Research Article

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF FRUITS OF *Tribulus terrestris* L. IN EXPERIMENTAL ANIMALS

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**ABSTRACT**

Aim: The present study aims at evaluating the analgesic and anti-inflammatory potential of the methanolic fruits extract of *Tribulus terrestris* in Swiss albino mice and Wistar rats. Methods: Tail flick latency was assessed by the analgesiometer, acetic acid induced writhing and carrageenan induced odema were evaluated by mercury displacement method using plethysmometer. Results: Methanolic fruits extract of *Tribulus terrestris* (MFTT) exhibited significant and dose dependent analgesic activity compared with the control (63.00%) and reduced abdominal writhing activity by acetic acid (59.39%). Furthermore, MFTT significantly (53.84%) reduced rat paw odema induced by subplantar injection of carrageenan. The test sample (500 mg/kg) significantly reduced odema induced by carrageenan with 53.84% inhibition. Conclusion: The present study provides basis for the traditional medicinal use of *Tribulus terrestris* for analgesic and anti-inflammatory activity and its significant impact on inhibition of edema formation.

**Keywords:** *Tribulus terrestris*, Tail flick, Acetic acid, Carrageenan, Analgesic activity, Anti-inflammatory activity.

**INTRODUCTION**

Medicinal plants in the form of therapy have been used as analgesics throughout history. Medicinal plants are the important sources of new chemical substances with potential therapeutics. The most important analgesic prototypes such as salicylic acid and morphine were originally derived from the plant sources. According to various medical literatures, several adverse reactions are known to be associated with the conventional nonsteroidal anti-inflammatory drugs, thereby limiting the widespread application of these agents. The study of plant species traditionally used as pain killers should still become a challenging research strategy in the search of novel analgesic and anti-inflammatory drugs.

In the modern era most of the commonly used drugs viz., opioids and non-steroidal anti-inflammatory drugs are no more use in the treatment of inflammatory diseases since their side effects. Hence there is a urge to search for new anti-inflammatory drugs from plants to cure inflammation. Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs. The currently used anti-inflammatory drugs may not be useful in all cases so there is increased focus on plant research and their active constituents.

Inflammation is a normal protective response to vascular tissue injury against aggressive agents such as pathogens, irritants, or damaged cells. It can be classified into two types such as acute and chronic, and involves a cascade of biochemical events comprising the local vascular system, the immune system, and different cell types found in the injured tissue. Acute inflammation is the immediate response and is mainly characterized by the increased movement of plasma and immune cells, such as neutrophils and macrophages, from the blood into the injured tissues. Chronic inflammation is associated with the progressive changes in the type of cells present at the site of the inflammatory reaction and is characterized by simultaneous destruction and healing of the injured tissue.

**MATERIALS AND METHODS**

**Chemicals**

Carrageenan (SD Fine Chem, Mumbai, India), Pentazocine, Aspirin, Indomethacin (Merck, Bangalore, India). All other chemicals used of analytical grade.

**Plant materials**

The *Tribulus terrestris* L was collected from different regions of Raichur district Karnataka, India and authenticated by Department of botany, Gulbarga University Gulbarga, Karnataka, India. A voucher specimen (No. HGUG 782) is preserved in the herbarium of Dept. of Botany, Gulbarga University.

**Preparation of extracts**

Collected plant materials were water cleaned, manually chopped into small pieces and air dried under shade for fifteen to twenty days. After drying, the plant materials were pulverized into fine powder by a grinding in machine and stored in dark airtight container till further use.

**Preliminary phytochemical screening**

The methanolic extract of the *Tribulus terrestris* fruits (MFTT) was studied for its preliminary phytochemical screening for the detection of secondary plant constituents such as saponins,
phenols, flavonoids, alkaloids and tannins, anthraquinones, carbohydrates, flavonoids, glycosides, tannins, saponins and terpenes.

