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STUDIES ON ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF *EUPHORBIA TIRUCALLI* L. LATEX

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ABSTRACT

The Aqueous, dichloromethane-methanol and pet. ether extract of the latex of *Euphorbia tirucalli* L. (Euphorbiaceae) was screened for analgesic and anti-inflammatory activities. Analgesic activity was compared with aspirin by tail-immersion and by acetic acid induced writhing methods. The anti-inflammatory activity against carrageenan induced paw edema in albino rats was compared with ibuprofen. In all the methods encouraging results were obtained for aqueous, dichloromethane-methanol and pet. ether extracts.

Key words: Analgesic, Anti-inflammatory, *Euphorbia tirucalli*, latex.

INTRODUCTION

Euphorbia tirucalli (Euphorbiaceae) is a succulent plant commonly distributed to tropical areas and rainforests in the Amazon, Madagascar and South Africa. The plant is commonly called as Vajradruma (Sanskrit), Indian tree spurge or Milk bush (English) and Bontakalli (Kannada). The latex is used as an application for warts, rheumatism, neuralgia and tooth ache[1]. Latex is also used as antimicrobial, antiparasitic, in treatments of coughs, cancer and other maladies as folk remedy[4]. The bark of this plant is used to treat fractures[1]. Literature review indicated that the analgesic and anti-inflammatory activity of latex of this plant has not been scientifically evaluated so far. Hence, the present study is aimed to find out the analgesic as well as anti-inflammatory activities of latex extract of *E. tirucalli.*

EXPERIMENTAL

Plant material: The fresh latex of *E. tirucalli* was collected from the region of

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Chitradurga, Karnataka and authenticated at Department of Botany, Sahyadri Science College, Shimoga.

Preparation of extract

The latex material was dried in shade and powdered mechanically. For the preparation of aqueous extract, the dried powdered latex was subjected to hot extraction and for the preparation of dichloromethane-methanol (1 : 1) and pet. ether extracts, the dried powdered latex was subjected to cold extraction. The solvent was removed completely over the water bath and finally dessicator dried.

Phytochemical screening

Standard methods (Evans and Trease; Vigar, 1984) were used for preliminary phytochemical screening of the extracts to know the nature of phytoconstituents present (Table 1).

Table 1.Qualitative analysis of phytochemicals of latex extracts.

Extracts	Alkaloid	Flavanoid	Steroid	Saponin	Cardiacglycoside	Phenols	Tannins	Anthraquinone
Aqueous	+	+	+	-	+	+	+	+
Dichloro-methane -methanol	+	+	+	-	+	+	+	+
Pet. ether	+	+	+	-	-	+	+	-

Animals

Healthy swiss albino mice weighing 25-30 g were obtained from the National Institute of Communicable Diseases, Bangalore, Karnataka and albino rats weighing between 120 to150 g were obtained from National Pharmacy College, Shimoga, Karnataka. They were maintained at standard housing conditions and fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum during the experiment.

Acute toxicity studies

The acute toxicity studies were carried out as per stair case method[5]. Accordingly

the LD_{50} of aqueous extract was found to be 3000 mg/Kg, LD_{50} of dichloromethane-methanol extract was found to be 1000 mg/Kg and that of pet.ether was found to be 300 mg/Kg. One tenth of this dose was selected for the evaluation of analgesic and anti-inflammatory activity.

Analgesic activity

E. tirucalli latex extracts were evaluated for analgesic activity in mice using tail immersion and acetic acid writhing methods.

Tail immerssion method

Albino mice of either sex were divided into 5 groups of 6 animals each. Extracts were orally administered. Group I served as control and received 1% tween 80 at the dose of 1 mL/Kg. Group II served as standard and received aspirin at a dose of 25 mg/Kg.

Group III received aqueous extract at a dose of 300 mg/Kg, Group IV received dichloromethane-methanol extract at a dose of 100 mg/Kg and Group V received pet. ether extract at a dose of 30 mg/Kg. Water was heated to 50.0 ± 1.0 ^{o}C before one hour of measurement of extract effect. The time taken for the animal to remove its tail out of water was recorded.

Acetic acid induced writhing method

Albino mice of either sex were divided into 5 groups of 6 animals each. Extracts were orally administered. Group I served as control and received 1% tween 80 at the dose of 1ml/Kg. Group II served as standard and received aspirin at a dose of 25mg /Kg.

Group III received aqueous extract at a dose of 300 mg/Kg, Group IV received dichloromethane-methanol extract at a dose of 100 mg/Kg and Group V received pet. ether extract at a dose of 30 mg/Kg. After 30 minutes of drug administration acetic acid (3% v/v) was administered to all group of animals at a dose of 0.1 mL/10 g intraperitonealy. The onset and severity of writhing response was noted for 10 minutes. The inhibition of pain response by drug treatment was noted[6,7].

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by measuring Carrageenan (Sigma) at a dose of 1 mL/Kg sub-cutaneous into the sub-plantar region of right hind paw. Male albino rats were grouped into 5 groups of 6 each. Group I served as control and received 1% tween 80 at the dose of 1 mL/Kg. Group II served as standard and received ibuprofen at a

dose of 40 mg /Kg. Group III received aqueous extract at a dose of 300 mg/Kg, Group IV received Dichloromethane-methanol extract at a dose of 100mg/Kg and Group V received pet. ether extract at a dose of 30 mg/Kg, one hour before carrageenan injection. Paw volume was measured before and two hours after the carrageenan injection using plethysmometer

RESULTS AND DISCUSSION

Analgesic activity

Table 2 shows the responses of mice to tail immersion. Treatment with 300 mg/Kg of aqueous extract, 100 mg/Kg of dichloromethane-methanol extract and 30 mg/Kg of pet. ether extract of *E. tirucalli latex* significantly ($p < 0.01$) protected animals from thermal stimuli.

