

Original Research Article

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Anticlastogenic Effect of *Euphorbia hirta* using *in vivo* Rodent Micronucleus Assay

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ABSTRACT

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Aqueous extracts of *Euphorbia hirta* were used to study the anticlastogenic activity by *in vivo* rodent micronucleus assay. Mice were administrated two doses, 250 mg/kg and 500 mg/kg orally for a period of seven days and at the end of the seventh day, cyclophosphamide (50 mg/kg) was given intraperitoneally. Bone marrow sample were analysed for cytotoxicity and micronucleus. Both the doses were not cytotoxic and clastogenic. There was a significant reduction in the micronucleus formation in bone marrow indicating the anticlastogenic activity of *E. hirta*.

Introduction

Euphorbia hirta (Tamil: Ammaan Pacharisi) is a common weed found in India, other tropical and subtropical countries. The plant has been used in traditional medicine to treat female disorders, respiratory ailments, especially cough, coryza, bronchitis and asthma, worm infestations in children and for dysentery, gonorrhoea, jaundice, pimples, digestive problems and tumours (Kirtikar *et al.*, 1991). The phytochemical constituents may act as anticlastogenic agents which play a crucial role in prevention of cancer (Marmot *et al.*, 2007). The current study was undertaken to evaluate the anticlastogenic effect of *E. hirta* using *in vivo* rodent bone marrow micronucleus assay.

Materials and Methods

Fetal calf serum (Himedia), Cyclophosphamide (Sigma-Aldrich), Methanol (Himedia), May Grunwald stain (Himedia) and Giemsa stain (Himedia) were used in this study. BALB/c mice (*Mus musculus*), Animals were housed six per cage and were acclimatized for one week at Centralized Laboratory Animal House, Madras Veterinary College, under controlled environment.

Commercial standard pellet diet and potable water were provided *ad libitum*. Thirty six male mice were divided randomly into six groups of six in each. The *in vivo* micronucleus procedure developed by Schmid

(1975) was followed. The aqueous extract of *E. hirta* was administered at 250 mg/kg BW (group 1 and 3) and 500 mg/kg BW (group 2 and 4) for seven days. Negative control group was administered with normal saline alone for seven days. Positive control group and treatment group-3 and group-4 were administered with single dose of cyclophosphamide at 50 mg/kg BW on 7th day. After 24 and 48 hours of cyclophosphamide / normal saline administration, bone marrow were collected in fetal bovine serum for the micronucleus assay. The animals were anesthetized and killed by cervical dislocation. The femurs were removed and cleared of all attached tissues. Both the ends of the femur were cut to expose the bone marrow canal. Using fetal bovine serum, the bone marrow contents were flushed and collected in 2 ml centrifuge tube. This suspension was centrifuged at 1000 rpm for 10 min and the supernatant was discarded. The sediment was resuspended in 0.1 ml of fetal bovine serum and this suspension was used to make smears on a microscopic glass slides and were air dried. The air dried smears were then fixed in methanol for 5-10 min. For each animal, about three to four slides were prepared.

Air dried, methanol fixed slides were stained with undiluted May-Grunwald stain for 5 min. Then slides were rinsed with distilled water for 2 min. Then the slides were stained with 10% Giemsa stain for 9 min. Again the slides were rinsed with distilled water for 2 min. The slides were air dried and observed in a microscope under oil immersion for micronucleus. 2000 polychromatic erythrocytes per animal were analysed for the presence of micronucleus and 200 erythrocytes were analysed for the ratio of polychromatic erythrocytes to normochromatic erythrocytes to determine the cytotoxicity and the results were analysed using SPSS Statistics 17.

Results and Discussion

The two doses, 250mg/kg BW and 500mg/kg BW of aqueous extract of *E. hirta*, administered orally did not induce cytotoxicity as indicated by the ratio of PCE/NCE compared to the negative control group. Further there is no significant increase in the frequency of MNPCE compared to that of negative control (Table 1). The aqueous extract of *E. hirta* at 250mg/kg BW and 500mg/kg BW showed significant dose dependent anticlastogenic activity at 24 and 48 hrs. There was a significant reduction in the micronucleus formation in bone marrow erythrocytes induced by the administration of cyclophosphamide (50mg/kg BW). Maximum inhibition was observed at 24 hrs followed by 48 hrs as shown in the Table 2. The present study indicates that the aqueous extract of *E. hirta* do not have any DNA damaging effect and it possess good DNA protective effect at the tested concentration. The aqueous extract of *E. hirta* showed excellent anticlastogenic activity against cyclophosphamide induced micronucleus formation in the erythrocytes, which acts by generation of free radicals that damage the DNA, RNA and proteins. The agents that has antioxidant properties acts as anticlastogens (Bronzetti *et al.*, 2003). In this study, the aqueous extract of *E. hirta* was found to inhibit the micronucleus formation which can be attributed to antioxidant property of *E. hirta* (Sharma *et al.*, 2008) (Subramanian *et al.*, 2011). Similar results were obtained in *in vivo* micronucleus assay with plants like *Boerhaviadiffusa* by Ravichandra (2009), *Tinosporacardifolia* by Chandrasekaran (2005), Silymarin by Kaleeswaran (2006), Citral (Rabbani *et al.*, 2005) which has excellent antioxidant properties. Rosmarinic acid revealed a significant reduction in micronucleus formation against DXR induced DNA damage which is attributed to the antioxidant activity by the phenolic compounds (Furtado *et al.*, 2008).

Table.1 Cytotoxic, clastogenic and anticlastogenic evaluation of *Euphorbia hirta* using rodent bone marrow micronucleus assay

Treatment	Dose (mg/kg)	24 hours		48 hours	
		MNPCE(Mean ± S.D)	P/E RATIO	MNPCE(Mean ±S.D)	P/E RATIO
		No.		No.	
NC	-	6.33 ± 1.52	0.94 ± 0.03	7.66 ± 1.15	0.96 ± 0.01
CP	50	49.66 ± 3.50	0.59 ± 0.04	28.00 ± 3.50	0.63 ± 0.10
Eh	250	8.66 ± 1.52	0.92 ± 0.01	6.00 ± 1.73	0.93 ± 0.01
Eh	500	10.00 ± 1.00	0.90 ± 0.01	6.66 ± 1.52	0.92 ± 0.01
Eh + CP	250 + 50	20.00** ± 2.08	0.73 ± 0.06	12.66* ± 0.57	0.77 ± 0.12
Eh + CP	500 + 50	16.66** ± 3.05	0.79 ± 0.07	11.00* ± 1.00	0.81 ± 0.01

NC- Negative control, Eh- *Euphorbia hirta*, CP- Cyclophosphamide, MNPCE- Micronucleated polychromatic erythrocytes, P/E ratio- Polychromatic/Total erythrocyte ratio

** P≤0.01 Significant at 1 percent, * P≤0.05 Significant at 5 percent

The study has provided a further scope for evaluation of aqueous extract of *E. hirta* as a possible chemo prophylactic agent.

It was concluded that the aqueous extract of *Euphorbia hirta* was anticlastogenic at the dose tested which paves way for further studies to be used as chemotherapeutic agent.

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