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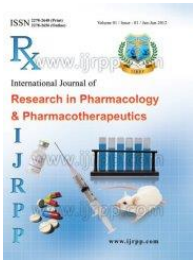

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Nephroprotective activity of heliotropium indicum in rifampicin induced nephrotoxicity rats

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ABSTRACT

The nephroprotective effect of whole plant of ethanolic extract of *Heliotropium indicum* was confirmed by our study. In Rifampicin treated group urea, uric acid and creatinine levels are increased in serum and urine whereas decreased total protein levels. In the present study, the whole plant of ethanolic extract of H*eliotropium indicum* significantly reduced the toxicant elevated levels of above mentioned parameters and increased in the levels of protein. Hence at this point it is concluded that the extract of H*eliotropium indicum* offers nephroprotection. Based on improvement in serum and urine marker levels, histopathological studies, level of antioxidant enzymes and presence of phytoconstituents, it is concluded that the ethnolic extract of H*eliotropium indicum* possesses nephron protective activity and thus supports the traditional application of the same under the light of modern science.

Keywords: Ethanolic extract of *Heliotropium indicum*, Nephrotoxicity, Rifampicin, Cystone.

INTRODUCTION

Kidneys are essential in urinary system and serve haemostatic functions such as regulation of electrolytes, maintenance of acid-base balance and regulation of blood pressure. They purify the blood by removing wastes from it and excreting them from the body through urine. Every day the kidneys filter about 180L of blood, about four times as much as the amount that passes through any organ. The kidney accomplishes these homeostatic functions both independently and in concert with other organs, particularly those of the endocrine system. Various endocrine hormones coordinate these endocrine functions include renin, angiotensin-II, aldosterone, antidiuretic hormone and atrial natriuretic peptide among others.

Rifampicin a potent drug is the main drug in the multidrug therapy for lung tuberculosis. It can induce hepatitis and nephritis in hypersensitive patient. Drug induced nephrotoxicity is often associated with marked elevation of biochemical parameters like urea, creatinine and uric acid in blood and urine and decrease in serum protein levels [1]. When the kidneys are exposed to a toxic agent either

accidentally or intentionally damage can occur in a number of different ways depending upon the agent. Toxins may create other substances or condition that result in the same cell death or nephrotoxicity. Nephrotoxic injury can lead to acute renal failure, in which the kidneys suddenly loss their ability to function or chronic renal failure, in which kidney function slowly deteriorates. If uncheck renal failure can result in death. At present there is no suitable allopathic medicine in the market to treat nephrotoxicity. Over 50 drugs have been reported as a cause of acute intestinal nephritis.

