ORIGINAL ARTICLE

Amelioration of Gentamicin-induced nephrotoxicity by *Allium cepa* extract in male Wistar rats

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ABSTRACT

Background and Objective: Gentamicin is a commonly used antibiotic in hospitalised patients. The nephroprotective value of *AlliumAllium cepa* has been recognised against various nephrotoxic agents. The aim of this study was to evaluate the nephroprotective effect of *AlliumAllium cepa* against gentamicin-induced nephrotoxicity in male Wister rats.

Methods: It was an experimental study design including 90 adult healthy Wistar rats of male gender. Group A (n = 30) was the control group while Group B (n = 30) was given intraperitoneal gentamicin at the dose of 100 mg/kg body weight/ day for 8 days. Group C was given gentamicin for 8 days followed by *AlliumAllium cepa* extract at a dose of 1 m*l*/kg body weight/day for a week. Serum creatinine, urea and electrolyte levels were measured after 15 days. A one-way ANOVA test followed by a post Hoc Tukey's test was applied to compare the means of parameters and to determine the significance of the difference between the groups.

Results: Significant differences in the serum urea and creatinine levels among the groups (p = 0.000) were observed. Post Hoc Tukey's test indicated that mice administered gentamicin had significantly elevated serum urea (130.70 ± 66.34 mg/dl) and creatinine levels (1.39 ± 0.64 mg/dl) compared to control mice (urea: 31.60 ± 9.26 mg/dl; creatinine: 0.453 ± 0.11 mg/dl) (p = 0.000). Gentamicin-administered mice that were given *Allium cepa* extract exhibited lower serum urea (61.30 ± 17.88 mg/dl) and creatinine levels (0.727 ± 0.22 mg/dl) than gentamicin-administered mice (130 ± 66.34, 1.39 ± 0.64, respectively). One-way ANOVA analysis revealed no significant differences in serum sodium levels among the groups (p = 0.784). However, significant differences were observed in serum potassium and chloride levels (p = 0.000 for both). Gentamicin-administered mice had a significant increase in serum potassium (5.79 ± 1.34 mEq/l) and chloride(105.93 ± 3.86) levels compared to control mice (p = 0.000). Furthermore, *Allium cepa* extract significantly (p = 0.000) reduced the increase in serum potassium (5.05 ± 0.26) and chloride levels (102.23 ± 2.59) caused by gentamicin.

Conclusion: Gentamicin administration resulted in significant elevations in serum urea, creatinine, potassium, and chloride levels, indicating nephrotoxicity and electrolyte imbalances. Co-administration with *Allium cepa* extract mitigated these adverse effects, suggesting its potential protective role against gentamicin-induced nephrotoxicity and associated electrolyte disturbances.

Keywords: *Allium cepa*, gentamicin, nephrotoxicity, protective agent, Wistar rats.

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Introduction

Nephrotoxicity is defined as rapid (hours to days) deterioration in kidney function due to the toxic effect of medications or chemicals resulting in the retention of nitrogenous wastes, primarily creatinine and urea[1]. Nephrotoxicity induced by gentamicin is present in 10%-15% of the cases of acute renal failure and approximately 10%-25% of patients prescribed gentamicin show raised levels of metabolic waste products subsequent to decreased glomerular filtration rate.[2] Although it is one of the most nephrotoxic aminoglycosides, it still constitutes the main viable remedial option against microbes resistant to other antibiotics.[3]

Mechanisms of gentamicin-induced nephrotoxicity can possibly be explained by its renal tubular, renal vascular and glomerular effects. Gentamicin is known to cause acute tubular necrosis. It can also cause constriction of renal vasculature decreasing blood supply and glomerular filtration rate. It is considered that oxidative stress plays a key role in generating this toxicity.[3]

Onion (*Allium* cepa) belongs to the Alliaceae family. It is being used as a herbal medicine for the treatment of various health issues. *Allium cepa* are a good source of phytochemicals which are non-nutrient plant compounds beneficial to human health. The main phytochemical of *A. cepa*, quercetin, is one of the most potent antioxidants.[4]

In previous literature, *A. cepa* bulb extract has shown nephroprotective effects against cyanide,[5] cadmium[6] and aspartame[7]-induced nephrotoxicities. It has been shown to remarkably improve serum urea and creatinine levels[6,7] and ameliorate serum electrolyte disturbances caused by renal impairment.[8]

Since Gentamicin is also a potent nephrotoxic agent that causes renal damage, this study might contribute to developing a new remedy in terms of a safe and efficacious agent against gentamicin-induced nephrotoxicity. Hence, the study was planned to determine the effects of *A. cepa* extract on the biochemical parameters of gentamicin-induced nephrotoxic rats and to compare it with the control group.

