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Research article ***Clinical research***

Nephro protective activity of *plumbago zeylanica* extract on gentamicin induced nephrotoxicity in rats

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ABSTRACT

Herbal medicine is the oldest form of healthcare known to mankind and most cultures have long folk medicine histories that include the use of plants. Nephrotoxicity is one of the most common kidney problems and occurs when the body is exposed to a drug or toxin that causes damage to the kidneys. To investigate the Nephroprotective activity of ethanol extract of *Plumbago zeylanica* 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 11th 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 *Plumbago zeylanica* 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.

Keywords: *Plumbago zeylanica*, Nephrotoxicity, Nephroprotective activity, Gentamicin

INTRODUCTION

Kidney

Anatomy and physiology of kidney

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[1, 2]. Hence kidney becomes one of the important targets for the toxicity of agents more than other organs in the body.

Mechanism of aminoglycoside induced nephrotoxicity

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, 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.

MATERIALS AND METHODS

MATERIALS

CHEMICALS REQUIRED

NaOH (Merck, Sura labs, Dilsukhnagar, Hyd). Formalin 10% (Merck, Sura labs, Dilsukhnagar, Hyd). Tween 80 2%(Merck, Sura labs, Dilsukhnagar, Hyd). Distilled water.

EXPERIMENTAL ANIMALS

Swiss Albino rats adult of either sex were obtained from Local vendor. 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±2ºc, humidity, 12 hours light and dark cycles fed with standard pellet diet (Hindustan lever, Bangalore) and adequate tap water.

METHODS

Acute Toxicity Studies

Animals will be fasted prior to dosing, food but not water should be withheld overnight. Following the period of fasting, the animals will be weighed and the test substance will be administered. After the substance is administered, food may be withheld for a further 3-4 hrs. As a dose is administered in fractions over a period, it may be necessary to provide the animals with food and water depending on the length of the period.

Three animals will be used for each step. The dose level used as the starting dose will be selected from one of the four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dose animals. The animals will be observed for behavioral changes for 4 hrs and 48 hrs for mortality.

In vivo model

Nephroprotective studies

Effect of Plumbago zeylanica 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: Ethanol extract of *Plumbago zeylanica* (200 mg/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 *Plumbago zeylanica* (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.

RESULTS

Preliminary phytochemical studies

Table 1: Results of the Preliminary Phytochemical Constituents present in ethanolic extract of *Plumbago zeylanica*.

Phyto-constituents	Plumbago zeylanica
Carbohydrate	Present
Tannins	Present
Flavonoids	Present
Saponins	Present
Alkaloids	Present
Glycosides	Present
Terpenes	Present
Phytosterols	Absent

Ethanolic extract of the whole plants of *Plumbago zeylanica* was subjected to various phytochemical tests, which showed the presence of carbohydrates, reducing sugars, glycosides, tannins, and flavonoids, Anthroquinone, Saponins, Alkaloids and Glycosides. In Gentamicin treated group of animals the concentration of serum urea and Creatinine were considerably increased than the normal animals (group 1) which indicates 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 2: Effect of 80 mg/kg/day intraperitoneal Gentamicin and *Plumbago zeylanica* oral on serum Creatinine; blood urea and serum uric acid in treated rats for 10 days

Group	Drug treatment	Serum Creatinine	Blood urea	Uric acid
1	5 ml/kg, i.p, NS	0.515±1.10126	22.151±1.510	5.1013±2.2136
2	80 mg/kg,i.p, Gentamicin	3.129±2.01536	114.51±1.206	5.269±2.219
3	80 mg/kg,i.p, Gentamicin+200 mg/kg	0.9216±2.0251**	54.321±1.109** *	4.281±0.2103*
4	80 mg/kg,i.p, Gentamicin+400 mg/kg	0.7521±0.08210** *	47.210±3.219** *	4.6216±0.5419* *
5	80mg/kg,i.p,gentamicin+Silymarin 25 mg/kg	0.7103±2.01649** *	46.210±1.316** *	3.5161±2.5126* *

Kidney weight

In Gentamicin treated group of animals weight of kidneys were considerably increased compared to normal animals (group1) and treating (group 4 & 5) with ethanol extract showed significant decrease ($p<0.001$) in kidney weight.

