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A Study on Assessment of Anti-inflammatory Activity of Ethanolic Extracts of Tamarind (*Tamarindus indica*) Seeds

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs in the world today to treat inflammatory conditions. 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 diseases. Therefore, there is resurgence to search for new antiinflammatory agents. Current study was conducted with the main purpose of evaluating the anti-inflammatory activity of the ethanolic extracts of seeds of *Tamarindus indica*. Anti-inflammatory activity of ethanolic extracts of seeds of *T. indica* at doses of 250 mg/kg, 500 mg/kg and 750 mg/kg was evaluated in Wistar albino rats in carrageenan induced paw edema animal model test. Inflammation was produced by administering 0.1 ml of 1% carrageenan into sub-plantar surface of rat hind paw to negative control group; 250 mg/kg (Group-A), 500 mg/kg (Group-B), 750 mg/kg (Group-C) ethanolic extracts of seeds of *T. indica* and Diclofenac sodium 10 mg/kg (positive control) was administered orally respectively, Results inferred that

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there was statistically significant ($p<0.01$) inhibition in hind paw edema volume of rats in positive control group at 2 h, 4 h and 6 h. At 2 h and 4 h time intervals there was a statistically significant inhibition of paw edema volume was observed in Group-B ($p<0.05$) and Group-C ($p<0.01$). However, paw edema volume was significantly ($p<0.01$) reduced in Group-A (250 mg/kg), Group-B (500 mg/kg), and Group-C (750 mg/kg) at 6 h. The anti-inflammatory effect of ethanolic seed extract of *T. indica* at the dose level of 750 mg/kg was comparable with that of positive control (Diclofenac sodium). In conclusion, results of the present study clearly demonstrated that ethanolic seed extract of *T. indica* exhibited antidepressant activity. Hence, ethanolic seed extract of *T. indica* could be considered for development natural anti-inflammatory drugs.

Keywords: Tamarindus indica, Seeds, Ethanolic extract, Anti-inflammatory, NSAIDs

Introduction

Inflammation is a body's immune system response to harmful stimuli associated with immune cells, molecular negotiators, and inflammatory cytokines. The exposure of pathogen, radiation, extremely high or low temperatures and autoimmune processes induce an inflammation.[1,2] Chronic inflammatory responses are related to the progression and manifestation of various inflammatory-related diseases, including rheumatoid arthritis, septic syndrome, cardiovascular diseases, cancer and neurodegenerative diseases.[3] Synthetic drugs commonly used for the treatment of pain and inflammation like non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids provide symptomatic and short-lived relief. Also, their long-term uses are associated with several serious adverse effects. Hence, the discovery of new and safe analgesic and antiinflammatory drug is needed. The healing power of tamarind is first mentioned in the traditional Sanskrit literatures.[4]

Tamarindus indica L. (Family: Leguminosae), commonly known as Tamarind, grows naturally in the tropical and sub-tropical regions of India.[5] *T. indica* is a long lived, medium to

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large in size, evergreen or semievergreen trees. *T. indica* trees are about 20-30m tall and 7m grith. The trunk forks about 1m above ground and multi-stemmed with branches widely spreading, drooping at the ends forming a beautifully spreading round crown (Figure 1).[6,7] Pods contain 1-10 seeds, irregularly shaped, flattened, rhomboid, with the center of each flat side of the seed marked with a large central depression. The seeds are very hard, shiny, reddish, or purplish brown (Figure 2).[6,8]

Figure 1: Showing *Tamarindus indica* plant

Figure 2: Showing seeds of *Tamarindus indica*

In the Indian system of medicine, tamarind has wide therapeutic application including inflammation, diabetes, constipation, indigestion and flatulency.[9] Throughout Southeast Asia, the tamarind fruit poultice is applied to foreheads of fever sufferers.[10] The seeds of *T. indica* are reported to possess pharmacological activities such as antidiabetic and hypoglycemic, antioxidant, antiulcer, anti-venom, hepatoprotective, antibacterial, inhibition of nitric oxide production and serine proteinase inhibitor.[11]

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Furthermore, seeds of *T. indica* are rich in phenolic compounds, polymeric tannins, and fatty acids flavonoids, saponins, alkaloids, and glycosides.[12,13] Flavonoids, tannins, saponins and alkaloids are responsible for ant-inflammatory and analgesic activity.[10] Therefore, the present research investigation has been designed to evaluate the anti-inflammatory activity of the ethanolic extracts of seeds of *T. indica* in carrageenan induced paw edema animal model study.

