International Journal of Phytomedicine 5 (2013) 350-355
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Anti-inflammatory studies of *Barringtonia acutangula* (Linn) fruits on wistar rats.

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Abstract

The study aims to evaluate the anti inflammatory activity of *Barringtonia acutangula* (Linn) fruit extracts in wistar rats. In this study fruits of *Barringtonia acutangula* were extracted with ethanol and purified water, these extracts were subjected to preliminary phytochemical analysis to identify their phytoconstituents. The ethanol and aqueous extracts were evaluated *in vivo* by using acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton-pellet induced granuloma and carrageenan induced air-pouch model in rats. The biochemical parameters like reduced glutathione (GSH), lipid peroxidation and catalase were also estimated as supportive studies. Acute toxicity studies were performed initially in order to ascertain the safety of ethanol and aqueous extracts. The ethanol extract reduced the inflammation more significantly than the aqueous extract in the carrageenan-induced rat paw oedema, cotton-pellet induced granuloma and carrageenan induced air-pouch model in rats. The phytochemical investigation of the ethanol fruit extract showed the presence of phytosterols, glycosides, flavonoids, alkaloids and carbohydrates. From the present study the ethanolic fruit extract of *Barringtonia acutangula* exhibited the anti-inflammatory effect by augmenting antioxidant defense system in the inflammation bearing rat, which is largely attributable to the additive or synergistic effect of its constituents.

Keywords: *Barringtonia acutangula,* Anti inflammatory activity; Antioxidants.

Introduction

Inflammation is typically a protective mechanism that is triggered in response to noxious stimuli, trauma or infection to guard the body and to hasten-up the recovery process. However, inflammation that is unchecked leads to chronic inflammatory disorders. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury [1]. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation. Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [2]. Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Although non steroidal anti-inflammatory drugs are the most commonly prescribed drugs in the world, their use as anti-inflammatory, antipyretic, antithrombotic and analgesic agents continues to be principally limited by their undesired side effects [3]. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

Barringtonia acutangula (Family: Lecythidaceae) an evergreen tree of moderate size is called as Hijja or Hijjala in Sanskrit. The fruit is spoken of as samudra-phala and various parts of this plant are used as a folklore medicine for curing various ailments like hemiplegia, pain in joints, eye diseases, stomach disorders, anthelmintic, diarrhoea, cough, dyspnoea, leprosy, intermittent fever, and spleenic disorders [4]. An aqueous extract of the bark is reported to be used in pneumonia, diarrhea, asthma and leaf juice is given for diarrhea [5]. Fruit is bitter, acrid, anthelmintic, emetic, expectorant and vulnerary. It is prescribed in gingivitis, as an astringent and tonic. Whole plant was reported to possess flavonols, phenolic acids, triterpenoids, tannins and steroidal compounds such as barringtogenic acid, tangulic acid and acutangulic acids. The fruit possessed saponins based on barringtogenol B, C and D [6]. The therapeutic potential of this plant were reported to be antitumor, antibiotic and it is reported to inhibit growth of Helicobacter pylori [7-9]. As there is no reference in literature to the anti-inflammatory studies of *Barringtonia acutangula* fruits it was considered worthwhile to evaluate the anti-inflammatory activity of *Barringtonia acutangula* fruits in animal models to validate its use in traditional medicine for the said activity.

Materials and Methods

Plant material

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The fruits of *Barringtonia acutangula* (Linn) were collected during January 2012 from Thalakona forest of Tirumala, Chittore district, Andhrapradesh state, India. The samples were authenticated by Dr. Madhava chetty, department of Botany, S.V. University College, Tirupati, India. A herbarium specimen has been deposited at the college for further reference.

Preparation of plant extracts

The fruits were dried in the shed and coarsely powdered. The powder was extracted with ethanol in a soxhlet apparatus for 72 h. The ethanol extract was evaporated in vacuo giving the residue (24%). Similarly the air dried fruits of *Barringtonia acutangula* were extracted with double distilled water by means of maceration. The extract was filtered, concentrated, and freeze dried to obtain crude aqueous extract with a yield of 10% (w/w).

Preliminary phytochemical analysis

The ethanol and aqueous extracts were subjected to Phytochemical screening according to the phytochemical methods described by Harborne [10].

Experimental animals

Adult Wistar strain rats (150 to 200 gm) were used for all the experiments in the present study. The animals were maintained under standard husbandry conditions in the animal house of the institute (temperature 25 ± 2 C) in a natural light-dark cycle and fed with standard rodent diet and water *ad libitum*. Ethical committee clearance was obtained from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No./IAEC/XII/01/SIPS/2011-2012).

