ORIGINAL ARTICLE

Anti-inflammatory activity of methanolic extract of *Bambusa vulgaris* leaves

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Bambusa vulgaris, commonly known as "Bamboo," possesses various pharmacological activities including anti-inflammatory activity. The present study is designed to investigate anti-inflammatory effect of methanolic extract of *B. vulgaris* (MEBV) on rats and mice. The anti-inflammatory effect is investigated employing acute inflammatory models: formaldehyde-induced paw edema, acetic acid-induced vascular permeability, subacute anti-inflammatory model: cotton pellet granuloma, estimation of plasma MDA and carrageenan-induced peritonitis. MEBV (100, 200 and 400 mg/kg, p.o) exhibited a dose-dependent and significant inhibition (P <0.01) in all the experimental models. Preliminary phytochemical screening revealed the presence of flavonoids, carbohydrates, glycosides, proteins, and alkaloids. The extract produces no mortality in the dose up to 2000 mg/kg, p.o. The results obtained suggest marked anti-inflammatory activity of the MEBV and support the traditional use of this plant in some painful and inflammatory conditions.

Key words: *Bambusa vulgaris,* poaceae, vascular permeability, granuloma, peritonitis, edema

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluids and blood cells. Though it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain, or aggravate many diseases.[1] However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use.[2] Hence, the development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary.

The leaves of *Bambusa vulgaris* have been used in Indian folk medicine to treat various inflammatory conditions. Other traditional uses are astringent, emmanogogue, vulnerary, and febrifuge to heal the wounds and also to control diarrhea in cattle[3]. Manna is a crystalline substance found inside the bamboo and leaves are used in ayurvedic medicine in ptosis and paralytic complaints.[3,4] Though the plant and its extracts have been used in the folklore medicine extensively, there is no scientific evidence for such activities available in established scientific journals of repute. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory potential of methanolic extract of *B. vulgaris* (MEBV) on formaldehyde-induced paw edema, acetic acid-induced vascular permeability, cotton pellet-induced granuloma, estimation of plasma MDA, and carrageenan-induced peritonitis experimental models.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *B. vulgaris* were collected at Kurnool, Andhra Pradesh, India. The leaves were taxonomically identified by Smt G. B. Rajya Lakshmi, Lecturer in Botany, K.V.R.Government Degree College for Women Kurnool, Andhra Pradesh, India. A voucher specimen (KU/UCPSc/17/2006) has been preserved in our laboratory.

Preparation of Plant Extraction

The collected leaves of *B. vulgaris* were shade dried and reduced to coarse powder using a mechanical grinder. The powdered material of the leaves was exhaustively extracted with methanol under the maceration process. The macerated mixture was filtered and evaporated to yield a green solid extract. The extract was stored in a desiccator and dilutions of the extract were made in 2% gum acacia for pharmacological studies.

Animals

Swiss albino mice of both sex weighing between 18 and 27 g and albino Wistar rats of either sex (180-200 g) were used for the present study. They were fed with standard, pellet diet, and water *ad libitum*. All animals were acclimatized for at least 1 week before the experimental session. All the experimental procedures were done following the

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guidelines of Institutional Animal Ethics Committee (IAEC).

Phytochemical Screening

The methanolic extract was screened for the presence of various phytoconstituents like steroids, alkaloids, tannins, flavonoids, and glycosides by employing standard phytochemical tests.[5]

Acute Toxicity Study

Acute oral toxicity study was performed in mice by following Organization for Economic Co-operation and Development (OECD) guidelines AOT No. 425.[6]

Chemicals and Drugs Used

Formaldehyde (S.D. Fine chemicals Ltd, Mumbai), acetic acid (Ranbaxy laboratories Ltd, Punjab), diclofenac sodium (Dr. Reddy Labs, Hyderabad), indomethacin (Sun Pharma, Mumbai), ibuprofen (Natco Pharma, Hyderabad), Evans blue (Sigma, St. Louis, MO, USA), gum acacia (Hi-media, Mumbai), and methanol (BDH, Mumbai). All other chemicals were of analytical grade and procured locally.

Formalin-induced Acute Inflammatory Model

Formalin 0.1 ml (2% in distilled water) was injected into sub plantar area of the left hind paw. The extract at doses of 100, 200, and 400 mg/kg or diclofenac sodium at a dose 10 mg/kg were given 1 hour prior to formalin injection.[7] The paw volume was determined by the plethysmographic method in order to measure the degree of inflammation as shown in Table 1.