**Experimental animals**

Swiss albino mice (19-30 g) and Wistar rats (180-220g) of either sex was used for the present study. The animals were procured from the listed suppliers of animal breeding center Mahaveera Enterprises A.F.Plot No: 9&18, S.No:127&150, Peerzadiguda, Ghatkesar Mandal. The animals were fed with standard pellet diet and water ad libitum. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each experiment. All the protocols were approved by the Institutional Animal Ethics Committee (IAEC) of the HKES’s MTRIPS Sedam road Guilbarga, Karnatuka, India (approval no. HKE MTRIPS 1948/po/Re/S/17/CPCSEA, Dated 23-02-2017) and conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Mice were used for the analgesic study while rats were used for the anti-inflammatory study.

**Oral acute toxicity study**

The acute toxicity was performed according to OECD guidelines. The wistar rats of either sex was used for this study. The animals were divided into four groups each group with three animals (n=3). The animals were fasted overnight and extracts were given orally to rats at a dose of 100, 300 and 1000 mg/kg body weight. The animals were observed continuously for its behavioral changes for first four hours and for mortality at the end of 24 h. The animals were examined daily till 10th day for any behavioral change or mortality. The animals were observed for toxic symptoms and death at 6, 12, 18 and 24 h and then daily for next 14 d (OECD, 2000). The study showed neither any observable toxic effects (agility, muscular tonus, tremors, convulsions, problem in breathing and water or food intake) nor any death following treatment, and hence, the procedure was repeated up to 5000 mg/kg. Animals were fasted overnight with water ad libitum and food was withheld for 3-4 hours after oral administration of the methanolic extract of fruits of *Tribulus terrestris*. First group of animals were treated with starting dose of 1000 mg/kg body weight of MFTT orally.

The LD₅₀ was determined using the graphical method in mice. Briefly, geometric doses of the extract (200–2000mg/kg) were administered i.p to 4 groups of mice each consisting of six animals. While the same in Wistar rats at 200, 500, 1000 and 2000 mg/kg body weight. Control group received normal saline (0.9 mL/kg i.p.). Signs of toxicity and mortality within 24-72 h were noted. Confirmatory test was carried out and the LD₅₀ was calculated from the graph of percent mortality against profit log dose of the extract. The LD₅₀ of methanolic extract was found to be 500 mg/kg therefore the LD₅₀ value is 500 mg/kg.

**Analgesic activity**

The acclimatized albino rats were weighed and grouped into 5 with 6 animals in each group. Selection was done randomly so as to assure equal distribution of sex, body weight etc. in each group. Then the animals were marked for proper identification and kept each group in separate cages. Each cage was labelled separately for group identification. The dose for each animal was calculated as per the body weight and was tabulated. The animals were overnight fasted without restricting water prior to the experiment. All the procedures were in strict accordance with “Guidelines for the care and use of laboratory animals”.

**Tail flick method**

The tail flick latency of rats was assessed by the analgesiometer with some modifications. Wistar albino rats of either sex was divided into five groups of six animals each. The tail of each rat was placed on the nichrome wire of an analgesiometer and the time taken by the animal to withdraw (flick) its tail from the hot wire was taken as a reaction time. The extract of MFTT at 200, 400 and 500 mg/kg were injected intraperitoneally using pentazocine (10 mg/kg, i.p.) as standard drug. Analgesic activity was measured after 30 min of administration of test and standard drug.

**Acetic acid induced writhing**

The acetic acid induced writhing test in the group of mice was performed according to the method described by Lu et al. with slight modifications. The swiss albino mice of either sex was divided into five groups containing six animals in each group. The first group served as control and the second group which received aspirin (100 mg/kg i.p) was used as standard while the 3rd to 5th group which received test samples at the conc. of 200, 400 and 500 mg/kg respectively as i.p. After one hour of drug administration writhing was induced by Acetic acid in distilled water (0.6%) at the dose of 10 ml/kg of body weight was injected intraperitoneally (i.p.). Immediately after acetic acid injection, counted the number of writhes or stretches (abdominal contraction, trunk twist response and extension of hind limbs) for 10 min. A reduction in the writhing number compared to the control was considered as evidence of analgesia. The intraperitoneal administration of acetic acid in mice caused abdominal cramps. It was reported that acetic acid causes inflammatory pain by inducing a permeability of capillaries. The percentage inhibition of writhing was calculated using the following formula.

$$\text{Percentage inhibition} = \frac{W_c - W_t}{W_c} \times 100$$

Where, $W_c$ = Mean values of number of writhing in control group, $W_t$ = Mean values of number of writhing in the test groups.

