

## Pharmacological Study of the Methanolic Whole Plant Extract of *Eclipta alba* Against Ischemic Reperfusion Injury on Kidney of Sprague Dawley Rats

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### ABSTRACT

Ischemia/Reperfusion (I/R) injury is one of the leading causes of acute kidney injury (AKI) which affects millions of people worldwide. I/R injury mainly occur after infraction, sepsis, and organ transplantation. The present study was conducted to evaluate the prophylactic effect of an ancient medicinal plant, *Eclipta alba* against experimentally induced bilateral renal I/R injury in rats. 15 male Sprague-Dawley rats were randomized into four groups which include control and two treatment groups which were treated with whole plant methanolic extract of *Eclipta alba* at graduated doses of 200 and 400 mg/kg/day through oral intubation for a period of 7 days. All the animals were subjected to ischemia for period of 45-min followed by 6 hours of reperfusion. At the end of reperfusion period, pathological alterations in kidney were evaluated by haematological and biochemical analysis in addition to the microscopical examination of kidney. Renal markers such as creatinine and blood urea nitrogen (BUN) were significantly decreased dose dependently in treatment groups when compared to control. Furthermore, there is an up regulation of haematological parameters such as Red blood cell count (RBC), White blood cell count (WBC) and Platelet count. Histopathology examination of I/R control group revealed severe haemorrhages and infarct characterized by tubular degeneration, glomerular tuft retraction, thickening of glomerular basement membrane and there is marked endothelial swelling in all parts of cortex and medulla. Whereas, treatment with *Eclipta alba* at graded doses ameliorated the pathological effects of ischemic insult and there is a marked improvement in the integrity of the anatomical structure of the kidney, thereby, establishing the potential protective effect of the extract against the ischemia – reperfusion injury.

### Keywords:

### INTRODUCTION

Being aware of the fact that kidneys perform diverse functions like filtration, absorption, reabsorption, and secretion they carry out huge accountability to maintain the physiological importance of their existence. Kidneys are vessel rich organs as like brain, heart, liver etc. and they receive relatively 20% of the total cardiac output with low oxygen extraction rate of about 10%<sup>1</sup>. This renal blood flow is 10–15 times greater than that in other organs regarding their weight<sup>2,3</sup>. The renal arteries arise from the relative sides of the abdominal aorta and gives rise to the dorsal and the ventral branches before entering the hilus of the kidney<sup>4,5,6</sup>. The regulation of the renal blood flow depends on both extra-renal and intra-renal factors. The extra renal-factors include sympathetic nerves, circulating agents etc and the intra-renal factors include pre-glomerular arterial myogenic response, tubulo-glomerular feedback, nitric oxide etc. The cortex is enriched with excessive blood flow conducive to produce an adequate glomerular filtration rate<sup>7</sup>.

Ischemia is referred to as an inadequate supply of blood to an organ that which usually occurs due to the obstruction of the arterial framework. Acute Kidney Injury is a

condition that occurs due to the damage of the renal tissue which is caused by the decreased kidney blood flow or modestly due to kidney ischemia<sup>8</sup>. Ischemia leads to progressive cell injury beginning with the functional changes in the cell and cell membrane<sup>9</sup>. This leads to enhanced capillary permeability, leakage of the cytosolic enzymes, edema formation and tissue destruction resulting in ischemia induced pathological processes<sup>10</sup>.

Ischemic Reperfusion Injury (IRI) is defined as the clinical complication caused due to the restoration of blood flow to an area which is previously under ischemic insult. It is interplay between biochemical, cellular and vascular endothelial factors<sup>11</sup>. Acute arterial occlusions and reperfusion result in severe metabolic complications. IRI is manifested by two phases namely ischemic or devascularisation phase and revascularization phase<sup>12</sup>. This injury is characterized by oxidant production, leucocyte-endothelial cell adhesion, complement pathway activation, platelet leucocyte aggregation, increased micro vascular permeability and decreased endothelium dependent relaxation<sup>13</sup>. IRI leads to various clinical conditions like kidney failure, inflammatory kidney disease, renal dysfunction, renal artery stenosis etc. The

