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SCREENING OF ANTINOCICEPTIVE ACTIVITY OF *EUPHORBIA FUSIFORMIS* BUCH-HAM

BK Ashok[1]*, Bhat Savitha D[2], Sorake Ravi Rao[3], B Ravishankar[4]

[1]Research Assistant, Pharmacology laboratory, IPGT & RA, Dhanvantri Mandir, Gujarat Ayurved University, Jamnagar, Gujarat, India

[2]Ph.D Scholar, Department of Dravyaguna, IPGT & RA, Dhanvantri Mandir, Gujarat Ayurved University, Jamnagar, Gujarat, India

[3]Reader, Department of Dravyaguna, Alva's Ayurveda Medical College, Mudabidri, Karnataka, India

[4]Director, SDM Research Centre for Ayurveda and Allied Sciences, Kuthpady, Udupi. Karnataka, India

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ABSTRACT

Antinociceptive activity of *Euphorbia fusiformis* root powder was investigated using acetic acid writhing, tail flick and formalin induced paw licking tests. The test was performed at two dose levels in Swiss albino mice and Wistar strain albino rats. Oral administration of *Euphorbia fusiformis* root powder did not produce any significant effect on acetic acid induced writhings, however in tail flick test it significantly raised pain threshold at both dose levels and is prolonged. Moreover, at high dose level it significantly inhibited the formalin induced paw licking responses at both the phases. The result of pharmacological tests performed in the present study suggests that *Euphorbia fusiformis* root possesses potent analgesic property which is mediated via central inhibitory mechanism. This could provide a rationale for the use of this plant in treatment of rheumatism in folk medicine.

KEYWORDS: *Euphorbia fusiformis*, Tail flick, Writhing, Analgesic, Formalin

***Corresponding author**

Ashok BK, Research Assistant, Pharmacology laboratory, IPGT & RA, Dhanvantri Mandir, Gujarat Ayurved University, Jamnagar, Gujarat, India Email: drashokbeekay@yahoo.co.in

INTRODUCTION

Plants and plant based medicaments have been employed since the dawn of civilization for prolonging life of man by combating various ailments. Indigenous people living on their traditional territory largely rely on medicinal plants for healthcare and they are therefore rich in ethnopharmacological knowledge. However, the meticulous scientific research is needed to evaluate their effectiveness in treatment of various diseases.

Euphorbia fusiformis Buch.-Ham. Ex. D.Don (Euphorbiaceae) is a rare medicinal plant found in Tropical Himalaya up to 1500 ft. from Garhwal to Nepal and also found in Konkan and Deccan Hills[1]. In Gujarat sate it is found in Dangs, Rajpippala, Chotaudaipur regions[2]. The ethnobotanical value of the plant refers to its recognized action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis[3,4]. The tribals of Waghai and adjacent forest regions of Dangs district of Gujarat are ethnic people who dwell in the forests. The majority of these tribal people have their own method of treatment for various diseases. They have their own physician called *Bhagat* who knows ample of information regarding the medicinal herbs of forests, especially their proper identification and utilization. The *Bhagats* use *Euphorbia fusiformis* drug in the name of *Ghate* for treatment of various abdominal disorders especially for tumors of abdomen. They also use tuberous root of this plant in the form of paste for application in rheumatism. In spite of its reputation in treating ailments like rheumatism, till date no pharmacological screening to support its analgesic activity has been reported. Hence the present study was under taken to evaluate analgesic activity of roots of *Euphorbia fusiformis* in experimental animals.

MATERIALS AND METHODS

Test Drug

The tuberous roots of *Euphorbia fusiformis* Buch-Ham. (Family - Euphorbiaceae) was identified with the help of Taxonomist and collected from Waghai forest in fully matured condition. The tuberous roots were made into

slices and shade dried for 12 days and then pulverized to fine powder (mesh no 80) and stored in airtight container for experimental purposes.

