Original Article

Evaluation of Toxicity Studies and Anti-inflammatory activity of *Terminalia bellerica* in Carrageenan-induced Paw Edema in Experimental Rats

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Abstract

Background: In traditional Indian system of medicine, *Terminalia* bellerica (TB) is known to possess several medicinal properties. Preclinical studies addressing safety profile and anti-inflammatory activity of TB extract (TBE) in experimental animals are lacking. The objective of the present study was to evaluate the toxicity studies and anti-inflammatory potential of hydroalcoholic fruit extract of TB in Wistar albino rats. **Methods:** Acute and subacute oral toxicity studies of TBE were evaluated in Wistar albino rats. TBE was administered at doses of 2000 and 1000 mg/kg in acute and subacute toxicity studies, respectively. Anti-inflammatory activity of TBE was evaluated in carrageenan-induced paw edema model. For the evaluation of anti-inflammatory activity, animals were divided into five groups (Group 1 – control, Group 2 – indomethacin 3 mg/kg, Group 3 – TBE 100 mg/kg, Group 4 – TBE 200 mg/kg, Group 5 – TBE 400 mg/kg). **Results:** No mortality and signs of toxicity were observed in both acute and repeated dose toxicity studies after oral administration of TBE up to the dose level of 2000 mg/kg. TBE showed a significant anti-inflammatory activity in carrageenan-induced paw edema model at 1, 3, and 5 h. A significant inhibition ($P < 0.01$) of paw edema as compared to control group was observed at doses of 100, 200, and 400 mg/kg at 1, 3, and 5 h. TBE showed comparable efficacy to indomethacin at 200 mg/kg. Maximum percentage inhibition was observed with TBE 200 mg/kg at 3 h (57.6%). **Conclusion:** The results indicate that hydroalcoholic extract of TB fruit is safe and exhibits a significant anti-inflammatory activity in experimental rats.

Keywords: Carrageenan, indomethacin, inflammation, paw edema, toxicity

Introduction

Acute inflammatory response is characterized by enhanced vascular permeability and cellular infiltration, leading to the development of edema, as a result of extravasation of fluid and proteins and accumulation of white blood cells (leukocytes) at the site of inflammation. Several inflammatory mediators are involved in producing inflammatory response, and one of the important mediators is prostaglandins (PGs) which are produced by the action of cyclooxygenase (COX) enzyme on arachidonic acid derived from the membrane phospholipids.[1] This acute inflammatory response correlates well with the symptoms such as joint pain in variety of arthritis such as osteoarthritis, rheumatoid arthritis (RA), and others. We have conventional drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs for the management of inflammatory conditions. However, treatment for arthritis remains unsatisfactory for many patients. Therefore, there is a need to search for alternative therapies that might improve outcomes in patients of arthritis.[2]

Complementary and alternative medicine (CAM) is attractive because it is generally perceived to be safe and "natural" in contrast to conventional drugs.[2-4]

In traditional system of medicine such as Ayurveda, Siddha, and Unani, use of medicinal plants for the treatment of diseases is well documented. One such genus is *Terminalia* belonging to *Combretaceae* family, which comprises of 200 species. Genus *Terminalia* covers all plants ranging from small shrubs to large deciduous trees up to a height of 75 m.[5]

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Terminalia bellerica (TB) is commonly known as "Belleric Myrobalan" in English and "Bahera" in Hindi. TB is a large tree about 50 m in height and 3 m in diameter. Active constituents present in plant are ellagic acid, tannic acid, glucoside, gallotannic acid, tannins, chebulagic acid, lignans, flavones, anolignan B, resins, etc., TB has known anti-inflammatory, antibacterial, wound healing, antioxidant, anti-nociceptive, nephroprotective, anti-arthritic, and hepatoprotective activities.[6,7]

Carrageenan-induced paw edema is one of the commonly used model for depicting acute inflammatory response.[8,9] Subplantar injection of carrageenan causes acute inflammatory reaction by inducing edema, erythema, and hyperalgesia. Inflammatory action is mediated via pro-inflammatory agents such as histamine, bradykinin, tachykinins, reactive oxygen species, and nitric oxide synthase.[9,10]

