ORIGINAL ARTICLE

Nephroprotective Effects of *Ipomoea Aquatica* (Kalmi shak) And Ramipril in Gentamicin Induced Nephrotoxic Rats - A Comparative Experimental Study

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Abstract

***Introduction**: Acute kidney injury is an important public health problem in patients treated with gentamicin. Kalmi shak and ramipril have nephroprotective effects that can alleviate gentamicin induced nephrotoxicity. A study was carried out on a group of rats to evaluate the nephroprotective effects of kalmi shak and ramipril with their comparison.*

***Methods**: An experimental study was conducted to demonstrate the effects of ethanolic extract of kalmi shak and ramipril on blood urea and serum creatinine levels as well as histopathological changes in gentamicin induced nephrotoxic rats and the nephroprotective effects were compared with kalmi shak and ramipril respectively.*

***Results:** Blood urea and serum creatinine levels were significantly higher than the normal in gentamicin treated group. There were significant differences in blood urea and serum creatinine levels as well as histopathological changes in gentamicin to kalmi shak and ramipril treated groups. Another test revealed no significant differences in blood urea and serum creatinine levels as well as histopathological changes between the kalmi shak and ramipril treated groups. Nephroprotective effects were observed with ethanolic extract of kalmi shak which was almost similar to that of ramipril.*

***Conclusion:** Kalmi shak and ramipril have nephroprotective effect that can alleviate gentamycin induced nephrotoxicity which is comparable to each other, though exact mechanism could not be confirmed by this study.*

***Keywords**: Gentamicin, Ramipril, ipomoea Aquatica, Kalmi shak, Nephrotoxicity, Blood Urea, Serum Creatinine, Histopathology*

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Introduction:

Acute kidney injury (AKI) is a common clinical problem associated with high mortality and can occur in normal renal function or patients with preexisting renal diseases.[1] It was defined as sudden and usually reversible loss of kidney function which develops over a period of days or weeks[2] and is characterized by accumulation of urea and other chemical substances in blood.[3] Metabolites of the drugs that are excreted through kidney cause cellular damage with histopathological changes including vascular congestion, cellular oedema, mononuclear cell infiltrations leading to kidney dysfunction.[4]

Gentamicin is an aminoglycoside antibiotic. It is used to treat gram negative organisms and a popular choice by clinicians[5] due to its low cost and is also one of the nephrotoxic drugs that produce renal tubular damage which

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limits its frequent clinical prescription[6]. Abolition of nephrotoxicity of gentamicin can increase its clinical use by the prescribers.

Gentamicin causes oxidative stress, apoptosis, necrosis, upregulation of transforming growth factor B and increase monocyte-macrophage infiltration as well as generates superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney which are responsible for acute kidney injury.[6]

Different studies showed that antioxidants abolish gentamicin induced nephrotoxicity in rats. Antioxidants improve histopathological injuries like tubular necrosis, tubular cell oedema and apoptosis in gentamicin treated rats.[7] Many vegetables and fruit species in Southeast Asia have nephroprotective effects against gentamicin induced nephrotoxicity.[8] Kalmi shak is a cheaper and easily available vegetable to treat gentamicin induced nephrotoxicity, which has potent antioxidant effect.[9,10]

Ramipril is an angiotensin converting enzyme inhibitor that inhibits the renin angiotensin aldosterone system. It has specific nephroprotective effect, probably because of the role of angiotensin II, which drives the underlying glomerular hyperfiltration.[11] Losartan is an angiotensin II receptor blocker and has nephroprotective effect on gentamicin induced nephrotoxic rats.[12]

In this background a scientific study of nephroprotective effect of kalmi shak should be explored and develop a natural source that is readily available with low cost. In this study we made an attempt to evaluate the nephroprotective effect of ethanolic extract of kalmi shak in gentamicin induced nephrotoxic rats and nephroprotective effects were compared with ramipril. This study was carried out in the department of Pharmacology & Therapeutics at Dhaka Medical College, Dhaka.

