

Antioxidant Activity of *Eclipta alba* L. in Normal Rat Liver

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ABSTRACT

The present study investigated the antioxidant potential of the ethyl acetate fraction of the aerial parts *Eclipta alba* L. Hassk (EA) which was widely used as hepatoprotective plant. EA was orally administered at doses of 50, 100 and 200 mg/kg (n=6) for 7 days in male Charles Foster rats. The extent of hepatoprotective potential of *E. alba* was studied by assessing the biochemical parameters like lipid peroxides (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), ascorbic acid and α -tocopherol in the liver of rats. Oral administration of the EA significantly decreased LPO and elevated the activity of antioxidant enzymes SOD, CAT, GPx and GR as well as endogenous levels of ascorbic acid and α -tocopherol in the liver of male Charles Foster rats. This study has revealed the significant antioxidant potential of *E. alba* in rat liver.

Keywords: comma separated keywords

Eclipta alba L. Hassk. (Asteraceae), commonly known as Bhingaraja (Sanskrit), Maka (Marathi), and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice [1]. The plant has been reported for the treatment of liver cirrhosis and infective hepatitis [2]. *E. alba* (EA) powder has been found to counteract carbon tetrachloride (CCl₄)-induced increase in liver weight, hepatic lipid peroxidation, liver γ -glutamyl transpeptidase, serum alanine transferase, serum alkaline phosphatase and serum albumin to globulin ratio in rats [3]. According to Ayurvedic text, EA is the main herb for hair and serosis; maintaining and rejuvenating hair, teeth, memory, sight and healing [4]. It also possesses an anti-aging property.

The plant has been reported to exhibit protective effect on CCl₄-induced acute liver damage by reducing centrilobular necrosis, hydropic degeneration and fatty change of the hepatic parenchymal cells [5]. Wagner *et al.* (1986) confirmed that the coumestan constituents of the plant, wedelolactone and demethylwedelolactone, are responsible for the potent antihepatotoxic activities in CCl₄-, galactosamine- and phalloidin-induced liver damage in rats. However, there are no reports on the effect of the plant on endogenous liver antioxidant system. On the basis of earlier reports [6], it was considered worthwhile to take the ethyl acetate fraction

of *Eclipta alba* (EA) for evaluating the antioxidant activity in liver. Hence, the present study aimed to assess the antioxidant potential of EA by estimating the activity of antioxidants (enzymatic and non-enzymatic) and levels of lipid peroxides on oral administration.

MATERIAL AND METHODS

Plant material

Aerial parts of *E. alba* L. Hassk. were procured and authenticated by Dr. Ashish Phadke, Head, Department of Draavyaguna (Herbal Pharmacology), Y.M.T. Ayurvedic Medical College, Khargar, Navi Mumbai, Mumbai, India. Specimen sample of *Eclipta alba* was preserved in the Pharmacognosy department of Bombay College of Pharmacy, Mumbai, India with the voucher no. 14/E. alba/ Majumdar/ Feb' 2004.

Animals

Inbred male Charles Foster rats (150-200 g) were used. Ethical clearance for the study was obtained from the Institutional Animal Ethics Committee of the Bombay College of Pharmacy. They were housed in standard environmental conditions and allowed free access to standard laboratory rat pellet diet and water *ad libitum*.

Table 1. Effect of EA administration on the antioxidant defense system in rat liver

Parameters	Control	EA 50 mg/kg	EA 100 mg/kg	EA 200 mg/kg
SOD activity ^a	1.35 ± 0.05	1.27 ± 0.04	2.36 ± 0.27*	3.17 ± 0.29**
CAT activity ^a	10.28 ± 0.66	12.89 ± 1.45	23.40 ± 1.40**	25.05 ± 2.91**
GPx activity ^a	0.35 ± 0.08	0.31 ± 0.05	0.61 ± 0.04*	4.69 ± 0.28**
GR activity ^a	9.51 ± 0.98	13.34 ± 1.84	25.91 ± 2.81**	29.72 ± 4.59**
Lipid peroxidation ^b	4.14 ± 0.44	1.44 ± 0.16**	1.11 ± 0.23**	0.44 ± 0.22**
Vitamin C ^c	0.72 ± 0.01	0.98 ± 0.10*	1.09 ± 0.01**	1.23 ± 0.02**
Vitamin E ^c	0.19 ± 0.02	0.27 ± 0.00*	0.39 ± 0.02**	0.41 ± 0.01**

All values are expressed as the mean ± SD, for 6 rats in each group. ^a Units (mg protein)⁻¹, ^b nmol of MDA (mg protein)⁻¹ and ^c µg (mg protein)⁻¹. **p* < 0.05, ***p* < 0.01, different from control-vehicle treated group evaluated by one-way analysis of variance test followed by Mann-Whitney *U*-test, or Kruskal Wallis's test, if necessary.

Preparation of the Fraction

The aerial parts of the plant *E. alba* were shade dried and the coarse powder (1 kg) was exhaustively extracted with methanol in a soxhlet extractor (yield: 15.7% w/w). The solvent was evaporated and the extract was suspended in water and heated on a steam bath (30 min, < 80° C). This methanolic extract was filtered under vacuum using Whatmann paper and air dried. The aqueous phase was partitioned with ethyl acetate five to six times. The organic fraction was dried with sodium sulphate and the solvent evaporated to yield ethyl acetate fraction of *Eclipta alba* (EA). The yield of the fraction was 2.26% (w/w).

