ORIGINAL ARTICLE

Nephroprotective Effect of Aqueous Extract of Carica Papaya Seeds

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ABSTRACT

Objective: To determine the nephroprotective effect of aqueous extract of *Carica papaya* seeds in Aminoglycoside induced acute nephrotoxicity in rats.

Study Design: Quasi Experimental study.

Place and Duration of Study: The study was conducted from April 2016 to March 2017 at "Department of Pharmacology and Therapeutics" and "Multidisciplinary Laboratory of Islamic International Medical College, Riphah International University", Islamabad in collaboration with National Institute of Health (NIH), Islamabad.

Materials and Methods: Thirty Sprague Dawley rats were divided into 3 groups i.e., A, B and C with 10 rats in each group. Group B and C were given Aminoglycoside; Gentamycin in 80 mg/kg) via intraperitoneal route once daily for 5 consecutive days to induce acute nephrotoxicity. At day 6th, nephrotoxicity was confirmed by measuring serum urea and creatinine. Aqueous extract of *Carica papaya* seeds (1000 mg/kg) was started once daily through oral route in group C for 5 consecutive days. All animals were given standard diet pellets manufactured at NIH.

Results: Mean serum urea and creatinine for Group A (Control Group) at day 0 was 24.70 mg/dL 5.16 and 0.750 mg/dL 0.1958 respectively. Mean serum urea and creatinine for Group B (Disease Control Group) was 78.60 mg/dL 3.921 and 1.920 0.1229 at day 6th. This suggested induction of nephrotoxicity by gentamycin. Mean serum urea and creatinine for Group C (Aqueous Extract Treated Group) at 11th day was 50.60 mg/dL 5.910 and 1.380 mg/dL 0.1932 after 5 days treatment with aqueous extract of *Carica papaya* seeds.

Conclusion: Aqueous extract of *Carica papaya* seeds has significant nephroprotective effects on aminoglycoside induced acute nephrotoxicity in rats.

Key Words: *Aminoglycoside, Carica papaya, Gentamycin, Nephrotoxicity.*

Introduction

A total of 25% of cardiac output is received by kidneys. They serve as main organs for maintenance of homeostasis of circulatory fluid. Kidneys also serve as primary organs for elimination and detoxification of xenobiotic elimination and detoxification.[1] Direct or indirect exposure of drugs and different chemicals to kidneys result in nephrotoxicity. Many drugs may result in acute or chronic renal failure.[2] Adverse reactions to drugs occur in approximately 6 % of admitted patients in hospitals and amongst these approximately 7 % patients suffer drug-related toxicities.[3] Nephrotoxic drugs may be responsible for 19% – 25% of acute kidney injury in critically ill patients receiving them.[4] Due to the intrinsic functions of kidneys for drug metabolism, concentration and excretion, they are vulnerable to toxicity due to drugs and their metabolites. Many patients suffer from drug-induced nephrotoxicity that can cause either acute injury to kidneys or chronic damage. Many widely used marketed drugs including anti-cancer drugs, antibiotics, immunosuppressants and radio contrast agents are nephrotoxic.[5] Nephrotoxicity is characterized by raised serum urea and creatinine levels.[6] Aminoglycosides used for the treatment of Gram-negative bacteria have been a vital component of antibiotic armamentarium. This is peculiar to their cost effectiveness and efficacy.[7] Aminoglycosides are not metabolized in the body and eliminated unchanged in the urine by glomerular filtration.[8] Due

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to their potent ability to cause nephrotoxicity and ototoxicity, dosage limitations of Aminoglycosides pertain.[9] Gentamycin, an important aminoglycoside antibiotic, is used widely due to its potent antibiotic activity against various gram-negative microorganisms.[10] However, its use is greatly limited by nephrotoxicity, accounting for 10 – 15 % of all cases of acute renal failure.[10] Aminoglycoside-induced nephrotoxicity is confirmed by rise in serum creatinine, serum urea, and marked decrease in glomerular filtration rate.[11] Drug induced nephrotoxicity at renal tubular level more commonly affects proximal tubular cells due to high exposure to drug and its metabolites during processes of drug concentration and reabsorption through the glomerulus. Mitochondria are damaged in tubular cells, tubular transport system is disturbed and there is increased oxidative stress due to generation of free radicals leading to cytotoxicity. Nephrotoxic drugs fiberize the kidney due to inflammation in glomerulus and proximal tubular cells and damage to surrounding renal cellular matrix.[12] Current research was aimed to see the effect of *Carica papaya* seeds on acute Drug-induced nephrotoxicity and to support it biochemically. Present study aims to find out the effect of extract of *Carica papaya* seeds in Aminoglycoside induced acute nephrotoxicity.