Materials and Methods

Collection of Seeds

The fresh seeds of *T. indica* were collected in and around Chikkaballapura. The seeds were sprayed with ethanol, and then shade dried at room temperature for 10 days. The dried seeds were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered seeds of *T. indica* was subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of ethanol. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator.[14,15] The extracts were preserved in airtight containers and stored at room temperature in desiccator and used throughout the study. Stock solution was freshly prepared daily in distilled water before dosing from which the different doses were administered by selecting the appropriate concentration.

Ethical Approval

The study was conducted by authorized, qualified and trained scientists & technicians in compliance with the guidelines laid down by the Institutional Animal Ethics Committee (IAEC) approved by the Committee for the Purpose of Control and Supervision of Experiments

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on Animals (CPCSEA), India. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental Animals

Healthy Wistar albino rats weighing between 150-200 g were used. They were maintained at 25ºC with relative humidity of 45 to 50% and under standard environmental conditions with 12:12 h light/dark cycle in polypropylene cages for one week before the experiments. The animals were fed with standard pellet feed and water was given *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.

Acute Oral Toxicity

All Wistar albino rats were free of any toxicity as per acceptable range given by the OECD guideline-423 up to the dose of 2000 mg/kg.[16] It was determined that the ethanolic seed extract of *T. indica* and the fractions were not mortal even at 2000 mg/kg dose. From this data and pilot study reports; three different doses 250, 500 and 750 mg/kg were selected for further study.

Anti-inflammatory Activity

Carrageenan-induced inflammatory model: 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.[17] The animals were divided in to five groups as follows;

Study Design

Groups	Treatment	No. of Animals / Group
Negative Control	Distilled water (10 ml/kg) + Carrageenan (0.1ml of 1% in normal saline)	6
Positive Control	Diclofenac sodium (10 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6

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Group-A	Methanolic extract of *T. indica* (250 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6
Group-B	Methanolic extract of *T. indica* (500 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6
Group-C	Methanolic extract of *T. indica* (750 mg/kg, p.o.) + Carrageenan (0.1 ml of 1% w/v in saline solution)	6

Selected doses of ethanolic seed extract of *T. indica* was 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. Recordings were taken up to 6 hours at 2 h, 4 h, and 6 h intervals. The % inhibition in paw volume was calculated by using following formula;[18]

% inhibition in paw volume = $100 \times (1 - Vt/Vc)$

Where,

Vt = mean paw volume in the drug treated group.

Vc = mean paw volume in control group

Statistical Analysis

The data are expressed as Mean. Data were subjected to statistical analysis using one-way ANOVA followed by Dunnett's multiple comparison *post-hoc* tests. $p \leq 0.05$ was considered as statistically significant.

Results

The results of the anti-inflammatory effect of ethanolic seed extract of T. indica and positive control drug was as represented in Figures 1, 2, and 3. Results depicted that there was statistically significant ($p<0.01$) inhibition in hind paw edema volume of rats in positive control group at 2 h, 4 h and 6 h. At 2 h and 4 h time intervals there was a statistically significant

