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Hepatoprotective and Curative Effect of *Eclipta prostrata* on CCl_4 Induced Hepatotoxicity in Albino Rats

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The combined antioxidant and hepatoprotective effect of *Eclipta prostrata* was evaluated against carbon tetra chloride (CCl_4) induced hepatic damage in wistar albino rats. Ethanolic extract from the *Eclipta prostrata* at a dose level of 200mg/kg of body weight was administered orally daily once for 15 days. The substantially elevated serum marker enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and the antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase were found due to CCl_4 treatment. The levels of above mentioned enzymes were brought to near normalcy after administration of plant extract. The biochemical parameters like total protein, total bilirubin were also restored towards normal levels. In addition *Eclipta prostrata* significantly decrease the liver weight of CCl_4 intoxicated rats. Silymarin at a dose level of 25mg/kg was used as a standard reference drug for comparison.. The results of this study strongly indicate that *Eclipta prostrata* is having a potent hepatoprotective action against CCl_4 induced hepatic damage in rats.

Key words: Hepatoprotective, Marker Enzymes, *Eclipta prostrata*, Carbon Tetra Chloride.

Liver, is a vital organ, it has a wide range of functions, such as detoxification, protein synthesis, and production of biochemicals necessary for digestion[1]. Hepatic damage is associated with some metabolic function[2]. Liver disease is still a worldwide health problem[3] Treatment option may vary depending on the causes of liver disease unfortunately, conventional or synthetic drugs used in the treatment of liver diseases, and sometimes can have serious side effect[4]. In modern medicine there are number of medicinal preparations in Ayurveda recommended for the treatment of liver disorder[5]. In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systemic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases[6]. Therefore an effective formulation has to be developed using medicinal plants, with proper pharmacological experiments and clinical trials.

The ethanolic extract of *Eclipta prostrata* was subjected to various assays in order to evaluate their hepatoprotective effect against CCl_4 toxicity in albino rats. This plant have traditional claim against liver disorders and all of them are scientifically evaluated for their potency individually[7]. The plant *E.prostrata* is available for the treatment of various liver disorders[8]. The Preliminary phytochemical analysis of the *Eclipta*

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prostrata reveals that the presence of flavonods, alkaloids, tannins,phenols, teroids, terpenoids, carbohydrate, protein and aminoacids.[9, 10] The activity of the *E.prostrata* against CCl_4 toxicity was compared with a well-known antihepatotoxic agent silymarin.[11,12] So the present study the antihepatotoxic property of *Eclipta prostrate* was tested against CCl_4 treated animal model by measuring the substantially elevated serum marker enzymes such as Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and the antioxidant enzymes such as glutathione peroxidase, superoxide dismutase and catalase were examined.

MATERIALS AND METHODS

The plant materials were collected from commercial medicinal shop at kumbakonam. The plant was identified and authenticated taxonomically by Dr. S. Kalavathy, Associate Professor, Department of Botany, Bishop Heber College, Trichy. The leaves of *E.prostrata* (1kg) were shade dried and pulverized to a coarse powder.

Extraction

Equal quantities of the powder was passed through 40-mesh sieve and exhaustively extracted with 95% (v/v) ethanol in Soxhlet apparatus at 60°C. The extract was evaporated under pressure until all the solvent had been removed and further removal of the water was carried out by freeze drying. The extract was stored in refrigerator; weighed amount was used for present investigation.

Phytochemical Analysis

Phytochemical analysis were carried out qualitatively and quantitatively to identify the presence and quantify various secondary metabolites such as flavonoids, alkaloids, saponins and tannins by HPLC.

Experimental animals

Male albino Wistar rats weighing 200–230 g were used in all experiments. Animals were maintained on 12 h light/dark cycle at approximately 27°C. The animals were fed on a commercial pelleted rat chow (Hindustan Lever Ltd, Mumbai) and water *ad libitum*. Experiment was performed according to ethical guidelines for the investigation of experimental pain in conscious animals. The animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature, acute toxicity study was carried out as per "up and down" or "stair case" method[13].

Experimental design

Hepatic injury was induced in rats by intraperitoneal administration of a single dose of 1 ml/kg CCl_4 along with olive oil in the ratio of 1:1 (v/v). Sylimarin, a known hepatoprotective agent was used as reference drug. Animals were grouped as follows

Group I. Control group, treated with saline (2.0 ml daily) for 15 days.

Group II. Treated with saline (2.0 ml daily) for 15 days followed by CCl_4 (1ml/Kg) on day 15.

Group III. Treated with ethanolic extract of *Eclipta prostrate* (200mg/kg) daily for 15 days followed by CCl_4 on day 15.

Group IV. Treated with sylimarin (25mg/kg) daily for 15 days followed by CCl_4 on day 15.

On the 15th day the animals were sacrificed and various biochemical parameters were analyzed.At the end of the treatment, blood samples of all animals were collected in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the assay.

Biochemical and statistical analysis

The serum bilirubin[14] ,protein[15], superoxide Dismutase[16,17], catalase[18] , Glutathione peroxidase[19], ALT [20], AST [20], ACP [2] and ALP[22] were estimated. All the enzymatic and biochemical assays were taken at particular nm using Shimadzu spectrophotometer. Values reported as Mean ± Standard Deviation. The statistical analysis was carried out using analysis of variance (ANOVA) followed by student T test, P values >0.001 were considered as significant.

