
Antihepatotoxic Activity of Euphorbia Antisyphilitica

S. SARAF¹, V.K. DIXIT², G.K. PATNAIK³ AND S.C. TRIPATHI³¹B.R.N. College of Pharmacy, Mandsaur 458 001, M.P.²Dept. of Pharmaceutical Sciences, Dr. Harisingh Gour Vishwavidyalaya, Sagar 470 003, M.P.³Pharmacology Divn., Central Drug Research Institute, Lucknow 200 001, U.P.

A medicinally important species of Euphorbiaceae was collected from the tribal locality and cultivated in medicinal plant garden of Dr. H.S. Gour University Sagar. It was identified as *Euphorbia antisiphilitica*. The ethanolic extract of the aerial parts of the plant has LD₅₀ of more than 1g/kg in mice. Petroleum ether and ethanolic extracts were tested for antihepatotoxic activity against thioacetamide and CCl₄ induced liver damage in rats. The active ethanolic extract was fractionated into chloroform soluble and insoluble fractions. The chloroform insoluble fraction was found to be active. Antihepatotoxic activity was evaluated using histopathological and serum parameters.

EUPHORBIA antisiphilitica Zucc. (Euphorbiaceae) is a herbal drug used among the tribals of Jhabua District of M.P., India, for the treatment of jaundice. Information collected from the tribal medicine men reveal that about 2 cm long stem of the plant if taken orally for three days removes yellow pigmentation completely in the patients of 'Peeliya' (Jaundice). The antihepatotoxic studies on extracts and fractions of ethanolic extract against CCl₄ and thioacetamide are described in this communication.

EXPERIMENTAL**Plant Material**

Aerial parts of the plant were collected during May-June 1988 from Jhabua District and grown in medicinal plant garden of the department. The identity of the plant as *Euphorbia antisiphilitica* Zucc. (Euphorbiaceae) was confirmed by the Department of Botany, Dr. H.S. Gour Vishwavidyalaya, Sagar.

Extraction

As the plant material is xerophytic in nature, drying in sunlight is difficult. The plant material was

therefore dried in the absence of sunlight. The powdered drug was extracted with petroleum ether (Yield 7.44% w/w of powder) and ethanol (yield 26.82% w/w of powder). The ethanolic extract was further fractionated into Chloroform soluble (yield 63.58% w/w of Ethanolic Extract) and chloroform insoluble fraction. (yield 36.42% w/w of Ethanolic Extract). Aqueous suspensions of the extracts and fractions were prepared with the help of 4% polyvinylpyrrolidone.

DETERMINATION OF LD₅₀

The LD₅₀ of the ethanolic extract was determined in Swiss albino mice (18-25 g) by using the method of Horn (1956). Doses of the extract were administered to groups of 4 mice each in geometric progression, starting with the dose of 464 mg/kg, i.p. and mortality was observed over a period of 24 h. The LD₅₀ with fiducial limits was determined from Horn's table.

ANTIHEPATOTOXIC STUDIES

The antihepatotoxic activity was determined using thioacetamide (TAA) model (Saraf and Dixit 1991; Gallagher et al., 1956) and carbon tetrachloride

* For correspondence

Table 1 : Effect of extracts of Euphorbia Antisyphilitica on serum parameters during thioacetamide induced hepatic injury in rats.

Groups.	SGOT U/L	SGPT U/L	ACP U/L	ALP U/L	GDH U/L
A. Normal.	96.32 ± 1.88	102.28 ± 9.11	20.88 ± 1.34	129.18 ± 5.54	2.84 ± 0.29
B. Control TAA.	248.68 ± 10.19	224.85 ± 13.42	30.82 ± 1.69	257.40 ± 12.79	21.71 ± 1.16
C. Ethanolic Extract.	112.11 ± 3.74* (89.64)	185.61 ± 5.86* (32.01)	23.68 ± 1.57* (78.65)	230.75 ± 10.79* (20.78)	14.25 ± 0.81* (39.53)
D. Petroleum Extract.	231.56 ± 14.73	219.27 ± 12.45	28.29 ± 2.68	248.40 ± 7.80	20.92 ± 1.65

Values are mean ± SEM Values in paranthesis indicate % inhibition.

* Significant at 95% confidence level (p< 0.05)

Table 2 : Effect of fractions of ethanolic extract of Euphorbia Antisyphilitica on serum parameters during thioacetamide induced hepatic injury in rats

Groups.	SGOT U/L	SGPT U/L	ACP U/L	ALP U/L	GDH U/L
A. Normal	114.72 ± 5.22	109.69 ± 6.65	22.91 ± 1.29	135.44 ± 7.29	3.50 ± 0.41
B. Control TAA.	201.94 ± 8.79	285.42 ± 10.82	39.17 ± 3.11	279.57 ± 11.56	17.83 ± 0.97
C. Chloroform Soluble Fraction	194.40 ± 10.95	280.22 ± 8.86	37.95 ± 2.02	269.68 ± 14.07	13.43 ± 0.90
D. Chloroform Insoluble Fraction.	124.19 ± 8.72*	115.10 ± 11.79	23.27 ± 1.34*	227.84 ± 11.82*	10.85 ± 0.86*

Value are mean ± Standard error. Values in Paranthesis indicate % inhibition.

