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Evaluation of Hepato-Renal and Haematological Protection of Ethanolic Extract of *Eclipta alba* in Experimental Rats

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Abstract: *Eclipta alba* has been widely used in the traditional medicine for the treatment of various liver and digestive ailments. In the present study, the effect of ethanolic crude extract of *E. alba* was investigated against body weight, organ weight, feed intake, nephritic markers, hepatic serum markers and haematological modification. The crude ethanolic extract of *E. alba* was obtained and the crude extract was administered to experimental rats. At the end of the treatment period, the level of feed intake, body and organ weight (liver and kidney) were determined. Further, serum enzyme markers, renal function tests and basic haematological screening were performed to analyse the effect of the plant extract. A marked rise was observed in the body weight and organ weight of the animals after intaking the feed. The hepatic serum markers were also observed in rising manner compared to the control group. The renal function markers were reduced significantly and the haematological levels were also increased. Our study showed significant medicinal effect of *E. alba* in experimental animals. The results also suggested that this ethanolic extract would be potential alternative for the management of hepatic and renal dysfunctions.

Keywords: *Hepatic markers, renal markers, hematological markers, ethanolic extract, Eclipta alba*

I. INTRODUCTION

Medicinal plants possess all the phytoconstituents which serves as a source of bioactive chemicals necessary for the significant pharmacological actions without any adverse effects. They can be used for the development of new classes of possibly safer drugs or medicines to cure various ailments. An increased resistance to present day available antibiotics has posed a problem worldwide due to the frequent use of antibiotics. The herbal drugs have always been used in healing various diseases because of the wide safety profile they provide. Important bioactive compounds contributing to the medicinal values in plants are alkaloids, glycosides, resins, gums, mucilages etc [1,2].

The search of plant-based products has completely modified the drug discovery programme as herbal compounds have shown a promising effect in therapeutics. The diversity in the disease causing ability of bacteria has always presented a challenge in the treatment of their infections [3]. Numerous plant-based substances show potential antitumour activity in several rodent and human cancer cell lines. Phytosignatures such as vitamins (A, C, E, K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to exhibit antioxidant activities [4].

Eclipta alba (L) is an annual herbaceous plant, commonly known as false daisy. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae. Various extracts of *E. alba* has exhibited potent antimicrobial activity [5,6], antioxidant power evaluated by DPPH and FRAP methods [7,8] and immunomodulatory properties [9]. *E. alba* possess various biological properties and used for the treatment of various disorders like memory disorders, general tonic, edema, rheumatic pain treatments, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders [10,11]. All the parts of *E. alba* contain various chemical constituents which have been used in different therapeutic cases. Major constituents are coumestans i.e. wedelolactone (I) and demethylwedelolactone (II), polypeptides, polyacetylenes, thiophene-derivatives, steroids, triterpenes and flavonoids [12]. Coumestans are known to possess estrogenic activity [13].

The decoction of leaves of *E. alba* is used in the treatment of jaundice and diabetes, and this plant is also used by the rural and tribals of India for treating diabetes and jaundice. Some studies showed hepatoprotective, anti-hyperlipidemic, *in vitro* anti-oxidant properties and attenuation of diabetic complications with *E. alba*. Some study showed that hepatoprotective activity of the extract of *E. alba* was by regulating the levels of hepatic microsomal drug metabolising enzymes [5].

Plants possess a wide variety of phytoconstituents such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins and lignins [14]. Various extracts of *E. alba* were subjected to analysis and identification of phytoconstituents responsible for the wide array of biological activities [15]. The extracts revealed the presence of various compounds like hydrazine carboxamide, naphthoquinones, glycine, carbamic acid etc., Hydrazine carboxamide is reported to possess antimicrobial activity and showed inhibition against some bacterial strains like *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and some fungal species, *Aspergillus niger, A flavus, Pencillium citrinum, Candida albicans* and *Monascus purpereus* [16], which is also exhibited in our study where the extracts were found to be highly antimicrobial in nature which may be attributed to the compounds present in various extracts [14].

Naphthoquinones are categorized under the category of phenolic compounds. They act by binding to DNA and proteins (enzymes) and inhibit the process of replication. They causes disturbance in cell and mitochondrial membranes leading to the interference with the respiratory chain electrons on the mitochondrial membranes which may be attributed to the anticancer activity of the plant [14,15]. Derivatives of naphthoquinone showed numerous biological activities such as antibacterial, antiviral, antitumour, cytotoxic, insect repellent, anti inflammatory and antipyretic properties [17]. Plants with such constituents were used for the treatment of malignant and parasitic diseases in China and countries of South America.

