Original Article

Evaluation of Histopathological Changes in Aminoglycoside Induced Nephrotoxicity and Protective Role of Citrullus lanatus Seeds (Ethanolic Extract) in Animal Model

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Abstract

Objective: To assess histopathological changes induced by aminoglycoside in nephrotoxicity and explore the protective role of Citrullus lanatus seeds in an animal model.

Material and Methods: The research, conducted from July 01, 2017, to December 31, 2018, at the Department of Pharmacology, King Edward Medical University Lahore, and UVAS, Lahore, received approval from the Institutional Review Board (IRB). The Animal Experimental Study involved 40 healthy albino Wistar rats, divided into five equal groups. Group A served as the normal control, receiving oral normal saline once daily. Group B, the disease control, received intraperitoneal (IP) injections of Gentamicin at 80mg/kg/day in two equally divided doses, with a 12-hour interval, for 14 days. Groups C and D received oral doses of Citrullus lanatus seed extract (CLSE) at 400 mg/kg and 600 mg/kg/day, respectively, concurrently with Gentamicin at 80mg/kg/day IP in two equally divided doses for 14 days. Histopathological changes such as tubular dilatation (outer and inner), tubular inflammation and tubular necrosis were compared in Aminoglycoside and Citrullus lanatus seeds extract treated groups.

Results: Groups C and D exhibited a significant decrease in tubular inflammation, tubular necrosis, and tubular dilatation, with a p-value < 0.001 compared to Group B.

Conclusion: The administration of CLSE in aminoglycoside-induced nephrotoxicity in albino rats significantly mitigated nephrotoxic effects by reducing inflammation, tubular necrosis, and tubular dilatation.

Keywords: nephrotoxicity, aminoglycosides, citrullus lanatus seeds, tubular inflammation, tubular necrosis, tubular dilatation.

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Introduction

Aminoglycosides, an important group of bactericidal antibiotics, were discovered in 1940 from the Streptomyces and Micromonospora genera of bacteria widely used in hospitalized patients. These antibiotics treat serious infections caused by aerobic gram-negative bacilli and are often combined with penicillins to combat gram-positive organisms.[1] Common examples include amikacin, gentamicin, tobramycin, and streptomycin.[2] Administered parenterally, aminoglycosides diffuse through the outer membrane of bacteria via porin channels. Their antibiotic function involves binding to the 30S ribosomal subunit, causing a misreading of the genetic code and depleting polysomes, rendering the bacterium incapable of synthesizing vital proteins for growth. The bactericidal effect is concentration and time-dependent, with metabolites rapidly excreted in the urine by glomerular filtration.[3] Major side effects include nephrotoxicity, ototoxicity, neuromuscular paralysis, and allergic reactions. Aminoglycoside-induced nephrotoxicity accounts for 58% of acute renal failure cases 4, with gentamicin being particularly nephrotoxic and associated

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with higher patient mortality. The mechanism involves binding to the megalin receptor in the proximal tubular epithelium, forming a complex that induces oxidative stress, leading to various cellular responses such as apoptosis, necrosis, and inflammation.[5]

Herbal products, known for their less adverse effects and cost-effectiveness, are being explored for their nephroprotective roles against aminoglycoside-induced oxidative stress. Various herbs, including Aloe vera, Silymarin, Kabab chini, Curcumin, Rosemary, Ginger, Propolis, black seeds, and Turmeric have been studied for their potential benefits. However, there is still a lack of a definitive treatment for gentamicin-induced nephrotoxicity.[6] Citrullus lanatus, a fruit from the Cucurbitaceae family, is being investigated for its potential nephroprotective effects due to its antioxidant and therapeutic components.[7] The study aims to explore the impact of Citrullus lanatus seeds on aminoglycoside-induced nephrotoxicity, considering the fruit's potential as a safe, cost-effective, and efficient nephroprotective agent when co-currently with aminoglycosides. The findings may contribute to the development of a new, affordable drug for nephroprotection.

Materials and Methods

The study was done from July 01, 2017, to December 31, 2018, at the Department of Pharmacology, King Edward Medical University Lahore, following approval from the Institutional Review Board (IRB) under reference number 165/RC/KEMU. Citrullus lanatus fruit was procured from the local fruit market in Lahore and underwent proper slicing to separate seeds. The identification process was carried out by authorized personnel from Government College University, Lahore. The Citrullus lanatus seed extract (CLSE) used in the study was prepared at PCSIR, Lahore. A dried concentrated extract weighing 28 grams was collected and mixed with 5 ml of distilled water in a tightly closed bottle, protected from sunlight, and stored at 4°C. This prepared extract was used throughout the experiment.

