

# Influence of ethanolic extract of *Borassus flabellifer* L. male flowers (inflorescences) on chemically induced acute- inflammation and poly arthritis in rats

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**ABSTRACT :** The present study was designed to investigate anti-inflammatory and antiarthritic activities of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L (Arecaceae). Nystatin-induced rat paw edema model was employed to investigate the anti-inflammatory activity and Freund's Complete Adjuvant (FCA) induced poly arthritis was used to screen antiarthritic potential of the extract. Various haematological and biochemical parameters like hydroxyproline, hexosamine, total protein content, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), lipid peroxidation and alkaline phosphatase (ALP) were also estimated as supportive studies. The extract at doses 200mg/kg b.w. and 400mg/kg b.w. and diclofenac sodium (standard) at 100mg/kg b.w. showed significant anti-inflammatory and antiarthritic activity, as compared to control ( $p < 0.0001$ ). The extract and standard drug also showed significant ( $p < 0.0001$ ) results for haematological and biochemical parameters. The results of the present study further confirm the use of *Borassus flabellifer* L. traditionally for the treatment of painful inflammatory conditions and in arthritic pain.

**Key words:** *Borassus flabellifer*, anti-inflammatory, antiarthritic, FCA, haematological & biochemical Parameters.

## INTRODUCTION

Rheumatoid arthritis (RA) is a kind of chronic inflammatory autoimmune disease<sup>1</sup>. Although a number of drugs (non-steroidal or steroidal anti-inflammatory agents and immunosuppressants) used in the treatment of RA have been developed over the past few decades, there is still an urgent need for more effective drugs with lower side effects<sup>2</sup>. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in body weight gain; during the acute period, hind paw and fore paw joint diameters increase<sup>3</sup>.

*Borassus flabellifer* L. (Arecaceae) is a tall palm found in hotter parts of India, wild as well as cultivated in most parts of India. It is a tall tree attaining a height of about 30m, with a black stem and crown of leaves at the top; leaves are 0.9-1.5m 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 fruits are stomachic, sedative, laxative and aphrodisiac in nature useful in hyperdipsia, dyspepsia, flatulence, skin diseases, haemorrhages, fever and general debility. The roots, leaves, toddy, inflorescence and juice of the plant are useful in bleeding, oedema and inflammatory reactions<sup>4-6</sup>. It has been reported that the methanolic extract from the male flowers of *Borassus 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<sup>7</sup>. It also has been documented to possess immunosuppressant property<sup>8</sup>.

Herbal medicines derived from the plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about

their mode of action is available. A bibliographic survey showed that there are no reports on the antiarthritic and anti-pyretic activity of *Borassus flabellifer*. This prompted us to investigate the effects of pharmacological activities of *Borassus flabellifer* in experimental models of the same.

## EXPERIMENTAL

### Plant Material

The male flowers (inflorescences) of *Borassus 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 (800g) 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. Nystatin was supplied by Nicholas Piramal, New Delhi, Freund's Complete Adjuvant (FCA) (Diclo Labs, USA), Hydroxyproline (Spectrochem Pvt. Ltd., Mumbai, India), Glucosamine (Sisco Labs, Mumbai, India), Pure Diclofenac Sodium was gifted by Dr. Reddy's Laboratories, Hyderabad, India.

### Experimental Animals

Swiss Albino Mice (25-30g) and Wister Albino Rats (180-210g) 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  $26 \pm 2^\circ\text{C}$  and relative humidity 44–56% and 10h light: 14h 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 (18-22g 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 4g/kg, p.o., during the 24h observation period. Based on the results obtained from this study, the dose for anti-

inflammatory activity was fixed to be 200mg/kg b.w. and 400mg/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, 10ml/kg p.o.). Group II served as Standard, received Diclofenac Sodium at dose of 100mg/kg b.w. Group III and IV served as test, received ethanolic extract at doses of 200mg/kg and 400mg/kg b.w. p.o. respectively.

### 1. Nystatin-induced inflammation in Rat paw<sup>9</sup>

The animals pretreated with extract or diclofenac sodium one hour before were injected with 0.1 ml of 6% suspension of Nystatin subcutaneously under plantar neurosis in right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) immediately after Nystatin application at 2, 4, 6, 24, 48, 72hr after the stimulus. Nystatin induces inflammation reaction by labilising the lysosomal membrane. Reduction in the paw volume compared to the vehicle-treated control animals was considered as anti-inflammatory response.