PATHOPHYSIOLOGY

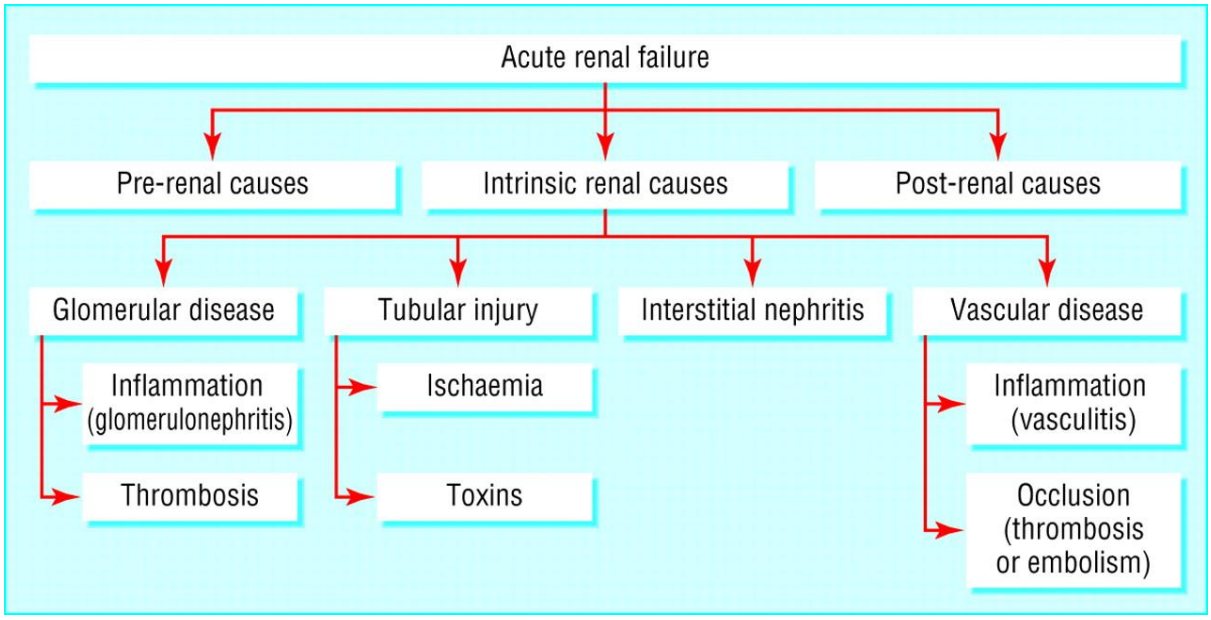

Fig 1: Pathophysiology of Acute renal failure

Table-1: Causes of acute renal failure

Pre renal	Renal	Post renal
Loss of body fluids due to excessive urination, bleeding, burns or other causes.	Injury to the renal tubules due to obstruction, surgery, medications such as NSAIDSs, Cyclosporine	Obstruction to the ureter due to calculi, clots, congenital defects or trauma during surgery or high impact injury.
Cardiac disorders that result in low output	Acute glomerulonephritis	Obstruction in the bladder due to cancer, administration of medications such as anticholinergic drugs
Conditions such as septic shock, liver failure, renal artery obstruction, renal vein thrombosis	Acute tubulointestinal nephritis	
Drugs such as NSAIDs, cyclosporine, ACE inhibitors that effect the normal function.	Disorders such as lymphoma, sarcoacidosis ,leukaemia	

PLANT PROFILE

In the recent years many researchers have examined the effects of plants used traditionally by herbalists to kidney function and treat diseases of kidney. Normally herbal plants are free from side effects/adverse effects and they are low cost effects, which will be beneficial for the people. Keeping in this view, we have selected *Heliotropium Indicum* based on the pharmacological information from Kolli

Hills, Nammakkal District, Tamilnadu which is traditionally used in various disorders including kidney diseases by tribes. *Heliotropium Indicum Linn,*commonly known as 'Indian heliotrope'. The name 'heliotrope'originates from the old idea that the inflorescence of these plants turned their rows of flowers to the sun. The meaning of '*helios*' in Greek is 'sun' and 'tropein' from where the word *'tropium'* is derived means'to turn' [2].

Scientific classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnaliopside
Order: Laminales
Family: Boraginaceae
Genus: Heliotropium L.
Species: *Heliotropium indicum L.*

Fig.2: Heliotropium Indicum plant

COMMON NAMES

Indian Heliotrope, Indian turnsole, Nagadanthi, Thel Kodukku. Medicinal uses of *Heliotropium indicum* comprises the use of juice of the leaves as an antiseptic and anti-inflammatory agent when applied to wounds, sores, boils and pimples on the face. Boiled with castor oil, it is applied to sores from scorpion bites and also locally used in treating ophthalmic infections. It is also used in the treatment of skin rashes and as a powerful expectorant. In Gambia the whole plant is buried for the fleshy tissues to decompose away leaving the fibre which is used to make false hair for women.

MATERIALS AND METHODS

Materials

Rifampicin was obtained as a gift sample from Microlabs Pvt.Ltd. Bangalore and Cystone from Himalaya Health Care, India. The kits for all biochemical estimation were purchased from Coreal Clinical Systems, Goa, India. The plant material of *Heliotropium indicum* was collected from the Kolli hills and Foot hill of Yarcaud, Tamil Nadu, India. It was authentified by Dr.K.Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupathi. The herbarium was prepared and submitted to the university.

