

## ORIGINAL ARTICLE

## PROTECTIVE ROLE OF GINSENG AGAINST GENTAMICIN INDUCED CHANGES IN KIDNEY OF ALBINO MICE

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**Background:** Use of gentamicin is now limited due to its toxic effects, mainly on kidney and vestibular system. Herbal products including ginseng has been reported to possess protective effects against drugs induced nephrotoxicity in experimental animals. The current investigation was designed to evaluate the effects of ginseng on gentamicin induced nephrotoxicity. **Methods:** Eighteen male albino mice of 6–8 weeks age, were divided into 3 groups. Group-A served as control and was given normal mouse diet; Group-B was given 80 mg/Kg/day of gentamicin intraperitoneally dissolved in 1 ml of distilled water for fifteen days. Group-C was given 80 mg/Kg/day of gentamicin intraperitoneally dissolved in 1 ml of distilled water along with 100 mg/Kg/day of ginseng orally dissolved in 1 ml of distilled water, also for fifteen days. At the end of the experiment, blood was drawn from each animal by cardiac puncture for renal function tests. Each animal was then sacrificed and kidneys removed for routine histological studies. **Results:** In group B, weight of the animals and kidneys decreased and there was significant increase in mean serum urea, creatinine and intraluminal diameter ( $p<0.001$ ) of proximal convoluted tubules as compared to the controls (group-A). Moderate to severe necrotic and degenerative changes in proximal convoluted tubules were seen in this group. When the Ginseng and gentamicin were given together (group-C), a statistically significant improvement in the mean body and kidney weight along with improvement in renal function tests and tubular diameter were seen ( $p<0.001$ ). **Conclusion:** It appears that Ginseng has some protective role against gentamicin induced nephrotoxicity.

**Keywords:** Gentamicin, ginseng, nephroprotective role, nephrotoxicity, serum urea, serum creatinine

## INTRODUCTION

Gentamicin is one of the common amino glycosides that have been used for the treatment of various bacterial infections. After oral administration, gentamicin is not very effective because it is not absorbed to an appreciable extent from the intestinal tract. The recommended routes of administration of gentamicin are intravenous, intramuscular, intraperitoneal or topical.<sup>1</sup> Its use is now limited due to its toxic effects, mainly on kidney and vestibular system.<sup>2</sup> Nephrotoxic effects of gentamicin treatment are due to its accumulation in renal cortical tubular epithelial cells.<sup>3</sup> Membranous structures that can be damaged by gentamicin include lysosomes, mitochondria, microsomes. Lysis of lysosomes containing gentamicin may release acid hydrolases which causes disruption of critical intracellular processes including mitochondrial respiration, electron transport chain, and microsomal protein synthesis.<sup>4</sup> It has been reported to stimulate the generation of reactive oxygen species<sup>5</sup>, form iron drug complex<sup>6</sup> that leads to renal damage.

Herbal products including ginseng has been reported to possess protective effects against drugs induced nephrotoxicity in experimental animals.<sup>7</sup> The mechanism by which ginseng exerts its activity is presumably through hypothalamus-pituitary-adrenal axis.<sup>8</sup> Tran *et al.* (2002) conducted an experimental study on guinea pigs and stated that ginseng may reduce

cell damage induced by toxic substances, act to stabilize cell membranes<sup>9</sup> and protect tissues from damage by inhibiting lipid peroxidation<sup>10</sup>. These effects may be due to the antioxidant nature of ginseng.<sup>11</sup> Blood urea and creatinine are raised significantly in gentamicin induced nephrotoxicity.<sup>12</sup> Treatment with ginseng had been reported to normalize values of raised blood urea and creatinine.<sup>13</sup> However, role of ginseng on nephrotoxic effects of gentamicin have not received proper attention. The current investigation was, therefore, designed to evaluate the effects of ginseng on gentamicin induced nephrotoxicity.

## MATERIAL AND METHODS

This study was an experimental Randomized Control Trial (RCT) conducted at the Experimental Research Laboratory of University of Health Sciences Lahore. Eighteen male albino mice 6–8 week old, weighing 20–25 gm each were procured from National Institute of Health, Islamabad. They were kept under controlled temperature (23–25 °C), humidity (60%), light and dark cycles of 12 hours each and allowed to acclimatize for one week. The animals were fed on standard mice diet and water *ad libitum* and were weighed at the start of experiment. The animals were then randomly divided into three groups, having six mice each. Group A served as control and were given 1 ml distilled water per day by mouth, in addition to water *ad libitum*. Group B

