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**Pusapati Chiranjeevi Raju**  
Research Scholar, Department  
of Biochemistry, Acharya  
Nagarjuna University,  
Nagarjuna Nagar, Guntur,  
Andhra Pradesh, India

**PVV Satyanarayana**  
Prof. Department of  
Chemistry, Acharya Nagarjuna  
University, Nagarjuna Nagar,  
Guntur, Andhra Pradesh,  
India

## Evaluation of nephroprotective activity of *Heliotropium indicum* in rifampicin induced nephrotoxicity

**Pusapati Chiranjeevi Raju and PVV Satyanarayana**

### Abstract

The nephroprotective effect of whole plant of ethanolic extract of *Heliotropium indicum* is experimentally evaluated by rat model. In the present study, the whole plant of ethanolic extract of *Heliotropium indicum* significantly reduced the toxicant elevated levels of urea uric acid creatinine parameters and increase in the levels of protein in blood and urine, the extract of *Heliotropium indicum* is found to reduce such changes in kidney histology induced by Rifampicin, 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. Based on improvement in serum and urine marker levels, histopathological studies, level of antioxidant enzymes and presence of phytoconstituents, it is concluded that the ethanolic extract of *Heliotropium indicum* possesses nephroprotective activity and thus supports the traditional application of the same under the light of modern science.

**Keywords:** *Heliotropium indicum*, Rifampicin, nephroprotective, nephrotoxicity

### Introduction

Kidneys are the organs that serve several essential regulatory roles in most animals, including vertebrates and some invertebrates. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid–base balance, and regulation of blood pressure. They serve the body as a natural filter of the blood, and remove wastes which are diverted to the urinary bladder. In producing urine, the kidneys excrete wastes such as urea and ammonium, and they are also responsible for the reabsorption of water, glucose, and amino acids. The kidneys also produce hormones including calcitriol, erythropoietin, and the enzyme rennin.

Rifampicin is a semi synthetic antibiotic derivative of rifamycin B. The chemical name for rifampin is 3-(4-methyl-1-piperazinyloxy) rifamycin. Rifampicin is not recommended for intermittent therapy. The patient should be cautioned against intentional or accidental interruption of the daily dosage regimen since rare renal hypersensitivity reactions have been reported when therapy was resumed in such cases. Haemolysis, haemoglobinuria, haematuria, renal insufficiency or acute renal failure have been reported which are generally considered to be hypersensitivity reactions.

The human beings are exposed to environmental, occupational and xenobiotics challenges due to modern life style. Free radicals are generated during the exposure to such stressful challenges. In addition the process of metabolism and excretion of xenobiotics may also generate free radicals. These free radicals bind covalently with the tissue macromolecules leading to the cell necrosis [1-4].

*Heliotropium indicum* Linn, commonly known as ‘Indian heliotrope’ is an annual plant that is a common weed in wet places and settled areas. It is native to Asia. It is very common in India and some parts of Africa and Bangladesh, but also found in other countries. Leaves, flowers, roots, stem and whole plant. Plants are astringent, emollient, vulnerary and diuretic.

### Correspondence

**PVV Satyanarayana**  
Prof. Department of  
Chemistry, Acharya Nagarjuna  
University, Nagarjuna Nagar,  
Guntur, Andhra Pradesh,  
India



*Heliotropium indicum* Linn

It is used as local application for ulcers, sores, wounds, gum boils, skin infections, stings of insects and rheumatism. Leaves are used for ring worm; juice is used in eye disease; decoction is used in fevers and urticaria. Roots are aphrodisiac; used for the cure of night blindness. Decoction of the root is used in coughs and fevers. Seeds are stomachic. The flowers are considered abortifacient in large doses. Aqueous and alcoholic extract of roots are oxytocic [5-8].