**Anti-inflammatory activity**

The anti-inflammatory activity of the methanolic extract of *Tribulus terrestris* in rats was determined by carrageenan according to the method described by Winder et al. with slight modifications. Wister Albino rats of either sex weighing 150-200 grams were divided into five groups of six animals each. The dosage of the drugs administered to the different groups was as follows. Group I - Control (normal saline 0.5 ml/kg), Group II - Indomethacin (5mg/kg) as drug control for assessing comparative pharmacological significance and Group III to V methanolic extract of *Tribulus terrestris* at 200, 400 and 500 mg/kg respectively. All the drugs were administered orally. Indomethacin served as the reference standard anti-inflammatory drug. After one hour of the drug administration 0.1 ml of 1% W/V carrageenan solution in normal saline was injected into the sub plantar tissue of the right hind paw of 18-h fasted rats to induce edema using a 30-gauge needle. The paw volume of the rats was measured by the mercury displacement method using plethysmometer at the successive intervals starting from 0 hours, 1 hours, 2 hours, 3 hrs. The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of drugs was studied. The relative potency of the drugs under investigation was calculated based upon the
percentage inhibition of the inflammation. The percentage inhibition (PI) at each time interval was calculated:

\[
\text{Anti-inflammatory activity} = \frac{(V_t - V_0) \text{ control} - (V_t - V_0) \text{ treated}}{(V_t - V_0) \text{ control}} \times 100
\]

Where, \( V_0 \) = Mean paw volume at 0 hours, \( V_t \) = Mean paw volume at a particular time interval.

Statistical analysis

The data was expressed as mean ± S.E.M. Statistical analysis was done using one way analysis of variance (ANOVA) followed by Dunnett's test. Results were considered significant at \( p<0.05 \).

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening revealed the presence of saponins, phenols, flavonoids, alkaloids and tannins, anthraquinones, carbohydrates, flavonoids, glycosides, tannins, saponins and terpenes in the methanolic extract of fruits of *Tribulus terrestris* L.

Oral acute toxicity study

A toxicity study conducted over 14 days showed no observable toxic effects (convulsion, ataxia, agility, dyspnea, water or food intake, diarrhea or diuresis) or any death. Administration of MFTT (5000 mg/kg, p.o) did not produce any toxic symptoms and mortality. Hence, the extract was found to be safe at the dose of 5000 mg/kg b.w. Therefore According to OECD – 423 guidelines the median lethal dose (LD\(_{50}\)) of extract was fixed for three doses as (200, 400 and 500 mg/kg b.w) for pharmacological studies.

Analgesic activity

**Tail flick method**

The results of tail flick test, presented in Table 1, showed that the MFTT extract had reaction time of 15.33 s (36.99%) at 200 mg/kg and 12.16 s (54.13%) at 400 mg/kg and 9.00 s (63.00%) at 500 mg/kg i.p doses. While the reaction times in vehicle control and pentazocine (5 mg/kg) group was 8.83 s and 24.33 s (63.70%), respectively. Thus significant activity was noted for the MFTT extract at 500 mg/kg. The maximum inhibition of 63.00% was observed at 9.00 s which is close to the standard drug (pentazocine 63.70%) treated group.

**Acetic acid induced writhing**

Effects of MFTT on acetic acid induced writhing were demonstrated in Table 2. Oral administration of MFTT in the 200, 400 and 500 mg/kg dose showed significant and dose dependent protection of acetic acid induced writhing in mice, as indicated by 34.51, 47.73 and 59.39% of writhing inhibition, respectively. Similarly positive control, Aspirin (150 mg/kg, p.o.) also exhibited significant writhing inhibition in mice (61.43%).

Anti-inflammatory activity

**Carrageenan induced rat paw edema method**

The anti-inflammatory activity of methanolic extract of fruits of *Tribulus terrestris* in acute experimental model was presented in Table 3 which explained that the results are comparable to that of a standard drug Indomethacin. The methanolic extract of fruits of *Tribulus terrestris* at 200, 400 and 500 mg/kg, p.o showed a dose-dependent, significant inhibition of carrageenan-induced rat paw edema from 1 hours to 3 hours following drug administration, compared to the control group. The maximum inhibition of paw edema by the MFTT was observed as 40.65, 52.74, and 53.84 % at the doses of 200, 400, and 500 mg/kg p.o.,respectively. Indomethacin 5 mg/kg p.o. showed a maximum inhibition of 59.34 % at 3 hours after its administration.

