Evaluation of Anti-inflammatory and Analgesic Activities of *Tamarindus indica* Seeds

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**ABSTRACT**

In this study, we investigated the effects of methanolic extract of *Tamarindus indica* (TI) seeds on anti-inflammatory and analgesic activities in vivo using rat as an animal model at the doses of 100 mg/kg, 200 mg/kg and 400 mg/kg body weight. The anti-inflammatory activities were investigated by utilizing carrageenan induced paw edema in rat. The analgesic activity was examined against tail immersion method in rats. The results showed that TI significantly \((p<0.01)\) reduced carrageenan induced paw edema in rats. In tail immersion method, methanolic extract of *Tamarindus indica* has shown significant \((p<0.01)\) increase in reaction time of tail in water maintained at 55°C indicating analgesic activity. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, saponins, glycosides and flavonoids. These results demonstrated that the methanolic extract of *Tamarindus indica* (TI) seed exhibited significant analgesic and anti-inflammatory activities.

**Keywords:** Analgesic, Anti-inflammatory, *Tamarindus indica*, Carrageenan.

**INTRODUCTION**

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agents as well as to remove the consequent necrosed cells and tissue and it is manifestation of the body's response to tissue damage and infection. The result of each inflammatory reaction may be beneficial (defense the body against agents deranging its homeostasis) or harmful (damage to surrounding tissues).\(^1\)

Pain and fever are being the most common complaints associated with inflammation. The NSAIDs used in the inflammatory conditions do not cure and remove the underlying cause of the disease but they only modify the inflammatory response to the disease. Large numbers of NSAIDs are available in the market with their advantages and disadvantages. Though there are standard drugs like Aspirin, Indomethacin, Phenylbutazone, etc., these drugs are not entirely free of side effects and have their own limitation.\(^1\) Thus there is still a need to develop newer and safer anti-inflammatory drugs. NSAIDs use is frequently limited by gastrointestinal side effects, ranging from dyspepsia to life threatening bleeding from ulceration. It is believed that NSAIDs by inhibiting COX pathway causes inhibition of prostaglandins synthesis, which are responsible for maintaining gastric mucosal integrity.\(^2\)

Herbal medicines used in Ayurveda remain the major source of health care for the world's population. WHO has recognized herbal medicine as an essential building block for primary health care of vast countries like India. Traditionally seeds of *Tamarindus indica* are being used in asthma, bronchitis, leprosy, tuberculosis, wounds, ulcers, inflammation, stomach algia, diarrhea, dysentry, burning sensation, giddiness, vertigo, and diabetes.\(^3\) It has been reported that seeds of *Tamarindus indica* are having antiulcer, anti-asthmatics, ant diabetic and antioxidant activity.\(^4-5\) Also seeds of *Tamarindus indica* are rich in phenolic compounds, polymeric tannins, and fatty acids flavonoids, saponins, alkaloids, and glycosides.\(^6-7\) Flavonoids, tannins, saponins and alkaloids are responsible for anti-inflammatory and analgesic activity.\(^8\)

Hence, considering the traditional claim, chemical constituents and reported activities of *Tamarindus indica*, the present study was planned to screen seed extract of *Tamarindus indica* for anti-inflammatory & analgesic activity.

**MATERIALS AND METHODS**
Collection and authentication of plant
The seeds of *Tamarindus indica* (TI) were collected from areas around Pune region. The seeds of *Tamarindus indica* (TI) were identified and authenticated by “Botanical Survey of India” Pune. (Voucher specimen no: KIROT AM-2).

Preparation of extract
After 10 days of drying under shed, the seeds were coarsely powdered using a mixer. The powder was sieved by 40 mesh sieve. The extract was prepared by using maceration method. About 500 g of dried powder of the seeds were extracted with 3500 ml methanol (1:7) for 72 h. The extract was concentrated and dried (yield - 8% w/w). The dried methanolic extract was kept in airtight container in desiccator and used throughout the study.

Experimental animals
Wistar albino rats weighing 150-200 g were housed in standard cages at room temperature 22±2°C and 50±5% relative humidity, under a light/dark cycle of 10/12 h, for 1 week before the experiments. Animals were provided with standard rodent pellet diet (Amrut, India), and water *ad libitum*. The animals were deprived of food for 24 hours before experimentation, but had free access to drinking water. All experiments were performed in the morning. Experimental protocols were approved by our Institutional Ethical Committee which follows guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) and complies with international norms of INSA (Indian National Science Academy).