The aim of the research is to find out new Nephroprotective agents from indigenous plants which are potent and non toxic agents. Normally herbal plants are free from side effects/adverse effects and they are low cost medicines, which will be beneficial for the people. Keeping in this view, we have selected *Heliotropium indicum* (family:) based on ethno pharmacology information from Kolli Hills, Namakkal District, Tamil Nadu which is traditionally used in various disorders including kidney diseases by tribes. The present study aimed at evaluates Nephroprotective of *Heliotropium indicum* against Rifampicin induced nephrotoxicity in rats with special reference to biochemical and histopathological studies.

## Materials and Methods

### Materials

Rifampicin was obtained as a gift sample from Microlabs Pvt.Ltd. Bangalore. The kits for all biochemical estimation were purchased from Coreal Clinical Systems, Verna Goa, India. The plant material of *Heliotropium indicum* was collected from the Kolli hills and Foot hill of Yercaud, Tamil Nadu, India.

### Preparation of extract

The plant material of *Heliotropium indicum* then pulverised to a coarse powder with mechanical grinder and the powder was passed through the sieve No 40.

### Extraction procedure

The coarse powder of *Heliotropium Indicum* was extracted with 1-1.5 liters of 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.

## Animals

Female wistar rats were procured and they were housed in microloan boxes with standard laboratory diet and water *ad libitum* at temperature of  $22^{\circ} \pm 2^{\circ} \text{C}$  and humidity of 45%-64%. The study was conducted after obtaining Institutional Animal Ethical Committee Clearance. (IAEC Number).

## Nephroprotective Studies

### Study Design of *Heliotropium indicum*

#### Grouping of Animals

Rats were divided into five groups each group consisting of six animals

**Group 1:** Normal control: Administered with equivalent volumes of 5ml/kg p.o. of normal saline (0.9%NaCl) for 2 weeks.

**Group 2:** Positive control: Received Rifampicin (1g/kg/bodyweight p.o.) every 72 hrs for 2 weeks to induce nephrosis.

**Group 3:** Standard: Received cystone (500mg/kg/bodyweight p.o.) and simultaneously administered after 30 min. Rifampicin (1g/kg/bodyweight p.o.) every 72 hrs for 2 weeks.

**Group 4:** Ethanolic extract of *Heliotropium indicum* (100mg/kg/body weight p.o.) and simultaneously administered after 30 min. Rifampicin (1g/kg/body weight p.o.) every 72 hrs for 2 weeks.

**Group 5:** Ethanolic extract of *Heliotropium indicum* (200mg/kg/body weight p.o.) and simultaneously administered after 30 min. Rifampicin (1g/kg/body weight p.o.) every 72 hrs for 2 weeks.

The study was carried out for 2 weeks. On 15<sup>th</sup> day all the animals were sacrificed under diethyl ether anaesthesia. Blood samples were collected, allowed to clot, serum was separated by centrifuging at 2500 rpm for 15 min. and analysed for various biochemical parameters.

### Assessment of kidney function

Biochemical parameters i.e., Estimation of urinary and Blood parameters like urea, Creatinine, uric acid and total proteins were analyzed 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 lipid peroxidation (LPO), superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). A portion of kidney was fixed in 10% formalin for histopathological studies.

### Histopathological studies

A histopathological study of kidneys was performed in histopathology laboratory by consultant histopathologist.

### Statistical analysis

The values were expressed as Mean  $\pm$  SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. P values < 0.05 were considered as significant [9-12].

**Results- Urine Values****Table 1:** Drug treatment

Group	Drug treatment	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
1	5ml/kg NS	29.28±0.58	25.75±0.49	0.14±0.06
2	1g/kg Rifampicin	74.52±0.49	50.23±0.32	1.96±0.31
3	500mg/kg Cystone+1g/kg Rifampicin	30.85±0.50***	34.54±0.34***	0.61±0.21***
4	100mg/kg HIEA+1g/kg Rifampicin	35.53±0.32***	37.25±0.51***	0.54±0.04***
5	200mg/kg HIEA+1g/kg Rifampicin	33.92±0.44***	26.09±0.41***	0.35±0.03***

