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Screening of *Terminalia bellirica* Fruits Extracts for its Analgesic and Antipyretic Activities

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Abstract

The study was designed to investigate the analgesic and antipyretic activities of ethanolic and aqueous extracts of ***Terminalia bellirica*** (family: Combrataceae) fruits (200 mg/kg, p.o.) in acetic acid-induced writhing, Eddy's hot plate method and brewer's yeast-induced fever models in mice and rats. Both extracts showed a significant decrease in the number of the writhes in acetic acid-induced writhing and increase in paw licking time to heat stimuli in the hot plate method. Both extracts showed a significant inhibition of elevated body temperature when compared to corresponding control. The results suggested that the ethanolic and aqueous extracts possessed significant analgesic and antipyretic activities.

Keywords: ***Terminalia bellirica***, analgesic activity, acetic acid-induced writhing, hot plate method, antipyretic activity, brewer's yeast-induced fever model.

1. Introduction

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states (Chattopadhyay *et al*., 2005 a). It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive (Chattopadhyay *et al*., 2005 b). Normal body temperature is regulated by a center in the hypothalamus that ensures a balance between heat loss and production. Fever occurs when there is a disturbance of this hypothalamic 'thermostat', which leads to the set-point of body temperature being raised. Once there has been a return to the normal set point, the temperature regulating mechanisms (dilatation of superficial blood vessels, sweating etc.) then operate to reduce temperature (Anonymous, 1976). Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PgE2 biosynthesis (Cheng *et al*., 2005). Analgesia is the inability to feel pain while still conscious. From the Greek an-, without + algesis, sense of pain.

In view of this, different therapeutic agents are employed like NSAIDS, Opiods etc. However, on chronic usage most of these agents produced several side effect including gastrointestinal, renal, hepatic, central nervous system and dermatological effects (Chaudhary, 2001).

Therefore, today a large section of world population relies on traditional remedies to treat plethora of disease due to their low cost, easy access and reduced side effects (Marino-Betlolo, 1980).

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Terminalia bellirica Roxb, belonging to Combrataceae family, commonly known as Belliric Myrobalan (Bahera in Hindi, one of the important constituents of Indian herbal preparation Triphala), is a deciduous tree found throughout Indian forests and plains. The Fruit is bitter, analgesic, astringent, brain tonic, expectorant and laxative (Chaudhary, 2008). It is chiefly used for fever, leprosy, diarrhea, and piles. The fruits exhibited bronchodilatory, antispasmodic, antiasthematic, hypoglycemic, wound healing, spermicidal activities; they contain tannin, β-sitosterol, gallic acid, ellagic acid, ethyl gallate, and chebulic acid in various proportions (Dhingra and Valecha, 2007). A survey of literature revealed that no scientific study on the analgesic and antipyretic activities has been reported on the fruits of the plant. Therefore, the present study was designed to evaluate the said activities of the fruit extracts of the ***T. bellirica***.

2. Materials and Methods

2.1. Collection of plant materials and preparation of extracts

The fruits of ***Terminalia bellirica*** Roxb were collected in the month of November from the local market of Etawah, Uttar Pradesh state, India, and were authenticated by Dr. Harish .K. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen was submitted at the Institute's Herbarium Department for future reference (AA 103). Shade dried fruits were ground to coarse powder. Powder was first defatted with pet. ether and then extracted with ethanol (80 %) which is further evaporated to dryness to obtain alcoholic extract. Aqueous extract were obtained by maceration for 24 hrs.

2.2. Drugs and chemicals

Ibuprofen was purchased from Cipla Pvt.Ltd, Pentazocine was purchased from Ranbaxy Pvt.Ltd, and Paracetamol was obtained from Zydus Cadilla Ltd. The solvents and the other chemicals of analytical grade were used and obtained from the institute's central store. Brewer's yeast was procured from a local source.

2.3. Phytochemical screening

Qualitative assay of ethanolic and aqueous extracts of ***T. bellirica*** fruits**,** for the presence of plant phytoconstituents such as flavonoids, phytosterol, alkaloids, glycosides, tannins and saponins, was carried out following standard procedure(Trease and Evans,2003).

2.4. Experimental Animals

Albino rats (160-180 g) and mice (40-50g) of either sex were procured from the institute's Animal House for experimental study. They were acclimated to laboratory conditions for seven days before the commencement of the experiments, and were allowed free access to standard dry pellet diet and water *ad libitum*. The experimental protocol was approved by the IAEC, for using animals in present study.

