ISSN: 2395-0536

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

EVALUTION OF NEPHROPROTECTIVE ACTIVITY OF *FICUS DALHOUSIAE* EXTRACT IN GENTAMICIN INDUCED NEPHRO TOXICITY IN WISTAR RATS

K.Sivaji*, K.Srinivas, K.Venkata Lakshmi, K.Eeswari, K.Lokeswari, K.Nageswari

Department of Pharmacology, JITS college of Pharmacy, Palakollu, Andhra Pradesh, India

ABSTRACT

To investigate the Nephroprotective activity of ethanol extract of *Ficus dalhousiae* on Gentamicin induced nephrotoxicity in male Wistar rats. In this model of nephrotoxicity, 30 adult male wistar rats (150-200gms) were evenly divided into 5 groups. Group-1 and Group-2 served as untreated and model controls respectively, while Group-3, 4 and 5 were the treatments groups which were simultaneously treated with standard, 200 and 400 mg/kg extract respectively, after each dose gentamicin (80 mg/kg, i.p.) for 10 day . On 11^{th} day, blood samples for biochemical parameters, while the rats kidneys for histology were obtained under inhaled diether anaesthesia. Gentamicin treatment caused nephrotoxicity as evidenced by marked elevation in blood urea, uric acid and creatinine. Co-administration of extract with Paracetanmol decreased rise in blood urea, uric acid and creatinine. Apart from these, histopathological changes also showed the protective nature of extract against Gentamicin induced necrotic damage of renal tissues. It was observed that the ethanol extract of conferred nephroprotective activity by histopathological and biochemical observation against Gentamicin induced nephrotoxicity in rats. In the near future could constitute a lead to discovery of a novel drug for treatment of drug induced nephrotoxicity.
Key words: *Ficus dalhousiae*, Gentamicin, nephrotoxicity

KEY WORDS: Naltrexone, oxycodone, bulk dosage form

Author for correspondence:
K.Sivaji,
Department of Pharmacology, JITS college of Pharmacy, Palakollu, Andhra Pradesh, India.
E mail: siva.bpharm09@gmail.com

INTRODUCTION

Kidney is an important excretory organ in the human body. The function of kidney is not only to excrete the metabolic waste products, but also to maintain the acid base balance and endocrine functions like erythropoietin production (which stimulates the bone marrow to produce red blood cells), active form of vitamin D (calcitriol or 1, 25 dihydroxy-vitamin D which regulates absorption of calcium and phosphorus from food, promoting formation of strong bone), renin

(which regulates blood volume and blood pressure). The kidney receives blood supply from the renal artery, the branch of abdominal aorta and the venous drainage occurs through renal vein. The urine formed in the kidney gets drained through ureter into the urinary bladder.Kidneys are situated retroperitonially in abdominal cavity and has outer cortex and inner hypertonic medulla.The structural and functional unit of the kidney is nephron. Each human kidney has approximately about 1.3 million nephrons. Each nephron has glomerulus and renal tubules. The glomerulus is formed by invagination of tuft of capillaries into the dilated blind end of the nephron (Bowman's capsule); the capillaries are supplied by an afferent arteriole and drained by an efferent arteriole. The blind end of the nephron continues as the proximal convoluted tubule of 15 mm long and 55nm diameter. The convoluted portion of the proximal tubule drain into the straight portion which forms the first part of the loop of henle. The loop of henle continues with ascending loop of henle and further as distal convoluted tubule which opens into the collecting duct (1).