**Table 2:** Serum Values

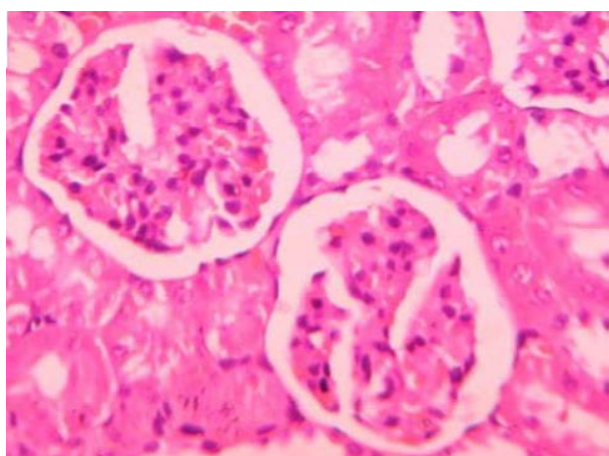
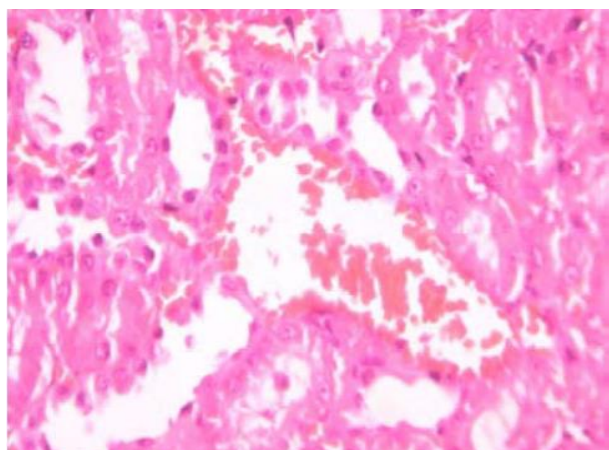
Group	Drug treatment	Urea (mg/dl)	Uric acid (mg/dl)	Total protein (mg/dl)	Creatinine (mg/dl)
1	5ml/kg NS	39.37±0.44	0.841±0.33	6.603±0.2	0.96±0.03
2	1g/kg Rifampicin	95.23±0.38	1.93±0.05	4.37±0.33	2.29±0.08
3	500mg cystone +1g/kg Rifampicin	30±0.57***	0.95±0.05***	6.16±0.28***	1.47±0.05***
4	100mg/kg HIEA+ 1g/kg Rifampicin	30.6±0.95***	0.71±0.05***	6.26±0.21***	0.71±0.05***
5	200mg/kg HIEA+ 1g/kg Rifampicin	27.16±0.94***	0.25±0.39***	6.8±0.36***	0.76±0.04***

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.

**Table 3:** Antioxidant parameter Values

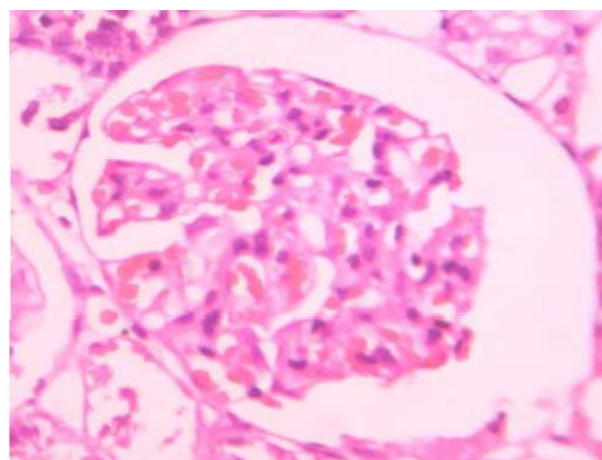
Group	Catalase ( $\mu\text{mole of H}_2\text{O}_2/\text{Sec/mg protein/ml}$ )	Lipid peroxidise (nmol MDA/ min $\times$ mg protein)	Glutathion peroxidise ( $\mu$ mole of oxidized GSH / min $\times$ mgprotein)	Superoxide dismutase (U/mg protein)
1	16.34±1.623	9.83±0.214	35.09±1.976	19.94±0.751
2	7.48±0.953	20.57±0.482	20.56±1.169	7.66±0.41
3	15.28±1.423***	14.86±0.404***	29.71±0.639***	17.23±0.623***
4	10.48±0.515 <sup>NS</sup>	18.73±0.487 <sup>NS</sup>	24.31±0.963 <sup>NS</sup>	11.71±0.656*
5	14.46±1.526*	15.97±0.818**	27.88±0.995*	15.91±0.79***

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. Ns; indicates no significant.

