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IN VIVO HEPATOPROTECTIVE ACTIVITY OF ACTIVE FRACTION FROM ETHANOLIC EXTRACT OF *ECLIPTA ALBA* LEAVES

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Abstract : The alcoholic extract of fresh leaves of the plant *Eclipta alba* (Ea), previously reported for is hepatoprotective activity was fractionated into three parts to chemically identify the most potent bioactive fraction. The hepatoprotective potential of the fraction prepared from extract was studied in *vivo* in rats and mice against carbon tetrachloride induced hepatotoxicity. The hepatoprotective activity was determined on the basis of their effects on parameters like hexobarbitone sleep time, zoxazolamine paralysis time, bromosulphaline clearance, serum transaminases and serum bilirubin. Fraction EaII (10–80 mg/kg, p.o.) containing coumestan wedelolactone and desmethylwedelolactone as major components with apigenin, luteolin, 4-hydroxybenzoic acid and protocateuic acid as minor constituents exhibited maximum hepatoprotective activity and is the active fraction for hepatoprotective activity of *Eclipta alba* leave. The acute toxicity studies have shown that like Ea, Fraction EaII also high safety margin.

Key words : *Eclipta alba* hepatoprotective activity CCl_4; hepatotoxicity coumestan wedelolactone desmethylwedelolactone acute toxicity

INTRODUCTION

Eclipta alba (L) Hassk. (Syn. *E prostata*. L., Asteraceae Bhringaraja) is a highly reputed plant in the Ayurvedic system of medicine for the treatment of liver ailments (1–2) we have previously reported significant *in vivo* hepatoprotective activity of ethanolic extract (Ea) from fresh leaves of *Eclipta alba* against carbon tetrachloride (CCl_4) induced liver injury (3–4).

Chemically, presence of a number constituents has been reported in this plant (5–7). Coumestan wedelolactone and desmethylwedelolactone have been identified as active components responsible for antihepatotoxic activity on the basis of

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in vitro studies on primary cultured rat hepatocytes against CCl_4, galactosamine and phalloidin induced cytotoxicity (8–9). These active components have also shown protection against phalloidin toxicity *in vivo* where the survival rate was considered the sole criterion of protection (8–9). However, these findings are not supported by effect on various makers of hepatoprotective activity *in vivo*. Further, the effect of other constituents present in *E. alba* leaf extract has also not been reported.

We have fractionated the alcoholic extract from *E. alba* leave (Ea) into three parts (EaI, EaII and EaIII) and of them were chemically analysed and pharmacologically evaluated to identify the biologically active fraction

METHODS

Preparation of extract and its fractions

Ethanolic extract of fresh leaves of *E. alba* (Ea) was prepared as described earlier (3). The dried extract (Ea) was suspended in hot water and insoluble portion was separated by filtration under vacuum to afford EaI. The remaining hot aqueous portion was extracted with equal volume of ethyl acetate for five times. Ethyl acetate portion was pooled, dried and distilled to afford light brown powder, EaII. The remaining aqueous portion on removal water over a rotary film evaporator at 45°C afforded EaIII.

Animals

Charles foster rats (100–200 g) and Swiss albino mice (25–30 g) of either sex bred in the Institute's Animals House were used. Mice were used for hexobarbitone induced sleep time, zoxazolamine sleep time, BSP clearance and acute toxicity studies whereas rats were used to study biochemical parameters in serum. Animals were kept on pellet diet (Lipton India Ltd. Bombay) and water *ad libitum* at constant temperature (25 ± 2°C), relative humidity (60–70%) and 12 h light/dark cycle.

Six groups with six animals each were taken for each test except for acute toxicity studies. The first four groups of animals were fed with different doses of test drug and CCl_4. The remaining two groups of animals were given CCl_4 alone and proportionate volume of liquid paraffin respectively and served as controls. The six groups of ten animals each were used for toxicity studies where one served as control and rest were given different doses of test drug.

