Original Article

Comparison of Nephroprotective Effect of *Daucus Carota Linn.* (Carrot) and Ramipril on Gentamicin Induced Nephrotoxicity in Rats – An Experimental Study

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ABSTRACT

Acute kidney injury (AKI) is an important public health issue in Bangladesh. There are different reasons behind this. Gentamicin is a commonly used antibiotic prescribed to cure infections and a potential cause of renal tubular damage. An available vegetable Daucus carota Linn. (Carrot) and ACE inhibitor, ramipril, have some nephroprotective properties that alleviates gentamicin induced acute kidney injury. A study was carried out on a group of rats to evaluate the nephroprotective effect of carrot in comparison with ramipril. An experimental study was conducted to demonstrate the effect of ethanolic extract of carrot on blood urea and serum creatinine levels as well as histopathological changes in gentamicin induced nephrotoxic kidneys of a group of rats and the results were compared with a nephroprotective drug ramipril. Blood urea and serum creatinine levels and histopathological changes revealed a significant difference with carrot which is comparable to ramipril in gentamicin induced nephrotoxicity in rats. Carrot has nephroprotective effect that can reduce gentamicin induced nephrotoxicity compared to ramipril.

Keywords: *Carrot, gentamicin, ramipril, nephrotoxicity, acute kidney injury*

Mugda Med Coll J. 2025; 8(1): 3-7
DOI: https://doi.org/10.3329/mumcj.v8i1.82809

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INTRODUCTION

Acute Kidney Injury (AKI) is a common clinical problem which is defined as sudden deterioration of renal function resulting in the retention of urea and other nitrogenous elements causing dysregulation of extracellular volume and electrolytes and associated with high mortality and can occur in normal renal function or patients with preexisting renal diseases[1]. A lot of prescribed drugs can cause acute kidney injuries. Drugs excreting through kidneys may cause cellular damage, resulting in kidney dysfunction[2]. Gentamicin is an aminoglycoside antibiotic which is effective against gram negative organisms and a popular choice for prescriber due to its low cost but is a nephrotoxic drug that can produce renal tubular damage[3]. Nephrotoxicity of gentamicin limits its frequent clinical prescription[4]. Amelioration of nephrotoxicity can increase its clinical use by the prescribers. Gentamicin induced nephrotoxicity includes oxidative stress, apoptosis, necrosis,

upregulation of transforming growth factor B, and increase monocyte macrophage infiltration by increasing the generation of superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species in the kidney[4]. Evidence showed that antioxidants can lower gentamicin induced nephrotoxicity in rats. Antioxidants may improve histological injuries such as tubular necrosis, tubular cell oedema, and apoptosis in gentamicin treated rats[5].

Several vegetables like carrot, which we intake in our daily life may exert nephroprotective effects[6,7]. Vitamin and phytonutrients such as carotenoids, phenolics may protect biological systems from the effects of oxidative stress. Carrot is abundant with phytonutrients including phenolics, polyacetylenes and carotenoids and also rich in â-carotene, ascorbic acid and tocopherol[8]. Carrot has nephroprotective, antioxidant, anticarcinogenic, immune enhancer, antidiabetic, antihypertensive, hepatoprotective, cholesterol and cardiovascular disease lowering effects[7] and improve kidney function on renal ischaemia reperfusion injury in rats [9].

Ramipril is an angiotensin converting enzyme inhibitor. It has specific nephroprotective effect due to angiotensin II driving action on glomerular hyperfiltration and prevents progression of earliest manifestation of renal damage like microalbuminuria and reduces macroproteinurea[10]. Based on those evidence, we proposed this experiment with ethanolic extract of carrot to evaluate its nephroprotective effects on gentamicin induced nephrotoxicity in rats and compare with the effect of ramipril, an ACE inhibitor drug.

