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Full Length Research Paper

Evaluation of anti-inflammatory activity of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) in experimental animals

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Borassus flabellifer **L. (Arecaceae) had been widely used for its reported biological activities in indigenous system of medicine. The present investigation was carried out to find the effect of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* for its anti-inflammatory activity in rodents. The anti-inflammatory activity was evaluated 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 serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), lipid per oxidation and alkaline phosphatase (ALP) were also estimated as supportive studies. Oral administration of the extract at the doses 150 and 300 mg/kg b.w. exhibited dose dependent and significant anti-inflammatory activity in acute (carrageenan- induced hind paw oedema, $p < 0.0001$) and chronic (cotton pellet granuloma and carrageenan-induced air-pouch models, $p < 0.0001$) of inflammation. The extract also showed significant ($p < 0.0001$) results for biochemical parameters. Hence, present investigation established some pharmacological evidences to support the folklore claim that *B. flabellifer* L. is used as anti-inflammatory agent.**

Key words: *Borassus flabellifer* L., inflorescences, male flowers, anti-inflammatory, ethanolic extract, SGOT, SGPT, lipid per oxidation, ALP.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflamemation (Mitchell and Cotran, 2000). 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 (Ialenti et al., 1995). Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflamemation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation (Di and Willoughby, 1971) whereas prostaglandins are detectable in the late phase of inflammation (Salvemini et al., 1996).

Drugs which are in use presently for the management of pain and inflammatory conditions are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs possess well known side and toxic effects. Moreover, synthetic drugs are very expensive to develop and whose cost of development ranges from 0.5 to 5 million dollars. On the contrary many medicines of plant origin had been used since long time without any adverse effects. Exploring the healing power of plants is an ancient concept. For centuries people have been trying to alleviate and treat dis-

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ease with different plant extracts and formulations (Cowan, 1999). It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper drugs. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (Ahmad et al., 1992). Screening of the plants for their biological activity is done on the basis of either their chemotaxonomic investigation or ethnobotanical knowledge for a particular disease. Identification of a particular compound against a specific disease is a challenging long process. Importance of the plant lies in their biologically active principles. There are two types of plant chemicals, primary metabolites such as sugars, proteins, amino acids, chlorophylls etc. The other category of chemicals is called secondary metabolites, which includes alkaloids, terpenoids, saponins and phenolic compounds. These chemicals exert a significant physiological effect on the mammalian system. A lot of references are available in the field of ethnomedicinal plants used as anti-inflammatory drugs. Bagul et al. (2005) have reported the antiinflammatory activity of two ayurvedic formulations containing 'guggul'. Bhattacharya et al. (2005) have reported anti-inflammatory potential of methanol extract of *Stepenia glabra* of Menispermaceae family. The extract depicted anti–inflammatory activity at the dose of 150 mg/kg body weight. Ammar et al. (1997) have revealed the anti-inflammatory activity of bioactive fractions isolated from seeds of *Trigonella foenum gracium* L., roots of *Glycyrrhiza glabra* L. and fruits of *Coriandrum sativum* L.

Borassus flabellifer L. (Arecaceae) is a tall palm attaining a height of about 30 m, with a black stem and crown of leaves at the top; leaves are 0.9 - 1.5 m in diameter, palmately fan shaped, petiole edges with hard horny spinescent serratures; flowers unisexual, male spadix branched, female spadix simple; fruits large, subglobose drupes, on the greatly enlarged perianth. The plant has been used traditionally as a stimulant, anti-laprotic, diuretic, antiphlogistic. The fruit is stomachic, sedative, laxative and aphrodisiac in nature. The roots and juice of the plant are useful in inflammatory reactions (Vaidyaratnam, 1994; Nadkarni, 1954; Kapoor, 2000). It has been reported that the methanolic extract from the male flowers of *B. flabellifer* was found to inhibit the increase of serum glucose levels in sucrose-loaded rats which may be due to presence of spirostane-type steroid saponins (Yoshikawa et al., 2007). It also has been documented to possess immunosuppressant property (Révész, 1999).

As there is no reference in literature to the anti-inflammatory aspects, it was considered worthwhile to study the anti-inflammatory activity of ethanolic extract of male flowers (inflorescences) of *B. flabellifer* in rodents.