Table 3: Effect of 80 mg/kg/day intraperitoneal Gentamicin and *Plumbago zeylanica* oral on kidney weight in treated rats for 10 days

Group	Drug treatment	Kidney weight (gm)
1	10 ml/kg, i.p, NS	0.465±0.0516
2	80 mg/kg,i.p, Gentamicin	0.786±0.0316
3	80mg/kg,i.p,gentamicin+200 mg/kg	0.651±0.0309***

4	80mg/kg,i.p,gentamicin+400 mg/kg	0.551±0.0128***
5	80mg/kg,i.p,Gentamicin+Silymarin	0.486±0.0093***

*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 4: Effect of 80 mg/kg/day intraperitoneal Gentamicin and *Plumbago zeylanica* oral on SGOT, SGPT, ALP in treated rats for 10 days

Groups	Drug treatment	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
	10 ml/kg, i.p, NS	44.81±3.21	43.10±1.62	31.56±3.19
	80 mg/kg,i.p,	123.14±2.34**	133.72±2.61***	90.24±3.06***
	80 mg/kg,i.p,	84.21±1.81**	91.03±2.19***	71.03±3.12**
	80 mg/kg,i.p,	64.89±2.61***	54.81±3.57***	51.31±1.10**
	80 mg/kg,i.p,	43.210±3.14***	45.18±1.21***	42.10±2.83***

Gentamicin induced Nephrotoxicity Histopathology

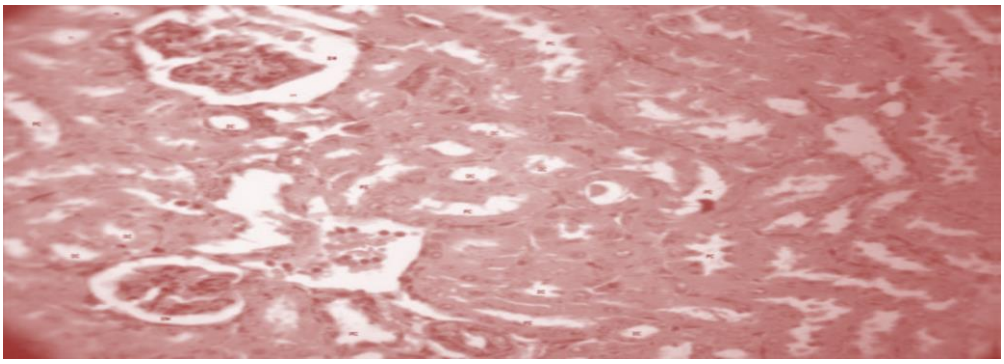

Fig 1: sectional representation of normal rat kidney showing normal glomeruli with an intact Bowman's capsule, proximal convoluted and distal convoluted tubules

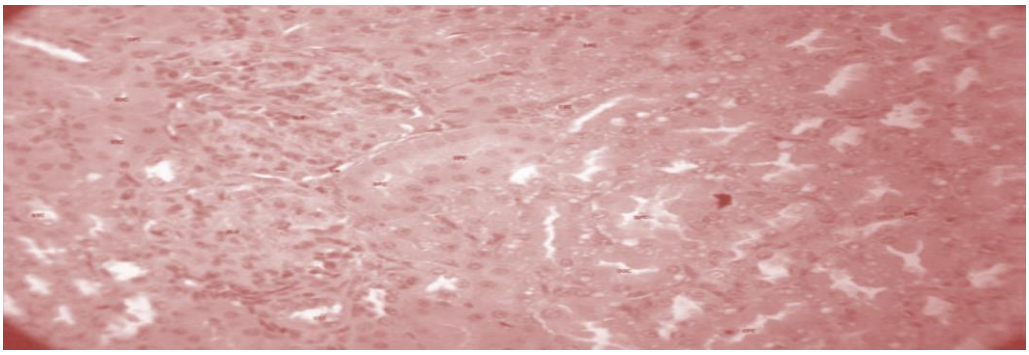

Fig 2: representative section Gentamicin–intoxicated rat kidney showing severe hydropic glomerular degeneration obliterated proximal convoluted tubular lumen and obliterated distal convoluted tubular lumen. The tubular lumens were completely obliterated and filled with fluid and casts