## ANTIARTHRITIC ACTIVITY

### 1. Freund's Adjuvant induced Poly arthritis<sup>10</sup>

Rats were injected, 0.1ml of FCA into the subplantar region of the right hind paw. Paw volume was measured by dislocation of the water column in a Plethysmometer (Ugo Basile, Italy) on day 0 (before administration of FCA) and at every 5 days during the treatment period ending on day 21. All the animals received either extract or diclofenac sodium or vehicle (1% CMC) orally depending upon their respective grouping for 21 consecutive days from the day of FCA injection. On 21<sup>st</sup> day, rats were anaesthetized using diethyl ether and oedematous tissues were isolated from the injected hind paw and were assayed for hydroxyproline<sup>11</sup>, hexosamine<sup>12-13</sup> and total protein content<sup>14</sup>. Blood was withdrawn from retro-orbital plexus of all the groups and various haematological and biochemical parameters were estimated.

## RESULTS

Rheumatoid arthritis is a chronic inflammatory disease affecting about 1% of the population in developed countries<sup>15</sup>. *Borassus flabellifer* has shown the persuasive protective effect against the acute and chronic inflammation induced various chemicals is here investigated for the protective effect against chronic inflammation and arthritis induced by CFA. The acute stage of arthritis is characterized by signs of hyperalgesia, lack of mobility and pause in body weight gain; during the acute period, hind paw and fore paw joint diameters increase<sup>16</sup>.

The result of ethanolic extract of *Borassus flabellifer* male inflorescences against nystatin-induced

paw oedema is shown in Table 1. The result shows that the extract at both the doses (200mg/kg b.w. and 400mg/kg b.w.) gave the significant ( $P<0.0001$ ) reduction of rat paw oedema from 2h to 72h when assessed at different time intervals. Diclofenac sodium, a COX-inhibitor at the dose of 100 mg/kg, also significantly ( $P<0.0001$ ) reduced the paw oedema.

The results of biochemical changes in nystatin-induced rat paw oedema are shown in Table 2. There was significant ( $P<0.0001$ ) decreased levels of SGPT, SGOT, ALP and Lipid peroxidation at either dose as compared to control.

The mean change in paw swelling was about  $1.90\pm0.02$  in the FCA induced control group on 21<sup>st</sup> day. *Borassus flabellifer* significantly ( $P < 0.0001$ ) reduced the mean change in paw swelling at 21<sup>st</sup> day evaluation and was found to be  $1.48\pm0.01$  and  $1.42\pm0.00$  in a dose dependent manner at 200 and 400 mg/kg b.w. respectively. However, the standard drug diclofenac sodium exhibited significant ( $1.32\pm0.02$ ,  $P < 0.0001$ ) protection as compared with the control group (Table 3).

In FCA induced arthritis, hematological and biochemical changes were investigated in blood and edematous tissue at the end of 21<sup>st</sup> day. The elevated levels viz. hydroxy proline, hexosamine and total protein observed in edematous tissue during FCA injection were significantly ( $P < 0.0001$ ) inhibited by the extract at both the doses and diclofenac sodium treatment. Diclofenac sodium (100mg/kg b.w.) treatment prevented both hematological as well as biochemical changes in blood and edematous tissue to a greater extent than extract (Table 4).

## 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 and arthritis so as to exploit them as herbal anti-inflammatory and antiarthritic agents.

The rat adjuvant arthritis is the most frequently used chronic inflammatory model<sup>17-18</sup> used in the screening of NSAIDs, steroids and immunosuppressive drugs<sup>19</sup>. The major limitation of this model is its inability to identify disease modifying antirheumatic drugs<sup>20</sup>.