Glycine is an essential amino acid used as a metabolic product for the growth of bacteria. In excess inhibits the growth of bacteria and used as a non-specific antiseptic agent. Esters of carbamic acid are used as muscle relaxants [17]. The phytoconstituents identified in the extracts of *E. alba* were found to be antibacterial, antitumor and antioxidant in nature so the plant can be used in herbal drug formulation as a cure to respiratory infections which arises due to bacterial pathogenecity, oxidative stress caused by the overproduction of free radicals that leads to the cancer when under uncontrolled conditions [17].

This plant known to possess important medicinal properties, as it is a rich source of anthraquinones, flavonoids, polysaccharides, sterols, phenolic compounds and coumarins. The present study was undertaken to evaluate the ethanolic extract of *E. alba* on body weight, organ weight, hepatic serum markers, nephritic markers and haematological modifications in the experimental animals.

II. MATERIAL AND METHODS

2.1. Herb collection and authentication

The leaves of *Eclipta alba* were collected from Lalgudi region of Tiruchirapalli, Tamilnadu. The leaves were identified and authenticated by the senior Botanist and Taxonomist. The specimen was deposited in our laboratory.

2.2. Extraction

The leaves of the plant were shadow dried and powdered coarsely. The powdered plant material (100grams) was macerated and extracted with ethanol (500ml) for 18 hours. Further the solvent was removed using rotary evaporator below 60^{o}C, kept in the desiccator for several days to completely remove the traces of solvent and dried extracts were separated and stored. The weight of the extract was found to be 5.9 grams and the percentage yield of ethanolic extract was found to be 5.9% w/w.

2.3. In vivo analysis

2.3.1. Acute toxicity test

The acute oral toxicity study of ethanolic extract of *E. alba* was performed according to the Organization for Economic Co-Operation and Development (OECD)-425 guidelines. Single dose of the extract 2000mg/kg, p.o., was administered in 24hours fasted rats (n=3) and rats were observed at 0, 30, 60, 120, 180, and 240min and then once a day for the next 14 days for any signs or symptoms of toxicity or abnormalities.

2.3.2. Extract administration

The crude ethanolic extract of leaves of *E. alba* was primarily experimented for increase in body and organs weight in experimental animals (Wister Albino rats) by feeding them orally in six group of animals [Group I – control; Group II – 400mg/kg of ethanolic crude extract; Group III – 800mg/kg of ethanolic crude extract; Group IV – 1200mg/kg of ethanolic crude extract; Group V – 1600mg/kg of ethanolic crude extract and Group VI – 2000mg/kg of ethanolic crude extract].

2.3.3. Hepatic marker analysis

The hepatic serum levels were estimated in the experimental animals by following the determination of marker enzyme levels including aspartate amino transferase [18], alanine aminotransferase [18], acid phosphatase [19] and alkaline phosphatase [20].

2.3.4. Nephritic marker analysis

The nephritic markers were also determined by Varley's method [21]. The separated serum samples were analysed for uric acid, urea and creatinine. Creatinine was determined by the alkaline picrate method, urea, by the urease-hypochlorite method and uric acid, by the uricase-peroxidase method [22] and all these methods were based on manufacturer's instructions.

2.3.5. Haematological investigation

For haematological investigations, the blood was collected from tail vein and used for estimation of haemoglobin (Hb) [23], red blood cell (RBC) count and White blood cell (WBC) count [24,25].

III. RESULTS

3.1. Acute toxicity study

The ethanolic extract of *E. alba* at the dose of 2000 mg/kg body weight did not exhibit any behavioural changes or symptoms of toxicity. Hence the drug was found to be safe up to the tested dose of 2000mg/kg body weight.

3.2. Effect on body and organ weight

The feed intake was increased upto 37.67g (2000mg/kg extract) compared to control animals (35.14g). The body weight of the experimental rats also increased upto 130.22g while administering 2000mg/kg of ethanolic extract. The control animals have the body weight of 127.43±1.27 (Table 1). The weight of the liver also improved from 12.59±0.20 (control) to 13.73±0.20 (2000mg/kg administered ethanolic extract). The kidney weight also markedly elevated upto 4.92±0.21 (Table 1).

