Gummalla Pitchaiah et al. / International Journal of Pharmacy & Therapeutics, 6(4), 2015, 194-198.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

RENOPROTECTIVE ACTIVITY OF *ZIZIPHUS JUJUBA* FRUIT EXTRACT IN GENTAMICIN-INDUCED NEPHROTOXIC RATS

Gummalla Pitchaiah*, Sravani K, Jasmini Prabhakar M, Hari Sravanth Reddy P, Anil Kumar T

Hindu College of Pharmacy, Amaravathi Road, Guntur-522002, Andhra Pradesh, India.

ABSTRACT

The present study was to evaluate ethanolic extract *Ziziphus jujuba* fruit for its protective effect in gentamicin-induced acute renal failure in rats. Male Wistar rats were divided into four groups. Group one is negative control fed with water, group two is treated with gentamicin(80 mg/kg) intraperitoneally for 8 days to induce nephrotoxicity, group three and four served as test groups and treated with *Ziziphus jujuba* 250mg/kg and 500mg/kg respectively concomitantly with gentamicin for 8days by oral route. Nephrotoxicity was assessed by measuring the abnormal levels of serum urea, creatinine, blood urea nitrogen (BUN),sodium and potassium. It was observed that treatment with *Ziziphus jujuba* fruit extract significantly protected rat kidneys from gentamicin-induced Nephrotoxicity by restoring the plasma urea, creatinine, BUN, sodium and potassium levels. In addition, treatment with *Ziziphus jujuba* protected from gentamicin induced microscopical changes in kidney structure. The results suggest that supplementation of *Ziziphus jujuba* fruit extract may be useful in reducing gentamicin induced nephrotoxicity in rats.

Key Words:- *Ziziphus jujuba*, Ethanolic extract, Gentamicin induced nephrotoxicity, Renopretective.

INTRODUCTION

Kidneys endowed with million units are termed as nephrons that act as natural sieves. You need a certain amount of water in your system – not too much, and not too little. The kidneys are instrumental in regulating the fluid balance in the body. In turn, kidneys help to control blood pressure, balance the acids and bases. Also, contribute to the production of hormones, such as Erythropoietin and Vitamin D. The kidneys provide the final common pathway for the excretion of many drugs and their metabolites, and therefore are frequently subjected to high concentrations of potentially toxic substances (Yukawa and Nakazawa, 1980; Asiiley *et al*., 2004). Drugs and their metabolites are taken up particularly in the renal medulla, which has relatively little vasculature compared with the cortex. As a result, direct toxic damage occurs, generally affecting the renal tubular cells and renal papillae. Nephrotoxicity of this type tends to be dose – dependent (Asiiley *et al*., 2004).

Drug induced renal failure is a well-recognized phenomenon, although the incidence of drug – induced renal disease remains uncertain. However, some reports suggest that between 5 - 20 percent of cases of acute renal failure can be directly attributed to drugs and chemicals, although minor damage may pass undetected. It is important to be aware of the types of drug that can induce renal impairment because, if suspected and acted on early, the damage to the kidney may be reversible (WHO, 1998). Aminoglycoside antibiotics have been widely used for gram-negative infections. However, their nephrotoxicity and their ototoxicity are major limitations in clinical use.

Corresponding Author

Gummalla Pitchaiah
Email:- drgumalla@gmail.com

Kidney failure induced by aminoglycoside antibiotics such as Gentamicin is often reversible, or partially reversible, unlike most of Gentamicin's side effects (Maliakel *et al*., 2008).

Medicinal plants have been found to be the source of biodynamic compounds of therapeutic values. Many plant products are rich in polyphenols including tannins and flavonoids, chemical structure, characteristics and widely recognized as naturally occurring antioxidants (Deepak and Shrivastava, 2008). Among the medicinal plants discovered by the ancestors, *Ziziphus jujuba* is one of the traditional medicinal plant that has been used for over the years. *Ziziphus jujuba* is commonly known as red date, belongs to the family Rhamnaceae. The fruit is employed as an antidote to aconite poisoning, abdominal pain in pregnancy and externally in poultice and applications for wounds (Preetiand Shalini, 2014). The aim of this study was to investigate the renoprotective activity of *Ziziphus jujuba* fruits in gentamicin induced nephrotoxicity in rats.

