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Research Article

Antinociceptive and Anti-Inflammatory Effects of Solvent Extracts of *Tagetes erectus* Linn *(Asteraceae)*

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Abstract

Purpose: *Traditionally, the leaves of Tagetes erectus L. are used in India for the alleviation of pain and inflammation. The objective of this study was to investigate the antinociceptive and anti-inflammatory activities of this plant material in an animal model.*
Methods: *The chloroform, methanol and ether extracts of the leaves of Tagetes erectus L. (family: Asteraceae) were tested against acetic acid-induced writhing in mice and carrageenan-induced paw oedema in rats in order to assess their antinociceptive and anti-inflammatory activities, respectively. The doses administered intraperitoneally (I.P.) ranged from 100 to 400 mg/kg body weight, and acetylsalicylic acid (ASA) and phenylbutazone were the reference standards for the antinociceptive and anti-inflammatory tests, respectively.*
Results: *The extracts showed antinociceptive and anti-inflammatory properties at doses between 200-400 mg/kg. They inhibited significantly (P < 0.005), in a dose-dependant manner, induced writhing reflexes in mice. The antinociceptive effect was comparable to that of ASA which served as the reference standard. Similarly, the extracts significantly (P <0.05) reduced carragenan-induced paw oedema in rats and the reduction in paw volume was comparable to that of the reference standard (phenylbutazone). It also increased pain threshold in the oedematous right hind limb paw of the rats.*
Conclusion: *The results obtained show that the extracts of Tagetes erectus L. (Asteraceae) has antinociceptive and anti-inflammatory properties. This finding provides a basis for the traditional use of the plant material.*

Keywords: *Tagetes erectus, Antinociceptive, Anti-inflammatory.*

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INTRODUCTION

Tagetes erectus L. (Asteraceae) has several therapeutic uses in traditional medicine across the world. The parts of the plant are of different therapeutic values. These include treatment of pain, inflammation, cancer and various gastro-intestinal disorders[1]. *Tagetes erectus L.* is rich in the xanthophylls, lutein, which occurs acylated with fatty acids[2,3]. Carotenoids, which are present, have excellent antioxidant properties while α- and β-carotene, xanthophylls and retinoids have been reported to inhibit some types of cancers[4,5]. Lutein also shows greater antioxidant activity than the other two common carotenoids, β-carotene and lycopene[6]. Other *Tagetes* species that share some of these therapeutic properties include: *T. patula*[7], *T. minuta*[8]. Arising from the traditional uses of *T. erectus* in India, we examined in this work the effects of three solvent extracts of the leaves of this plant for their antinociceptive and anti-inflammatory activities in experimental animal models.

MATERIALS AND METHODS

Drugs

Acetylsalicyclic acid, phenylbutyzone, carrageenan, chloroform, methanol, petroleum ether were purchased from Fine Chem Industry, Mumbai, India.

Plant material

Tagetes erectus L. collected from garden in 2008. The botanical identity of the sample was confirmed by Dr. P. G. Diwakar, Joint Director of Botonical Survey of India, Pune, and assigned the voucher no. BSI/WC/Tech./2008/485. The voucher specimens were deposited in the herbarium of the Botanical Survey of India, Pune

Animals

Albino mice (20 – 30 g) and Wistar albino rats (180–200 g) of both sexes, bred in the Animal House of Pharmacology Department, S.G.R.S. College of Pharmacy, Saswad, Pune, were maintained at room temperature 25 ± 2 °C in 12h dark–light cycle.

Preparation of extracts

Leaves of *tagetes erectus L.* (150 g) were dried at 40 °C for 1 week and pulverised. The powder was packed into the thimble of a Soxhlet extractor and refluxed continuously for 6 h. The solvent - either petroleum ether (PEE), chloroform (CE) or methanol (ME)) - was changed at the end of every 6 h. The solvent was removed by distillation on a boiling water-bath at atmospheric pressure and then under reduced pressure in a rotary evaporator. Before administration, ME extract was reconstituted by dissolving in water while PEE and CE extracts were suspended in 3% gum acacia solution.

Toxicity study

Eighty mice were divided into eight groups of ten animals each. One group served as a control and received 0.9 % NaCl alone (10 ml/kg) given intraperitoneally (i.p.), while the remaining seven groups were treated with increasing doses of the aqueous extract: 50, 100, 200, 400, 600, 800 and 1000 mg/kg (i.p.), respectively. The mortality rate within a 24 h period was determined and the LD50 was estimated according to the method described by Miller and Tainter[9]. Based on the results of the acute toxicity test, doses of 100, 200 and 400 mg/kg were chosen for other tests.

