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Research Article

EVALUATION OF THE ANALGESIC AND ANTIPYRETIC ACTIVITIES OF ETHANOLIC EXTRACT OF MALE FLOWERS (INFLORESCENCES) OF *BORASSUS FLABELLIFER* L. (ARECACEAE)

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ABSTRACT

Analgesic and antipyretic effects of ethanolic extract of male flowers (inflorescences) of *Borassus flabellifer* L. (Arecaceae) were investigated at doses 150mg/kg b.w. and 300mg/kg b.w. using acetic-acid induced writhing, hot-plate, tail-clip, formalin and yeast-induced pyrexia tests. Oral administration *Borassus flabellifer* ethanolic extract (BFEE) produced significant ($P<0.0001$) reduction in no. of writhes induced by acetic-acid. Moreover, in hot-plate test, BFEE significantly ($P<0.0001$) raised the pain threshold at different time of observation (0-60min) in comparison with control. In tail-clip test also the extract caused a significant ($P<0.0001$) inhibition of pain at both the doses used. There was a significant dose-dependent inhibition of both phases of the formalin induced pain response in mice. Tested on yeast-induced pyrexia in rats, BFEE significantly ($P<0.0001$) reversed hyperthermia at either dose. The results of pharmacological tests performed in the present study suggest that BFEE possesses potent analgesic and antipyretic effects.

Key words: *Borassus flabellifer*, Male flowers (inflorescences), Analgesic activity, Antipyretic activity

INTRODUCTION

Pain is an unpleasant sensation no doubt, but on the whole it is usually beneficial to man (or animal). It is mainly a protective mechanism for the body, occurs whenever any tissues are being damaged, and it causes the individual to react to remove the pain stimulus[1].

Typically, it is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause (e.g. trigeminal neuralgia), or persistent long after the precipitating injury has healed (e.g. phantom limb pain). It can also occur as a consequence of brain or nerve injury (e.g. following a stroke or herpes infection).

With many pathological conditions, tissue injury is the immediate cause of the pain, and this results in the local release of a variety of chemical agents, which are assumed to act on the nerve terminals, either activating them directly or enhancing their sensitivity to other forms of stimulation[2].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states[3]. It is the body's natural function to create an environment where infectious agents or damaged tissues cannot survive. Normally, the infected or damaged tissue initiates the enhanced formation of proinflammatory mediators (cytokines, such as interleukin 1β, α, β, and TNF- α), which increase the synthesis of prostaglandin E2 (PgE2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature[4]. When body temperature becomes high, the temperature regulatory system, which is governed by a nervous feedback mechanism, dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the

internal temperature by vasoconstriction. High fever often increases faster disease progression by increasing tissue catabolism, dehydration, and existing complaints, as found in HIV[5]. Most of the antipyretic drugs inhibit COX-2 expression to reduce the elevated body temperature by inhibiting PGE2 biosynthesis[6]. These synthetic agents irreversibly inhibit COX-2 with a high selectivity and are toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles.

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, heamorrhages, fever and general debility. The roots and juice of the plant are useful in inflammatory reactions. The ash obtained by burning the inflorescence is a good antacid antiperiodic, and is useful in heart burn, spleenomegaly and in bilious fever[7-9]. 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[10]. It also has been documented to possess immunosuppressant property[11].

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 analgesic and anti-pyretic activity of *Borassus flabellifer*. This prompted us to investigate the effects of pharmacological activities of *Borassus flabellifer* in experimental models of algesia and pyrexia.

MATERIALS AND METHODS

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.

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 ± 2°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 150mg/kg b.w. and 300mg/kg for dose dependent study.

Analgesic 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 Indomethacin or Morphine (10mg/kg b.w.) or Paracetamol (200mg/kg b.w.) Group III and IV served as test, received ethanolic extract of *Borassus flabellifer* L. Male flowers (BFEE) at doses of 150mg/kg and 300mg/kg b.w. p.o. respectively.

Writhing test

The test was carried out according to Koster R et al[12]. Animals were administered orally with Indomethacin (10mg/kg b.w.) as the standard drug, BFEE (150mg/kg and 300mg/kg b.w.) and vehicle. Thirty minutes after treatment, the mice were given an intraperitoneal (i.p.) injection of 0.6% v/v acetic acid in a volume of 10ml/kg to induce the characteristic writhings. The no. of writhings occurring between 5 and 15 min. after acetic acid injection was recorded. The response of the extract treated animals was compared with that of control.

Hot-plate test

Mice were placed on an aluminium hot plate kept at 55±0.5ºC for a maximum time of 30s[13]. Reaction time was recorded when the animals licked their fore and hind paws and jumped; at before (0) and 15, 30, 45 and 60 min after i.p. administration of (BFEE) at doses of 150mg/kg and 300mg/kg b.w. to different groups. Morphine (10mg/kg b.w.) as the standard drug.