was given 80 mg/Kg/day of gentamicin intraperitoneally dissolved in 1 ml of distilled water for fifteen days. Group C was given 80 mg/Kg/day of gentamicin intraperitoneally dissolved in 1 ml of distilled water along with ginseng orally at the dose of 100 mg/Kg/day dissolved in 1 ml of distilled water for fifteen days. The body weight of each animal was recorded twice weekly and also at the end of the experimental period when each animal was taken out of cage and euthanized under chloroform before 2 ml of blood was taken in 5 ml disposable syringe by cardiac puncture. Serum was separated and stored at -20 °C for measuring urea and creatinine. Both metabolites were measured by using commercially available kits of "Human Company". Each animal was then sacrificed, kidneys removed and examined for gross changes, 2 mm<sup>3</sup> pieces were taken for routine histology and fixed in 10% formalin. Sections were cut at five micron on a motorized microtome and stained with H and E.

Intra-luminal diameter of proximal convoluted tubules was measured using ocular micrometer. The tubules from ten randomly selected fields in the cortex were assessed for tubular necrosis, cellular vacuolation, condition of their lumen and scored as:

- Normal (-)= No tubular necrosis
- Mild (+)= Less than quarter of the total number of proximal tubule (<25%)
- Moderate (++)= When there was involvement of less than half of the tubules (26–50%)
- Severe (+++)= When more than half of the proximal tubules showed necrosis (>50%)

Mean scores of histological changes were calculated and the frequency of histological changes in renal tubules was expressed in percentage.

The data was analysed using SPSS version 17. Mean±SE is given for quantitative variables. One way ANOVA was used to compare the groups and Tukey post hoc test was used for detail analysis. Fisher Exact Test was applied to observe association between qualitative variables. Differences between groups were considered to be statistically significant, if  $p < 0.05$ .

## RESULTS

All animals of groups A and C were healthy and active; however, the animals of group B showed irritating behaviour. The mean body weight of the animals at the start of experiment was 23.66±0.33, 24.33±0.42 and 24.66±0.42 gm for groups A, B and C respectively. ANOVA showed that there was no difference between groups weight. The body weights decreased significantly after treatment with gentamicin however, it improved when gentamicin and ginseng were given together (group C). The Similar changes were seen in the kidney weights. The difference in the

kidney weight between groups A and C and groups B and C was not significant. Serum urea and creatinine increased ( $p < 0.001$ ) after treatment with gentamicin while they came towards control groups in group C but were still different from group B. These data have been summarized in Table-1.

Examination of renal cortex from group A (Control) showed normal renal corpuscles. Glomerular capillaries were observed in all sectional profiles. The cortical tubules made the bulk of parenchyma and mainly consisted of proximal and distal convoluted tubules in addition to collecting tubules (CT). Proximal convoluted tubules (PCT) were lined by simple cuboidal epithelium, having prominent brush borders and acidophilic cytoplasm. Distal convoluted tubules (DCT) were identified on account of simple cuboidal epithelium, clearly defined and wider lumen, than those of the PCT, closely packed nuclei per section (Figure-1).

In group B, the proximal convoluted tubules in cortex were dilated and showed patchy necrosis, loss of brush border, presence of cellular debris and accumulation of inflammatory exudates within their lumen. The epithelial cells of proximal convoluted tubules showed hydropic changes with cytoplasmic vacuolations at some places. Some of the tubules exhibited desquamated epithelial cells in their lumina. The nuclei of these cells were swollen and karyolytic (Figure-2).

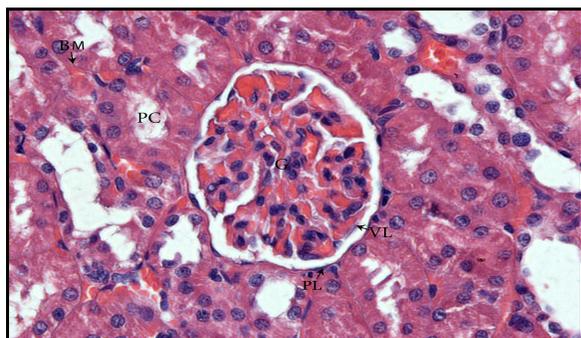
Kidneys from group C showed less nephrotoxic effects compared to those in group B. The difference was statistically significant when the effects as tubular necrosis, inflammatory cellular infiltration (Figure-3 and 4) of group C were compared with those in group B ( $p < 0.05$ ). However, in group C, there were areas of interstitial haemorrhage and vascular congestion as compared to those in groups A and B (Figure-3).