MATERIALS AND METHODS

Plant material collection and authentication

The fruits of *Ziziphus jujuba* were collected from local market and authenticated by Prof.K.Madhava chetty,Ph.D., Reasearch officer-Botany,Sri Venkateswara University,Tirupathi.

Preparation of ethanolic extract

The fruits were deseeded, dried under shade for 15 days and ground to coarse particle size. Weighted quantity (100 g) of powder was extracted by ethanolic extraction by soaking in 1L of 90% ethanol for 72 hours at ambient temperature. The mixtures were then filtered and concentrated under reduced pressure in vacuum at 30°C using a rotary evaporator which was stored at -4°C until required for use. Aliquot portions of the crude extract residue were weighed and dissolved in distilled water for use on each day of the experiment (Kokate, 1991).

Preliminary phytochemical investigation

Phytochemical tests were carried out to find out the presence of phytoconstituents viz flavonoids, saponins, terpenoids, carbohydrates, tannins etc.

Experimental Animals

Male albino rats of Wistar strain weighing between 180-200 g were obtained from animal house of Hindu College of Pharmacy and used for the study. They were housed in standard cages at room temperature (25 ± 2^{o}C) with relative humidity (55 ± 5 %) and 12/12 h light/ dark cycle. The animals were provided with standard pellet diet (Amrut Laboratories Pranava Agro Industries Ltd. Hyderabad) and water *ad libitum*. They were adapted to the environment for one week prior to experimental use.

Acute Oral Toxicity study

Acute oral toxicity of etahnolic extarct of *Ziiziphus jujuba* was performed according to OECD Guideline 423 (Ecobichon, 1997). Two groups of each were used for the study. Group I served as control and received distilled water. Group II received single oral dose of *Ziziphus jujuba* (2000 mg/kg). The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for 14 days. Food consumption was monitored daily and body weights were recorded weekly.

Experiment design

After acclimatization, the animals were divided randomly into four groups (n=6), and placed in metabolic cages separately. Group I:Control animals received with standard diet and water.GroupII: Received intraperitoneal injection of gentamicin (80 mg/kg Bodyweight) daily for 8 days. Group III& IV: Test animals received gentamicin (80 mg/kg body weight) and co-treatment with ethanolic extract of *Ziziphus jujuba* fruits at a dose levels of 250 mg/kg and 500mg/kg body weight for 8days respectively by oral route. Injections of gentamicin were made daily at 10:00 hours to minimize the circadian variation in nephrotoxicity.

Serum preparation and Biochemical analysis

Twenty-four hours after the last injection, the animals were anaesthetized using anaesthetic ether prior to the dissection. Blood was collected without EDTA in the test tubes. The blood was allowed to clot by standing at room temperature for 30 minutes and then centrifuged at 3000 rpm for 10minutes. Serum (supernatant) was separated and used for the analysis of urea, creatinine, blood urea nitrogen (BUN), sodium and potassium using commercially available kit procedure in Semi Auto Analyzer (Lesely, 2005).

Histological evaluation

Kidney specimens were taken and preserved in 10% neutral formalin solution. The fixed specimens were trimmed, dehydrated in ascending grades of alcohol, cleared in xylene. The specimens were embedded in paraffin boxes, sectioned at 4-6 microns thickness and stained with Hematoxylen and Eosin (H&E) and then examined microscopically (Carleton *et al*., 1976).

Statistical Analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance was carried out and the individual comparisons of the group mean values were done using Dunnett's test.

RESULTS

Preliminary Phytochemical Investigation

The results of the preliminary phytochemical screening of the ethanolic extract *Ziziphus jujuba* fruit gave positive results for Carbohydrates, Saponins, Alkaloids, Proteins, Flavonoids, Triterpenoids and Tannins.