In the resting adult, the kidney receives 1.2 to 1.3 liters of blood per minute. Glomerular filtrate is formed by the blood in the glomerular capillaries by hydrostatic and osmotic pressure gradients. The glomerular membrane permits free passage of neutral substances with particle size up to 4nm in diameter and excludes such with diameter greater than 8nm like albumin. Approximately 120 ml of ultra filtrate is formed each minute, yet only 1 ml per minute of urine is produced. Therefore, greater than 99% of glomerular filtrate is reabsorbed. In the proximal convoluted tubule approximately 65% of filtrated solutes are reabsorbed and is highly permeable to water. In the loop of Henle there is reabsorption of Na+, Cl–, H2O and urea, about 25% of the filtrate is reabsorbed in this site. The distal convoluted tubule transports Na+ and Cl– and is impermeable to water. The collecting duct system of the kidney is an area of fine control of ultra filtrate composition and volume, where final adjustment in electrolyte composition is made by the action of mineralocorticoid (aldosterone) and antidiuretic hormone (ADH). The hyper tonicity of medullary interstitium plays an important role in concentrating the urine. Thus urine is formed by three processes that are glomerular filtration, tubular reabsorption and tubular secretion. Kidney not only excretes the metabolic substances, but also toxic agents from the body(2). Hence kidney becomes one of the important targets for the toxicity of agents more than other organs in the body.

Kidney toxicity induced by nephrotoxic agents

The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood. In addition, there is failure of regulation of fluid and electrolyte balance along with endocrine dysfunction.

Chronic renal failure (CRF) is an irreversible deterioration in the renal function which classically develops over a period of years, leading to loss of excretory metabolic and endocrine functions. Various causes of renal failure has been attributed like hypertension, diabetes mellitus, antineoplastic agents like Cyclophosphamide, Vincristin, Cisplatin etc. Acute renal failure (ARF) refers to the sudden and usually reversible loss of renal function which develops over a period of days or weeks. There are many causes of acute renal failure which could be pre-renal (55%), renal (40%), or post renal (5%). Among the renal causes of acute renal failure, acute tubular necrosis is more common accounting for 85% of incidence. Acute tubular necrosis occurs either due to ischemia or toxins. The toxin can be either exogenous or endogenous. The exogenous agents are radio contrast agents, cyclosporine, antibiotics, chemotherapeutic agents, organic solvents, and acetaminophen and illegal abortifacients (3, 4).

Gentamicin induced renal injury

In 1982, aminoglycosides were described as the cornerstone of therapy against majority of aerobic Gram-negative organisms, responsible for serious sepsis. They still retain their position in the clinical armamentarium. Presently, amino glycosides are used in many types of infections, such as Gram-negative urosepsis and in febrile granulocytopenic patients, because of their established anti-pseudomonal activity. Gentamicin is an extensively used

aminoglycoside. One of the limiting side effects of amino glycoside is nephrotoxicity. The incidence of nephrotoxic reaction in aminoglycoside treated patients varies from 10-20% (5). Aminoglycosides induced nephrotoxicity manifests clinically as non-oliguric renal failure with slow rise in serum creatinine and hypo-osmolar urinary output. Several days of treatment with aminoglycosides are necessary for the manifestation of clinical signs of toxicity. However much before these clinical manifestations, signs of tubular dysfunction such as low molecular weight proteinurea, enzymeurea, phospholipidurea and excretion of casts can be detected. The later stages of renal failure are often associated with oligouria. A small fraction (3-5% of the total injected dose) is taken up by the proximal tubular cells of kidney by endocytosis after binding to the brush border membrane. The endocytosed drug is sequestered into the secondary lysosomes, where the pH is acidic (about 5.4). At this pH aminoglycosides are fully protonated and bind to the negatively charged phosphatidylinositol or phophatidylerine. This binding causes the inhibition of the activities of lysosomal phospholipases, resulting in a lysosomal phospholipidosis characterized by the "myeloid bodies" visible on electron microscopy. Although several other mechanisms involving other subcellular organelles, such as mitochondria, plasma membranes, microsomes, etc[20] have been proposed, the lysosomal phospholipidosis appears to be the key event in the development of nephrotoxicity due to aminoglycosides. When the phospholipid overloading reaches a threshold limit, it somehow triggers cell necrosis by a mechanism that is poorly understood at present. Although there is no direct evidence for a causal relationship between phospholipidosis and cell necrosis[21], such relationship is logical in view of the fact that lysosomal phospholipidosis is the only major subcellular alteration demonstrated in intact tubular cells of animals that were administered at low therapeutic doses of these drugs. Solez et. al proposed that four factors could be involved in the renal failure observed in patients with acute tubular necrosis, namely: (1) afferent arteriolar vasoconstriction leading directly to decreased glomerular filtration; (2) back-leak of glomerular filtrate through the damaged tubular epithelium; (3) tubular obstruction by cellular debris or casts; and (4) reduction in capillary surface area available for glomerular filtration or reduction in glomerular capillary permeability. The other mechanism attributed to gentamicin induced renal impairment is free radical generation. The kidney provides the principal excretory route for elimination of aminoglycoside antibiotics from the body. Pharmacokinetic studies in both experimental animals and humans have demonstrated that these are not metabolized and excreted primarily by glomerular filtration. The majority of experimental evidence suggests that net absorption of aminoglycosides occurs in the proximal tubules by means of a high capacity transport system; there is little direct evidence for marked luminal concentration increase of aminoglycosides along with proximal tubule. Net drug secretion may occur in the early proximal tubule at high doses, while net secretion may occur in the late proximal tubule of juxtamedullary nephrons may occur at low doses. This nephronal heterogeneity in drug handling explains most features observed in the experimental setting. The combined processes of luminal reabsorption and basolateral uptake account for the high renal cortical tissue levels achieved in the experimental animals and humans. Once taken up by renal tubular cells, aminoglycosides reside in a poorly exchangeable pool, with tissue half lives exceeding those observed in serum by over a hundred fold (6).