Various animal models are used to assess mediators of inflammation and screening of anti-inflammatory agents.[10,11] For the evaluation of vascular changes associated with acute inflammatory response, carrageenan-induced paw edema serves as the convenient model.[8-10]

CAM has gained importance for the treatment of inflammatory disorders such as RA because of the safety issues of the conventional drugs. Furthermore, there is a need of add-on drugs to address the inadequate control of symptoms or disease progression in inflammatory disorders.[3,4]

TB is one such potential anti-inflammatory drug which might be useful in culminating the symptoms of inflammatory disorders without any safety concerns. However, preclinical studies addressing anti-inflammatory activity of TB are lacking. Therefore, the aim of this study was to evaluate the safety profile and anti-inflammatory potential of hydroalcoholic fruit extract of TB in Wistar albino rats.

Methods

Chemicals

Carrageenan and indomethacin were procured from Sigma Chemical Company, St. Louis, MO, USA.

Plant extract

Hydroalcoholic fruit extract of TB (referred as TB extract [TBE] in this article) was provided by Natural Remedies Pvt. Ltd., Bangalore, India, Batch No: RD16180. The total tannic acid (43.89% w/w), gallic acid (3.58% w/w), and ellagic acid (1.48% w/w) content present in TBE were determined by high-performance liquid chromatographic analysis.

Animals

Thirty adult Wistar albino rats were used in the study. Rats were housed in clean polypropylene cages with free access to food and water *ad libitum* at a temperature of 25°C ± 2°C and 55%–65% relative humidity, and 12 h light/dark cycle was maintained. The protocol was approved by the Institutional Animal Ethics Committee (IAEC), All India Institute of Medical Sciences, New Delhi, India, with IAEC No-950/IAEC/16. Following study was performed according to the "Guidelines for Care and Use of Animals in Scientific Research" (India National Science Academy 1998, Revised 2000).[12]

Toxicity studies

Acute oral toxicity of TB was evaluated according to the Organization for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals-425.[13] Five female Wistar albino rats (weighing 140–170 g) were given TBE at a dose of 2000 mg/kg. Animals were observed daily for a period of 14 days. Animals were monitored for behavioral change, weakness, aggressiveness, discharge from eyes and ears, weight loss, diarrhea, and loss of appetite.

Repeated dose toxicity was evaluated according to the OECD-407 guidelines.[14] In this part, twenty Wistar albino rats were divided into two groups (n = 10 in each group, i.e., five male and five female rats in each group). Group 1 received distilled water (normal control) at 1 ml/kg and Group II received TBE at a dose of 1000 mg/kg for 28 days. Body weight was monitored weekly, and organ weight and hematological, biochemical, and histopathological parameters were observed at the end of the study.

Carrageenan-induced paw edema

Five groups (n = 6 in each group) of female Wistar albino rats were used in this study. Before the experiment, rats were fasted overnight with free access to water. On the 1st day, baseline paw volumes were measured using a plethysmometer (Ugo Basile 7140). Following were administered:

- Group I – Control group was given distilled water
- Group II was given indomethacin (3 mg/kg per orally)
- Groups III, IV, and V were given TBE orally at a dose of 100, 200, and 400 mg/kg, respectively.

After the administration of drug/vehicle, 1 h later, 0.1 ml of 1% carrageenan was administered in the subplantar region of the left hind limb of rat. Thereafter, paw volume was measured with plethysmometer as described by Nair *et al*. at 0, 1, 3 and 5 h.[15]

Percentage inhibition of edema is calculated using the formula:
$(1 – Vt / Vc) \times 100$

Where Vt = change in paw volume of rat given test drug and Vc = change in paw volume of rat given control.

Statistical analysis

All data were represented as mean ± standard error of the mean. Comparison among the different groups was done by applying one-way analysis of variance, followed by Tukey's multiple comparison test (GraphPad InStat; Version 3.05). $P < 0.05$ was considered as statistically significant.

