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Analgesic and Anti-Inflammatory Effect of Ethanol Extract of *Tribulus Terrestris* L.

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ABSTRACT

We evaluate the analgesic and anti-inflammatory potential of ethanol extract of *Tribulus terrestris* L. (TT) leaves on Wistar rats. Analgesic and anti-inflammatory potential of the ethanol extract of the TT leaves at doses of 50, 100 & 200 mg/kg was evaluated against the standard drug indomethacin at a dose of 20 mg/kg, p.o. Adult Wistar rats of either sex of six 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. Ethanol extract of TT 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, TT 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 TT ($p < 0.01$). However, no gastric lesions were observed in TT treated rats indicating the safety of test drug. The ethanol extract of TT showed significant analgesic and anti-inflammatory potential in different animal models.

Keywords: analgesic, anti-inflammatory, indomethacin, gastric mucosa.

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INTRODUCTION

Inflammation is one of the oldest known diseases of mankind and affects a large population of the world. No substantial progress has been made in achieving a permanent cure of inflammation and wounds. The search of screening and development of drugs for analgesic and anti-inflammatory is an everlasting problem. There is much hope 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 [1].

The practice of plants, plant extracts or plant-derived pure chemicals to manage disease become a therapeutic modality, which has stood the test of time. As assumed 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 traditional 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, Kampo (Japan) and Greco-Arab or Unani/Tibb (South Asia) [2, 3].

Tribulus terrestris L. (TT; Zygophyllaceae), also known as puncture vine or small caltrops has immense importance in oriental medicine because they are used as an aphrodisiac, diuretic and anthelmintic, as well as to treat coughs and kidney failure [4]. TT reported to have antimicrobial, antihypertension, diuretic, antiacetylcholine, haemolytic activity, to stimulate spermatogenesis, libido and antitumor activity and effects on cardiovascular system [5]. Plants TT have 10 to 60 cm high, annual herb, with pinnate leaves and yellow flowers [6]. The plant can be found in arid climate regions around the world as in southern USA, Mexico, Spain, Bulgaria, India, and China [7].

Inflammation is a pathophysiological response of living tissue to injuries that causes to the local accumulation of plasmic fluid and blood cells, characteristically redness, swelling, pain, and heat. The complex events and mediators concerned in the inflammatory reaction may induce, maintain or aggravate many diseases. However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses. Consequently, development of newer and more substantial anti-inflammatory drugs with lesser side effects is necessary [8]. Thus, during the past decades many researchers have focused on medicinal plants with fewer side-effects for patients to develop anti-inflammatory and analgesic drugs. The present study was undertaken to investigate the analgesic and anti-inflammatory potential of TT leaves in different animal models.

MATERIAL AND METHODS

Plant

The leaves of TT were collected from Chidambaram, Cuddalore, Tamil Nadu, India. The plant was identified and authenticated by Prof. Dr. R. Selvaraj, 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 leaves of TT 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 yield of the ethanol extract of TT was 19.5%^{W}/w. The extract of TT was freeze dried and stored at –80°C until further use. The dried mass (yield=50.2 g) was diluted with normal saline and used in experiments.

Preliminary phytochemical screening

Petroleum ether, chloroform, ethanol and aqueous extracts of TT was subjected to preliminary phytochemical screening for their presence or absence of active constituents utilizing standard method of analyses [8].

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 ethanol extract of TT leaves

The dried plant material (100 g) TT leaves 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 ethanol extract.

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 [10]. 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- TT-I (50 mg/kg, p.o.); group IV- TT-II (100 mg/kg, p.o.) & group V- TT-III (200 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 [10]. 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: TT–I (50 mg/kg, p.o.); group IV: TT–II (100 mg/kg, p.o.) & group V: TT–III (200 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 ± 1ºC. 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 [11]. The animals were divided in to 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: TT–I (50 mg/kg, p.o.); group IV: TT–II (100 mg/kg, p.o.) & group V: TT–III (200 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 ethanol extract of TT 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 ethanol extract of TT 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: TT (200 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 [12].

Statistical analysis

All the values are expressed as mean ± 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

Alkaloids, Carbohydrates, Cardiac Glycosides, Flavonoids, Saponins, Tannins and Proteins were found to be present in ethanol extract while Steroids were absent in all extracts of TT. Chloroform extracts of TT also showed the presence of Alkaloids.

Analgesic activity

Effect of ethanol extract of TT leaves 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 ethanol extract of TT leaves at doses of 50, 100 and 200 mg/kg on acetic acid-induced writhing in rats over the score of control group ($p < 0.05$). The effect of ethanol extract of TT leaves on acetic acid-induced writhing test was comparable to indomethacin (Table 1).

Table 1. Effects of ethanol extract of TT leaves on acetic acid-induced writhing in rats.