The overall aim of proposed study is to explore the application of traditional medicinal plants of India. To explore the possibilities of traditional uses of the drug with proper chemical and pharmacological profiles. Screening of various extracts for *in-vivo* Nephro-protective activity on experimentally induced Nephrotoxicity (Gentamicin) in rats

MATERIALS AND METHOD (7-12)

Collection of plant material

Ficus dalhousiae used for the present studies was collected from Chitoor district of Andhra pradesh. The plant was identified, confirmed and authenticated by comparing with voucher specimen available at Survey of medicinal plants & collection unit, Department of Botany, Sri Venkateswara University, Tirupathi by Field Botanist Dr. Madhav shetty. The bark was cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanichal grinder. The resulting powder was then used for extraction.

Preparation of ethanolic Extract

The powdered drug was dried and packed well in Soxhlet apparatus and extracted with 1500 ml of ethanol for seven days. The extract was concentrated and dried using Rotary flash evaporator. It was kept in desiccators' until used.

Experimental animals

Swiss Albino rats adult of either sex were obtained from Mahaveer enterprises, Hyd(169/CPCSEA/1999). The rats were divided randomly into 5 groups of 6 rats each for each model. Each rat that weighed between 180-200 gm was housed separately (Four rats per cage). The animals were left for 48 hrs to acclimatize to the animal room conditions. They were maintained in standard laboratory conditions of temperature 22 ± 2^{o}c, humidity, 12 hours light and dark cycles fed with standard pellet diet (Hindustan lever, Bangalore) and adequate tap water.

Effect of *Ficus dalhousiae* on gentamicin-induced nephrotoxicity

Experimental design: Rats will be divided into five groups, each group consisting of six animals. Group 1: Control with normal saline (5 ml/Kg); Group 2: Gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days; Group 3: Hydro alcoholic extract of Ficus dalhousiae (200mg/kg/body weight, p.o) and simultaneously administered gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days; Group 4: Ethanol extract of Ficus dalhousiae (400mg/kg/body Weight,p.o.) and simultaneously administered gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days; Group 5: Silymarin (25mg/kg/body Weight, p.o.) and simultaneously administered gentamicin (80 mg/kg/body weight, i.p.), daily for 10 days. At the end of experimental period, all the animals will be sacrificed under diethyl ether anesthesia. Blood samples will be collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of kidney function

Biochemical parameters i.e., Estimation of Blood urea, Creatinine and uric acid were analyzed according to the reported methods. The kidney was removed, weighed and morphological changes were observed. A portion of kidney was fixed in 10% formalin for histopathological studies

Statistical analysis of data

Results were expressed as mean ± S.E.M. The statistical difference between the groups in the term of the mean rate of wound healing was calculated in terms of ANOVA mean ± S.E.M. The difference was considered significant if $P< 0.05$.