Results

Toxicity studies

In acute toxicity study, TBE at a dose of 2000 mg/kg did not produce any signs of toxicity or mortality. Hence, oral lethal

dose of 50% of TBE was found to be more than 2000 mg/kg. In repeated dose toxicity study also, no mortality and no signs of toxicity were observed. No significant changes were observed in the body weight or organ weight of the study animals [Tables 1 and 2]. Furthermore, no abnormality was detected with respect to the hematological and biochemical parameters [Tables 3 and 4]. No pathological changes or abnormalities were observed on histopathological examination of various organs of experimental animals after administration of TBE at a dose 1000 mg/kg (maximum dose in the repeated dose toxicity study) [Figure 1].

Anti-inflammatory potential of *Terminalia bellerica* extract in carrageenan-induced paw edema

Maximum edematous inflammation was seen at 3 h. Standard drug indomethacin (3 mg/kg) and TBE (100, 200, and 400 mg/kg) were administered 1 h before carrageenan injection. Indomethacin showed maximum inhibition of 63.8% at 3 h and declined to 55.4% after 5 h. TBE at a dose of 200 mg/kg showed maximum inhibition of 57.6% at 3 h. No significant difference was observed in edema inhibition in the standard group and TBE 200 mg/kg group. Tables 5-7 describe the results of this section.

Discussion

CAM can be useful when given along with conventional therapy in inflammatory disorders. CAM can be helpful in alleviating side effects or improving patient's symptoms.[3]

Several CAM therapies have shown to play a role in the treatment of arthritis. Mostly, such therapies are being used adjunctively and are not meant to replace conventional treatment or standard of care.[3,4] The objective of the present study was to evaluate the safety profile and explore the evidence on anti-inflammatory potential of TB (a form of CAM) in acute model of inflammation.

In both acute and repeated dose toxicity studies, hydroalcoholic extract of TB fruits (TBE) proved to be safe up to a dose of 2000 mg/kg as no toxicity signs were observed with respect to laboratory parameters and histopathology.

TB is an active component of an Ayurvedic formulation, named "Triphala" which is used as an antioxidant, immunomodulatory, antimicrobial, hypolipidemic, analgesic, anti-inflammatory, purgative, and anti-mutagenic agent. Other components of this preparation are *Terminalia chebula* and *Emblica officinalis*.[16]

Carrageenan-induced paw edema model best depicts the acute inflammatory condition. Inflammation induced by carrageenan is a biphasic process. The initial phase (0–1 h) is mediated by the release of histamine, 5-hydroxytryptamine, and bradykinin and it is not completely inhibited by NSAIDs – indomethacin and aspirin. The second phase (1–6 h) is associated with maximum swelling and it is mediated by PGs, COX-2, and neutrophil infiltration.[8-10]

Standard drug indomethacin showed inhibition of 62.3%, 63.8%, and 55.4% at 1, 3, and 5 h, respectively. Hydroalcoholic extract of TB at a dose of 100 mg/kg showed inhibition of edema-50.9%, 54.9%, and 54.6% at 1, 3, and 5 h, respectively. At a dose of 200 mg/kg, the percentage of edema inhibition was 52.5%, 57.6%, and 52.3% at 1, 3, and 5 h, respectively, and at 400 mg/kg edema inhibition was 54.1%, 56.7% and 50.8% at same time points. In both standard and treatment groups, peak inhibition of edema was observed at 3 h. Indomethacin (COX inhibitor) showed maximum effect at 3 hours which can be attributed to the fact that during the second phase of inflammation (1-6 h), COX is one of the important primary mediator.[17] Moreover, Reddy *et al*.[18] have proved that gallic acid component of TB is a selective and reversible COX-2 inhibitor.

