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ANALGESIC AND ANTIPYRETIC ACTIVITIES OF ETHANOLIC EXTRACT OF *BAMBUSA ARUNDINACEA* LEAVES

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ABSTRACT: To study the analgesic and antipyretic activities of ethanolic extract of *Bambusa arundinacea* leaves (EEBA) in rats by using models tail immersion test, hot plate test and brewer's yeast pyrexia model. **Methods:** The dried powdered leaves were extracted with ethanol and the resultant extract was subjected for phytochemical investigation to identify different phytoconstituents. Analgesic activity of the ethanolic extract of *B. arundinacea* leaves at a dose of 100mg/kg b.w., p.o. and 200mg/kg b.w., p.o. were evaluated against the standard drug Diclofenac sodium at a dose of 10mg/kg b.w., i.p. by tail immersion test and hot plate test. Antipyretic activity of EEBA (100 mg/kg and 200 mg/kg) was assessed by using brewer's yeast pyrexia model in rats against the standard drug Paracetamol (150 mg/kg, p.o.). **Results:** Preliminary phytochemical investigations showed the presence of flavonoids, alkaloids, steroids, carbohydrates, proteins and amino acids, tannins and phenolic compounds as major secondary metabolites. The results showed that in tail immersion and hot plate test, EEBA has shown significant ($P < 0.05$) increase in the latency period at both high and low doses indicating analgesic activity. EEBA (100 mg/kg and 200 mg/kg) also significantly ($P < 0.0001$) reduced brewer's yeast induced pyrexia in rats. **Conclusion:** These results demonstrated that the ethanolic extract of *Bambusa arundinaceae* leaves exhibited significant analgesic and antipyretic activities at 100 mg/kg and 200 mg/kg in dose dependent manner.

INTRODUCTION: A variety of diseases and injuries are most often presented with fever and pain. Synthetic drugs, *i.e.* Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed drugs for their management but significant gastrointestinal complications like bleeding, perforation, obstruction, peptic ulcers, renal disorders etc. have limited their use [1-3].

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Selective COX-2 inhibitors have some benefits on lowering such side effects while risk of cardiovascular adverse effects demands important concern [4-7]. Narcotic analgesics are also associated with social abuse and other side effects like addiction, psychological dependency, sedation, tolerance, respiratory depression and constipation [8]. The negative consequences of pain are that it affects overall quality of life and working status of individual and the society [9].

In addition, currently available analgesic drugs are not so effective in subsiding pain, only contributing to 50% relief in about 30% of the patients in some cases which suggest dire requirement for effective

analgesics [10]. With such type of shortcomings and other associated problems with analgesics, search for newer drugs to treat pain and fever is going on. Alternative medicines from medicinal plants are important options in this regard as around 25% of all currently available synthetic medicines are directly or indirectly based on medicinal plants because they have lesser side effects and are economic [11].

Bambusa arundinacea belongs to the family Poaceae (Graminae), commonly known as Baans, is a tall and thorny tree. A bamboo stem consists of nodes (which is strong and provides structural integrity for the plant) and internodes (which are hollow for most of the bamboo plants). At the node are one or more buds (depending on the species) which produce side branches. Traditional use of bamboo varies from region to region and people to people [12-14].

The various parts of this plant contain resins, silica, cynogenetic glycosides, betain, oxalic acid, reducing sugar, benzoic acid, albuminoids, waxes, arginine, cysteine, histidine, isoleucine, phenylamine, leucine, threonine, methionine, lysine, valine, riboflavin, tyrosine, niacin, thiamine, gluteline, protein, choline, betain, proteolytic enzyme, urease and nuclease [15].

Different parts of *B. arundinacea* such as root, shoot, leaf, flower and seed showed antidiabetic [16], anthelmintic [17], anti-inflammatory [18], estrogenic [19], antiulcer [18], antimicrobial [20], antifertility [21], wound healing [22], anti-arthritic activity [23], antioxidant [24] anti-thyroid [25] and antiamnesic activities [26]. Seeds are unpleasant, laxative, and said to be useful in urinary discharge and strangury [27]. The root (burnt root) is applied to ringworms, bleeding gums and aching joints. Bark is used for skin eruptions. Leaf is febrifuge, emmenagogue, bechic, antileprotic, and also used in haemoptysis [28]. This study was aimed to evaluate the analgesic and antipyretic activities of ethanolic extract of *Bambusa arundinacea* (EEBA) in rats by using models tail immersion test, hot plate test and brewer's yeast pyrexia model.

