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Anti-inflammatory and analgesic activity of *Barringtonia racemosa* Roxb. fruits

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The ethanolic extract of *Barringtonia racemosa* Roxb. (Lecythidaceae) fruits were screened for its anti-inflammatory and analgesic effects in experimental animals. The extract showed significant inhibition of carrageenan/formalin induced paw oedema at the three doses used in the study. The activity of the extract was comparable to that of Indomethacin, the standard anti-inflammatory drug. *B. racemosa* ethanolic extract also showed significant inhibition of acetic acid induced writhing in mice at 125, 250 and 500 mg/kg doses almost comparable to the standard analgesic drug, acetyl salicylic acid.

Keywords: *Barringtonia racemosa,* Anti-inflammatory, Analgesic, Indomethacin, Acetyl salicylic acid.

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Introduction

Plants have long provided mankind with medicine, with natural products once serving as the source of all drugs[1]. The rural population of the country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost[2]. Inflammatory and infectious diseases are among those treated using traditional medicine. Inflammation is the response of living tissues to injury; acute and chronic inflammations are a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair[3]. It is known that acute inflammatory response consists of three main vascular effects, namely vasodilation and consequent increased vascular flow; increased vascular permeability and leucocytes migration. Histamine and 5-Hydroxy tryptamine are usually responsible for eliciting the immediate response of inflammation in rats whereas kinins and prostaglandins mediate the more prolonged delayed onset responses[4]. Anti-inflammatory agents exert their effects through a spectrum of different modes of action[5].

Barringtonia racemosa Roxb. (Family-Lecythidaceae) is a moderate evergreen tree with drooping branches found on the west coast of India from Konkan southwards, the Sundarbans, Assam and the Andaman Islands[6] (Plate 1). The plant has a wide range of therapeutic applications. The roots have de-obstructant and cooling properties. The fruits are efficacious in cough, asthma and diarrhoea. The seeds are used in colic and opthalmia. The bark and the leaves are used for rat and snake bites, on boils and in gastric ulcer[7,8]. In certain remote areas of Kerala, these seeds are being used to treat cancer like diseases[9]. Secondary metabolites such as diterpenes, triterpenoids and flavonoids (including polyphenols), steroids and saponins have previously been isolated from *B. racemosa*[10]. The fruit kernels contain two sapogenins namely, barringtogenol and barringtogenic acid[11].

Materials and Methods

Plant material

B. racemosa Roxb. fruits were collected from Kannur, Kerala. It was authenticated by Dr N Mohanan, plant taxonomist of the Institute. A voucher specimen of the plant was deposited at the Herbarium of the Institute (TBGT 57038 dated 25/01/2009).

Preparation of plant extract

The fruits were washed, sliced to small pieces, shade dried and powdered. The powder (100 g) was extracted with 1000 ml 95% ethanol overnight, at room temperature with constant stirring. The filtrate was then concentrated and the solvent was evaporated

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Plate 1—*Barringtonia racemosa*

under reduced pressure in a rotary evaporator. The crude extract was referred to as BR. For oral administration BR was reconstituted in 0.5% Tween-80 to concentrations of 125 mg/kg, 250 mg/kg and 500 mg/kg.

Experimental animals

Wistar albino rats (150-250 g) and Swiss albino mice (20-30 g), of either sex, obtained from the Institute's Animal House were used for the studies. They were housed under standard laboratory conditions and were fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water, *ad libitum*. All animal experiments were carried out according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee (Registration No. 25-1/99/ AWD 176/ CPCSEA dtd 29/09/1999).

