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## Analgesic and Anti-inflammatory Effect of Ethanolic Extract of *Tabernaemontana divaricata* L. Flowers in Rats

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### ABSTRACT

Ethanolic extract of *Tabernaemontana divaricata* L. (TD) flowers were evaluated for analgesic and anti-inflammatory effects on Wistar rat model. Analgesic and anti-inflammatory potential of TD flowers at doses of 125, 250 & 500 mg/kg was evaluated against the standard drug indomethacin at a dose of 20 mg/kg, p.o. Wistar rats of either sex of 6 numbers in each group was undertaken for study and evaluated by acetic acid-induced writhing, hot plate reaction time, carrageenan-induced hind paw edema and safety test on gastric mucosa method. Ethanolic extract of TD showed anti-nociceptive effect in acetic acid-induced writhing characterized by a significant decrease in the number of writhings in rats ( $p < 0.01$ ). In hot plate test, TD showed nociceptive reaction towards thermal stimuli in rats and a significant increase in the reaction time was observed ( $p < 0.01$ ). The test drug significantly inhibited the carrageenan-induced hind paw edema in rats that is indicative of the anti-inflammatory effect of TD ( $p < 0.01$ ). However, no gastric lesions were observed in TD treated rats indicating the safety of test drug. The ethanolic extract of TD showed significant analgesic and anti-inflammatory effects in different animal models.

**Keywords:** *Tabernaemontana divaricata*, flowers, indomethacin, carrageenan.

### INTRODUCTION

*Tabernaemontana divaricata* L. (TD; Apocynaceae) is a general garden plant in Southeast Asia and other tropical countries. It has been reported as a wealthy source of various alkaloids, with various pharmacological properties [1]. It has been used in conventional rejuvenation remedies in Thailand [2]. In Thai herbal medicine, these remedies are thought to improve memory. In addition, local people in America, Africa and Continental Asia have used this plant as a central nervous system stimulant [3].

Inflammation is one of the oldest known diseases of mankind affecting a large population of the world. No considerable progress has been made in achieving a permanent cure of inflammation and wounds. The search of screening and development of drugs for wound healing is a serious problem. There is much expectation of finding anti-inflammatory drugs from native plants, as these are still used in therapeutics despite the progress made in conventional chemistry and pharmacology for producing effective drugs [4].

The practice of plants, plant extracts or plant-derived pure chemicals to deal with disease become a therapeutic modality, which has stood the test of time. As understood by the World Health Organization (WHO), about three-

quarters of the world population depends upon traditional remedies (mainly herbs) for the health care of its people. The conventional medicines also some time called as, herbal or natural medicine existed in one way or another in different cultures/civilizations, such as Egyptians, Western, Chinese, Kambo (Japan) and Greco-Arab or Unani/Tibb (South Asia) [5, 6].

The present study was undertaken to investigate the analgesic and anti-inflammatory effects of TD flowers in different animal models.

## MATERIALS AND METHODS

### *Plant*

The flowers of TD were collected from Chidambaram, Cuddalore, Tamil Nadu, India. The plant was identified and authenticated by Chief Botanist, Department of Botany, Annamalai University, Annamalai Nagar Chidambaram, Cuddalore, Tamil Nadu, India. A voucher specimen has been kept at the herbarium of the University.

### *Preparation of extract*

The flowers of TD were dried in shade, powdered and passed through a 40-mesh sieve. Dried powder (500 g) was taken and subjected to successive extraction with petroleum ether, chloroform, ethanol and water in soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 60 °C without vacuum) and dried completely in desiccators and weighed. The extract of TD was freeze dried and stored at -80°C until further use. The dried mass (yield=20 g) was diluted with normal saline and used in experiments.

### *Preliminary phytochemical screening*

Petroleum ether, chloroform, ethanolic and aqueous extracts of TD was subjected to preliminary phytochemical screening for their presence or absence of active constituents utilizing standard method of analyses [7].

### *Drugs and chemicals*

Carrageenan and indomethacin were procured from Sigma-Aldrich, St. Louis, MO, USA. Acetic acid was procured from Pure Chem. Ltd., India.

