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Research

Nephroprotective Activity Of *Heliotropium Indicum* Extract On Gentamicin Induced Nephrotoxicity In Rats

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Abstract

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 *Heliotropium indicum* 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 *Heliotropium indicum* 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: Nephroprotective activity, *Heliotropium indicum* and Gentamicin.

INTRODUCTION

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. Kidney is particularly prone for the action of nephrotoxins because of following reasons.

1. Kidney receives 25% of the cardiac output hence high levels of toxins are delivered to the kidney.
2. The large surface area of tubular epithelium provides sites for toxicin interaction and uptake.
3. The availability of specific transport mechanisms that mediate cellular uptake.
4. The normal concentrating mechanisms of the kidney can increase the concentration of
5. the toxins.
6. Due to the presence of the metabolic processes in the renal tubular cells, nephrotoxins an release toxic components and induce damage.

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.

The renal failure is fundamentally categorized into acute and chronic renal failure (Herfindal et al) [2,3]. 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[2,4].

Nephrotoxic agents: Drugs, diagnostic agents and chemicals are well known to be nephrotoxic. The important nephrotoxic agents are mentioned here under:

Antineoplastic agents
Alkylating agents: Cisplatin, Cyclophosphamide, Nitrosoureas: Streptozotocin, Carmustine, Lomustine, Semustine.
Antimetabolites: High dose Methotrexate, Cytosine, Arabinose, High dose 6-thioguanine, 5-Flurouracil
Antitumor antibiotics: Mitomycin, Mithramycin, Doxorubicin
Biological agents: Recombinant Leukocyte A, Interferon.

Antimicrobial agents
Tetracycline, Acyclovir, Pentamidine, Sulphadiazine, Trimethoprim, Rifampicin, Amphotericin B
Aminoglycosides: Gentamicin, Amikacin, Kanamycin, Streptomycin, Toberamycin, Neomycin, Dibekacin.
Heavy metals: Mercury, Arsenic, Lead, Bismuth
Miscellaneous: Radio contrast agents
NSAIDS: Paracetamol, Ibuprofen, Indomethacin, Aspirin etc.

Nephrotoxic agents can produce damage either by directly reacting with cellular macromolecules and membrane components or from metabolism within the tubular cells to toxic products. The agents which cause direct toxicity are heavy metals like Hg, Pb which interact with sulphydryl groups, organic cations such as spermine, cationic amino acids, amino glycosides, which interacts with membrane phospholipids, polyene antibiotics like amphotericin B which interacts with membrane cholesterol. Fluoride and oxalates produced by hepatic metabolism of methoxyflurane, intermediates of cisplatin, cystine conjugates, cephalodrine, and acetaminophen induce damage by their metabolites.

These toxic metabolites mainly include free radicals[5]

The nephrotoxins damage specific segment of the nephron to a greater extent than the other segments. The proximal tubule is the most commonly affected, because of the presence of inducible type of microsomal mixed function oxidases (cytochrome P450) which have been implicated in the toxic activation of various agents. This segment is also rich in glutathione and glutathione metabolizing enzymes. The other common sites which can be affected are renal medulla, distal tubule and Loop of Henle. The renal medulla is affected mainly by polyene antibiotics and cyclosporine and that of distal tubule dysfunction is mainly due to non steroidal anti-inflammatory agents, cyclosporine, pentamidine, trimethoprim, sulphamethaxozole, amphotericin, aminoglycoside antibiotics, lithium, and demeclocycline.

The functional manifestations of nephrotoxicity can occur at several levels like tubular function abnormalities such as potassium, magnesium and sodium wasting, concentrating defects and reduction in glomerular filtration. However, there are no ideal clues to the occurrence or localization of tubular cell injury. The nephrotoxin induced changes in the tubule cells may be lethal or sub lethal[5,6].

Mechanisms of drugs induced renal damage
a) Free radical production[7,8].
b) Disturbance of renal tubule cell energy metabolism[9].
c) Disrupted cell calcium homeostasis[10].
d) Alteration of membrane phospholipid metabolism[11,12].
e) Disruption of cellular monovalent cation volume and pH dependant degradation[13,14].
f) Disruption of cytoskeleton[13].
g) Abnormalities of cell proteases[14].
h) Abnormalities of protein and nucleic acid synthesis[15,16,17].
i) Distruption of lysosomal function.

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% [19].

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.

MATERIALS AND METHODS

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

Equipments

- Microscope
- Centrifuge
- Animal weighing balance
- Mechanical stirrer

Apparatus required

- Burette
- Pipette
- Conical flask
- Petridish
- Surgical equipments

Collection of plant material

Heliotropium indicum used for the present studies was collected Local market. The bark was cut into small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical 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.

Qualitative phytochemical screening

The following tests were carried out on the standardized herbal extract to detect various Phytoconstituents present in them.