Induction of hepatic injury drug treatment

CCl_4 diluted (1:4) with liquid paraffin was given orally as hepatotoxicant. The dose of CCl_4 was 0.5 ml/kg. p.o. for hexobarbitone induced sleep time, zoxazolamine sleep time whereas 1.5 ml/kg, p.o. was used for BSP retention and biochemical parameters in serum.

Stock aqueous suspension (5% in 0.2% acacia gum) of all the three fraction were prepared separately and further diluted with distilled water as per requirement. Each fraction was evaluated at four different doses on the basis of their presence in Ea, the parent extract. The doses of these fraction were-EaI (50, 100, 200 and 400 mg/

kg, p.o.), EaII (10,20, 40 and 80 mg/kg, p.o.) and EaIII 62.5, 125, 250 and 500 mg/kg, p.o.).

Hexobarbitone induced sleep time

The single dose of the drug and hexobarbitone (60 mg/kg, i.p.) were given to mice 1 h before and 2 h after CCI4 (0.5 ml/kg, p.o.). respectively. The time between loss of the righting reflex and its recovery was taken as duration of hexobarbitone induced sleep time (10).

Zoxazolamine induced paralysis time

The Single dose of test drug and zoxazolamine (70 mg/kg, i.p.) were given to mice 1 h before and 2 h after CCl_4 0.5 ml/kg, p.o.) respectively and paralysis time was recorded (10).

Bromosulphalein retention

The test drug was fed to mice daily for seven days at 24 h interval and single dose of CCl_4 (1.5 ml/kg, p.o.) intoxication was done 6 h after the last dose of test drug. Bromosulphalein (BSP, 100 mg/kg, i.v.) was injected 18 h after CCl_4 administration and blood collected from orbital sinus in heparinised tubes exactly 30 min later and the plasma dye concentration was determined (11).

Biochemical parameters in serum

The schedule of treatment of test drug and CCl_4 intoxication in rats was the same as described for BSP retention. The blood was collected from individual animal from the orbital sinus 18 h after CCl_4 administration and serum activities of glutamine-pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT) (12) and bilirubin (13) were determined.

Hepatoprotective activity

The hepatoprotective activity (3), expressed as hepatoprotection percentage (H) was calculated using the equation:

$$H = \left(1 - \frac{T-V}{C-V}\right) \times 100$$

Where 'T' is mean value of group treated with test drug and CCl_4 alone and 'V' is the mean value of control animal.

Observational screening and acute toxicity

Different groups of ten mice each fed with 10, 30, 100, 300 and 1000 mg/kg, p.o. of EaII and one group with the same number of mice served as control. The animal were observed for 1 h continuously and then hourly for 4 h for any gross behaviour changes (14). General motor activity, writhing, convulsion, response to tail pinching, gnawing, piloerection, pupil size, fecal output, feeding behavior etc.; and further up to 72 h for any mortality.

Statistical analysis

The data were statistically analysed using one-way ANOVA analysis (15). The significance value for control and multiple test groups by Dunnett's 't' test (16). The P value of 0.05 or less was taken as the

criterion of significance. The regression analysis was used to calculate regression coefficient (Slop b), correlation coefficient (r) with its P value and ED_{50} with 95% confidence limit (CL) from the log dose and percent inhibition of elevated values (17).

RESULTS

Chemical analysis and identification of the three fraction EaI, EaII and EaIII showed presence of polyacetylenes and thiophene derivatives, stigmasterol and its β-glucopyranoside, β–amyrin and atleast seven other sterols/triterpenoids in the fraction EaI (yield 4.2%). Wedelolactone (I) and desmethylwedelolactone (II), apigenin (IV), 4-hydroxybenzoic acid and protocateuic acid were present in the second fraction EaII (yield 0.6%). The third fraction, EaIII (yield 12%), contained small quantity of phenolic glycosides including desmethylwedelolactone. glucoside, luteolin 7–0-glucoside, a few uncharacterised glycosides and polypeptides beside inorganic material.