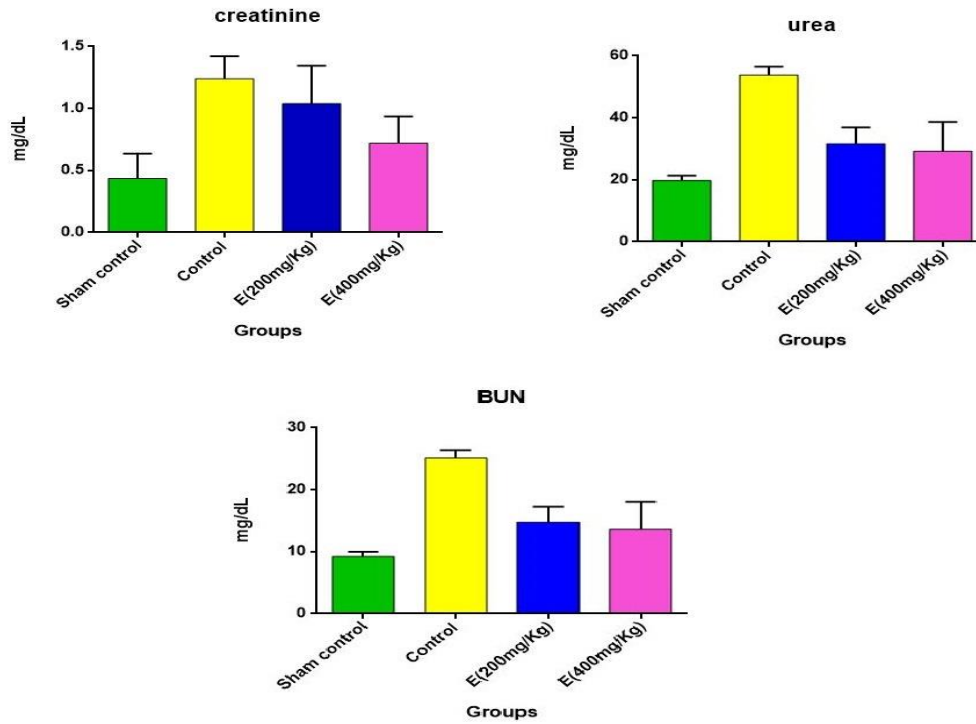


Figure 1: Biochemical parameters.

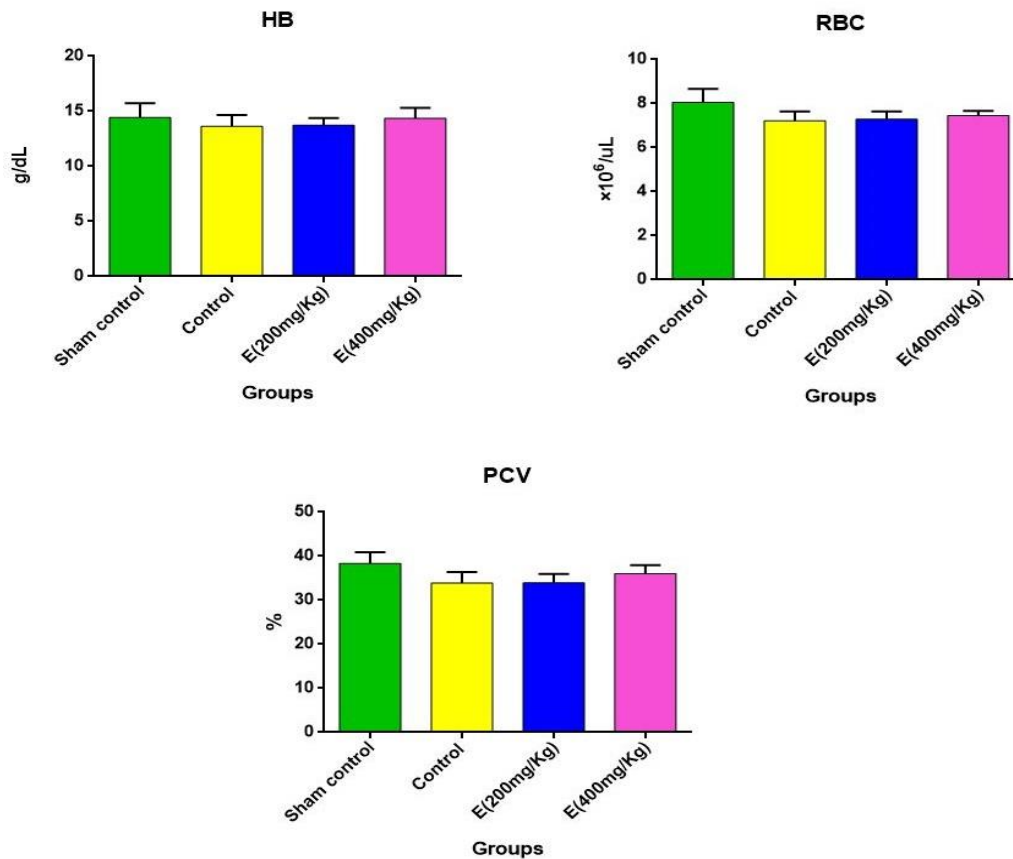


Figure 2: Haematology parameters.

therapeutic strategies of IRI include ischemic pre-conditioning, controlled reperfusion, ischemic post-conditioning, anti-oxidant therapy, complement therapy, stem cell therapy etc.