**Histopathological Studies****Fig 1.8.a:** Group-1**Fig 1.8.b:** Group-1**Normal saline - 5ml/kg (p.o) for 2 weeks daily (Group - I)****Microscopy**

Section studied shows renal parenchyma with intact normal architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels are dilated and congested (Figure.(b)) within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium.

**Significant observation:** Some blood vessels show congestion.

**Fig 1.8.c:** Group-II

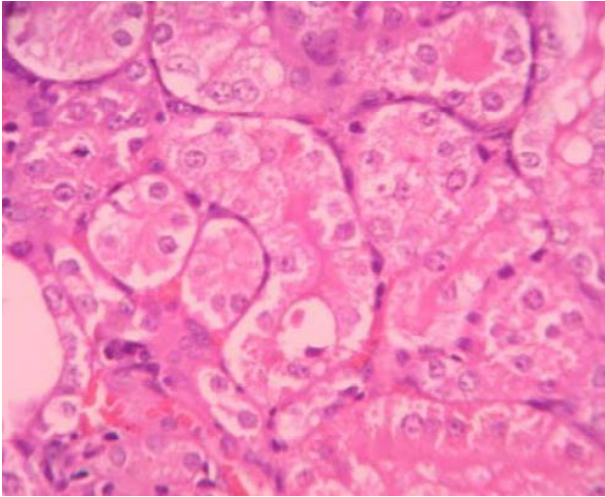


Fig 1.8.d: Group-II

**Rifampicin – 1g/kg (p.o) every 72 hrs for 2 weeks (Group – II)**

**Microscopy:**

Section studied shows renal parenchyma with intact architecture. There is seen diffuse glomerular congestion (Figure. (c)) occasional tubular casts (Figure. (d)), focal hydropic degeneration of the tubular epithelial cells and peritubular congestion. Some of the tubules show partial desquamation of the epithelial cells. Also seen are blood vessel congestion and scattered mononuclear inflammatory cell infiltrations within the interstitium compared to group-I.

**Significant observation:** Diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion.

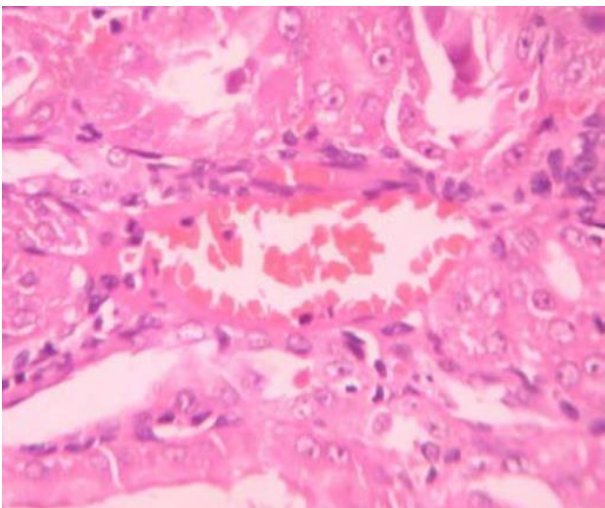


Fig 1.8.e: Group-III

**Cystone (500mg/kg) p.o and simultaneously administered after 30 min. Rifampicin (1g/kg) p.o. every 72 hrs for 2 weeks (group-III)**

**Microscopy:**

Section studied shows renal parenchyma with intact architecture. The glomerular and tubular changes appear unremarkable. Some blood vessels are dilated (Figure (e)) within the interstitium compared to group-II.