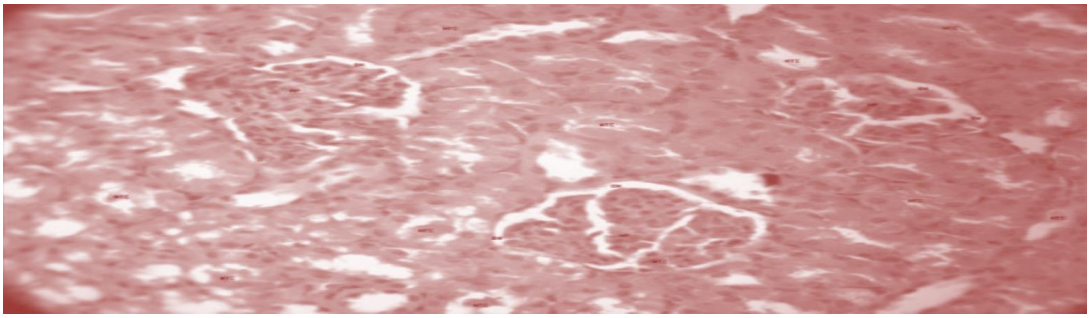

Fig 3: Sectional representation of 200 mg/kg/day, Gentamicin–intoxicated rat kidney showing mesengial proliferation with thinning out of the Bowman's capsule. There is mild tubular cast deposition interposed with normal proximal convoluted tubule and distal convoluted tubule

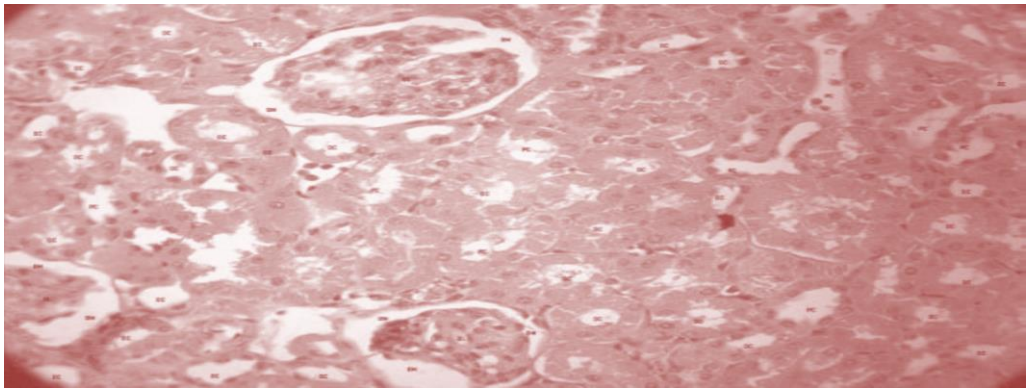

Fig 4: Sectional representation of 400 mg/kg/day Gentamicin–intoxicated rat kidney showing normal glomeruli encapsulated by normal Bowman's capsule. There is no obvious tubular cast deposition

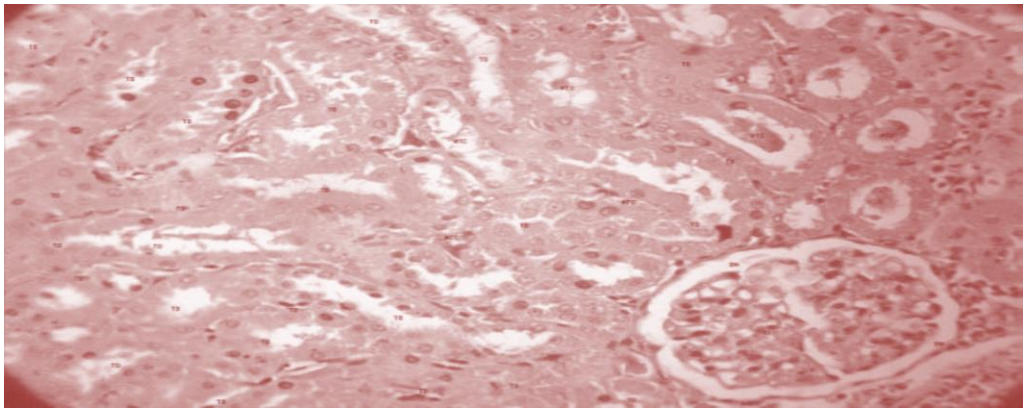

Fig 5: sectional representations of 25 mg/kg/day Gentamicin–intoxicated rat kidney showing moderate tubular degeneration w i t h normal glomeruli and Bowman's capsule.