Methods

This randomised controlled trial was conducted at the Department of Physiology, Akhtar Saeed Medical and Dental College, Lahore, Pakistan, from November 1, 2017, to December 31, 2019. All animal procedures were reviewed and approved by the Ethical Review Committee of Akhtar Saeed Medical College, Lahore. Using non-probability consecutive sampling, 90 healthy adult male Wistar rats, aged 6-8 weeks and weighing 180-200 g, were randomly selected from the Veterinary Institute and Research Centre, Lahore. The sample size was determined to achieve 90% power and a 5% significance level, with an initial calculation suggesting 10 rats per group. To enhance validity, this was increased to 30 rats per group, totaling 90 across three groups. Inclusion criteria encompassed healthy male Wistar rats within the specified age and weight range, while those with any observable physical irregularities or deformities were excluded.

Animals were kept at the animal house of Akhtar Saeed Medical College, Lahore. The rats were kept in standard-sized steel cages in a 12-hour light/dark cycle (lights were kept on from 7 am to 7 pm). They were kept at a temperature close to 26ºC, 50% humidity and adequate ventilation.[9] After 1 week of acclimatisation, they were randomly divided into three groups of 30 each.

Group A: (control, *n* = 30) were given distilled water 1 ml/100 gm body weight/day by oral gavage tube for a week followed by intraperitoneal injection of distilled water for the next 8 days.

Group B: (experimental 1, *n* = 30) were given distilled water 1 ml/100 gm body weight/day by oral gavage tube daily for a week followed by i.p injection of gentamicin at a dosage of 100 mg/kg body weight/day for the next 8 days.[10]

Group C: (experimental 2, *n* = 30) were given *A. cepa* extract at the dosage of 1 ml/100 gm body weight/day by oral gavage tube daily for 1 week[5,11] followed by administration of i.p injection of gentamicin daily for the next 8 days. *Allium cepa* extract was continued throughout the period.

Fresh red *A. cepa* bulbs were purchased from the local market. These were washed thoroughly in water and dried in air, and the bulbs were then peeled off. 200 g of fresh *A. cepa* bulbs were crushed in the grinding machine. As a result, the paste formed was allowed to settle and then squeezed and filtered through a fine cloth. The extract was stored below 4ºC until used. *Allium cepa* extract was given by oral gavage tube in a dose of 1 ml/100 gm body weight per day.[7,12]

Terminal blood sampling was done 24 hours after administering the last dose of gentamicin.[13] Biochemical assays were performed at 'Biochemistry Research Lab', University of Lahore, Lahore. International standards and protocols were followed for handling various chemicals, kits, laboratory equipment and machines. Manufacturer's instructions were followed for assays.

Serum urea, creatinine, sodium, potassium and chloride were measured at the end of the treatment period (15th day) in all groups.

Serum urea levels were quantitatively determined using the Urea kit from MTD Diagnostics Srl, Italy (LOT 2017178), which employs the Berthelot reaction - a colorimetric method where urea is hydrolysed by urease into ammonia and carbon dioxide; the ammonia then reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside to yield a green chromophore. Serum creatinine was measured using the Jaffe method with the RANDOX kit from the UK (Cat. No. 510/CR524); in this method, creatinine reacts with picric acid in an alkaline solution to form a reddish-coloured complex, known as the Janovski complex, which can be measured colorimetrically.[14] Serum chloride levels were estimated using a colorimetric method,[15] while serum sodium and potassium concentrations were determined via flame photometry using the Flame Photometer F-100.[16]

Statistical analysis

Descriptive analysis was carried out using database software and statistical program PASW18 (formerly SPSS) to determine mean, standard deviation and 95% confidence interval. One-way ANOVA test followed by post Hoc Tukey's test to compare the levels of parameters and to determine the significance of difference between the groups. p value ≤ 0.05 was considered significant.