* Significant at 95% confidence level (p<0.05)

(CCl₄) model (Bassi 1960; Recknagel, 1967) of acute hepatotoxicity.

Male sprague - Dawley rats (125-150 g) maintained under uniform laboratory conditions for at least 10 days were used throughout the studies. They were fed on pellet diet (Hindustan Lever Ltd., India) and water **adlibitum**.

(i) TAA - INDUCED HEPATOTOXICITY

Albino rats were divided into normal control and treated groups of 8 rats each. The animals of the normal group were fed a normal diet and were given the vehicle for 5 days. The animal of the control group were administered TAA (100 mg/kg b.w.) subcutaneously on the 4th day as 1% solution in isotonic saline,

Table 3 : Effect of Ethanoloic extract of Euphorbia Antisyphilitica and its fractions on serum parameters against carbon tetrachloride challenge in rats.

Groups.	SGOT U/L.	SGPT U/L	ACP U/L	ALP U/L	GDH U/L
A. Normal.	46.75 ± 7.04	33.00 ± 4.84	160.42 ± 6.21	3.24 ± 2.90	5.58 ± 0.29
B. Control CCl ₄	93.00 ± 6.38	145.25 ± 11.70	273.81 ± 8.52	4.43 ± 0.69	4.51 ± 0.43
C. Ethanolic Extract.	64.75 ± 6.38* (61.08)	88.24 ± 5.31* (50.78)	174.00 ± 12.83* (88.02)	3.93 ± 0.35* (42.02)	4.87 ± 0.35* (33.64)
D. Chloroform Soluble Fraction	83.75 ± 7.50 (20.00)	119.50 ± 15.11 (22.94)	209.00 ± 9.09 (57.16)	4.29 ± 0.14 (12.37)	4.68 ± 0.36 (15.88)
E. Chloroform Insoluble Fraction	62.00 ± 3.56* (67.02)	75.25 ± 3.86* (62.36)	188.00 ± 10.33* (75.68)	3.92 ± 0.27* (42.85)	5.15 ± 0.19* (59.81)

Values are mean ± Standard error. Values in paranthesis indicate % inhibition.

* Significant at 95% confidence level (P 0.05)

in the scapular region. The animals of the treated group were administered the respective extract/fraction orally for 5 days. The daily dose consisted of 200 mg/kg. b.w. Animals were injected with TAA (100 mg/kg) on the 4th day. The animals of all the groups sacrificed 24 h. after the TAA administration. The blood was collected by cardiac puncture and the serum was separated for studying serum enzyme levels.

(ii) CCl₄ - INDUCED HEPATOTOXICITY

Rats were divided into 5 groups (A,B,C,D, and E) of 8 rats each. Animals of group A (Normal group) were given only the vehicle. Group B (CCl₄ control group) was administered at 0,8 and 16 h with vehicle (i.p.) and a single subcutaneous dose of CCl₄ received three doses of respective extract/fraction (200 mg/kg b.w) at 0,8 and 16 h and single subcutaneous dose of CCl₄ half an hour after the administration of first dose of respective extract/fraction. Rats sacrificed 24 h after the administration of CCl₄ and the blood was collected for studying serum enzyme lev-

els. Slices of liver were also obtained simultaneously for histopathological studies.

SERUM PARAMETERS

Blood collected by cardiac puncture was allowed to coagulate and the serum separated. Different serum parameters were estimated by standard methods. The serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) levels were determined using the method of Reitman and Frankel (1957). The serum alkaline phosphatase (SALP) and serum acid phosphatase (SACP) were determined using the method of kind and king (1954). The serum bilirubin (SBRN) was estimated by Malloy and Evelyn (1937) method. Serum albumin (SALB) was estimated by the method of Miyada et al (1972). The method of king et al (1962) was employed for the determination of Glutamate dehydrogenase (GDH).

HISTOPATHOLOGICAL PARAMETERS

The liver slices were fixed in Bouin's aqueous solution (Picric acid : formaldehyde : glacial acetic

acid 75:20:5) and embedded in paraffin by employing the standard technique 5 μ thick sections were cut and stained with hematoxylin-eosin (Galigher and Kozdoff 1971).

STATISTICAL ANALYSIS

Results of serum parameters estimation are presented as mean \pm SEM and percent inhibition against hepatotoxin by the test extract or fraction. The percentage was calculated by considering the enzyme level difference between rats treated with hepatotoxin and control rats as 100 percent reversal. The variation present in a set of data was analyzed through one way analysis of variance (ANOVA). The difference among the means have been analyzed by the least significant difference (LSD) at the 95% ($p < 0.05$) confidence level (Armillage *et al.* 1985).

