Research Article

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Evaluation of Anti-nociceptive and Anti-inflammatory Activities of *Tagetes erecta* Linn Leaves.

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To evaluate the activities of *Tagetes erects* upon pain (Antinociception) and inflammation in rodent models. The antinociceptive activity of Hydro alcoholic extract of *Tagetes erecta* (250 &500 mg/kg) was studied using acetic acid induced writhing and hot plate method in mice. The anti-inflammatory activity of Tagetes erecta was studied in rats by Carrageenan induced paw edema. Hydro alcoholic extract of *Tagetes erecta* revealed significant antinociceptive with both the models. The activity was statistically similar to aspirin but less potent than morphine. The *Tagetes erecta* extract also revealed significant anti-inflammatory activity. This effect was statically similar to the non steroidal anti-inflammatory drug Diclofenac sodium. The results of the study showed the justification of the use of the plant in the treatment of inflammatory disease conditions, and the active chemical constituents when isolated will be added to the present anti-inflammatory agents.

Key words: *Tagetes erecta,* Anti-nociceptive, Anti- inflammatory activity.

INTRODUCTION

Inflammation is a pathophiosological response of inflammation tissues to a variety of hostile agents including in infectious organisms, toxic chemical substances, physical injury or tumors growth leading to local accumulation of Plasmic fluid and blood cells [1].The clinical treatment of inflammatory diseases is dependent on drugs which belongs either to the non-steroidal or steroidal chemical therapeutics. The nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin and ibuprofen inhibit early steps in the biosynthesis pathway of prostaglandins by inhibition of COX enzymes and are the main drugs used to reduce the untoward consequences of inflammation [2]. However, the side effects of the currently available anti-inflammatory drugs pose a major problem in their clinical use. For instance, NSAIDs cause several serious adverse effects like gastric injury and ulceration, renal damage, and bronchospasm due to their non-selective inhibition of both isoforms of the COX enzyme [3]. The use of steroidal drugs as anti-inflammatory agents are also becoming highly controversial due to their multiple side effects [4]. Therefore, a demand arises for the development of newer anti-inflammatory agents from natural sources with more potent activity and with minor side effects as substitutes for chemical therapeutics.

Tagetes erecta of the family compositae is commonly found in parts of India, Asia, Africa and America. It is known as Marigold [5]. The leaves are reported to be effective against piles, Kidney troubles, muscular Pain, ulcers, wound and earache [6]. The herbs are used for the treatment of inflammatory conditions as a household remedy on experimental basis. The objectives of this study were to evaluate the anti nociceptive and anti inflammatory activities of *Tagetes erecta* in mice and rats.

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MATERIALS AND METHODS

Leaves of *Tagetes erecta* Linn were collected during the month of November from the local area of Burdwan District, West Bengal, India, and authenticated by Udayan Sarkar, Dept of Botany, Sonamukhi College. A voucher specimen (No-SC341/07) was deposited at the college. The plants were dried in shade around 10 days and the crude drug were subjected to pulverizations and passed through sieve no.40.

The powder (75g) was packed into a Soxhlet apparatus for extraction by Hydro alcoholic solvent (350ml alcohol (95%) and 150ml water) at 60^0 C to 70^0 C for 24 hour. The extract was dried at 75^0C in water bath for hours when a solid mass was obtained. After concentrated preparation, the dried powder extract was stored at 4^o C. The yields of the hydro alcoholic extract were found to be 9.5%.

Animals: Wistar albino rats (150-250g) and Albino mice (20-30g) of either sex were used for the activity study and procured from the Bioneeds animal house, Dhavas pet, Tumkur. The animals were housed in Poly propylene cages and maintained at 24^0C ± 2^0 C under 12 hr light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. The standard diet supplied by Pranav agro industries Ltd. Sangli. The experimental protocol was approved by the Institutional animal ethics committee,(No.997/C/06/ CPCSEA). As per the requirement of committee for the purpose of control & Supervision on animal (CPCSEA) New Delhi.

Table 1: Effect of Tagetes erecta extra ct on Acetic acid-induced writhing in mice.