This is an important observation as TBE has shown comparable effect to the standard drug indomethacin with respect to peak inhibition of COX-2, which translates into its potential utility in inhibiting the important inflammatory mediator in RA. NSAIDs are widely employed in the symptomatic treatment of rheumatic diseases characterized by chronic joint pain and other forms of acute pain.[19] Indomethacin is invariably used NSAID in patients of arthritis to control the inflammation and related symptoms such as pain and joint stiffness; however, some patients cannot tolerate the drug-related adverse effects of

Table 1: Effect of Terminalia bellerica on body weight of rats over a period of 28 days

Drug treatment	Change in body weight (g)			
	7th day	14th day	21st day	28th day
Group I (control, male)	4.80±0.5	4.8±1.2	3.56±1.1	4.32±0.6
Group II (control, female)	4.16±0.7	5.72±0.3	4.70±0.7	3.10±0.3
Group III (TBE, male)	2.86±0.9	10.30±3.5	3.28±1.3	3.0±1.2
Group IV (TBE, female)	6.42±2.1	6.80±1.7	7.14±1.14	5.20±0.8

TBE: *Terminalia bellerica* extract

Table 2: : Effect of Terminalia bellerica on organ weight of rats

Drug treatment	Organ weight (g)					
	Liver	Kidney	Heart	Brain	Testis	Ovaries
Group I (control, male)	3.42±0.03	0.77±0.01	0.35±0.02	1.87±0.04	1.17±0.03	-
Group II (control, female)	3.33±0.02	0.75±0.02	0.38±0.01	1.91±0.02	-	0.06±0.001
Group III (TBE, male)	3.36±0.02	0.78±0.03	0.38±0.01	1.91±0.06	1.20±0.05	-
Group IV (TBE, female)	3.39±0.05	0.74±0.02	0.36±0.01	1.92±0.04	-	0.06±0.003

TBE: ***Terminalia bellerica*** extract

Table 3: Effect of *Terminalia bellerica* on hematological parameters

Drug treatment	Hematological parameters					
	RBC count (1,000,000/mm^3)	WBC count (1000/mm^3)	PT count (1000/mm^3)	Hb (g/dl)	BT (s)	CT (s)
Group I (control, male)	7.8±0.58	10.4±0.6	830.4±29.89	12.40±0.3	724.4±8.1	232.6±5.4
Group II (control, female)	7.0±0.7	12.5±0.7	829.4±45.80	12.28±0.3	736.4±12.2	231.6±11.9
Group III (TBE, male)	8.0±0.23	13.3±0.9	791.4±80.36	12.56±0.7	725.6±7.8	240.0±7.1
Group IV (TBE, female)	7.2±0.86	11.5±0.6	812.0±47.20	12.48±0.6	736.4±15	235.8±10.3

BT: Bleeding time, CT: Clotting time, Hb: Hemoglobin, PT: Platelet, RBC: Red blood corpuscles, TBE: *Terminalia bellerica* extract, WBC: White blood corpuscles

Table 4: Effect of *Terminalia bellerica* on biochemical parameters

Drug treatment	Biochemical parameters					
	Glucose (mg/dl)	Creatinine (mg/dl)	AST (IU/L)	ALT (IU/L)	TG (mg/dl)	HDL (mg/dl)
Group I (control, male)	103.60±5.53	0.45±0.12	11.64±2.26	10.13±1.97	46.38±2.45	51.04±5.57
Group II (control, female)	104.40±8.07	0.53±0.13	15.52±2.44	13.29±2.24	45.0±2.81	43.76±1.57
Group III (TBE, male)	122.60±5.06	0.61±0.17	9.28±1.37	13.67±2.37	46.34±3.74	62.40±5.07
Group IV (TBE, female)	112.80±4.96	0.47±0.12	10.91±1.29	7.05±1.35	37.13±3.30	57.40±2.94

ALT: Alanine aminotransferase, AST: Aspartate transaminase, HDL: High-density lipoprotein, TBE: *Terminalia bellerica* extract, TG: Triglycerides