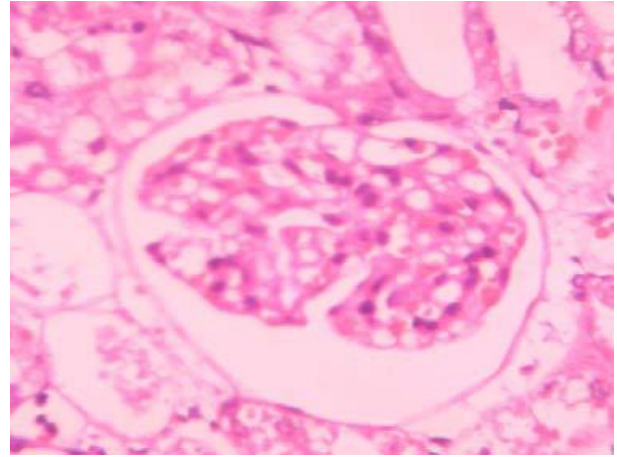


Fig 1.8.f: Group-IV

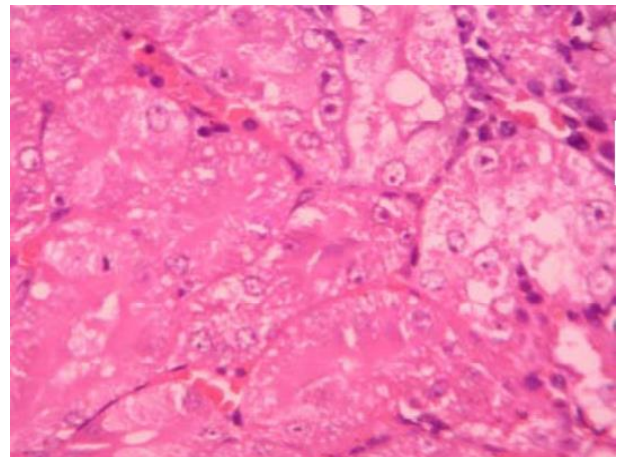


Fig 1.8.g: Group-IV

**Ethanol extract of *Heliotropium indicum* (100mg/kg/body weight p.o.) and simultaneously administered after 30 min. Rifampicin (1g/kg/body weight p.o.) every 72 hrs for 2 weeks (group – IV).**

**Microscopy:** Section studied shows renal parenchyma with intact architecture. There are seen focal glomerular congestion (Figure. (f)), few the tubular epithelial cells show hydropic degeneration (Figure. (g)) and peritubular congestion. Also seen are few scattered mononuclear inflammatory cell infiltrations within the interstitium.

**Significant observation:** Focal glomerular congestion, Peritubular congestion, Focal hydropic degeneration of tubular epithelial cells.

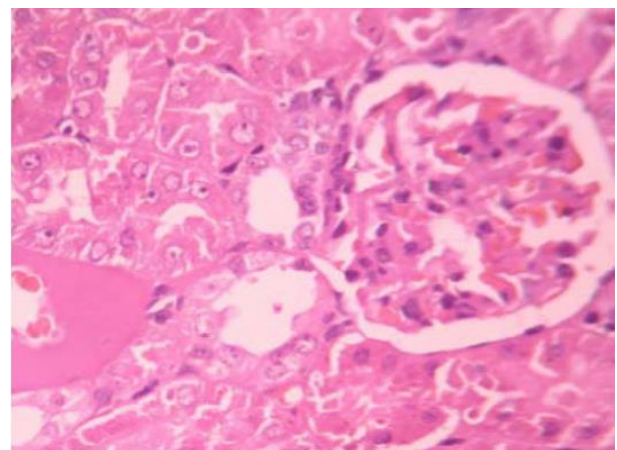
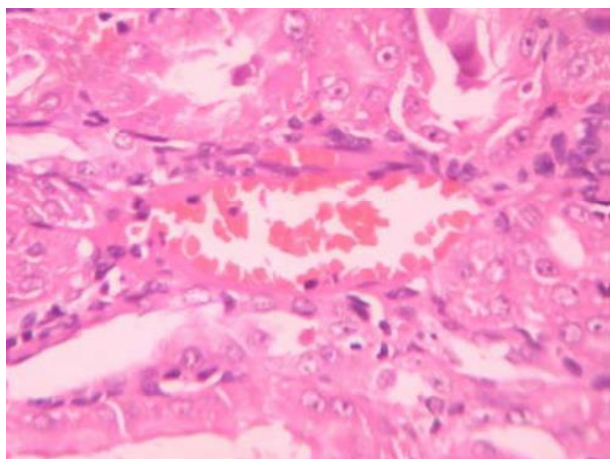