Results

Effect of Allium cepa extract on serum urea and creatinine levels

There was a significant difference in serum urea and serum creatinine levels among groups A (control), B (gentamicin) and C (gentamicin + *Allium cepa*) as estimated by one-way ANOVA ($p = 0.000$) (Table 1).

Post Hoc Tukey's test showed that group B had raised serum urea and serum creatinine levels as compared to group A ($p = 0.00$). Group C had significantly ($p = 0.014$) raised serum urea levels and significantly ($p < 0.026$) raised serum creatinine levels as compared to group A. Group B had significantly ($p = 0.000$) raised serum urea and creatinine levels as compared to group C (Table 2).

There was no statistically significant difference in serum sodium levels ($p = 0.784$) among the groups A, B and C as determined by one-way ANOVA, whereas, there were statistically significant differences in serum levels of potassium ($p = 0.000$) and serum chloride ($p = 0.000$) among groups A, B and C. (Table 3).

Post Hoc Tukey's test showed that group B had an insignificant ($p = 0.990$) rise in serum sodium level, and significantly raised serum potassium and serum chloride levels as compared to group A ($p = 0.000$). Group C had a rise in serum sodium level ($p = 0.787$), potassium ($p = 0.024$) and chloride ($p = 0.022$) levels as compared to group A. Group B had an insignificant ($p = 0.858$) rise in serum sodium level, a significant ($p = 0.002$) raised serum potassium and a highly significant ($p = 0.000$) raised serum chloride levels as compared to group C (Table 4).

Discussion

Gentamicin is an important anti-bacterial drug. Its main dose-limiting adverse effect is nephrotoxicity which causes reduction of dose or stoppage of the treatment.[3] The present study was conducted to investigate whether *A. cepa* administration could provide protection against gentamicin-induced nephrotoxicity or not.

Results of the present study showed raised serum urea and creatinine levels following Gentamicin administration which are indicators of acute kidney injury and reduced glomerular filtration.[17] This was in line with the view that gentamicin causes acute tubular necrosis and reduces glomerular filtration by multiple processes such as blockage of nephron by necrotic epithelial cell debris (increased intratubular pressure), back-leak of fluid into the interstitium (reduced tubular flow), direct renal vascular constriction and activation of the renin-angiotensin system. These findings are correlated biochemically with raised serum urea and creatinine levels.[3]

In the present study, raised serum urea and creatinine levels occurred with the dosage of 100 mg/kg body weight/day given for 8 consecutive days. In a study by Udupa and Prakash[18], comparison between a low dose of 30 mg/kg body weight/day and 100 mg/kg body weight/day of gentamicin in rats showed that on the eighth day, a low dose of gentamicin

Table 1. *Comparison of serum urea and creatinine levels among study groups.*

Parameters	Group A ($n = 30$)	Group B ($n = 30$)	Group C ($n = 30$)	p-value
Serum urea (mg/dl)	31.60 ± 9.261	130.70 ± 66.349	61.30 ± 17.887	0.000*
Serum creatinine (mg/dl)	0.453 ± 0.1106	1.393 ± 0.6443	0.727 ± 0.2288	0.000*

Group A = Control, Group B = Gentamicin, Group C = Gentamicin + *Allium cepa*, values are presented as mean ± SD, *$p <$ 0.001 - highly significant.

Table 2. *Comparison of serum urea and creatinine levels between Groups by post Hoc Tukey's test.*

Parameters	Group	Group	p value
Serum urea	Group A	Group B	0.000*
		Group C	0.014
	Group B	Group C	0.000*
Serum creatinine	Group A	Group B	0.000*
		Group C	0.026
	Group B	Group C	0.000*

Group A = Control, Group B = Gentamicin, Group C = Gentamicin + *Allium cepa*
*$p < 0.001$ - highly significant.

Table 3. *Comparison of serum electrolyte levels among groups A, B and C by one-way ANOVA.*

Parameters	Group A (*n* = 30)	Group B (*n* = 30)	Group C (*n* = 30)	*p* value
Serum sodium (mEq/l)	141 ± 2.865	141 ± 3.305	141 ± 2.583	0.784
Serum potassium (mEq/l)	4.483 ± 0.3905	5.790 ± 1.3402	5.050 ± 0.2583	0.000*
Serum chloride (mEq/l)	100 ± 2.971	105.93 ± 3.868	102.23 ± 2.596	0.000*

Group A = Control, Group B = Gentamicin, Group C = Gentamicin + *Allium cepa*
Values are presented as mean±SD*$p < 0.001$ - highly significant.

