

Full Length Research Paper

Anti-nociceptive, anti-inflammatory and antipyretic effects of the methanolic extract of *Bombax buonopozense* leaves in rats and mice

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Accepted 5 January, 2010

Methanolic extract of *Bombax buonopozense* was evaluated for possible anti-nociceptive, anti-inflammatory and anti-pyretic activities in mice and rats. Acetic acid-induced abdominal constriction test in mice and formalin test in rats were used to investigate the antinociceptive effect of the extract. Studies were carried out on yeast-induced pyrexia and egg albumin-induced anti-inflammatory activity in rats. The extract produced a significant decrease in acetic acid-induced writhing in mice and inhibition of late phase of the formalin pain test in rats. The methanolic extract of *B. buonopozense* leaf also produced a potent antipyretic effect and significant inhibition of egg albumin-induced anti-inflammatory activity in rats. The result suggests that *B. buonopozense* contains biologically active substances with potential values for the treatment of fever, painful and inflammatory conditions.

Keywords: *Bombax buonopozense*; analgesic, inflammation, pyrexia.

INTRODUCTION

Bombax buonopozense P. Beauv. (*Bombaceae*) is a large tropical tree that grows to 40 m in height with large buttress roots that can spread 6 m. The bark is covered with large conical spines, especially when young, but shedding them with age to some degree. The branches are arranged in whorls, the leaves are compound and have 5 to 9 leaflets and 5 to 25 secondary veins. The individual leaflets have entire margins and are also large. The underside of the leaflets may be glabrous or puber-

clous (Beentje and Sara, 2001). It is widely distributed in Africa, from Ghana to Sierra Leone, Uganda and Gabon. Common vernacular names include: Vabga (Dagbani language in Ghana), Kurya (Hausa language in Northern Nigeria). Many parts of the plant are used for medicinal purposes: as food, as a source of clothing fibre, as building materials, as cotton wool and as dye. The fruits are eaten by animals such as the water chevrotain (Dubost, 1984). A decoction of the leaves is used for feverish conditions, pains and muscle aches. Root decoction is used as antimicrobial and for stomach-aches.

Due to the limitations of opioid and non-steroidal anti-inflammatory drug (NSAID) therapy, there is a continuous search for new analgesic. In view of this and on account of the usefulness of some painful and inflammatory conditions which has not been scientifically verified, this current study was aimed at investigating possible anti-nociceptive, anti-inflammatory and antipyretic activities of

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Abbreviations: NSAID, Non-steroidal anti-inflammatory drug; i.p, intraperitoneally; ASA, acetylsalicylic acid; s.c, subcutaneously; PG, prostaglandin; TNF, tumor necrosis factor.

the methanolic extract of the leaf in rats and mice.

MATERIALS AND METHODS

Plant collection

The leaves of *B. buonopozense* were collected from Suleja, Niger State, Nigeria, by Mallam Muazzam Ibrahim an ethnobotanist in the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial Area, Abuja, Nigeria. The plant was identified and authenticated by Mrs. Grace Ugbae a taxonomist in the same department. Voucher specimen (No.6402) was deposited in the herbarium of National Institute for Pharmaceutical Research Development (NIPRD).

Preparation of plant extract

The plant material was dried, pulverized into a dry powder and macerated with 70% methanol in water for 72 h with constant shaking. The resultant mixture was filtered using Whatman (No.1) filter paper and the filtrate was concentrated using rotary evaporator and dried on a water bath to give a yield of 7.7% (w/w). The extract was reconstituted in water at appropriate concentrations for the various experiments conducted.

Animals

Adult Wistar rats (180 to 250 g) and Swiss albino mice (18 to 25 g) of either sex were used in the various experiments. The animals were all obtained from Animal Facility centre (AFC), Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and development (NIPRD), Abuja, Nigeria. The animals were kept in cages and housed under standard condition of 12: 12 h light/dark cycle and were fed with NIPRD formulated food and had water *ad libitum*.

Drug and chemicals

The drugs and chemical used were methanol (riedel-dehaen, German), formaldehyde (M&B, England), glacial acetic acid (Searle, England), drugamol (Drugfield, Nigeria), aspirin (Sigma, USA) and Triton X-100 (Lubley, West Yorkshire, England).

Phytochemical screening

Standard screening tests (Trease and Evans, 1989) for detecting the presence of different constituents were employed in screening the crude extract. Secondary metabolites tested include alkaloids, tannins, saponins, sterols, terpenes, flavoids, anthraquinones and carbohydrates.