For individual rats, specific doses of CLSE were calculated, namely 400 mg/kg and 600 mg/kg, respectively. These doses were administered through a feeding gauge of no. 16. Nephrotoxicity was induced in rats by administering Gentamicin sulfate at a dose of 80 mg/kg intraperitoneally (IP) in two equally divided doses for 14 days at 12-hour intervals. A total of 40 male adult healthy albino rats, weighing between 150-200 grams, were procured from the local market and randomly divided into five groups, each containing eight rats. Group A served as the healthy control group, receiving 0.5 ml of distilled water orally once daily with a 16-gauge feeding tube. Group B, the disease control group, received Gentamicin at a dose of 80 mg/kg/day IP in two equally divided doses at 12-hour intervals for the duration of 14 days. In the study, Citrullus lanatus seed extract (CLSE) was administered to rats in Group C at a dosage of 400 mg/kg body weight orally once daily, concurrently with gentamicin at a dose of 80 mg/kg/day intraperitoneally (IP) in two equally divided doses for a duration of 14 days. Similarly, rats in Group D received CLSE at a dosage of 600 mg/kg body weight orally once daily, concurrently with gentamicin at a dose of 80 mg/kg/day IP in two equally divided doses for the same 14-day period. Group E rats received the Citrullus lanatusseeds ethanolic extract in the dose of 600 mg/kg/day body weight orally with a 16 gauge feeding tube once daily along with 0.5 ml of normal saline I.P for 14 days. Euthanization occurred 24 hours after the last dose, and rats were sacrificed on day 15 through slaughtering. Kidneys were removed, weighed, and sliced for further analysis. The kidney slices were fixed in 10% formalin, and standard H & E staining was employed for slide preparation. The slides were then examined under a microscope to assess renal changes, including proximal tubular dilatation, necrosis, and inflammation. Semi-quantitative grading of histopathological changes was done as following!

– No pathological change (absence of all parameters defined above)

+ Mild (less than 25% of the tissue affected)

++ Moderate (25 – 50 % of the tissue affected)

+++ Severe (more than 50 % of the tissue affected

The data underwent statistical analysis using GraphPad Prism version 8. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests was employed for group comparisons. A significance level of $p < 0.05$ was considered statistically significant.

Results

Size of kidney (cm): At day-14, width of the kidneys were taken to observe the size of kidneys and no significant difference in length and width of the rats' kidneys among the groups was observed. (Table-1) Inflammation: At day-14, there was significant difference in inflammation among the groups at day 14 with p-value <0.01. (Table-2) (Fig-1,2&3) Tubular necrosis: There was also significant difference among the groups at day 14 with p-value <0.01 in tubular necrosis. (Table-3) (Fig 1,2 &3) Tubular dilatation [diameter (μm^2)]. A significant difference was seen among the groups for outer

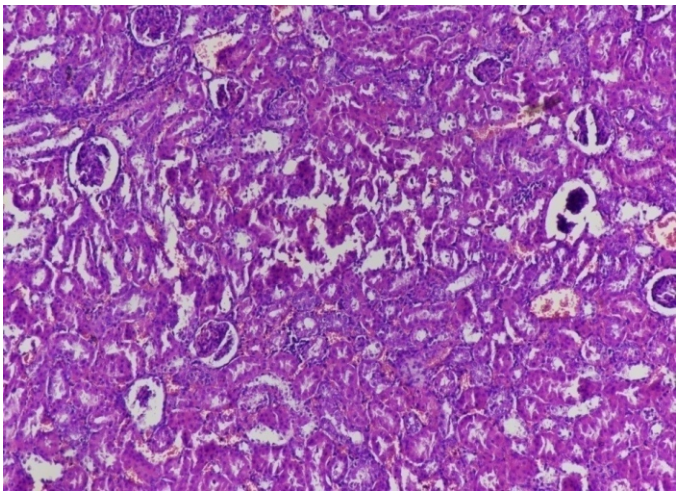

Fig 1: *Group B (positive control) Photomicrograph showing increased tubular dilatation, severe inflammation and necrosis (10 x 10; H&E)*

tubular dilatation with p-value 0.02 and for inner tubular diameter with p-value 0.03 **(Table-1) (Fig-1,2 &3)**

