Research Article

EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF TAMARIND (*TAMARINDUS INDICA* L.) SEEDS

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ABSTRACT

Objectives: The aim of this study is to evaluate the *in vivo* anti-inflammatory and analgesic activities of methanol extract of *Tamarindus indica* L. (METI) seeds in different experimental models in rats and mice and its antioxidant potential *in vitro*.

Materials and Methods: Anti-inflammatory activity of methanol extract of *T. indica* (METI) was accessed in carrageenan induced paw edema and cotton pallet induced granuloma model in rats (n=6), where it was compared with indomethacin. Analgesic activity was evaluated using hot plate method and tail immersion method in mice (n=6), where it was compared with diclofenac. The *in vitro* antioxidant activity was determined by using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging, nitric oxide radical scavenging activity and reducing power assay. In addition to this the ulcerogenicity of the extract was tested using ethanol induced ulcer model in rats.

Results: METI showed significant reduction in paw thickness in carrageenan induced acute inflammation, and cotton pellet induced chronic inflammation. The extract produced significant analgesic activity ($p < 0.05$ and $p < 0.01$) at both the doses studied. METI at 100 µg/ml exhibited significant reducing power, DPPH free radical scavenging and nitric oxide radical scavenging activity.

Conclusion: The results obtained demonstrated that METI has potential health benefits as it showed dose dependant anti-inflammatory activity, central analgesic activity and lack ulcerogenicity. These activities of METI are attributed to its antioxidant mechanism and presence of tannins and flavonoids which themselves are responsible for antioxidant potential.

Keywords: *Tamarindus indica* L. seeds, Anti-inflammatory, Analgesic, Antioxidant.

INTRODUCTION

Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents. Inflammation is the body's effort to inactivate or destroy invading organisms, remove irritants and set the stage for tissue repair [1]. Synthetic drugs commonly used for the treatment of pain and inflammation like non-steroidal anti-inflammatory drugs 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 anti-inflammatory drug is needed. The healing power of tamarind is first mentioned in the traditional Sanskrit literatures [2]. *Tamarindus indica* L. (Family: Leguminosae), commonly known as Tamarind, grows naturally in the tropical and sub-tropical regions of India [3]. It is widely found throughout the tropical belt, from Africa to south Asia, northern Australia, throughout Southeast Asia, Taiwan and China [4].

In the Indian system of medicine, tamarind has wide therapeutic application including inflammation, diabetes, constipation, indigestion and flatulency [5]. Throughout Southeast Asia, the tamarind fruit poultice is applied to foreheads of fever sufferers [6]. The seeds of *T. indica* are reported to possess pharmacological activities such as antidiabetic and hypoglycemic, antioxidant, anti-ulcer, anti-venom, hepatoprotective, antibacterial, inhibition of nitric oxide production and serine proteinase inhibitor [7]. Fruits and leaves of *T. indica* are reported with antiasthamtic, hepatoprotective and antimicrobial activities [8].

The tamarind leaves are one of the constituents of an Ayurvedic formulation called Kottamchukkadi Taila which is applied topically and massaged for the treatment of rheumatism, body stiffness, pain, inflammation and disorders due to Vata dosha [9]. Folkloric uses include application of leaf poultice on inflamed ankles and joints to reduce swellings and pain. Tamarind fruit pulp is applied topically on inflammatory swellings and rheumatism to relieve pain [10, 11]. *T. indica* bark is reported to possess anti-inflammatory, analgesic and wound healing activity [12].

The seeds are reported to contain polymeric tannins and polyphenolic compounds like (+) catechin, procyanidin B$_2$, (-) epicatechin, procyanidin trimer, procyanidin tetramer, procyanidin pentamer, procyanidin hexamer, taxifolin, apigenin, eriodictyol, luteolin and naringenin [13]. Despite its traditional use, no scientific study has yet reported the anti-inflammatory activity of the tamarind seeds. This, therefore, forms the basis of the current study which evaluates the anti-inflammatory effect and mechanism of methanolic extract of tamarind seeds. Also, considering the strong association between inflammation and oxidative damage, the current study evaluates the *in vitro* antioxidant activity of the methanol extract of tamarind seeds. The main side effect and limitation of synthetic anti-inflammatory and analgesic compounds is its ulcerogenic property. Hence, we also investigated the ulcerogenicity of methanolic extract of tamarind seeds.

