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Original Article

INVESTIGATION OF ANALGESIC ACTIVITY OF LEAVES PART OF THE *TRIANTHEMA PORTULACASTRUM* (L) IN STANDARD EXPERIMENTAL ANIMAL MODELS

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Abstract

The ethanol extract of Trianthema portulacastrum belonging to the family of Aizoaceae was evaluated by acetic acid induced writhing and hot plate methods to assess analgesic activity. It was found that the extract caused an inhibition on the writhing response induced by acetic acid in a dose dependent manner. Dose of 250 mg/kg EETP and Aspirin could block the writhing response by 50.92% and 67.68% ($p<0.001$), respectively. It was also indicated that the EETP showed significant antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug Aspirin treated controls, suggesting the central activity of EETP.

Key words: *Trianthema portulacastrum*, Analgesic activity, Acetic acid induced writhing and hot plate method.

INTRODUCTION

Trianthema portulacastrum (family: Aizoaceae) known as Satodo (Vaidya, 1982) in Gujarat, is historically valued as a vegetable by poor people on the Indian subcontinent. An annual/perennial herb with regular flower is found throughout India, Leaves are rarely polygonous in cymes (or) fascicles, rarely solitary. The plant is bitter, hot, alexiteric, analgesic, stomachic, laxative, alterative, cures "kapha", heart diseases and diseases of the blood, inflammations and piles. The root applied to the eye cures, corneal ulcers, itching, dimness of sight and night blindness (Ayurveda) mild purgative cathartic and abortion (Chopra *et al*, 1956). The bitter and nauseous roots are given in combination with Zinger as a cathartic (Nadkarni, 1976). when fresh it is some what sweet. The juice of the leaves dropped in to the nostrils relieves migraine. The plants are considered useful in obstructions of the liver and spleen (Singh and Dixit, 1991, Javid Ahmed, 2000), uteralgia, cough and diuretic. It has significant hepatoprotective activity against paracetamol and thio-acetamide intoxication in rats (Kumar *et al*, 2004), asthma and amenorrhea. The present study showed that *EETP* exhibit analgesic activity by hot plate and acetic acid induced writhing methods and anti inflammatory activity against carrageenan and serotonin induced paw oedema and cotton pellet induced granuloma methods.

MATERIALS AND METHODS

Collection of plant

Preparation of Extract

Trianthema portulacastrum was collected at surrounding of Erode, (Tamil Nadu) and authenticated by Dr. S. Rajan, (Botanical Survey of Medicinal Plant Unit, Ooty), the plant specimen was preserved in the department of Pharmacognosy in our institutions for future references.

Chemical and reagents

The chemicals used in the present study were Aspirin (Micro lab, Hosur)

Preparation of Extract

The dried powdered plant material was extracted with ethanol in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (yield 19.6 %).

The phytochemical profile was performed as described by (Wagner *et al*, 1984). The presence of alkaloid (Dragendroff reagent and Mayer's reagent), flavonoids (shinoda test), steroids (liberman Burchard test) and terpenes (Vanillin sulfuric acid reagent) were analyzed. The extract showed positive test for alkaloids, steroids and tannins. The extract at the different doses of 125 and 250 mg/kg was suspended in aqueous Tween 80 solution (2%).

Animals

Swiss albino mice of either sex weighing between (18-22 gm) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet with *water ad libitum*.

Toxicity Study

An acute toxicity study relating to the determination of LD_{50} was performed. Albino Swiss mice of either sex weighing 20-25 g were used. The animals were fed standard pellet diet (Hindustan Lever Ltd., Calcutta) and given water *ad libitum*. The animals were kept fasting for 18 hr before the commencement of experiments. The test compounds (dissolved in propylene glycol) at different doses were injected intraperitoneally to different groups of mice, each group containing 10 animals. The animals are observed and the number of deaths recorded after 24 and 72 hr The $LD_{50,}$ the dose killing half of the animals, is determined by the conventional graphic method of Litchfield and Wilcoxon (1949). The ratio of the median lethal dose to the median effective dose (LD_{50}: ED_{50}) is known as the therapeutic index.

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EVALUATION OF ANALGESIC ACTIVITY

Acetic Acid Induced Writhing Response In Mice

Swiss albino mice of either sex were used and divided into four groups of six animals. Writhing test determined according to the method of turner with slight modification (Turner, 1960). The animals were treated with *EETP* (125 and 250 mg/kg) and Aspirin (200 mg/kg) injected intraperitoneally 1 hr prior to the injection of acetic acid while control group received vehicle. Writhing was induced by 10 ml/kg of acetic acid solution (0.6%) intraperitoneally (IP). Ten minutes after acetic acid injection, the mice were placed in a transparent box and the number of writhes was counted for a period of 10 minutes. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. A significant reduction in the number of writhes by drug treatments as compared to vehicle treatment animals, which was considered as a positive analgesic response and the percentage inhibition of writhing was calculated and evaluated statistically. Standard drug Aspirin was used as reference standard.