METHODS

This experimental study was carried out in the Department of Pharmacology and Therapeutics, Dhaka Medical College, Dhaka, Bangladesh, 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., Bangladesh. Carrots, which DACB accession number was 45085, were collected from a grocery 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 the Opsonin Pharmaceuticals Ltd., Bangladesh.

The rats were divided into four groups (A, B, C & D) comprising 7 rats in each group. All the 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 gentamicin treated group. Nephrotoxicity was induced by intraperitoneal injection of gentamicin at a dose of 100mg/kg body weight for 9 days (following Sodimbaku et al.)[11]. The rats were allowed for the usual diet for the same duration and then sacrificed on day 10. Group C was treated with ethanolic extract of carrot (400mg/kg/day) (following Sodimbaku et al.)[11] 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) (following Mavrakanas et al.)[12] by gastric intubation and gentamicin (100mg/kg/day) intraperitoneally for 9 days with usual rat diet and then sacrificed on day 10.

On the 10th day, after sacrificing the rats, blood samples were collected through cardiac puncture and sent to the Department of Biochemistry of the same institution for biochemical analysis. The rats were then sacrificed, and kidneys were dissected and stained with haematoxylin & eosin (H&E) for histopathological examination done in the Department of pathology of the same institution.

All results were appropriately recorded in computer. Statistical analysis was done by using Statistical Package for Social Sciences (SPSS) version 17.0 for Windows. The variables were expressed as mean±SD. Unpaired student †s t-test and ANOVA (Post-Hoc) test were done for comparisons as applicable. A p-value <0.05 was considered as significant.

The study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka, Bangladesh.

RESULTS

Table-I shows that the mean blood urea and serum creatinine levels were higher in group B than that of group A; the difference was statistically significant ($p<0.001$). Table-II shows that the mean blood urea and serum creatinine levels were higher in group B as compared to group C and D; those differences were statistically significant ($p<0.001$). However, no differences were observed between group C and group D ($p>0.05$).

Table-I: *Effect of gentamicin on blood urea and serum creatinine level in group B as compared to control (group A)*

Groups	Blood urea(mg/dl)		Serum creatinine(mg/dl)	
	mean±SD	p-value	mean±SD	p-value
A (Control) (n=7)	21.43±2.15	<0.001*	0.49±0.05	<0.001*
B (Gentamicin) (n=7)	84.43±6.87		3.37±0.55	

Unpaired Student's-t test was used to reach p-value; *= significant.

Table-II: *Comparison of blood urea and serum creatinine levels among group B, group C and group D*

Groups	Blood urea(mg/dl)		Serum creatinine(mg/dl)	
	mean±SD	p-value	mean±SD	p-value
B (Gentamicin)(n=7)	84.43 ± 6.87	B vs. C <0.001*	3.37 ± 0.56	B vs. C <0.001*
C (Carrot)(n=7)	64.57±4.27	B vs. D<0.001*	1.85±0.13	B vs. D<0.001*
D (Ramipril)(n=7)	53.85±11.55	C vs. D>0.05	1.67±0.26	C vs. D>0.05

In histopathological examination, in group A, rat kidneys showed a typical appearance of glomeruli, tubules and interstitium. No inflammatory cell infiltration or vascular changes were observed (Fig. 1). However, in group B, rat kidneys showed moderate to severe distortion of renal architecture, e.g., glomerular congestion, early tubular necrosis and interstitial infiltration with lymphocytes (Fig. 2). In group C, rat kidneys showed mild changes with preservation of renal architecture; however, only few areas having glomerular congestion and interstitial infiltration with few lymphocytes were observed (Fig. 3). In group D, rat kidneys showed preserved renal architecture with very few areas with glomerular congestion and interstitial infiltration with occasional lymphocytes (Fig. 4).