MATERIAL AND METHODS

Experimental animals

Adult Wistar albino rats (weighing between 180-200 g) and Swiss mice (weighing between 25-30 g) of either gender were used. They were procured from the National Institute of Biosciences (NIB), Pune. The animals were kept in cages at 25°C, under a 12:12 h light-dark cycle, with free access to standard pellet diet and water *ad libitum*. They were allowed to acclimatize to the laboratory conditions for a period of one week and fasted overnight prior to the experiment whenever needed. The study was approved by Institutional Animal Ethical Committee (IAEC) and experimental procedures were conducted in accordance with the regulations of CPCSEA (884/PO/ac/05/CPCSEA).

Drugs and Reagents

The seeds of *T. Indica* were purchased from the local market of Pune region of Maharashtra in the month of September. The plant was identified and authenticated by Mr. Diwakar, Botanical Survey of India, Pune. Voucher specimen (TAMIMAG6) has been deposited in the herbarium of BSI, Pune for future reference. Carrageenan was procured from Analab fine chemicals, Mumbai. All other

chemicals and reagents used were of analytical grade (SRL Mumbai, E. Merck India).

Extraction of Plant material

The extraction was performed using maceration technique [14]. The coarse powder of tamarind seeds (100 g) was subjected to maceration for 72 h at room temperature using 500 ml methanol. The extract was filtered and the solvent was evaporated under vacuum to obtain powdered residue.

Acute Toxicity Study

The methanolic extract of *Tamarindus indica* seeds was tested for its acute toxicity in rats. Acute toxicity studies were performed according to OECD (Organization for Economic Co-operation and Development) guidelines OECD 423. To determine short term toxicity, the adult female Wistar rats were starved overnight and were administered with extract orally in increasing dose levels of 300, 500, 2000, 5000 and 8000 mg/kg body weight. The mortality and general behavior of the animals were observed periodically for 48 h. The animals were observed individually after dosing periodically for 24 h with special attention during first two hours and then intermittently thereafter, for a total period of 14 days. The animals were observed for the signs of toxicity which include changes in eyes and mucous membrane, skin, fur and behavior pattern. Attention was given to parameters like grooming, hyperactivity, convulsions, tremors, salivation, lethargy, diarrhea, loss of righting, reflex, sleep and coma [15, 16].

Preliminary Phytochemical Screenings

The preliminary phytochemical analysis of methanolic extract of *T. indica* seeds was performed as per the standard methods given by Trease and Evans (1983) [17]. A series of chemical tests were carried out viz. Molisch's, Fehling's, Benedict's test for carbohydrates; Biuret and Million's test for proteins; Salkowski and Liebermann-Buchard's test for steroids; Borntrager's test for anthraquinone glycosides; foam test for saponins; Shinoda and alkaline test for flavonoid glycosides; Dragendorff's, Mayer's, Hagger's and Wagner's test for alkaloids; ferric chloride, lead acetate and potassium dichromate test for tannins and phenolics [18].

Evaluation of anti-inflammatory activity

Carrageenan-induced Acute Inflammatory Model

Wistar rats (180-200 g) of either sex were divided into four groups comprising of six animals each. Group I with carrageenan was kept as control and Group II was treated with drug-indomethacin (10 mg/kg). Group III and IV were treated with different concentrations of METI (300 and 600 mg/kg) orally. One h after extract administration, acute inflammation was induced in the hind paw with single sub-planter injection of 0.1 ml of % w/v of carrageenan suspension in 0.9% normal saline [19].

The paw thickness upto the tibio-tarsal articulation of all animals was measured using a plethysmometer and recorded up to 6 h. The % inhibition in paw volume was calculated by using following formula,

% inhibition in paw volume = $100 \times (1 - V_t/V_c)$

Where,

V_t = mean paw volume in the drug treated group.

V_c = mean paw volume in control group [20, 21].