Haffner's tail clip method

A metal artery clip was applied to the root of the mouse's tail to induce pain[14]. A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 10s were discarded. The responsive mice were allotted to groups of six animals each. The tail clip was applied 60min after oral administration of extract (150mg/kg b.w and 300mg/kg b.w.), morphine (10 mg/kg b.w.). Whereas vehicle treated group served as control.

Formalin test

The method used was similar to that described previously[15]. 20µl of 1% formalin was injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5min after formalin injection (first phase) and 15–

30min after formalin injection (second phase). Extract (150mg/kg b.w. and 300mg/kg b.w.) and Indomethacin (10 mg/kg b.w.) were administered 60min, before formalin injection. Control animals received the vehicle.

Antipyretic activity

Antipyretic activity was measured by slightly modifying the method described by Adams et al[16]. Rats were fasted overnight with water *ad libitum* before the experiments. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10ml/kg) into the animal's dorsum region. 17h after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250 MC, Sato keiryoki Mfg.). Only rats that showed an increase in temperature of at least 0.7℃ were used for experiments. BFEE (150mg/kg and 300mg/kg b.w.), aspirin (200mg/kg b.w.) or vehicle were administered orally and the temperature was measured at 1, 2, 3, 4, and 5h after treatment.

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 effects of extract on acetic-acid induced writhes in mice have been shown in Table 1. BFEE at either dose (150mg/kg b.w and 300mg/kg b.w.) produced a significant ($P<0.0001$) decrease in no. of writhes in comparison with the control group. Indomethacin (10mg/kg b.w.) also showed significant ($P<0.0001$) decrease in no. writhes.

Table 1 : Effect of ethanololic extract of *Borassus flabellifer* male flowers on acetic-acid induced writhes in mice

Treatment	Dose (mg/kg)	No. of writhes (Mean ± S.E.M)	Inhibition (%)
Control	---	47.67±2.69	---
Standard	10	12.67±1.11[c]	73.42
BFEE 150	150	30.67±2.84[c]	35.66
BFEE 300	300	19.33±1.56[c]	59.45

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, Data were analyzed by using One-way ANOVA followed by Dunnett's test, Standard: Indomethacin (10mg/kg b.w.)
BFEE 150: Ethanolic extract at dose 150mg/kg b.w., BFEE 300: Ethanolic extract at dose 300mg/kg b.w.

Hot-plate test was also assayed to characterize the analgesic activity of the BFEE. The results presented in Table 2 show that the oral administration of the BFEE at doses 150mg/kg b.w and 300mg/kg b.w. significantly ($P<0.0001$) raised the pain threshold at different time of observation (0-60min) in comparison with control. Morphine (10mg/kg b.w.), used as standard drug, also produced a significant analgesic effect during all the observation times when compared with control values ($P<0.0001$).

Table 2 : Effect of ethanololic extract of *Borassus flabellifer* male flowers on mice subjected to the hot-plate test

Treatment	Dose (mg/kg)	Reaction time in seconds at time (minutes)				
		0	15	30	45	60
Control	---	7.71±0.33	7.75±0.34	8.07±0.13	9.03±0.18	8.62±0.21
Standard	10	7.77±0.37	11.15±0.37[c]	13.67±0.44[c]	18.21±0.60[c]	20.90±0.40[c]
BFEE 150	150	7.73±0.28	9.28±0.40[a]	11.26±0.26[c]	12.95±0.34[c]	14.88±0.32[c]
BFEE 300	300	7.78±0.39	10.10±0.37[c]	12.10±0.16[c]	15.19±0.24[c]	17.58±0.29[c]

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
Data were analyzed by using One-way ANOVA followed by Dunnett's test, Standard: Morphine (10mg/kg b.w.), BFEE 150: Ethanolic extract at dose 150mg/kg b.w., BFEE 300: Ethanolic extract at dose 300mg/kg b.w.

The effect of BFEE on tail clip test is shown as in Tables 3. The extract caused a significant (P<0.0001) inhibition of pain at both the doses used (150mg/kg b.w and 300mg/kg b.w.), Morphine (10mg/kg b.w.), a standard drug, was highly effective (P<0.0001).

Table 3 : Effect of ethanololic extract of *Borassus flabellifer* male flowers on tail-clip test in mice

Treatment	Dose (mg/kg)	Reaction time (in Sec)	Inhibition (%)
Control	---	1.17±0.12	----
Standard	10	7.65±0.27[c]	84.70
BFEE 150	150	2.74±0.24[c]	57.27
BFEE 300	300	3.75±0.24[c]	68.80

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
Data were analyzed by using One-way ANOVA followed by Dunnett's test
Standard: Morphine (10mg/kg b.w.),BFEE 150: Ethanolic extract at dose 150mg/kg b.w.
BFEE 300: Ethanolic extract at dose 300mg/kg b.w.

There was a significant dose-dependent inhibition of both phases of the formalin induced pain response in mice (Table 4), with a more potent effect on the second than the first phase. Morphine (10mg/kg b.w.) also inhibited both phases of the pain significantly (P<0.0001) when compared to control group.

