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Antisecretory and analgesic activities of *Terminalia* bellerica

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This study describes the antisecretory and analgesic activities of the crude extract of *Terminalia bellerica* (Tb.Cr). *T. bellerica* extract inhibited the castor oil-induced intestinal fluid secretion in mice at the dose range of 300 - 1000 mg/kg. The extract also dose-dependently (50 - 100 mg/kg) reduced the numbers of acetic acid-mediated writhes in mice. These results indicate that *T. bellerica* exhibit antisecretory and anti-nociceptive effects, hence justifying its medicinal use in diarrhea and pain.

Key words: Terminalia bellerica, intestinal secretion, analgesia.

INTRODUCTION

Terminalia bellerica Roxb. (Combretaceae) commonly known as "belleric myrobalan" and locally as "bahera" is a large deciduous tree, found throughout central Asia and some other parts of the world. Its fruit is used in folk medicine to treat asthma, cancer, colic, diarrhea, dysuria, headache, hypertension, inflammations and pain. The plant is reported to known to contain termilignan, thannilignan, anolignan B, gallic acid, ellagic acid, β-sitosterol, arjungenin, belleric acid, bellericosidem, flavonoids and tannins. *T. bellerica* possesses antioxidant, antispasmdic, bronchodilatory, hypercholesterolemic, antibacterial, cardioprotective, hepatoprotective, hypoglycemic and hypotensive properties (Gilani et al., 2008; Khan and Gilani, 2008).

MATERIALS AND METHODS

Plant material and preparation of crude extract

The fruits of *T. bellerica* were bought from a local market in Dhaka (Bangladesh) and the sample voucher (TB-FR-10-95-30) was submitted to the herbarium of Aga Khan University, Karachi. About 432 g of fruits were crushed, then soaked in 70% aqueous-methanol, filtered and concentrated with rotary evaporator to obtain crude extract, yielding 9.25%.

Chemicals and animals

Acetic acid, diclofenic sodium and loperamide were purchased from Sigma Chemicals Co, St Louis, MO, USA. Castor oil was obtained from KCL Pharma, Karachi, Pakistan. All chemicals used were of the analytical grade available. Animals used in this study; Balb-C mice (20 - 25 g) of either sex and local breed were housed at the animal house of the Aga Khan University, maintained at 23 - 25 °C and was give standard diet and tap water *ad libitum*. Experiments performed complied with the rulings of the institute of laboratory animal resources, commission on life sciences, national research council (1996) and approved by the ethical committee of the Aga Khan University.

Intestinal fluid accumulation

Intestinal fluid accumulation was studied by the enteropooling assay. Different groups of overnight fasted mice were treated with increasing doses of extract intraperitoneally, 1 h before the administration of castor oil (10 ml/kg, p.o.). The mice were sacrificed 30 min later by cervical dislocation and the entire intestine was removed and weighed with care (Capasso et al., 2002). The results were expressed as (Pi/Pm) x 1000 where Pi is the weight (g) of the intestine and Pm is the weight of the animal (Gilani et al., 2005).

Anti-nociceptive study

Male adult mice were used in this study according to a method described previously (Koster et al., 1959; Bashir and Gilani, 2008). After 30 min of plant extract administration, mice were given an i.p. injection of 0.7% (v/v) acetic acid solution (volume of injection 0.1 ml/10 g b.w). The mice were placed individually into glass beakers

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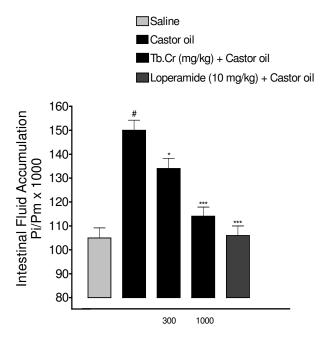


Figure 1. Inhibitory effect of the crude extract of *T. bellerica* (Tb.Cr) and loperamide on the castor oil-stimulated fluid accumulation in small intestine of mice. Results shown are mean \pm SEM of 5 animals for each experimental group. Intestinal fluid accumulation is expressed as Pi/Pm x 1000 (g) where Pi is the weight of the small intestine and Pm is the weight of mouse. [#]P < 0.001 vs. saline group, *P < 0.05 and ****P* < 0.001 vs. castor oil group, student's *t*-test.