Animals were fasted overnight with free access to water prior to each experiment.

2.5. Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by the 'up and down' method (425 OECD, 2001). The animals were fasted overnight and the next day extracts of the ***T. bellirica*** Roxb fruits dissolved in normal saline were administered orally at different dose levels. Then the animals were observed continuously for 3 hours for general behavioral, neurological, and autonomic profiles, and then every 30 minutes for the next three hours, and finally till death after 24 hours.

3. Pharmacological evaluation

3.1. Acetic acid induced writhing in mice

The mice were divided into four groups of six mice in each. To each group 0.1 ml of 1% acetic acid was injected intraperitonially (Purnima *et al*., 2009). The control group mice received saline solution (0.9% w/v, NaCl) 2 ml/kg and standard group of mice received 40 mg/kg Ibuprofen. The test groups of mice were treated orally with 200 mg/kg of the ethnolic and aqueous extracts respectively, 60 min before acetic acid injection. The Number of writhes (abdominal muscle contraction), stretching of the hind limbs and trunk twisting were counted for 10 min after acetic acid injection. Percent inhibition was determined for each experimental group as follows: Percent inhibition = $(N\text{-}N^{t}/N) \acute{}100$, where N is the average number of writhing of control per group, and N^{t} is the average number of writhing of test per group.

3.2. Thermal stimulus-induced pain (hot plate test) in rats

The rats were divided into four groups of six each mice in each. The test was carried out using Eddy's hot plate apparatus (Eddy and Leimbach, 1953). The temperature was set at $55\pm1^{0.}$ Rats were placed on a hot plate and recorded the reaction time in second for licking of hind paw or jumping with cut-off time of 15 s., following the administration of the test extracts (ethanolic and aqueous at 200 mg/kg), reference standard Pentazocine (5 mg/kg) and control saline vehicle at 0, 30, 90 and 180 min.

3.3. Brewer's yeast-induced pyrexia

Albino rats were divided into four groups of six rats in each group. Fever was induced by injecting 20 mg/kg (subcutaneous) of 20% suspension of Brewer's yeast in normal saline below the nape of the neck (Somezeet Panda *et al.,* 2009)**.** Initial rectal temperature was recorded. After 18h, animals that showed an increase of $0.3\text{-}0.5^{0}$ in rectal temperature were selected. The test extracts (200mg/kg) reference standard Paracetamol (150 mg/kg) and control saline vehicle were administered orally. The rectal temperature was measured with clinical thermometer at 0, 0.5,1, 2 and 3 hours post dosing.

3.4. Statistical analysis

The results are presented as Mean ± SEM. Statistical analysis of data was performed using Student's't' test to study the differences amongst the means.

4. Results

4.1. Preliminary phytochemical screening

Phytochemical studies revealed that fruits extracts of ***T.bellirica*** contains phytosterol, alkaloids, flavonoids, glycosides, saponins and tannin.

4.2. Acute toxicity studies

The result of acute toxicity study in rats indicated that the ethanolic and aqueous extracts did not produce any significant changes in the behavioural or neurological responses up-to 2000 g/kg b. wt.

4.3. Analgesic activityIn acetic acid-induced writhing test, T.bellirica fruits extracts (200 mg/kg, p.o.) reduced writhing counts significantly Table 1.

Table 1. Effect of ***Terminalia bellirica*** fruits extracts on acetic acid induced writhing in mice.

Treatment	Dose (mg/kg)	Mean No. of Writhing±S.E.M (10Mins.)	% Inhibition
Control	0.2 ml	61.60±4.26	…….
Ibuprofen	40	15.20±1.20***	75.33
Ethanolic	200	23.60±1.94***	61.89
Aqueous	200	27.60±2.14**	55.20

Values are Mean±S.E.M. (n=6) Significance vs. control group: **P<0.01, ***P<0.001

In the hot plate method, the ethanolic and aqueous extracts at a dose of 200mg/kg showed significant increase in reaction time i.e. 7.15 s and 6.81 s, respectively at 30 min. when compared to control (5.15 s) Table 2. These increases were found to be statistically significant (**P<0.01, ***P<0.001). Hence the ethanolic extract was found to be more effective compared to aqueous extract.