Fig 1.8.h: Group-V



**Fig 1.8.i:** Group-V

**Ethanollic extract of *Heliotropium indicum* (200mg/kg/body weight p.o.) and simultaneously administered after 30 min. Rifampicin (1g/kg/body weight p.o.) every 72 hrs for 2 weeks (group –V).**

#### **Microscopy:**

Section studied shows renal parenchyma with intact architecture. The glomerular and tubular changes appear unremarkable. Some of the blood vessels are dilated compared to group-IV and congested (Figure. (i)) within the interstitium. Also seen are few scattered mononuclear inflammatory infiltration is seen within the interstitium.

**Significant observation:** Some blood vessels show congestion.

#### **4. Discussion**

Rifampicin is a potent drug and is the main drug in the multidrug therapy for lung tuberculosis. It can induce hepatitis and nephritis in hypersensitive patient. Thus Rifampicin induced nephrotoxicity is well established experimental model of drug induced renal injury. Many animal experiments have demonstrated overwhelmingly, the positive correlation between oxidative stress and nephrotoxicity. 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. In the present study drug induced nephrotoxicity by administration of Rifampicin. This toxicity characterized by marked elevation in the circulating levels of urea, creatinine and uric acid in blood and urine and decrease in total protein levels and histological features of interstitial nephritis in group 2 rats when compared to untreated (group 1) rats. However these changes were attributed by pre-treatment with oral administration of ethanollic extract of *Heliotropium indicum* for 2 weeks. Oral administration of plant extract significantly decreased the urea, uric acid and creatinine where as total protein levels are elevated in both treatment group compare to toxicant group. 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. It was established that rifampin-induced Acute tubular interstitial nephritis have demonstrated the occurrence of circulating antirifampin antibodies and immunoglobulin G (IgG) deposits along the tubular basement membrane as well as containing immunoglobulin light chains in tubular lumens. Rifampicin is known to decrease the activities of catalase, glutathione peroxidase and the reduced level of glutathione. Therefore it is no doubt to assume that the nephroprotection showed by *Heliotropium* ethanollic extract is mediated through its potent antioxidant effect. A relation between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. In Rifampicin treated rats there was a significant increase in lipid peroxidation products (MDA) 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 antioxidant enzymes. In histopathological study of saline treated group showing some blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. Rifampicin treated group showing diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion. While treatment group (100 mg/kg) shows Focal glomerular congestion, Peritubular congestion, Focal hydrophic degeneration of tubular epithelial cells and treatment group (200 mg/kg) 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 histopathological results *Heliotropium* ethanollic extract at dose of 100 mg/kg have partial protective effect while *Heliotropium* ethanollic extract at dose of 200 mg/kg have protective effect on Rifampicin induced nephrotoxicity. The findings suggest the potential use of ethanollic extract of *Heliotropium indicum* 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.

#### **5. Conclusion**

Based on improvement in serum and urine marker levels, histopathological studies, level of antioxidant enzymes and presence of phytoconstituents, it is concluded that the ethanollic extract of *Heliotropium indicum* possesses nephroprotective activity and thus supports the traditional application of the same under the light of modern science.

#### **6. Acknowledgement**

I take this privilege and pleasure to acknowledge the contributions of many individuals who have been inspirational and supportive throughout my work undertaken and endowed with the precious knowledge to see success in my endeavour.

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