Group & Dose	No. of Writhing	Percentage Inhibition[1]
Control	41.4 ±2.5*	-
Tagetes erecta(250mg/kg)	15.2 ± 3.1*	63.2
Tagetes erecta(500mg/kg)	7.1±2.5**	82.8
Aspirin (100mg/kg)	6.9±1.8**	83.3
Morphine(10mg/kg)	3.1±1.2**	92.5
Tagetes erecta(500mg/kg) + Naloxone (5mg/kg)	19.1±1.3**	53.4

Values are expressed as mean±SEM, Number of animals (N) =6,
[1] Percentage Inhibition compare to control
**=P<0.01= very significant,*=P<0.05= significant

Preliminary Phytochemical screening:

The extracts of *Tagetes erecta* Linn *were* subjected to qualitative analysis for the various Phytoconstituents like Alkaloids, Carbohydrates, Glycosides, Phytosterols, Saponins, Tannins, Proteins, Amino acids and Flavonoids[8, 9].

Determination of acute toxicity (LD_{50})

An acute toxicity study was carried out for determination of LD_{50} of hydro alcoholic extract of leaves of Tagetes erecta Linn in albino mice of either sex weighing 20-30 g. The animals were fasted overnight prior to the experiment Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies. The extract administered by intraperitonially. The animals were continuously observed for 24 h to detect changes in autonomic or behavioral responses. Mortality in each group was observed for 7 days [10, 11].

Table2: Evaluation of analgesic activity of hydro alcoholic extract of Tagetes erecta by hot plate method.

Treatment	Increase in reaction time(sec) Different time intervals (hr)					
	0.5	1	2	3	6	24
Control	0.623 ± 0.07	0.704 ± 0.15	0.266 ± 0.16	0.498 ± 0.17	0.503 ± 0.13	0.4842 ± 0.19
Morphine (5mg/kg)	5.493 ± 0.73 **	9.476 ± 0.50 **	6.976 ± 0.64 **	5.241 ± 0.5 **	2.491 ± 0.45**	0.7242 ± 0.19 **
Extract treated (250mg/kg)	2.415 ± 0.58	3.177 ± 0.49 **	2.618 ± 0.37 * *	1.808 ± 0.24 *	0.493 ± 0.07 *	0.2700 ± 0.06*
Extract treated (500mg/kg)	5.281 ± 1.19 **	6.544 ± 0.45 **	4.406 ± 0.25 **	2.729 ± 0.16 **	1.083 ± 0.20**	0.6608 ± 0.31**

Values are expressed as mean±SEM, Number of animals (N) =6,
**=P<0.01= very significant,*=P<0.05= significant

Evaluation of analgesic activity Acetic acid induced writhing: The Antinociceptive activity of *Tagetes erecta* was assessed using writhing test [12]. Acetic acid solution (10ml /kg 0.6%) was injected i.p rout & the construction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 0.5h beginning 5 min after acetic acid injection. The extract was administered (250&500 mg)

0.5h before the acetic acid injection. Antinociceptive activity was expressed as the percentage inhibition of abdominal constriction between control animals &mice treated (n=6) with the extract using the ratio.

(Control mean –Treated mean) x100/control mean. In attempt to investigate the participation of the opioid system in this plant extract separate group of mice (n=6) were pretreated with non specific opioid receptor antagonist, naloxone (5mg/kg i.p) injected 15 min before the administration of the extract [13].

Hot Plate Method: [14]

Mouse of either sex (20-30gm) was fasted for 18 h. They were then divided into four groups (n=6). Analgesic activity was assessed using the hot plate analgesiometere that uses heated metal plate (with adjustable temperature). A 15-s cutoff to

Table No 3:- Anti- inflammatory activity of crude extract *Tagetes erecta* by Carrageenan induced rat paw oedema.