MATERIAL AND METHODS:

Plant material: The matured leaves of *Bambusa arundinaceae* used for the present studies were collected from local market, Lucknow, India and its identification and authentication were done from National Botanical Research Institute (Council of Scientific and Industrial Research), Lucknow-226001, India (Ref. No: NBRI/CIF/rb-4/411/2013). Soon after authentication, all leaves were shade dried until they were free from the moisture. Finally leaves were subjected to size reduction to get coarse powder and then passed through Sieve No. 40 to get uniform powder. The resulting powder was then used for extraction.

Preparation of ethanolic extract: The powdered plant material was subjected to Soxhlet extraction with ethanol for 6 h at 50 °C. The resulting crude extract after evaporation of the ethanol was washed with petroleum ether, chloroform and ethyl acetate successively. Percentage yield of the obtained crude extract was calculated [19]. The crude extract was subjected to phytochemical investigation and pharmacological screening for its analgesic and antipyretic activities.

Preliminary phytochemical screening: EEBA were subjected to various chemical tests for determination of various phytochemical constituents present in it, according to standard protocols [29].

Animals: Male albino wistar rats weighing between 160-180 g were procured from the Central Drug Research Institute Lucknow, India. They were housed in polypropylene cages (22.5 × 37.5 cm^2) and maintained under standard laboratory environmental conditions; temperature 25 ± 2 °C, 12 h light: 12 h dark cycle and 55 ± 10% relative humidity with free access to standard pellets and water, *ad libitium*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures [Hygia/M.Pharm./02/2013-14].

Acute toxicity study: The acute toxicity studies were conducted as per Organization for Economic Cooperation and Development (OECD) guidelines 425 for testing of chemicals for acute oral toxicity

[30]. Male rats (n = 6) treated with different doses of EEBA (50, 250, 500, 1000 and 2000 mg/kg, p.o.), while the control group received saline (10 ml/kg, i.p.). All the groups were observed up to 6 h for any gross effect and then mortality rate was observed after 24 h of treatment.

Analgesic Activity:

Tail-immersion test: The tail withdrawal response was determined by immersing the lower 3.5 cm of the animals tail into a cup freshly filled with water from a large bath at a constant-temperature of 55 ± 0.5 °C until the typical response was observed. A 25 s cutoff was imposed to avoid tail damage by heat. The animals were randomly divided into four groups, each group contains six rats. Group I received normal saline (10 ml/kg, i.p.). Group II was given standard drug diclofenac sodium (10 mg/kg b.w., i.p.). Groups III and IV received EEBA at oral dose of 100 mg/kg and 200 mg/kg respectively. After the treatment, analgesic activity was measured at 0, 15, 30, 60, 90 and 120 minutes after administration of EEBA, diclofenac sodium and normal saline [31].

Hot plate test: Animals were subjected to pre-testing on a hot plate (Eddy's Hot Plate) maintained at 55 ± 0.1 °C. Animals having latency time greater than 15 sec on hot plate during pre-testing were debarred. Animals were randomly divided into four groups, each group contains six rats. The group I was treated with saline (10 ml/kg, i.p.), group II with diclofenac sodium (10 mg/kg b.w., i.p.), and Group III and IV were treated with oral doses of 100 and 200 mg/kg of EEBA respectively. Diclofenac sodium was used as reference drugs for comparison. After 30 min of dose administration, rats were dropped inside the cylinder onto the hot plate and the latency time (time for which rat remains on the hot plate without licking or flicking of hind limb or jumping) was recorded in seconds. In order to prevent the tissue damage the cut off time of 30 sec was set for all animals. The latency time was recorded for each group at 0, 30, 60, 90 and 120 min following drug administration [32, 33].