RESULTS AND DISCUSSION

The LD₅₀ of the ethanolic extract was found to be more than 1000 mg/kg i.p. in albino mice.

It was observed that the ethanolic extract at 200 mg/kg dose protected the liver from the TAA intoxication whereas the petroleum ether extract was found to be inactive. The ethanolic extract had definite anti-hepatotoxic activity, since it lowered the different serum enzyme levels significantly, **Table 1**.

The chloroform insoluble fraction of the ethanolic extract offered greater protection wherein serum parameters were remained almost normal. The chloroform soluble fraction on the other hand was found to be inactive **Table 2**.

The administration of CCl₄ elevated the serum enzymes significantly. The ethanolic extract (200 mg/kg b.w.) lowered the elevated serum parameters significantly. The ethanolic extract and its chloroform soluble and insoluble fractions showed apparent anti-hepatotoxic activity against CCl₄ challenge. The ethanolic extract and its chloroform insoluble fraction exhibited better activity. The chloroform insoluble portion in the major contributor to the activity in



Fig 1 : Representative photomicrograph of liver of normal rats (250). (IV) -Intralobular Vein; HC-Hepatic Cell; N - Nucleus, BC-Bile Capillary).

ethanolic extract as evidenced from the significant lowering of different serum parameters **Table 3**.

HISTOPATHOLOGICAL PARAMETERS

Liver slices of normal rats showed that the hepatic cells are radially placed and each cell has a large spherical nucleus and granular cytoplasm. The blood vein and bile capillaries were found to be clear without any injury to their walls **Fig.1**.

It was observed that CCl₄ causes a heavy destruction of the overall arrangement of liver cells because most of the cells were found in a ruptured state and without cytoplasm. Space formation and high degree of vacuolation was also seen in the liver **Fig. 2**.

Pretreatment with ethanolic extract showed that the liver is normal. The hepatic cells were found to be clear with prominent nucleus. No damage was observed in the intralobular vein, which has normal structures **Fig. 3**.

The pretreatment with chloroform soluble fraction of ethanolic extract failed to demonstrate and protective activity. The liver appeared to be normal after the pretreatment with chloroform insoluble fraction. The cells were found to be arranged in the form of hepatic

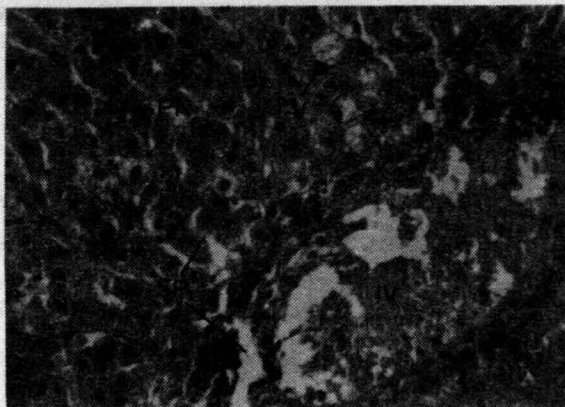


Fig 2 : Representative photomicrograph of liver of rats intoxicated with carbon tetrachloride (250). (IV - Intralobular Vein, BC - Bile Capillary, PN-Pycnotic Nuclei; V-Vacuolation; SP - Space Formation; WC - Without Cytoplasm Cell; DE - Degenerated Nuclei).

cords and the vein and bile capillaries were also found to be normal.

The selected experimental toxins, Carbon tetrachloride and thioacetamide have entirely different mechanisms of action. In CCl₄ poisoning an initial lesion results in the leakage of cytoplasmic enzymes and mitochondrial damage is a late manifestation.

Whereas, in thioacetamide poisoning, mitochondrial damage occurs early in the course of the injury (Rees and Sinha, 1960) The ethanolic extract and its chloroform insoluble fraction were found to be effective against both the toxins which substantiate the traditional claim about the hepatoprotective activity of the *E. antisiphilitica*.

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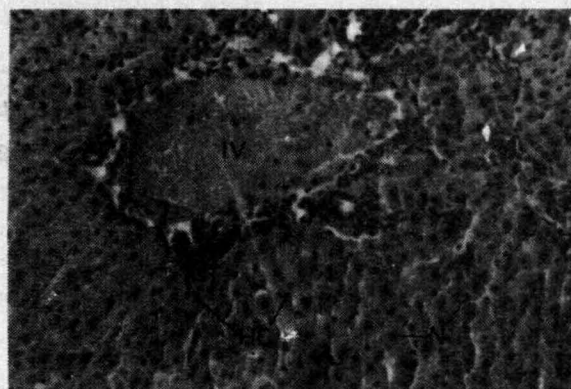


Fig 3 : Representative photomicrograph of liver of rats treated with ethanolic extract and intoxicated with carbon tetrachloride (250). (IV - Intralobular Vein; HC - Hepatic Cells; N - Nuclei; BC - Bile Capillary).

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