Hot Plate Reaction Time In Mice

The hot-plate test was assessed on groups of six mice. The temperature of metal surface was maintained at 55±1°c. Latency to discomfort reaction (forepaw licking or jumping) was determined by the method of (Turner, 1960). The cut-off time was 20 seconds. The latency was recorded before and after the *EETP* (125 and 250 mg/kg) and standard drug administered by IP route. The prolongation of the latency times compared with values of the control was used for statistical comparison. Aspirin (200 mg/kg, IP) was used as reference standard.

Statistical Analysis

The experimental results were expressed as mean ± SEM. Data were assessed by the method of analysis of ANOVA followed by student's *t*-test, $p<0.05$ was considered as statistically significant.

RESULTS

Acute s toxicity study

When the mice were observed for the behavioral changes after intraperitoneally administration of a single dose of the extract, none of the mice exhibited any abnormal behavioral responses at lower doses. These include inactiveness, loss of appetite, slow movement and dizziness, erection of hairs and hypothermia. But, those mice, which received higher, dose or above showed slight toxic symptoms, which are, reflect the LD_{50}. However, the greater a drug's therapeutic index, the less likely is the fatalities that will follow an accidental overdose.

Analgesic studies

The effect of the *EETP* on writhing response of mice was shown in Table 1. It was found that the extract caused an inhibition on the writhing response induced by acetic acid in a dose dependent manner. Dose of 250 mg/kg *EETP* and Aspirin could block the writhing response by 50.92% and 67.68%, respectively. As shown in Table 2 *EETP* showed significant antinociceptive action in hot plate reaction time method in mice. This effect was comparable to that of standard drug Aspirin treated controls, suggesting that central activity of *EETP*.

The present study establishes the analgesic activity of the *EETP* in standard experimental animal models. The effect of *EETP* at the dose of 125 and 250 mg/kg showed significant analgesic activity. The analgesic test used in the present study was chosen in order to test different nociceptive stimuli, namely cutaneous thermic (hot plate) and chemical visceral (writhing) stimuli9 (Lavine and Taiwo, 1994). In acetic acid induced abdominal writhing and it causes analgesia by liberating endogenous substances and many others excite pain to the nerve ending (Fields, 1987). According to the percentage of inhibition on the number of writhes obtained with different doses of *EETP*, it was found that the intensity of the analgesic effect was similar to that of the Aspirin. Aspirin and related drugs can inhibit cyclooxygenase in peripheral tissues, thus interfering with mechanism transduction in primary afferent nociceptors (Raj, 1996). The prostaglandin amplifies the pain mechanism and enhances vascular permeability whilst the leukotrienes contract smooth muscles blood vessels. And it leads to enhance the vascular permeability and mediate proinflammatory and allergic response (Bisgaard, 2000 and Bley *et al*, 1998). Results of the present study shows that all the doses of the *EETP* produces significant antinoceptive effect that may be due to blockade or release of endogenous substances that excite pain nerve endings similarly to aspirin and other NSAIDs.

Table 1: Effect of ethanol extract of *Trianthema portulacastrum* extract on acetic acid induced writhing test

Treatment	Dose (mg/kg)	Number of writhing	Percentage of inhibition
Control	-	36.4 ± 2.36	-
Aspirin	200	11.4 ± 1.02[a]	67.68
EETP	125	22.4 ± 1.19[b]	36.36
EETP	250	17.3 ± 0.98[a]	50.92

Values shown are mean ± SEM (n = 6). [a]$p<0.01$, [b]$p<0.05$, experimental groups were compared with control

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Table 2: Analgesic activity of ethanol extract of *Trianthema portulacastrum* leaves extract on hot plate method

Treatment	Dose (mg/kg)	Reaction Time (sec)			
		15 min	30 min	60 min	90 min
Control	-	2.43 ± 0.23	3.05 ± 0.27	2.86 ± 0.22	3.69 ± 0.22
Aspirin	2	3.35 ± 0.28	5.29 ± 0.46	7.22 ± 0.68[a]	7.58 ± 0.58[a]
EETP	125	3.15 ± 0.23	5.37 ± 0.51	7.76 ± 0.74[a]	8.45 ± 0.65
EETP	250	3.58 ± 0.22	5.51 ± 0.52	8.43 ± 0.58[b]	10.66 ± 1.85[b]

Values shown are in mean ± SEM (n = 6). [a]p<0.001, [b]p<0.01, Experimental groups were compared with control.

The hot plate method described by Woolfe and Mac Donalod, (1944) was followed. This test has been found to be suitable for evaluating centrally but not in the peripherally acting analgesic. The validity of this test has been shown even in the presence of substantial impairment of motor performance (Plummer *et al*, 1991). The present study findings indicate that the *EETP* may be centrally acting.

It can also be concluded that this analgesic activity of the drug could be attributed to phenoliccompounds, terpenoids and steroids, which are present in *Trianthema portulacastrum* .

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