Anti-pyretic activity:

Brewer's yeast pyrexia model:

The antipyretic activity was evaluated with fever induced by Brewer's yeast following the established method [34, 35] in rats with some modifications. At zero hour, the basal rectal temperature of each rat was recorded using clinical digital thermometer. Pyrexia was induced by subcutaneous injection of 15% w/v suspension of Brewer's yeast in distilled water at a dose of 10 ml/kg body weight. After 18 h of Brewer's yeast injection the rise in rectal temperature was recorded and only animals showing an increase in temperature of at least 0.6 °C (or 1°F) were selected for the study.

The animals were randomly divided into four groups, each group contains six rats. Group I received normal saline orally. Group II was given standard drug paracetamol at the dose of 150 mg/kg perorally. Groups III and IV received EEBA at oral dose of 100 mg/kg and 200 mg/kg respectively. After the treatment, the temperature of all the rats in each group was recorded periodically at 0 h, 1 h, 2 h, 3 h, 4 h and 5 h of drugs administration.

Statistical analysis: All values were expressed as mean ± S.E.M. and data were analyzed by Graph Pad Prism using One-way analysis of variance followed by dunnett's test. $P < 0.05$ was considered significant.

RESULTS:

Preliminary phytochemical screening:

Preliminary phytochemical investigations showed the presence of flavonoids, alkaloids, steroids, carbohydrates, proteins and amino acids, tannins and phenolic compounds as major secondary metabolites.

Estimation of acute toxicity: EEBA found safe at all tested doses (up to 2000 mg/kg) and did not show any noxious symptom in rats like sedation, convulsions, diarrhoea and irritation. During the 48 h assessment, no mortality was found.

Analgesic Activity:

Tail-immersion test: EEBA 100 mg/kg and 200 mg/kg exhibited a dose dependent increase in latency time and inhibited pain sensation in a pattern similar to standard drug diclofenac sodium at all the time interval measured (0, 15, 30, 60, 90 and 120 minutes) after administration (**Table 1**).

TABLE 1: EFFECTS OF EEBA ON TAIL-IMMERSION TEST IN RATS

Group	Dose/Route	Latency time in seconds					
		0 min	15 min	30 min	60 min	90 min	120 min
Control	10 ml/kg, i.p.	2.27 ± 0.13	2.32 ± 0.13	2.65 ± 0.23	3.0 ± 0.09	3.0 ± 0.29	3.0 ± 0.08
Diclofenac sodium	10 mg/kg, i.p.	2.55 ± 0.20	4.25 ±0.22*	6.8 ± 0.23*	7.6 ± 0.26*	8.5 ± 0.30	7.02 ± 0.11
EEBA	100 mg/kg p.o.	2.6 ± 0.17	4.1 ± 0.18*	5.5 ± 01.23*	5.7 ± 0.23*	7.4 ± 0.29	7.25 ± 0.22
EEBA	200 mg/kg p.o.	2.8 ± 0.08	4.0 ± 0.04*	5.0 ± 0.07*	5.0 ± 0.07*	7.8 ± 0.08	8.2 ± 0.21

Data are expressed as mean ± S.E.M.; *n* = 6. *Significant difference (*$P < 0.05$) in comparison to control.

Hot plate test: EEBA 100 mg/kg and 200 mg/kg exhibited a dose dependent increase in latency time and inhibited pain sensation in a pattern similar to standard drug diclofenac sodium at all the time interval measured (0, 15, 30, 60, 90 and 120 minutes) after administration (**Table 2**).

TABLE 2: EFFECTS OF EEBA ON HOT PLATE TEST IN RATS

Group	Dose/Route	Latency time in seconds					
		0 min	15 min	30 min	60 min	90 min	120 min
Control	10 ml/kg, i.p.	2.85 ± 0.31	2.87 ± 0.33	2.8 ± 0.31	2.8 ± 0.31	2.77 ± 0.27	2.95 ± 0.34
Diclofenac sodium	10 mg/kg, i.p.	2.5 ± 0.28	9.1±0.41**	11.5 ±0.28**	10.0±0.04**	8.0 ± 0.38	6.3 ± 0.34
EEBA	100 mg/kg, p.o.	2.6 ± 0.24	6.0 ± 0.40	8.05 ± 0.37	8.47 ± 0.27	6.25 ± 0.25	4.87 ± 0.31
EEBA	200 mg/kg, p.o.	2.75 ± 0.25	6.25±0.25*	8.22 ± 0.13*	9.32 ± 0.23*	7.4 ± 0.20	5.4 ± 0.18

Data are expressed as mean ± S.E.M.; *n*=6. *Significant difference (*P <0.05 and **P < 0.001) in comparison to control

Anti-pyretic activity:

Brewer's yeast pyrexia model: EEBA 100mg/kg and 200 mg/kg significantly (P <0.0001) attenuated hyperthermia in rats. The inhibition was dose dependent. EEBA at 200 mg/kg showed maximum antipyretic effect and returned body temperature to normal levels (P < 0.0001) (**Table 3**).