Methods:

This experimental study was carried out in the department of Pharmacology and Therapeutics, Dhaka Medical College from July 2016 to June 2017. Twenty eight healthy Wistar Albino rats of both sexes weighing from 180 to 250 grams were collected from ICDDR'B. Gentamicin was purchased from a pharmacy which was manufactured by Opsonin Pharmaceuticals Ltd. Kalmi shak, which DACB accession number was 45091, were collected from a super shop and identified and authenticated by National Herbarium, Mirpur, Dhaka. Ethanol extracts were prepared in Drug Research Laboratory of Centre for Advanced Research of Sciences (CARS) of Dhaka University. Ramipril was purchased from a pharmacy which was manufactured by Opsonin Pharmaceuticals Ltd.

The experimental study was designed to demonstrate the effect of ethanolic extract of kalmi shak and ramipril on blood urea and serum creatinine levels as well as histopathological changes on gentamicin induced nephrotoxic rats and to compare the nephroprotective effects of ethanolic extract of kalmi shak and ramipril. The rats were divided into four groups (A, B, C & D) comprising 7 rats in each group. Rats were treated for nine days.

Group A was served as control group. Rats were given a standard diet for 9 days and were sacrificed on day 10.

Group B was gentamycin treated group. Nephrotoxicity was induced by intraperitoneal injection of gentamicin at a dose of 100mg/kg body weight for 9 days.[13,14] The rats were allowed the usual diet for the same duration and then sacrificed on day 10.

Group C was treated with ethanolic extract of kalmi shak (500mg/kg/day)[9] by gastric intubation and gentamicin (100mg/kg/day) intraperitoneally for 9 days with usual rat diet and then sacrificed on day 10.

Group D was treated with ramipril (1mg/kg/day)[15] by gastric intubation and gentamicin (100mg/kg/day) intraperitoneally for 9 days with usual rat diet and then sacrificed on day 10.

On day 10, blood samples were collected through cardiac puncture and sent to the department of biochemistry of Dhaka Medical College for biochemical analysis. The rats were then sacrificed and kidneys were dissected and stained with haematoxylin & eosin for histopathological examination. All results were appropriately recorded in computer. Statistical analysis was done by SPSS version 17 software. The variables were expressed as mean±SD. 'Unpaired student †s t test, ANOVA test and Post Hoc test were done for comparison of means.

Results:

Obtained data on blood urea and serum creatinine of the four different groups (A, B, C & D) were separately recorded and analyzed. Data were expressed as mean ± SE and presented accordingly in tables of a variable of the specific group. The results of the interventional groups were compared with that of the control.

At first, the data on groups A and B were compared to observe the effect of gentamicin on their blood urea and serum creatinine level as well as histopathological changes.

Secondly, the data on blood urea and serum creatinine level as well as histopathological changes of groups B, C, and D were compared to observe the effect of gentamicin, kalmi shak and ramipril respectively.

Finally, the blood urea and serum creatinine level as well as histopathological changes of group C and D were compared to observe the effects of kalmi shak and ramipril in gentamicin treated rats.

Table I

Effects of Gentamicin on blood urea and serum creatinine level in group B at day 10

Groups	N = 7	Blood urea level (mg/dl)		Serum Creatinine level (mg/dl)	
		Mean ± SD	p value	Mean ± SD	p value
A		21.43 ± 2.15	<0.001	0.49 ± 0.05	<0.001
B		84.43 ± 6.87		3.37 ± 0.55	

*unpaired t- test

Table I showed that mean blood urea and serum creatinine levels were higher in group B than in group A. This difference was statistically significant ($p<0.001$). An unpaired t test was done to find out the difference between individual groups.

Table II

Comparison of blood urea and serum creatinine levels between different groups (group B with C and D) at day 10

Groups	N = 7	Blood urea level (mg/dl)		Serum creatinine level (mg/dl)	
		Mean ± SD	p-value	Mean ± SD	p-value
B		84.43 ± 6.87	<0.001	3.37 ± 0.56	<0.001
C		68.43 ± 3.36		1.96 ± 0.10	
D		53.85 ± 11.55		1.67 ± 0.26	

*ANOVA test was done

Table II showed that mean blood urea and serum creatinine level was higher in group B and lower in group C and D. These difference was statistically significant ($p<0.001$). ANOVA test was done to determine the difference between individual groups.

Table III

Comparison of blood urea and serum creatinine level between group B and groups C and D at day 10

Groups	Mean± SE in blood urea level (mg/dl)	p value	Mean ± SE in blood creatinine level (mg/dl)	p value
Group B vs. C	16.00 ± 2.89	<0.004	1.41 ± 0.21	<0.004
Group B vs. D	30.57 ± 5.08	<0.001	1.70 ± 0.23	<0.001

*Post Hoc test (Games-Howell) was done

Table III revealed that there was a highly significant difference in blood urea and serum creatinine level of group B compared with group C and group D.