Acute oral toxicity study and selection of doses
The acute toxicity of methanolic extract of *Tamarindus indica* (TI) seeds was determined as per the Organization of Economic Co-Operation and Development (OECD) guideline no. 423 (Acute toxic class method). It was observed that the ethanolic extract and the fractions were not mortal even at 2000 mg/kg dose. Hence, 1/10th (200 mg /kg) of this dose was selected for this study. [9]

Anti-inflammatory activity

Carrageenan-induced paw edema test [10]
Inflammation was produced by administering 0.1 ml of (1%) carrageenan into sub-plantar surface of rat hind paw. Albino rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided into five groups (n=6 each) viz.

- **Group I**: Distilled water (10 ml/kg) + Carrageenan (0.1ml of 1% in normal saline)
- **Group II**: Diclofenac sodium (10mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)
- **Group III**: Methanolic extract of *Tamarindus indica* (100 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/w in saline solution)
- **Group IV**: Methanolic extract of *Tamarindus indica* (200 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/w in saline solution)
- **Group V**: Methanolic extract of *Tamarindus indica* (400 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/w in saline solution)

In this experiment, all drugs were given orally. One hour after drug treatment 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 h, 2 h, 3 h, 4 h, 5 h and 24 h. Results were expressed as,

\[
\text{Edema Volume} = V_t - V_c
\]

\[
V_c = \text{Paw volume in ml at time } t, \text{ after carrageenan administration.}
\]

\[
V_p = \text{Paw volume in ml before carrageenan administration.}
\]

\[
\text{Inhibition rate} \% = \frac{E_c - E_t}{E_c} \times 100
\]

\[
E_c = \text{edema volume of control group.}
\]

\[
E_t = \text{edema volume of treated group.}
\]

Various hematological changes were measured at 5th h after carrageenan injection. Animals were anaesthetized by ether and blood was withdrawn by retro orbital plexus by using fine glass capillary and collected into epindorf tubes and estimated for hematological parameters such as ESR, Hemoglobin, Total WBC, RBC, Lymphocytes, and Neutrophils. The average values between treated animals and control group were calculated for each time interval and evaluated statistically.

Analytical activity

Tail immersion method in rats [11]
Analytical activity was assessed by Tail immersion method. The wistar rats of either sex weighing 150-250 g were fasted overnight with *ad libitum* access to water. The animals were divided into five groups (n=6 each) viz.

- **Group I**: Distilled water (10 ml/kg)
- **Group II**: Pentazocine (30 mg/kg)
- **Group III**: Methanolic extract of *Tamarindus indica* (100 mg/kg, p.o.)
- **Group IV**: Methanolic extract of *Tamarindus indica* (200 mg/kg, p.o.)
- **Group V**: Methanolic extract of *Tamarindus indica* (400 mg/kg, p.o.)

The animals are allowed to adapt to the cages for 30 min before testing. The distal part of the tail of each animal was marked (5 cm). This marked part of the tail was immersed in a beaker of freshly filled water of exactly 55°C. Within a few seconds the rat reacted by withdrawing the tail. The time taken to withdraw the tail was noted as reaction time. A cut-off time of 10 seconds was maintained at 55°C to prevent tissue damage. After respective drug treatment, tail of each animal was immersed in a beaker of freshly filled water of exactly 55°C and reaction time was measured at 0, 15, 30, 45, and 60 min, respectively.

Statistical analysis
The values expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one way ANOVA followed by Dunnett’s test to verify the significant difference if any among the groups. *p*<0.05*, *p*<0.01** and *p*<0.001*** were considered significant.

RESULTS

Anti-inflammatory Effect of methanolic extract of *Tamarindus indica* on carrageenan induced hind paw edema in rats
There was a significant reduction in the paw volume observed with Diclofenac sodium treated group showed significant inhibition (*p*<0.01) of paw edema from 1st h to 24th h as compared to control group. Groups treated with the methanolic extract of *Tamarindus indica* at dose of 100 mg/kg showed significant decrease in paw edema volume from at 5th h (*p*<0.05) and 24th h (*p*<0.01) compared to control group.