Cotton Pellet Induced Chronic Inflammatory Model

Wistar rats (180-200 g) of either sex were divided into four groups comprising of six animals each. The cotton pellets weighing 25±1 mg were sterilized in an autoclave (Lab hosp, Mumbai, India). Chronic phase of inflammation was induced by implanting sterile cotton pellet subcutaneously in the groin region of each rat under thiopental sodium (25 mg/kg) anesthesia. Control group received vehicle. Group II was treated with indomethacin (10 mg/kg, p.o.) whereas Group III and IV were treated with METI (300 and 600 mg/kg, p.o.) respectively for seven consecutive days from the day of cotton pellet implantation. On 8th day, the animals were anaesthetized and blood was collected by cardiac puncture. The animals were sacrificed and pellets along with the granuloma mass were collected carefully and made free from extraneous tissue.

The wet pellets were weighed for wet weight and dried in an incubator at 60°C to a constant weight, after that the dried pellets were weighed again. The granulation tissue formation and exudate formation was calculated using following formula,

Measure of granuloma tissue formation = Constant dry weight – Initial weight of pellet.

Measure of exudate formation = Wet weight – Constant dry weight of pellet [22, 23].

Evaluation of Analgesic Activity

Hot Plate Method

The analgesic activity of the given drug was determined by the basal reaction time [24]. A total of 24 mice of either sex were divided into four groups. Group I was kept as control, administered with distilled water (10 ml/kg) and Group II was treated with standard drug-diclofenac (9 mg/kg). Group III and IV were treated with two different concentrations of METI (300 mg/kg and 600 mg/kg body weight) orally 30 min prior to the start of the experiment. The heated hot plate, maintained at 55±0.5°C was used to induce pain. Mice (six per group) were individually placed on the hot plate into a transparent glass cylinder (diameter 20 cm). Before the treatment, the reaction time of each animal (paw licking or jumping) was recorded. The reaction time was recorded at 1, 2, 3 and 4 h following the administration of METI and diclofenac. In order to minimise damage to the animal paw, the cut off time for latency was taken as 25 sec [25, 26, 27].

Tail Immersion Method

24 mice of either sex were divided into four groups. Group I was kept as control, administered with distilled water (10 ml/kg) and Group II was treated with standard drug- diclofenac (9 mg/kg). Group III and IV were treated with two different concentrations of METI (300 mg/kg and 600 mg/kg body weight) orally 30 min prior to the start of the experiment. This method involved immersing tail of mice up to 5 cm length in a water bath maintained at 51±0.2°C. Reaction of mice by withdrawing the tail was considered as the reaction time. The reaction time for each animal was recorded with a stopwatch at 1, 2, 3 and 4 h after administration of test products. The cut off time to prevent tissue damage was put at 20 sec [26, 28].

Determination of *In Vitro* Antioxidant Activity of METI

Determination of DPPH scavenging activity of METI

The effect of METI was estimated by using the method described by Dasgupta and Bratati (2004). A solution of 4 mg 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in methanol (0.004%) was prepared and 3 mL of solution was mixed with 0.1 mL methanolic solution of METI each from concentrations 20-100 μg/ml. The reaction mixture was mixed thoroughly and left in the dark at room temperature for 30 min. Absorbance of the mixture was determined spectrophotometrically at 517 nm. Ascorbic acid prepared at same concentration was used as the reference drug. The experiment was conducted in triplicate. The percent inhibition in scavenging DPPH radicals was calculated using the formula,

$$\% \text{ inhibition} = [(A_o - A_e)/A_0] \times 100$$

Where,

A_o = Absorbance without extract.

A_e = Absorbance with extract [29, 30].

Determination of nitric oxide scavenging activity of METI

Nitric oxide scavenging activity of METI was determined by slightly modified procedure of Saha *et al.* (2008) [31]. Butylated hydroxyl tolune (BHT) was used as the standard drug [32].

Determination of reducing power of METI

Reducing power of different extracts of *T. indica* L. seeds was evaluated by method of Kumaran and Karunakaran (2007) [30].

Anti-ulcerogenic activity in ethanol induced ulcer model

Male Wistar rats were divided into four groups comprising of six animals in each group. Group I served as control which received vehicle, orally Group II was administered with omeprazole 30 mg/kg orally, while Group III and Group IV were test groups which received METI 300 mg/kg and 600 mg/kg orally respectively. The animals were fasted for 16 h and 1 h after the respective treatments, 99.5% ethanol was given orally to induce gastric ulcers and animals were sacrificed after 1 h. The stomachs were removed and cut opened along the greater curvature and the gastric mucosa were washed with normal saline and scored according to the scale. The scoring of ulcer was performed using magnifying hand lens.

