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ORIGINAL/RANDOMIZED CONTROL TRIAL ARTICLE

FICUS CARICA L. (ANJIR) LEAF EXTRACT EFFECTS ON NEPHROTOXIC CHANGES IN GENTAMICIN TREATED ALBINO MICE

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ABSTRACT
Current randomized controlled experimental study was carried out to investigate the beneficial effects of leaf extract of Ficus carica L. (Anjir) leaf extract in gentamicin induced nephrotoxicity. Thirty mice were divided into three groups of 10 mice each. Group A served as control and was given distilled water orally for 8 days. Group B and C were experimental and were treated with gentamicin 200 mg/kg/day intraperitoneally and gentamicin 200 mg/kg/day intraperitoneally with Ficus carica L. (Anjir) leaf extract 400 mg/kg/day orally respectively for a period of 8 days. After 24 hours of experimental period, kidneys of each mouse were dissected and processed for microscopic examination. The histological examination of group A showed normal renal architecture whereas histopathalogical examination of group B showed fragmented tubular basement membrane, tubular degeneration, tubular cast with interstitial inflammation and congestion. Histological examination of group C showed minimal change in renal histology as compared to group B. Gentamicin is a nephrotoxic drug and Ficus carica L. leaf extract has nephroprotective effects against gentamicin induced nephrotoxicity due to its antioxidant properties.

Keywords: Gentamicin, Ficus carica (Anjir), Nephrotoxicity.

INTRODUCTION

Nephrotoxicity has been well known adverse effect of aminoglycoside antibiotics for many years. During the past 6 to 8 years, a number of research scientists have produced a large experimental data that helped us understand the pathogenesis of nephrotoxicity.[1] Gentamicin is one of the most commonly used antibiotic for the treatment of gram negative bacterial infections.[2] Gentamicin is potentially effective drug against gram positive bacterial infections and also used in the treatment of infected wounds, burns and skin lesions.[3]

Gentamicin induced kidney damage leads to a significant rise in serum urea, creatinine and malondialdehyde levels.[4] Gentamicin treated renal sections showed nephrotoxic changes only in cortical areas, whereas renal tubular necrosis was primarily observed in proximal convoluted tubules and rarely, interstitial infiltrate of mononuclear cells were also noticed[5]. Gentamicin induced nephrotoxicity showed increased lipid peroxidation in renal cortical area and in vitro production of hydrogen peroxide.[6] At ultrastructural level of study, gentamicin showed formation of myeloid bodies and cellular necrosis in focal manner.[7]

Ficus being the largest genera of angiosperms, with almost 800 species of terrestrial trees, shrubs, hemi-epiphytes, climbers and creepers occurs worldwide.[8] *Ficus* is considered highly important because of its economical and nutritional value[9]. *Ficus carica* L. is a small or moderate sized deciduous tree with spreading branches, having pear shaped fruit of variable size and colour.[10]

Antioxidant effects of *Ficus carica* L. leaf extract have been reported against hepatotoxicity induced by various agents,[9,11] and *Ficus carica* L. fruit extract has proven equally effective against gentamicin induced nephrotoxicity.[12]

Antioxidant effects of *Ficus carica* L. fruits and leaves reduced the raised uric acid, urea nitrogen, creatinine and blood glucose levels due to presence of polyphenoles and flavonoids.[13] Nephroprotective effect of *Ficus carica* L. leaf extract has not been tried on gentamicin induced nephrotoxicity, therefore present study was designed to observe the histological and structural changes in mice kidney produced by gentamicin and effects of *Ficus carica* L. leaf extract on these changes.

MATERIALS AND METHODS

Animals

This was a randomized controlled study conducted at the experimental research laboratory of University of Health Sciences UHS, Lahore, Pakistan. Thirty healthy adult male albino mice, having mean weight 30 gm ± 5 gm, were procured from Veterinary Research Institute, Lahore. The animals were kept under controlled room temperature 23 ± 2°C and humidity (50 ± 5%)

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with 12 hours night and day cycle. The experiment was started after acclimatization for one week. The animals were weighed and examined daily to assess their state of health.