Preparation of extract

The plant material of *Heliotropium indicum* was dried under shade and then pulvarised to a coarse powder with mechanical grinder and the powder was passed through the sieve No 40. The coarse powder was extracted with ethanol by continuous hot soxhlet apparatus for 48 hrs after completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The dried extract was stored in dessicator.

Pharmacological evaluation

Animals

Female Wister strain rats (150-200g) were used for the study. The studies were carried out in accordance with the guidelines given by Committee for the purpose of Control and Supervision of Experiments on animals (CPCSEA), New Delhi (India). The Institution Animal Ethical Committee of SICRA labs Pvt Ltd, Hyderabad, approved the protocol (769/2011/CPCSEA).

Nephroprotective studies: Grouping of animals [3]

Group I-Rats treated with normal saline
Group II-Rats treated with rifampicin 1g/kg p.o
Group III-Rats treated with cystone 500mg/kg p.o
Group IV-Rats treated with Heliotropium ethanolic extract 150mg/kg p.o
Group V-Rats treated with Heliotropium ethanolic extract 250mg/kg p.o

Rats were randomly divided into 5 groups (n=6).Group-I rats served as control. Group-II rats were treated with rifampicin1g/kg p.o. served as negative control. Group-III rats were treated with cytosine 500mg/kg p.o. served as standard. Group-IV and Group-V were treated with Heliotropium ethanolic extract (HIEA) 150 and 250mg/kg p.o. respectively. Standard cystone and Heliotropium ethanolic extract were administered for 14 days and simultaneously administered after 30 min. Rifampicin every 72 hrs for 2 weeks. On 15thday urine was collected and all the animals were sacrificed under diethyl ether anaesthesia. Blood samples were collected and serum was separated by centrifuging at 2500 rpm for 15min. and analysed for various biochemical parameters.

Assessment of kidney function

Biochemical parameters i.e., estimation of urinary and serum parameters like urea [4], creatinine [5], uric acid [6] and total proteins [7] were analysed according to the reported photometric methods. The kidney was removed, weighed and morphological changes were observed. A 10% of kidney homogenate was used for antioxidant studies such as catalase [8], lipid peroxidation (LPO) [9], glutathione peroxidase (GPx) [10] and superoxide dismutase (SOD) [11]. The kidney was removed, weighed and morphological changes were observed. A portion of kidney was fixed in 10% formalin for histopathological studies.

RESULTS

Phytochemical screening

The phytochemical analysis revealed the presence of alkaloids, carbohydrates and glycosides, phytosterols, fixed oils and fats, phenolic compounds and tannins, flavonoids, terpenoids, proteins and amino acids.

Table-2: Data showing phytochemical screening

Phytoconstituents	Present Or Absent
Carbohydrates	+
Glycosides	+
Gums and mucilage	–
Proteins and amino acids	+
Saponins	+
Tannins	+
Steroids	+
Flavonoids	+
Alkaloids	+
Triterpenoids	+
Fixed oils and fats	+

STATISTICAL ANALYSIS

Data are expressed as mean±SEM of six observations, representing the effect on biochemical parameters of urea, uric acid and creatinine in urine. Group I,III,IV and V are compared with Group II using one way ANOVA followed by Dunnett's test.*P<0.05; **P<0.01.

Urine parameters estimation values

Table-3: Effect of HIEA on urine, urea, uric acid and creatinine in rifampicin induced nephrotoxicity

Group	Drug treatment	Urea(mg/dl)	Uric acid (md/dl)	Creatinine(mg/dl)
I	Control	29.28±2.5**	25.75±5.3**	0.41±2.3**
II	Negative control	74.52±3.2	50.23±4.1	1.96±2.7
III	Standard	30.85±4.7**	34.54±2.5**	0.61±3.6**
IV	150mg/kg HIEA	35.53±3.0*	37.25±2.1*	0.54±5.2*
V	250mg/kg HIEA	33.92±4.3**	26.09±2.9**	0.35±3.4**

Serum parameter estimation values

Table-4: Effect of HIEA on serum urea, uric acid, total protein and creatinine in rifampicin induced nephrotoxicity