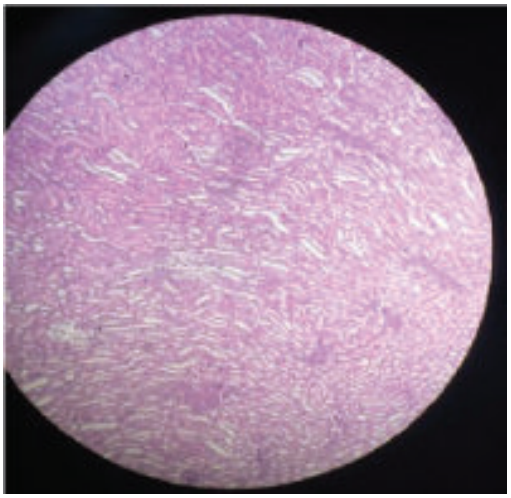

Fig. 1: *In group A, rat kidneys showing a typical appearance of glomeruli, tubules and interstitium; no inflammatory cell infiltration or vascular changes seen (H&E stain; ×100).*

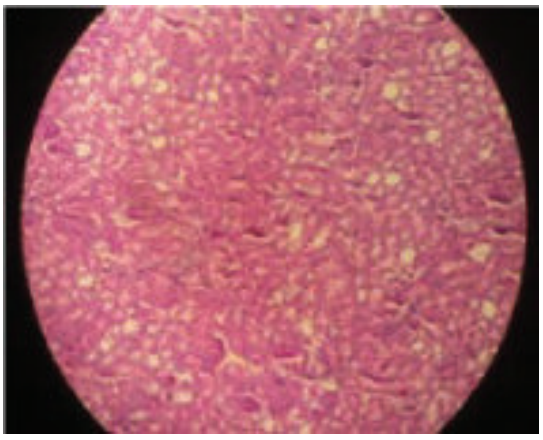

Fig. 2: *In group B, rat kidneys showing moderate to severe distortion of renal architecture with glomerular congestion, early tubular necrosis and interstitial infiltration with more lymphocytes seen (H&E stain; ×100).*

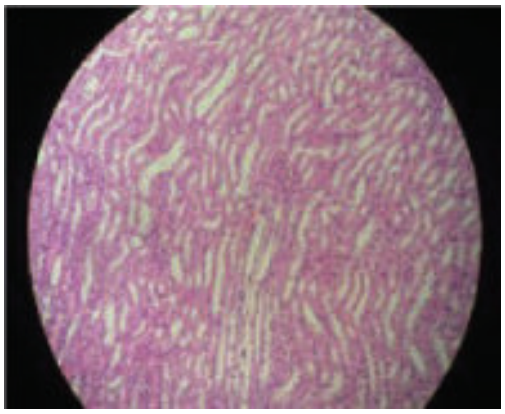

Fig. 3: *In group C, rat kidneys showing mild changes with preservation of renal architecture; only few areas having glomerular congestion and interstitial infiltration with few lymphocytes seen (H&E stain; ×100).*

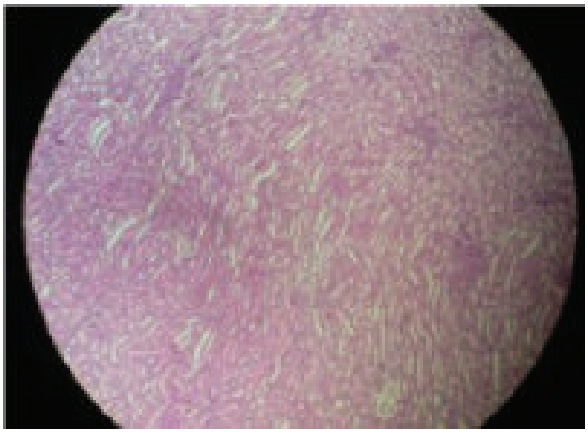

Fig. 4: *In group D, rat kidneys showing preserved renal architecture with very few areas with glomerular congestion and interstitial infiltration with occasional lymphocytes seen (H&E stain; ×100).*

DISCUSSION

In the present study, group A was the control group, where the rats were given a standard rat diet for nine days. Nephrotoxicity was generated by intraperitoneal injection of gentamicin at a dose of 100mg/kg body weight in this study in group B (following Sodimbaku et al.)[11]. The nephroprotective effects were compared with ethanolic extract of carrot and oral ramipril. Group C rats were treated with ethanol extract of carrot (400mg/kg/day)[11] by gastric intubation for nine days along with gentamicin (100mg/kg/day) intraperitoneally and standard rat diet, Group D were treated with ramipril (1mg/kg/day) (following Mavrakanas et al.)[12] by gastric intubation along with gentamicin (100mg/kg/day) intraperitoneally and standard rat diet for the same period.