Group & Dose	Increase in paw Volume(mm)±SEM AND % Oedema inhibition relative to control at every 30 min till 180min.					
	Time after Carrageenan injection					
	30min	60min	90min	120min	150min	180min
Control (Tragacanth1%)	0.169±0.0012	0.281±0.221	0.373±0.031	0.435±0.015	0.401±0.032	0.380±0.0010
Diclofenac sodium(10mg/kg)	0.131 ±0.005** (25.4%)	0.178 ±0.0011** (36.6%)	0.211 ±0.200** (43.4%)	0.196 ±0.0082*** (54.9%)	0.132 ±0.0049*** (67.1%)	0.070 ±0.0011*** (81.5%)
Extract treated (250mg/kg)	0.144 ±0.004* (14.8%)	0.186 ±0.0156* (33.81%)	0.245 ±0.285* (34.82%)	0.238 ±0.0065** (45.29%)	0.198 ±0.0036** (50.63%)	0.159 ±0.0013*** (56.56%)
Extract treated (500mg/kg)	0.126 ±0.0025** (25.5%)	0.181 ±0.168** (35.5%)	0.221 ±0.185** (40.7%)	0.201 ±0.0136*** (53.8%)	0.140 ±0.0052** (65.09%)	0.081 ±0.0012*** (79.0%)

Values are expressed as mean±SEM, Number of animals (N) =6,
***p<0.001= very very significant**=P<0.01= very significant, *=P<0.05= significant.

prevent damage to the animal's paw. The temperature of the hot-plate was then regulated to 45±1ºC. Each mouse was placed on the hot-plate in order to obtain the animal's response to electrical heat-induced nociceptive pain stimulus (licking of the fore paws and eventually jumping). The time taken for each mouse to jump out (i.e., reaction time) was noted and recorded in seconds. Each mouse served as its own control. Thus, before treatment, its reaction time was determined thrice at 0, 20, 40 min intervals. The mean of these three determinations constituted the 'initial reaction time'—that is, reaction time before treatment of the mouse. Baseline values were determined before drug administration in each animal. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second receive standard drug, morphine (5 mg/kg i.p.) as suspension. The *Tagetes erecta* was administered by i.p. route at 250 mg/kg to third group and 500 mg/kg to fourth group as suspension. 30 min after treatment the reaction time was again evaluated, at 0.5, 1, 2, 3, 6 and 24 h. This final 'test' mean reaction time value represented 'after-treatment reaction time' (Ta) for each treated mouse. This 'test' reaction time value (Ta) was subsequently used to determine percentage protection, using (T_b) as basal reaction time, by applying the formula:

$$\%\text{protection} = \frac{\text{Test reaction time}}{\text{Basal reaction time}} \times 100 = \times 100 \frac{Ta}{Tb}$$

Evaluation of anti-inflammatory activity: Carrageenan induced paw edema: [15]

Rats of either sex (150-250gm) were fasted for 18 h. They were then divided into four groups (n=6). Before any treatment, volume of the right paw of each animal was determined thrice at 0, 20, 40 min intervals using a venire caliper. The mean of these three determinations constituted the Vo, basal volume. The first group which served as control was administered with aqueous 1% tragacanth suspension. The second group receive standard drug, Diclofenac (10 mg/kg) orally as suspension. The *Tagetes erecta* was administered orally at 250 mg/kg to third group and 500 mg/kg to fourth group as suspension. 30 min minutes later, paw edema was induced by subplantar injection of 0.1 ml Carrageenan (0.1%) into the plantar surface of the right hind paw. 30 min after treatment the paw volume was again measured, at 30, 60, 90,120, 150 and 180 min. This final 'test' volume value represented 'after-treatment paws volume' (V_t) for each treated rat. This 'test' mean paw volume value (V_t) was subsequently used to determine percentage protection. These individual records allowed to find out the variation of edema (Vt − Vo) for each group. Percentages of inhibition (*I* %) in each treated group was determined using the following formula:

$$I\% = 100 - [B \times 100]/A,$$

Where *A* is the mean variation of edema (Vt − Vo) for the control group and *B* is the (Vt − Vo)

for the treated groups with extracts or compounds.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnet comparison test. The values are expressed as mean ± SEM and $p<0.05$ was considered significant.

RESULTS

Results of Phytochemical screening: The Preliminary Phytochemical investigation of the Hydro alcoholic extract leaves of, *Tagetes erecta* Linn shows that it contains Saponins (Triterpenoids), essential oil

Acute toxicity studies

The acute toxicity studies of the Hydro alcoholic extract of leaves of *Tagetes erecta* Linn were found to be non-lethal up to dose of 2 g/kg body weight of the animals so that $1/8^{th}$ and $1/4^{th}$ (i.e. 250 mg/kg and 500 mg/kg orally) was selected .