MATERIAL AND METHODS

Plant material

The male flowers (inflorescences) of *B. flabellifer* L. (Arecaceae) were collected from various parts of Uttar Kannada district, Karnataka during November to December and were authenticated from Mr. Shivanand Bhat, Department of Botany, Government Arts and Science College, Karwar, Karnataka, India. The selected parts of the plant were then dried in shade at temperature between 21 - 30°C for 15 to 30 days, after which these parts were chopped and ground. Finally extraction was carried out by the following procedure.

Preparation of the extract

The powdered crude drug of male flowers (800 g) was subjected for extraction process by maceration with 90% ethanol at room temperature for 7 days. The extract was filtered and concentrated to dryness at room temperature to avoid the decomposition of natural metabolites. The yield was found to be approximately 5.18% w/w.

Chemicals

All the drugs used in this study were of pharmaceutical grade. Carrageenan was supplied by Sigma Chemicals Company, St. Louis, USA. SGOT, SGPT and ALP standard kits were procured from Span Diagnostics, Surat, India. Pure Diclofenac Sodium was gifted by Dr. Reddy's Laboratories, Hyderabad, India.

Experimental animals

Swiss albino mice (25 – 30 g) and Wister albino rats (180 – 210 g) of either sex were used in the study. They were procured from Venkateshwara Enterprises, Bangalore, Karnataka, India. They were randomly distributed into groups and housed in cages (6 per cage) and maintained under standard conditions at 26 ± 2°C and relative humidity 44 – 56% and 10 h light: 14 h dark cycles each day for one week before and during the experiments. All animals were fed the standard rodent pellet diet (Amrut, India) and water *ad libitum*. This project was cleared by Institutional Animal Ethical Committee.

Acute toxicity studies

Swiss albino mice of either sex (18 – 22 g weight) were used for acute oral toxicity study. The study was carried out as per the guidelines set by OECD and no adverse effects or mortality were detected in the mice up to 4 g/kg, p.o., during the 24 h observation period. Based on the results obtained from this study, the dose for anti-inflammatory activity was fixed to be 150 mg/kg b.w. and 300 mg/kg for dose dependent study.

Anti-inflammatory activity

The animals were divided into four groups (n = 6). Group I served as Control, received the vehicle only (1% Carboxymethylcellulose, CMC, 10 ml/kg p.o.). Group II served as Standard, received Diclofenac Sodium at dose of 100 mg/kg b.w. Group III and IV served as test, received ethanolic extract at doses of 150 and 300 mg/kg b.w. p.o. respectively.

Carrageenan induced paw oedema

The test was used to determine the anti-inflammatory activity of the extract by the method of Winter et al. (1962). The animals pretreated with extract or diclofenac sodium one hour before were injected with 0.1 ml of 1% carrageenan (in 1% CMC) solution into

Table 1. Effect of ethanololic extract of *Borassus flabellifer* male flowers on carrageenan induced rat paw oedema.

Groups	Dose (mg/kg)	Paw Volume (ml)				
		0 h	1 h	3 h	6 h	12 h
Control	1% CMC	1.205±0.009916	1.730±0.03759	2.302±0.01138	2.060±0.01571	1.637±0.02028
Standard	100	1.192±0.02212	1.590±0.03550[a]	1.212±0.01537[c]	1.135±0.01544[c]	1.173±0.009888[c]
Alc 150	150	1.198±0.007491	1.638±0.02651	1.525±0.01648[c]	1.498±0.007032[c]	1.507±0.01926[c]
Alc 300	300	1.185±0.02487	1.640±0.04074	1.352±0.01621[c]	1.288±0.007923[c]	1.267±0.01256[c]

Standard: Diclofenac sodium (100 mg/kg b.w.), Alc 150: Ethanolic extract at dose 150 mg/kg b.w., Alc 300: Ethanolic extract at dose 300 mg/kg b.w. Each value is the Mean ± S.E.M. for 6 rats [a]P < 0.05; [b]P < 0.01; [c]P < 0.0001 compared with control.

the sub-plantar region of right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) immediately after carrageenan application at 0, 1, 3, 6 and 12 h after the stimulus. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

Cotton pellet-induced granuloma

The test was performed on the rats using the cotton pellet induced granuloma method. 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 extract or diclofenac sodium or vehicle (1% CMC) orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion (Winter et al., 1962). On the 8th day, animals were anesthetized again and cotton pellets were removed and dried to constant mass.