In adjuvant arthritis, bacterial peptidoglycan and muramyl dipeptide are responsible for its induction<sup>18, 20</sup>. It occurs through cell mediated-autoimmunity by structural mimicry between mycobacteria and cartilage

proteoglycans in rats<sup>21</sup>. The anti-inflammatory effect of Diclofenac (an NSAID) is mediated chiefly through inhibition of COX and prostaglandin production<sup>22</sup>. Dexamethasone (a steroid anti-inflammatory drug) exerts anti-inflammatory effects through profound inhibitory effects on peripheral leucocytes and its suppressive effects on the inflammatory cytokines and on other lipid and glucolipid mediators of inflammation. This is achieved through interaction with specific receptor proteins in target tissues to regulate the expression of corticosteroid-responsive genes, thereby changing the levels and array of proteins synthesized by the various target tissues<sup>23</sup>. Hence, this could be the possible mechanism involved in the anti-inflammatory and antiarthritic activities of the ethanolic extract of *Borassus flabellifer*.

There is increasing evidence that lysosomal enzymes play an important role in the development of acute and chronic inflammation<sup>24-27</sup>. 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<sup>27</sup>. So, we can assume that our drug extract might be acting by either inhibiting the lysosomal enzymes or stabilizing the membrane.

In Freund's Adjuvant Arthritic rat model, treatment with ethanolic extract of *Borassus flabellifer* showed significant inhibitory effect on injected hind paw edema and maximum inhibition was observed on the 21<sup>st</sup> day (Table3). In the present study the increased lymphocyte count and migration of leucocytes into inflamed area of arthritic rats were significantly prevented with the treatment of the ethanolic extract of *Borassus flabellifer* and the standard drug as reflected from the significant decrease in total WBC count<sup>26</sup>. The erythrocyte sedimentation rate (ESR) level which was markedly elevated in arthritic control group of rats was decreased significantly with ethanolic extract of *Borassus flabellifer* at higher dose (400mg/kg) and the effect was comparable to standard drug (Table 4). The increased hexosamine in treatment with *Borassus flabellifer* contributes towards its defensive property in treatment of pain and inflammation.

## CONCLUSION

This report clearly showed that ethanolic extract of fruits of *Borassus flabellifer* significantly inhibited the adjuvant-induced arthritis, and had preferable chronic anti-inflammatory along with antinociceptive effect with the long-time administration.

**Table1: Effect of ethanolic extract of *Borassus flabellifer* male flowers on nystatin-induced rat paw oedema**

Groups	Dose (mg/kg)	Mean change in Paw Volume (ml)					
		2h	4h	6h	24h	48h	72h
Control	1%CMC	2.63±0.06	2.88±0.04	2.65±0.02	2.17±0.02	2.01±0.03	1.93±0.02
Standard	100	1.51±0.04 <sup>c</sup>	1.33±0.02 <sup>c</sup>	1.24±0.01 <sup>c</sup>	1.30±0.02 <sup>c</sup>	1.18±0.01 <sup>c</sup>	1.03±0.01 <sup>c</sup>
Alc 200	200	2.35±0.05 <sup>b</sup>	2.21±0.03 <sup>c</sup>	2.11±0.03 <sup>c</sup>	2.21±0.02	2.10±0.02 <sup>a</sup>	1.96±0.02
Alc 400	400	1.79±0.04 <sup>c</sup>	1.63±0.02 <sup>c</sup>	1.51±0.01 <sup>c</sup>	1.68±0.03 <sup>c</sup>	1.5±0.01 <sup>c</sup>	1.38±0.01 <sup>c</sup>

Standard: Diclofenac sodium (100mg/kg b.w.), Alc 200: Ethanolic extract at dose 200mg/kg b.w.,

Alc 400: Ethanolic extract at dose 400mg/kg b.w.

Each value is the Mean ± S.E.M. for 6 rats

<sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.0001 compared with control**Table 2: Effect of ethanolic extract of *Borassus flabellifer* male flowers on various biochemical changes in nystatin-induced paw oedema in rats**

Groups	Dose (mg/kg)	SGOT (U/ml)	SGPT (U/ml)	Lipid peroxidation	Alkaline Phosphate (U/ml)
Control	1% CMC	99.17±3.4	58.17±1.77	100	80.00±2.20
Standard	100	58.33±2.18 <sup>c</sup>	22.33±1.28 <sup>c</sup>	48.50±1.47 <sup>c</sup>	48.50±1.47 <sup>c</sup>
Alc 200	200	83.83±1.85 <sup>c</sup>	42.17±1.13 <sup>c</sup>	78.83±1.77 <sup>c</sup>	65.67±1.22 <sup>c</sup>
Alc 400	400	73.17±1.92 <sup>c</sup>	29.17±1.16 <sup>c</sup>	66.50±1.58 <sup>c</sup>	54.50±1.33 <sup>c</sup>

Standard: Diclofenac sodium (100mg/kg b.w.), Alc 200: Ethanolic extract at dose 200mg/kg b.w.,

Alc 400: Ethanolic extract at dose 400mg/kg b.w.