Table 4 : Effects of the ethanololic extract of *Borassus flabellifer* male flowers on formalin-induced pain in mice

Treatment	Dose (mg/kg)	0–5 min	% Inhibition	15–30 min	% Inhibition
Control	---	98.67±2.84	----	94.33±1.764	----
Standard	10	74.83±2.27[c]	28.46	27.50±2.045[c]	70.84
BFEE 150	150	89.33±1.62[a]	9.46	55.67±1.585[c]	40.98
BFEE 300	300	79.50±2.11[c]	19.42	43.17±1.167[c]	54.23

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
Data were analyzed by using One-way ANOVA followed by Dunnett's test, Standard: Morphine (10mg/kg b.w.)
BFEE 150: Ethanolic extract at dose 150mg/kg b.w., BFEE 300: Ethanolic extract at dose 300mg/kg b.w.

Tested on yeast-induced pyrexia in rats, BFEE significantly reversed hyperthermia at either dose (150mg/kg b.w and 300 mg/kg b.w.). Time of peak effect obtained were 1 to 3h after oral administration. The standard drug, Paracetamol (200mg/kg b.w.) also suppressed hyperthermia induced by yeast significantly ($P<0.0001$) during all the observation times when compared with control values (Table 5).

Table 5 : Effect of ethanololic extract of *Borassus flabellifer* male flowers on brewer's yeast induced pyrexia in rats

Treatment	Dose (mg/kg)	Mean ± S.E.M. Rectal temperature (°C)					
		0h	1h	2h	3h	4h	5h
Control	---	37.02± 0.11	37.10± 0.10	37.07± 0.08	37.05± 0.11	37.07± 0.16	37.02± 0.21
Standard	200	37.10± 0.10	35.82± 0.08[c]	35.72± 0.13[c]	35.80± 0.09[c]	35.75± 0.15[c]	35.92± 0.06[c]
BFEE 150	150	37.08± 0.12	36.72± 0.10[a]	36.57± 0.10[b]	36.68± 0.11[a]	36.72± 0.13	36.70± 0.18
BFEE 300	300	37.13± 0.12	36.37± 0.10[c]	36.15± 0.06[c]	36.45± 0.09[b]	36.43± 0.12[a]	36.68± 0.07

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
Data were analyzed by using One-way ANOVA followed by Dunnett's test
Standard: Paracetamol (200mg/kg b.w.), BFEE 150: Ethanolic extract at dose 150mg/kg b.w.
BFEE 300: Ethanolic extract at dose 300mg/kg b.w.

DISCUSSION

The data presented here suggests that the BFEE possesses anti-nociceptive and antipyretic activities. The extract at the doses tested was shown to possess anti-nociceptive activity evident in all the nociceptive models, signifying it possesses both central and peripherally mediated activities. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics[17]. In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response[18]. The method has also been associated with prostanoids in general, that is, increased levels of PGE2 and PGF2α in peritoneal fluids[19], as well as lipoxygenase products[20]. The significant reduction in acetic acid-induced writhes by BFEE suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

The hot-plate and tail-clip tests are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level[21]. The significant increase in pain threshold produced by BFEE in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems[22-25]. The analgesic effect produced by

the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain.

The extract gave a similar effect on the formalin test inhibiting both the first and the second phase. Formalin test is biphasic, and measures pain of both neurogenic (first phase) and of inflammatory origin (second phase). The first phase (0 – 5min) being a result of direct stimulation of nociceptors measures centrally mediated effects and is insensitive to anti-inflammatory agents while the second phase (15 – 30 min) which is qualitatively different from the first phase is dependent on peripheral inflammation and changes in central procession due to chemical mediators release from damaged cells that stimulate nociception and thus induced pain [26]. In general, the test measures the response to a long lasting nociceptive stimulus similar to clinical pain[27] and is recommended as a tool in basic pain research for studying the mechanisms of analgesic agents because of its connection to tissue injury. Agents that act primarily on the CNS inhibit both phases equally while peripherally acting drugs inhibit the late phase. The ability of BFEE to inhibit both phases of the formalin test indicates its involvement in both central and peripherally mediated activity, probably by prostaglandin synthesis inhibition, as well as central inhibition mechanism.

Fever may be due to infection or one of the sequele of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are the agents, which reduce the 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 elevates and a drug like paracetamol does not influence body temperature when it is elevated by the factors such as exercise or increase in ambient temperature. Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a lower temperature[28]. The present results show that BFEE possesses a significant antipyretic effect in yeast-provoked elevation of body temperature in rats, and its effect is comparable to that of paracetamol (standard drug). So inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol[29]. Also, there are several mediators or multi-processes underlining the pathogenesis of fever. Inhibition of any of these mediators may bring about antipyresis[30].

CONCLUSION

The results obtained in this study indicate that BFEE possesses potent analgesic and antipyretic properties, which are mediated via peripheral and central inhibitory mechanisms. This could provide a rationale for the use of this plant in fever, pain and inflammatory disorders in folk medicine.

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