The following scale was used: 0 = normal stomach, 0.5 = red coloration, 1 = spot ulcer, hemorrhagic, 1.5 = streak and 2 = ulcers.

The results were expressed in % protection,

% Protection = (Ulcer index of control - Ulcer index of test / Ulcer index of control) × 100 [32, 33, 34].

Statistical Analysis

The statistical significance was compared between control and experimental groups by one way analysis of variance (ANOVA) followed by appropriate post hoc test (Dunnet's multiple comparison test) using Graph pad in Stat software; $P < 0.05$ was considered as statistically significant. All results were presented as Mean± SEM of six animals.

RESULTS

Extraction

The yield of methanolic extract of tamarind seeds was 17.9 % w/w.

Phytochemical Screening of *Tamarindus indica*

Phytochemical analysis revealed the presence tannins, flavonoids, anthraquinone glycosides, alkaloids, phenolic compounds, saponins and steroids in the methanolic seed extract of *T. indica*.

Acute Toxicity Study

As suggested by OECD guidelines, the test animals were observed individually, after dosing at once during first 30 min, periodically during the first 24 h with special attention during first 2 h. The test animals did not exhibit any visible change and survived beyond recommended duration of observation with 8000 mg/kg. Hence, METI was safe up to 8000 mg/kg.

Carrageenan-induced Acute Inflammatory Model

Carrageenan-induced acute inflammatory model paw edema was significantly reduced at 3 and 4 h by 23.86 and 15.21% in animals treated with METI 600 mg/kg b. wt. when compared with control group. At dose of 300 mg/kg b. wt., METI produced 25.95 &19.17% inhibition at 3 and 4 h respectively [Table 1]**.** The maximum inhibition percentage by standard (indomethacin, 10 mg/kg) group at 3 h was 35.22%.

Table 1: Effect of METI (300 mg/kg and 600 mg/kg) in carrageenan induced paw edema in rats

Groups	Dose (mg/kg)	Paw volume (ml)					
		0.5 h	1 h	2 h	3 h	4 h	6 h
Control	--	1.33± 0.04	1.41± 0.03	1.74± 0.13	1.67± 0.1	1.89± 0.11	1.88± 0.12
Indomethacin	10	1.13± 0.02[ns]	1.26± 0.03*	1.28± 0.04**	1.08± 0.02**	1.28± 0.05**	1.59± 0.05*
METI	300	1.30± 0.06[ns]	1.19± 0.03**	1.52± 0.02*	1.55± 0.09**	1.63± 0.04*	1.56± 0.06*
METI	600	1.19± 0.01[ns]	1.16± 0.03**	1.37± 0.06*	1.23± 0.06**	1.57± 0.06*	1.59± 0.05*

Values are expressed in mean±SEM (n=6); *$P<0.05$,**$P<0.01$ as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison. METI: methanolic extract of *T. indica* seeds.

Table 2: Effect of METI (300 mg/kg and 600 mg/kg) on weight of cotton pellets in cotton pellet granuloma model in rats

Group	Dose (mg/kg)	Dry weight (mg)	Moist weight (mg)	Transudative weight
Control	--	77.65±2.19	298.95±9.1	221.3±6.91
Indomethacin	10	62.31±1.5**	221.03±12.7**	158.72±11.2**
METI	300	60.18±2.3**	226.88±11.9**	116.7±9.6**
METI	600	59.53±4.2**	245.1±14.5*	185.57±10.3*

Values are expressed in mean±SEM (n=6); *$P<0.05$,**$P<0.01$ as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison. METI: methanolic extract of *T. indica* seeds.

Cotton Pellet Induced Chronic Inflammatory Model

Treatment with METI (300 mg/kg and 600 mg/kg) to rats showed a significant (p<0.01) inhibition in the dry weight of cotton pellet compared to control group and percentage inhibition was found to be 22.49 & 23.33 respectively. Treatment with reference standard i.e. indomethacin (10 mg/kg) also showed significant inhibition in cotton pellets granuloma formation (36.74%) as compared to control group [Table 2].

