

Studies on Anti-inflammatory, Analgesic and Antipyretic Properties of Methanol Extract of *Caesalpinia bonducella* leaves in Experimental Animal Models

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Received October 10, 2003; Revised November 12, 2003; Accepted November 13, 2003

This paper is available online at <http://ijpt.iuims.ac.ir>

ABSTRACT

The methanol extract of *Caesalpinia bonducella* leaves were investigated for anti-inflammatory, analgesic and antipyretic activity at the doses of 50, 100 and 200 mg/kg, body weight. The experimental paradigms used were carrageenan, dextran, histamine induced pedal edema and cotton pellet induced granuloma for anti-inflammatory activity, while hot plate and acetic acid induced writhing methods were used to assess analgesic activity. Yeast-induced hyperpyrexia was used to evaluate the antipyretic activity. In acute phase inflammation, a maximum inhibition 50.6% ($P < 0.05$), 51.1% ($P < 0.05$) and 52.3% ($P < 0.05$) was noted at the dose of 200 mg/kg after 3 h of treatment with methanol extract of *Caesalpinia bonducella* (MECB) in carrageenan, dextran and histamine induced pedal edema respectively. In the chronic model (cotton pellet induced granuloma) the MECB (200 mg/kg) and standard drug (Indomethacin 10 mg/kg) showed decreased formation of granuloma tissue by 51.8% ($P < 0.05$) and 56.6% ($P < 0.05$) respectively. The extract also produced significant ($P < 0.01$) analgesic activity in both paradigms. In addition, MECB potentiated the morphine and aspirin induced analgesia. A significant ($P < 0.01$) reduction in hyperpyrexia in rat was also produced by the extract. This study exhibits that the methanol extracts of leaves of *C. bonducella* possess anti-inflammatory, analgesic and antipyretic activities.

Keywords: *Caesalpinia bonducella*, Anti-inflammatory, Analgesic, Antipyretic

Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be induced, 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]. Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary.

Caesalpinia bonducella (L.) Roxb. Fever nut; bonduc nut (Family: Caesalpinaceae) commonly known as Nata Karanja (Hindi), is a prickly shrub found throughout the hotter regions of India, Myanmar and Sri Lanka [3]. The leaves of *C. bonducella* are traditionally used

for the treatment of inflammation and toothache [4]. The topical anti-inflammatory activity of *C. bonducella* leaves has been reported [5, 6]. It has also been found to possess multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial [7], anticonvulsant [8], anti-anaphylactic and antidiarrheal [9], antiviral [10], antiasthmatic [11], antiamebic and antiestrogenic [12]. Currently we have reported the hepatoprotective and antioxidant properties of this plant [13].

However, no work has been reported on the anti-inflammatory effects on acute and chronic phases of inflammation, analgesic and antipyretic activity of *C. bonducella*. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory, analgesic, and antipyretic potential of methanol extract of *Caesalpinia bonducella* (MECB) in experimental animal models.

MATERIALS AND METHODS

Plant material and extraction

The plant grows in all textures of mildly acid to alkaline soil. Annual rainfall in the areas where *C. bonducella* grows in Puerto Rico ranges from 750 mm to 1800 mm. The plants grow from sea level to 850 m in elevation in India. The plant *Caesalpinia bonducella* was collected from Kolli Hills of Tamilnadu, India. The plant material was taxonomically identified by the Botanical Survey of India, Kolkata, India. A Voucher specimen (No.GMS-2) has been preserved in our laboratory. The leaves were dried under shade and then powdered with a mechanical grinder and stored in an airtight container. The dried powder material of the leaves was extracted with methanol (Yield 8.78%), in a soxhlet apparatus. Phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. MECB was dissolved in 10% propylene glycol prior to administration.

Animals

Swiss albino mice of both sex weighing between (18-22 g) and Albino Wistar rats of the either sex (180-200 g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet supplied by Hindustan lever Ltd. Kolkata, India, and water *ad libitum*.

Chemicals and Drugs used

Carrageenan (S. D. Fine Chemicals Limited, Bombay), histamine (Sigma, USA), dextran (Sigma, USA) were used in the present study and indomethacin (Recon, Bangalore), aspirin (USV, Bombay), paracetamol (IPCA, Bombay), and morphine (M.M. Pharma, New Delhi) were used as the standard drugs.

Anti-inflammatory models

Carrageenan-induced paw oedema in rats. The rats were divided into 5 groups (n = 6). The different groups were treated with MECB (50, 100 and 200 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) and control vehicle per oral and the paw volume was measured at 0 h and 3 h after carrageenan injection using a plethysmometer [14]. The animals were pretreated with the extract 1 h before the administration of carrageenan. Acute inflammation was produced by the subplanter administration of 0.1 ml of (1%, w/v) carrageenan in normal saline in the right paw of the rats. The ratio of the anti-inflammatory effect of MECB was calculated by the following equation: anti-inflammatory activity (%) = $(1 - D / C) \times 100$, where D represents the percentage difference in paw volume after MECB was administered to the rats, and C represents the percentage difference of volume in the control groups [15].