RESULTS AND DISCUSSION

In the present study ethanolic extract of *Eclipta prostrata* were evaluated for hepatoprotective activity using CCl_4 in rat model. Necrosis or membrane damage releases the AST, ALT into circulation, hence it can be measured in the serum. High levels of AST indicates liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury, AST catalyses the conversion of alanine to pyruvate

Table 1 . Quantitative analysis of secondary metabolites of the *Eclipta prostrata* using HPLC

S. No.	Secondary Metabolits	Amout Mg/Kg
1.	Total Alkaloids	0.55
2.	Total Flavonoids	1.97
3.	Tannin	0.06
4.	Lignin	0.08
5.	Phenol	0.11
6.	Sterol	0.06
7.	Saponin	0.15
8.	Quinones	0.06
9.	Coumarins	0.04
10.	Terpenoids	0.03
11.	Vitamins	98

and glutamate and is released in a similar manner. Phytochemical analysis results showed qualitatively and quantitatively identified the presence of various secondary metabolites such as flavonoids, alkaloids, saponins and tannins were studied (Table 1). Therefore ALT is more specific to the liver, and is thus a better parameter for detecting liver injury. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [23]. Serum ALP, ACP, bilirubin and total protein levels on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary pressure[24].

Administration of CCl_4 caused a significant elevation of enzyme levels such as AST, ALT, ALP, ACP, total bilirubin and decrease in total protein when compared to control (Table 2). There was a significant restoration of these enzyme levels

Table 2. The effect of ethanolic extract of *Eclipta prostrata* on biochemical parameters and marker enzymes of liver

Parameter	Group1	Group2	Group3	Group4
Total protein (mg/dl)	7.31±0.06	5.4± 0.003*	6.8±0.22**	7.1±0.13*
AST (IU/L)	119.7±1.3	383.7±1.82*	289.2±1.20**	245.4±2.3**
ALT (IU/L)	37.39±3.2	133.9±1.94*	85.1±1.55**	79.8±4.7**
ACP (IU/L)	15.07±0.81	87.3±7.9*	35.7±1.44**	33.6±2.4**
ALP (IU/L)	110.9±0.031	339±0.77*	216.0±0.12**	175.7±0.9**
Total Bilirubin (mg/dl)	0.43±0.04	1.6±0.77*	0.63±0.06**	0.57±0.01**
SOD (units /mg ptn)	77.03±3.91	45.87±0.50*	81.07±0.77**	88.34±2.54**
CAT(nmoles of H_2O_2 utilised /mt/mg ptn)	293.73±13.05	147.73±5.78*	283.2±11.92***	268.27±6.465**
Glutathione peroxidase(nmoles of GSH/ mt/ mg ptn)	0.993±0.07	0.61±0.02*	0.9±0.06**	0.95±0.03***

Values are mean ±SD. Stastistical significant test for comparison was done by ANOVA, followed by T test (n=6) *p< 0.05 vs control, **p< 0.01 vs control. ***p< 0.001 vs control

Table 3. Antioxidant activity of ethanolic extract of *Eclipta prostrata* using Hydrogen peroxide assay and phospho molybdate method

S. No.	Concentration (mg/ml)	% of Inhibition	
		H_2O_2	Phospho molybdate
1.	50	0.4991	0.5432
2.	100	0.6089	0.5925
3.	150	0.7513	0.6639
4.	200	0.7954	0.8010

on administration of the plant extract and also by silymarin at a dose of 25mg/kg. The reversal of increased serum enzymes in CCl_4 induced liver damage by the extract may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity [25]. Effective control of ALP, ACP, total bilirubin and total protein levels points towards an early improvement in the secretary mechanism of the hepatic cells. The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been distributed by a hepatotoxin. Both silymarin and the plant extract decreased CCl_4 induced elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells[26.]

Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in liver injury[27]. SOD has been reported as one of the most important enzymes in the antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus diminishing the toxic effect caused by this radical. In *Eclipta prostrata* causes a significant increase in hepatic SOD activity and thus reduces reactive free radical induced oxidative damage to liver. CAT is widely distributed in all animal tissues, red cells and liver. CAT decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals [28]. Therefore reduction in the activity of CAT may result in a number of deleterious effects due to the assimilation of superoxide radical and hydrogen peroxide (Table 3). Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. It removes free radical species such as hydrogen peroxide, superoxide radical and maintains membrane protein thiol. Also it is substrate for glutathione peroxidase[29]. Decreased level of GSH is associated with an enhanced lipid peroxidation in CCL_4 treated rats. Administration of *Eclipta prostrata* significantly increased the level of GPX. Preliminary phytochemical studies reveal the presence of flavonoids and phenols in extract posses various activities such as hepatoprotective activity[30], antioxidant activity, antidiabetic activity, antifungal and antibacterial activity[30.31].

In conclusion the *E.prostrata* ethanolic extract afforded protection from CCl_4 induced liver damage the protections against liver damage by the plant were found comparable to silymarin [32,33]. Possible mechanism that may be responsible for the protection of CCl_4 induced liver damage by *E.prostrata* may be it could act as a free radical scavenger intercepting those radicals involved in CCl_4 metabolism by microsomal enzymes. By trapping oxygen related free radicals the extract could hinder their interaction with polyunsaturated fatty acids and would abolish the enhancement of lipid peroxidative processes[34, 35, 36]. It is well documented that flavonoids and glycosides are strong antioxidants[37, 38]. Antioxidant principles from herbal resources are multifaceted in their effect and provided enormous scope in correcting the imbalance through regular intake of a proper diet. Hence we conclude the administration of *E.prostrata* is a promising hepatoprotective agent and this hepatoprotective activity of the plant may be due to its antioxidant chemicals present in it.

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