Effect of *Ziziphus jujube* on serum biochemical parameters

Intraperitoneal injection of gentamicin (GM) in a dose 80 mg/kg/day for 8 days to rats caused nephrotoxicity manifested by significant ($P < 0.05$) increases in serum urea, creatinine and blood urea nitrogen. A significant reversion of elevated levels of serum urea, creatinine and blood urea nitrogen (BUN) in groups treated with ethanolic extract of *Ziziphus jujuba* fruit as compared to gentamicin treated group and these results were tabulated in table 1.

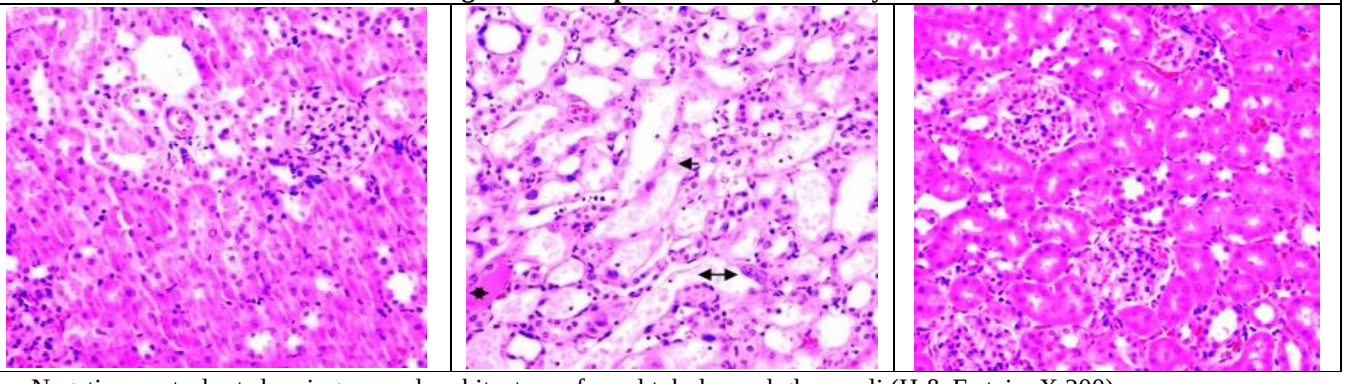
Effect of *Ziziphus jujuba* on serum sodium (Na^+) and potassium (K^+) levels

Intraperitoneal injection of gentamicin to rats for 8 days caused significant decrease in serum levels of sodium (Na^+) and potassium (K^+) electrolytes when compared to normal rats. Oral administration of ethanolic extract of *Ziziphus jujuba* concomitantly with gentamicin normalized the decreased levels of Na^+ and K^+ electrolytes in the serum when compared with gentamicin treated rats and the results were tabulated in table 2.

Effect of *Ziziphus jujuba* on renal histology

Histological examination revealed loss of brush border, vacuolation, and desquamation of epithelial cells in renal tubular epithelium with marked necrosis in gentamicin treated rats. In contrast, treatment with *Ziziphus jujuba* significantly improved gentamicin nephrotoxicity histologically and were shown in figure 1.

Fig 1. Microscopic evaluation of kidney



A. Negative control rat showing normal architecture of renal tubules and glomeruli (H & E stain, X 200)
B. Gentamicin-nephrotoxic rat showing marked necrosis of renal tubules (Arrows) (H & E stain, X200).
C. Nephrotoxic rat given orally the high dose of *Ziziphus Jujuba* showing only mild congestion of intertubular blood capillaries (H & E stain, X 200).

Table1. Effect of *Ziziphus jujuba*fruit extract on serum urea,creatinine and BUN

S.no	Treatment	Urea (mg/dl)	Creatinine (mg/dl)	BUN (mg/dl)
1	Control	22.42 ± 2.04	0.94 ± 0.01	24.40±0.97
2	Gentamycin treated	46.08 ± 4.05[a]***	4.05 ± 0.06[a]***	76.11±5.92
3	*Ziziphus jujuba*250mg/kg	32.10 ± 0.30*	2.12 ± 0.01[b]*	46.03±2.81**
4	*Ziziphus jujuba*500 mg/kg	23.58 ± 0.13**	1.36 ± 0.03[b]**	32.57±3.27***

Values are expressed as mean±SEM, One way ANOVA followed by Dunnett's 't' test, (n=6) in each group (*P value <0.05, **P value <0.01& ***P value <0.001).