Acute toxicity studies

The acute toxicity LD₅₀ was estimated in mice intraperitoneally (i.p) following Lorke (1983) method. Mice of either sex were fasted overnight and the evaluation of the LD₅₀ was carried out in 2 stages. In the first stage, 3 groups of 3 mice each were treated with the extract at doses of 10, 100 and 1000 mg/kg, i.p in order to determine the range in which the LD₅₀ falls. In the second stage, another 4 groups of 3 mice each were further treated with the

extract at doses of 125, 250, 400 and 600 mg/kg. Animals were observed for 24 h for signs and symptoms of toxicity after been treated. The number of deaths in each group within 24 h was recorded and the final LD₅₀ values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where death occurred).

Acetic acid-induced writhing test in mice

The peripheral analgesic activity of methanolic extract of *B. buonopozense* leaves was determined by inhibitory effect of the extract in the acetic acid induced writhing method of Koster et al. (1959). The pre-screened albino mice employed for this study were divided into five groups of six each (n = 6) and pre-treated as follows: Groups I, II and III received 25, 50 and 20 mg/kg of the extract, respectively; group IV received 150 mg/kg of acetylsalicylic acid (ASA) and group V which served as the control, received distilled water in appropriate volumes, all were administered i.p. After the 30 min of the test dose extract/standard/vehicle, each of the mice was injected i.p with acetic acid of 0.7% at a dose of 10 ml/kg to create pain sensation. Each mouse was placed in transparent observation box. After a five-min lag period of the post-administration of acetic acid, the number of abdominal constrictions was counted for each mouse for 10 min. The percentage of pain protection was calculated:

$$\text{Percentage of inhibition} = \frac{\text{Control mean} - \text{Treated mean}}{\text{Control mean}} \times 100$$

Formalin induced nociception in rats

The study was carried out using rats adopting the method of Dubuisson and Dennis (1977) as modified by Tjolsen et al. (1992). Adult Wistar rats of either sex (200 to 250 g) were divided into five groups of six each and group I which served as negative control received distilled water in appropriate volumes. Group II received standard drug ASA (150 mg/kg), while groups III, IV and V received 25, 50 and 100 mg/kg of the extract, respectively, all administered i.p. Thirty minutes after this treatment, 50 µl of 2.5% formalin was subcutaneously (s.c) injected into the sub planter surface of the left hind paw. The animals were then placed in an observation chamber and monitored for 60 min, recording severity of nociceptive responses based on the following scale: 0 = Rats walked or stood firmly on injected paw; 1 = the injected paw was favoured or partially elevated; 2 = the injected paw clearly lifted of the floor; 3 = the rat licked, chewed or shook the injected paw.

Antinociceptive effect was determined in two phases: Early phase (first 10 min) and at every 5 min between the next (10 to 60 min) interval for late phase.

Anti-inflammatory activity

The test was conducted using a modified method of Winter et al. (1963) as described by Akah and Nwabie (1994). The rats were divided into five groups of six rats of either sex and pre-treated as follows: Group I which serve as control received distilled water (10 ml/kg i.p.), group II, III and IV received 25, 50 and 100 mg/kg of the extract i.p, respectively, while group V received acetylsalicylic acid (150 mg/kg i.p.). After 30 min of post drug administration, oedema was induced by sub-planter injection of 0.1 ml of fresh raw egg albumin in the left hind paw. Oedema volume was measured with a letica (spain) digital plethysmometer (LE7500) (calibrated with 0.1% Triton X-100), with readings taken at 20 min intervals, that is, at 20, 40, 60, 80, 100 and 120 min after albumin administration.

Table 1. Effect of methanolic extract of *B. buonopozense* on acetic acid-induced writhing in mice.

Extract	Dose	No of writhing (mean + S.E.M)	Percentage inhibition
Control	10 ml/kg	20.24 + 1.02	
	25 mg/kg	7.33 + 1.88	63.78
<i>B. buonopozense</i>	50 mg/kg	2.92 + 1.36	85.57
	100 mg/kg	2.17 + 1.42	89.28
ASA	150 mg/kg	3.67 + 0.08	81.88

The results given are mean + S.E.M; number of animal used (n = 6); *P < 0.05 as compared to control groups.

Activity on yeast-induced pyrexia

The method described by Al-Ghamdi (2001) was adopted to determine the antipyretic activity of the extract in rats. The rats were injected subcutaneously (s.c.) with 10 ml/kg of 15% suspension of yeast (Danbaoli) to induce pyrexia. The rectal temperature of each animal was taken before and 24 h after injection using a clinical thermometer (Geon corp. U.S.A). Rats that did not show a minimum increase of 0.5°C in temperature at 24 h after yeast injection were discarded. Thirty selected rats were grouped into five and treated as follows: Group I received distilled water and 10 ml/kg drugamol, respectively, all administered i.p. The rectal temperature of each rat was again recorded at thirty minutes interval for 120 min.