Group	Drug treatment	Urea(mg/dl)	Uric acid (md/dl)	Total protein(mg/dl)	Creatinine (mg/dl)
I	Control	39.37±3.1**	0.841±2.4**	6.603±4.3**	0.96±3.8**
II	Negative control	75.23±2.9	1.93±5.2	4.37±8.6	2.29±4.4
III	Standard	30±5.9	0.95±6.9**	6.16±4.8**	1.47±3.6**
IV	150mg/kg HIEA	30.6±6.2*	0.71±7.4*	6.26±5.3*	0.76±8.9*
V	250mg/kg HIEA	27.16±8.3*	0.42±3.9**	6.8±6.7**	0.71±7.7**

All values are expressed as Mean ± SEM, N = 6 animals in a group. One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test *P<0.001 as compared to positive control group.

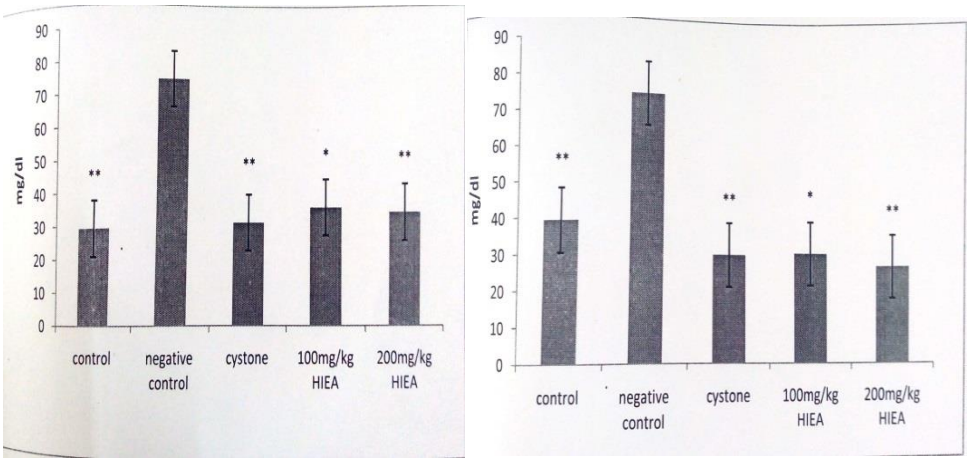

Fig.3: Effect of HIEA on Urea in Urine and Serum in Rifampicin induced nephrotoxicity

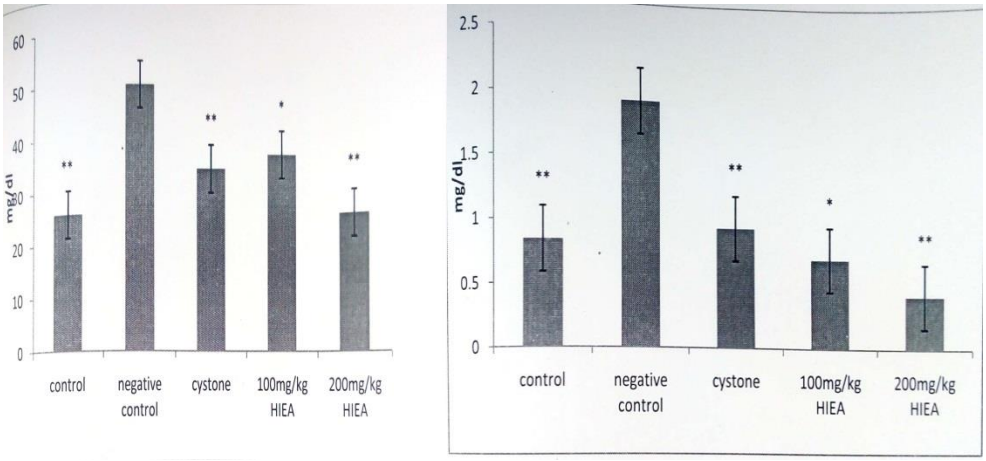

Fig.4: Effect of HIEA on Uric acid in Urine and Serum in Rifampicin induced nephrotoxicity