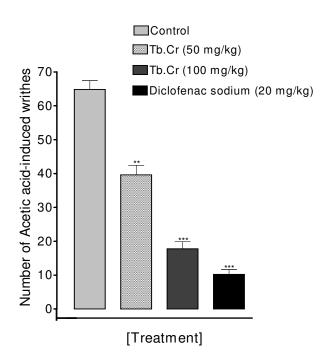


Figure 2. Inhibitory effect of the crude extract of *T. bellerica* (Tb.Cr) and diclofenic sodium on the acetic acid-induced writhes in mice. Values shown are mean \pm SEM, n=5. **P < 0.01 and ***P < 0.001 compared to control. Control animals were treated with vehicle (saline, 10 ml/kg).

and 5 min were allowed to elapse. The number of writhes produced in these animals was counted for 20 min. For scoring purposes, a writhe was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb. Control animals received normal saline (10 ml/kg, i.p.).

Statistical analysis

The data expressed are mean \pm standard error of mean (SEM) and analyzed by using GraphPad program (GraphPAD, San Diego, CA, USA). The results were compared using the student's t-test with P < 0.05 noted as significantly different.

RESULTS

Effect on intestinal fluid accumulation

When tested against castor oil-induced intestinal fluid accumulation in mice, *T. bellerica* crude extract exhibited dose-dependent (300 - 1000 mg/kg) antisecretory effect (Figure 1). Intestinal fluid accumulation in the saline treated group was 105.0 ± 4.2 g, while with castor oiltreated group it was 150.0 ± 4.2 g (P < 0.001 vs. saline group). The extract at the doses of 300 and 1000 mg/kg reduced the castor oil-induced fluid accumulation to 134.0 ± 4.3 g (P < 0.05 vs. castor oil group) and $114.1 \pm$ 3.8 g (P < 0.001 vs. castor oil group), respectively. Loperamide at the dose of 10 mg/kg decreased the intestinal fluid accumulation to 106.0 \pm 4.0 g (P < 0.001 vs. castor oil group) as shown in Figure 1.

Effect on acetic acid -mediated writhing

T. bellerica crude extract dose-dependently (50 - 100 mg/kg) reduced the number of writhes evoked by acetic acid in mice (Figure 2). The number of writhes in control group were 65 ± 3.0 (mean \pm SEM, n = 5). At the doses of 50 and 100 mg/kg, the extract reduced the number of writhes to 40 ± 3.0 (P < 0.01, n = 5) and 18 ± 2.0 (P < 0.001, n = 5) respectively. Diclofenic at the dose of 20 mg/kg decreased the number of acetic acid-mediated writhes to 10 ± 1.0 (P < 0.001, n = 5) as shown in Figure 2.

DISCUSSION

Based on the medicinal use of *T. bellerica* in diarrhea, it was tested for the possible protective effect against castor oil-induced intestinal secretion in mice. The plant extract dose-dependently suppressed the castor oil-stimulated intestinal fluid accumulation, like that caused by positive control drug, loperamide, thus showing antisecretory effect and explains the *T. bellerica* use in gut hypersecretion. For the anti-nociceptive activity, acetic acid induced writhing test was employed. Various peripheral analgesic drugs such as diclofenac sodium,

ibuprofen and aspirin have been reported to inhibit acetic acid induced writhing (Okpo et al., 2001). In this study, the tested plant extracts reduced the nociception induced by acetic acid. Abdominal writhing in response to acetic acid is postulated to be mediated through stimulation of local peritoneal receptors related to prostanoid system, indicating increased levels of lipoxygenase products as well as prostaglandins in peritoneal fluid (Deraedt et al., 1980). The inhibitory effect of the plant extracts against acetic acid induced writhing, suggests that it may have occurred through inhibition of lipoxygenase and/or cycloxygenase pathways. The flavonoids are known for their antisecretory and analgesic actions (Di Carlo et al., 1993; Bukhari et al., 2007) and the presence of such compounds in T. bellerica (Khan and Gilani, 2008) is likely to account for their observed effects. In conclusion, the present study, by reporting the antisecretory and analgesic activities of T. bellerica, contribute towards evidencebased phytomedicine, as well as validate its effectiveness in diarrhea and pain.

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