Acetic Acid-induced Vascular Permeability Test

Whittle's method was used with some modifications.[8] In brief, male mice weighing 20-27 g were fasted for 10 hours prior to the experiment and were given the test doses, standard drug, and vehicle orally. Each animal was given an intravenous injection of 1% solution of Evans blue at a dose of 0.1 ml/10 g at 30 minutes after the oral treatment. The vascular permeability inducer, 0.1 ml/10 g of 0.6% acetic acid in saline, was injected intraperitoneally at 30 minutes after Evans blue injection. After 20 minutes, the mice were killed by dislocation of the neck and 10 ml of normal saline was injected intraperitoneally, after which the washing solution was collected in tubes and then centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatant was read at 610 nm with a spectrophotometer. The control group was treated similarly except that they received an oral dose of vehicle alone. The vascular permeability was expressed in terms of the amount of total dye (μg/ mouse) that was leaked into the intraperitoneal cavity.

Cotton Pellet-induced Granuloma

The cotton pellet-induced granuloma in rats was studied according to the method D'Arcy *et al*. (1960).[9] The animals were divided into five groups of six animals in each group. The rats were anaesthetized and sterile cotton pellets weighing 10 ± 1 mg were implanted subcutaneously into both sides of the groin region of each rat. Group 1 served as control and received the vehicle (2% gum acacia). The extract of *B. vulgaris* at the concentrations of 100, 200, and 400 mg/ kg body weight was administered orally to groups 2, 3, and 4, respectively for seven consecutive days from the day of cotton pellets implantation. Group 5 received the standard drug, indomethacin (10 mg/kg body weight) for the same period. On 8th day, the animals were anaesthetized and the pellets together with granuloma tissues were carefully removed and made free from extraneous tissues. The wet pellets were weighed and then dried in an oven at 60°C for 24 hours to constant weight, after that the dried pellets were weighed again. Increment in the dry weight of the pellets was taken as a measure of granuloma formation. The anti-proliferative effect of MEBV was compared with control.

Plasma MDA (malondialdehyde) Estimation

After 7 days of drug treatment in the cotton pellet-granuloma method, 3–5 ml of blood was collected from inner canthus of eye from each animal using capillary tube, in a vial containing EDTA as an anticoagulant. Plasma was separated by centrifugation at 3000 rpm for 10 minutes. It was stored at –20°C and used to estimate MDA levels. The reduced levels of MDA were taken as indicator of anti-lipoperoxidative activity which can be taken as the index of reduced oxidative stress.

Carrageenan-induced Peritonitis

Inflammation was induced by the modified method of Griswold *et al*. 1987.[10] Male Swiss albino mice weighing 20–25 g were divided into five groups (n = 6). Group 1

Table 1: Effect of the methanolic extract of *Bambusa vulgaris* leaves on formaldehyde-induced rat paw edema

Group	Dose (mg/kg)	Increase in paw volume (ml)					
		1 hour	2 hours	3 hours	4 hours	5 hours	24 hours
Control	—	0.305 ± 0.011	0.345 ± 0.00	0.374 ± 0.010	0.304 ± 0.004	0.230 ± 0.005	0.200 ± 0.003
Diclofenac sodium	10	0.208 ± 0.003**	0.221 ± 0.005**	0.203 ± 0.006**	0.209 ± 0.003**	0.142 ± 0.002**	0.110±0.003**
B. vulgaris	100	0.257 ± 0.012	0.257 ± 0.007**	0.246 ± 0.007**	0.221 ± 0.007**	0.208 ± 0.003*	0.179 ± 0.003**
B. vulgaris	200	0.246 ± 0.013**	0.243 ± 0.005**	0.209 ± 0.006**	0.209 ± 0.006**	0.184 ± 0.006**	0.131 ± 0.002**
B. vulgaris	400	0.203 ± 0.008**	0.210 ± 0.004**	0.202 ± 0.010**	0.217 ± 0.004**	0.174 ± 0.004**	0.117 ± 0.010**

Values are expressed as mean ± S.E.M. (n = 6). **Experimental groups were compared with control (P < 0.01).

served as control and treated with 2% gum acacia. Group 2 served as standard and was dosed with indomethacin (10 mg/kg, p.o.) and group 3–5 were administered with MEBV at the doses of 100, 200, 400 mg/kg, p.o., respectively. The standard drug and extract doses were administered orally 1 hour prior to the induction of peritonitis. After 1 hour, carrageenan (0.25 ml, 0.75% w/v in saline) was injected intraperitonially. Four hours later, the animals were sacrificed by cervical dislocation and 2 ml of Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS) was injected into the peritoneal cavity. Following a gentle massage, peritoneal exudates were removed. The total leukocyte count was determined in a Neubauer chamber and the differential cell count was determined.[11,12] The percentage of leukocyte inhibition was calculated using the following formula:

% of leukocyte inhibition (% L. I) = (1− T/C) × 100

Where 'T' represents the leukocyte count of the treated group and 'C' represents the leukocyte count of the treated control group.