IRI leading to Acute Renal Failure results in increased mortality and morbidity<sup>14</sup> and is physiologically determined by calcium overload, pH paradox, and reactive oxygen species (ROS) overproduction. The cellular effects

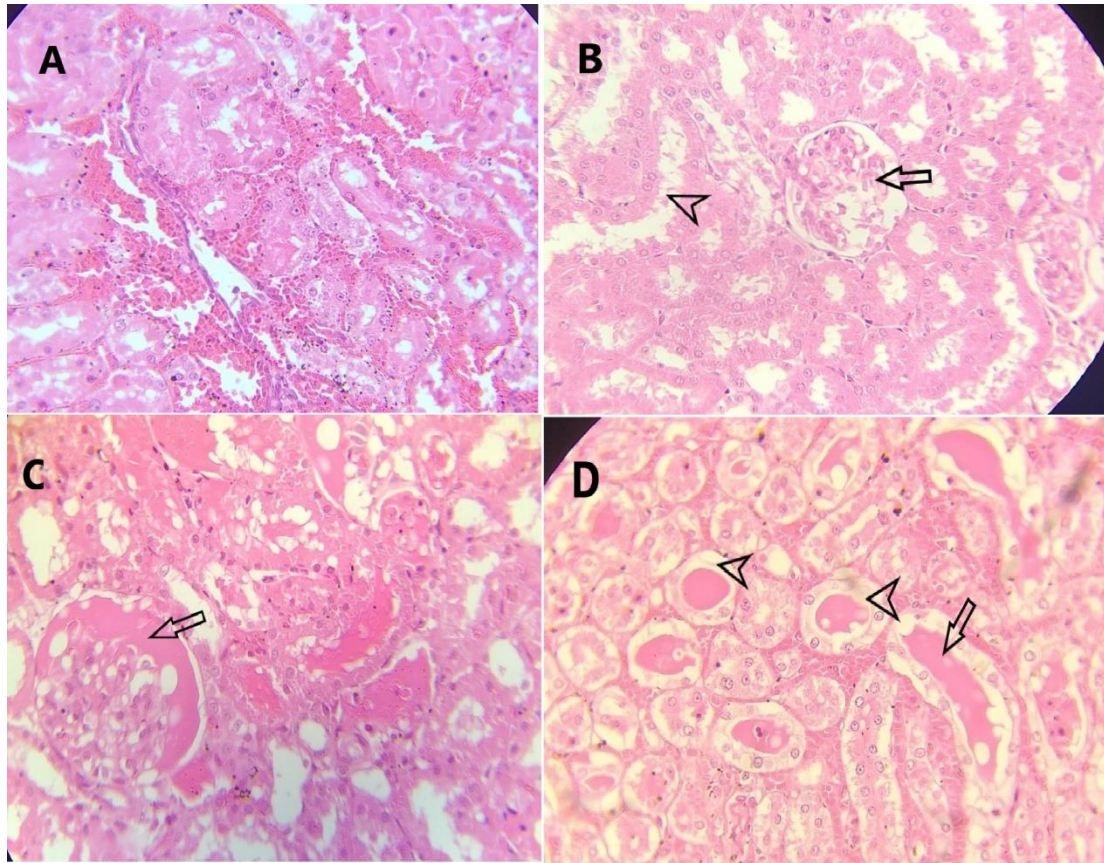


Figure 3: Histopathology of renal cortex sections of I/R control (H&E X100).

A-Severe hemorrhages; B-Showing glomerular retraction (arrows) and tubular degeneration (pointed arrows); C-Showing thickening of glomerular basement membrane (arrow); D-Showing cast formation (arrows) and loss of brush border (pointed arrows); E-Showing endothelial swelling (arrow) and infiltration (pointed arrows).

Phytochemicals	Tests	Methanolic Extract of <i>Eclipta alba</i>
Alkaloids	Mayer's Test	-
	Wagner's Test	+
	Hager's Test	-
Terpenoids	Salkowski's Test	+
Flavonoids	Shinoda's Test	+
	Zinc Hydrochloride Test	+
Carbohydrates	Alkaline Reagent Test	+
	Molisch Test	+
	Fehling's Test	+
Glycosides	Killer – Kiliani's Test	+
Tannins	FeCl <sub>3</sub> Test	-
Saponins	Froth Test	-
Aminoacids	Ninhydrin Test	-

of ischemia include altered membrane potential, altered ion distribution (increase of intracellular calcium or sodium ions), cellular swelling, cytoskeletal disorganization, increased hypoxanthine, decreased adenosine triphosphate (ATP), decreased phosphocreatine, decreased glutathione, cellular acidosis etc<sup>15</sup>. After the ischemic damage, the reperfusion increases the oxygen

levels and extracellular pH levels which are dangerous for the cells that have already undergone ischemic damage. The further increase in the cytoplasmic and mitochondrial calcium overloads causes cell structure impairment and cell death whereas the further increase in the oxygen levels generates more ROS that contributes to the damage of membranes and cytoskeleton<sup>16</sup>. This overall picture initiates the cell death programmes like apoptosis, necrosis, autophagy<sup>17,18</sup>, organ dysfunctions and innate immune responses<sup>19</sup>.