Groups treated with the methanolic extract of *Tamarindus indica* at dose of 200 mg/kg showed significant decrease in paw edema volume 3rd , 4th, 5th , 24th h (*p*<0.01) and showed
significant decrease in paw edema volume at 2nd h (p<0.05) compared to control group. Groups treated with the methanolic extract of Tamarindus indica at a dose of 400 mg/kg showed significant decrease in paw edema volume at 1st hr (p<0.05), and 2nd, 3rd, 4th, 5th and 24th h (p<0.01) when compared to control group. The results of this work are shown in Table 1.

Effect of methanolic extract of Tamarindus indica on percentage inhibition of carrageenan induced hind paw edema in rats:

Inhibition of carrageenan induced hind paw edema in rats by Diclofenac sodium started at 1st hr and which was maintained up to 24th hr. Diclofenac sodium at the dose of 10 mg/kg at 1st, 2nd, 3rd, 4th, 5th and 24th hr has shown 35.08, 67.47, 40.09, 50.00, 55.23 and 66.82% inhibition, respectively. At 2nd hr, the methanolic extract Tamarindus indica at the dose of 100, 200 and 400 mg/kg has shown 2.68, 13.44 and 31.18% inhibition, respectively. At 3rd hr, the methanolic extract Tamarindus indica at the dose of 100, 200 and 400 mg/kg has shown 4.05, 20.27 and 33.33% inhibition, respectively.

At 4th hr the methanolic extract Tamarindus indica at the dose of 100, 200 and 400 mg/kg has shown 5.46, 23.94 and 36.13% inhibition, respectively. At 5th hr the methanolic extract Tamarindus indica at the dose of 100, 200 and 400 mg/kg has shown 11.29, 27.19 and 38.91% inhibition, respectively. At 24 hr the methanolic extract Tamarindus indica at the dose of 100, 200 and 400 mg/kg has shown 37.98, 46.15 and 52.88% inhibition, respectively. The results of this work are shown in Table 2.

Effect of methanolic extract of Tamarindus indica on various hematological parameters at 5th h during carrageenan induced hind paw edema in rats:

With increase in inflammation, there is marked increase in the ESR count Total WBC, Lymphocytes, Neutrophils and RBC count in control group. The following observation was made:

**ESR:** Animals treated with diclofenac sodium, there was significant (p<0.01) restoration of ESR. There was significant restoration in ESR count in animals treated with Tamarindus indica at the dose of 100 and 200 mg/kg p.o. (p<0.05) and Tamarindus indica 400 mg/kg p.o. (p<0.01) showed significant restoration of ESR.

**Hemoglobin:** There was no significant change in hemoglobin count neither by Diclofenac sodium nor by Tamarindus indica at all doses.

**Total WBC:** Animals treated with Diclofenac sodium, there was significant (p<0.01) restoration of total WBC count. Tamarindus indica at the dose of 200 and 400 mg/kg p.o (p<0.01) and at the dose of 100 mg/kg (p<0.05) showed significant restoration of WBC.

**Lymphocytes:** Animals treated with diclofenac sodium, there was significant (p<0.01) restoration of Lymphocytes. Tamarindus indica 200 mg/kg p.o. (p<0.05) and 400 mg/kg p.o. (p<0.01) has shown significant restoration in Lymphocytes count respectively.

**Neutrophils:** Animals treated with diclofenac sodium, there was significant (p<0.01) restoration of Neutrophils. There was no significant restoration of neutrophils count was found at the dose of Tamarindus indica 100 and 200 mg/kg p.o. But Tamarindus indica at the 400 mg/kg p.o. has shown significant (p<0.01) restoration in Neutrophils count.
Table 1: Effect of methanolic extract of Tamarindus indica on carrageenan induced hind paw edema in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Volume of paw edema (ml) [Mean±SEM] (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Group I (Control)</td>
<td>0.62±0.03</td>
</tr>
<tr>
<td>Group II (Standard)</td>
<td>0.68±0.04</td>
</tr>
<tr>
<td>Group III (TI-100)</td>
<td>0.65±0.03</td>
</tr>
<tr>
<td>Group IV (TI-200)</td>
<td>0.67±0.028</td>
</tr>
<tr>
<td>Group V (TI-400)</td>
<td>0.70±0.029</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM, (n=6). The data was analyzed by using One way ANOVA followed by Dunnett’s test. **p<0.01, *p<0.05, where Group II, III, IV & V were compared with Group I