Preparation of Gentamicin Solution

Solution was made by dissolving 1200 mg of gentamicin in 60 ml of distilled water; thus, each ml of solution contained 20 mg of gentamicin. This stock solution was stored in the refrigerator at 3-4°C and administered at the dose of 200 mg/kg/bw to each mouse daily.

Preparation of *Ficus carica* L. Leaf Extract

The extract was prepared at PCSIR laboratories, Lahore. The leaves were powdered after shade drying. 5 g of *Ficus carica* L. leaf powder was soaked in 1000 ml of ethanol for 24 hours and then filtered which was evaporated on Rotary evaporator (Edolph, Germany) at 40°C. 2400 mg of this concentrated extract was dissolved in 60 ml of distilled water; thus, each ml of solution contained 40 mg of the extract. This stock solution was administered orally at the dose of 400 mg/kg/bw to each mouse daily.

Experimental Procedure

The animals were randomly placed in three groups A, B, and C containing 10 animals in each by using lottery method. Group A served as control and received distilled water orally, Group B received gentamicin 200 mg/kg/day for 8 days intraperitoneally and Group C was given *Ficus carica* L. leaf extract at a dose of 400 mg/kg/day orally and after 3 hours; gentamicin at a dose of 200 mg/kg/day intraperitoneally for 8 days[9]. The experimental protocol was completed on 8th day and on 9th day, the animals were anaesthetized; blood samples were collected by performing cardiac puncture. The kidneys of all animals were removed, weighed and small pieces of 3-5mm size were excised and preserved for histological examination. H&E and PAS stained histological sections were observed for tubular parameters (tubular degeneration, condition of the tubular basement membrane and presence of tubular cast) and interstitial parameters (interstitial inflammation & interstitial congestion).

Statistical Analysis

The data was entered and analyzed using SPSS 18.0 (Statistical Package for Social Sciences). Mean ± SEM was given for quantitative variables. One way ANOVA (Analysis of Variance) was applied to observe differences among groups. Post Hoc Tukey test was applied to observe mean differences among the groups. Chi-square and Fisher's exact test was applied to observe associations between qualitative variables. A *p-value* of ≤ 0.05 was considered as statistically significant.

RESULTS

Weight of Mice

At the end of experiment, we observed statistically significant difference ($p < 0.001$) in weight of mice of group B which were receiving gentamicin as compared to group A receiving distilled water. When compared to group B statistically significant difference ($p < 0.001$) was found in weight of mice of group C receiving gentamicin and *Ficus carica* L. leaf extract. Statistically insignificant difference ($p < 0.001$) was observed among groups A and C which were receiving distilled water and gentamicin and *Ficus carica* L. leaf extract respectively (Table 1).

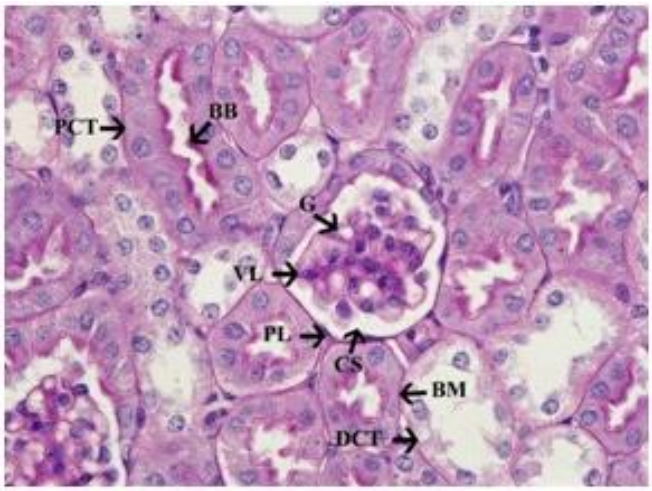

Fig. 1: *Photomicrograph of the cortex of the kidney from group A showing Bowman's capsule containing visceral (VL) and parietal layers (PL) separated by capsular space (CS) and enclosing the glomerulus (G). Proximal convoluted tubule (PCT) lined by cuboidal epithelium with strongly PAS positive brush border (BB). Distal convoluted tubules (DCT) are identified by larger lumen and simple cuboidal epithelium. Basement membrane (BM) is strongly PAS positive. PAS stain X 400.*