Group	Treatment	No of writhes in 20 min
I	Acetic acid control (10 ml/kg)	10.40 ± 0.28
II	Indomethacin (20 mg/kg)	4.10 ± 0.15**
III	TT-I (50 mg/kg)	8.10 ± 0.34**
IV	TT-II (100 mg/kg)	6.43 ± 0.42**
V	TT-III (200 mg/kg)	4.98 ± 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.

Effect of ethanol extract of TT leaves 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, ethanol extract of TT at doses of 50, 100 and 200 mg/kg showed a significant increase in the reaction time at 30, 60 and 90 min, respectively ($p < 0.05$). The effect of ethanol extract of TT leaves on reaction time was comparable to the standard drug, indomethacin (Table 2).

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Table 2. Effect of ethanol-extract of TT leaves on hot plate reaction time in rats.

Group	Treatment	Reaction time (s)		
		30 min	60 min	90 min
I	Control (10 ml/kg)	2.72 ± 0.21	3.59 ± 0.27	4.12 ± 0.44
II	Indomethacin (20 mg/kg)	9.53 ± 0.32**	9.67 ± 0.41**	9.35 ± 0.40**
III	TT-I (50 mg/kg)	3.12 ± 0.37*	5.56 ± 0.50**	6.62 ± 0.44**
IV	TT-II (100 mg/kg)	3.52 ± 0.22*	5.75 ± 0.30*	7.51 ± 0.44**
V	TT-III (200 mg/kg)	4.13 ± 0.30*	6.56 ± 0.31*	7.69 ± 0.35**

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.

Anti-inflammatory activity

Effect of ethanol extract of TT leaves on carrageenan-induced hind paw edema in rats

The ethanol extract of TT at doses of 50, 100 and 200 mg/kg showed a significant reduction in the paw volume at 1st, 3rd and 5th hr as compared to control group ($p < 0.01$). The effect of ethanol extract of TT leaves on paw volume (edema) was comparable to the standard drug, indomethacin (Table 3).

Table 3. Effect of ethanol extract of TT leaves on carrageenan-induced hind paw edema in rats

Group	Treatment	Increase in paw volume (ml.) after carrageenan administration			
		0 hr	1st hr	3rd hr	5th hr
I	Carrageenan control (10 mg/kg,)	0.91 ± 0.02	1.96 ± 0.03	1.86 ± 0.03	1.72 ± 0.03
II	Indomethacin (20 mg/kg)	0.90 ± 0.2	1.36 ± 0.05**	1.04 ± 0.04**	1.02 ± 0.02**
III	TT-I (50 mg/kg)	0.92 ± 0.03	1.56 ± 0.03 **	1.71 ± 0.02**	1.57 ± 0.02**
IV	TT-II (100 mg/kg)	0.93 ± 0.03	1.44 ± 0.05**	1.44 ± 0.02**	1.42 ± 0.02**
V	TT-III (200 mg/kg)	0.90 ± 0.02	1.38 ± 0.07**	1.22 ± 0.03**	1.17 ± 0.03**

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.

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

This method was adopted to assess the safety of ethanol extract of TT leaves on gastric mucosa of rats using the higher dose of the test drug. In this method the TT (200 mg/kg) caused no ulcers at all as shown in Table 4.

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

Group	Treatment	Ulcer index
I	Indomethacin (20 mg/kg)	4.60
II	TT (200 mg/kg)	0

n=6

DISCUSSION

The observations of present study are shown in Table 1-4. It indicates that the ethanol extract of TT possesses analgesic and anti-inflammatory potential and the effects are comparable to that of standard. Among the doses, TT (200 mg/kg) higher dose was found to be more effective than TT (50 mg/kg) lowest dose. The abdominal constriction response induced by acetic acid is a sensitive method to establish peripherally acting analgesics [10]. The response is thought to involve local peritoneal receptors. The mean score for writhing was decreased significantly by treatment with ethanol extract of TT. In hot plate test, nociceptive reaction towards 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 [13]. A significant increase in the reaction time at various dose levels of ethanol extract of TT leaves (50, 100 & 200 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 TT exerts analgesic effect similar to non-steroidal anti-inflammatory drugs. Thus the anti-nociceptive activity shown by TT in ethanol 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 incriminated as one of the important causes of gastritis and gastric ulceration (peptic ulcers). The gastric lesions produced are the result of prostaglandin inhibitory effect of anti-inflammatory agents, resluted 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. In view of this, the drug was investigated for the gastric irritation potential also. The results of the study revealed that no gastric irritation sign was observed with TT administration. Thus, the test drug TT leaves may be considered safer for use as compared to indomethacin, which although having well anti-inflammatory and analgesic activity produces gastric ulcers. The ability of the ethanol extract of leaves TT 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 traditional 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 ethanol extract of TT leaves possess analgesic and anti-inflammatory properties, which are probably 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 TT involved was not determined in the present study, this is likely to be the focus of another study.

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