Dextran-induced paw oedema in rats. The animals were treated in a manner similar to that of carrageenan induced paw oedema model; dextran (0.1 ml, 1% w/v in normal saline) was used in place of carrageenan [14].

Histamine-induced paw oedema in rats. The anti-inflammatory activity of the MECB was measured with histamine (phlogistic agents) which acts as mediator of inflammation. The paw oedema was induced in rats by subplanter injection of 0.1 ml (1% w/v) of freshly prepared histamine solution, and the paw oedema was measured [16].

Cotton pellets-induced granuloma. The rats were divided into five groups (n = 6). After shaving the fur, the rats were anaesthetized and 10 mg of sterile cotton pellets were inserted, one in each axilla. The MECB (50, 100 and 200 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) and control vehicle were administered orally for 7 consecutive days from the day of cotton pellet implantation. The animals were anaesthetized on the eighth day and cotton pellets were removed surgically and made free from extraneous tissues. The pellets were incubated at 37°C for 24 h and dried at 60°C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation [16].

Analgesic activity

MECB at the dose of 50, 100 and 200 mg/kg and combination of above doses of extract with the standard drug aspirin 100 mg/kg (Acetic acid induced writhing response in mice) and morphine 5 mg/kg (Hot plate reaction time in mice) were administered to eight groups of six mice in each paradigm.

Acetic acid-induced writhing test. Acetic acid solution (15 mg/ml) at the dose of 300 mg/kg body weight was injected (i.p.) and the number of writhes during the following 30 min period was observed [17]. A significant reduction in the number of writhes by drug treatment as compared to vehicle treated animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated. Aspirin (100 mg/kg, i.p.) was used as standard.

Hot plate reaction time in mice. Mice were screened by placing them on a hot plate maintained at $55 \pm 1^\circ\text{C}$ and the reaction time in seconds for hind paw licking or jumping were recorded [17]. Only mice which reacted within 15 sec and which did not show large variation when tested on four separated occasions, each 15 min apart, were used in this study. Morphine (5 mg/kg, i.p.) was used as standard. The time for hind paw licking or jumping on the heated plate of analgesimeter was taken as the reaction time.

Induction of Yeast-induced pyrexia

Rats were divided into five groups of six rats in each and were trained to remain quiet in a restraint cage. A thermister probe was inserted 3-4 cm deep into the rectum and fastened to the tail by an adhesive tape and the temperature was measured on a thermometer. The normal body temperature of each rat was measured rectally at predetermined intervals and recorded. Fever was induced by a subcutaneous injection of 20 ml/kg body wt. of 20% w/v yeast suspended in methyl cellulose solution [18]. Rats were then returned to their housing cage. After 24 h of yeast injection, the animals were again restrained in individual cages for the recording of their

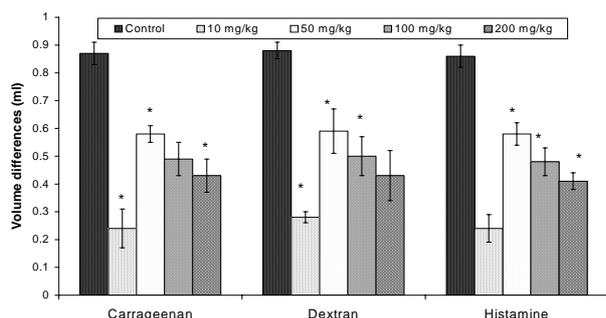


Fig 1. Effect of MECB and Indomethacin on Carrageenan, Dextran and Histamine induced rat paw oedema. Difference of means of edema volume (ml) between control and treatment values at different doses \pm S.E.M. Variation compared to the control animals. ANOVA followed by Student's t-test * $P < 0.05$.

rectal temperatures as described previously. Then MECB was administered orally at doses of 50, 100 and 200 mg/kg body wt. to three groups of animals respectively. 10% Propylene glycol (5ml/kg, body wt.) was administered orally to the control group of animals. The fifth group of animals received the standard drug paracetamol (150 mg/kg, body wt.) orally. Rats were restrained for recording of their rectal temperatures at intervals of one hour, after the drug administration.

Toxicity study

For toxicity studies the test compounds in the range of doses 100-1600 mg/kg were administered in five groups of 10 mice respectively. The mortality rates were observed after 72 hours. The LD_{50} was determined using the graphical methods of Litchfield and Wilcoxon [19].

Statistical analysis

The experimental results were expressed as the mean \pm S.E.M. Data were assessed by the method of analysis of ANOVA followed by student's t-test. P value of < 0.05 was considered as statistically significant.

