenerative changes. by the end of 6wks another two rats were sacrificed to see the changes. By the end of 9wks all the 6 rats were sacrificed to see the changes.

- ⊙ All animals were fed standard rat chow and were provided tap water to drink *ad libitum*. All animals were weighed before the injections. The animals were anaesthetized with ether inhalation.
- ⊙ Blood samples were collected from retro-orbital plexus for biochemical investigations like blood urea, uric acid, creatinine, serum Na, K, Ca, determination. Bilateral periumbilical vertical incisions were made. Right and left kidneys were removed quickly and weighed and preserved in 10% formalin.
- ⊙ **Histopathological examination:**

Anterior half of Kidneys from all three groups were fixed in 10% neutral buffered formalin and processed to paraffin wax. 5 microns Sections are stained with Haematoxylin and Eosin , Massons trichrome, and Periodic Acid Schiff and are examined under light microscope at 100 and 400 magnification.

### III. Results

Table (1): Comparative Nephrotoxic effects of gentamicin and nephroprotective effects of different doses of Boerhavia diffusa (Punarnava) on some biochemical parameters in rats.

	Group-I CONTROL- SALINE	Group-II 400mg B.diff/kg.b.w	Group-III 80mg gen./kg.b.w	Group-IV 80mg gen./kg.b.w for 10days and 400mg/kg.b.wt punarnava from 11th day for 9 weeks	Group-V 80mg gen./kg.b.w for 10days and 800mg/kg.b.wt punarnava from 11th day for 9 weeks
PARAMETERS	MEAN+SD	MEAN+SD	MEAN+SD	MEAN+SD	MEAN+SD
žUrea (mg/dl)	16.8+0.43	16.76+0.45	74.3+1.46	38.23+4.45	22.16+2.15
žCreatinine (mg/dl)	0.46+0.02	0.46+0.29	2.14+0.21	1.21+0.23	0.67+0.07
žUric acid (mg/dl)	0.36+0.05	0.37+0.04	1.05+0.11	0.68+0.07	0.51+0.03
žNa (meq/L)	125.53+1.44	124.62+2.26	117.97+1.93	119.6+1.56	121.9+1.72
žK (meq/L)	4.06+0.08	4.11+0.11	5.58+0.43	5.06+0.21	4.5+0.22
žTotal calcium (mg/dl)	9.21+0.45	9+0.25	7.7+0.50	8.07+0.26	8.66+0.46

Mean , Standard deviation and one way ANOVA was done to know the significance. All The Values Are Significant Between And Within Groups At .001

- ⊙ Values are expressed as means  $\pm$  S.D. By using one way ANOVA the results are significant .

Group-III –1 rat out of 4 rats which are not treated after inducing gentamycin toxicity for 10 days , died on the fourth day and remaining 3 rats were sacrificed on the eight day. Mean and S.D are similar to that of group-III and are excluded from the table.

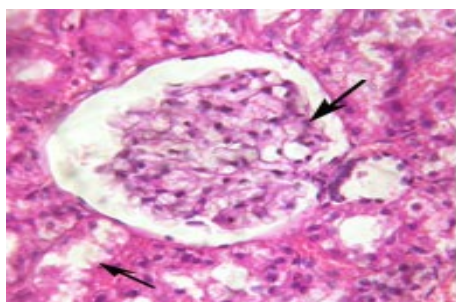
#### 3.1 Histopathological Observations

##### 3.1.1 Group-I –6 rats treated with normal saline(0.9% Nacl)

Male albino rats with intake of normal saline showed normal architecture of renal glomeruli with intact bowmans capsule. Brush bordered cuboidal epithelium lining the proximal convoluted tubules. Simple cuboidal epithelium lining the distal convoluted tubules .Macula densa is very prominent(fig.1).

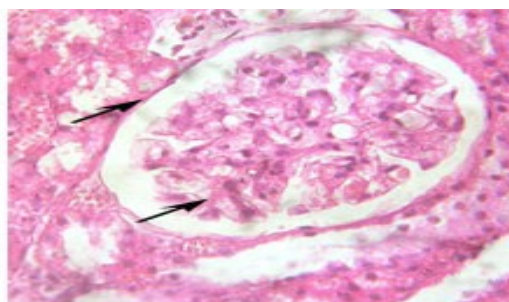
##### 3.1.2 Group-II- rats treated with punarnava 400mg/kg b.w

The cytoarchitecture of kidneys showed normal structure of glomeruli, distinct proximal and distal convoluted tubules(fig.2).