Determination of total phenolic content of EA

The assay of total phenolic content of EA was determined as per the procedure stated by Randolph (1995) in Association of Agricultural Chemist [7].

Acute toxicity evaluation of the fraction

Acute toxicity studies were performed according to Organization for Economic Co-Operation and Development (OECD)-423 guidelines [8].

Experimental procedure

Young adult Charles Foster rats were divided into four groups. Group 1 served as control and groups 2, 3 and 4 were administered a dose of 50, 100 and 200 mg/kg 0.3% carboxymethyl cellulose suspension of EA in distilled water respectively by oral gavage once daily between 10.00 am and 11.00 am for 7 days. Animals were sacrificed 1 h after the last drug or vehicle administration on day 7. A 10% liver homogenates prepared in ice-cold 20 mM phosphate buffer (pH 7.0) was incubated with 0.1% tritonX-100 for 1h at 5°C and centrifuged (20000×g, 20 min, 4°C). The supernatants were used for measuring activity of antioxidant enzymes such as CAT [9], SOD [10], GPx [11] and GR [12]. The results were expressed as units/mg protein. Further, 10% liver homogenates prepared in KCl (150 mM) was used for determination of LPO [13], ascorbic acid [14] and α-tocopherol [15]. The protein content of the rat liver homogenate was determined by the method of Biuret reaction using Bovine serum albumin (BSA) as a standard [16].

Statistical analysis

All results were evaluated by one-way analysis of variance test followed by Mann-Whitney *U*-test or Kruskal Wallis's test, if necessary. Significance was set at *p* < 0.05.

RESULTS AND DISCUSSION

The results are shown in Table 1.

Effects of EA on enzymatic antioxidant system

The present study revealed that oral administration of EA for 7 days at a dose of 100 mg/kg and 200 mg/kg in rats significantly elevated the activity of the enzymes involved in scavenging reactive oxygen species such as superoxide dismutase and catalase. This indicated that EA improved the enzymatic antioxidant status in rat liver since it is known that a marked increase in SOD and CAT activity can offer first line protection against the damaging effects of superoxide radicals in the liver [17]. EA administration for 7 days at a dose of 100 mg/kg and 200 mg/kg resulted in improved activity of enzymes GPx and GR reflecting elevated levels of GSH in rat liver, which may be one of the key mechanisms mediating the potent hepatoprotective action of *E. alba*. However no significant change in the enzyme levels were observed in the groups treated with 50 mg/kg.

Effects of EA on non-enzymatic antioxidant system

EA treatment significantly inhibited lipid peroxides in rat liver in a dose-dependent manner. This proves the potent antioxidant potential of EA, since it is known that oxygen radicals produce peroxidation of lipids leading to disruption of cell membrane and structural damage to the tissue [18]. EA was also found to significantly improve the levels of cellular antioxidants vitamin E and vitamin C in rat liver. Vitamin E is the first antioxidant present in the cell membrane that counteracts the entry of free radicals in the cells and ultimately gets transformed into tocopheroxyl radicals [19]. Vitamin C acts as a co-antioxidant by regenerating α-tocopherol from α-tocopheroxyl radical produced during scavenging of reactive oxygen species (ROS) [20]. Although vitamin E levels were elevated distinctly only at 100 and 200 mg/kg dose, vitamin C levels in the

liver homogenates were significantly elevated in all the three groups treated with 50, 100 and 200 mg/kg. Thus, EA imparted antioxidant activity by elevating the levels of endogenous antioxidants vitamin E and vitamin C.

These observations are consistent with the conclusion that the ethyl acetate fraction of *E. alba* improves both enzymatic and non enzymatic antioxidant status in rat liver. Further acute toxicity studies of EA at a dose of 2 g/kg p.o. revealed no evident toxicity or mortality, thereby confirming the traditional safety profile of *E. alba* as per the citation [1].

Increased oxidative stress has been suggested to play a major role in liver damage due to cirrhosis of liver [21]. Hence, when the normal levels of antioxidants are not enough for the eradication of free-radical-mediated injury, then administration of antioxidant compounds has a potential role to play in reducing liver damage. Several phenolic components in plants like flavonoids [22], coumarins [23] and anthraquinones [24] are reported to exhibit antioxidant activity. Thus, the potent antioxidant activity expressed by the fraction of *E. alba* might be due to the presence of 40.95 % of phenolic compounds as per our finding, predominantly the coumestan derivatives, wedelolactone and demethylwedelolactone and also triterpenes and flavonoids [6].

Earlier studies have reflected that *E. alba* expressed hepatoprotective activity by regulating hepatic lysosomal enzymes [25]. Our study suggests that hepatoprotective action of *E. alba* may be also due to its antioxidant potential, thus justifying its use in liver tonics as a prophylactic supplement for combating oxidative-stress-induced liver damage. The result of this investigation is of importance, since *E. alba* is widely used in India for the traditional treatment of liver disorders.

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