Table 2. Analgesic activity by tail immersion method.

Treatment	Dose	Reaction time (s)		Inhibition percentage
		Before treatment (0 min)	After treatment (60 min)	
Control (Tween 80)	1 mL/Kg	5.67 ± 0.42	6.00 ± 0.58	-
Aspirin	25 mg/Kg	5.17 ± 0.40	9.33 ± 0.42**	80.23%
Aqueous	300 mg/Kg	5.67 ± 0.42	8.83 ± 0.40**	57.67%
Dichloromethane – Methanol	100 mg/Kg	5.17 ± 0.31	7.83 ± 0.40**	51.80%
Pet ether	30 mg/Kg	5.50 ± 0.34	8.17 ± 0.48**	48.48%

**$p < 0.01$

The percentage of inhibition of pain of the animals by the extracts from the thermal stimuli were compared to that of aspirin 25 mg/Kg. The inhibition were 57.67%, 51.80% and 48.48% respectively for aqueous, dichloromethane-methanol and pet.ether extracts. aqueous extract showed the significant analgesic activity, when compared to the

dichloromethane-methanol and pet.ether extracts.

Table 3 shows the responses of mice to acetic acid induced writhing. Treatment with 300 mg/Kg of aqueous extract, 100 mg/Kg of dichloromethane-m ethanol extract and 30 mg/Kg of pet.ether extract of *E. tirucalli latex* significantly ($p < 0.01$) reduced the number of writhes. The inhibitions were 55.15%, 48.69% and 44.84%, respectively for aqueous, dichloromethane-methanol and pet.ether extracts.

Table 3. Analgesic activity by acetic acid induced writhing method.

Treatment	Dose	Number of Writhings (30 min)	Percentage inhibition
Control (1% acetic acid)	1 mL/100 mg	13.0 ± 0.89	-
Aspirin	25 mg/Kg	3.17 ± 0.31**	75.61%
Aqueous	300 mg/Kg	5.83 ± 0.48**	55.15%
Dichloromethane Methanol	100 mg/Kg	6.67 ± 0.33**	48.69%
Pet ether	30 mg/Kg	7.17 ± 0.31**	44.84%

**$p < 0.01$

The analgesic activity of latex extract of *E. tirucalli* was studied for its central and peripheral activities. The analgesic activity of *E. tirucalli* latex against acute pain was moderate as compared to the potent inhibitory activity of aspirin. Aspirin leads to a relief from pain by suppressing the formation of pain inducing substances in the peripheral tissues[10]. Prostaglandin and bradykinin were suggested to play an important role in the pain process[11]. Therefore, it is likely that *E. tirucalli* latex extracts might suppress the formation of these substances and exert its analgesic activity in tail immersion and acetic acid induced writhing test. In present study, *E. tirucalli* latex extracts significantly increased reaction time in tail immersion method suggesting its central analgesic activity and lessening the number of writhes in acetic acid induced writhing method suggesting its peripheral analgesic activity. In both methods; aqueous extract showed high analgesic activity among the *E. tirucalli* latex extracts. The analgesic activity may be due to the presence of flavanoids in the latex extracts.

Anti-inflammatory activity

The results obtained with 300 mg/Kg of aqueous extract, 100 mg/Kg of dichloromethane-methanol extract and 30 mg/Kg of pet. ether extract of *E. tirucalli latex* on carrageenan induced rat hind paw edema are shown in Table 4. The extracts significantly ($p < 0.01$) inhibited the inflammatory edema. The effect of inhibition by all the extracts was compared to that of ibuprofen. The inhibition was more in aqueous than dichloromethane-methanol and pet. ether extracts.

Table 4.Anti-inflammatory activity by carrageenan induced paw edema method.

Treatment	Dose	Volume of paw edema			Percentage of paw edema
		Before carrageenan	After carrageenan	After drug treatment (2hrs)	
Control (Tween 80)	1 mL/Kg	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	-
Ibuprofen	40 mg/Kg	0.11 ± 0.01	0.14 ± 0.01	0.09 ± 0.01**	35.71%
Aqueous	300 mg/Kg	0.12 ± 0.01	0.14 ± 0.01	0.10 ± 0.01**	28.5%
Dichloro methane - Methanol	100 mg/Kg	0.11 ± 0.01	0.13 ± 0.01	0.10 ± 0.01**	23.0%
Pet. ether	30 mg/Kg	0.11 ± 0.01	0.13 ± 0.01	0.10 ± 0.01**	23.0%

**$p < 0.01$

Carrageenan induced paw edema is a useful model to detect oral action of anti-inflammatory agents. It is described as biphasic[9]. The initial phase is attributed to the release of histamine, serotonin and kinin. The second phase is related to release of prostaglandin like substances. The significant anti-inflammatory effect of latex extracts of *E. tirucalli* was compared to that of ibuprofen, which could be related to its histamine, serotonin, kinin and prostaglandin inhibitory activities. Hence our present study revealed that *E. tirucalli* latex extracts showed significant ($p < 0.01$) anti-inflammatory activity. The anti-inflammatory activity may be due to the presence of flavanoids in the latex extracts

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