In this study, we found that the mean blood urea and serum creatinine levels were 21.43±2.15 mg/dl and 0.49±0.05 mg/dl in our control group (group A), which were almost similar with a previous study done by Quadir et al.[13]. Accumulation of gentamicin tends to be a pathological event in gentamicin inducing nephrotoxicity and subsequent renal dysfunction[14]. The renal injury manifests as migration of monocytes and macrophages to the site of injury[15,16]. Studies reported the role of reactive oxygen species in implicating the pathogenesis of gentamicin induced nephrotoxicity[17]. Gentamicin intoxicated 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]. Serum urea and creatinine were found to be increased as an indicative of parenchymal tissue injury[18,19]. In this study, in gentamicin treated rats (group B) the mean blood urea and serum creatinine levels were observed 84.43±6.87 mg/dl and 3.37±0.55 mg/dl respectively ($p<0.001$, as compared to the control, group A); histopathological examination revealed moderate to severe distortion of renal architecture including glomerular congestion, early tubular necrosis and interstitial infiltration with more lymphocytes. Similar reports were found in the study done by Saha & Choudhury[20].

In our study, the mean blood urea and serum creatinine levels in group C were 64.57±4.27 mg/dl and 1.85±0.13 mg/dl ($p<0.001$, as compared to group B); histopathologically observed mild to preserved renal architecture, few areas of glomerular congestion and infiltration of interstitium by few lymphocytes, which signifies that carrot has nephroprotective effect in gentamicin induced nephrotoxic rats. Similar observations were reported by Tavafi & Ahmadvand[5] and Sodimbaku et al.[11].

We also observed that the mean blood urea and serum creatinine levels in group D were 53.85±11.55 mg/dl and 1.67±0.26 mg/dl ($p<0.001$, as compared to group B); histopathologically observed preserved renal architecture with very few areas with glomerular congestion and interstitial infiltration with occasional lymphocytes, which signifies that ramipril, an ACE inhibitor, has nephroprotective effect in gentamicin induced nephrotoxic rats. Similar observations were reported by Darwish & El-Lateef[21]. Evidence suggests the renoprotective potential of the angiotensin-converting enzyme inhibitors (ACEIs), e.g., ramipril in nephropathies of almost any aetiology[19]. Overall, increased blood urea and serum creatinine levels were prevented and preserved histological architecture near to normal was evident in experimentally nephrotoxic groups treated with ethanolic extract of carrot and ramipril respectively. Antioxidants present in carrot can improve histological injuries such as tubular necrosis, tubular cell oedema, and apoptosis in gentamicin treated rats[6]. Our results suggest that carrot acts as a beneficial nephroprotective agent. However, in this study, sample size was small, and gender variation was not evaluated due to time and budget constraint. Moreover, we used drug component (ramipril) and herbal product (ethanol

extract of carrot) to influence the biological system, which has its own limitations like individual variation and interference with the response to the system. Hence, the result obtained in this experiment may differ somewhat from the exact effect. We recommend further studies with lager sample size and gender specific evaluation to validate our claims.

CONCLUSION

Our data suggests that the ethanolic extract of carrot has a nephroprotective effect on gentamicin induced nephrotoxic rats. Concurrent administration of ethanolic extract of carrot ameliorates the pathological implications of gentamicin induce nephrotoxicity largely as ramipril. Further studies are warranted to identify and characterize more precision before establishing it in clinical settings.

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