RESULTS AND DISCUSSION

In gentamicin treated group of animals the concentration of serum urea and creatinine were considerably increased than the normal animals (group 1) whichindicates severe nephrotoxicity. Treating (group 4 & 5) with ethanol extract of showed significant decrease ($p<0.001$) in concentration of serum urea and creatinine compared to gentamicin treated group 2. Nevertheless the concentration of uric acid not so much considerably increased in the gentamicin treated groups (group 2) than control group (group1). Treatment with ethanol extract of significantly ($p<0.05$) decreases the uric acid levels in group 4 & 5 ($p<0.01$) compared to gentamicin treated group (group 2) (Table-1-3).

Table 1: Effect of 80 mg/kg/day intraperitoneal gentamicin and Ficus dalhousiae oral *on* serum creatinine; blood urea and serum uric acid in treated rats for 10 days

Group	Drug treatment	Serum creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
1	5 ml/kg, i.p, NS	0.681±0.05309	22.622±1.783	4.0233±0.4233
2	80 mg/kg,i.p, gentamicin	1.261±0.03701	118.76±5.981	5.136±0.273
3	80 mg/kg,i.p, gentamicin+200 mg/kg	0.8566±0.0417***	54.932±6.196** *	3.933±0.2693*
4	80 mg/kg,i.p, gentamicin+400 mg/kg	0.7441±0.04849** *	49.962±4.204** *	3.5733±0.1719* *
5	80 mg/kg,i.p, gentamicin+Silymarin 25 mg/kg	0.7041±0.03849** *	47.762±4.204** *	3.2533±0.1719* *

N=6 animals in a group; Values are expressed as Mean ± SEM;
*: p<0.05, **p<0.01, p<0.001 vs Toxicant Control. ns indicate no significant.

Table 2: Effect of 80 mg/kg/day intraperitoneal gentamicin and Ficus dalhousiae oral on **kidney weight in treated rats for 10 days**

Group	Drug treatment	Kidney weight (gm)
1	10 ml/kg, i.p, NS	0.567±0.0136
2	80 mg/kg,i.p, gentamicin	0.712±0.0138
3	80 mg/kg,i.p, gentamicin+200 mg/kg	0.6±0.0146***
4	80 mg/kg,i.p, gentamicin+400 mg/kg	0.567±0.0099***
5	80 mg/kg,i.p, gentamicin+silymarin mg/kg	0.546±0.0078***

N=6 animals in a group; Values are expressed as Mean ± SEM;
*: p<0.05, **p<0.01, p<0.001 vs Toxicant Control. ns indicate no significant.

Table 3: Effect of 80 mg/kg/day intraperitoneal gentamicin and Ficus dalhousiae oral on **SGOT, SGPT, ALP in treated rats for 10 days**

Group	Drug treatment	SGPT levels (U/L)	SGOT levels	ALP levels (U/L)
A	10 ml/kg, i.p, NS	42.6.8±1.23	45.25±1.36	34.56±1.56
B	80 mg/kg,i.p, gentamicin	123.45±1.45**	136.19±3.48***	92.52±2.77***
C	80 mg/kg,i.p, gentamicin+200 mg/kg	89.38±0.87**	92.45±1.76***	73.74±1.38**
D	80 mg/kg,i.p, gentamicin+400 mg/kg	65.26±2.14***	55.38±1.45***	51.38±1.54**
E	80 mg/kg,i.p, gentamicin+silymarin mg/kg	45.4 7±1.31***	48.18±1.57***	44.47±1.67***

N=6 animals in a group; Values are expressed as Mean ± SEM;
*: p<0.05, **p<0.01, p<0.001 vs Toxicant Control. ns indicate no significant.

In histopathological study of Normal group showing some blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. Gentamicin treated group showing diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion. While treatment group show glomerular congestion,Peritubular congestion, Focal hydrophic degeneration of tubular epithelial cells and treatment group(400 mg/kg, Group IV) shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. From histopatological results we can conclude that EFD extract at dose of 200 mg/kg have partial protective effect while EFD extract at dose of 400 mg/kg have protective effect on Gentamicin induced nephrotoxicity. The findings suggest the potential use of ethanol extract of EFD a therapeutically useful nephroprotective agent. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases (Fig-1-5).