The cellular effects that contribute to the pathophysiology of IRI contemplatively involves alterations in the purine metabolism<sup>20-25</sup>, acidosis<sup>26,27</sup>, alterations in cell volume<sup>28,29</sup>, disturbances in calcium metabolism<sup>30-32</sup>, phospholipid degradation<sup>33-35</sup>, alterations in the maintenance of cell viability by amino acids and glutathione<sup>36,37</sup>, oxidant injury<sup>38-40</sup>, damage due to proteases in lysosomes and cytosol<sup>41-43</sup>, damage in the cytoskeleton and cell polarity<sup>44-46</sup>, production of heat shock protein<sup>47-50</sup>, inflammatory mediators, endothelial and epithelial injury followed by repair that can be either adaptive or maladaptive<sup>51</sup> pertains to the expression of the ischemic cell damage within the kidney<sup>52</sup>.

Medicinal plants have set a historical dogma in the prevention of various challenging diseases and disorders. They set a provocative bench mark in research and therapy in view of their valid curative properties. The therapeutic

Table 1: The EGTI histology scoring system.

Tissue type	Damage	Severity	Score
Tubular	No damage	-	0
	Loss of Brush Border (BB) in less than 25% of tubular cells. Integrity of basal membrane.	Minimal	1
	Loss of BB in more than 25% of tubular cells, thickened basal membrane	Mild	2
	(Plus) Inflammation, Cast formation, Necrosis up to 60% of tubular cells	Moderate	3
	(Plus) Necrosis in more than 60% of tubular cells	Severe	4
Endothelial	No Damage	-	0
	Endothelial Swelling	Mild	1
	Endothelial Disruption	Moderate	2
	Endothelial Loss	Severe	3
Glomerular	No damage	-	0
	Thickening of Bowman's capsule	Mild	1
	Retraction of glomerular tuft	Moderate	2
	Glomerular fibrosis	Severe	3
Tubulo/Interstitial	No damage	-	0
	Inflammation, haemorrhage in less than 25% of the tissue	Minimal	1
	(Plus) necrosis in less than 25% of tissue	Mild	2
	Necrosis up to 60%	Moderate	3
	Necrosis more than 60%	Severe	4

Table 2: Clinical sign examination.

A. No.	Dose	Days														
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	2000 mg/kg	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6		N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

A. No. = Animal number; N = Normal.

Table 3: Body weight (gm).

Group	Day 0	Day 7	Day 14
<i>Eclipta alba</i> (2000 mg/kg)	128.47 ± 2.90	134.37 ± 3.73	141.90 ± 4.01

potential and the lead strategy of these plants signify their purport in medicinal research and novel therapeutic outcomes. The natural product based drug discovery in spite of its high complexity provides broad interdisciplinary research approaches<sup>53,54</sup>. Computational methods of discovery aid the rationalization of biological activity of the natural products<sup>55</sup>.

*Eclipta alba* is an annual herb commonly known as False Daisy. It is familiarly known as Bringraj (King of Hair) belonging to the family Asteraceae. The plant is erect or prostrate, much branched, rooted at the nodes. Leaves are opposite, sessile and lanceolate. The flowers are small,

white and yellow in colour arranged in clusters. The dry fruit is formed by the fusion of carpels. Roots are well developed, cylindrical and grayish<sup>56</sup>. It is found as a common weed ascending up to 6000ft<sup>57</sup>. The genus name comes from the Greek word meaning "deficient" with reference to the absence of bristles and awns on the fruits<sup>58</sup>. It is grown widely in tropical and subtropical regions of South America, Africa, and Asia. The plant contains wide range of active principles which include coumestans, alkaloids, flavanoids, glycosides, and triterpenoids. The leaves contain stigmaterol,  $\beta$ -terthienylmethanol, wedelolactone, demethylwedelolactone and demethyl wedelolactone-7-glucoside. The roots give hentriacontanol and heptacosanol<sup>59</sup>. The plant is well documented for its pharmacological and ethnomedicinal properties<sup>60</sup>.