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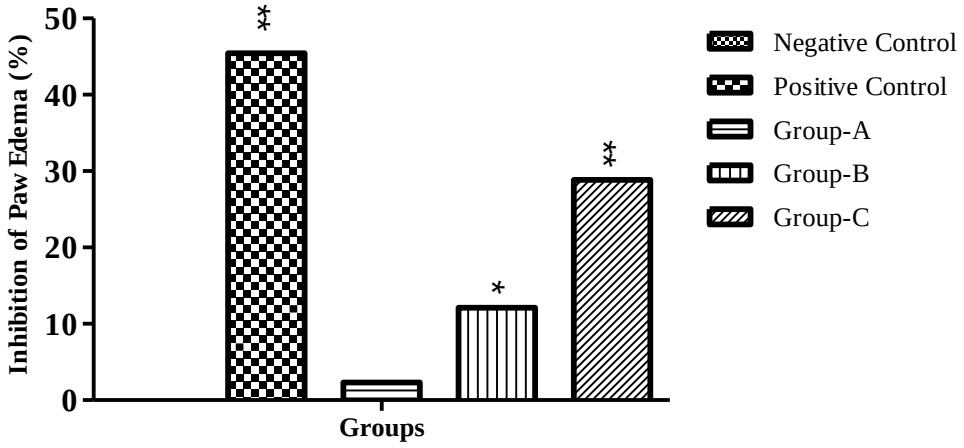
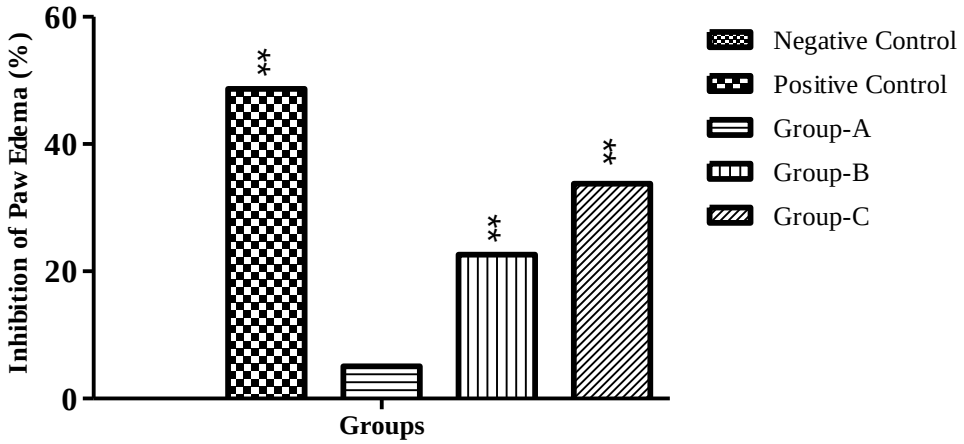
inhibition of paw edema volume was observed in Group-B ($p<0.05$) and Group-C ($p<0.01$). However, paw edema volume was significantly ($p<0.01$) reduced in Group-A, Group-B, and Group-C at 6 h. The anti-inflammatory effect of ethanolic seed extract of *T. indica* at the dose level of 750 mg/kg was comparable with that of positive control (Diclofenac sodium).

Figure 1: Effect of ethanolic seed extract of *T. indica* on inhibition of carrageenan induced hind paw edema in rats at 2 h



*p<0.05 and **p<0.01 as compared to negative control group based on one-way ANOVA followed by Dunnett's multiple comparison *post-hoc* test

Figure 2: Effect of ethanolic seed extract of *T. indica* on inhibition of carrageenan induced hind paw edema in rats at 4 h



*p<0.05 and **p<0.01 as compared to negative control group based on one-way ANOVA followed by Dunnett's multiple comparison *post-hoc* test

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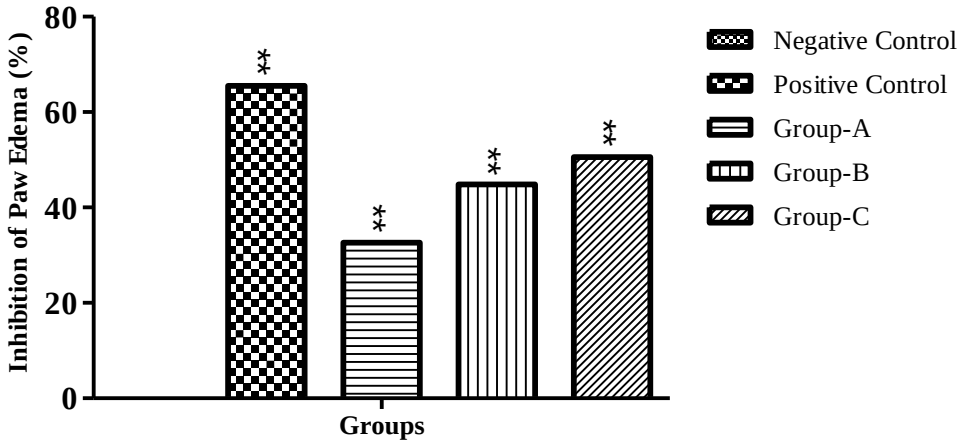
Figure 3: Effect of ethanolic seed extract of *T. indica* on inhibition of carrageenan induced hind paw edema in rats at 6 h