Antinociceptive activity

Chemical-induced (acetic acid) writhing method

Three different groups of mice received 100, 200 and 400 mg/kg orally of *the extract.* Sixty minutes after extract administration, 0.1 ml of 1% v/v acetic acid was injected (i.p.). The number of abdominal contractions over a period of 20 min was noted. Acetysalicylic

acid (ASA, 100 mg/kg, orally) was used as positive control. Significant reduction in the number of abdominal contraction ($P < 0.05$) compared to the control (that received 0.3 ml normal saline) was considered as antinociceptive action[10].

Hot plate (thermal) method

The mice were first treated with different doses of *the extract* (100, 200 and 400 mg/kg, orally). One hour later, they were placed on Eddy's hot plate maintained at 55±1 °C. The time taken by the animals to lick the fore or hind paw or jump out of the plate was taken as the reaction time. ASA (100 mg/kg orally) was used as the reference drug.

Anti-inflammatory activity

Carragennan-induced paw oedema

Acute inflammation was produced by injecting 0.1 ml of 1 % carrageenan into the plantar surface of rat hind paw. The extracts (100, 200 and 400 mg/kg, orally) and phenylbutazone (PBZ, 100 mg/kg, orally) as reference drug, were administered 60 min before carrageenan injection. The paw volume was measured at 0, 0.5, 1, 2, 3 and 4h plethysmometrically (Ugo Basile 7140)[11].

Statistical analysis

The data are presented as mean ± SEM and subjected to one way analysis of variance (ANOVA), followed by Students 't' test. $P < 0.05$ was considered significant.

RESULTS

The yield was 6.2 % (PEE), 7.4 % (CE) and 6.3 % (ME). The orally administered extracts (obtained with CE, ME and PEE, respectively) significantly reduced pain induced by acetic acid writhing responses, as shown in Table 1. The number of writhing reflexes in treated mice decreased significantly ($P < 0.05$) and was comparable

to ASA. No significant change in thermal stimuli was found (Table 1).

In the oedema test, shown in Table 2, there was a gradual increase in oedema paw volume of rats in the control group. However, in the test groups, the three extracts showed a significant reduction in the oedema paw volume. CE extract exhibited a dose-related inhibition of hind paw oedema between 2 and 4 h with the inhibitory effect highest at 400 mg/kg. Equipotent effects were demonstrated by PEE and ME extracts which were comparable to PBZ (reference drug, 100 mg/kg orally) with as high as 76 % inhibition of oedema formation.

DISCUSSION

Of the several traditional claims of the usefulness of *T. erectus L.,* pain and inflammation are the most cited in literature[1]. This, therefore, influenced the focus of this investigation on the evaluation of the antinociceptive and anti-inflammatory activity of the plant extract.

Most of the so-called peripheral analgesics possess anti-inflammatory properties and, in some cases, also antipyretic activity besides analgesia. For many of them, the mode of action has been elucidated as an inhibition of cyclooxygenase in the prostaglandin pathway. Nevertheless, new peripheral analgesics have to be tested not only for their *in vitro* activity on cyclooxygenase but also for their *in vivo* activity. The most commonly used methods for measuring peripheral analgesic activity are the acetic acid induced writhing tests in mice[11]. Pain is induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called writhing. The test is suitable to detect analgesic activity although some psychoactive agents also show activity. An irritating agent such as phenylquinone or acetic acid is injected intraperitoneally into mice and the stretching reaction evaluated. The reaction is not specific for the irritant.

Table 1: Effect of the extracts of *T. erectus L.* on chemical- and thermal-induced pain response in mice

Treatment	Dose (mg/kg)	Writhing response (min)	Hot plate reaction time (s)
Control (saline)	0.5 ml	55.3 ± 1.7	5.5 ± 2.1
Chloroform extract (CE)	100	38.0 ± 4.9*	4.4 ± 0.8
	200	37.2 ± 3.8*	5.4 ± 1.3
	400	36.0 ± 1.0*	5.6 ± 1.2
Methanolic extract (ME)	100	34.4 ± 2.2*	14.4 ± 4.4
	200	29.6 ± 5.7*	8.2 ± 1.6
	400	26.4 ± 6.1*	16.8 ± 2.3**
Petroleum ether extract (PEE)	100	21.8 ± 5.0*	4.4 ± 0.6
	200	18.4 ± 4.4*	12.4 ± 3.6
	400	12.8 ± 2.4*	13.3 ± 2.8
ASA	100	32.0 ± 2.5*	16.2 ± 2.9

Values represent mean ± S.E.M. (n = 5); *P <0.05.