DISCUSSION

Drug induced Nephrotoxicity are often associated with marked elevation in blood urea, serum Creatinine and acute tubular necrosis. So these biochemical parameters have been used to investigate drug induced Nephrotoxicity in animal and man. In the present study drug induced Nephrotoxicity were established by single daily of the Gentamicin for 10 days. This toxicity characterized by marked elevation in the circulating levels of blood urea, serum Creatinine and histological features of tubulonephritis in the model control(group 2) rats when compared to untreated(group 1) rats. However these changes were attributed by concomitant treatment with single daily graded doses of ETG extract for 10 days. Oral administration of plant extract significantly decreases the urea and Creatinine level in both treatment group compare to toxicant group. Apart from the direct nephrotoxic effect of Gentamicin in group 2 rats, the acute elevation in the measured biochemical parameters could also be attributed to increased catabolic state of the rats due to the prolong anorexia associated with Gentamicin Nephrotoxicity. In renal diseases, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and Creatinine levels in serum was taken as the index of Nephrotoxicity. Creatinine derives from endogenous sources by tissue Creatinine breakdown. Thus serum urea concentration is often considered a more reliable renal function prediction than serum Creatinine. Anyhow the level of uric acid is nonsignificantly increased in the toxicant group when compared to control. Oral administration of plant extract significantly decreases the uric acid level in both treatment group compare to toxicant group.

It was established that Gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR.

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 EPZ extracts at dose of 200 mg/kg have partial protective effect while EPZ extract at dose of 400 mg/kg have protective effect on Gentamicin induced Nephrotoxicity.

The findings suggest the potential use of ethanol extract of EPZ 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.

During hepatic and Nephro damage, cellular enzymes like AST, ALT and ALP present in the liver cells leak into the serum, resulting in increased concentrations. Gentamicin administration for 10 days significantly increased all these serum enzymes.

In the current study treatment of rats with ethanolic extract of roots of *Plumbago zeylanica* significantly ($p<0.05$ in 200mg/kg b.wt. and $p<0.01$ in 400mg/kg b.w.) decreased the levels of SGPT in serum which is an indication of nephroprotective activity.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. Nephro toxicity elevated the SGOT levels in serum due to the damage to the tissues producing acute necrosis, such as severe viral hepatitis & acute cholestasis. Alcoholic liver damage and cirrhosis can also associate with mild to moderate elevation of transaminase. In the current study treatment of animals with ethanolic extract of leaves of *Plumbago zeylanica* significantly ($p<0.05$) decreased the levels of SGOT in serum which is an indicative of nephroprotective activity.

In case of toxic kidney, alkaline phosphatase levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by parenchymal or duct cells.

In the current study treatment of animals with ethanolic extract of *Plumbago zeylanica* significantly ($p<0.05$ in 200mg/kg b.w. And $p<0.001$ in 400mg/kg b.w) decreased the levels of ALP in serum as an indication of nephroprotective activity.

CONCLUSION

The present study was undertaken to scientifically evaluate the nephroprotective activity of the ethanolic extract of *Plumbago zeylanica*. The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavanoids, glycosides, saponins, tannins, phenols in EEPZ. The administration of Gentamicin during experimentation is effectively induced apoptosis and necrosis, which was similar to acute renal failure in human. Therefore it is an effective and an ideal model for Nephrotoxicity research. The evaluation of renal parameters on nephrotoxic rats with EEPZ showed significantly elevate the attenuated Kidney weight, Creatinine clearance, Blood urea, Uric acid, SGOT, SGPT and ALP significant reduce in elevated serum creatinine level, which supports its Nephroprotective activity. The Gentamicin induced rats showed elevated levels of serum blood urea which was significantly decreased with treatment of EEPZ, which proves it having Nephroprotective activity. Histopathological studies on isolated kidney revealed that the EEPZ, reversed the kidney damage and also restored normal kidney architecture. In summary, the fruit pulp of *Plumbago zeylanica* in an ethanolic extract showed statistically significant nephroprotective activity. The plant extract proved to have nephroprotective potentials may because of its known flavonoid contents and antioxidant properties. There is a scope for further investigation on the histopathology of liver and spleen and clinical studies that are required to elucidate the active phytoconstituents with potent nephroprotective activity.

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