**fig.1**

**fig.1 showing normal glomeruli and PCT with brush bordered cuboidal epithelium in G-I rats**

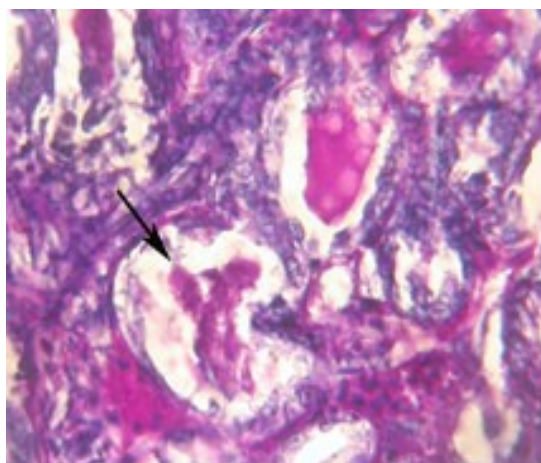


**fig.2**

**fig.2 showing normal glomeruli and continuous Bowman's capsule in G-II rats**

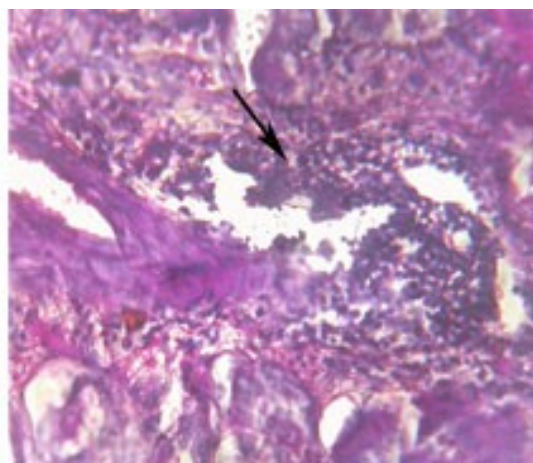
### **3.1.3 Group-III –rats induced with gentamycin 80mg/kg b.w**

The use of the periodic acid-Schiff reaction confirmed that these apoptotic cells were almost exclusively found in proximal tubules causing obstruction of PCT'S. Tubular basement membrane is interrupted. Glomerular congestion, disruption of glomerular capillaries, vacuolar degeneration of tubular epithelial cells is observed with hyaline cast formation is observed in PCT. Atrophic glomeruli are present effecting half of the cortical region(fig.3). Lymphocytic infiltration has increased(fig.4) . Rats induced with gentamycin 80mg/kg b.w for 10 days and not treated showed atrophic glomeruli and hyaline casts in PCT'S. There is no self regeneration.



**fig.3**

**fig.3 showing atrophic glomeruli effecting half of the cortical region in G-III rats, 10x40, PAS.**



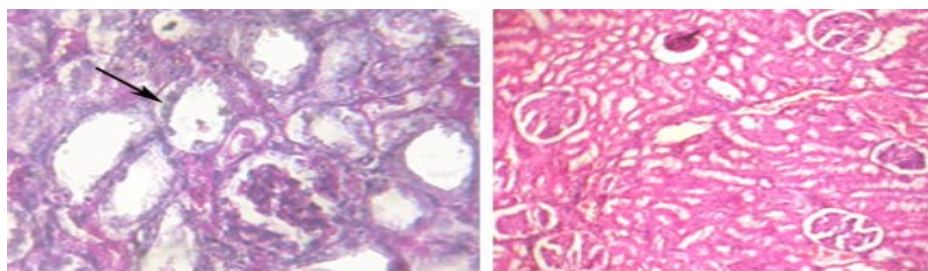
**fig.4**

**fig.4 showing peritubular lymphocytic infiltration in G-III rats in G-III rats, 10x40, PAS.**

### **3.1.4 Group-IV- rats induced with 80mg/kg.bw gentamycin and treated with 400mg/kg.bw of punarnava**

After 3wks of treatment , the renal microscopic details did not show any regeneration in kidneys of two rats. By the end of 6 wks , the PCT'S are lined with cuboidal epithelium and are clear without any hyaline cast(fig.5) and regeneration of luminal epithelium. By the end of 9wks , the renal architecture showed 50% improvement in regeneration of glomerular tuft and distinct epithelium formation of proximal convoluted tubules(fig.6).