The plant is pharmacologically proven for various activities like antimicrobial and anti-oxidant activity<sup>61</sup>,

Table 4: Gross pathology examination

A.No.	Dose	External	Internal
1		NAD	NAD
2		NAD	NAD
3	2000	NAD	NAD
4	mg/kg	NAD	NAD
5		NAD	NAD
6		NAD	NAD

A. No. = Animal number; NAD = No abnormality detected

Table No. 5: Effect of *Eclipta alba* on Renal I/R injury induced changes in biochemical parameters

Group	Creatinine (mg/dL)	Urea (mg/dL)	BUN (mg/dL)
Sham control	0.44 ± 0.20	19.78 ± 1.57	9.24 ± 0.73
Control	1.24 ± 0.18	53.80 ± 2.68	25.14 ± 1.25
<i>Eclipta alba</i> (200 mg/kg)	1.04 ± 0.30	31.60 ± 5.32***↓	14.77 ± 2.49***↓
<i>Eclipta alba</i> (400 mg/kg)	0.72 ± 0.22**↓	29.20 ± 9.42***↓	13.64 ± 4.40***↓

\*\*\*\* p< 0.0001, \*\*\* p< 0.001, \*\* p<0.01, \*p<0.1 ns – non-significant

analgesic and anti-inflammatory activity<sup>62</sup>, hepatoprotective activity<sup>63-65</sup>, treatment of alopecia and hair growth<sup>66</sup>, proteolytic and hemorrhagic activity<sup>67,68</sup>, anticancer activity<sup>69,70</sup>, anti-hyperglycemic activity<sup>71</sup>, neuropharmacological activity<sup>72,73</sup>, anti-ulcer activity<sup>74,75</sup>, anti-allergic activity<sup>76</sup>, anti-HIV activity<sup>77</sup>, immunomodulatory activity<sup>78,79</sup>, anti-hyperlipidemic activity<sup>80</sup>.

The present study was investigated carefully so as to evaluate the nephroprotective activity of the methanolic extract of the whole plant of *Eclipta alba* against the renal stroke induced by the ischemic reperfusion injury in rats by means of hematological, biochemical and histopathological investigations.

## MATERIALS AND METHODS

### Chemicals and Instruments

#### Chemicals

Sodium carboxy methyl cellulose (CMC Na, Sigma-Aldrich), Ethylenediaminetetraacetic acid potassium salt (K<sub>2</sub> EDTA, Fischer Scientific), Diethyl ether (SRL chemicals), Thiopentone sodium, 70% Ethanol, Normal Saline, 10% Neutral buffered formalin.

#### Assay Kits

Creatinine and Urea Estimation Kits.

#### Instrumentation

Digital balance, Automated Haematology Analyzer XP-100 (Sysmex), micropipettes (20 µL, 50 µL and 1000 µL), Centrifuge (REMI Motors Ltd., Mumbai), Semi-Autoanalyser (Optima S, Lab India Health Care). Heparinised capillary tubes.

#### Plant Collection and Extract Preparation

*Eclipta alba* herbs were collected from the local areas in and around Vishakhapatnam and were authenticated by the

Table No. 6: Effect of *Eclipta alba* on Renal I/R injury induced changes in Haematological parameters

Group	RBC (×10 <sup>6</sup> µL)	Hemoglobin (gm/dL)	PCV(%)
Sham control	8.05 ± 0.62	14.43 ± 1.32	38.37 ± 2.55
Control	7.21 ± 0.44	13.64 ± 1.03	33.90 ± 2.48
<i>Eclipta alba</i> (200 mg/kg)	7.29 ± 0.35	13.72 ± 0.65	33.92 ± 2.02
<i>Eclipta alba</i> (400 mg/kg)	7.46 ± 0.20	14.36 ± 0.94	36.00 ± 1.97

\*\*\*\* p< 0.0001, \*\*\* p< 0.001, \*\* p<0.01, \*p<0.1 ns – non-significant

Department of botany, Andhra University. The extract was prepared according to the WHO protocol. The whole plant materials were shade dried and powdered. The whole powdered material was extracted by overnight soaking method by using methanol as a solvent (v/v). The content was filtered through Whatmann filter paper No. 1. Extraction procedure was repeated and the fractions were pooled, dried and stored in dark bottle at 4° C for further use.

#### Animals

Swiss albino mice weighing 25–30 g and adult albino rats of *Sprague Dawley* strain, weighing 150–200 g procured from Mahaveera enterprises, Hyderabad were used. The rats were housed in polypropylene cages at room temperature (25±3°C) with 12 hours light and dark cycle during the acclimatization period as well as the experimental period. They were fed with a balanced diet and tap water *ad libitum*. The food was withdrawn 18–24 hours before the experiment though water was continued. The study protocol was approved by the Institutional Animal Ethical Committee.

#### Phytochemical Analysis of the plant

The plant contains valid range of coumestans, alkaloids, flavanoids, glycosides, polyacetylenes, triterpenoids, saponins and others. The qualitative and quantitative estimation of the phytochemical constituents was described as below:

#### Quantification of Flavonoid content:

The sample is mixed with the aluminium chloride reagent and the mixture is incubated for 10-min. The absorbance was measured at 440 nm and the flavonoid content was expressed in mg/gm of quercetin.

#### Quantification of phenolic content

Volumetric Titration: The sample was titrated against potassium permanganate and the content of the unknown phenols was expressed in terms of standard equivalents.