Table 2. Effect of *Ziziphus jujuba* fruit extract on serum sodium (Na^+) and potassium (K^+) levels.

S. no.	Treatment	Na^+ (MEq/L)	K^+ (Meq/L)
1.	Control	132.10±6.16	5.15±0.7
2.	Gentamicin treated	110.55±5.27	2.12±0.27
3.	*Ziziphus jujuba* 250mg/kg	119.55±5.19	3.54±0.75*
4.	*Ziziphus jujuba*500 mg/kg	127.75±8.24*	4.82± 1.3**

Values are expressed as mean±SEM, One way ANOVA followed by Dunnett's 't' test, (n=6) in each group (*P value <0.05, and **P value <0.01).

DISCUSSION

Gentamicin is widely used aminoglycoside antibiotic, possessing significant nephrotoxic potential in man and experimental animals (Falco *et al*., 1969). Intraperitoneal injection of gentamicin (GM) to rats for 8 days in this study induced acute nephrotoxicity evident by significant increase in serum urea, creatinine and blood urea nitrogen associated with decreased serum levels of Na+ and K+ as well as marked tubular necrosis upon histopathological examination. These serum biochemical changes and histopathological alterations agreed with nephrotoxic potential of gentamicin supporting previous reports (Kumar *et al*., 2000; Abdel-Raheem *et al*., 2009; Yaman and Balikci, 2010). The specificity of GM to induce renal toxicity was attributed to its deposition and accumulation in the renal convoluted tubules and lysosomes leading to cytotoxicity (Laurent *et al*., 1999). There is an accumulating body of evidence supporting the concept that gentamicin was reported to enhance the generation of reactive oxygen species (ROS). Further, Reactive oxygen species may produce cellular injury and necrosis through several mechanisms including peroxidation of membrane lipids, protein, denaturation and DNA damage (Yukawa and Nakazawa, 1980).

Urea, creatinine and blood urea nitrogen are the end products of catabolism freely filtered by the glomerulus, passively high concentration in urine. The serum levels of urea, creatinine and blood urea nitrogenare commonly accepted measure of renal function in clinical medicine. In the present study, oral supplementation of *Ziziphus jujuba* restored the increased levels of urea, creatinineand blood urea nitrogen induced bygentamicin in rats (Lesely and Levey 2005; Perrone 1992; Cohen and Lemann 1991). In addition, acid, bases and salts are collectively called electrolytes. Electrolyte imbalance can leads to serious consequences as it affects the homeostasis of the body, which are good indicators of kidneys function. In the present study, Gentamicin treated rats significantly lowered potassium (K+) and sodium (Na+) levels, when compared with normal control rats. (Akpabio *et al*., 2012). Further, administration of *Ziziphus jujuba* restored the normal level of sodium and potassium in gentamicin treated rats. Further, nephroproetective ability of *Ziziphus jujuba* was supported by mitigation of histopathological alterations kidneys induced by GM in rats.

A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. It has been demonstrated that fruits of *Ziziphus jujube* contains some antioxidative ingredients both in vivo and in vitro (Taati *et al*., 2011, Esteki *et al*., 2012). Therefore, it is not unreasonable to assume that the nephroprotection shown by *Ziziphus jujuba* fruit extract in gentamicin induced nephrotoxicity is mediated through its potent antioxidant effects and this antioxidant activity of might have contributed to its nephroprotective effect by inhibiting gentamicin-induced oxidative stress. Furher, Flavonoids, tannins and polyphenols are potent water-soluble antioxidants which prevent oxidizing cell damage, suggesting antioxidant properties. The therapeutic potential of antioxidants in controlling degenerative diseases with marked oxidative damage from reactive oxygen species or free radicals have been reported (Akpabio *et al*., 2012). Hence, the nephroprotective activity of *Ziziphus jujuba* may be due to the phytochemical constituents such as flavonoids, tannins and other phenolic compounds present in it.

