



Antinociceptive and anti-inflammatory activities of *Tagetes Erectus* Flowers in animal models.

Guno Sindhu Chakraborty*

SVKM'S, NMIMS University, School of Pharmacy and Technology Management, Shirpur
Campus, Shirpur, Maharashtra, India

Abstract

In the present study potential of chloroform extract of *Tagetes Erectus* (Family-Compositae) flowers on antinociceptive, behavioral study and anti-inflammatory effects using various animal models. The dried, powdered flowers of *Tagetes Erectus* were extracted successively with petroleum ether (60-80° C) and chloroform in soxhlet apparatus. The chloroform extract yield (20.6 % w/w with respect to dry powdered plant material) was selected for all experimental procedure. Two models were employed to investigate the effects on nociception; tail immersion and hot plate method in Swiss albino mice and anti-inflammatory effect were investigated by employing the carrageenan induced paw edema test in adult Wistar albino rats. Behavioral study was investigated by elevated plus maze method in Swiss albino mice. Results were revealed that the TECE was found significant antinociceptive effect ($p < 0.001$) at dose levels of 100, 200 and 400 mg/kg, orally in mice and produced a remarkable anti-inflammatory effect ($p < 0.001$) at the same dose levels used in rats. Behavioral study of the TECE has no significant effect when used orally. Thus from the above findings it can be concluded that, TECE possessed remarkable antinociceptive effect and anti-inflammatory effect but no anxiolytic effect on animal models.

Key words: *Tagetes Erectus*, Chloroform extract, inflammation, antinociceptive, paw edema.

Introduction

Plants are the essential and integral part in Complementary and Alternative medicine and due to this they develop the ability for the formation of secondary metabolites. Plants are the best source of active secondary metabolites which are beneficial to mankind in treating many diseases [1]. Many plant origin drugs have been reported with biological properties like Analgesic, Antiinflammatory, Antioxidant, hypoglycemic agents and many more. Thus the present investigation was aimed at evaluating the Antinociceptive and anti-inflammatory activities of *Tagetes Erectus* Flowers in animal models.

Tagetes Erectus belonging to the family Compositae is a medicinal plant used in Indian System for Medicine for curing various diseases like Analgesic, Antiinflammatory, Antioxidant, hypoglycemic agents and many more. The flowers are used in kidney troubles and in muscular pains and are applied on boils and carbuncles. Infusion of plant is used against rheumatism, cold and bronchitis [2]. In unani medicine, a confection of tender flowers and purified sugar is prescribed in anuria, retention of urine and kidney troubles. The flowers contain pigments as Quercetagenin and quercetagenin [3]. From the literature cited very few works has been carried out in this plant. Thus it was thought worthwhile to explore this plant for its therapeutic activity.

Materials and Methods

Tagetes Erectus flowers were collected during the month of September from the rural garden of Shirpur, Maharashtra and it was identified and authenticated from the standard resources. The collected plant were washed and air dried under shade, cut, powdered by a mechanical grinder and passed through 40 mesh sieve and stored in a closed vessel for future use.

Preparation of Extracts

The dried powdered flowers of *Tagetes Erectus* (1 kg) were extracted successively with petroleum ether (60-80° C) in soxhlet apparatus. A yellowish orange colored petroleum ether extract was obtained. The same were extracted with chloroform (18 h) to produce a pale yellowish orange semisolid mass. These extracts were dried and concentrated by evaporating the solvent completely under vacuum at the range of boiling points of solvent using rotary evaporator. The chloroform extract yield (20.6 % w/w with respect to dry powdered plant material) was selected for all experimental procedure [4].

Preliminary Phytochemical Analysis

The extracts were subjected to preliminary phytochemical screening for detection of major chemical groups [5]. In each case test 10% w/v solution of the extract in chloroform was used. The results showed the presence of steroids, flavonoids, phenolic substances, sterols and fatty substances were present in both the extracts.

Experimental Animals

Adult wistar albino rats weighing 180-200 g and swiss albino mice of either sex between 18-22 g were used for the experiments. All the animals were housed in standard polypropylene cages at room temperature of 30±2°C, 12 h light and dark cycle and 60-65% relative humidity and had a free access to food and water *ad libitum*. The animals were used for the experiment after an acclimatization period of one week. The acute toxicity was done as per the protocol mentioned by OECD guidelines [6] and it was found that the dose upto 2000mg/kg was significant and no deaths of animals were observed. The protocol was approved by Institutional Ethics committee

Antinociceptive Activity

Animals were divided in to five groups of six mice in each. Group-I, serves as a control, received 0.025% w/v CMC, 10 ml/kg, orally, Group II-IV, animals received TECE at doses of 100, 200 and 400 mg/kg, orally and Group V animals were treated with positive control Ibuprofen.