Table 1: Effect of ethanol crude extract of *E. alba* on body weight, organ weight and feed intake in experimental rats

Experimentation	Observation of variations in the body and organ weight in experimental rats					
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
Feed intake (g)	35.14 (±) 2.90	35.79 (±) 2.00	35.90 (±) 2.44	36.49 (±) 2.06	37.18 (±) 2.59	37.67 (±) 2.75*
Body weight (g)	127.43 (±) 1.27	127.77 (±) 1.97	128.44 (±) 1.76	129.27 (±) 1.39	129.88 (±) 2.32	130.22 (±) 1.66*
Liver weight (g)	12.59 (±) 0.20	12.66 (±) 0.26	12.76 (±) 0.16	12.84 (±) 0.11	13.63 (±) 0.25	13.73 (±) 0.20*
Kidney weight (g)	4.22 (±) 0.08	4.33 (±) 0.15	4.41 (±) 0.30	4.64 (±) 0.30	4.77 (±) 0.35	4.92 (±) 0.21*
Values are expressed as mean±S.D (n=6). Statistically significant are expressed as *p<0.05 The values of Group II, III, IV, V and VI are significant in comparison to the values of Group I.						
Group I – Control; Group II – 400mg/kg methanolic crude extract; Group III – 800mg/kg; Group IV – 1200mg/kg; Group V – 1600mg/kg; Group VI – 2000mg/kg)						

3.3. Effect on Hepatic marker analysis

The hepatic marker enzyme levels are elevated and markedly increased compared to the control animals. The maximum elevation was observed in the 2000mg/kg administration of ethanolic extract of *E. alba*. The amino transferase enzymes (aspartate and alanine) increased from 61.80±1.07 and 26.59±1.21 to 62.86±1.12 and 27.94±1.43 respectively. The phosphatise group of enzymes (acid and alkaline) also significantly increased from 15.54±1.30 and 172.73±1.42 to 16.13±1.04 and 174.71±1.21 respectively while administering 2000mg/kg body weight of experimental rats (Table 2).

Table 2: Effect of ethanol crude extract of *E. alba* on amino transferases and phosphatases enzymes in experimental rats

Experimentation (µ moles of pyruvate liberated/ mg protein/ hour)	Observation of variations in the animo transferases and phosphatise enzyme levels in experimental rats					
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
Aspartate amino transferases	61.80 (±) 1.07	61.89 (±) 1.02	61.93 (±) 1.28	62.18 (±) 1.12	62.60 (±) 1.05	62.86 (±) 1.12*
Alanine amino transferases	26.59 (±) 1.21	26.70 (±) 1.32	26.87 (±) 1.28	27.19 (±) 1.39	27.57 (±) 1.26	27.94 (±) 1.43*
Acid phosphatase	15.54 (±) 1.30	15.67 (±) 1.33	15.74 (±) 1.39	15.86 (±) 1.07	15.94 (±) 1.46	16.13 (±) 1.04*
Alkaline phosphatase	172.73 (±) 1.42	172.98 (±) 1.54	173.32 (±) 1.12	173.67 (±) 1.49	174.34 (±) 2.14	174.71 (±) 1.21*
Values are expressed as mean±S.D (n=6). Statistically significant are expressed as *p<0.05 The values of Group II, III, IV, V and VI are significant in comparison to the values of Group I.						
Group I – Control; Group II – 400mg/kg methanolic crude extract; Group III – 800mg/kg; Group IV – 1200mg/kg; Group V – 1600mg/kg; Group VI – 2000mg/kg)						

3.4. Effect on Nephritic markers

The nephritic marker levels are reduced and significantly decreased compared to the control animals. The maximum reduction was observed in the 2000mg/kg administration of ethanolic extract of *E. alba*. The urea

level decreased from 5.78±1.51 mg/dL to 5.62±1.13. The uric acid level also significantly decreased from 4.38±1.14 mg/dL to 4.29±1.16 and creatinine level get reduced from 0.70±0.19 to 0.57±0.18 mg/dL while administering 2000mg/kg body weight of experimental rats (Table 3).

Table 3: Effect of ethanol crude extract of *E. alba* on nephritic markers in experimental rats

Experimentation mg/dL	**Observation of variations in the nephritic marker levels in experimental rats**					
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
Urea	5.78 (±) 1.51	5.87 (±) 1.49	5.62 (±) 1.13	5.66 (±) 1.35	5.70 (±) 1.14	5.74 (±) 1.12*
Uric acid	4.38 (±) 1.14	4.64 (±) 1.27	4.49 (±) 1.14	4.38 (±) 1.10	4.29 (±) 1.16	4.36 (±) 1.08*
Creatinine	0.70 (±) 0.19	0.76 (±) 0.17	0.65 (±) 0.19	0.57 (±) 0.18	0.67 (±) 0.16	0.72 (±) 0.16*
Values are expressed as mean±S.D (n=6). Statistically significant are expressed as *p<0.05 The values of Group II, III, IV, V and VI are significant in comparison to the values of Group I.						
Group I – Control; Group II – 400mg/kg methanolic crude extract; Group III – 800mg/kg; Group IV – 1200mg/kg; Group V – 1600mg/kg; Group VI – 2000mg/kg)						

3.5. Effect on haematological investigation

The haematological investigations were also well analyzed in this study are elevated and significantly increased compared to the control animals. The maximum elevation was observed in the 2000mg/kg administration of ethanolic extract of *E. alba.* The haemoglobin (Hb) level increased from 11.86±0.75 (control - Group I) to 12.03±0.68 (Group VI). The White blood cell (WBC) level also significantly increased from 7.44±1.11 to 7.96±0.19 mm^3 and red blood cell (RBC) level get elevated from 6.68±0.80 to 7.50±0.95 mm^3 while administering 2000mg/kg body weight of experimental rats (Table 4).