Acute toxicity studies

The acute toxicity of ethanolic and aqueous extracts of *Barringtonia acutangula* fruits were determined as per the OECD guideline no. 423 (Acute toxic class method) [11]. Based on the results obtained from this study, the dose for anti inflammatory activity was fixed to be 200 mg kg^{-1} b.w. and 400 mg kg^{-1} b.w. for dose dependent study.

Air pouch model of inflammation

Adult Wistar strain rats (150 to 200 gm) were used for all the experiments in the present study. Air-pouch was produced according to the method described by Salvemini *et al* [12]. Briefly, rats were anesthetized and air cavities were produced by subcutaneous injection of 20 mL of sterile air into the intrascapular area of the back (that is, 0 day). An additional 10 mL of air was injected into the cavity every 3rd day (3rd and 6th day) to keep the space open. On the 7th day, 2 mL of 1% solution of carrageenan dissolved in saline was injected directly into the pouch to induce an inflammatory response. The rats were orally pre-treated with either vehicle or test substances or diclofenac sodium 2 h prior to the injection of carrageenan. The second dose of treatment was repeated after 24 h of the first treatment. 48 h after carrageenan injection, the rats were anesthetized with ether and the pouch was carefully opened by a small incision. The volume of exudates was collected and measured. An aliquot of the exudates was used for quantification of leukocyte concentration using a haemocytometer and differential cell count was performed using a manual cell counter after staining with Wright's stain. The results were expressed as the total number of neutrophils and monocytes.

Carrageenan induced rat hind paw edema

To study the anti inflammatory activity of *Barringtonia acutangula* fruit extracts against carrageenan induced rat hind paw edema, the method of Winter *et al* was followed [13]. The animals were divided into four groups of six animals each. One group served as a standard (Diclofenac sodium) and another group served as control (1% CMC) and rest of the groups were used for the test substances. The animals pretreated with test substances or diclofenac sodium one hour before were injected with 0.05 mL of 1% carrageenan (in 1% CMC) solution into the sub-plantar region of right hind paw. The volume of the injected paw was measured with a plethysmograph immediately. The paw volume was again measured after 3 h. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response and the percentage inhibition of oedema was calculated using the formula (1).

Inhibition (%) = (1- Vt / Vc) x 100 (1)

Where Vt is Mean volume of the test drug and Vc is Mean volume of the control

Biochemical estimations

Biochemical changes in carrageenan induced paw oedema were estimated. The rats were anaesthetized under light ether anaesthesia and Liver was removed and subjected for homogenization and aliquots of the homogenate were suitably processed for the assessment of reduced glutathione (GSH), Catalase and lipidperoxidation. GSH was estimated by the method of Moran *et al* [14], Catalase activity was assayed according to the method of Cohen *et al* [15] and lipid peroxidation by the method of Ohkawa *et al* [16]. The % inhibition of lipid peroxidation by the test or standard drug was calculated by using following formula (2).

[(A-B)/B] x 100 (2)

Where A is Control group and B is Test or Standard group.

Cotton pellet-induced granuloma

The test was performed on the rats using the cotton pellet induced granuloma method described by Winter *et al* [13]. The rats were anesthetized under light ether and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 ± 1 mg) was inserted in the groin area. All the animals received either test substances or diclofenac sodium or vehicle (1% CMC) orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion. On the 8th day, animals were anesthetized again and cotton pellets were removed and dried to constant mass.

Statistical analysis

The experimental results were expressed as mean ± S.E.M. Results were analyzed by the one- way ANOVA followed by Tukey-kramer post hoc multiple comparison test using Graph pad InStat version 3.00. P value of <0.05 was considered as statistically significant.

Results

Phytochemical screening

The preliminary phytochemical screening showed the presence of various Phytoconstituents in the ethanol and aqueous extracts which are listed in Table 1.

Table 1: Phytochemical screening of ethanol and aqueous extracts.

S.No.	Components	Ethanol	Aqueous
1	Alkaloids	+	-
2	Carbohydrates	+	+
3	Phytosterols	+	-
4	Fixed oils and fats	-	+
5	Saponins	-	-
6	Phenolic compounds tannins		-
7	Proteins and amino acids	-	-
8	Gums and Mucilages	-	-
9	Volatile Oil	-	-
10	Flavanoids	+	-
11	Glycosides	+	+

+ denotes the presence of the respective class of compounds.
- denotes the absence of the respective class of compounds.