Inhibition of neutrophil migration was calculated by the following equation:

Inhibition of neutrophil migration = 100 − {(N T/NC) × 100}

where NT = neutrophil counts of treated groups and NC = neutrophil counts of control groups.

Statistical analysis

The experimental results were expressed as the mean ± SEM. Data were assessed by the method of analysis of ANOVA followed by Dunnett's *t*-test. *P* value of <0.05 was considered statistically significant.

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of the plant extract revealed the presence of flavonoids, carbohydrates, glycosides, proteins, and alkaloids.

Test for Acute Toxicity

In the acute toxicity study, no mortality was observed during the 24-hour period at the doses tested and the animals showed no stereotypical symptoms associated with toxicity, such as convulsion, atoxia, diarrhea, or increased diuresis.

Anti-inflammatory Activity

Formaldehyde-induced rat paw edema

In the formaldehyde-induced paw edema method, the oral administration of MEBV in graded doses (100, 200, and 400 mg/kg) produced a significant reduction in paw volume in a dose-dependent manner in comparison to control. The maximum effect was seen in the oral dose of 400 mg/kg that showed a significant (P <0.01) reduction as 46% in paw volume in comparison to control. The anti-inflammatory activity in this dose of the test drug was comparable to diclofenac (10 mg/kg, p.o.,). The maximum anti-inflammatory effect was observed in 3 hours in all the doses of test drug.

Peritoneal capillary permeability test

The vascular permeability test is one of the acute inflammatory models. As shown in Table 2, the dye leakage induced by acetic acid was significantly inhibited by 34.1% and 39.5% in response to 400 mg/kg of MEBV and 10 mg/kg of indomethacin, respectively (compared with the control group). The anti-inflammatory activity of MEBV was less effective than the standard drug. However, the anti-inflammatory effect was statistically significant compared with the control group.

Cotton pellet granuloma

The effects of MEBV and indomethacin on the prolifeative phase of inflammation are summarized in Table 3. It was seen that MEBV was responsible for anti-inflammatory effect, which would be calculated depending on the moist

Table 2: Effect of the methanolic extract of *Bambusa vulgaris* leaves on acetic acid-induced vascular permeability in mice

Group	Dose (mg/kg)	Amount of dye leakage (OD) (%)	Inhibition
Control	-	1.38±0.035	
Indomethacin	10	0.835 ± 0.018**	39.5
B. vulgaris	100	1.078 ± 0.031**	21.9
B. vulgaris	200	1.02 ± 0.05**	26.1
B. vulgaris	400	0.91 ± 0.015**	34.1

Values are expressed as mean ± S.E.M. (n = 6). **Experimental groups were compared with control (P < 0.01).

Table 3: Effect of the methanolic extract of *Bambusa vulgaris* leaf on cotton pellet-induced granuloma in rats

Treatment	Dose (mg/kg)	Weight of cotton pellet (mg) (wet)	% Inhibition	Weight of cotton pellet (mg) (dry)	% Inhibition
Control	—	142.0 ± 1.91		41.5 ± 2.11	
Indomethacin	10	75.67 ± 1.75**	46.71	23.83 ± 0.95**	42.58
B.vulgaris	100	103.0 ± 2.24**	27.46	33.17 ± 1.42**	20.07
B.vulgaris	200	91.17 ± 1.83**	35.8	26.0 ± 1.18**	37.35
B.vulgaris	400	89.33 ± 3.48**	37.09	25.33 ± 1.76**	39.0

Values are expressed as mean ± S.E.M. (n = 6). **Experimental groups were compared with control (P < 0.01).

and dry weight of cotton pellets. According to these results, the antiproliferative effects of MEBV (400 mg/kg b.w.) and indomethacin (10 mg/kg b.w.) were calculated as 37.09% and 46.71 % (P <0.01), respectively. After they were dried, the antiproliferative effects were calculated on the basis of dry weight pellets; the inhibition of inflammation by MEBV and indomethacin was established as 37% and 42.58% (P < 0.01), respectively.

MDA levels in plasma

In oxidative stress model, MEBV (400 mg/kg x 7 days, orally) produced a significant (P <0.01) reduction in plasma MDA levels that was 37.75% in comparison to diseased control. However, standard drug reduced greater reduction of MDA levels (52.5%) as shown in Figure 1.

Carrageenan-induced pleurisy

The MEBV also inhibited peritoneal leukocyte migration at the rate of 38, 55.8, and 77.6% at the doses of 100, 200, and 400 mg/kg, respectively, whereas the inhibition produced by indomethacin (10 mg/kg) was found to be 60.7% in carrageenan-induced peritonitis model as shown in Table 4. The inhibition of neutrophils infiltration of MEBV was 32.7, 54.3, and 64.9%, respectively, whereas indomethacin shows 65.1%.