Carrageenan induced air-pouch model

The rats were divided into four groups (n = 6). Air-pouch was produced according to the method described by Salvemini et al. (1996). 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 extract 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 exudate 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.

Biochemical estimations

In earlier experiments, especially carrageenan and histamine induced paw oedema; the biochemical changes observed were maximum at 6 h as compared to 12 and 24 h. Hence, biochemical changes in carrageenan induced paw oedema were estimated at 6 h only. Where as in case of cotton-pellet induced granuloma biochemical changes were estimated on 8th day.

The rats were anaesthetized under light ether anaesthesia and blood samples were collected by retro-orbital plexus route for biochemical estimation. Serum was separated and SGOT, SGPT, ALP were determined by the colorimetric method (Reitmen and Frankel, 1957; Woessner, 1961) using standard kits.

Liver was removed and subjected for homogenization to measure liver per oxidation by the method of Ohkawa et al. (1979). The % inhibition of lipid per oxidation by the test or standard drug was calculated by using following formula;

[(A-B)/B] x 100

Where; A: Control group, B: Test or Standard group

Statistical analysis

Results are expressed as Mean ± S.E.M. The difference between experimental groups was compared by One-way Analysis of Variance (ANOVA) followed by Dunnett's test. The results were considered statistically significant when P < 0.0001.

RESULTS

The ethanolic extract of *B. flabellifer* male inflorescences was evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in Table 1, 2 and 3. The ethanolic extract on carrageenan induced paw oedema in rats is shown in Table 1. The result obtained indicates that the extract found to have significant (P < 0.0001) anti-inflammatory activity in rats. The extract at the test doses 150 and 300 mg/kg b.w. reduced the oedema induced by carrageenan by 33.75 and 41.26% respectively at 3 h, whereas the standard drug showed 47.35% of inhibition as compared to the control group (results not shown).

The ethanolic extract was screened for cotton pellet-induced granuloma in rats and the results are shown in Table 2. The extract exhibited 32.81 and 41.70% inhibition of granuloma formation at the doses 150 and 300 mg/kg b.w respectively, whereas diclofenac sodium showed 53.64% when compared to control.

The activity of the extract on carrageenan-induced air-pouch in rat is shown in Table 3. The extract dose-dependently elicited significant (P < 0.0001) reduction in excudate volume and infiltration of neutrophils and monocytes into the air-pouch compared to control group. Diclofenac sodium at a dose of 100 mg/kg b.w. also showed signifi-

Table 2. Effect of ethanololic extract of *Borassus flabellifer* male flowers on cotton-pellet granuloma in rats.

Groups	Dose (mg/kg b.w.)	Granuloma dry weight (mg)	% inhibition
Control	1% CMC	55.03±1.029	--
Standard	100	25.51±1.154^{c}	53.64
Alc 150	150	36.97±1.543^{c}	32.81
Alc 300	300	32.08±0.9478^{c}	41.70

Standard: Diclofenac sodium (100 mg/kg b.w.), Alc 150: Ethanolic extract at dose 150 mg/kg b.w., Alc 300: Ethanolic extract at dose 300 mg/kg b.w. Each value is the Mean ± S.E.M. for 6 rats $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ compared with control.

Table 3. Effect of ethanololic extract of *Borassus flabellifer* male flowers on leukocyte infiltration and excudate volume in carrageenan-induced air-pouch inflammation.

Groups	Dose (mg/kg)	Excudate volume (ml)	Neutrophils (x 10^{6} cells)	Monocytes(x 10^{6} cells)
Control	1% CMC	3.987±0.1228	287.5±10.69	123.9±2.423
Standard	100	0.8850±0.06845^{c}	104.2±3.899^{c}	59.66±3.385^{c}
Alc 150	150	2.048±0.1039^{c}	179.9±4.755^{c}	93.61±4.121^{c}
Alc 300	300	1.338±0.08089^{c}	136.7±4.979^{c}	74.71±2.944^{c}

Standard: Diclofenac sodium (100 mg/kg b.w.), Alc 150: Ethanolic extract at dose 150 mg/kg b.w., Alc 300: Ethanolic extract at dose 300 mg/kg b.w. Each value is the Mean ± S.E.M. for 6 rats $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ compared with control.