DISCUSSION

In the present study, an attempt was made to evaluate the analgesic and anti-inflammatory activity of the methanolic extract of fruits of *Tribulus terrestris* in experimental models. The use of traditional medicine is widespread, and plants still present a large source of structurally novel compounds that might serve as leads for development of novel drugs16. The present investigation was carried out to scientifically evaluate the traditional claim of *Tribulus terrestris* as anti-inflammatory. On acute oral toxicity the extract was found to be safe up to 5000 mg/kg. Phytochemical screening showed the presence of saponins, phenols, flavonoids, alkaloids and tannins, anthraquinones, carbohydrates, flavonoids, glycosides, tannins, saponins and terpenes. Majority of the plants having flavonoids as their bioactive constituent(s) have been shown to possess analgesic and anti-inflammatory activities2. Flavonoids, saponins and tannins have been shown to exert analgesic effect on acetic acid induced writhing test15. The constituents obtained after screening of methanolic extract of *Tribulus terrestris* confers with the previous reports for their analgesic and anti-inflammatory activity.

The effects of methanolic extract of *Tribulus terrestris* were evaluated on pain induced by acetic acid. The intraperitoneal administration of acetic acid in mice caused abdominal cramps. The abdominal constriction may be related to sensitization of nociceptive receptors to prostaglandins induced by acetic acid, which is a sensitive procedure to establish peripherally acting analgesics.

Carrageenan induced paw oedema which is a classical model of acute inflammation has been widely used in the study of steroid and non steroid anti-inflammatory drugs16. Carrageenan-induced inflammation has a significant predictive value for anti-inflammatory agents acting by inhibiting the mediators of acute inflammation17. Carrageenan is a family of linear sulphated polysaccharides extracted from the red seaweed marine alga *Chondrus crispus*. Lambda carrageenan is used in animal models of inflammation to test anti-inflammatory activity because dilute carrageenan solution (1-2%) causing swelling and pain8. The edema produced by sub plantar injection of carrageenan in rat hind paw is biphasic over 4 or more hours. In the present study, Methanolic extract of *Tribulus terrestris* presumed to have ethnomedical use for treating painful inflammatory conditions similar to NSAIDs18. The first phase (1 hour) involves the release of serotonin and histamine and the second phase (> 1 hour) is mediated by cyclooxygenase products. Continuity between the two phases is provided by kinin19.

The methanolic extract of *Tribulus terrestris* significantly inhibited the edema formation in both the first and second phases. In the present investigation, Indomethacin, an anti-inflammatory drug was used as a standard to reduce acute inflammatory response in terms of swelling. In comparison with control group, treatment with *T. terrestris* (500mg/kg) reduced swelling significantly which showed marked percentage inhibition of 53.84 at 3rd hr after carrageenan injection. This suggests its effect on inflammation in the second phase of carrageenan induced
inflammation. Furthermore, the second phase is sensitive to most clinically effective anti-inflammatory drugs. The MFTT was found to significantly inhibit carrageenan induced rat paw edema in the late phase regulated by prostaglandins and leukotrienes. Our results are in accordance with Jaya and Raju (2008) who have reported about anti-inflammatory and antibacterial activity of methanolic extract of *T. terrestris* [21]. Baburao et al (2009) who showed a dose-dependent inhibition of rat paw volume in carrageenan-induced inflammation in rats [22], and Oh et al (2012) have reported that the ethanolic extract of *T. terrestris* inhibits the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in lipopolysaccharide stimulated RAW 264.7 cells. It also suppressed the expression of proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-4 in macrophage cell line. Thus, the ethanolic extract of *T. terrestris* inhibits the expression of mediators related to inflammation and expression of inflammatory cytokines, which has a beneficial effect on various inflammatory conditions [23]. Hong CH et al (2002) have reported that the fruits of *T. terrestris* contains active principles such as phytoestrogens, flavanoids, alkaloids, glycosides, steroidal saponins of the furanostere type, which produce anti-inflammatory effects. The inhibitors of prostaglandin biosynthesis and nitric oxide production have been considered as potential anti-inflammatory agents and inhibition of COX-2 activity [24].