Materials and Methods

A quasi experimental study including 30 Sprague Dawley rats was performed in the "Department of Pharmacology and Therapeutics" and "Multidisciplinary Laboratory of Islamic International Medical College, Riphah International University", Islamabad in collaboration with National Institute of Health (NIH), Islamabad for duration of one year.

A total of 30 Sprague Dawley rats were acquired and divided into 3 groups; A, B and C with 10 rats each through random selection via balloting method. Authorization of the study was made by the Ethics Review Committee of Islamic International Medical College (Riphah International University) and was implemented within the frame of rules specified by the National Institute of Health for animal experiments. Rats weighing more than 300 grams and with normal baseline serum urea and creatinine levels were included in the experiment. All rats were served with standard food pellets manufactured at the Animal house of NIH, Islamabad according to the recommendations agreed by the universities federation for animal welfare, alongside fresh water supply of 250 mL in inverted water bottles anchored on the enclosures in a particular inclined position.

Group A was taken as control group throughout the experiment. Group B and C (10 rats each) were given Aminoglycoside drug Gentamycin (80 mg/kg) via intraperitoneal route for 5 consecutive days for induction of acute nephrotoxicity.[11] Blood samples were collected via tail vein and serum urea and serum creatinine were measured at day 6 to see that nephrotoxicity has developed.[13] After the confirmation of nephrotoxicity on day 6, Group C was started with administration of Aqueous Extract of *Carica papaya* seeds (1000 mg/kg) dissolved in distilled water for 5 consecutive days.[14] After terminal sampling through cardiac puncture serum urea and creatinine were measured again at Day 11 to see the effect of aqueous extract of *Carica papaya* seeds on renal function.

Carica papaya plants were grown locally. Seeds were collected from ripened fruits of papaya. Seeds were air dried. The dried seeds of *Carica papaya* were identified and authenticated by herbarium section of National Agricultural Research Centre (NARC) Islamabad. Coarse powder was made. In 1.0 L of distilled water, approximately 200 g powder was soaked. The mixture was kept for 48 hours with intermittent shaking. After 48 hours, the extract was filtered using Whattman filter paper no.1. The filtrate was dried via rotary evaporator through evaporation. The extraction yielded 5.61 w/w dry matter. The extract was then stored in a refrigerator at 4°C till usage.[15]

The data was analyzed using Microsoft Excel 2010 and SPSS 20. Multiple comparisons were done through Tuckey test and group mean differences were observed. A p-value of <0.05 was considered as statistically significant.

Results

At day 6 mean value of serum urea for Group A (Control Group) was 24.70 mg/dL 1.633. Mean serum urea for Group B (Disease Control Group) and Group C were 81.00 mg/dl ± 1.247 and 82.70 mg/dl ± 2.587. There was significant difference (p Value less than 0.05). This suggested induction of acute nephrotoxicity by aminoglycoside drug gentamycin.

Mean serum urea for Group C (Aqueous Extract Treated Group) at day 11 was 50.60 mg/dL±1.869 after 5 days treatment by aqueous extract of *Carica papaya* seeds. There was significant difference (p Value <0.05) which supported the nephroprotection by the herb.

Table I: Mean Values of Serum Urea of All Groups at Day 0, 6 And 11

Study Groups	Serum Urea on Day 0 Means±SD	Serum Urea on Day 6 Means±SD	Serum Urea on Day 11 Means±SD
Group A (control group)	24.90 ± 1.633	24.70 ± 1.633	24.70 ± 1.633
Group B (Disease Control)	24.70 ± 1.760	81.00 ± 1.247***	78.60 ± 1.240
Group C (Aqueous Extract Treated Group)	24.50 ± 1.821	82.70 ±2.587***	50.60 ± 1.869***
p-value	0.924	< 0.05	< 0.05

Mean value of serum creatinine for Group A (Control Group) was 0.750 mg/dL 0.0619. Mean value of serum creatinine for Group B (Disease Control Group) and Group C were 1.980 ± .0467 and 2.030 ± .0667 respectively. There was significant difference (p Value .000). This suggested induction of nephrotoxicity by aminoglycoside drug gentamycin. Mean serum Creatinine for Group C (Aqueous Extract Treated Group) was 1.380 ± .0611 after 5 days treatment by aqueous extract of *Carica papaya* seeds.