Effect of *Tagetes erecta* on the acetic acid induced writhing

As shown in Table 1. Hydro alcoholic extract of *Tagetes erecta* (250&500mg/kg i.p) showed a significant dose- dependent reduction with approximately 63% and 83% inhibition respectively. Maximum inhibition at the dose of 500mg/kg. Which was statistically similar to standard drug Aspirin (100mg/kg).Morphine (10mg/kg) showed the most potent inhibition. The mechanism of the *Tagetes erecta* induced Antinociception was investigated using the opioid receptor antagonist, naloxone. The antinociceptive effect induced by the Hydro alcoholic extract of *Tagetes erecta* (500mg/kg) was significantly antagonized by pretreatment with naloxone in the writhing test (Table 1).

Effect of analgesic activity of hydro alcoholic extract of *Tagetes erecta* by hot plate method.

The antinoceptive effect of *Tagetes erecta* by the hot plate method was produced dose dependently on mice and was showed in table 2. At the dose of 250 mg/kg was not significant after 30 min of its administration but its 500 mg/kg dose produced significant ($P<0.01$) effect. The *Tagetes erecta* showed peak effect 120.61% and 155.35% at the dose of 250 mg/kg and 500 mg/kg respectively at 60 min after treatment while morphine was also showed peak effect 197.22% at 60 min in comparison with control. Comparatively the effect of high dose of *Tagetes erecta* was lesser than the effect of morphine. The *Tagetes erecta* was showed significant analgesic affect up to 3 h after the treatment whereas morphine was showed significant effect up to 6 h.

Anti-inflammatory effect of hydro alcoholic extract of Tagetes erecta

The extract as well as Diclofenac sodium showed anti- inflammatory activity. The extract was dose- dependent and found to be statiscally significant at the higher concentration 500mg/kg. (Table-3). The anti-inflammatory activity of Diclofenac sodium, a standard reference drug, was also found to be very significant. In the Carrageenan induced rat paw oedema test for acute inflammation, the extract of *Tagetes erecta* in dose of 250 mg/kg and 500mg/kg. Body weight showed significantly inhibition of Oedema respectively at end of 180min.

DISCUSSION

Administered of Tagetes erecta extracts showed sigficant antinociceptive activity in the hot-palte and acetic acid-induced writhing tests (Table 1and 2).These Results indicate that the plant extract possesses centrally and peripherally mediated antinociceptive properties. Carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the anti-edematous effect of natural products [16]. Carrageenan is a polysaccharide known to activate the Hageman factor and to liberate kallikrein from its inactive precursor prekallikrein [17]. Acute inflammation of Carrageenan involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, histamine, bradykinins, leucotrienes and serotonine, all of which also cause pain and fever. Inhibition of these mediators from reaching the injured site or from bringing out their pharmacological effects will normally ameliorate the inflammation and other symptoms.

Development of edema in the paw of the rat after injection of carrageenan is a biphasic event [18]. The initial phase observed during the first hour is attributed to the release of histamine and serotonin [19]. The second phase (2-5 hrs after injection) of edema is due to the release of prostaglandins, protease and lysosome [20]. Based on this, the *Tagetes erecta*

has effect on both the phases since its effect was showed effect after 30 min of carrageenan injection that is the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and also up to 3 h i.e. the second phase, this effect may be by either inhibition of phospholipase A_2 (PLA_2) activity or cyclooxygenase pathway and by blocking the release of vasoactive substances (histamine, serotonin and kinins). Both the doses of extract showed significant protection against edema. It shows maximum effect at 90min which is comparable to the standard. The effect could also be related to inhibition of kinins release, which has been implicated as major pro inflammatory mediators in addition to histamine and prostaglandins [21].

CONCLUSION

The above findings justify the hydro alcoholic extract of *Tagetes erecta* contain bioactive constituents with antinociceptive and anti-inflammatory activities, and further support the etanomedical claim of the use of the plant in the management of inflammatory conditions.

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