### Table 1: The analgesic activity (Tail flick test) of methanolic crude extract of fruits of *Tribulus terrestris*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Tail flick (Reaction time in s)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>-</td>
<td>24.33 ± 0.08</td>
<td>-</td>
</tr>
<tr>
<td>Group II</td>
<td>Standard (Pentazocine)</td>
<td>10</td>
<td>8.83 ± 0.90</td>
<td>63.70</td>
</tr>
<tr>
<td>Group III</td>
<td>MFTT</td>
<td>200</td>
<td>15.33 ± 1.0*</td>
<td>36.99</td>
</tr>
<tr>
<td>Group VI</td>
<td>MFTT</td>
<td>400</td>
<td>12.16 ± 0.56**</td>
<td>54.13</td>
</tr>
<tr>
<td>Group V</td>
<td>MFTT</td>
<td>500</td>
<td>9.00 ± 0.38***¥</td>
<td>63.00</td>
</tr>
</tbody>
</table>

**MFTT**: Methanolic extract of fruits of *Tribulus terrestris*

The values are mean ± SEM (n=6 animals in each group). Statistical significance analysed by one way ANOVA followed by Dunnett’s test *p<0.05, **p<0.006, ***p<0.001 as compared to control group. ¥-Non significant difference compared to group II.

### Table 2: The analgesic activity (acetic acid-induced writhing test) of methanolic crude extract of fruits of *Tribulus terrestris*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (Mg/Kg)</th>
<th>No. of Writhings</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>32.83 ± 1.04</td>
</tr>
<tr>
<td>Group II</td>
<td>Asprin</td>
<td>150</td>
<td>12.66 ± 0.90</td>
<td>61.43</td>
</tr>
<tr>
<td>Group III</td>
<td>MFTT</td>
<td>200</td>
<td>21.50 ± 0.58</td>
<td>34.51</td>
</tr>
<tr>
<td>Group VI</td>
<td>MFTT</td>
<td>400</td>
<td>17.16 ± 1.2</td>
<td>47.73</td>
</tr>
<tr>
<td>Group V</td>
<td>MFTT</td>
<td>500</td>
<td>13.33 ± 0.65</td>
<td>59.39</td>
</tr>
</tbody>
</table>

**MFTT**: Methanolic extract of fruits of *Tribulus terrestris*

The values are mean ± SEM (n=6 animals in each group). Statistical significance analysed by one way ANOVA followed by Dunnett’s test p<0.05, as compared to control group.

### Table 3: The anti-inflammatory activity (carrageenan induced rat paw oedema model) of methanolic crude extract of fruits of *Tribulus terrestris*

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Paw edema volume (mL.) Time (hrs)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Control</td>
<td>--</td>
<td>1.05±0.03</td>
<td>2.06±0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>Standard (Indomethacin)</td>
<td>5</td>
<td>1.68±0.02</td>
<td>26.96</td>
</tr>
<tr>
<td>Group III</td>
<td>MFTT</td>
<td>200</td>
<td>1.62±0.04</td>
<td>30.33</td>
</tr>
<tr>
<td>Group IV</td>
<td>MFTT</td>
<td>400</td>
<td>1.56±0.02</td>
<td>39.32</td>
</tr>
<tr>
<td>Group V</td>
<td>MFTT</td>
<td>500</td>
<td>1.54±0.02</td>
<td>45.37</td>
</tr>
</tbody>
</table>

**MFTT**: Methanolic extract of fruits of *Tribulus terrestris*

The values are mean ± SEM (n=6 animals in each group). Statistical significance analysed by one way ANOVA followed by Dunnett’s test p<0.05 as compared to control group.

**CONCLUSION**

Based on the results obtained it is concluded that the methanolic fruits extract of *Tribulus terrestris* at the dose of 500 mg/kg b.w exhibited potent anti-inflammatory and analgesic effect. This shows its significant impact on inhibition of edema formation.

**ACKNOWLEDGEMENT**

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