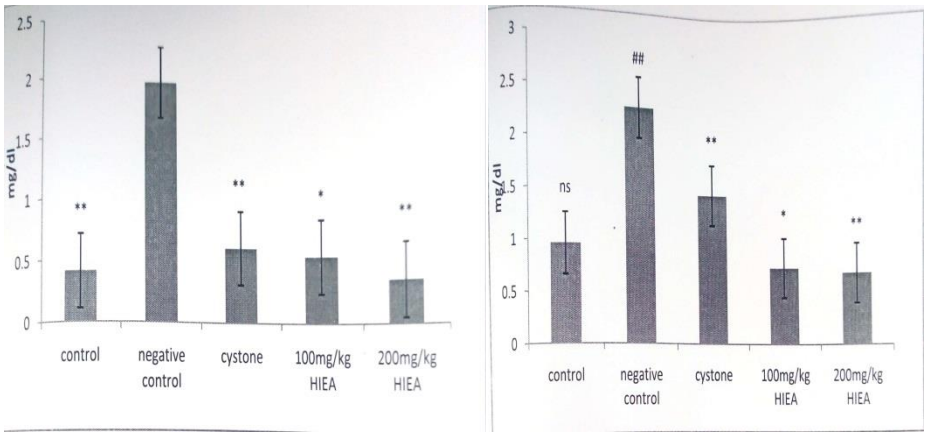

Fig.5: Effect of HIEA on Creatinine in Urine and Serum in Rifampicin induced nephrotoxicity

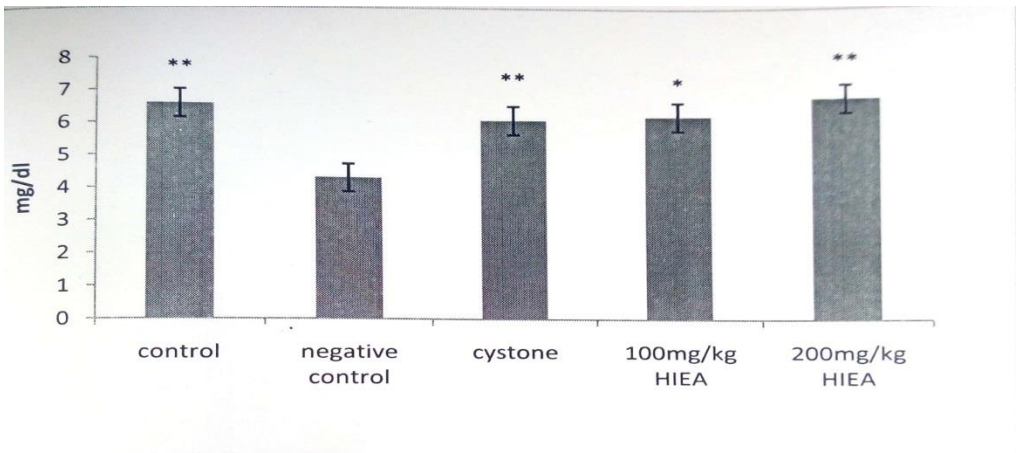

Fig.6: Effect of HIEA on Total protein in Serum in Rifampicin induced nephrotoxicity

Antioxidant studies

Rifampicin many animal experiments have demonstrated that the positive correlation between oxidative stress and nephrotoxicity. In the present study drug induced nephrotoxicity by administration of Rifampicin. This toxicity characterised by marked elevation in the circulating levels of urea, creatinine and uric acid in blood and urine whereas decrease in total protein levels and histological features of interstitial nephritis in Group II rats when compared

to Group I rats. However these changes were attributed by pre-treatment with oral administration of HIEA for 2 weeks. HIEA, in particular, reversed oxidant – anti oxidant imbalance and has good hydroxyl radicle scavenging activity.