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

Oedema was induced in rats according to the method of Winter *et al* (1962)[12]. The animals were divided into five groups of six animals each and fasted overnight. Group 1, the carrageenan control group was administered orally, 1 ml 0.5% Tween-80, Group 2 received 1 ml Indomethacin (10 mg/kg, standard) in normal saline, while Groups 3, 4 and 5 received BR at various concentrations(125, 250 and 500 mg/kg), prepared in 0.5% Tween-80. After 30 min., BR administration and 0.1 ml of 1% carrageenan in normal saline (Sigma Chemicals Company, USA) was injected into the right hind paw under the plantar aponeurosis of all the animals. The hind paw volume was measured just before and 3 h after carrageenan injection using a plethysmometer. The difference in the paw volumes indicated the degree of inflammation. The anti-inflammatory activity of BR was estimated as the degree of oedema inhibition.

Formalin-induced paw oedema

Oedema was induced in rats according to the method of Winter *et al*[12]. Overnight fasted rats were divided into five groups of six animals each. Group 1 received 0.5% Tween-80, p.o. (formalin control).Group 2 received Indomethacin (10 mg/kg, standard) in saline, while Groups 3, 4 and 5 received BR extract at various concentrations (125, 250 and 500 mg/kg) reconstituted in 0.5% Tween-80, respectively. Formalin 20 µl of 1% was injected into the right hind paw, under the plantar aponeurosis of all the animals, 30 min after drug administration[13]. The hind paw volume was measured using a plethysmometer just before and 3 h after formalin injection. The difference in the paw volumes indicated the degree of inflammation. The anti-inflammatory activity of BR was estimated as the degree of oedema inhibition.

Analgesic activity

Acetic acid-induced writhing test in mice

Analgesia of BR was assessed by the writhing test in mice[14]. Mice were divided into five groups of six animals each. Group 1, acetic acid control group received p.o, a single dose of 0.5 ml of 0.5% Tween-80. Group 2 was administered p.o, a single dose of 1 ml standard (acetyl salicylic acid 25 mg/kg), as per Bose e*t al*[15]. Groups 3, 4, 5 received (p.o), a single dose of 0.5 ml of 125, 250 and 500 mg/kg BR, respectively. All groups received i. p., 0.5% aqueous solution of acetic acid (0.25 ml) 20 min post drug administration. The number of writhes (full extension of the hind limb) per animal was recorded during 20 min period beginning 5 min after the injection of acetic acid.

Anti-lipid peroxidation studies

Anti-lipid peroxidation effect of BR was studied *in vitro* following the modified method of Yoshiuki *et al* (1981)[16] and Masao *et al* (1993)[17]. Briefly, 2 g of rat liver tissue was sliced and homogenized with 150 m*M* KCl-Tris HCl buffer (*p*H 7.2). The reaction mixture was composed of 0.25 ml liver homogenate, Tris-HCl buffer (*p*H 7.2), 0.1 m*M* ascorbic acid (A.A), 0 4 m*M* $FeCl_2$ and 0.05 ml of various concentrations of BR extract (50, 100 and 150 µg/ml).

The mixture was incubated at 37ºC for 1 h in capped tubes. Then, 0.05 ml of 0.1N HCl, 0.2 ml of 9.8% sodium dodecyl sulphate (SDS), 0.9 ml distilled water and 2 ml of 0.6% thiobarbituric acid (TBA) were added to each tube and the tubes were vigorously shaken. The tubes were placed in a boiling water bath at 100ºC for 30 min. After cooling, 5 ml of butanol was added and centrifuged at 1500 rpm for 20 min. The supernatant was collected and the absorbance was measured at 532 nm.

Behavioural and toxic effects

Four groups of 10 mice each were administered p.o, 250, 500, 1000 and 1500 mg/kg of BR. The animals were observed continuously for 1 h for any gross behavioural changes, symptoms of toxicity and mortality, if any, and intermittently for the next 6 h and then again 24 h after dosing with BR.

Statistical analysis

The analysis was carried out using the Students't'-test (Snedecor and Cochran., 1980)[18]. Results were reported as mean ± S.D and the 't' test was used to evaluate difference between groups with $P \leq 0.01$, considered as significant.