*p<0.05 and **p<0.01 as compared to negative control group based on one-way ANOVA followed by Dunnett's multiple comparison *post-hoc* test

Discussion

Inflammation is a complex process initiated by several factors ranging from bacterial infection and chemical injury to environmental pollution that result in cell injury or death.[19,20] NSAIDs are the most commonly used drugs in the world today. 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 diseases. There is a market need for orally active molecules that can treat inflammatory disease processes, rather than just the symptoms, more effectively than currently available drugs. Therefore, there is resurgence to search for new antiinflammatory. Hence, in the current study we aimed to assess the anti-inflammatory activity of the ethanolic extracts of seeds of *T. indica*.

Carrageenan induced paw edema has been widely used to screen natural products with anti-inflammatory potentials.[21] In our study, there was statically significant reduction in hind paw edema volume of rats was observed following treatment of rats with ethanolic seed extract

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T. indica when compared with control group. Furthermore, the anti-inflammatory effect of ethanolic seed extract of *T. indica* at the dose level of 750 mg/kg was comparable with that of positive control (Diclofenac sodium). Moreover, maximum inhibition of paw edema volume was observed at 6 h time interval at all the dose levels of ethanolic seed extract of *T. indica.*

Carrageenan induced paw edema test model basically reflects the action of prostaglandins involved in the inflammation process induced by carrageenan. Oedema formation in paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase blood flow.[22] Subplanter injection of carrageenan in the rat hind paw induces inflammation in two distinct phases namely: the first phase (0-2 h) which involves release of histamine and 5-hydroxytryptamine and second phase (2-6 h) which involves release of the inflammatory mediators like prostaglandins, leukotrienes, polymorphonuclear cells and bradykinins. These two phases are linked with kinin release.[23,24] However, synthetic anti-inflammatory agents such as aspirin, indomethacin and diclofenac are known to mediate their anti-inflammatory action via inhibition of second phase of inflammatory response. Since ethanolic seed extract of *T. indica* showed maximum reduction in paw edema during second phase, it may be stated that ethanolic seed extract of *T. indica* might have mediated its anti-inflammatory action by inhibiting the release of mediators like prostaglandins, leukotrienes, polymorphonuclear cells and bradykinins.[25]

Studies reported in the literature revealed that phytochemical evaluation of tamarind seed extract has been reported on the presence of many active components including procyanidin B2, catechin, rutin, embelin, arecatannin B1, D-threo-isocitric acid and galactosyl glycerol.[26] Procyanidin, catechin, rutin and embelin have been revealed to be an effective anti-oxidant and anti-inflammatory properties, which are capable of inhibiting oxidative stress.[27-29] Moreover, polyphenols and tannins display antioxidant, antibacterial, and anti-inflammatory action.[30] Therefore, this study results of anti-inflammatory activities of ethanolic seed extract

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of *T. indica* could be accredited to the phytochemicals present in ethanolic seed extract of *T. indica.*

Conclusion

The results of present study clearly demonstrated that ethanolic seed extract of *T. indica* exhibited antidepressant activity. Hence, ethanolic seed extract of *T. indica* could be considered for development natural anti-inflammatory drugs. However, further studies are recommended to elucidate the exact mechanism of action of particular phytochemical responsible for anti-inflammatory activity of *T. indica* seeds.

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