Table 2. Effect of ***Terminalia bellirica*** fruits extracts on Thermal stimulus induced pain (Hot Plate Test) in Rats.

Treatment	Dose (mg/kg)	Reaction time in second at (Mins)			
		0	30	90	180
Control	0.2ml	4.12± 0.85	5.15± 0.75	5.00±0.35	5.50±0.46
Pentazocine	5.00	5.60 ±0.92	7.25 ±0.80***	10.83 ± 0.56***	13.92 ±0.77***
Ethanolic	200	5.35±0.48	7.15 ±0.37***	9.10 ± 0.50***	12.27±0.59***
Aqueous	200	5.10±0.24	6.81± 0.20**	8.72±0.31***	10.14 ±0.37***

Values are Mean ±S.E.M. (n=6) Significance vs. control group: **P<0.01, ***P<0.001

The present investigations suggested that both extracts showed a significant analgesic effect in chemical and mechanical induced pain models.

4.4. Antipyretic activity

The results of the antipyretic effect of the test compounds (ethanolic and aqueous), standard (Paracetamol 150 mg/kg), and control are depicted in Table 3. The Paracetamol as well as ethanolic extract at dose of 200 mg/kg started showing effective antipyretic activity after 1h of post dosing; while aqueous extract 200 mg/kg reduced temperature after 2 h, when compared with control. Antipyretic activity was observed up to 3 h after paracetamol and test extracts administration.

Table 3. Effect of ***Terminalia bellirica*** fruits extracts on Brewer's yeast-induced pyrexia in Rats.

Treatment	Dose (mg/kg)	Rectal temperature in °C at time (h)				
		0	0.5	1	2	3
Control	0.2ml	37.13±0.50	37.19 ±0.10	37.21 ± 0.11	37.50 ± 0.11	37.42 ± 0.13
Paracetamol	150	37.11±0.15	37.01 ±0.34	36.68 ±0.44***	36.10±0.17***	35.81±0.11***
Ethanolic	200	37.10 ± 1.0	37.05 ±0.23	36.78 ±0.05***	36.90 ± 0.67**	36.61±0.23***
Aqueous	200	37.41 ±0.22	37.21 ±0.31	37.20 ± 0.06	36.89 ± 0.72**	37.01 ±0.63*

Values are Mean±S.E.M. (n=6) Significance vs. control group: *P<0.05, **P<0.01, ***P<0.001

5. Discussion

Acetic acid-induced writhing and Eddy's hot plate induced thermal stimulation are models of pain that mainly involve peripheral and central mechanisms, respectively. Analgesic effect observed in these two models with 200 mg/kg ethanolic and aqueous extracts of ***T. bellirica*** indicates the involvement of both peripheral and central mechanisms. The acetic acid-induced writhing has been associated with an increased level of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products (Derardt *et al*., 1980). The present results revealed that a significant reduction in acetic acid-induced writhing, and increase reaction time to heat stimuli strongly suggests that the mechanism of both extracts may be linked partly to cyclooxygenase and/or lipoxygenase inhibition (Franzotti *et al*., 2002).

In addition, the flavonoids are known to inhibit prostaglandin synthetase (Ramaswamy *et al*., 1985 a). Since prostaglandins are involved in pain perception and are inhibited by flavonoids, it could be suggested that reduced availability of prostaglandins by flavonoids present in titled plant might be responsible for its analgesic effect.

It is well known that most of the anti-inflammatory and analgesic drugs possess antipyretic activity. Both extracts markedly decreased the rectal temperature of pyretic rats. This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on the pathogenic fever induced by the administration of a yeast injection. Its etiology includes the production of prostaglandins in central nervous system which is the final common pathway responsible for fever induction (Howard, 1993). In general, NSAIDS produce their antipyretic action through the inhibition of prostaglandin synthetase within the hypothalamus (Clark, 1975; Zeal, 1975). Therefore, it appears that the antipyretic action of the titled plant may also be related to the inhibition of prostaglandin synthesis in hypothalamus by present flavanoids in both extracts of ***T.bellirica*** (Ramaswamy *et al*., 1985 b).

Thus, the present study concludes that the ethanolic and aqueous extracts have analgesic and antipyretic activities in rats and mice at the doses 200 mg/kg, respectively. However, further study needs to be carried out for the isolation and identification of specific phytoconstituents present in titled plant.

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