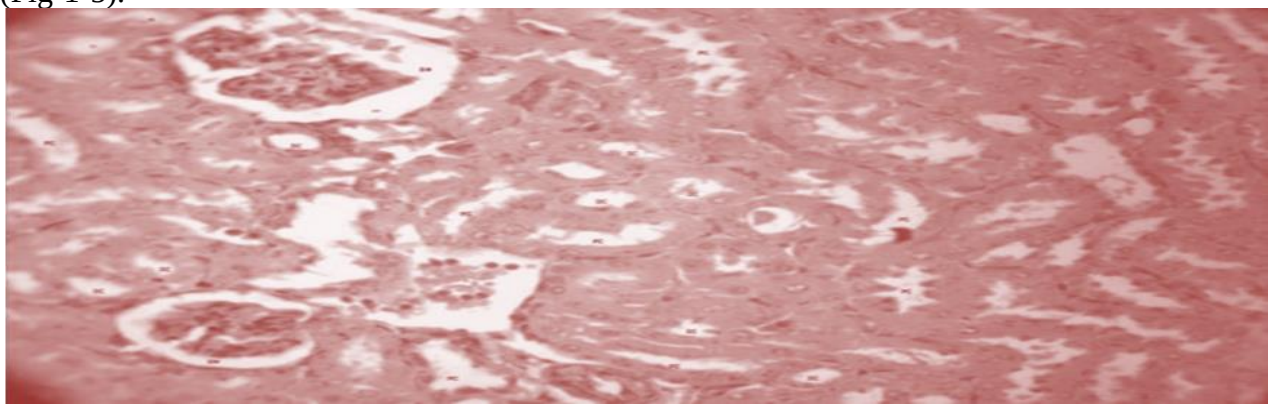

Fig-1 A sectional representation of normal rat kidney showing normal glomeruli with an intact Bowman's capsule, proximal convoluted and distal convoluted tubules

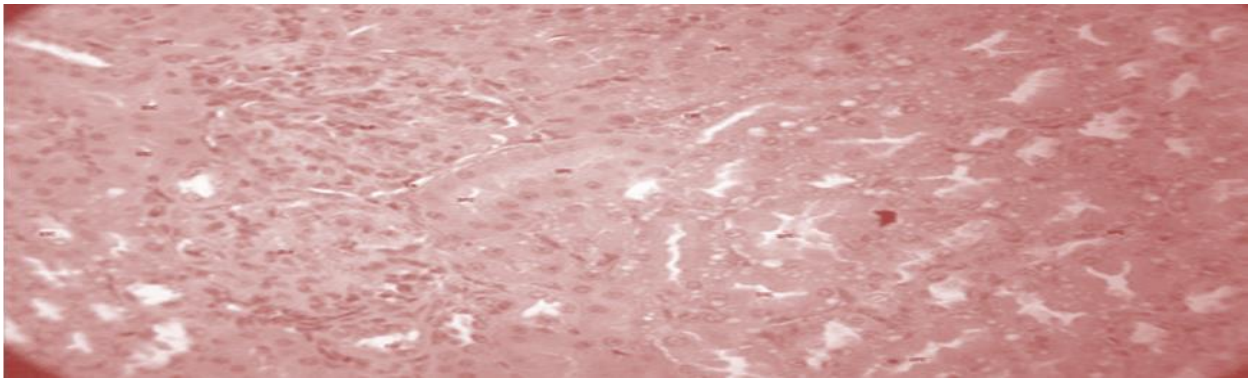

Fig-2 A representative section gentamicin–intoxicated rat kidney showing severe hydropic glomerular degeneration obliterated proximal convoluted tubular lumen and obliterated distal convoluted tubular lumen. The tubular lumens were completely obliterated and filled with fluid and casts