Histopathological studies

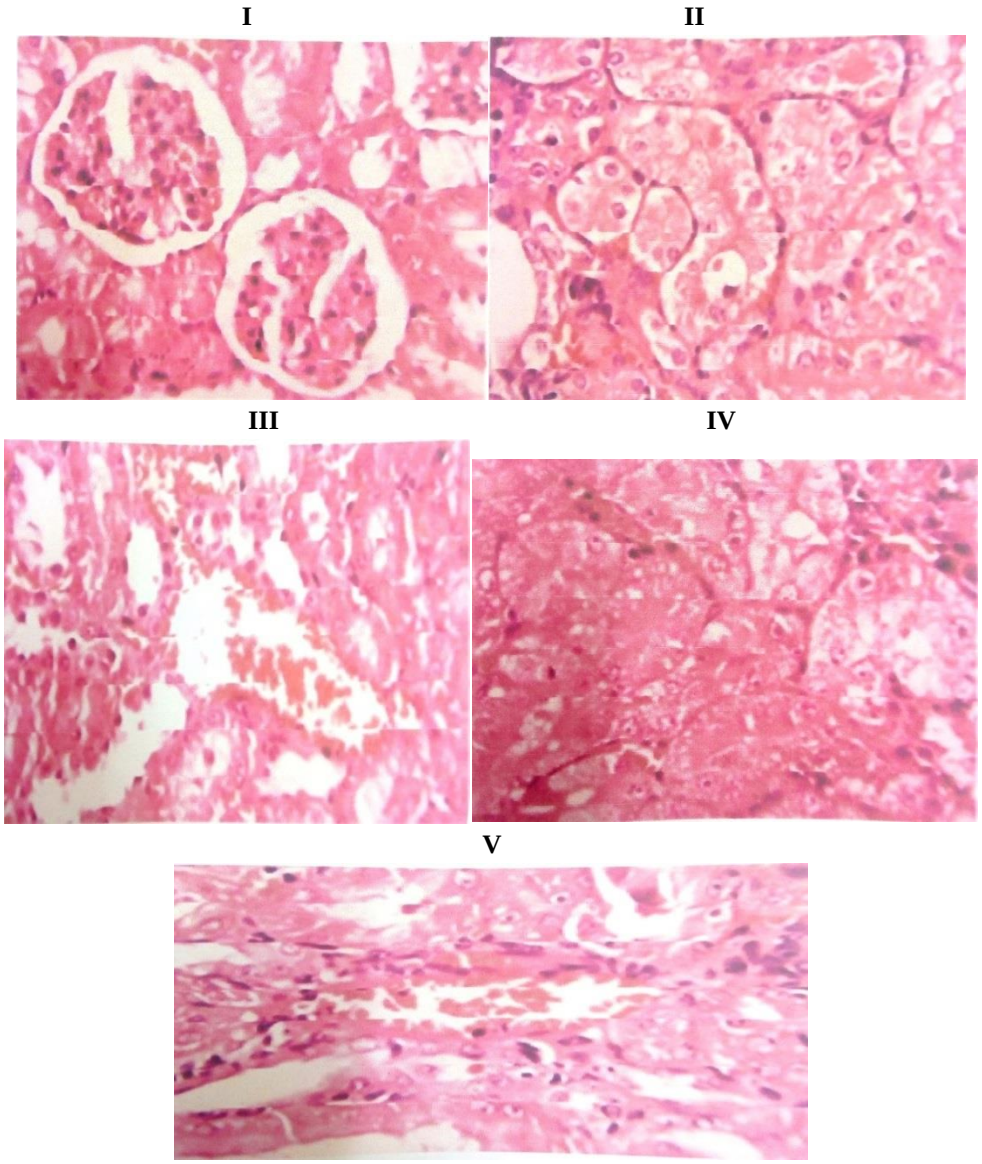

Fig.7- Histopathological changes occurred in rats during Rifampicine intoxication and prevention by the treatment with ethanolic extract of *Heliotropium indicum* (Group I-V)

Significant observations

- **Group I:** Some blood vessels show congestion.
- **Group II:** Diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion
- **Group III:** Some blood vessels are dilated within the interstitium compared to group-II
- **Group IV:** Focal glomerular congestion, Peritubular congestion, Focal hydrophobic degeneration of tubular epithelial cells.
- **Group V:** Some blood vessels show congestion.