Table 2: Effect of methanolic extract of Tamarindus indica on percentage inhibition of carrageenan induced hind paw edema

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 h</th>
<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
<th>5h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group II (Standard)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Group III (TI-100)</td>
<td>35.08</td>
<td>46.77</td>
<td>49.09</td>
<td>50.00</td>
<td>55.23</td>
<td>66.82</td>
<td></td>
</tr>
<tr>
<td>Group IV (TI-200)</td>
<td>0.87</td>
<td>2.68</td>
<td>4.05</td>
<td>5.46</td>
<td>11.29</td>
<td>37.98</td>
<td></td>
</tr>
<tr>
<td>Group V (TI-400)</td>
<td>9.64</td>
<td>13.44</td>
<td>20.27</td>
<td>23.94</td>
<td>27.19</td>
<td>46.15</td>
<td></td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM, (n=6). The data was analyzed by using One way ANOVA followed by Dunnett’s test. **p<0.01, *p<0.05, where Group II, III, IV & V were compared with Group I

Table 3: Effect of methanolic extract of Tamarindus indica on various hematological parameters at 5th h during carrageenan induced hind paw edema

<table>
<thead>
<tr>
<th>Haematological parameters</th>
<th>Control</th>
<th>Standard</th>
<th>TI-100</th>
<th>TI-200</th>
<th>TI-400</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR (mm/h)</td>
<td>4.95±0.55</td>
<td>2.13±0.49**</td>
<td>3.75±0.13*</td>
<td>3.63±0.15*</td>
<td>2.73±0.10**</td>
</tr>
<tr>
<td>Haemoglobin (%)</td>
<td>11.90±0.73</td>
<td>13.15±0.74</td>
<td>11.71±0.52</td>
<td>11.76±0.62</td>
<td>12.17±0.50</td>
</tr>
<tr>
<td>Total WBC Count (cu.mm)</td>
<td>91.07±6.330</td>
<td>45.87±0.789</td>
<td>6860.±3.689</td>
<td>6100.±3.536**</td>
<td>5390.±2.089**</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>63.16±1.70</td>
<td>47.16±1.88</td>
<td>58.66±1.94</td>
<td>54.83±1.68*</td>
<td>52.00±2.17**</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>43.66±1.56</td>
<td>37.66±1.98</td>
<td>41.66±1.22</td>
<td>41.00±1.96</td>
<td>38.00±1.06**</td>
</tr>
<tr>
<td>RBC (million/cu.mm)</td>
<td>7.15±0.85</td>
<td>2.92±0.26**</td>
<td>5.24±0.45**</td>
<td>4.24±0.45**</td>
<td>3.49±0.57**</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM, (n=6). The data was analyzed by using One way ANOVA followed by Dunnett’s test. **p<0.01, *p<0.05, where Group II, III, IV & V were compared with Group I

Table 4: Effect of methanolic extract of Tamarindus indica on Tail immersion in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0 min.</th>
<th>15 min.</th>
<th>30 min.</th>
<th>45 min.</th>
<th>60 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>4.83±0.46</td>
<td>5.08±0.41</td>
<td>7.58±0.54</td>
<td>8.0±0.41</td>
<td>5.58±0.41</td>
</tr>
<tr>
<td>Group II (Standard)</td>
<td>5.18±0.31</td>
<td>10.73±0.43**</td>
<td>12.61±0.50**</td>
<td>11.48±0.39**</td>
<td>10.23±0.5**</td>
</tr>
<tr>
<td>Group III (TI-100)</td>
<td>5.66±0.35</td>
<td>9.00±0.50</td>
<td>7.58±0.54</td>
<td>10.10±0.48*</td>
<td>9.63±0.39</td>
</tr>
<tr>
<td>Group IV (TI-200)</td>
<td>5.03±0.30</td>
<td>7.00±0.43*</td>
<td>11.77±0.41**</td>
<td>9.67±0.19**</td>
<td>7.20±0.31*</td>
</tr>
<tr>
<td>Group V (TI-400)</td>
<td>4.90±0.34</td>
<td>5.53±0.42**</td>
<td>10.09±0.38**</td>
<td>8.23±0.25**</td>
<td>6.86±0.27**</td>
</tr>
</tbody>
</table>