Each value is the Mean ± S.E.M. for 6 rats

<sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.0001 compared with control**Table 3: Effect of ethanolic extract of *Borassus flabellifer* male flowers on FCA induced poly arthritis in rats**

Groups	Dose (mg/kg)	Mean change in Paw Volume (ml)				
		Day 0	Day 5	Day 10	Day 15	Day 21
Control	1% CMC	1.07±0.02	1.43±0.02	1.76±0.02	1.88±0.02	1.90±0.02
Standard	100	1.02±0.02	1.23±0.03 <sup>c</sup>	1.34±0.02 <sup>c</sup>	1.39±0.03 <sup>c</sup>	1.31±0.02 <sup>c</sup>
Alc 200	200	1.02±0.02	1.36±0.02	1.49±0.02 <sup>c</sup>	1.53±0.01 <sup>c</sup>	1.48±0.01 <sup>c</sup>
Alc 400	400	1.02±0.03	1.29±0.02 <sup>b</sup>	1.41±0.02 <sup>c</sup>	1.46±0.01 <sup>c</sup>	1.42±0.00 <sup>c</sup>

Standard: Diclofenac sodium (100mg/kg b.w.), Alc 200: Ethanolic extract at dose 200mg/kg b.w.,

Alc 400: Ethanolic extract at dose 400mg/kg b.w.

Each value is the Mean ± S.E.M. for 6 rats

<sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.0001 compared with control

**Table 4: Effect of ethanololic extract of *Borassus flabellifer* male flowers on various haematological and biochemical parameters in FCA induced poly arthritis in rats**

Parameter	Control	Standard	Alc 200	Alc 400
<b>ESR (mm)</b>	8.25±0.3	1.62±0.15 <sup>c</sup>	6.50±0.18 <sup>c</sup>	4.83±0.27 <sup>c</sup>
<b>Total WBC (cmm)</b>	10409±249.7	5951±150.6 <sup>c</sup>	8973±107.8 <sup>c</sup>	7990±76.3 <sup>c</sup>
<b>Lymphocytes (%)</b>	67.83±1.4	30.17±1.9 <sup>c</sup>	56.17±1.7 <sup>c</sup>	45.17±1.6 <sup>c</sup>
<b>Neutrophils (%)</b>	60.83±1.7	69.67±2.2 <sup>a</sup>	84.33±2.3 <sup>c</sup>	81.00±2.2 <sup>c</sup>
<b>Hb (%)</b>	79.17±1.9	77.50±2.2	82.17±2.5	81.17±1.6
<b>RBC (million/cmm)</b>	4.15±0.10	4.66±0.12 <sup>b</sup>	3.95±0.07	3.90±0.11
<b>Total Protein (g%)</b>	8.98±0.27	4.36±0.18 <sup>c</sup>	7.08±0.12 <sup>c</sup>	5.65±0.16 <sup>c</sup>
<b>Hydroxyproline (µg/g)</b>	450.0±23.23	130.8±7.52 <sup>c</sup>	306.3±9.45 <sup>c</sup>	203.8±9.84 <sup>c</sup>
<b>Hexosamine (µg/g)</b>	1573±91.47	536.8±37.25 <sup>c</sup>	1025±36.38 <sup>c</sup>	870.5±65.13 <sup>c</sup>

Standard: Diclofenac sodium (100mg/kg b.w.), Alc 200: Ethanolic extract at dose 200mg/kg b.w.,

Alc 400: Ethanolic extract at dose 400mg/kg b.w.

Each value is the Mean ± S.E.M. for 6 rats

<sup>a</sup>P < 0.05; <sup>b</sup>P < 0.01; <sup>c</sup>P < 0.0001 compared with control**REFERENCES**

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