Histological Examination

Histological examination of kidneys of control group A showed normal renal architecture. No histological change in glomeruli, proximal and distal convoluted tubules and interstitium was seen (Table 2, Fig. 1). Histopathological examination of group B mice which received gentamicin only, exhibited marked degeneration in proximal convoluted tubules. Fragmented tubular basement membrane and proteinacoeus cast in the lumen were observed. In interstitium, severe inflammation and congestion was noticed (Table 2, Fig. 2). In histological sections from group C, nephrotoxic effects were less evident as compared to group B. Tubules showed areas of patchy necrosis and degeneration with few areas of mild congestion and mild inflammatory reaction in interstitium as compared to group B. PAS

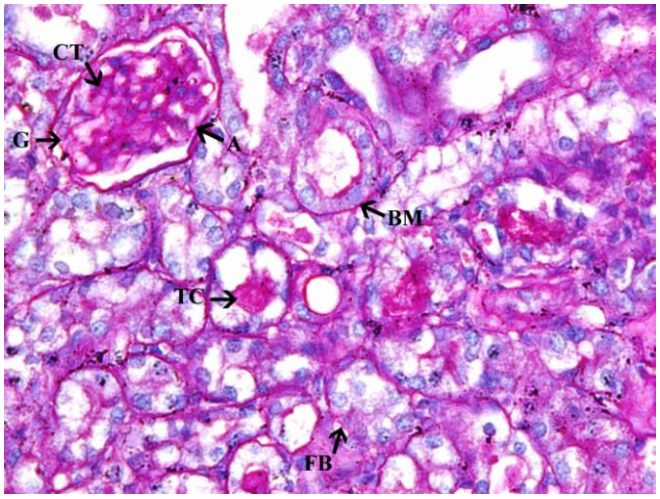

Fig. 2: *Photomicrograph of cortex of kidney from group B showing glomerulus (G); with capillary wall thickening (CT) and adhesions (A) between capillary tuft and Bowman's capsule. Tubules showing cast (TC) and foci of fragmented basement membrane (FB). Basement membrane (BM) is strongly PAS positive. PAS stain X 400.*

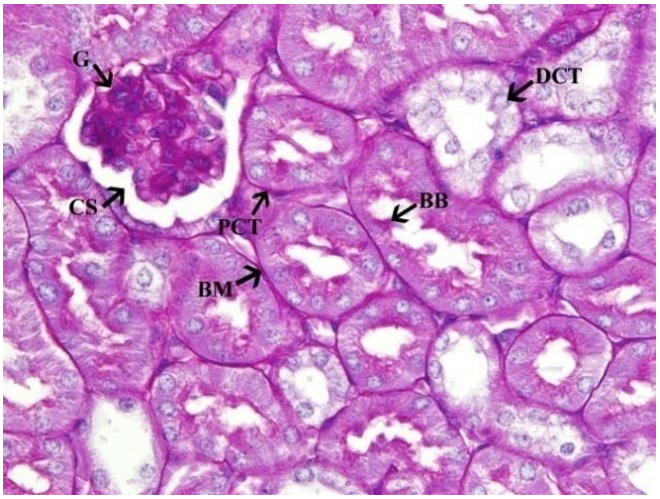

Fig. 3: *Photomicrograph of the cortex of the kidney from group C showing normal looking renal corpuscle enclosing glomerulus (G) within Bowman's capsule, containing visceral and parietal layers separated by capsular space (CS). Proximal convoluted tubule (PCT) lined by cuboidal epithelium with strongly PAS positive brush border (BB). Distal convoluted tubules (DCT) are identified by larger lumen and simple cuboidal epithelium. Basement membrane (BM) is strongly PAS positive. PAS stain X400.*

stained section from group C showed intact tubular basement membrane and absence of casts in the tubular lumen (Table 2, Fig. 3).

Table 1: *Showing Multiple Comparisons of Mean Values of Weight of Mice Among Groups A, B and C.*

Parameter	*Comparison among groups*		*Mean Difference (I-J)*	*Standard Error (S.E)*	*p-value*
	Groups (I)	*Groups of Comparison (J)*			
Weight of animal in grams	Group A	Group B	4.600*	1.082	0.001
		Group C	-2.400	1.082	0.138
	Group B	Group A	-4.600*	1.082	0.001
		Group C	-7.000*	1.082	0.000
	Group C	Group A	2.400	1.082	0.138
		Group B	7.000*	1.082	0.000

*p-value ≤ 0.05 is considered statistically significant.