### *Preparation of ethanolic extract of TD flowers*

The dried plant material (100 g) TD flowers were extracted three times by refluxing with distilled water for 8 hrs and the filtered extract was evaporated on a water bath to get a viscous ethanolic extract.

For dosing, the ethanolic extract of TD was uniformly suspended in 1% carboxymethyl cellulose (CMC) dissolved in water and administered orally (p.o.).

### *Experimental animals*

The study was conducted after obtaining institutional ethical committee clearance (160/1999/CPCSEA). Wistar rats (100–150 g; 4–6 weeks old, either sex) were maintained under controlled conditions of light (12 h/12 h), temperature (26±2 °C) and relative humidity (44–56%) for one week before and during the experiments. The animals had access to standard laboratory feed (Gold Mohur, Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*.

### *Analgesic activity*

#### *Acetic acid-induced writhing test*

Analgesic activity was assessed by abdominal writhing test using acetic acid [8]. The animals were divided into six groups (n=6 each) viz.: group I- acetic acid control (normal saline, 10 ml/kg, p.o.); group II- indomethacin solution (20 mg/kg, p.o.); group III- TD-I (125 mg/kg, p.o.); group IV- TD-II (250 mg/kg, p.o.) & group V- TD-III (500 mg/kg, p.o.).

In the writhing test, 0.2 ml of 0.6 % acetic acid solution was injected intraperitoneally and the number of writhes were counted starting 5 min after injection for a period of 20 minutes.

**Hot plate reaction time**

Analgesic activity was assessed by hot plate latency assay [8]. The animals were divided into six groups (n=6 each). The animals were divided into six groups (n=6 each) viz.; group I: control (normal saline 10 ml/kg, p.o.); group II: indomethacin (20 mg/kg p.o.); group III: TD-I (125 mg/kg, p.o.); group IV: TD-II (250 mg/kg, p.o.) & group V: TD-III (500 mg/kg, p.o.).

Rats from each group were placed on the hot plate after drug administration. Then reaction time for the animal to lick the paw or jump from the hot plate was taken as the latency (s). This was repeated at 60 and 90 minutes from the exact time given. The average of the latency was determined from the six rats in each group. The temperature of the hot plate was maintained at  $55 \pm 1^\circ\text{C}$ . The cut off time was kept at 20 seconds.

**Anti-inflammatory activity****Carrageenan-induced hind paw edema**

Inflammation was induced by administering 0.1 ml of (1%) carrageenan into sub-plantar surface of rat hind paw [9]. The animals were divided into six groups (n=6 each) viz.; group I: carrageenan control (normal saline 10 ml/kg, p.o.); group II: indomethacin (20 mg/kg p.o.); group III: TD-I (125 mg/kg, p.o.); group IV: TD-II (250 mg/kg, p.o.) & group V: TD-III (500 mg/kg, p.o.).

In this method, all drugs were given orally. One hour later all animals were injected with 0.1 ml of 1% Carrageenan solution in the sub-plantar aponeurosis of left hind paw and the paw volume was measured plethysmometrically at 1 hr, 3 hr and 5 hr. Indomethacin (20 mg/kg, p.o.) as standard and ethanolic extract of TD administered by the intragastric route 1 hr before administration of carrageenan.

**Safety of drugs on gastric mucosa of rats (ulcer index)**

This method was performed to assess the safety of ethanolic extract of TD on the gastric mucosa of rats. In this method, the animals were divided into two groups (n=6 each) viz.: group I: indomethacin (20 mg/kg p.o.) & group II: TD (500 mg/kg, p.o.).

In the present method, higher doses of drugs were given orally. After 5 hours of administration animals were sacrificed by an overdose of ether vapors. Then the stomachs were removed and opened. The sum of length of lesions was evaluated for ulcer index score 1, 2 & 3 for erosions 1 mm or less, 1 mm to 2 mm & more than 2 mm respectively. The overall score was divided by a factor of 10 and designated as ulcer index [10].