The results are expressed as mean ± SEM, (n=6). The data was analyzed by using One way ANOVA followed by Dunnett’s test. **p<0.01, *p<0.05, where standard, TI-100, TI-200, TI-400 groups were compared with Control group

In carrageenan induced paw edema model, the effect of Tamarindus indica was observed on various haematological parameters. During inflammation, hematological parameters like ESR, Total WBC, neutrophils count, lymphocyte count and RBC were increased. ESR is an estimate of the suspension stability of RBC's in plasma, related to the number of size of red cells and to the relative concentration of plasma proteins especially fibrinogen and the α and β globulins. Increases are an indication of active but obscure disease processes. The acute phase proteins in ESR and C-reactive protein share the property of showing elevations in the concentration in response to stress or inflammation that occurs like infection, injury, and surgery and tissue necrosis. So in inflammation condition, ESR is elevated. Increase in the erythrocyte sedimentation rate is an indication of active but obscure disease process which elevate in response to stress, inflammation and cell necrosis. WBC count seems to be raised in control group. In inflammatory condition there is a mild to moderate rise in WBC count due to release of IL-β inflammatory response. IL-β increases the production of both granulocyte and macrophages colony stimulating factor. RBC count in the control group raised marginally which may be due to excess presence of iron in blood and high serum iron with normal iron store. Increased RBC is suggestive of polycythemia and a positive erythropoietic effect. Lymphocytes have been reported to play a central role in the pathogenesis of rheumatoid arthritis. These cells comprise the majority of the lymphoid cells found in the rheumatoid synovium. In arthritic condition there is a moderate elevation in lymphocyte count indicating inflammation of synovial fluid.[13-16] Neutrophils infiltrating into an arthritic joint can release proteolytic enzymes and reactive oxygen species which can increase inflammation and accelerate the destruction of the bone and cartilage.[15]

The methanolic extract of Tamarindus indica showed the significant decrease in levels of ESR, total WBC count, lymphocytes, neutrophils and total RBC count. Inhibition of increased lysosomal enzymes with methanolic extract of Tamarindus indica showed its ability to stabilize lysosomal membrane. Thus decrease in all the hematological parameters seems to contribute its anti-inflammatory activity of methanolic extract of Tamarindus indica.

The methanolic extract of Tamarindus indica was also evaluated in the tail immersion test for its analgesic activity. Tail immersion method is type of thermal stimulant and induces centrally mediated pain at the supraspinal level. This method is supraspinally mediated and has selectivity for centrally acting analgesics.[16] In this method increase in the reaction
time is considered for evaluating central antinociceptive activity. This method is used to differentiate between central and peripheral analgesics. The centrally acting analgesics increase the reaction time in the tail immersion model. In the present study, methanolic extract of *Tamarindus indica* exhibited significant increase in reaction time to thermal stimuli indicating analgesic activity. This further support to the use of *Tamarindus indica* in analgesic activity due to the inhibition of release of endogenous inflammatory mediators like histamine, serotonin, prostaglandin and inhibition of nociceptive transmission i.e. inhibition of ascending and descending pathways of pain by acting on a opioid µ receptor and inhibition of neurotransmission.

Furthermore, *Tamarindus indica* extract shown presence of chemical constituents like flavonoids, saponins, alkaloids and glycosides.

Form the results obtained in the present study, it can be concluded that methanolic extract of *Tamarindus indica* seeds possesses significant anti-inflammatory and analgesic effect which may be due to its inhibition of inflammatory mediators like histamine, serotonin, prostaglandin, bradykinin and leukotriene, membrane stabilising activity, and antioxidant activity. The methanolic extract of *Tamarindus indica* showed presence of flavonoids, saponins and tannins compounds which may be further supported its potential for anti-inflammatory and analgesic activity.

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