Table 2: *Showing Comparison of Histopathological Parameters among Groups A, B and C.*

Parameter		*Group*			*p-value*
		A	*B*	*C*	
Tubular Degeneration	Absent	10 (100.0%)	0 (0.0%)	0 (0.0%)	< 0.001
	Mild	0 (0.0%)	0 (0.0%)	10 (100.0%)	
	Severe	0 (0.0%)	10 (100.0%)	0 (0.0%)	
Tubular Casts	Absent	10 (100.0%)	2 (20.0%)	8 (80.0%)	< 0.001
	Present	0 (0.0%)	8 (80.0%)	2 (20.0%)	

Tubular Basement Membrane	Normal	10 (100.0%)	0 (0.0%)	8 (80.0%)	< 0.001
	Fragmented	0 (0.0%)	10 (100.0%)	2 (20.0%)	
Interstitial Inflammation	Absent	8 (80.0%)	0 (0.0%)	3 (30.0%)	< 0.001
	Mild	2 (20.0%)	0 (0.0%)	7 (70.0%)	
	Moderate	0 (0.0%)	2 (20.0%)	0 (0.0%)	
	Severe	0 (0.0%)	8 (80.0%)	0 (0.0%)	
Interstitial Congestion	Absent	8 (80.0%)	0 (0.0%)	6 (60.0%)	< 0.001
	Present	2 (20.0%)	10 (100.0%)	4 (40.0%)	

DISCUSSION

The extent of gentamicin induced renal toxicity depends upon age, sex and species of the experimental animal, as well as on the dose and duration of the therapy used.[14]

In the present study, statistically significant reduction in the body weight of mice in group B was observed in comparison with other groups. This finding is in agreement with reports earlier with statistically significant decrease in body weight was observed in gentamicin treated animals when compared to control group.[4,6]

In the present study, renal tubules showed severe tubular degeneration and fragmented tubular basement membrane in group B animals. These findings were similar to other such studies where gentamicin treated animals showed proximal renal tubular necrosis and degeneration of epithelial cells due to oxidative damage.[15,16] Our study revealed the presence of proteinaceous casts in tubular lumen of group B animals when compared to other groups. Another research reported similar findings including extensive necrosis and presence of tubular casts in gentamicin treated animals.[17] Studies reported similar evidences of accumulation of necrotic cellular debris in the tubular lumen of rats with gentamicin induced renal toxicity.[18]

Histological examination of kidneys of group B animals treated with gentamicin showed infiltration of inflammatory cells with congestion of blood vessels as compared to other groups of the study which showed nearly normal interstitium. Our results are similar to findings of other researchers of congested blood vessels and increased inflammatory cells in gentamicin treated rats.[19]

Significant improvement in tubular changes was noticed when gentamicin and *Ficus carica* L. leaf extract simultaneously administered to the animals of group C in comparison to group B. Significant difference of tubular degeneration and fragmented basement membrane was observed among groups. It was markedly reduced in animals of group C as compared to group B. Histologically group C animals showed normal tubules without any proteinaceous casts, few inflammatory cells and normal looking blood vessels in interstitium in contrast to group B.[20]

It is ***concluded*** that the present study investigated *Ficus carica* L. leaf extract effects on nephrotoxic changes produced by gentamicin in adult male albino mice. The results of the present study clearly indicate that *Ficus carica* L. leaf extract has effectively corrected the histopathological changes induced by gentamicin in the animals.

RECOMMENDATIONS

Current study was a time limited study so we were not able to observe the effect of *Ficus carica* L. (Anjir) leaf extract on other organs and systems of the mice. Since *Ficus carica* L. (Anjir) leaf extract has more potential being a rich source of antioxidants, it deserves further intensive study both in humans and animals to explore the mechanism of action of the active ingredients in health and diseases at the cellular and molecular level.

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Authors' Contribution

AG: Study conduction & Drafting. SWK: Photomicrographs & Review. NA: Statistical Analysis & Review.

Disclosure

There had been no disclosure.

Conflict of Interest

There is no conflict of interest.

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