Statistical analysis

Results are expressed as mean ± standard error of mean (SEM). The data was analyzed using student's t-test and 2 ways ANOVA. P < 0.05 was considered significant.

RESULTS

Phytochemical screening

Phytochemical analysis of the crude extract gave a positive reaction for each of the following secondary metabolites: Flavonoids, tannins, saponins, alkaloids, terpenes, sterols and carbohydrates.

Acute toxicity tests

The behavioural signs of toxicity exhibited by mice that received 125 mg/kg extract are respiratory discomfort and increased abdominal contraction. The intraperitoneal LD₅₀ of *B. buonopozense* extract in mice was estimated to be 633 mg/kg.

Acetic acid-induced writhing in mice

The results show that *B. buonopozense* significantly (P < 0.05) and dose-dependently reduced the number of acetic acid-induced abdominal constrictions in mice. The effect of the extract at 50 and 100 mg/kg was superior to the effect of aspirin at 150 mg/kg (Table 1).

Formalin-induced pain in rats

The results show that *B. buonopozense* reduced formalin paw nociception and paw licking in both phases. The effect was however more pronounced in the second phase (64.35) with 100 mg/kg (Table 2).

Albumin-induced paw oedema test

The results show that the extract caused inhibition of albumin-induced oedema over a period of 120 min. The effects were dose-dependent (P < 0.05). Maximum percentage inhibition in the oedema with 100 mg/kg of methanolic extract was 59.71% (Table 3).

Yeast-induced pyrexia

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 24 h of administration. Treatment with *B. buonopozense* extract decreased the rectal temperature of the rats in a dose dependent manner. The antipyretic effect started from the first 30 min and was maintained for 120 min, after administration of the extract. The result obtained from both the standard and methanolic extract of *B. buonopozense* treated rats were compared with the control group and a significant reduction in the yeast induced elevated rectal temperature was observed (Table 4).

DISCUSSION

Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer and vascular diseases (Collier et al., 1968). In various traditional medical systems, a number of natural products are used to relieve the symptoms of pain and inflammation. The methanolic extract of *B. buonopozense* leaf with three different dose levels exhibited a significant antinociceptive activity in different animal models of pain. Nociception can be induced by injection of irritants into the peritoneal cavity of mice. The animals react with a characteristic stretching behavior which is called

Table 2. Effect of methanolic extract of *B. buonopozense* on formalin induced pain in rats.

Extract	Dose (ml/kg)	Early phase (0 to 10min)		Late phase (15 to 60min)	
		Score of pain	Percentage inhibition	Score of pain	Percentage inhibition
Control	10	2.58 + 0.08		2.3 + 0.10	
	25	1.93 + 0.10	25.19	1.45 + 0.06	36.10
<i>B. buonopozense</i>	50	1.52 + 0.10	41.09	1.05 + 0.23	54.35
	100	1.32 + 0.07	48.84	0.82 + 0.07	64.35
ASA	150	1.05 + 0.08	59.30	0.98 + 0.07	57.39

The results given are mean + S.E.M; number of animal used (n = 6); *P < 0.05 as compared to control groups.

Table 3. Effect of methanolic extract of *B. buonopozense* on paw oedema in rats.

Extract	Dose (ml/kg)	Paw oedema volume after (min)						
		0	20	40	60	80	100	120
Control	10	1.33+0.08	2.28+ 0.17	2.35+0.08	2.34+0.09	2.52+0.10	2.52+0.11	2.73+0.11
	25	1.1+0.07	1.68+0.07	1.59+0.05	1.55+0.04	1.62+0.07	1.48+0.04	1.47+0.05
<i>B. buonopozense</i>	50	1.11+0.08	1.52+0.05	1.59+0.08	1.42+0.05	1.38+0.05	1.35+0.04	1.37+0.03
	100	1.07+0.06	1.31+0.06	1.32+0.09	1.17+0.05	1.16+0.02	1.11+0.07	1.1+0.02
ASA	150	1.26+0.04	1.94+0.16	1.85+0.26	1.73+0.23	1.63+0.12	1.48+0.16	1.38+0.16

The results given are mean + S.E.M; number of animal used (n = 6); *P < 0.05 as compared to control groups.

Table 4. Effect of methanolic extract of *B. buonopozense* on yeast induced pyrexia in rats.