Tubular Necrosis: Significant association was observed between percentages of tubular necrosis and various groups (Fisher Exact test:  $p < 0.001$ ). In group B, proximal tubular necrosis was moderate (++) in 1 animal, severe (+++) in remaining 5 animals with the percentages of 16.66 and 83.33 % respectively. In group C, proximal tubular necrosis was mild (+) in 1 animal, moderate (++) in 4, severe (+++) in remaining 1 animal with the percentages of 16.66, 66.66, and 16.66 % respectively showing significant improvement in the condition of kidney parenchyma.

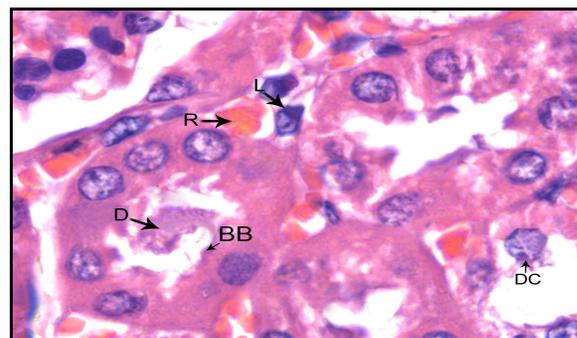
Luminal diameter of proximal convoluted tubules in group B was noticeably increased as compared to groups A and C. Significant difference ( $p < 0.001$ ) was observed in the tubular luminal diameter among all the groups (Table-1).

**Table-1: Mean body and kidney weight, serum urea, creatinine and tubular diameter of animals treated with gentamicin (80 mg/Kg/day) intraperitoneally and ginseng (100 mg/Kg/day) orally for 15 days. (Mean±SE)**

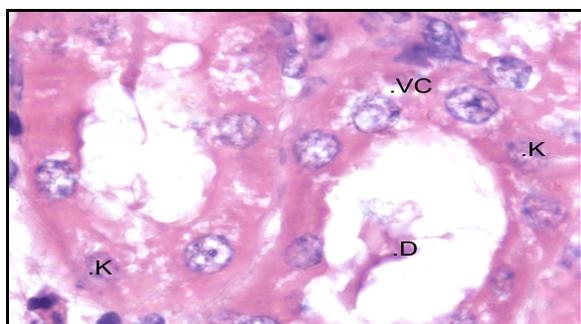
Parameter	Group A	Group B	Group C	p
Body weight (g)	25.50±0.50	17.16±0.30	21.83±0.47	<0.001
Kidneys weight (g)	0.39±0.001	0.37±0.005	0.38±0.004	<0.001
Serum urea (mg/dl)	34.73±0.84	66.40±0.54	47.73±0.69	<0.001
Serum creatinine (mg/dl)	0.53±0.04	1.41±0.08	0.68±0.08	<0.001
Mean Tubular Diameter (µm)	41.45±0.04	60.71±1.20	48.13±0.64	<0.001



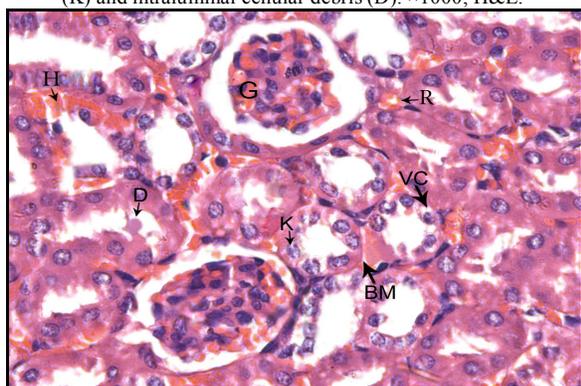
**Figure-1: Section of kidney from group A,** showing glomerulus (G), parietal layer (PL), and visceral layer (VL), proximal convoluted tubule (PC) lined with cuboidal epithelium and intact basement membrane (BM). ×400; H&E.



**Figure-4: A section of kidney from group C,** showing cortical tubules containing luminal cellular debris (D), their epithelial cells bear brush borders (BB), dropping out cell (DC), lymphocyte (L) and red blood cells (R) are also seen. ×1000; H&E.



**Figure-2: A section of kidney from group B,** showing damaged and dilated tubule with desquamating epithelium having cytoplasmic vacuolations (VC), karyolitic nuclei (K) and intraluminal cellular debris (D). ×1000; H&E.