CONCLUSION

In conclusion, that the ethanolic extract of *Ziziphus jujuba* was able to produce considerable alleviation from the nephrotoxic action of gentamicin in male albino rats and hence exhibits marked renoprotective activity and can be deemed to be a good bioagent for the treatment of acute renal injury induced by nephrotoxins. However, further investigations should be conducted in order to better characterize the attenuation of gentamicin-induced nephrotoxicity by *Ziizphus jujuba.*

ACKNOWLEDGMENTS

Authors are sincerely thanking the Management, Principal and Animal ethical committee, Hindu College of Pharmacy for their support during the study.

REFERENCES

Abdel-RaheemIT, Abdhal-Ghany A A and MohamedGA. Protective effects of Quercetin against gentamicin-induced nephrotoxicity in rats. *Biol Pharm Bull*, 32(1), 2009, 61.

Akpabio UD, Udo UE, Akpankpan AE. Evaluation of phytochemical, proximate and mineral element composition of stem of Costus afer. *Asian J Plant Sci Res*,2,2012, 607-12.

Asiiley C. Renal failure - how drugs can damage the kidney. *Hospital Pharmacist*, 11, 2004, 48-53.

Carleton H. Carleton's Histological technique. The 4 Edition, London, Oxford University press, New York, Toronto, 1976, 56-66.

Cohen EP and Lemann J. The role of the laboratory in evaluation of kidney function. *Clinical chemistry*, 1991, 37/6, 785-796.

Deepak A and Shrivastava A. "Indigenous herbal medicines - tribal formulations and traditional herbal practices". Aavishkar publishers distributor, 2008, 440.

Ecobichon DJ. The basis of toxicity testing. CRS Press, New York, 1979, 43-49.

Esteki T and Urooj A. Antioxidant components and activity in the peel of *Ziziphus jujuba* Mill. *Journal of Pharmacy Research*, 5(5), 2012, 2705-2709.

Falco FG, Smith HM, Acieri GM. Nephrotoxicity of aminoglycosides and gentamicin. *J Infect Dis*, 119, 1969, 406-409.

Kleinknecht D and Fillastre JP. La Tolerance Rénale des Antibiotiques. Paris, Masson Pub, 1973, 89.

Kokate CK. Practical Pharmacognosy. Nirali Prakasham, Pune, 1991.

Kumar KV, Naidu MUR, Shifow AA and Ratnakar KS. Probucol protects againstgentamicin-induced nephrotoxicity in rats.*Indian J Pharmacol*, 32, 2000, 108.

Laurent G, Kishore BK, TulkensPM. Aminoglycoside-induced renal phospholipidosis and nephrotoxicity. *Biochemical Pharmacology*, 40, 1999, 2383-2385.

Lesely AS and Levey AS. Measurements of Kidney function. *Medical Clinical North America*, 89, 2005, 457-473.

Maliakel DM, Kagiya TV, Nair CK. Prevention of cisplatin-induced nephrotoxicity by glucoside of ascorbic acid and α-tocopherol. *Exp Toxicol Pathol*, 60, 2008, 521–527.

Perrone RD Madias NE, Levey AS. Serum creatinine as an Index of Renal function: New insights into old concepts. *Clinical chemistry*, 38/10, 1992, 1933-1952.

Preeti and Shalini T. Ziziphus Jujuba: A Phytopharmacological Review. *International Journal of Research and Development in Pharmacy and Life Sciences*, 3(3), 2014, 959-966.

Research Guidelines for evaluating the safety and efficacy of herbal medicines, World Health Organization, Regional Office for the Western Pacific, Manila, 1993 (ISBN92 9061 110 3).

Taati M, Alirezaei M, Meshkatalsadat MH, Rasoulian B, KheradmandA, NeamatiSh. Antioxidant effects of aqueous fruit extract of *Ziziphus jujuba* on ethanol-induced oxidative stress in the rat testes. *Iranian Journal of Veterinary Research*, 12(1), 2011, 34.

Yaman I andBalikciE. Protective effects of Nigella sativa against gentamicin nephrotoxicity in rats. *Experimental and Toxicologic Pathology*, 62, 2010, 183 190.

Yukawa O and Nakazawa T. Radiation induced lipid peroxidation and membrane bound enzymes in liver microsomes. *Int J Radial Biol*, 37, 1980, 621.