Table 4. *Comparison of serum electrolyte levels between groups.*

Parameters	Group	Group	*p* value*
Serum potassium	Group A	Group B	0.000*
		Group C	0.024
	Group B	Group C	0.002
Serum chloride	Group A	Group B	0.000*
		Group C	0.022
	Group B	Group C	0.000*

Group A = Control, Group B = Gentamicin, Group C = Gentamicin + *Allium cepa*
*$p < 0.001$ - significant (Post Hoc Tukey's test).

did not elevate serum urea and creatinine significantly but there was rise in these parameters in 100 mg/kg body weight/day treated rats.[18] In the study by Moghadam et al.[19], gentamicin caused raised serum urea and creatinine levels and it was related to the necrosis of epithelial cells in proximal tubules and deposition of necrotic debris in tubular lumen.[19] Similarly, Hajihashemi et al.[17] found that the gentamicin-treated group showed a rise in serum urea and creatinine and a reduction in creatinine clearance. This reduced glomerular filtration rate was related to increased renal vascular resistance and reduced renal blood flow, which was mediated by the generation of reactive oxygen species.[17]

In the present study, no significant changes were seen in serum sodium levels among the groups while serum potassium and chloride levels were raised in gentamicin-treated rats. Different studies have shown different results regarding serum electrolytes in gentamicin-treated rats. Gentamicin, administered in a similar dosage (100 mg/kg), caused no change in serum sodium in the study by Hajihashemi et al.[17] Fractional excretion of sodium was found to be increased in the gentamicin-treated group. Likewise, in a study by Kanna et et al,[20] there was no change in serum sodium level by gentamicin despite evidence of acute kidney injury. In contrast, in the study by Hajishami et al.,[17] serum sodium level was raised by gentamicin.[5] There was no rise in serum potassium and chloride levels in a study by Berkovitch et al.[21] However, Hajihashemi et al. [17] found raised serum potassium and chloride levels which were in accordance with the results of the present study. However, Mahmoud and Farag[22] found rather fall in serum sodium and potassium levels in gentamicin treated group.

Allium cepa juice extraction method used in the present study has been used previously by many researchers.[7,13] In the present study, *A. cepa* was able to attenuate many of the biochemical alterations induced by gentamicin in rat kidneys. Studies have assessed *A. cepa* for its nephroprotective efficacy against other agents[5,7] on account of its anti-oxidant activity on aspartame-treated renal injury.[7] Similar to the results of the present study, *A. cepa* supplement in cyanide-administered rats ameliorated the elevated level of serum urea and creatinine.[5] Thus, *Allium cepa* extract has a potential role in scavenging the free radicals injury to the renal tissue.

Limitations of the study

The experiment was conducted for a limited time period and long-term effects of *A. cepa* extract on renal functions were not assessed. The effect of diet and environmental factors was overlooked which could impact renal function.

Conclusion

It may be concluded that the concurrent use of *A. cepa* extract with gentamicin has ameliorative effects on gentamicin-induced nephrotoxicity which is well correlated with the levels of serum urea and creatinine.

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The authors have no acknowledgments to declare.

List of abbreviations
ANOVA	Analysis of variance
ip	Intraperitoneal
gm	Gram
ml	Milliliter
SPSS	Statistical Package for the Social Sciences

Conflict of interest
None to declare.

Grant support and financial disclosure
None to disclose.

Ethical approval
The ethical approval of the present study was granted by the Ethical Review Committee of Akhtar Saeed Medical College, Lahore, Pakistan, vide Letter # AMDC-279, dated 1-11-2017.

Authors' contributions
JS: Conceived the study, designed the methodology and drafting of manuscript and critical intellectual input.
MT: Collection and assembly of data, drafting of the manuscript, data analysis and interpretation of results.
SA: Reviewed and revised the manuscript critically for important intellectual content, drafting of manuscript and acquisition of data.
MJJS, SJ and CN: Acquisition of data, literature search and drafting of manuscript with critical intellectual input.
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