Detection of carbohydrates

Small quantity of the extract was dissolved in distilled water and filtered. The filterate was subjected to

Molisch's test: To the filtrate few drops of alcoholic α-napthol was added and 2ml of conc sulphuric acid was added slowly through the slides of the test tube. No purple colored ring was formed at junction of the two layers, which indicates absesnce of carbohydrates.

Fehling's test: Small portion of the extract was treated with fehling's solution I and II and then heated on water bath. No brick red colored precipitate was formed, which indicates absence of carbohydrates.

Barfoeds test: Small portion of the extract was treated with barfoed's reagent. No red precipitate formed, which indicates absence of carbohydrates.

Test of starch

A small amount of powdered drug was treated with diluted iodine solution. No blue color was observed, which indicates absence of starch.

Detection of proteins and amino acids

Small quantity of extract was dissolved in few ml of water and was subjected to million's, biuret and ninhydrin test.

Million's test: The extract was treated with million's reagent. No white precipitate was produced, shows the absence of proteins and free aminoacids.

Biuret test: To the extract equal volume of 5%w/v NaOH and four drops of 1%w/v $CuSO_4$ solution were

added. No pink or purple color was formed indicating the absence of proteins.
Ninhydrin test: The extract was treated with ninhydrin reagent. No purple color was produced, indicating the absence of proteins.

Detection of phenolic compounds and tannins
The decoction were diluted with distilled water and filtered. The filtrates were treated with following reagent.

Ferric chloride test: The filtrate was treated with 5% of ferric chloride solution. No black precipitate was found in the decoction of the plant, indicating the absence of tannins and phenolic compounds.

Test with Lead acetate Solution: Few ml of filtrate were treated with lead acetate solution. No white precipitate was produced in the decoction of plant.

Gelatin test: To the filtrate of decoction, add 1ml of 1% solution of gelatin. No white precipitate was seen, which indicates absence of tannin in plant.

Test for phytosterols
Small quantity of decoction were dissolved in 5ml of chloroform separately. Then these chloroform layer subjected to,

Salkowski test: To 1ml of the above prepared chloroform solutions, few drops of conc H_2SO_4 was added. Red color produced in the lower layer, shows the presence of phytosterols.

Libermann – Burchards test: The above chloroform solution was treated with few drops of conc. H_2SO_4 followed by 1ml of acetic anhydride solution. Green color was produced, shows the presence of phytosterols.

Test for fixed oils and fats
Spot test: A small quantity of extract was pressed between two filter papers. Oil stain was observed, show presence of fixed oils.

Saponification: Few drops of 0.5N alcoholic potassium hydroxide was added to extract along with a few drops of phenolphthalein. The mixture was heated on a water bath for about 1 – 2 hours. Formation of soap or a partial neutralization of alkali indicated the presence of fixed oils and fats.

Test for alkaloids
Small amount of extract was stirred with a few ml of dil HCl and filtered. The filtrate was tested with various alkaloidal reagents such as Mayer's, Dragendroff's, Wagner's and Hager's reagent.

Mayer's test: To the small amount of filtrate add few drops of Mayer's reagent. A white color precipitate was formed, indicating the presence of alkaloids.

Dragendroff's test: (potassium bismuth iodide) To the small amount of filtrate add few drops of Dragendroff's reagent. An orange red color precipitate was formed, indicating the presence of alkaloids.

Wagner's test: To the small amount of filtrate add few drops of Wagner's reagent. A brown color precipitate was formed, indicating the presence of alkaloids.

Hager's test: (picric acid): To the small amount of filtrate add few drops of Hager's reagent. An yellow crystalline precipitate was formed, indicating the presence of alkaloids.

Test for glycosides
A small amount of the extract was hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to

Legal's test: To the hydrolysate 1ml pyridine few drops of sodium nitroprusside solution was added and then made alkaline with NaOH solution. Pink color was obtained showing the presence of glycosides.

Balget's test: To a solution of extract sodium picrate solution was added. Yellowish orange color was obtained showing, the presence of glycosides.

Borntrager's test: Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Pink color was observed in ammoniacal layer, confirms the presence of glycosides.

RESULTS

Preliminary phytochemical studies

Table 1: Results of the Preliminary Phytochemical Constituents present in ethanolic extract of *Heliotropium indicum*.

Phyto-constituents	Heliotropium indicum
Carbohydrate	Present
Tannins	Present
Flavonoids	Present
Saponins	Present
Alkaloids	Present
Glycosides	Present
Terpenes	Present
Phytosterols	Absent

Ethanolic extract of the whole plants of *Heliotropium indicum* was subjected to various phytochemical tests, which showed the presence of carbohydrates, reducing sugars, glycosides, tannins, 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 *Heliotropium indicum* 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	0.515±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 (*Heliotropium indicum*)	0.9216±2.0251**	54.321±1.109** *	4.281±0.2103*
4	80 mg/kg,i.p, Gentamicin+400 mg/kg (*Heliotropium indicum*)	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 *Heliotropium indicum 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 (*Heliotropium indicum*)	0.651±0.0309***