Histopathology of cotton pallet granulomatous tissue

Figure 1 reveals the histopathological findings of granulomatous tissue. Section from control group shows fibromuscular tissue comprising of neutrophils, lymphocytes, macrophages, giant cell and few plasma cells with proliferating blood vessels and dense infiltration by acute inflammatory cells. Rats treated with indomethacin (10 mg/kg) showed absence of necrosis and presence of diffusely arranged lymphocytes, macrophages and few plasma cells. METI treated groups (300 mg/kg and 600 mg/kg) showed diffused infiltration by lymphocyte, macrophages, few plasma cells with multinucleated giant cells and congested vessels. No evidences of necrosis were found.

Hot Plate Method

Oral administration of METI (300 mg/kg and 600 mg/kg) and diclofenac (9 mg/kg) produced a significant (p<0.05 and p<0.01) increase in the reaction time of mice to thermal stimulation compared to control [Table 3].

Tail Immersion Method

In tail immersion test, administration of METI (300 mg/kg and 600 mg/kg) as well as diclofenac (9 mg/kg) significantly (p<0.05 and p<0.01) increased the latency time, compared to the control [Table 4].

Determination of *In Vitro* Antioxidant Activity of METI

DPPH Scavenging activity of METI

Using DPPH, the free radical scavenging activities of METI and ascorbic acid were observed to be concentration dependant [Figure 2]. At 1000 μg/ml, the scavenging effect of METI was calculated to be 71.97% whereas the scavenging effect of ascorbic acid was 94.2%.

Nitric oxide scavenging activity of METI

METI and BHT significantly ($p<0.01$) scavenged nitric oxide radicals at variable concentrations (20-100 μg/ml), shown in Figure 3. At 1000 μg/ml, % inhibition of nitric oxide radicals by METI was calculated to be 79.61% while by BHT it was found to be 60.42%.

Reducing power of METI

As plotted in Figure 4, the reducing power in decreasing order was as follows, ascorbic acid (2.1 μg/ml) > METI (1.50 μg/ml).

Antiulcerogenic activity

The groups of animals treated with METI (300 mg/kg and 600 mg/kg) showed less ulceration in the stomach after 16 h of fasting compared to control (Table 5).
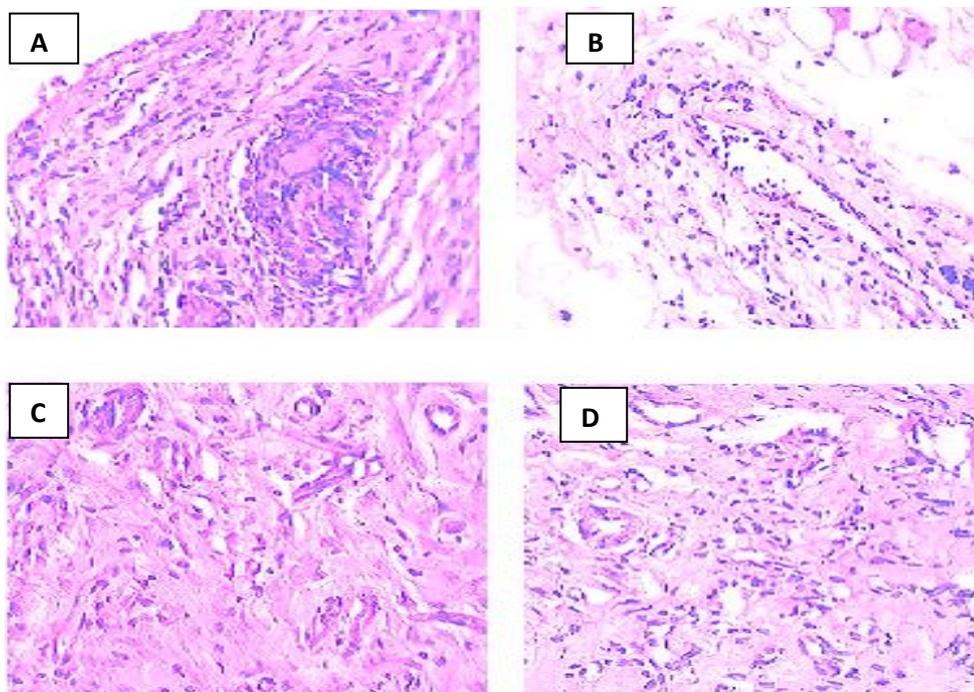
Fig. 1: Effect of METI on cotton pellet induced granuloma model in rats.