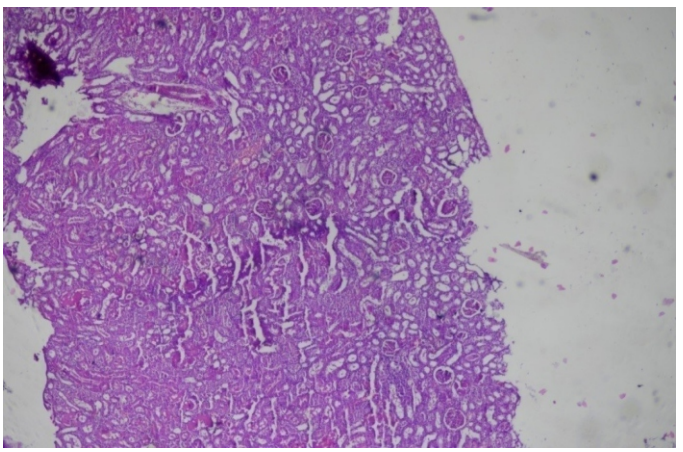

Fig 2: *Group C (low dose Citrullus lanatus seeds extract prophylactic group). Photomicrograph showing moderate to severe inflammation, moderate tubular dilatation*

Table 1: *Comparison of size (width-cm) among groups A, B, C, D, and E.*

	Group-A (n=8)	Group-B (n=8)	Group-C (n=8)	Group-D (n=8)	P-value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Size of kidneys (width-cm)	0.59±0.07	0.58±0.07	0.58±0.07	0.57±0.07	0.99
Tubular dilatation (Outer)	1972.73±1.26	1384.29±0.36	1547.54±0.28	2042.28±48.93	0.02
Tubular dilatation (Inner)	699.10±0.62	508.23±0.25	493.52±0.35	811.31±42.91	0.03

Group A = normal control, Group B = disease control, Group C = low dose, Group D = high dose.

Table 2: *Comparison of inflammation among groups A, B, C, D, and E (Chi-Square Test)*

Inflammation	Group -A (n=8)	Group-B (n=8)	Group-C (n=8)	Group-D (n=8)	value
	n (%)	n (%)	n (%)	n (%)	
No pathological change	8 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Mild	0 (0.0%)	0 (0.0%)	2 (25.0%)	6 (75.0%)	<0.01
Moderate	0 (0.0%)	2 (25.0%)	3 (37.5%)	2 (25.0%)	
Severe	0 (0.0%)	6 (75.0%)	3 (37.5%)	0 (0.0%)	

**P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001, ns=not significant*

Group A=normal control, Group B = disease control, Group C=low dose, Group D =high dose.

Table 3: *Comparison of tubular necrosis among groups A, B, C, D, and E (Chi-Square Test)*

Tubular necrosis	Group-A (n=8)	Group-B (n=8)	Group-C (n=8)	Group-D (n=8)	-value
	n (%)	n (%)	n (%)	n (%)	
No pathological change	8 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
Mild	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (37.5%)	<0.01
Moderate	0 (0.0%)	3 (37.5%)	4 (50.0%)	3 (37.5%)	
Severe	0 (0.0%)	5 (62.5%)	4 (50.0%)	2 (25.0%)	

**P-value < 0.05, ** P-value < 0.01, *** P-value < 0.001, ns = not significant*

Group A = normal control, Group B = disease control, Group C = low dose, Group D = high dose.

and moderate to severe necrosis (10 x 10; H&E)