4	80mg/kg,i.p,Gentamicin+400 mg/kg (*Heliotropium indicum*)	0.551±0.0128***
5	80mg/kg,i.p, Gentamicin + Silymarin mg/kg	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 *Heliotropium indicum oral* on SGOT, SGPT, and ALP in treated rats for 10 days

Groups Group	Drug treatment	SGPT levels (U/L)	SGOT levels (U/L)	ALP levels (U/L)
A	10 ml/kg, i.p, NS	44.81±3.21	43.10±1.62	31.56±3.19
B	80 mg/kg,i.p, Gentamicin	123.14±2.34**	133.72±2.61***	90.24±3.06***
C	80 mg/kg,i.p, Gentamicin+200 mg/kg	84.21±1.81**	91.03±2.19***	71.03±3.12**
D	80 mg/kg,i.p, Gentamicin+400 mg/kg	64.89±2.61***	54.81±3.57***	51.31±1.10**
E	80 mg/kg,i.p, Gentamicin+Silymarin mg/kg	43.210±3.14***	45.18±1.21***	42.10±2.83***

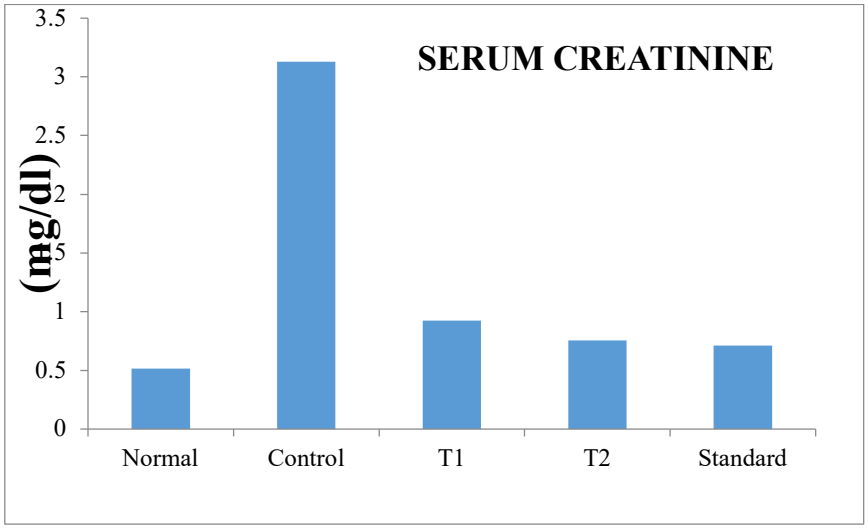

Fig 1: Effect of 80 mg/kg/day intraperitoneal Gentamicin and *Heliotropium indicum* oral on serum creatinine; in treated rats for 10 days

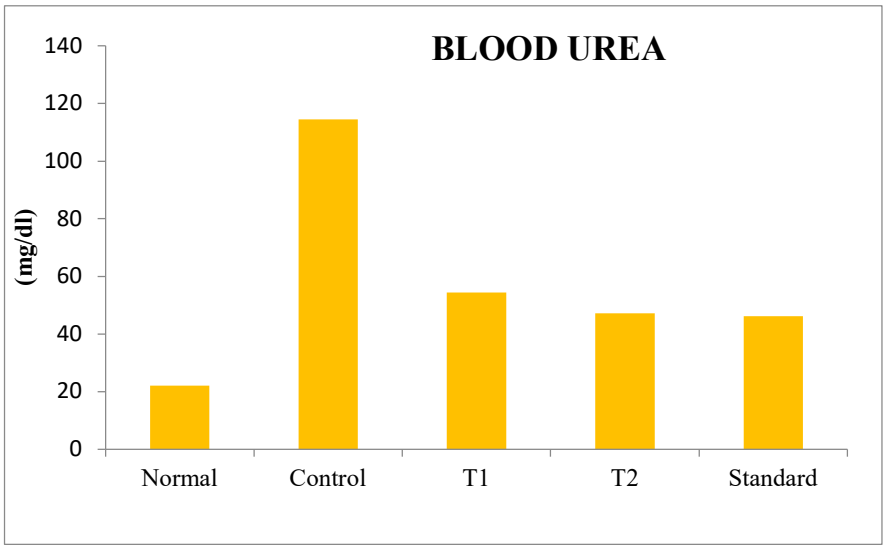

Fig 2: Effect of 80 mg/kg/day intraperitoneal Gentamicin and *Heliotropium indicum* oral on blood urea in treated rats for 10 days

DISCUSSIONS

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 *Heliotropium indicum* 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 *Heliotropium indicum* 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 *Heliotropium indicum* 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 *Heliotropium indicum*. The phytochemical investigation revealed the presence of carbohydrate, alkaloids, flavanoids, glycosides, saponins, tannins, phenols in EEHI. 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 EEHI 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 EEHI, which proves it having Nephroprotective activity.

Histopathological studies on isolated kidney revealed that the EEHI, reversed the kidney damage and also restored normal kidney architecture. In summary, the fruit pulp of *Heliotropium indicum* 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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