#### Colorimetry

The Folin–Ciocalteu reagent reduces the samples containing polyphenols and thereby produces a blue coloured complex. Sample and standard (gallic acid) were mixed with the reagent and sodium bicarbonate. The mixture is incubated for 30-min. The absorbance was measured at 765 nm and the phenolic content was expressed in mg gallic acid equivalents.



Table No. 7: EGTI scoring of histopathology findings

Tissue type	Severity	Frequency of severity (n=5)			
		Sham Control	Control	<i>Eclipta alba</i> (200mg/Kg)	<i>Eclipta alba</i> (400mg/Kg)
Tubular	Minimal	0	0	2	3
	Mild	0	1	1	1
	Moderate	0	2	1	1
	Severe	0	2	1	0
Endothelial	Mild	0	0	1	3
	Moderate	0	2	3	2
	Severe	0	3	1	0
Glomerular	Mild	0	1	3	3
	Moderate	0	3	2	0
	Severe	0	1	0	0
Interstitial	Minimal	0	1	1	4
	Mild	0	3	3	0
	Moderate	0	1	1	0
	Severe	0	0	0	0

Lead acetate Test: 50 mg sample is dissolved in distilled water and then mixed with 3 mL of 10% lead acetate and a bulky white precipitate is formed.

#### Quantification of Saponins

Saponification Test: Sample was mixed with 0.5 N alcoholic KOH and a drop of phenolphthalein. The mixture is heated for 2 hrs. The formation of soap indicated the presence of fixed oils and fats.

#### Acute Toxicity Tests

According to the OECD test guideline 423, Swiss albino mice were randomly 77selected, marked and kept in cages for at least 7 days prior to dosing so as to allow acclimatization to laboratory conditions. The animals were fasted prior to dosing without food for 3 to 4 hours. The animals were weighed and the test substance was administered using an oral gavage. The limit test was performed at one dose level of 2000 mg/kg body weight with six animals (three animals per step). The animals were observed individually after dosing periodically during the first 24-hours and daily thereafter for a total period of 14 days. The signs of toxicity were observed carefully.

#### Induction of Ischemic-Reperfusion Injury

Ischemia-Reperfusion induced acute kidney injury (IR-AKI) is widely used as a model of AKI in rodents. AKI may result from the necrosis and apoptosis of renal epithelial cells. The present study was performed by using bilateral renal pedicle clamping of 45-min followed by 6-hour reperfusion in order to induce IR-AKI. Renal ischemia – reperfusion (IR) injury is induced via ventral (laparotomy) or dorsal (retro-peritoneal) approaches. The present study followed dorsal approach since it is less traumatic allowing faster recovery and improved survival.

#### Procedure

All the rats were anesthetized by using 50mg/kg intraperitoneally (i.p) of Thiopentone sodium.

The surgical site was clipped to keep hair from contaminating the incision site and made aseptic by using Betadine solution swab stick.

Kidney location was palpated through the skin. Dorsal skin is cut along the midline of rat (approximately 2.5 cm) using scissors and forceps.

A small incision was made through the right flank muscle and fascia above the kidney and exteriorized the right kidney.

Carefully dissected the upper and lower poles of the kidney and 4-0 silk suture was tied around right pedicle and another small suture was placed at the place of knot before tying, it was used as knot releaser after the end of ischemic period.

A small incision was made through the left flank muscle and fascia above the kidney and exteriorized the left kidney and the left renal pedicle is tied as described above. Both kidneys were covered with the skin and the skin incisions were in turn covered with saline soaked gauze. Timer was used for different clamp times.

At the end of indicated time, both kidneys were exteriorized, and release the knot by using the knot releasers and gently pushed back into the retroperitoneal space.

Animals in the sham control were surgically prepared, but, the renal pedicles were not occluded.

After the end of the reperfusion period, all the rats were anaesthetized by using diethyl ether and the blood samples were collected by puncturing the retro-orbital plexus by using heparinised capillary tubes. After collecting blood samples all the rats were euthanized by overdose of thiopentone sodium and both kidneys were harvested and stored in 10% neutral buffered formalin for further histopathological analysis.

#### Experimental Design

Animals were grouped into 4 with 5 animals in each group. *Group A -Sham Control:* The rats were fed on standard diet.

*Group B -Vehicle Control:* The rats were fed on standard diet and were treated with 0.9% sodium carboxy methyl cellulose orally for 7 days daily.