DISCUSSION

The development of edema in the rat paw after the injection of formalin is a biphasic event. The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin-like substance[13] and the second accelerating phase of swelling due to the release of prostaglandin-like substances. Inhibition of edema observed in the formalin model may be due to the ability of MEBV to inhibit these chemical mediators of inflammation.

Acetic acid-induced vascular permeability in a mouse model is a commonly used vascular permeability assay.[14] The inflammatory response is a physiological characteristic of vascularized tissue. [15] MEBV reduced the intensity of the peritoneal inflammation produced by acetic acid, indicating that it has the ability to inhibit the permeability of small blood vessels in the process of acute inflammation.

The cotton pellet granuloma method is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the cotton pellet correlates with the transuda; the dry weight of the pellet correlates with the amount of the granulomatous tissue.[16] Administration of MEBV (100, 200, and 400 mg/kg b.w.) and indomethacin (10 mg/kg b.w.) appears to be effective in inhibiting the moist weight of cotton pellet. On the other hand, the MEBV effect on the dry weight of the cotton pellet was almost near to that of indomethacin. These data support the hypothesis of the greater effect of the MEBV on the inflammation mediators in the immediate response of inflammation in rats. This effect may be due to the cellular migration to injured sites and accumulation of collagen.

The present study showed a significant reduction in MDA levels by MEBV. The oxidative stress is the condition where reactive oxygen species (ROS) generation exceeds endogenous antioxidant defense,[17] and it is well-known that in chronic and sub-acute inflammation, ROS play an important role in modulating the extent of inflammatory response and consequent tissue and cell injury.[18] MDA is a metabolic product of lipidperoxidation, the level of which is increased in oxidative stress. Therefore, reduction of oxidative stress by anti-lipoperoxidative activity might possibly be the mechanism of anti-inflammatory action of MEBV in the sub-acute inflammation model.

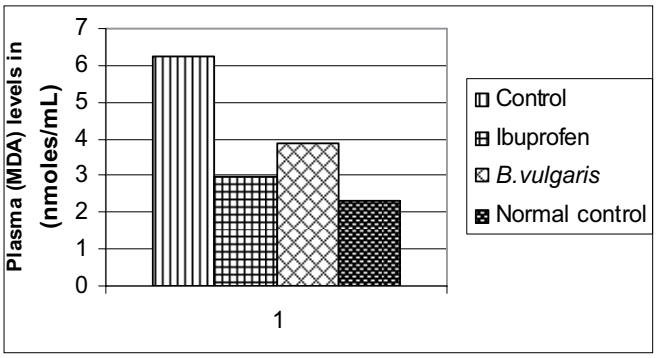

Figure 1: Effect of methanolic extract of *Bambusa vulgaris* leaves on oxidative stress by plasma estimation of malondialdehyde (MDA)

Table 4: Effect of methanolic extract of *Bambusa vulgaris* leaves on leukocytes migration and neutrophils migration in peritoneal exudation in carrageenan-induced mice

Group	Dose (mg/kg)	Leukocytes (10^5/mL)	Leukocyte inhibition	Neutrophils (10^5/mL)	% inhibition of neutrophil migration
Control		4.07±0.08	-	2.45±0.1	-
Indomethacin	10	1.6±0.14**	60.7	0.85±0.03**	65.3
B.vulgaris	100	2.53±0.03**	38.0	1.65±0.05**	32.7
B.vulgaris	200	1.8±0.09**	55.8	1.12±0.08**	54.3
B.vulgaris	400	0.91±0.04**	77.6	0.86±0.02**	64.9

Values are mean ± S.E.M. (n=6). **Experimental groups were compared with control (P < 0.01).

Intraperitonial injection of carrageenan leads to inflammation of the peritoneum resulting from macrophages in the carrageenan insulated tissue. Interleukin-1, a pro-inflammatory cytokine, induces accumulation of polymorphonuclear cells by a variety of processes including adhesion and cell mobility.[19] Leukocyte aggregation is a fundamental event during inflammation. Cell migration occurs as a result of much different process including adhesion and cell mobility.

The phytochemical analysis of the extract revealed that it contains flavonoids, carbohydrates, glycosides, proteins, and alkaloids. Of these, flavonoids and alkaloids are well known for their ability to inhibit pain and inflammation. Flavonoids also have anti-inflammatory properties due to their inhibitory effects on enzymes involved in the production of the chemical mediators of inflammation.[20] Finally, we may conclude that these results support the traditional use of this plant in some inflammatory and painful conditions and confirm the presence of active chemical compounds related to these activities.

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