Animals

Wistar strain albino rats (200 ± 20g) and Swiss albino mice (24 ± 4g) of either sex were procured from the animal house attached to pharmacology lab, IPGT & RA, Gujarat Ayurved University, Jamnagar. They were housed in large spacious polypropylene cages and fed with Amrut brand rat pellet feed supplied by Pranav Agro Industries and tap water given *ad libitum*. The animals were acclimatized for at least one week in lab condition before commencement of the experiment in standard laboratory conditions 12 ± 01 hour day and night rhythm, maintained at 25 ± 3°C and 40 to 60 % humidity. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number; IAEC 04-05/01/MSc.01).

Dose Selection and Schedule

The dose selection was done on the basis of body surface area ratio by referring to the standard table of Paget and Barnes (1964)[5]. On this basis the rat dose was found to be 90 mg/kg and for mouse130 mg/kg. The test was carried out at two dose levels viz., TED (Therapeutically equivalent dose) and TED × 02 (180mg/kg for rat and 260mg/kg for mouse). The test drug was suspended in tap water and administered orally to animals with the help of rubber catheter.

Acetic Acid Induced Writhing Test

Swiss albino mice of either sex were grouped into four groups of 6 mice each. To the first group tap water was administered to serve as control. Second and third groups were administered with the test drug TED (130mg/kg) and TED × 2 (260mg/kg) respectively. The fourth group was taken as reference standard group and administered with aspirin (20mg/kg p.o.). The test drugs were administered to overnight fasted animals and exactly one hour later acetic acid in the dose of $10mlkg^{-1}$ (3% v/v Solution) was injected intraperitonially to each mouse[6]. Analgesic effect was recorded by counting the number of writhing syndrome after the injection of acetic acid for a period of 30 minutes. The writhing syndrome is characterized by intermittent contraction of abdominal muscles, extension of hind limbs and twisting of trunk. Effect of drug on incidence and number of writhing syndrome was noted.

Radiant Heat Test

The animal groupings and test drug administration were similar to acetic acid induced writhing test, except the reference standard drug in this test Pentazocine(20mg/kg) was used. The latency of tail flick response (TFL) was measured with the help of tail flick analgesiometer (INSIF-Ambala). Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the radiant heat source. The tail withdrawal from the heat (TFL) is taken as the end point[7]. A cut off period of 15 sec was observed to avoid damage to the tail. To obtain baseline value the tail flick response was measured three times in each animal initially. Then the test drugs were administered to respective groups and tail flick response was recorded at 30, 60, 120, 180 and 240 minutes.

Formalin Induced Hind Paw Licking

Animal grouping and test drug administration are similar to acetic acid induced writhing test, however rats were used in this test. Indomethacin (10mg/kg orally) was used as standard drug. Pain was induced by injecting 0.1 ml of 3% formalin in distilled water in subplantar region of right hind paw and the duration of paw licking as an index of nociception was counted in periods of 0 to 10 minutes (Early phase) and 20 to 30 minutes (Late phase)[8].

STATISTICAL ANALYSIS

Student's t test for unpaired data has been used for analyzing the data generated during the study. A 'P' value less than 0.05 is considered as statistically significant and the value of $P<0.01$ or $P<0.001$ is considered statistically highly significant.

RESULTS

Test drug at both the dose levels did not show any significant response to acetic acid writhing syndrome (**Table 1**). However, at both dose levels test drug significantly increased the TFL at 60, 120, 180 and 240minutes in comparison to control group. Mice pre-treated with pentazocine significantly increased TFL at 30 minute and non-significantly at 60minutes onwards (**Table 2**).

Test drug only at high dose level significantly decreased the paw licking response at both early phase and late phase and the observed effect is almost equal to indomethacin treated group (**Table 3**).

DISCUSSION

The present study was carried out to evaluate antinociceptive activity of *Euphorbia fusiformis* (Euphorbiaceae) root powder in different models of pain. The mechanism for testing analgesic was selected such that both centrally and peripherally mediated effects were investigated. The acetic acid induced abdominal constriction and tail flick methods elucidated peripheral and central activity, respectively, while the formalin test investigated both.