All the three fractions namely EaI, EaII and EaIII were investigated, four doses each, for their hepatoprotective activity, CCl_4 significantly increased ($P<0.01$) the values of all the parameters studied. Administration of different doses of EaII showed dose dependent reduction of CCl_4 induced values of these Parameters. The ED_{50} value of hepatoprotection in case of hexobarbitone sleep time ($b = 37.52$, $r = 0.72$, $P<0.001$) was <10 mg/kg, p.o., the minimum dose used. The same for zoxazolamine paralysis time ($b = 57.71$,

TABLE I: Hepatoprotective effect of Eall against CCl_4 induced hepatotoxicity.

Parameters / Groups	*Control (vehicle treated)*	*CCl_4 treated*	*EaII+CCl_4 treated (mg / kg, p.o.)*			
			10	*20*	*40*	*80*
Hexobarbitone Sleep time (min)	30.0±1.9	98.0±6.0[ad]	58.0±4.3[bd] (58.8)	50.0±3.0[bd] (70.6)	45.0±4.0[bd] (77.9)	34.0±2.9[bd] (94.1)
Zoxazolamine paralysis time (min)	20.8±1.3	92.0±5.0[ad]	72.5±3.7[bd] (27.4)	58.3±3.8[bd] (47.3)	50.0±4.2[bd] (59.0)	34.0±3.8[bd] (81.5)
BSP retention (mg%)	4.3±0.70	26.60±1.7[ad]	17.3±0.5[bd] (41.9)	15.6±1.4[bd] (49.5)	10.2±1.1[bd] (73.7)	7.0±0.8[bd] (88.1)
Serum GPT (units)[c]	84.6±8.2	325.1±14.7[ad]	267.0±9.9[be] (24.2)	243.60±19.3[bd] (33.9)	218.8±13.3[bd] (44.2)	171.30±12.5[bd] (63.9)
Serum GOT (units)[c]	174.7±19.5	664.7±30.0[ad]	626.0±16.9[bf] (7.9)	582.0±24.9[bf] (16.9)	549.4±18.8[be] (23.5)	506.7.0±3.8[bd] (32.2)
Serum bilibrubin	0.4±0.01	1.5±0.1[ad]	1.02±0.1[bd] (42.7)	0.79±0.1[bd] (42.7)	0.5±0.01[bd] (86.6)	0.3±0.0[bd] (98.9)

Values represent the mean ± S.E of six animals in each group. Values with in parentheses indicate percent hepatoprotection.

[a] Difference in relation to control (vehicle treated) group.

[b] Difference in relation to CCl_4 treated group.

[c] Each unit is μ mol pyruvate/min/L.

[d, e] and [f] $P<0.01$, $P<0.05$ and $P>0.05$ respectively (Dunnett's 't' test).

r = 0.85, P<0.001) and BSP retention (b = 53.96, r = 0.85, P<0.001) were 24.22 (95% CL = 20.28–28.93) and 15.96 (95% CL = 13.39–19.02) mg/kg, p.o. respectively. The ED_{50} value of hepatoprotection for serum GPT (b = 43.0, r = 66, P<0.001), GOT (b = 26.48, r = 0.67, P<0.001) and bilirubin (b = 63.54, r = 0.80, P<0.001) were 44.33 (95% CL = 34.25–57.37), >80 and 12.23 (95% CL = 9.96–15.01) mg/kg, p.o. respectively (Table I).

The hepatoprotective activity of fractions EaI and EaIII was much less as compared to EaII and therefore instead of detailed result of these fractions the ED_{50} values of these fractions has been summarised in Table II.

TABLE II: Hepatoprotective effect of different fractions (EaI, EaII and EAIII) from alcoholic extract of *Ecipta alba* leaves.