Table 4: Effect of Ethanolic crude extract of *E. alba* on haematological investigations in experimental rats

Experimentation	**Observation of variations in the nephritic marker levels in experimental rats**					
	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
Haemoglobin (%)	11.86 (±) 0.75	11.70 (±) 0.79	11.48 (±) 0.70	11.62 (±) 0.75	11.74 (±) 0.73	12.03 (±) 0.68*
White blood cells (mm^3)	7.44 (±) 1.11	6.57 (±) 0.61	6.65 (±) 0.61	6.94 (±) 0.57	7.34 (±) 0.46	7.96 (±) 0.19*
Red blood cells (mm^3)	6.68 (±) 0.80	6.60 (±) 0.71	6.66 (±) 0.75	7.31 (±) 0.71	7.45 (±) 0.89	7.50 (±) 0.95*
Values are expressed as mean±S.D (n=6). Statistically significant are expressed as *p<0.05 The values of Group II, III, IV, V and VI are significant in comparison to the values of Group I.						
Group I – Control; Group II – 400mg/kg methanolic crude extract; Group III – 800mg/kg; Group IV – 1200mg/kg; Group V – 1600mg/kg; Group VI – 2000mg/kg)						

IV. DISCUSSION

The objective of the present study was fulfilled that the protective effect of crude ethanolic extract of *E. alba* (400, 800, 1200, 1600 and 2000mg/kg body weight) against experimental rat models. The ethanolic extract (2000mg/kg) dose-dependently increased the body and organ weights. The hepatic markers get increased significantly and nephritic markers reduced. The haematological investigations and its observation showed marked increase in all parameters.

Literature survey reveals that ethanolic extract of *E. alba* is a rich source of triterpenoids, phenolics, and steroids. Hepatoprotective activity of *E. alba* is evident by regulating the levels of hepatic microsomal drug metabolising enzymes. Similarly hepatic lesions caused by CCl4 were improved by treatment with both ethanolic extracts of *E. alba* [26,27]. The hepatoprotective effect of the ethanol/water (1:1) extract of *E. alba* was studied at subcellular levels in rats against CCl4 induced hepatotoxicity. The loss of hepatic lysomal acid phosphatase and alkaline phosphatase by CCl4 was significantly restored by *E. alba* at the end of the study period. The study showed that hepatoprotective activity of *E. alba* was regulating the levels of hepatic microsomal drug metabolising enzymes [15,26].

Antioxidants are compounds that help to inhibit many oxidation reactions caused by free radicals, which damage to the cells and tissues. As antioxidants play an important role in inhibiting and scavenging

radicals thereby providing protection to humans against infection and the degenerative diseases. However, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for medicinal materials to replace synthetic antioxidant [28].

E. alba offers a remarkable activities for curing of many diseases. It has a wide range of chemical constituents. Clinical investigations have been done on pharmacological activities like hepatotoxicity, proliferative, diabetic, hypolipedemic etc [17]. Some studies it is revealed a novel mechanism by which this particular chloroform fraction of *E. alba* inhibits growth of breast cancer cells *in vitro* and *in vivo*, and involves specific activation of mitochondrial apoptotic pathways and localization of robustly upregulated Hsp60 in the ER [29].

A combination of ethanolic extract of *E. alba* leaves and *Piper longum* demonstrated better hepatoprotective action against CCl4-induced hepatotoxicity in rats [30, 31]. Serum marker enzymes like alanine aminotransferase (ALT/GOT), aspartate aminotransferase (AST, also known as GOT), acid phosphatase (AP), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), and 5'-nucleotidase were elevated with carbon tetrachloride treatment, which were restored towards normalization by the combined extract. At the same time, changes in biochemical parameters like total protein, total bilirubin, total cholesterol, triglycerides, and urea were restored to near normal levels with the combined extract [31]. Thus the present study proved that *E. alba* provided a foot print of having hepato-renal protection.

V. CONCLUSION

In this study, the ethanolic extract of *E. alba* influencing in the restoring of the hepatic and nephritic markers. Further, pharmacological and chemical studies are required to explore the mechanism of action of active ingredient(s) responsible for the antioxidant activity observed.

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