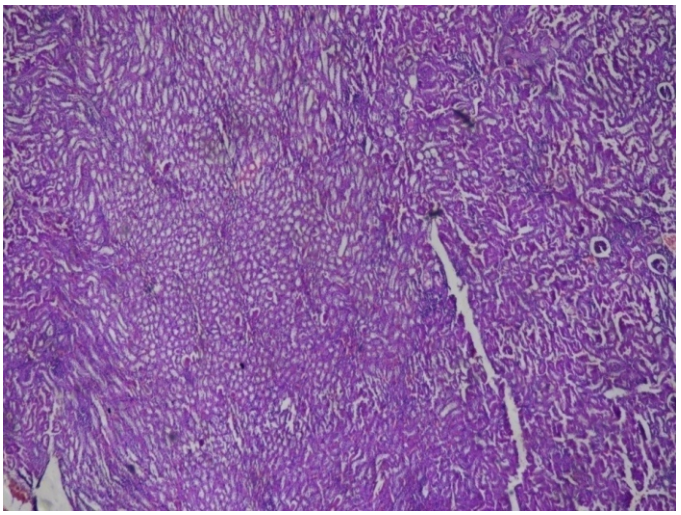

Fig 3: *Group D (high dose Citrullus lanatus seeds extract prophylactic group). Photomicrograph showing moderate to mild inflammation, mild tubular dilatation and mild necrosis (10 x 10; H&E)*

Discussion

Nephrotoxicity in albino rats was induced using gentamicin as it is documented one of the most nephrotoxic drug among aminoglycosides. On day 14 of the study, the length of kidneys across all groups was consistent, leading to the utilization of kidney width (cm) to assess the impact of gentamicin and Citrullus lanatus seed extract on kidney size. No statistically significant change in kidney width was observed when comparing groups B, C, D, and E to the normal control group A. Gross examination revealed that in the positive control group B, kidneys appeared slightly globular and pale with a shiny surface and whitish spots, indicating nephrotoxic effects of gentamicin (acute renal injury), as described in a previous study. In contrast, normal control group A and group E kidneys were red in color. This could suggest that the insignificant change in kidney size may be attributed to the protective use of Citrullus lanatus seeds.[8] Microscopically, positive control group B exhibited severe inflammation in 75% of rats and moderate inflammation in 25% after induction of nephrotoxicity by gentamicin. In group C, where 400 mg/kg of Citrullus lanatus seed extract was given concurrently with gentamicin, 25% of rats showed mild inflammation, 37.5% showed moderate inflammation, and 37.5% showed severe inflammation. Increasing the extract dose to 600 mg/kg in group D resulted in 75% of rats showing mild inflammation and 25% showing moderate inflammation. These findings support the anti-inflammatory properties of the extract, attributed to the presence of antioxidants that counteract oxidative injury and inflammation caused by gentamicin.[9] No signs of inflammation were observed in normal control group A, and in group E, where only the extract was given, no inflammation was noted, consistent with a previous study.[8]

Regarding tubular necrosis, normal control group A showed no necrosis at day 14. Positive control group B exhibited severe necrosis in 62.5% of rats and moderate necrosis in 37.5% after nephrotoxicity induction. In group C, 50% had moderate tubular necrosis, and 50% had severe necrosis. Group D showed 37.5% mild necrosis, 37.5% moderate necrosis, and 25% severe necrosis. Statistically significant results indicated a reduction in tubular necrosis after using Citrullus lanatus seed extract, potentially due to antioxidative effects of vitamin E and flavonoids present in the extract.[10] Group E showed no tubular necrosis, aligning with a previous study concluding that Citrullus lanatus seed extract does not cause tubular necrosis in healthy rats.

At day 14, tubular dilatation significantly decreased in positive control group B compared to normal control group A. However, in groups C and D, tubular dilatation increased when Citrullus lanatus seed extract was administered concurrently with gentamicin, suggesting the potential of the extract to reverse tubular dilatation to normal, possibly due to its antioxidant effect. This aligns with a previous study where tubular damage due to gentamicin was reversed by the antioxidant effect of Kabab chini.[11] In group E, the effect of Citrullus lanatus seed extract on tubular dilatation was statistically insignificant compared to normal control group A, consistent with a study showing no significant histological findings in the kidneys of normal rats given Citrullus lanatus seeds.[9]

Conclusion

The present study confirms the nephroprotective effect of Citrullus lanatus seeds extract. This beneficial effect of Citrullus lanatus seeds might be due to their antioxidant potential. This study may be helpful for development of economical safe and efficacious nephroprotecive agent. Further researches are required to explore the active constituents responsible for nephroprotective effect.

Conflict of Interest: *None*
Funding Source *None*

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Authors Contribution

NY: Conceptualization of Project
INY, AEZ: Data Collection
MUT: Literature Search
FAK: Statistical Analysis
SMNZ: Drafting, Revision
HT: Writing of Manuscript