**Statistical analysis**

All the values are expressed as mean  $\pm$  S.E.M. The statistical significance was determined by ANOVA followed by Dunnett's test. Values  $p < 0.05$  was considered as significant.

**RESULTS****Preliminary Phytochemical Screening**

Ethanolic extract of TD flowers showed the presence of alkaloids, carbohydrates, flavonoids, saponins, and terpenes while proteins, amino acids, phenols, glycosides, fixed oils, volatile oils steroids and tannins were found to be absent.

**Analgesic activity****Effect of ethanolic extract of TD flowers on acetic acid-induced writhing in rats**

A significant decrease in acetic acid-induced writhing test was observed in 20 min observation. The score for writhing was significantly decreased by ethanolic extract of TD flowers at doses of 125, 250 & 500 mg/kg on acetic acid-induced writhing in rats over the score of control group ( $p < 0.05$ ). The effect of ethanolic extract of TD flowers on acetic acid-induced writhing test was comparable to indomethacin (Table 1).

**Effect of ethanolic extract of TD flowers on hot plate reaction time in rats**

A significant raise in the reaction time on hot plate was observed at 30, 60 and 90 min. In comparison to control group, ethanolic extract of TD at doses of 125, 250 & 500 mg/kg showed a significant increase in the reaction time at 30, 60 and 90 min, respectively ( $p < 0.05$ ). The effect of ethanolic extract of TD flowers on reaction time was comparable to the standard drug, indomethacin (Table 2).

**Anti-inflammatory activity****Effect of ethanolic extract of TD flowers on carrageenan-induced hind paw edema in rats**

The ethanolic extract of TD at doses of 125, 250 & 500 mg/kg showed a significant reduction in the paw volume at 1<sup>st</sup>, 3<sup>rd</sup> and 5<sup>th</sup> hr as compared to control group ( $p < 0.01$ ). The effect of ethanolic extract of TD flowers on paw volume (edema) was comparable to the standard drug, indomethacin (Table 3).

**Assessment of the safety of test drugs on gastric mucosa of rats**

This method was adopted to assess the safety of ethanolic extract of TD flowers on gastric mucosa of rats using the higher dose of the test drug. In this method the TD (500 mg/kg) caused no ulcers at all as shown in Table 4.

**Table 1. Effects of ethanolic extract of TD flowers on acetic acid-induced writhing in rats.**

Group	Treatment	No of writhes in 20 min
I	Acetic acid control (10 ml/kg)	12.00 ± 0.45
II	Indomethacin (20 mg/kg)	5.00 ± 0.14**
III	TD-I (125 mg/kg)	9.00 ± 0.32**
IV	TD-II (250 mg/kg)	7.56 ± 0.54**
V	TD-III (500 mg/kg)	5.56 ± 0.21**

Values are expressed as mean ± S.E.M. (n = 6),

\*\* $p < 0.01$ , compared with acetic acid control, ANOVA followed by Dunnett's test.

**Table 2. Effect of ethanolic-extract of TD flowers on hot plate reaction time in rats.**

Group	Treatment	Reaction time (s)		
		30 min	60 min	90 min
I	Control (10 ml/kg)	3.00 ± 0.13	4.05 ± 0.23	5.10 ± 0.42
II	Indomethacin (20 mg/kg)	10.00 ± 0.40**	9.95 ± 0.65**	9.68 ± 0.43**
III	TD-I (125 mg/kg)	5.00 ± 0.37*	6.05 ± 0.45**	7.00 ± 0.41**
IV	TD-II (250 mg/kg)	4.50 ± 0.24*	5.65 ± 0.32*	6.50 ± 0.43**
V	TD-III (500 mg/kg)	3.50 ± 0.30*	5.14 ± 0.25*	6.00 ± 0.30**

Values are expressed as mean ± S.E.M. (n = 6),

\* $p < 0.05$ , \*\* $p < 0.01$ , compared with control, ANOVA followed by Dunnett's test.