TABLE 3: EFFECTS OF EEBA ON BREWER'S YEAST-INDUCED PYREXIA IN RATS

Group	Dose/Route	Rectal temperature (°C) after yeast induction						
		0 h	19h	20h	21h	22h	23h	24h
Control	10 ml/kg, i.p.	37.27 ± 0.08	39.15 ± 0.04	39.2 ± 0.07	39.24 ± 0.08	39.23 ± 0.09	39.02 ± 0.01	38.23 ± 0.09
Paracetamol	150 mg/kg, p.o.	36.18 ± 0.1	38.25 ± 0.06	37.37 ± 0.07***	39.24 ± 0.08***	39.23 ± 0.07***	37.08 ± 0.04***	37.05 ± 0.01***
EEBA	100 mg/kg, p.o.	37.23 ± 0.08	39.17 ± 0.02	38.4 ± 0.09	38.27 ± 0.1	38.20 ± 0.08	38.12 ± 0.08	38.07 ± 0.04
EEBA	200 mg/kg, p.o.	37.25 ± 0.06	38.32 ± 0.08	37.37 ± 0.08***	37.3 ± 0.12***	37.25 ± 0.11***	37.13 ± 0.07***	37.07 ± 0.04***

Data are expressed as mean ± S.E.M.; *n* = 6. *Significant difference (**P < 0.001 and **P < 0.0001) in comparison to control

DISCUSSION: The aim of this study was to evaluate the analgesic and antipyretic activities of ethanolic extract of *Bambusa arundinacea* leaves (EEBA). For this purpose the phytochemical analysis of the ethanolic extract was studied as well. The phytochemical analysis showed the presence of flavonoids, alkaloids, steroids, carbohydrates, proteins and amino acids, tannins and phenolic compounds in the crude extract.

The present study revealed that ethanolic extract of *Bambusa arundinacea* leaves (EEBA) possessed significant dose dependent analgesic and antipyretic activities in experimental animals. The analgesic activity of ethanolic extract of *Bambusa arundinacea* leaves was evaluated using tail immersion test and hot plate test models of analgesia. The antipyretic activity of ethanolic extract of *Bambusa arundinacea* leaves was evaluated using brewer's yeast pyrexia model.

Two different analgesic testing models were employed with the objective of identifying central analgesic effect (hot plate and tail immersion method) of the test extract. In the analgesic testing models, EEBA 100 mg/kg and 200 mg/kg

markedly exhibited a dose dependent increase in latency time and inhibited pain sensation in a pattern similar to standard drug diclofenac sodium. It is well established that thermal nociceptive tests are more sensitive to opioid μ-agonists [36]. The data produced in the present study suggest that there is involvement of μ-opioid receptor in the analgesic activity of EEBA, from which the central involvement of EEBA could be assumed.

In the antipyretic testing model, EEBA 100 mg/kg and 200 mg/kg markedly decreased elevated body temperature but not in control animals. Brewer's yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, particularly PGE_2 appears to be a final pathway responsible for fever production induced by several pyrogens [34]. Most of the NSAIDS show the antipyretic activity by inhibiting the prostaglandin synthesis. It is therefore suggested that the antipyretic effect of EEBA occurs in a similar fashion as paracetamol.

CONCLUSION: To conclude, the ethanolic extract of *Bambusa arundinacea* leaves (EEBA) was evidenced as a natural safe remedy for the treatment of pain and pyrexia. The observed pharmacological activities might have been accredited to the presence of active constituents like flavonoids, alkaloids, steroids, carbohydrates, proteins and amino acids, tannins and phenolic compounds. Further studies are in progress to isolate and identify the compounds which are responsible for these activities.

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CONFLICT OF INTEREST: There is no conflict of interest.

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