Air pouch model of inflammation

The effect of ethanol and aqueous fruit extracts of *Barringtonia acutangula* in carrageenan induced air pouch model of inflammation in rats is shown in Table 2. The ethanolic fruit extract of *Barringtonia acutangula* significantly reduced the carrageenan induced inflammation in the air pouch than the aqueous extract. The ethanol extract dose-dependently elicited significant ($P < 0.05$) reduction in exudate volume and infiltration of neutrophils and monocytes into the air-pouch compared to control group. Diclofenac sodium at a dose of 100 mg kg^{-1} b.w. also showed significant ($P < 0.05$) result.

Cotton pellet-induced granuloma

The ethanol and aqueous extracts of *Barringtonia acutangula* fruits were screened for cotton pellet induced granuloma in rats and the results are shown in Table 3. The ethanol extract exhibited 16.51% and 29.98% inhibition of granuloma formation at the doses 200 and 400 mg kg^{-1} b.w respectively and the aqueous extract exhibited 6.20% and 16.10% inhibition of granuloma formation at the doses 200 and 400 mg kg^{-1} b.w respectively, whereas diclofenac sodium showed 54.36% when compared to control group.

Carrageenan induced rat hind paw oedema

The effect of ethanol and aqueous extracts of *Barringtonia acutangula* in carrageenan induced paw oedema in rats is shown in Table 4. The result obtained indicates that the ethanolic extract found to have significant ($P < 0.05$) anti-inflammatory activity in rats than the aqueous extract. The ethanol extract at the test doses 200 and 400 mg kg^{-1} b.w. reduced the oedema induced by carrageenan by 60.95% and 71.57% respectively at 3 h and the aqueous extract reduced the oedema by 33.68% and 43.49% respectively, whereas the diclofenac sodium at a dose 100 mg kg^{-1} b.w. showed 91.73% of inhibition as compared to the control group.

Table 2: Effect of ethanol and aqueous extracts of *Barringtonia acutangula* fruits on leucocyte infiltration and exudate volume in carrageenan induced air pouch inflammation.

Groups	Dose (mg kg^{-1})	Excudate volume (mL)	Neutrophils (x 10^6 cells)	Monocytes (x 10^6 cells)
Control	1 % CMC	4.65±0.159	362.13±2.45	149.25±1.56
Standard	100	1.78±0.053***	92.25±0.85***	42.55±1.98***
ETEX200	200	3.79±0.092***	323.23±2.12***	136.833±0.71***
ETEX400	400	3.25±0.121***	234.32±1.64***	124.1666±1.29***
AQEX200	200	4.45±0.093	358.95±1.96	146.54±0.89
AQEX400	400	4.15±0.035*	352.16±1.35**	141.43±0.99**

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanol extract 200 mg kg^{-1} b.w. ETEX 400: Ethanol extract 400 mg kg^{-1} b.w., AQEX200: Aqueous extract 200 mg kg^{-1} b.w, AQEX400: Aqueous extract 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats *P<0.05; **P < 0.01; ***P < 0.001 compared with control.

Table 3: Effect of ethanol and aqueous extracts of *Barringtonia acutangula* fruits on cotton-pellet induced granuloma in rats.

Groups	Dose (mg kg^{-1})	Granuloma dry weight (mg)	Inhibition
Control	1 % CMC	70.456±0.23	-
Standard	100	32.153±0.199***	54.36
ETEX200	200	58.825±0.15***	16.51
ETEX400	400	49.335±0.19***	29.98
AQEX200	200	66.0883±0.14	6.20
AQEX400	400	59.1133±0.21	16.10

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanol extract 200 mg kg^{-1} b.w. ETEX 400: Ethanol extract 400 mg kg^{-1} b.w., AQEX200: Aqueous extract 200 mg kg^{-1} b.w, AQEX400: Aqueous extract 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats.
**P < 0.01; ***P < 0.001 compared with control.

Table 4: Effect of ethanol and aqueous extracts of *Barringtonia acutangula* fruits on carragenan induced paw oedema in rats.