Parameters	*ED_{50} values (mg/kg, p.o.)*		
	EaI	*EaII*	*EaIII*
Hexobarbitone sleep time	122.60	<10.00	>500
Zoxazolamine paralysis time	133.42	24.22	>500
BSP retention	129.02	15.96	>500
Serum GPT	>400	44.33	>500
Serum GOT	>400	>80.00	>500
Serum bilibrubin	131.45	12.23	406.11

EaI, EaII, and EaIII were evaluated for their effects on various parameters at doses 50, 100, 200 and 400; 10, 20, 40 and 80 and 62.5, 125, 250 and 500 mg/kg, p.o. respectively.

EaII did not produce any apparent behavioural changes or mortality in mice up to 1000 mg/kg, (data not included).

DISCUSSION

Eclipta alba is a well known plant of medicinal importance and reported for hepatoprotective activity also (1–4) but the active principle responsible for its hepatoprotective activity *in vivo* was not known. A number of chemical constituents have been reported previously from the plant leaves and it was natural to get these constituents during extraction and fractions.

Chemical analysis and identification of the fraction showed presence of polyacetylenes and thiophene derivatives (6, 18–20), stigmasterol and its β glucopyranoside (21), β-amyrin and atleast seven other sterols/ triterpenoids in the Fraction EaI (yield 4.2%). Wedelolactone and desmethylwedelolactone, apigenin, luteolin, 4-hydroxybenzoic acid and protocateuic acid (5, 8) were present in the second fraction EaII (yield 0.6%). The third fraction EaIII (Yield 12%) contained small quantity of phenolic glycosides (7, 22) including desmethylwedelolactone. Glucoside, luteolin 7-0-glucoside, a few uncharacterised glycosides and polypeptides beside inorganic material.

The fraction EaII containing coumestan wedelolactone and desmethylwedelolactone as major components with some other minor constituents like apigenin, luteolin, 4-hydroxybenzoic acid and protocateuic acid showed dose-dependent significant activity in preventing CCl_4 induced liver injury. The protection of hepatocytes from CCl_4 damage may be based on its ability to

improve the functional status of hepatic drug metabolising enzymes. This is evident from shortening of the hexobarbitone sleep time (23), zoxazolamine paralysis time (24) and restoration of the CCl_4 impaired excretory capacity of hepatocytes as judged from BSP retention (11) (Table I). The hepatoprotective activity of EaII is also supported by its ability to significantly reduce the level of serum GPT, increased due to leakage of this cellular enzyme into plasma by CCl_4 induced hepatic injury (25–26). Like serum GPT, elevated values of serum GOT are also reduced/restored by hepatoprotective drugs. However, in the present study, there was definite reduction in serum level of GOT but statistically non-significant at lower doses. An increase in serum bilirubin is an indication of hepatic damage (27). The ability of EaII to reduce/counteract CCl_4 induced increase in serum bilirubin further supports its hepatoprotective potential (Table I). Observational screening and acute toxicity studies have shown that like Ea (3), fraction EaII also has a high safety margin.

EaI, a mixture of polyacetylenes thiophene derivatives, stigmasterol and other oleane triterpenoids with some unidentified compounds also elicited activity on limited parameters such as hexobarbitone induced sleep time, zoxazolamine induced paralysis, BSP retention and serum level of bilirubin. The activity of EaI is probably due to presence of phytosterols and oleane triterpenoids like stigma sterol and β-amyrin, which tend to increase the hepatoprotective activity in some cases (28) while in other these are directly responsible for protection (29). The fraction EaIII, which contained mostly inorganic material with small quantity of phenolic glycosides, showed no effect except for very low effect on serum level of bilirubin (Table II).

These studies have led us to conclude that the fraction EaII containing coumestan wedelolactone and desmethylwedelolactone as the major constituents is responsible for *in vivo* hepatoprotective activity of *E. alba* that supports the earlier *in vitro* finding (8). However, some contribution of fraction EaI containing phytosterols and oleane triterpenoids like stigmasterol and β-amyrin can't be ruled out for hepatoprotective activity of *E. alba*.

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