RESULTS

Anti-inflammatory studies

The anti-inflammatory potential of MECB (50, 100 and 200 mg/kg) against various experimental animal models exhibited significant ($P < 0.05$) anti-inflammatory activity. The effects of MECB and indomethacin on the inflammation induced by carrageenan, dextran, histamine and cotton pellet induced granuloma are summarized in Fig 1 and Fig 2.

As shown in Fig 1, MECB showed maximum inhibition of 50.6% at the dose of 200 mg/kg after 3 h of treatment in carrageenan induced paw oedema, whereas the standard drug (Indomethacin 10 mg/kg) showed 72.4% of inhibition ($P < 0.05$). In case of dextran induced paw oedema, the MECB showed significant inhibition (33.0%, 43.2% and 51.1%) in a dose dependent manner as compared with control. The MECB showed 52.3% of inhibition at the dose of 200 mg/kg whereas indomethacin showed 72.1% of inhibition in histamine induced

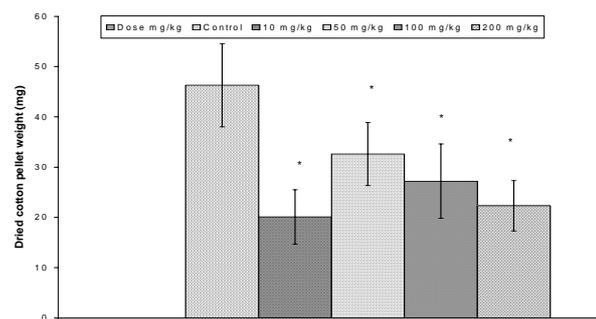


Fig 2. Effect of MECB and Indomethacin on the proliferative phase of inflammation in rats. Difference of means of dried cotton pellet weight (mg) between control and treatment values at different doses \pm S.E.M. Variation compared to the control animals. ANOVA followed by Student's t-test * $P < 0.05$.

paw oedema. In the chronic model (cotton pellet induced granuloma), the MECB (50, 100 and 200 mg/kg) and indomethacin showed decreased formation of granuloma tissue at 29.6%, 41.3%, 51.8% and 56.6% respectively (Fig 2).

The effect of MECB on acute (carrageenan, dextran and histamine) and chronic (cotton pellet induced granuloma) phase of inflammation, a maximum inhibition 69.9%, 74.9%, 72.5 and 91.5% respectively was noted at the dose of 200 mg/kg when compared with standard drug.

Acetic acid-induced writhing in mice

The results presented in Table 1, shows that MECB at the doses of 50, 100 and 200 mg/kg and aspirin (100 mg/kg) exhibited significant ($P < 0.01$) inhibition of the control writhes at the rate of 16.5%, 27.8%, 48.8% and 66.0% respectively when compared to that of control. In addition, MECB at the above mentioned doses, potentiated the analgesic activity of aspirin shown by further decreasing the writhing response when given in combination.

Hot plate reaction time in mice

As shown in Table 2, the MECB produced significant ($P < 0.01$) analgesic activity at all the tested doses when compared to the that of control. Additionally, MECB at different doses potentiated the analgesic activity of the standard drug (Morphine 5 mg/kg).

Yeast-induced hyperpyrexia

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with the MECB at the dose of 50, 100 and 200 mg/kg, body weight decreased the rectal temperature of the rat in a dose dependent manner. The antipyretic effect started from the first hour and was maintained for 4 h, after administration of the extract. The result obtained from both the standard and MECB treated rats were compared with the control group and a significant reduction in the yeast induced elevated rectal temperature was observed (Table 3).

Table 1. Effects of MECB and aspirin on writhing induced by acetic acid in mice.

Treatment	Dose (mg/kg)	Number of writhes (per 30 min) ^a	Inhibition (%)
Control	-	32.33±2.71	-
MECB	50	27.00±2.27 ^b	16.5
MECB	100	23.33±1.14 ^b	27.8
MECB	200	16.54±1.53	48.8
Aspirin	100	11.00±1.40 ^b	66.0
MECB+Aspirin	50+100	10.52±1.53	67.5
MECB+Aspirin	100+100	9.14±0.72 ^b	71.7
MECB+Aspirin	200+100	6.66±0.46 ^b	79.4

^a The results given are mean±S.E.M.; number of animal used (n=6)^b $P < 0.01$ Experimental groups were compared with control

Table 2. Effects of MECB and morphine on hot plate reaction time in mice.