Table 4. Effect of ethanololic extract of *Borassus flabellifer* male flowers on various biochemical changes in carrageenan-induced paw oedema in rats.

Groups	Dose (mg/kg)	SGOT (U/ml)	SGPT (U/ml)	Lipid peroxidation	Alkaline Phosphate (U/ml)
Control	1% CMC	115.7±5.777	80.83±3.092	100	84.67±1.542
Standard	100	68.00±2.251^{c}	51.00±1.770^{c}	63.00±1.238^{c}	61.50±1.335^{c}
Alc 150	150	90.33±1.745^{c}	65.17±1.537^{c}	82.83±1.014^{c}	74.17±1.249^{c}
Alc 300	300	80.83±1.167^{c}	57.50±1.258^{c}	71.67±0.8819^{c}	69.50±0.4282^{c}

Standard: Diclofenac sodium (100 mg/kg b.w.), Alc 150: Ethanolic extract at dose 150 mg/kg b.w., Alc 300: Ethanolic extract at dose 300 mg/kg b.w. Each value is the Mean ± S.E.M. for 6 rats $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ compared with control.

Table 5. Effect of ethanololic extract of *Borassus flabellifer* male flowers on various biochemical changes in cotton-pellet induced granuloma in rats.

Groups	Dose (mg/kg)	SGOT (U/ml)	SGPT (U/ml)	Lipid peroxidation (%)	Alkaline Phosphate (U/ml)
Control	1% CMC	105.7±1.667	67.67±1.256	100.0	85.00±1.461
Standard	100	69.83±1.195^{c}	28.33±1.542^{c}	66.17±1.815^{c}	53.83±1.400^{c}
Alc 150	150	87.17±1.905^{c}	56.33±1.308^{c}	85.50±1.432^{c}	69.67±1.820^{c}
Alc 300	300	77.67±1.145^{c}	46.33±1.626^{c}	74.33±1.542^{c}	60.83±1.167^{c}

Standard: Diclofenac sodium (100 mg/kg b.w.), Alc 150: Ethanolic extract at dose 150 mg/kg b.w., Alc 300: Ethanolic extract at dose 300 mg/kg b.w. Each value is the Mean ± S.E.M. for 6 rats $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.0001$ compared with control.

ficant ($P < 0.0001$) result.

The results of biochemical changes in carrageenan-induced rat paw oedema and cotton-pellet induced granuloma is shown in Table 4 and 5. There was significant ($P < 0.0001$) decrease in the levels of SGPT, SGOT, ALP and Lipid peroxidation is seen in all the models as compared to their respective control groups.

DISCUSSION

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 polymerphonuclear 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 (Higgs et al., 1984; Vane, 1971).

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 (Hernandez et al., 2002). Our results revealed that administration of ethanolic 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 (Lowry et al., 1958; Castro et al. 1968). Chronic inflame-mation 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 (Della et al., 1968; Alcaraz and Jimenez, 1988). The ethanolic extract of *B. flabellifer* male inflorescences 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 (Bhattacharya et al., 1992; Swingle, 1974). Thus, in this model the extract significantly reduced infiltration of macrophages, monocytes, neutrophils and others. These results indicate that the extract may alter the action of endogenous factors that are involved in the migration of these substances to the site of inflammation.

There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation (Anderson et al., 1971; Shen, 1967; Weissmann, 1967; Jannoff and Zweifach, 1964). Most of the anti-inflammatory drugs exert their beneficial effects by inhibiting either release of these enzymes or by stabilizing lysosomal membrane, which is one of the major events responsible for the inflammatory process (Nair et al., 1988). So, we can assume that our drug extract might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane.

From the above studies it is quite apparent that the ethanolic extract possesses significant anti-inflammatory activity. The study justifies its use in inflammation as suggested in the folklore medicines.

Conclusion

Thus, it can be concluded that the ethanolic extract of male flowers (inflorescences) of *B. flabellifer* possess anti-inflammatory activity. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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