Table II: Mean Values of Serum Creatinine of All Groups at Day 0, 6 And 11

Study Groups	Serum Creatinine on Day 0 Means±SD	Serum Creatinine on Day 6 Means±SD	Serum Creatinine on Day 11 Means±SD
Group A(control group)	0.750 ± .0619	0.750 ± .0619	0.750 ± .0619
Group B(Disease Control)	0.780 ± .0611	1.980 ± .0467***	1.920 ± .0389
Group C(Aqueous Extract Treated Group)	0.770 ± .0559	2.030 ± .0667***	1.380 ± .0611***
p-value	0.988	< 0.05	< 0.05

Discussion

Kidneys function as main organ to detoxify and eliminate xenobiotics. Xenobiotics which accumulate in renal tubular cells can damage the kidneys acutely.[1] Acute kidney injury (AKI) being a common clinical complication possesses high mortality and morbidity. According to the statistics, the incidence rate of chronic Kidney Disease followed by an episode of AKI is 7.8 per 100 patients per year. The rate of End Stage Renal Disease (ESRD) is 4.9 per 100 patients per year. Gentamycin, an important aminoglycoside antibiotic, has potent antibiotic spectrum but its clinical use has been greatly reduced due to nephrotoxicity that accounts for 10 to 15 % of all cases of acute renal failure in drug induced kidney damage.[10] Gentamycin due to its cost effectiveness and high potency and more liability to cause toxicity in kidneys is widely used for inducing nephrotoxicity in experimental models.[13] Baseline serum urea and serum creatinine were measured at Day 0 i.e., beginning of the experiment. Gentamycin was administered intraperitoneally to Group B and Group C for five consecutive days. At day 6 Mean serum urea and serum creatinine were again measured. Mean serum urea and creatinine of Group B (Disease Control Group) and Group C were significantly raised when compared with the Control Group A suggesting that gentamycin produced acute nephrotoxic changes in the kidney and altered the renal function. After measuring the serum markers; Group C was started with administration of aqueous extract of *Carica papaya* seeds dissolved in distilled water via oral route. The aqueous extract was administered for five consecutive days. At Day 11, again serum markers were measured and the values of serum urea and serum creatinine were found to have been reduced than Day 6 in Group C which received aqueous extract of *Carica papaya* seeds. There was significant difference statistically between the Disease Control Group i.e., Group B and the Aqueous Extract treated Group i.e., Group C (p Value less than 0.05). The difference in serum markers showed nephroprotection by *Carica papaya* seed extract.

Gentamycin resulted in increased levels of serum markers i.e serum urea and creatinine in Group B and Group C after five days intraperitoneal administration of Drug in dose of 80 mg/kg when

compared to Group A i.e., Control Group. These findings were found to be in accordance with findings of a study by (Ajami M et al; 2010), in which nephrotoxicity was induced in male Wister rats via Gentamycin in same dose and there was resultant increase in levels of serum markers i.e., serum urea and creatinine.[13] After the induction of nephrotoxocity, the protective effect of aqueous extract of *Carica papaya* seeds was observed when 1000 mg/kg dose of Aqueous extract of *Carica papaya* seeds was administered to Group C. This was consistent with the study performed by umana et al., 2013) in which same dose of Chloroform extract of *Carica papaya* seeds was utilized in rats for toxicity studies.[16] In current study aqueous extract preparation of *Carica papaya* seeds was utilized which revealed improvement in levels of serum urea and serum creatinine in Group C after previous induction of nephrotoxicity by Gentamycin. The results indicated that Aqueous extract of *Carica papaya* seeds possess the potential of nephroprotection in Gentamycin induced acute nephrotoxicity. However, the cost of experiment limited the study of phytochemical constituents of the extract preparation. The duration of experiment can however be prolonged in further studies to assess complete reversal of nephrotoxicity and to study other extract preparations of *Carica papaya* too.

Conclusion

Aqueous extract of *Carica Papaya* seeds have significant protective effect as shown by the improvement of kidney function after Gentamycin induced acute renal injury.

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