DISCUSSION

Rifampicin is one of the widely used anti tubercular agent in the treatment of tuberculosis. In spite of its clinical usefulness, there are complications in using this drug as it causes nephrotoxicity and hepatotoxicity. The mechanism of nephrotoxicity is due to rifampicin might be due to the occurrence of circulating antibodies and immunoglobulin G (IgG) deposits along the tubular basement membrane as well as containing immunoglobulin light chains in tubular lumens.

Rifampicin is a potent drug and is the main drug in the multidrug therapy for lung tuberculosis. It can induce hepatitis and nephritis in hypertensive patient. Thus Rifampicin induced nephrotoxicity is well established experimental model of drug induced renal injury. Drug induced nephrotoxicity is often associated with marked elevation of biochemical parameters like urea, creatinine and uric acid in blood and urine and decrease in serum protein levels. So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animal and man.

Oral administration of plant extract significantly decreased the urea, uric acid and creatinine whereas total protein levels are elevated in both treatment groups compare to Group II. 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.

Rifampicin is known to decrease the activities of Catalase, SOD and Glutathione peroxidase whereas increase in activity of Lipid peroxidase. Therefore it is no doubt to assume that the nephroprotection showed by Heliotropium ethanolic extract is mediated through its potent antioxidant effect.

In Rifampicin treated rats there was a significant increase in lipid peroxidation products suggesting that the involvement of oxidative stress. In addition triterpenoids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity. The presence of Triterpenoids could be the reason of protection by the extract might be due to its ability to activate anti-oxidant enzymes.

In histopathological study of saline treated group showing some blood vessels are dilated and congested with in the interstitium. Rifampicin treated group showing diffuse glomerular congestion, Tubular casts, peritubular congestion, epithelial desquamation, Blood vessel congestion. While Group IV shows Focal glomerular congestion, peritubular congestion, Focal hydrophilic degeneration of tubular epithelial cells and Group V shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen with in the interstitium.

SUMMARY AND CONCLUSION

In the present study, the extract of *Heliotropium indicum* treated Rifamficin group animals were found to reduce glomerular, peritubular and blood vessel congestion and presence of inflammatory cells in kidney section histology induced by Rifamficin indicating nephroprotection.

Further documented reports reveal that plant material containing phenols, flavonoids, triterpenoids, alkaloids and saponins offers organ protection by virtue of their free radical scavenging activity. Phytochemical analysis showed the presence of phenols, flavonoids, triterpenoids, alkaloids and saponins as phytoconstituents. Hence the role of these phytoconstituents as free radical scavengers and consequent nephroprotection cannot be ruled out.

REFERNCES

[1]. Cooper K, Bennett WM. Nephrotoxicity of common drugs used in clinical practice. Arch Intern Med, 147, 1987, 1213-20.

[2]. Chugh KS, Singhal PC. Acute renal failure in the tropic. In: Asian manual of nephrology. Seamic Tokyo, 1981, 180-7.

[3]. Shelke TT, Kothari R. Nephroprotective activity of ethanolic extract of dried fruits of *Pedalium murex Linn*. J. Cell Tissue Res., 9, 2009, 1687-90.
[4]. Young DS. Effects of drugs on Clinical Lab. Tests, AACC Press. 4, 1995.
[5]. Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH-Books Verlagsgesell Schaft, 1998, 208-14.
[6]. Tietz, N.W., Clinical guide to laboratory tests, 3rd Ed, (W.B. Saunders eds. Philadelphia USA), 1995, 624.
[7]. Christensen, S.E., Proteins. Clinical Chemistry: Concepts and Application, Anderson, S.C., Cockayne, S. (W.B. Saunders eds. Philadelphia USA), 188, 1983.
[8]. Tiez, N.W., Clinical guide to laboratory tests. 3rd Ed, (W.B. Saunders eds. Philadelphia USA), 518, 1995.
[9]. Ohkawa H., Ohishi N., Yagi K., Assay for lipid peroxidation in animal tissue by thiobarbituric acid reaction, *Anal Biochem*., 95, 1979, 51-58.
[10]. Rotruck JT, Pope AL, Ganther HL, Swanson AB. Selenium: biochemical role as a component of glutathione peroxidase. *Science*, 179, 1973, 588-590.
[11]. Marklund A., Marklund G. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*, 47, 1974, 469-474.