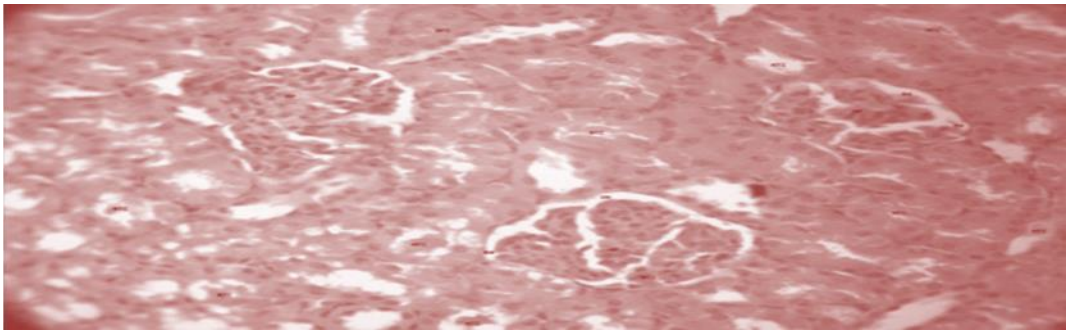

Fig-3 A sectional representation of 200 mg/kg/day, gentamicin–intoxicated rat kidney showing mesengial proliferation with thinning out of the Bowman's capsule. There is mild tubular cast deposition interposed with normal proximal convoluted tubule and distal convoluted tubule

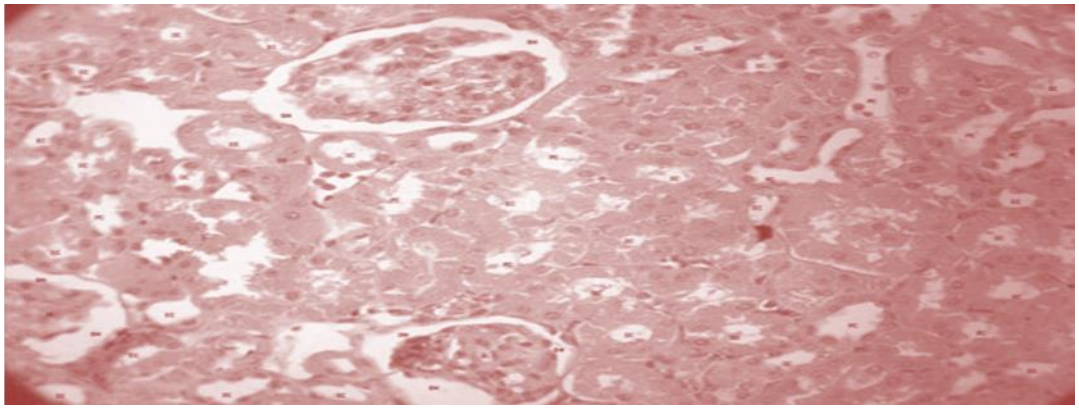

Fig-4 A sectional representation of 400 mg/kg/day gentamicin–intoxicated rat kidney showing normal glomeruli encapsulated by normal Bowman's capsule. There is no obvious tubular cast deposition

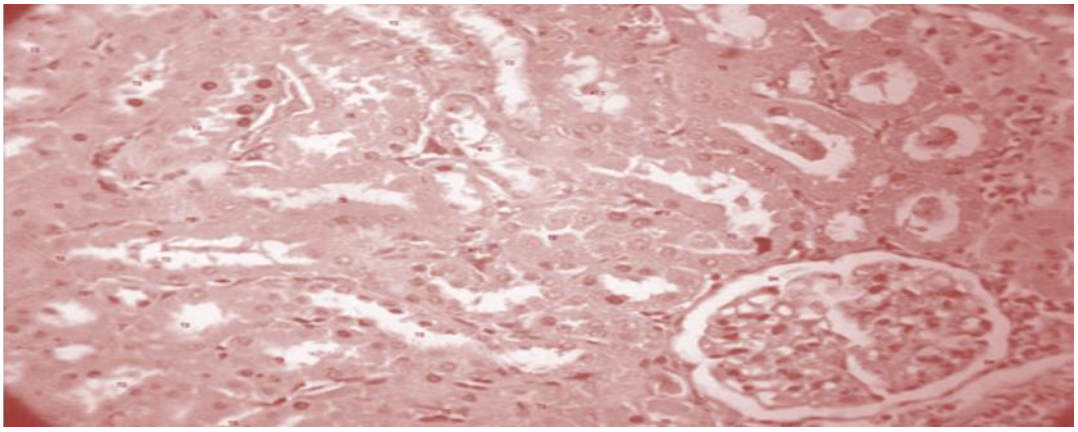

Fig-5 A sectional representation of 25 mg/kg/day gentamicin–intoxicated rat kidney showing moderate tubular degeneration with normal glomeruli and Bowman's capsule.