Control: Section of granuloma tissue showing proliferating blood vessels and dense infiltreation by both acute and chronic inflammation with necrosis. (B) Indomethacin: Section of granuloma tissue showing fibromuscular tissue with infiltration by polymorphs and macrophages with few congested vessels and fat cells. No evidence of necrosis. (C) METI (300 mg/kg): Section of granuloma tissue showing acute inflammation with giant cells, lymphocyte, macrophages and few plasma cells. (D) METI (600 mg/kg): Section of granuloma tissue showing moderate acute inflammation with diffuse infiltration by lymphocyte, macrophages, few plasma cell, also show multiple small congested vessels with giant cells.

Table 3: Effect of METI (300 mg/kg and 600 mg/kg) on hot plate induced pain in mice

Group	Dose (mg/kg)	Latency period (sec)				
		0 h	1 h	2 h	3 h	4 h
Control	--	9.55±1.50	7.90±0.68	10.68±2.02	11.07±2.01	9.05± 1.38
Diclofenac	10	36.02±5.63 ns	41.42±5.08*	53.69±3.14**	57.24±13.60**	49.21±10.30**
METI	300	31.08±7.02 ns	58.76±11.66**	60.58±11.40**	39.00±2.27 ns	56.02±5.76**
METI	600	29.06±5.45 ns	36.97±7.11 ns	64.05±7.75**	61.82±11.01**	64.03±10.8**

Values are expressed in mean±SEM (n=6); *P<0.05,**P<0.01 as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison. METI: methanolic extract of *T. indica* seeds.

Table 4: Effect of METI (300 mg/kg and 600 mg/kg) on tail immersion method induced pain in mice

Group	Dose (mg/kg)	Latency period (sec)				
		0 h	1 h	2 h	3 h	4 h
Control	--	2.95± 0.56	2.393± 0.54	3.735± 0.82	5.073± 0.82	2.398± 0.17
Diclofenac	9	4.36± 0.53	6.995± 1.10*	6.456± 1.39 ns	12.838± 1.25*	9.378± 1.0**
METI	300	2.95± 0.30	7.486± 1.33*	6.59± 0.60 ns	12.691± 1.74*	10.936± 1.67**
METI	600	6.88± 1.97	8.703± 1.13**	10.388± 0.07*	10.02± 1.81*	10.906± 2.37**

Values are expressed in mean±SEM (n=6); *P<0.05,**P<0.01 as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison. METI: methanolic extract of *T. indica* seeds.
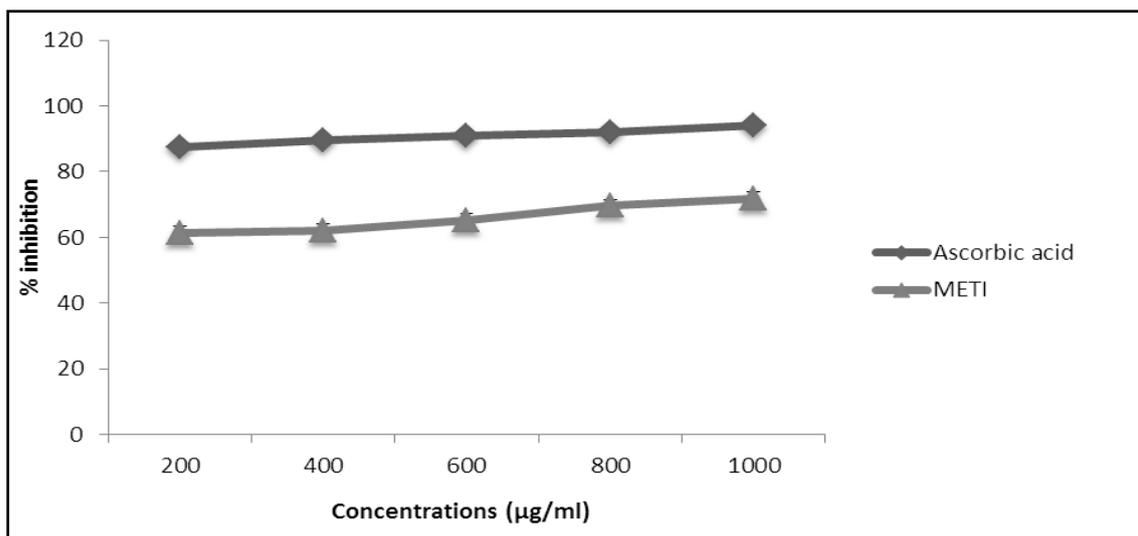
Fig. 2: DPPH radical scavenging activity of different extracts of T. indica seeds.