**Figure-3: Photomicrograph of histological section of kidney from group C,** most tubules have smudged appearance, showing glomerulus (G), karyolitic nuclei (K) cytoplasmic vacuolations (VC) intact basement membrane (BM), intra luminal cellular debris (D), interstitial haemorrhage (H) and congested blood vessel containing red blood cells (R). Stain H&E ×400.

## DISCUSSION

Our findings show that in group B, gentamicin produced statistically significant loss of body and kidney weight when given alone. This decrease is probably due to the anorexia and partial renal failure leading to acidosis as reported earlier.<sup>14,15</sup> However, when gentamicin was given concomitantly with the ginseng, there was an improvement both in the body and kidney weight in the group C animals. When the body and organ weights of group C were compared with those of groups A and B, the difference compared to group A was statistically insignificant ( $p>0.05$ ); however, the difference was still significant ( $p<0.05$ ). These results show that although some amelioration in body and organ weights occurred after giving ginseng, complete reversal of the toxic effects of the gentamicin were not achieved. May be a higher dose or treatment for a longer time would have completely ameliorated the toxic effects of the gentamicin on kidney. Further, studies are suggested in this direction.

As reported in the results that gentamicin had increased the serum urea and creatinine when given alone. However, like body and organ weights, these parameters also showed improvement in group C animals. This improvement seem to presumably be due to the amelioration of gentamicin induced oxidative injury to the tubular system. Lipsky *et al*<sup>16</sup> had earlier reported similar toxic effects of gentamicin<sup>16</sup> and Yokozawa *et al*<sup>13</sup> reported the improvement in these effects by ginseng treatment.

The fact that ginseng prevented toxic effects of gentamicin was observed in histological studies also.

Kacew, (1989) had earlier reported that gentamicin caused tubular necrosis and loss of brush borders.<sup>17</sup> Our observations corroborate the findings and those of Morsy (2002) and Fatima (2003), who found that ginseng alleviated the deleterious effects of pesticides, ochratoxin and prophenophos on kidney tissue that had been grossly altered after treatment with these pesticide.<sup>18,19</sup>

Kosek *et al*<sup>20</sup> reported that gentamicin accumulated in renal cortex due to its reabsorption in proximal convoluted tubules causing degeneration and necrosis of the epithelial cells of the tubules similar to those observed in the current investigation. However in group C, the luminal diameter of proximal convoluted tubules improved as compared to group B, and was comparable to those in group A. This finding is also in accord with those observed by Fatima<sup>19</sup>, who reported that treatment of rats with ginseng alleviated deleterious effects of (insecticide) on renal tubules.

The probable mechanism by which gentamicin causes tubular damage is reported to be lipid peroxidation and oxidative injury.<sup>21</sup> Gentamicin enters proximal tubular cells, by interaction between cationic drug and anionic phospholipids of cell membrane as a first step, causing iron release from renal cortical mitochondria and forming iron drug complex, a potent catalyst of free radicals formation.<sup>22</sup> Reactive oxygen species (ROS) attack DNA and cause renal damage by inducing mesangial cell contraction and altering the filtration surface area leading to decrease in glomerular filtration rate. Gentamicin treatment induced a strong accumulation of oxidants in the kidney and inhibited protein synthesis and DNA replication.<sup>4</sup> Gentamicin is the treatment of choice for serious infections caused by *Streptococcus pyogenes*; since nephrotoxicity is its major side effect, therefore, an antidote which is cheap and effective shall provide impunity for its use in the cases where its use appear to be imperative.

The mechanisms involved in ginseng's protective effects are mainly its antioxidant, anti-inflammatory and anti-proliferative properties.<sup>23</sup> Beneficial effects of ginseng are due to the presence of phenolic acids and flavonoids which are responsible for increase in renal blood flow<sup>24</sup> and elimination of free radicals, thus protecting from gentamicin induced oxidative injury. Ginseng protects cell organelles of rats from lipid peroxidation induced by various toxins<sup>25</sup> and cause increase in amount of ribosome in rough endoplasmic reticulum, reflecting its ability to synthesize protein<sup>26</sup>. These findings may conduce improvements in renal function.

Several antioxidants that scavenge or interfere with production of reactive oxygen species (ROS) have been used successfully to ameliorate gentamicin nephropathy.<sup>27</sup> It is evident from our work that gentamicin caused tubular necrosis, increase in serum

urea and creatinine. However these effects were mostly ameliorated in the animals simultaneous treated with ginseng indicating that considerable protection is afforded by ginseng however, detailed studies are needed to confirm and study the mechanism of action of ginseng in amelioration of nephropathy.

## CONCLUSION

Ginseng has some protective role against gentamicin induced nephrotoxicity.

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