Table IV

Comparison of blood urea and serum creatinine level between group D with group C

Groups	Mean± SE in blood urea level(mg/dl)	p value	creatinine level (mg/dl) Mean ± SE in serum	P value
Group D vs. C	14.57 ± 4.55	0.102	0.29 ± 0.11	0.175

*Post Hoc test (Games-Howell) was done

Another Post Hoc test (Games-Howell) was done to compare blood urea and serum creatinine levels between groups D and C; no significant difference was observed (Table IV).

Histopathological observations

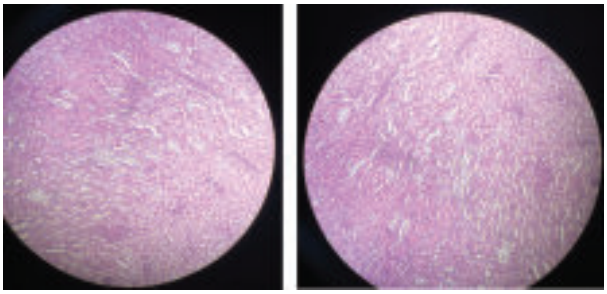

Figure 1: Microscopic photograph of group A

In group A the normal appearance of glomeruli, tubules and interstitium.

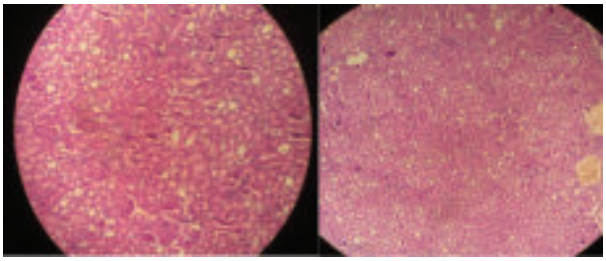

Figure 2: Microscopic photograph of group B

In group B there are moderate to severe distortion of renal architecture as well as glomerular congestion, early tubular necrosis and the interstitium was infiltrated with many lymphocytes.

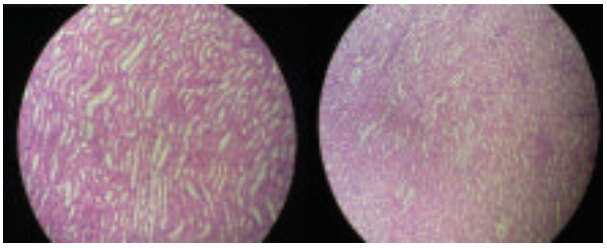

Figure 3: Microscopic photograph of Group C

Group C showed mild to moderate distortion of renal architecture with few to some areas with glomerular congestion and the interstitium infiltrated by few to some number of lymphocytes.

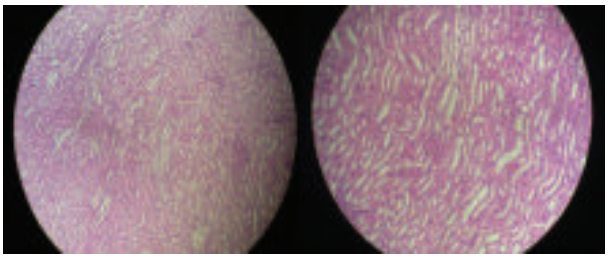

Figure 4: Microscopic photograph of Group D

Group D showed renal architecture with very few areas with glomerular congestion and the interstitium infiltrated with occasional number of lymphocytes.

Discussion:

The study was carried out to evaluate the nephroprotective effect of ethanolic extract of kalmi shak with a comparison to ramipril on experimentally induced nephrotoxic rats. Nephrotoxicity was generated by the intraperitoneal injection of gentamicin.