Results

B. racemosa (BR) at the three doses used in the study (125, 250 and 500 mg/kg) significantly inhibited the carrageenan induced paw oedema. At 125 and 250 mg/kg doses, there was 66.6 and 70% inhibition and at 500 mg/kg dose, 75% inhibition was observed, at 3 h after carrageenan injection. Indomethacin (10 mg/kg) produced 78.33% inhibition of oedema (Table 1).

BR at the three doses used in the study significantly inhibited the formalin induced paw oedema in rats. At 125 and 250 mg/kg doses, there was 61.6% and 75% inhibition of oedema. At 500 mg/kg dose, 81.66% inhibition was obtained, at 3 hours after formalin injection. Indomethacin (10 mg/kg) produced 83.3% inhibition of oedema (Table 2).

Intraperitonial injection of acetic acid produced 73.5 ± 9.6 writhes in the control group. BR at all the three doses used in the study significantly inhibited acetic acid induced writhing response in mice, dose dependently. Writhing response inhibition at the doses 125 and 250 mg/kg were 68.02 and 79.5%, respectively. The extent of writhing response inhibition at 500 mg/kg dose was 91.8% which is comparable to acetyl salicylic acid, the positive control used in the study, with an inhibition of 93.1% (Table 3). BR at 150 µg/ml showed the highest percentage of inhibition of $FeCl_2$-AA stimulated rat liver lipid peroxidation *in vitro*. There was significant increase in malondialdehyde (MDA) in $FeCl_2$-AA treated rat liver homogenate, compared to the normal one without $FeCl_2$-AA (Table 4). BR was found to be effective in decreasing MDA production *in vitro* in rat liver homogenate treated with $FeCl_2$-AA treated mixture showing its anti-lipidperoxidant effects.

In the behavioural and toxicity studies, the mice did not show any gross behavioural changes and no mortality occurred within 24 h with the four doses of BR tested. The LD_{50} of BR was therefore greater than 1500 mg/kg.

Table 1—Effect of *B. racemosa* (BR) on carrageenan (CG)-induced paw oedema in rats

Treatment	Oral dose (mg/kg)	Difference in paw volume at 3 h (ml)	Percentage inhibition of oedema (%)
CG Control (0.5% Tween-80)	–	0.60	–
CG + Indomethacin	10	0.13 ± 0.05**	78.33
CG + BR	125	0.20 ± 0.10**	66.6
CG + BR	250	0.15 ± 0.04**	70.0
CG + BR	500	0.13 ± 0.05**	75.0

Values are the mean ± SD, n = 6; $^{**}P \leq 0.01$ compared to CG control.

Table 2—Effect of *B. racemosa* (BR) on formalin (FN)-induced paw oedema in rats

Treatment	Oral dose (mg/kg)	Difference in paw volume at 3 h (ml)	Percentage inhibition of oedema (%)
FN Control (0.5% Tween-80)	–	0.60 ± 0.06	–
FN + Indomethacin	10	0.10 ± 0.01**	83.3
FN + BR	125	0.23 ± 0.06**	61.6
FN + BR	250	0.15 ± 0.05**	75.0
FN + BR	500	0.11 ± 0.03**	81.7

Values are the mean ± SD, n = 6; $^{**}P \leq 0.01$ compared to FN control

Table 3—Effect of *B. racemosa* (BR) on acetic acid (AA)-induced writhing response in mice

Treatment	Oral Dose (mg/kg)	Mean number of writhes in 30 min	Percent inhibition of writhes
AA Control (0.5% Tween-80)	–	73.5 0± 9.6	–
AA + Acetyl salicylic acid	25	5.00 ± 3.3**	93.19
AA + BR	125	23.50 ± 3.7**	68.02
AA + BR	250	15.00 ± 0.65**	79.50
AA + BR	500	7.00 ± 3.3**	91.80

Values are the mean ± SD, n=6; $^{**}P \leq 0.01$ compared to AA control

Table 4—Inhibitory effect of *B. racemosa* (BR) on $FeCl_2$-ascorbic acid (AA) –induced lipid peroxidation in rat liver homogenate *in vitro*