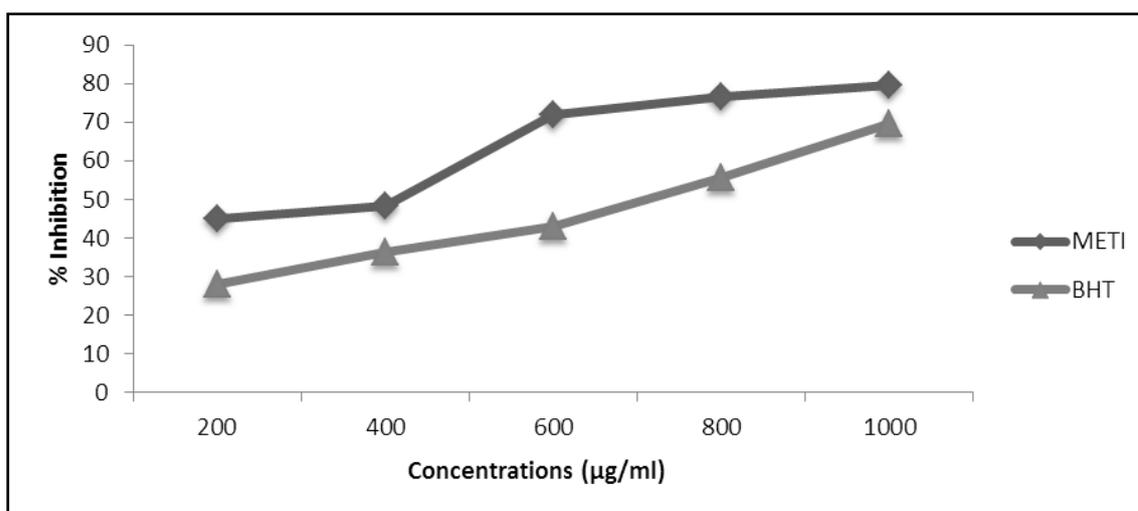METI: methanolic extract of T. indica seeds, ASC: ascorbic acid.

Fig. 3: Nitric oxide scavenging activity of different extracts of *T. indica* seeds.

METI: methanolic extract of *T. indica* seeds, BHT: butylated hydroxyl tolune
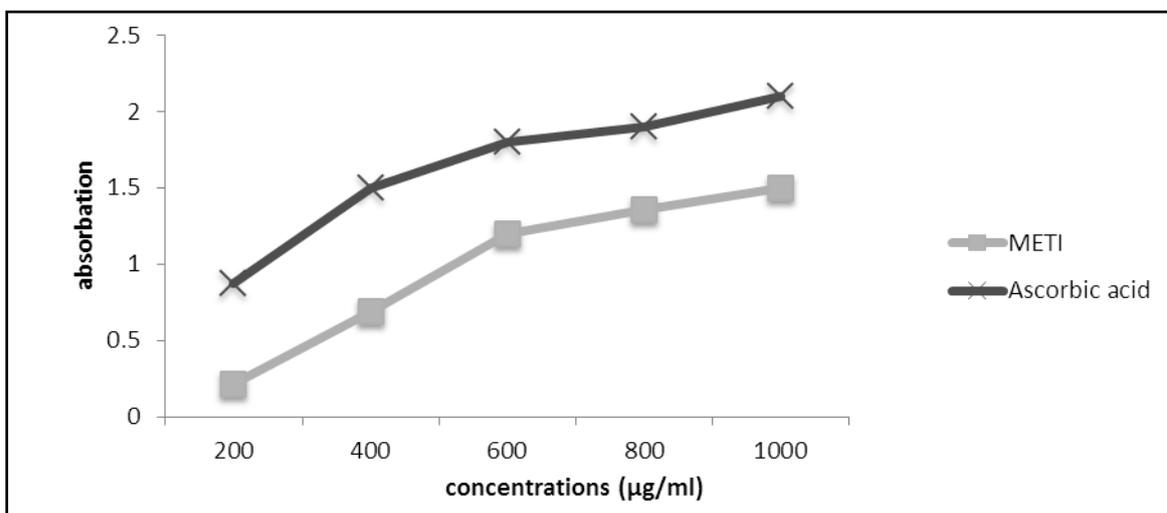

Fig. 4: Reducing power of different extracts of *T. indica* seeds.