Treatment	Dose (mg/kg)	Mean latent time ^a	
		Initial	After 30 min
Control	-	8.74±0.72	8.14±0.56
MECB	50	9.23±0.53 ^b	13.34±0.93
MECB	100	9.57±0.89 ^b	14.25±1.22
MECB	200	9.12±0.63	15.74±1.26
Morphine	5	9.74±0.87 ^b	19.17±1.33
MECB+Morphine	50+5	9.37±0.51 ^b	21.37±1.57
MECB+Morphine	100+5	8.83±0.32 ^b	23.68±1.32
MECB+Morphine	200+5	8.93±0.62	27.53±1.21

^a The results given are mean±S.E.M.; number of animal used (n=6)^b $P < 0.01$ Experimental groups were compared with control

Test for acute toxicity

The leaf extract was found to be non-toxic up to doses of 1.6 g/kg and did not cause any death of the animals tested.

DISCUSSION

The MECB was evaluated for its anti-inflammatory activity in acute and chronic models. A significant ($P < 0.05$) anti-inflammatory activity was observed for MECB in carrageenan, dextran, histamine induced oedema and cotton pellet-induced granuloma models.

Carrageenan-induced rat paw oedema has been used as an inflammation model in order to investigate the anti-inflammatory effect of drug [20]. There are two phases of carrageenan-induced inflammatory reaction: early or first phase and later or second phase. It has been proposed that early phase results from histamine, serotonin and bradykinin liberation while late phase is associated with the release of prostaglandin [21]. In carrageenan induced paw oedema the MECB showed maximum inhibition of 50.6% at the dose of 200 mg/kg after

3 h of drug treatment. Dextran induced paw oedema is known to be mediated both by histamine and serotonin. Dextran induces fluid accumulation, which contains little protein few neutrophils, where as carrageenan induces protein rich exudation containing large number of neutrophil [22]. The MECB also exhibited significant anti-inflammatory activity in dextran induced paw oedema. Histamine is one of the important inflammation mediators and it is a potent vasodilator substance and increases the vascular permeability [23, 24]. This study showed that all the doses of MECB effectively suppressed the oedema produced by the histamine, which indicates that the extract exhibit its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandin might be involved in inflammation.

From these results, it is suggested that anti-oedematogenic effects of the MECB on carrageenan, dextran and histamine induced oedema may be related to inhibition of inflammation mediator formation.

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation [25, 26]. Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or granuloma form [26]. Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation [27]. The MECB showed significant ($P < 0.05$) anti-inflammatory activity in cotton pellet induced granuloma and thus found to be a effective in chronic inflammatory condition.

In order to distinguish between the central and peripheral analgesic action of MECB, acetic acid induced writhing responses in mice were used to examine the effect. This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test, the animals react with characteristic stretching behavior, which is called writhing. It was found that MECB significantly ($P < 0.01$) inhibited the acetic acid induced writhing response and potentiated the analgesic activity of aspirin as well. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that MECB produced analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandins.

Table 3. The effect of MECB and paracetamol on yeast-induced pyrexia in rats.

Treatment	Rectal temperature (°C) ^a					
	After yeast injection at			After drug administration		
	0 h	24 h	1 h	2 h	3 h	4 h
Control Vehicle 5ml/kg	37.9±0.02	39.9±0.03	39.8±0.02	39.9±0.05	39.9±0.05	39.9±0.04
Paracetamol 150mg/kg	37.9±0.02 ^b	39.9±0.03 ^b	38.9±0.02	38.3±0.02 ^b	37.8±0.04 ^b	37.7±0.03
MECB 50mg/kg	37.7±0.04 ^b	39.8±0.03 ^b	39.5±0.02	38.7±0.02 ^b	38.4±0.04 ^b	38.1±0.06 ^b
MECB 100 mg/kg	37.6±0.02 ^b	39.7±0.04	39.3±0.05 ^b	38.5±0.05	38.1±0.03 ^b	37.8±0.06 ^b
MECB 200 mg/kg	37.7±0.07	39.8±0.06	39.7±0.02 ^b	38.2±0.03 ^b	37.7±0.05	37.6±0.04 ^b

^a The results given are mean±S.E.M.; number of animal used (n=6)^b $P < 0.01$ Experimental groups were compared with control

The hot plate method was originally described by Woolfe and Mac Donald [28]. This test has been found to be suitable for the evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance [29]. The present findings of the study indicate that the MECB may be centrally acting.

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are drugs, which reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. In fever this set point is elevated and a drug like paracetamol do not influence body temperature when it is elevated by factors such as exercise or increases in ambient temperature [30]. The present result show that the MECB possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats and its effect is comparable to that of paracetamol.

Based on the results of the present study it can be concluded that MECB has potential activity against both acute and chronic phases at a dose range of 50-200 mg/kg. b.w. Of the three doses the dose of 200 mg/kg is found to be more potent and efficacious towards the anti-inflammatory, analgesic and antipyretic activity, when compared with control and the activity is in dose dependent manner. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism(s) of action.

ACKNOWLEDGEMENT

One of the authors R. Sambath Kumar is grateful to AICTE, New Delhi, India, for providing financial support to this work.

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