Table 5: Absolute paw volume before and after administration of study drugs

Drug treatment	Drug dose PO	Baseline	Mean paw volume (ml)		
			1 h	3 h	5 h
Group I (disease control)	1 ml/kg	0.76±0.02	1.38±0.13	2.01±0.08	2.08±0.09
Group II (indomethacin)	3 mg/kg	0.72±0.01	0.93±0.04**	1.13±0.04**	1.31±0.06**
Group III (TBE)	100 mg/kg	0.74±0.02	1.04±0.04*	1.25±0.06**	1.35±0.06**
Group IV (TBE)	200 mg/kg	0.76±0.02	1.06±0.04*	1.26±0.03**	1.39±0.03**
Group V (TBE)	400 mg/kg	0.72±0.02	1.02±0.03*	1.23±0.03**	1.38±0.04**

*P<0.05, **P<0.01. PO: Per oral, TBE: *Terminalia bellerica* extract

Table 6: Mean change in paw volume after administration of study drugs

Drug treatment	Drug dose PO	Change in paw volume (ml)		
		1 h	3 h	5 h
Group I (disease control)	1 ml/kg	0.61±0.13	1.13±0.15	1.32±0.08
Group II (indomethacin)	3 mg/kg	0.23±0.02**	0.41±0.03**	0.59±0.03**
Group III (TBE)	100 mg/kg	0.30±0.02**	0.51±0.05**	0.60±0.07**
Group IV (TBE)	200 mg/kg	0.29±0.03**	0.48±0.01**	0.63±0.03**
Group V (TBE)	400 mg/kg	0.28±0.01**	0.49±0.01**	0.65±0.01**

**P<0.01. PO: Per oral, TBE: *Terminalia bellerica* extract

NSAIDS (similar to conventional NSAIDs).[20] Although very much effective in relieving pain and inflammation, indomethacin, like other NSAIDs, has safety concerns due to significant risk of gastrointestinal, cardiovascular, renal, and hepatic toxicity with the chronic use.[20,21] Moreover, TBE acts through the same mechanism (COX inhibition) and might prove as a potential anti-inflammatory agent. At the same time, this demands further investigation (in the form of well-designed clinical studies) of anti-inflammatory potential TBE in arthritic patients.

Although indomethacin showed maximum inhibition of paw edema, no significant difference was observed in percentage inhibition of paw edema between indomethacin and TBE. This shows the potential of TBE as an alternative and efficacious agent for controlling the inflammation and troublesome symptoms of arthritis. Similarly, it also gives the indication of potential use of TBE as an adjunct treatment to the conventional therapies, to increase the effectiveness of the therapy and to decrease the adverse effects (by decreasing the dose of the conventional drugs such as indomethacin).

Hence, this study proved that no significant difference was observed in edema inhibition in standard group and TBE 200 mg/kg group. TB has the potential to be used as an anti-inflammatory agent in acute inflammations and its potential could further be explored.