**Table 3. Effect of ethanolic extract of TD flowers on carrageenan-induced hind paw edema in rats**

Group	Treatment	Increase in paw volume (ml.) after carrageenan administration			
		0 hr	1 <sup>st</sup> hr	3 <sup>rd</sup> hr	5 <sup>th</sup> hr
I	Carrageenan control (10 mg/kg, )	0.91 ± 0.03	2.00 ± 0.02	1.90 ± 0.02	1.73 ± 0.04
II	Indomethacin (20 mg/kg)	0.90 ± 0.1	1.30 ± 0.03**	1.10 ± 0.03**	1.12 ± 0.03**
III	TD-I (125 mg/kg)	0.94 ± 0.02	1.50 ± 0.04**	1.75 ± 0.03**	1.40 ± 0.04**
IV	TD-II (250 mg/kg)	0.92 ± 0.02	1.40 ± 0.04**	1.48 ± 0.04**	1.30 ± 0.03**
V	TD-III (500 mg/kg)	0.91 ± 0.03	1.36 ± 0.05**	1.28 ± 0.05**	1.22 ± 0.05**

Values are expressed as mean ± S.E.M. (n = 6),

\* $p < 0.05$ , \*\* $p < 0.01$ , compared with carrageenan control, ANOVA followed by Dunnett's test.

**Table 4. Assessment of the safety of test drugs on gastric mucosa of rats.**

Group	Treatment	Ulcer index
I	Indomethacin (20 mg/kg)	4
II	TD (500 mg/kg)	0

n=6

**DISCUSSION**

The observations of present study are shown in Table 1-4. It indicates that the ethanolic extract of TD possesses analgesic and anti-inflammatory effects which are comparable to that of standard. Among the doses, TD (500 mg/kg) higher dose was found to be more effective than TD (125 mg/kg) lowest dose.

The abdominal constriction response produced by acetic acid is a sensitive method to establish peripherally acting analgesics [8]. The response is thought to involve local peritoneal receptors. The mean score for writhing was decreased significantly by treatment with ethanolic extract of TD.

In hot plate test, nociceptive reaction against thermal stimuli in rats is a well-established model for detection of opiate analgesic as well as several types of analgesic drugs from spinal origin [11]. A significant raise in the reaction time at various dose levels of ethanolic extract of TD flowers (125, 250 & 500 mg/kg) was observed at 30 min, 60 min and 90 min increased the reaction time in a dose dependent manner which is comparable to indomethacin. These findings suggest that the TD exerts analgesic effect similar to non-steroidal anti-inflammatory drugs. Thus the anti-nociceptive activity shown by TD in ethanolic extract on hot plate and acetic acid-induced writhing test might possess centrally and peripherally mediated anti-nociceptive properties.

Anti-inflammatory agents have broadly been implicated as one of the important causes of gastritis and gastric ulceration (peptic ulcers). The gastric lesions formed are the result of prostaglandin inhibitory effect of anti-inflammatory agents, resulted in the cyclo-oxygenase pathway of arachidonic acid metabolism. Prostaglandins generated through cox-1 enzyme pathway have got a gastroprotective role and inhibition of cyclo-oxygenase results in the depletion of both the cox-1 and cox-2 enzymes. Considering this, the drug was investigated for the gastric irritation potential also. The results of the study exposed that no gastric irritation sign was observed with TD administration. Thus, the test drug TD flowers may be considered safer for use as compared to indomethacin, which although having well anti-inflammatory and analgesic activity produces gastric ulcers. The capability of the ethanolic extract of flowers TD to suppress abdominal writhes, increase pain threshold latency, inhibition of the phases of carrageenan-induced inflammation confirms the analgesic and anti-inflammatory properties. These findings justify conventional use of this plant in the treatment of pain and other inflammatory conditions and validate its claim of being used for the said purpose in folklore medicine.

#### CONCLUSION

It can be concluded that ethanolic extract of TD flowers possess analgesic and anti-inflammatory properties, which are possibly mediated via prostaglandin synthesis as well as central inhibitory mechanisms which may be of potential benefit for the management of pain and inflammatory disorders. Although the mechanism of TD involved was not determined in the present study, this is likely to be the focus of another study.

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