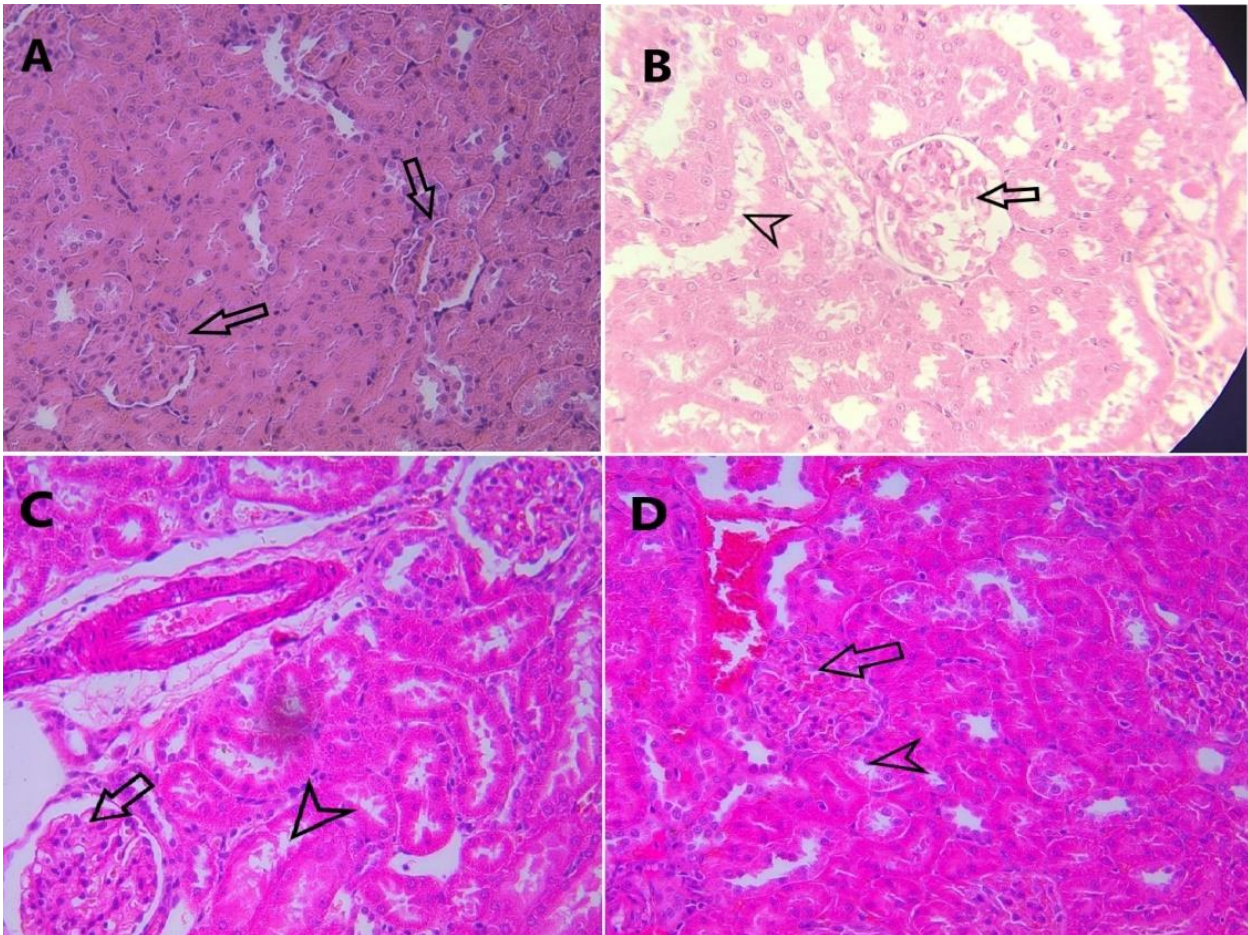


Figure 4: Effect of *Eclipta alba* on Histopathological changes induced by I/R injury (H&E X100).

A(Sham control) -Normal architecture of renal cortex with intact glomerulus; B(I/R control) -Severe degenerative changes included glomerular retraction (arrows) and tubular degeneration (pointed arrows); C(*Eclipta alba* 200 mg/kg) - Attenuated degenerative changes with mild glomerular retraction (arrow) and tubular degeneration (pointed arrows) in comparison to control; D(*Eclipta alba* 400 mg/kg) –marked improvement with minimal glomerular retraction (arrow) and tubular degeneration (pointed arrows) in comparison to I/R control.

**Group C – Low dose MEE:** The rats were administered with 200 mg/Kg body weight of methanolic extract of *Eclipta alba* in 0.9 % Sod. CMC vehicle orally for 7 days daily.

**Group D – High dose MEE:** The rats were administered with 400 mg/Kg body weight of methanolic extract of *Eclipta alba* in 0.9 % Sod. CMC vehicle orally for 7 days daily.

#### Assessment of Haematological Parameters

The haematological parameters were estimated by collecting blood samples from the rats on the 8<sup>th</sup> day by means of puncture in the retro-orbital plexus and then the samples were subjected to haematology analyser.