Groups	Dose (mg kg^{-1})	Mean oedema volume 0-3h	% Inhibition
Control	1 % CMC	0.985±0.01	-
Standard	100	0.0815± 0.0024***	91.73
ETEX200	200	0.3846±0.0194***	60.95
ETEX400	400	0.28±0.0257***	71.57
AQEX200	200	0.6533±0.03229	33.68
AQEX400	400	0.5566±0.0492	43.49

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanol extract 200 mg kg^{-1} b.w. ETEX 400: Ethanol extract 400 mg kg^{-1} b.w., AQEX200: Aqueous extract 200 mg kg^{-1} b.w, AQEX400: Aqueous extract 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats.
* P<0.05, **P < 0.01; ***P < 0.001 compared with control.

Biochemical estimations

The results of biochemical changes in carrageenan induced rat paw oedema are shown in Table 5. Treatment with ethanol and aqueous extracts of *Barringtonia acutangula* fruits decreased the levels of lipid peroxidation and increased the levels of GSH and catalase. The results obtained with ethanol extract were found to be more significant (P < 0.05) than the aqueous extract as compared to control groups.

Table 5: Effect of ethanol and aqueous extracts of *Barringtonia acutangula* fruits on various biochemical changes in carrageenan induced rat paw oedema.

Groups	*Dose (mg/kg)*	*GSH (ng mg-1 protein)*	*Lipid peroxidation (%)*	*Catalase (µg mg-1 protein)*
Control	1 % CMC	3.65±0.06	98.15±1.29	28.50±0.48
Standard	100	4.62±0.09***	59.35±1.89***	40.05±0.27***
ETEX200	200	4.34±0.02**	84.85±2.53**	30.92±0.32***
ETEX400	400	4.56±0.12***	65.28±3.35***	36.50±0.26***
AQEX200	200	3.92±0.24	89.08±2.85	28.22±0.17
AQEX400	400	4.05±0.09	79.95±1.22***	29.66±0.19

Standard: Diclofenac sodium (100mg kg^{-1} b.w.), ETEX200: Ethanol extract 200 mg kg^{-1} b.w. ETEX 400: Ethanol extract 400 mg kg^{-1} b.w., AQEX200: Aqueous extract 200 mg kg^{-1} b.w, AQEX400: Aqueous extract 400 mg kg^{-1} b.w. Each value is the Mean ± S.E.M for 6 rats.
* P<0.05, **P < 0.01; ***P < 0.001 compared with control.

Discussion

The results of the present investigations revealed that the ethanol extract of *Barringtonia acutangula* possess significant anti-inflammatory activity against acute inflammatory models like; carrageenan induced paw oedema and chronic models like; cotton-pellet induced granuloma and carrageenan induced air-pouch model in rats in a dose dependent manner. In spite of tremendous development in the field of synthetic drugs during recent era, they are found to have some or other side effects, whereas plants still

hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents. The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthesis) to prostaglandins, which are major components that induce pain and inflammation [17, 18].

It is well known that carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3–4 h after carrageenan injection), kinin and prostaglandins are involved [19]. Our results revealed that administration of ethanolic fruit extract inhibited the oedema starting from the first hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

The cotton-pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of the pellets correlates with transuda, the dry weight of the pellet correlates with the amount of granulumatous tissues [20]. Chronic inflammation occurs by means of the development of proliferate cells. These cells can be either spread or in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma which results from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fibers and suppressing mucopolysaccharides [21, 22]. The ethanolic extract of *Barringtonia acutangula* fruits showed significant anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

In order to assess the efficacy of the extract against proliferative phase of inflammation, we selected carrageenan- induced air-pouch model in which tissue degradation and fibrosis occurs. During the repair process of inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels occurs, which are the basic sources of forming a highly vascularised reddish mass, termed granulation tissue [23, 24]. Thus, in this model the extract significantly reduced infiltration of macrophages, monocytes, neutrophils and others. These results indicate that the ethanol extract of *Barringtonia acutangula* fruits may alter the action of endogenous factors that are involved in the migration of these substances to the site of inflammation.

From the above studies it is quite apparent that the ethanol extract of *Barringtonia acutangula* fruits possesses significant anti-inflammatory activity by augmenting antioxidant defense system in the inflammation bearing rat.

Conclusion

The ethanolic fruit extract of *Barringtonia acutangula* showed anti-inflammatory property similar to those observed for non-steroidal anti-inflammatory drugs. The anti-inflammatory activity of ethanolic fruit extract is largely attributable to the presence of alkaloids, carbohydrates, phytosterols, flavonoids and glycosides. The present investigation offers scientific evidence to the folkloric accounts of the use of fruits.

Acknowledgement

The author thanks to the Department of animal sciences, University of Hyderabad, Hyderabad for providing the facilities necessary to carry out the work.

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