METI: methanolic extract of *T. indica* seeds, ASC: ascorbic acid.

Table 5: Effect of METI on ethanol induced ulcers in rats.

Group	Dose (mg/kg)	Ulcer index
Control	--	20.16±0.92
Omeprazole	30	1.33±0.16**
METI	300	6.50±1.25**
METI	600	3.66±0.33**

Values are expressed in mean±SEM (n=6); *P<0.05, **P<0.01 as compared to control. Data analysed by One-way ANOVA test followed by Dunnet's multiple tests for comparison. METI: methanolic extract of *T. indica* seeds.

DISCUSSION

In the present study, the anti-inflammatory, analgesic and antioxidant activities of METI were evaluated using different standard methods. Carrageenan induced paw edema has been widely used to screen natural products with anti-inflammatory potentials [22, 36]. This 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 [37]. Sub-planter 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 [38, 39]. 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. 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 METI showed maximum reduction in paw edema during second phase, it may be stated that METI might have mediated its anti-inflammatory action by inhibiting the release of mediators like prostaglandins, leukotrienes, polymorphonuclear cells and bradykinins [40].

In the present study, cotton pellet granuloma model was used to evaluate the transudative and proliferative components of the chronic inflammation [41]. The decrease in the size of the granuloma observed by METI may be via events inhibition of granulocyte infiltration, preventing generation of collagen fibres and by suppression of mucopolysaccharides. Histopathology of granulomatous tissue further confirmed the anti-inflammatory potential of METI. METI treated groups showed absence of necrosis, reduction in dense infiltration inflammatory cells and exudate compared to toxic control.

It is well known that antioxidant potential plays an important role in the anti-inflammatory activity [42, 43, 44]. It has been reported that the neutralization of free radicals by antioxidants and radical scavengers eases inflammation [45, 46]. Bearing this strong association between inflammation and oxidation, it is possible that anti-inflammatory of METI is partly mediated via its antioxidant mechanism. Tannins and flavonoids have been reported to exhibit significant inhibitory activities on nitric oxide implicated in physiological and pathological process as chronic inflammation [47].

In the present study, Eddy's hot plate method and tail immersion methods were used to evaluate analgesic activity of METI. The validity of these tests has been shown even in the substantial impaired motor performances of animals. Hot plate method is based on the mice paw sensitivity to heat at temperatures not damaging the skin which is observed as jumping, paw licking and paw withdrawal [48]. While in case of tail immersion method, nociceptive response is noted as withdrawal of tail from hot water. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripherally acting analgesics do not generally affect theses responses [24]. In the present study, METI was found to affect jumping, withdrawal or paw licking response which makes it evident that it is centrally acting. This suggests implication of μ receptors in the analgesic effect. The significant analgesic activity exhibited by METI suggests an advantage in the use of the extract as an anti-inflammatory agent.

The major limitation of most of the NSAIDs is the ulcerogenic activity which is due to inhibition of prostaglandin synthesis. The results of ethanol induced ulceration in rats suggests that METI has no such side effects and can hence prove to be comparatively more effective than the present synthetic preparation.

Reports from previous studies suggest that tannins and flavonoids inhibit prostaglandin synthesis [49]. The presence of tannins and flavonoids in the METI as evident from the results of our preliminary phytochemical analysis, appears to inhibit prostaglandin synthesis and exerts the anti-inflammatory effect. Also tannins are reported to possess protein precipitating and vasoconstricting effect [50]. We can thus conclude that METI possess significant anti-inflammatory and analgesic activity probably mediated through inhibition of prostaglandin synthesis which might be attributed to presence of tannins and flavonoids. While absence of ulcers by METI might be due to presence of tannins which prevented damage to gastric mucosa through its astringent action.

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