CONCLUSION

In the present study, the extract of Ficus dalhousie significantly reduced the toxicant elevated levels of above mentioned serum markers and increase in the levels of protein. Hence, at this point it is concluded that the extract of Ficus dalhousie offers nephroprotection. In Gentamicin treated animals there will be found glomerular, peritubular and blood vessel congestion and result in presence of inflammatory cells in kidney sections. The same is observed in case of humans who are suffering from major kidney disorders. In the present study, the extract of Ficus dalhousie treated group animals were found to reduce such changes in kidney histology induced by Gentamicin and acetaminophen, indicating nephroprotection. Further documented reports reveal that, plant material containing phenols, flavonoids, alkaloids and saponins offers organ protection by virtue of their free radical scavenging activity. The extract under study upon phytochemical analysis showed the presence of aforementioned phytoconstituents. Hence, the role of these phytoconstituents as free radical scavengers and consequent nephroprotection cannot be ruled out. Gentamicin induced nephrotoxicity was significantly prevented by concomitant with ethanolic extract of Ficus dalhousie. Reduction in elevated biochemical parameter levels like serum SGPT, SGOT, ALP after treatment with ethanolic extract of Ficus dalhousie confirmed the nephro-protective effect of extract under study.

REFERENCES

1. Arthur Guyton C. Text book of medical physiology. 10thEd. Harcourt publisher International Company, Singapore; 2000:264-379.el. Year 1997, Page No. 486-491.
2. Herfindal ,Gourley.Textbook of therapeutic drug and diseasemanagement.7thEdn. CharcilLivingstone,London;2000:425-36.
3. Barry M, Brenner, Floyd C, Rector. The kidney 6th Ed. W.B.Saunders Company, Philadelphia; 2000:3-67.
4. Paul Munson L. Principles of pharmacology, Basic concepts and clinical applications.Chapmanan d Hall IT Pan international Thomson publishing company, NewYork; 685.
5. Best, Taylors. Physiological basis of medical practice.11th Ed.Williams and Wilkins.
6. London; 1984:451-544.
7. Goodman, Gilman's.The pharmacological basis of therapeutics.10th Ed.Megrow
8. Syed SafiullahGhori et al. Gastro Protective Effects Of Ficus Dalhousiae Miq Roots Ethanolic Extract In Indomethacin And Cold Restraint Stress Induced Ulcers Int. Res. J. Pharm. 2014, 5 (9)
9. Ghori et al. Anti-Inflammatory Activity Of *Ficus Dalhousiae* Miq Roots Ethanolic Extract In Wistar Albino Rats Asian J Pharm Clin Res, Vol 8, Issue 1, 2015, 117-119
10. Ghori et al. Antihyperglycaemic Effect Of *Ficus Dalhousiae* Miq Leaf Ethanolic Extract In Alloxan–Induced Diabetic Rats Int J Pharm Pharm Sci, Vol 6, Issue 9, 132-136
11. Pasha et al. (2014) Evaluation of Hypolipidemic Activity Of Methanolic Extract of Ficus Dalhousiaemiq. On Triton-X And High Fat Diet Induced Hyperlipidemia In Albino rats ejpmr, 2014, 1(1), 183-191
12. Syed Safiullah Ghori et al Amelioration of carbon tetrachloride- and paracetamol-induced hepatotoxicity in rats by Ficus dalhousiae Bangladesh J Pharmacol 2014; 9: 588-594
13. Syed Safiullah Ghori et al. Wound Healing Effect of *Ficus Dalhousiae Miq* Stem Bark Ethanolic Extract In Excision And Incision Wounded Wistar Rats RJPBCS 6(3) Page No. 427-432.