Table 2: Anti-inflammatory properties of the extracts of *T. erectus* leaves and phenylbutazone (PBZ) on carrageenan-induced oedema in the right hind-limb paw of rats

Treatment	Dose (mg/kg)	Paw volume (mL)			
		1 h	2 h	3 h	4 h
Control (saline)	-	0.44 ± 0.12	0.72 ± 0.08	0.88 ± 0.18	0.92 ± 0.18
Chloroform extract (CE)	100	0.52 ± 0.07	0.70 ± 0.04	0.42 ± 0.02*	0.38 ± 0.02*
	200	0.52 ± 0.06	0.32 ± 0.05*	0.30 ± 0.18*	0.18 ± 0.18*
	400	0.12 ± 0.02*	0.00 ± 0.0*	0.10 ± 0.0*	0.0 ± 0.0*
Methanol extract (ME)	100	0.32 ± 0.06	0.24 ± 0.02*	0.24 ± 0.05*	0.20 ± 0.02*
	200	0.14 ± 0.07	0.26 ± 0.05*	0.26 ± 0.02*	0.26 ± 0.09*
	400	0.26 ± 0.07	0.20 ± 0.05*	0.32 ± 0.09*	0.44 ± 0.07*
Petroleum ether extract (PEE)	100	0.30 ± 0.11	0.32 ± 0.07*	0.20 ± 0.02*	0.10 ± 0.04*
	200	0.34 ± 0.09	0.38 ± 0.1*	0.28 ± 0.07*	0.20 ± 0.08*
	400	0.26 ± 0.02	0.16 ± 0.06*	0.20 ± 0.09*	0.18 ± 0.06*
Phenylbutazone (PBZ)	100	0.20 ± 0.02*	0.21 ± 0.09*	0.23 ± 0.07*	0.14 ± 0.05*

Values are mean ± S.E.M. (n = 5), *P <0.05 of the difference between the left and the right hind paws

The extracts showed significant antinociceptive effect in acetic acid-induced writhing response. This is a clear indication of very potent antinociceptive activity against pain stimuli. The antinociceptive effect of the extracts could be mediating through peripheral mechanisms rather than central as the extracts did not show any significant analgesic activity when evaluated by the hot plate method. Painful stimuli can consist of direct stimulation of the efferent sensory nerves or stimulation of pain receptors by various means such as heat or pressure. The role of endogenous peptides such as enkephalins and endorphins gives more insight into brain processes and the action of central analgesics. In the peripheral system, analgesic agents inhibit cyclooxygenase in the prostaglandin pathway[11]. This may explain explain the antinociceptive activity of the extract and thus the rationale for the traditional use of the plant. In this regard, PEE and ME extracts were more potent than CE in all the models used.

Other properties exhibited by the plant extracts are anti-inflammatory effects. They showed a potent suppressant activity on the acute inflammatory model of carrageenan-induced paw oedema in rats. The anti-inflammatory principles of PEE and CE are probably non-polar. Non-polar substances are more effective in chronic inflammation, while ME, which contains polar substances, are more effective in acute inflammation[12]. The plant leaf is said to contain flavonoids and terpenoids as its major constituents[13]. Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction to eliminate or limit the spread of injurious agent as well as necrosed cells. Acute inflammation can be conveniently described as a vascular and cellular event. In vascular events, alteration in the macrovasculature is the earliest response to tissue injury. These alterations include haemodyanamic changes such as transient vasoconstriction, persistent progressive vasodilation, followed by local hydrostatic pressure, stasis, leucocytes migration and vascular changes in which accumulation of oedema fluid. In cellular events, phagocytosis, that is, engulfment of solid particulate material by cells, causes the inflammation. Chronic inflammation causes tissue destruction brought by activated macrophages by release of variety of biological active substances[14]. It would appear that the extracts had a suppressive effect on these events.

CONCLUSION

The results of this investigation reveal that the leaves of *T. erectus L.* have antinoceciptive and anti-inflammatory activities and this may provide the basis for its use in traditional medicine.

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