Extract	Dose (ml/kg)	Rectal temperature (°C)					
		-24	0.0 h	0.5 h	1.0 h	1.5 h	2.0 h
Control	10	37.16	38.11	38.63	39.10	39.38	39.58
	25	36.10	37.85	36.29	36.10	36.06	35.50
<i>B. buonopozense</i>	50	36.18	37.17	36.11	35.70	35.02	34.52
	100	36.97	37.25	36.58	35.68	35.64	34.07
Drugamol	20	36.52	37.61	36.08	36.02	35.59	35.11

The results given are mean + S.E.M; number of animal used (n = 6); *P < 0.05 as compared to control groups.

abdominal constriction. The acetic acid induced abdominal constriction method is very sensitive and able to detect antinociceptive effects of compounds and dose levels that may appear inactive in other methods like the tail-flick test (Bentley et al., 1981). Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response (Bentley et al., 1983). The method has been associated with increase levels of prostaglandin E₂ (PGE₂) and PGF₂ in peritoneal fluids (Deraedt et al., 1980) as well as lipoxigenase products (Dhara et al., 2000). The results of the acetic acid-induced abdominal constriction test strongly suggest that one of the mechanisms of action of the extract may be linked to lipoxigenases and or cyclo-oxygenases.

Formalin produces a distinct biphasic response and act differently in the early and late phases of pain and can be used to elucidate the mechanisms of pains and analgesia (Tjolsen et al., 1992). Centrally acting drugs such as

narcotics, inhibit both phases of pain equally (Shibata et al., 1989), while peripheral acting drugs such as aspirin, hydrocortisone, oxyphenbutazon and dexamethazone inhibit only the late pain phase (Hunnskaar and Hole, 1987; Yuh-fung et al., 1994). The first phase is believed to be a direct result of stimulation of nociceptors in the paw, while the second phase may reflect the inflammation process and at least to some degree, the sensitization of central nociceptive neurons (Coderre et al., 1990; Coderre and Melzack, 1992). The activity of the extract on both early and late phases of pain therefore suggests the possible involvement of the central mechanism in the pain inhibition. Also, the late phase of formalin test involves peripheral inflammatory process and since the extract was able to inhibit this phase involving inflammation, it might also mean an involvement of the peripheral mechanism in anti-nociceptive effect. The effect of the extract was more observed on late pain

phase.

The extract also caused marked inhibition of albumin induced hind-paw oedema in rats. Albumin-induced oedema is a biphasic response; the early phase is mediated through the release of histamine, serotonin and kinins, while the late phase is related to the release of prostaglandins and mediated by bradykinin, leucotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. The anti-inflammatory activity shown by the extract over a period of 2 h was quite similar to that exhibited by the group treated with standard drug. Therefore, it involves a complex array of enzyme activation, mediator release extravasations of fluid, cell mitigation, tissue breakdown and repair (Vane and Botting, 1995). These results indicate that the extract acts on both early and late phases of inflammation. Late phase activity might probably be involved with arachidonic acid metabolites which produce an edematous response by mobilization of the neutrophils (Just et al., 1998). The co-existence of both anti-nociceptive and anti-inflammatory effects observed with the extract is well-documented for various non-steroidal anti-inflammatory drugs (NSAIDs), especially the salicylates and their cogenes (Famaey, 1983). This method is an *in vivo* model of inflammation used to screen agents for acute inflammatory effect (Akah et al., 1993; Akah and Nwabie, 1994; Amos et al., 2002). Flavonoids isolated from some medicinal plants have been proven to possess anti-nociceptive and/or anti-inflammatory effects (Duke, 1992). It is therefore possible that the anti-nociceptive and anti-inflammatory effects observed with this extract may be attributable to its flavonoid component, which was shown to be present during photochemical analysis.

The extract produced a significant reduction in yeast induced pyrexia in rats in a dose dependent manner. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β , α , β and tumor necrosis factor (TNF)- α) which increase synthesis of PGE₂ near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (Spacer and Breder, 1994). 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 pyrexia, this set point is elevated and drug like drugamol do not influence body temperature when it is elevated by factors such as exercise or increase in ambient temperature (Goodman and Gilman, 1996). The present result shows that the methanolic extract of *B. buonopozense* possesses a significant antipyretic effect in yeast provoked elevation of body temperature in rats and its effect is comparable to that of drugamol.

Based on the result obtained, it can be concluded that the methanolic extract of *B. buonopozense* possesses potential activity against both early and late phases at

dose range of 25 to 100 mg/kg body weight. However, 100 mg/kg was observed to be more potent and efficacious toward the anti-inflammatory, analgesic and antipyretic activity when compared with control and activity is dose-dependent. More studies are however, necessary to elucidate the exact mechanism(s) of action.

ACKNOWLEDGEMENT

The authors are grateful for the secretarial assistance of Ngozi Anyalewechi A.

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