Group	Concentration (µg/ml)	MDA	MDA inhibition (%)
Normal control	–	0.98 ± 0.01	–
$FeCl_2$-AA control	–	2.42 ± 0.09	–
$FeCl_2$-AA + BR	50	0.82 ± 0.03**	66.1
$FeCl_2$-AA + BR	100	0.66 ± 0.05**	72.7
$FeCl_2$-AA + BR	150	0.60 ± 0.07**	75.2

Values are the mean ± SD, n=3; $^{**}P \leq 0.01$ compared to $FeCl_2$-AA control

Discussion

In the present study, ethanolic extract of *B. racemosa* Roxb. fruits showed significant dose dependent anti-inflammatory and analgesic activity. Carrageenan-induced inflammation is useful to detect orally active anti-inflammatory agents[19]. It is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandins and slow reacting substances that peak at 3 h[20]. The carrageenan paw oedema test produces a non-specific inflammation that results from the sequential action of several mediators. Development of oedema induced by carrageenan is commonly correlated with the early exudative stage of inflammation, one of the important processes of the inflammatory pathology[21]. BR showed maximum inhibition of inflammation (75%) at the dose of 500 mg/kg after 3 h of drug treatment. This is almost equal to that produced by the standard (Indomethacin, 10 mg/kg).

The mechanism of the anti-inflammatory effect of BR in formalin-induced paw oedema in rats may depend on the neutralization of active globulins which are non-steroidal anti-inflammatories[22]. It is well established that the non-steroidal anti-inflammatory drugs exert their effect by the inhibition of prostaglandin synthesis[23]. After 3 h, 125, 250 and 500 mg/kg doses of BR showed 61.6%, 75.0% and 81.7% inhibition, respectively against formalin-induced paw oedema.

A large number of herbal drugs used in the indigenous system of medicine possess a variety of actions on the central nervous system. Acetic acid induced writhing test is widely used method for the evaluation of peripheral antinociceptive activity[24]. Acetic acid is an irritating agent which stimulates the local peritoneal receptors to induce pain with characteristic abdominal constrictions when injected into the peritoneal cavity[25]. In the present investigation, the analgesic activity of the ethanol extract of BR was demonstrated by acetic acid induced writhing in mice. In this model, BR extract markedly reduced the number of mouse abdominal constrictions at all the three doses used in the study. Hence, it can be concluded that the BR extract showed a dose dependent inhibition of acetic acid induced writhing in mice.

$FeCl_2$-ascorbic acid mixture is known to stimulate lipid peroxidation in microsomes and mitochondria of rat liver *in vitro*[17]. It is evident that non-enzymatic lipid peroxidation occurs during experimental inflammation in rats. Lipid peroxides may be pro-inflammatory and can damage the tissues directly[26]. Reactive oxygen species and free radicals are involved in a variety of pathological events. A potential mechanism of oxidative damage is the nitration of tyrosine residues of proteins, peroxidation of lipids and degeneration of DNA and oligonucleotide fragments[27]. Any compound, natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of damage caused by nitric oxide, reactive nitrogen species or by superoxides[28]. In the present study, BR prevented the rise in lipid peroxides (MDA

production) showing its significant anti-lipid peroxidant effect.

The anti-inflammatory and analgesic effects exhibited by BR in the present study may be due to the presence in BR of the sapogenins namely, barringtogenol and barringtogenic acid[11]. Saponins and sapogenins are known to cause significant anti-inflammatory and analgesic effects[29,30]. Further, studies are on in our laboratory to decipher the exact nature of the phytocompounds responsible for the anti inflammatory and analgesic effects of BR and its mechanism of action.

Conclusion

The present study indicated that *B. racemosa* fruits have potent anti-inflammatory and analgesic action and therefore, it can be used for the development of a safe herbal drug for anti-inflammatory and analgesic conditions

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