28 healthy adult Wistar Albino rats of both sex weighing 180-250 grams were selected for the study. Rats were divided into 4 groups (A, B, C, D) comprising 7 rats in each group. They were treated for 9 days. Group A served as the control group where the rats were given a standard rat diet for 9 days. Group B was gentamicin treated group and intraperitoneal administration of gentamicin (100mg/kg/day), and a standard rat diet were given for 9 days to them. Group C rats were treated with ethanol extract of kalmi shak (500mg/kg/day) by gastric intubation for 9 days, along with gentamycin (100mg/kg/day) intraperitoneally and standard rat diet. Lastly, group D rats were treated with ramipril (1mg/kg/day) by gastric intubation for the same period, along with gentamicin (100mg/kg/day) intraperitoneally and standard rat diet. On day 10, blood samples were collected and blood urea and serum creatinine levels were measured. At the same day the rats were sacrificed and the kidneys were preserved and prepared for histopathological study.

In this study, we found that the mean blood urea and serum creatinine level was 21.43 ± 2.15 and 0.49 ± 0.05 in group A which is almost similar with an earlier study.[16]

Accumulation of gentamicin leads to pathological events in gentamicin induced nephrotoxicity and subsequent renal dysfunction.[17] Gentamicin induced nephrotoxicity is functionally evident by the elevated serum levels of urea, creatinine and structurally characterized by tubular necrosis, glomerular atrophy, mononuclear cell infiltration, intertubular haemorrhage, and hyaline casts.[18,19] In this study, we found that the mean blood urea and serum creatinine level was 84.43 ± 6.87 and 3.37 ± 0.56 respectively with a p value of <0.001, and histopathologicaly there were severe distortion of renal architecture, glomerular congestion, early tubular necrosis and interstitial infiltration with more lymphocytes in gentamicin treated rats in group B which is statistically significant and also reported by another study.[20]

In current study, we found that mean blood urea and serum creatinine level in group C were 68.43 ± 3.36 SD and 1.96 ± 0.10 SD respectively with a p value of <0.001 which is

statistically significant and histopathologicaly there were mild to moderate distortion of renal architecture, few to some areas of glomerular congestion and infiltration of interstitium by few to some number of lymphocytes. Kalmi shak has nephroprotective effect in gentamicin induced nephrotoxic rats which was also observed by other studies.[9,10]

We also observed that mean blood urea and serum creatinine level in group D were 53.85 ± 11.55 SD and 1.67 ± 0.26 SD with a p value of <0.001 which is statistically significant. Losartan has nephroprotective effect of gentamicin induced nephrotoxic rats which was observed in a study.[12] In this study, we tried to find out the nephroprotective effect of ramipril and found that it has nephroprotective effects in gentamicin induced nephrotoxic rats.

In our study, we found that the difference between mean blood urea and serum creatinine level in group C and group D were 14.57 ± 4.55 SE and 0.29 ± 0.11 SE with a p value of 0.102 and 0.175 respectively which is statistically insignificant and in histopathologically there were mild to moderate distortion of renal architecture, few to some areas of glomerular congestion and infiltration of interstitium by few to some number of lymphocytes with kalmi shak treated rats and in ramipril treated rats showed preserved renal architecture with very few area of glomerular congestion and infiltration of interstitium by very few lymphocytes. The nephroprotective effect of kalmi shak was demonstrated in gentamicin induced nephrotoxic rats and comparable with the nephroprotective effect of ramipril. Increased blood urea and serum creatinine levels were significantly protected and histological architecture observed almost normal in experimentally nephrotoxic group when treated with ethanolic extract of kalmi shak and ramipril.

Antioxidants can inhibit or abolish gentamicin induced nephrotoxicity in rats. They improve histological injuries such as tubular necrosis, tubular cell edema and apoptosis in gentamicin treated rats.[7]

This study observed that the ethanolic extract of kalmi shak has nephroprotective effect on gentamicin induced nephrotoxic rats which is comparable with ramipril. Results of this study suggest that kalmi shak may be an effective, easily available and cheap nephroprotective agent. However, more studies would be necessary to validate the claim and explore the mechanism of action for application in human population.

Study Limitation:

1. Sample size was small and gender variation could not be evaluated.
2. Modern drugs and herbal products were used to influence the biological system. Natural system has certain limitations like individual variation and interference in response to the system which might have interfered with results. So, results obtained in this study may differ somewhat from the exact effect.

Conclusion:

Despite limitations, interpretation of results obtained in this study was made cautiously and carefully. Co administration of kalmi shak with gentamicin abolish the gentamicin induce nephrotoxicity as like as ramipril. Further studies are needed to identify and characterize before being establish it in clinical setting.

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None

Conflicts of Interest:

There are no conflicts of interest.

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