In the acetic acid induced writhing model the constrictions induced by acetic acid in mice results from an acute inflammatory reaction with production of PGE2

and PGF2α in the peritoneal fluid[9]. Administration of test drug at both dose levels failed to inhibit acetic acid induced writhings, this may be due to lack of prostaglandin inhibitory effect.

Tail flick model which is thermal induced nociception indicates narcotic involvement which is sensitive to opioid μ receptors[10] which focuses mainly on changes above the spinal cord level. The significant increase in pain threshold produced by *Euphorbia fusiformis* at both doses suggests involvement of central pain pathways.

An important feature of the formalin test in rodents is that animals show two phases of antinociceptive behavior[11]. The first phase (0 – 10min) which is neurogenic and result of direct stimulation of nociceptors measure centrally mediated effects and is insensitive to anti-inflammatory agents while the second phase (20 – 30 min) is of inflammatory origin which is dependent on peripheral inflammation and changes in central procession due to chemical mediators release from damaged cells[12]. Drugs with analgesic effect may inhibit or decrease the paw licking in both or either of the phases.

Euphorbia fusiformis only at high dose level significantly inhibited the formalin induced paw licking responses at both the phases so as indomethacin which is a non-selective cyclooxygenase inhibitor. Further, ability of *Euphorbia fusiformis* root powder to inhibit both the phases of formalin induced pain response in addition to tail flick latency further indicates the involvement of central opioidergic mechanisms as the antinociceptive activity.

CONCLUSION

The results obtained in this study indicate that *Euphorbia fusiformis* root possesses potent analgesic property, which is mediated via central inhibitory mechanism. This could provide a rationale for the use of this plant in treatment of rheumatism in folk medicine.

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Table 1: Effect of *Euphorbia fusiformis* on acetic acid writhing syndrome

Treatment	Number of writhing episodes	% inhibition
Control	32.66 ± 6.51	--
E.fusiformis (130mg/kg)	33.33 ± 6.60	--
E.fusiformis (260mg/kg)	31.83 ± 5.02	02.54
Aspirin (20mg/kg)	17.33 ± 1.54*	46.93

* P< 0.05 (Compared with control group)

Table 2: Effect of *Euphorbia fusiformis* on tail flick response

Treatment	Initial TFL (sec.)	TFL after drug administration (sec.)				
		30 min	60 min	120 min	180 min	240 min
Control	3.67 ± 0.42	3.11 ± 0.20	3.15 ± 0.178	3.55 ± 0.432	3.16 ± 0.27	3.35 ± 0.251
E.fusiformis (130mg/kg)	2.98 ± 0.43	2.71 ± 0.19	3.63 ± 0.177	4.73 ± 0.238*	4.53 ± 0.427**	4.48 ± 0.356*
E.fusiformis (260mg/kg)	3.42 ± 0.38	3.68 ± 0.34	4.60 ± 0.337***	4.71 ± 0.519	4.66 ± 0.365***	4.68 ± 0.456
Pentazocine (20mg/kg)	2.10 ± 0.20	8.50 ± 1.09***	3.80 ± 0.79	2.83 ± 0.30	2.66 ± 0.33	2.33 ± 0.33

** P< 0.01, ***P < 0.001 (Compared with control group)

Table 3: Effect of *Euphorbia fusiformis* on formalin induced paw licking response

	Number of paw lickings			
Treatment	0-10 min	% inhibition	20-30 min	% inhibition
Control	12.16 ± 1.35	-	11.66 ± 1.17	-
E.fusiformis (90mg/kg,po)	11.66 ± 0.84	04.11	10.00 ± 1.61	02.91
E.fusiformis (180mg/)	8.16 ± 1.04*	33.00	7.33 ± 1.42*	37.13
Indomethacin (10mg/kg)	08.33 ± 00.67*	31.50	07.00 ± 00.52**	40.00

* P< 0.05, **P < 0.01 (Compared with control group)