#### Assessment of Serum Biochemical Parameters

The rats were fasted overnight and the blood was collected by retro-orbital puncture using diethyl ether as anaesthetic agent. The samples were centrifuged to obtain serum. The collected serum is analysed for markers of renal impairment like creatinine, urea, BUN by using commercial enzymatic kits and a Semi-autoanalyser.

#### Histological Analysis

After the surgical IRI, the animals were sacrificed and the kidneys were collected in formalin solution. These kidney

tissues were then dehydrated in ascending grade series of alcohol and embedded in paraffin. Renal tissue specimens were cut into slices of 5  $\mu$ m thickness using a Histo-range microtome followed by staining with haematoxylin and eosin. These histological sections were carefully observed under electron microscope.

The IRI induced AKI insult was quantified and evaluated by means of Endothelial Glomerular Tubular and Interstitial Scoring system (EGTI Scoring System) represented in Table No. 1. The numerical scoring was based on the severity of the damage to the particular tissue type as shown in the following table:

#### Statistical Analysis

All the data was expressed as the mean  $\pm$  SD. Results were analysed by using ONE WAY ANOVA followed by Dunnet's Multiple Comparison Test through Graph Pad Prism and analysis was considered to be significant.

## RESULTS AND DISCUSSION

### Results

#### Acute Toxicity Studies

The methanolic extract of *Eclipta alba* at dose level of 2000 mg/Kg produced no mortality and adverse changes

in clinical sign examination (Table No.2). All the animals showed normal growth throughout the observation period (Table No. 3) and no changes were observed during gross pathology examination (Table No. 4). Therefore the selected doses are likely to be 200 mg/ Kg and 400 mg/Kg therapeutic lowest and highest doses respectively.

#### *Effect of MEE on Creatinine levels*

The effects of the selected doses of MEE were studied on creatinine levels in animals subjected to ischemia - reperfusion injury. The percentage protection in the renal marker of the blood serum treated with 200 mg/Kg of MEE was 16.13 and that with 400 mg/kg of extract was 41.94 respectively.

#### *Effect of MEE on Urea levels*

The pre-treatment of MEE at graded doses 200 mg/Kg and 400 mg/Kg produced a significant decrease in the serum urea levels and the percentage protection offered was 41.26 at 200 mg/Kg and 45.72 at 400 mg/Kg.

#### *Effect of MEE on BUN levels*

The effect of MEE on the serum blood urea nitrogen in the animals that suffered IRI. This pertained to a significant change when compared to the control group. The percentage protection in BUN levels in 200 mg/Kg treated group was 41.25 and that in 400 mg/Kg was 45.74.

#### *Effect of MEE on Hematological parameters*

The protective effect of the minimum (200 mg/Kg) and maximum (400 mg/Kg) doses of the MEE were studied on various hematological parameters like Red Blood Cell (RBC) count, Hemoglobin and Packed Cell Volume (PCV) and the percentage increase in the RBC count in the doses under study was 1.1 and 3.47 and that in the hemoglobin levels was 5.9 and 5.27 where as in case of PCV it was 0.06 and 6.19 respectively.

#### *Histopathological Findings*

The architecture of glomeruli, proximal and distal convoluted tubules of the treated groups was compared with the control group. The renal I/R injury revealed multifocal histological changes in the cortex and medulla (Fig No. 3). The tubular degeneration, glomerular retention, necrosis, desquamation of tubular epithelial cells was mild in the animals subjected to the pre-treatment of 200 mg/Kg and 400 mg/Kg doses of MEE when compared to the control group (Table No.7 and Fig No. 4).

## **DISCUSSION**

The kidney being a complex tissue was extremely vulnerable to I/R injury. The tissue injury and the activation of necrotic pathways attenuate the renal function with apparent decrease in the glomerular filtration rate and the clearance of metabolic by-products. The protection against renal I/R injury was significant in the groups of animals that were pre-treated with graded doses of MEE i.e., 200 mg/Kg and 400 mg/ Kg.

The protection was remarkable in the metabolic, hematological examinations that were performed on creatinine, urea, BUN, RBC count, hemoglobin (Hb) levels and PCV. The target extract claims for its abundant polyphenols that render prophylactic protection against the injury caused by ischemia- reperfusion.

The Histopathological investigations on the renal tissue similarly revealed a marked improvement in the integrity of the anatomical structure of the kidney thereby establishing the potential protective effect of the extract against the ischemia – reperfusion injury.

## **CONCLUSION**

The present study revealed that methanolic whole plant extract of *Eclipta alba* was potential to offer protection against the renal ischemia- reperfusion injury and improved the architecture of the renal tissue by decreasing the levels of the metabolic markers, increasing the hematological parameters and comprehensively safeguarding the renal tissue. The extensive approach of the study may therefore elucidate the supplementary benefits of the plant in renal health care and medicine.

## **ACKNOWLEDGEMENTS**

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