**fig.5**

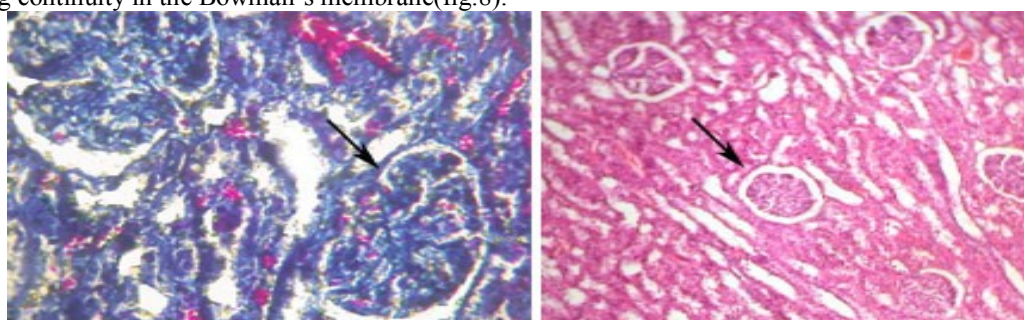
**fig.6**

**fig.5 showing PCT'S without any hyaline cast, 10x40, PAS.**

**fig.6 showing 50% improvement in regeneration of glomerular tuft , 10X10, H&E**

### **3.1.5 Group-V- rats induced with 80mg/kg.bw gentamycin and treated with 800mg/kg.bw of punarnava**

By the end of 3wks the lymphatic infiltration in the proximal convoluted tubules disappeared indicating anti-nephrotoxic effect of punarnava. By the end of 6wks , hyaline cast disappeared and by the end of 9wks, 80% of glomeruli have regained their normal structure and enclosed by continuous bowman's capsule(fig.7) showing continuity in the Bowman's membrane(fig.8).



**fig.7**

**fig.8**

**fig.7 showing glomeruli having normal structure, 10x40, Massons Trichrome**

**fig.8 showing continuity in the Bowman's membrane, 10x10, H&E.**

## **IV. Discussion**

*Acute renal failure* is characterized by disorders in some biochemical parameters in gentamicin treated rats .Rats treated with 80mg/kg b.w. Gentamicin produced increase in the concentration of serum urea, creatinine and uric acid. These results confirmed that gentamicin produced nephrotoxicity as previously reported by Ali *et al.*, 2003[7], Goto, 2004[8] and Heibashy *et al.*, 2009[9]. More than half of proximal tubules showing desquamation of necrosis but involved tubules easily found, complete or almost complete tubular necrosis. Serum electrolytes were disturbed in GM treated rats as compared with control animals. Lower value of serum sodium indicated inability of kidney to conserve sodium and chloride(Heibashy & Abdel Moneim (1999)[10] and Heibashy *et al.* (2009[9]). Gentamicin treated rats show tubular epithelial damage with intense granular degeneration involving > 50% of renal cortex. Some of the tubular epithelium contains tubular casts as observed by K.VIJAY KUMAR *et al* 2000[11]). Gentamicin renal cell damage as induced by tubular necrosis ie, marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the tubular lumen and infiltration of inflammatory cells in the interstitium was confirmed on histopathological examination by Shirwaikar A *et al* 2003[12]. In the present study shrunken glomeruli and glomerular atrophy is observed by gentamycin induced renal damage (80mg/k.g. b.wt for 10 days) are in harmony with the above authors.

Surendra K 2011[13] investigated the effects of pre-treatment of aqueous extract of *B. diffusa* root (200 – 400 mg/kg/day) in repeated dose acetaminophen nephrotoxic rats for 14 days. pre-treatment with *B. diffusa* extract protected against degenerative changes renal cortical architecture in the experimental rats. Antioxidant enzymes such as Glutathioneperoxidase, Reduced glutathione, Vitamin C and Catalase were elevated in animals treated with *Boerhaavia diffusa* aqueous extract which showed that the aqueous extract of *Boerhaavia diffusa* leaves significantly reduced the nephrotoxicity induced by mercuric chloride(T.Indhumathi *et al* 2011[14]). The herb is a diuretic that acts on the glomeruli of the kidney and also protects the kidney from being damaged (Rawat *et al*, 1997[15]).

Post -treatment with *B. diffusa pure* extract (400mg/kg.b.wt and 800mg/kg.bwt for 9wks exhibited anti-nephrotoxic effects(curative) against degenerative changes of renal cortical architecture and also significant normal values of biochemical parameters.

### V. Conclusion

- Daily intraperitoneal injection of rats with 80 mg gentamicin /kg b.w for 10 days caused a serious harmful effects on renal function tests.
- Treatment with *B. diffusa pure* extract (400mg/kg.b.wt and 800mg/kg.bwt for 9wks exhibited anti-nephrotoxic effects(curative). Histopathological findings indicate rejuvenation of necrotic cell in kidney
- Thus, it could be suggested that gentamicin must be given in the lowest effective therapeutic doses in patients with normal kidney function.
- Also, gentamicin therapy should be accompanied with administration of punarnava which will nullify the nephrotoxic effect of gentamycin.
- *Boerhaavia diffusa* (Punarnava) even though given in larger doses will not have any side effects .

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Note: This study is a partial work of my Ph.D research , titled “An experimental anatomical study on the effectiveness of punarnava (*Boerhaavia diffusa*) on gentamycin induced renal failure in albino rat and to estimate the drug induced damage and revival of renal tissue” . Part of this work has been published in iosrjdm journals with gentamycin nephrotoxicity.

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