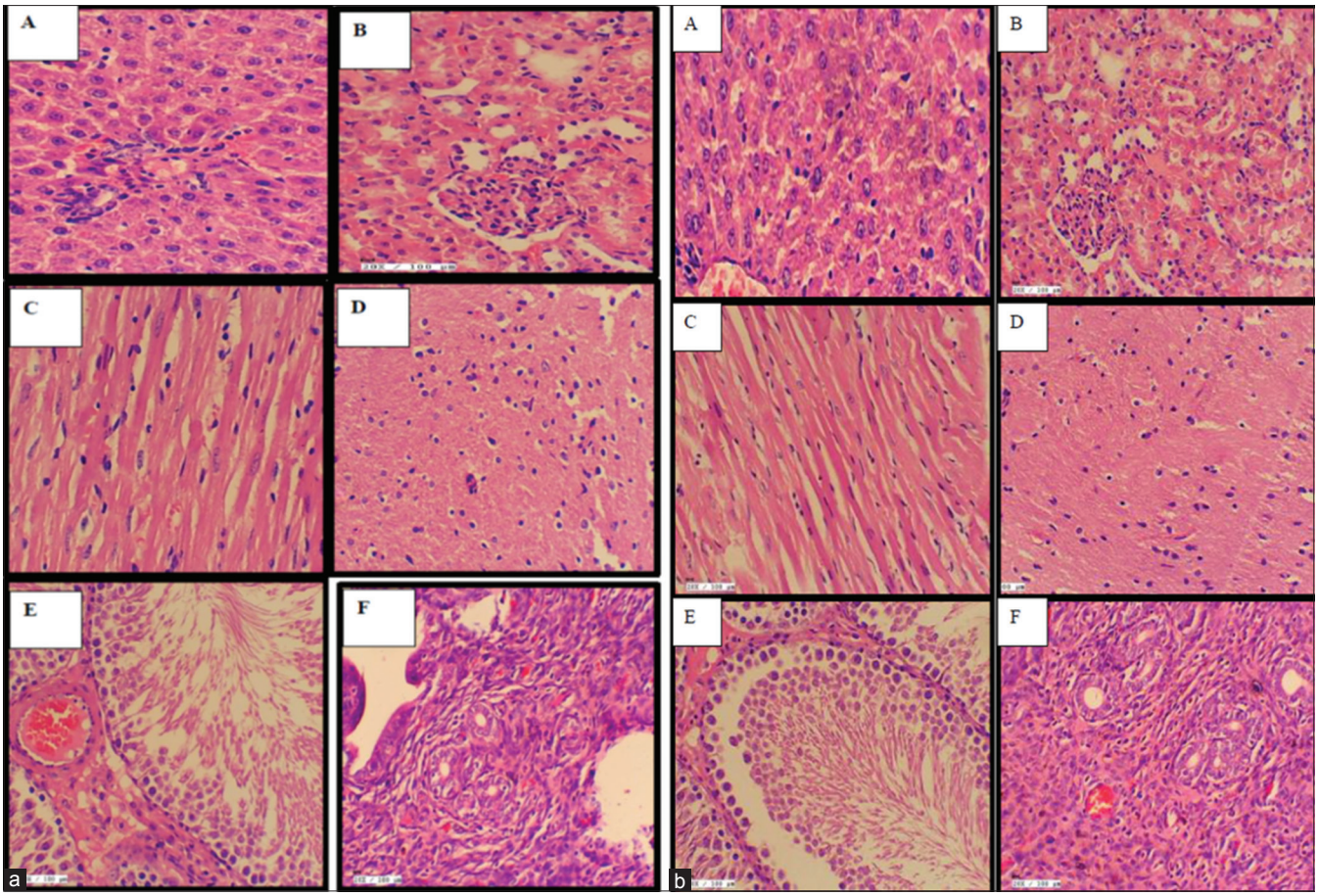

Figure 1: (a) Organ histopathology of normal control experimental rats (×20 magnification). A = Liver, B = Kidney, C = Heart, D = Brain, E = Testis, F = Ovary. (b) Organ Histopathology of Experimental rats after administration of *Terminalia bellerica* (1000 mg/kg) for 28 days (×20 magnification). A = Liver, B = Kidney, C = Heart, D = Brain, E = Testis, F = Ovary

Table 7: Percentage inhibition of paw edema after administration of study drugs

Drug treatment	Drug dose PO	Percentage inhibition		
		1 h	3 h	5 h
Group I (disease control)	1 ml/kg	-	-	-
Group II (indomethacin)	3 mg/kg	62.3	63.8	55.4
Group III (TBE)	100 mg/kg	50.9	54.9	54.6
Group IV (TBE)	200 mg/kg	52.5	57.6	52.3
Group V (TBE)	400 mg/kg	54.1	56.7	50.8

PO: Per oral, TBE: *Terminalia bellerica* extract

Conclusion

Our study concludes that the hydroalcoholic extract of TB fruits is both safe and efficacious anti-inflammatory agent in experimental model of acute infl ammation. These findings support the potential use of TB for the management of inflammatory conditions such as arthritis and need for further clinical evaluation.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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