Table 1. Antinociceptive Activity of *Tagetes Erectus* by tail immersion method

Groups	Dose	Pretreatment	Post Treatment after 4 hours
Group I Negative Control	10 ml	1.6 ± 1.00	1.6 ± 1.11
Group II TECE	100 mg/kg	1.62 ± 1.50	4.19 ± 1.00
Group III TECE	200 mg/kg	1.79 ± 0.7	5.2 ± 1.12*
Group IV TECE	400 mg/kg	1.89 ± 1.02	6.9 ± 1.19*
Group V Positive Control	50 mg/kg	1.79 ± 1.12	13.9 ± 1.00

Results are expressed as mean ± S.E.M. * P < 0.001 significantly different from control; paired t- test (n=6).

Table 2. Antinociceptive Activity of *Tagetes Erectus* by hot plate method

Groups	Dose	Paw licking time in seconds (90)	Paw jumping time in seconds (90)
Group I Negative Control	10 ml	2.6 ± 0.11	2.5 ± 1.11
Group II TECE	100 mg/kg	5.82 ± 1.50*	6.7 ± 1.00*
Group III TECE	200 mg/kg	5.79 ± 0.12*	7.7 ± 1.12**
Group IV TECE	400 mg/kg	5.01 ± 1.02**	6.9 ± 1.19**
Group V Positive Control	50 mg/kg	6.09 ± 1.12**	8.3 ± 1.00**

Results expressed as mean ± S.E.M. *P < 0.05, ** P < 0.001, significantly different from control; paired t- test (n=6).

Anti-inflammatory Activity

Animals were divided in to four groups of six rats in each. Group-I, serves as a control, received 0.025% w/v CMC, 10 ml/kg, orally, Group II-IV, animals received TECE at doses of 100, 200 and 400 mg/kg, orally and Group V animals were treated with positive control.

Table 3. Anti-inflammatory Activity of *Tagetes Erectus* by carrageenan induced paw edema in albino rats.

Groups	Dose	Percentage of inhibition of paw edema after carrageenan injection	
		2h	4h
Group I Control	10 ml	32.6 ± 4.11	42.5 ± 5.11
Group II TECE	100 mg/kg	75.82 ± 9.52**	90.7 ± 1.09*
Group III TECE	200 mg/kg	45.78 ± 1.12**	56.7 ± 1.12**
Group IV TECE	400 mg/kg	40.01 ± 1.02**	62.9 ± 4.19**
Group V Positive Control	50 mg/kg	96.09 ± 2.12**	120.3 ± 1.23**

Results expressed as mean ± S.E.M. *P< 0.05, ** P< 0.001, significantly different from control; paired t-test (n=6).

Results and Discussion

The effects of TECE were used to investigate the activity in animal models by two methods of Tila immersion test and hot plate test. Results of analgesic study showed that *Tagetes erectus* increases the reaction time in Tail immersion method. At 4 hours, the mean reaction time for a dose of 200 mg/kg and 400 mg/kg were 5.2 ± 1.12 and 6.9 ± 1.19 respectively as compared to 1.6 ± 1.11 for the control and 13.9 ± 1.00 for standard drug. The similar activity was observed for hot plate method. The results are shown in Table 1 and 2. In case of anti-inflammatory activity the dose of 200 mg/kg and 400 mg/kg were found to be highly significant when it was compared with the control and the standard drug which are shown in Table 3.

The results were statistically significant and the p value was found to be $P < 0.001$, which was considered significant.

The antinociceptive action of all the tested compounds was clearly evident by a dose dependent reduction in tail immersion and hot plate test. In this regard, it is interesting that many flavonoids isolated from various plants exhibit potent analgesics and anti-inflammatory action [7, 8]. Ibuprofen inhibits COX in peripheral tissue and interferes the pain transduction in primary afferent nociceptors. The analgesic action of TE may be attributed to the blockade of pin pathway or the release of endogenous substances that react pain nerve endings similarly to ibuprofen.

Carrageenan- induced paw edema model is the best method for screening of anti-inflammatory activity of herb extract. The edema production is related with the presence of kinins and prostaglandins [9].

Conclusion

In the present report revealed the anti-inflammatory and anti-nociceptive ability of TE, suggesting TE may be used for the treatment of inflammation and pain. These methods which were selected for the study was to have a check on the centrally mediated effects

of the extract. It has been observed that the most potent groups were the opiate which were associated with undesirable side effects and has an addiction towards it. But on the contrary the NSAID are notorious for the ulcerogenic activity [10]. Thus it was interesting to see that flavonoids isolated from plant exhibits potent analgesics and anti-inflammatory actions. Thus from the present investigation it can be said that TECE at doses 100,200 and 400 mg/kg, p.o. widely used acute inflammatory model for studying anti-inflammatory agent and was found to be statistically significant ($p < 0.001$) antinociceptive.

References

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