

## Anti-Inflammatory, Analgesic and Antipyretic Activities of the Ethanol Extract of the Seeds of *Buchholzia coriacea* in Experimental Animals

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### Abstract

*The effects of the ethanol extract from Buchholzia coriacea seed on inflammatory response using fresh egg albumin- induced paw edema in rats were examined. Antinociceptive activity of the extract using writhing test with acetic acid-induced pain in mice and the antipyretic activity in yeast- induced fever in rats were also examined. Oral administration of the extract (at the dose of 400mg/kg and in a dose dependent manner) significantly ( $p < 0.05$ ) decreased the paw oedema induced by fresh egg albumin in rats. The extract (at 400mg/kg) also significantly ( $p < 0.05$ ) inhibited yeast-induced pyrexia in rats when compared to the control. The extract was also found to possess significant ( $p < 0.05$ ) dose dependent antinociceptive response in mice at 800 mg /kg. The results support the use of Buchholzia extract for the treatment of inflammatory disorders.*

**Keywords:** Anti-Inflammatory, Analgesic, Antipyretic, Ethanol extract, *Buchholzia coriacea*, Rat

### Introduction

*Buchholzia coriacea* (Fam. Cappariaceae) is a perennial shrub which is prevalent in tropical Africa (Hutchinson and Dalziel, 1954). The seeds of the plant are traditionally used in Nigeria and other West African countries for the management of inflammatory disorders, bacterial infections, rheumatism and as an anthelmintic (Moser *et al.* 2007; Ajaiyeoba *et al.*, 2001). The seeds are edible and used as condiment, stimulant and as aphrodisiac in some West African countries (Moser *et al.*, 2007). Inflammation is a co-ordinated response of the host to numerous noxious stimuli such as infection, chemical and physical injury and antigen- antibody interactions (Bloom, 1981).

Inflammation is characterized by redness, swelling, heat, pain, and loss of function (Bloom, 1981). Chemical mediators of inflammation include histamine, cytokines, bradykinin, eicosanoids (prostaglandin  $E_2$ , thromboxane  $A_2$  and leukotrienes), and platelet activating factor. These proinflammatory agents when released at the sites of injury exert their characteristic inflammatory response which includes dilation of vascular smooth muscle and smooth muscle spasm (Bloom, 1981). Anti- inflammatory agents are drugs which inhibit or reduce the inflammatory response.

The high cost, side effects and drug interaction associated with some conventional anti-inflammatory drugs make their use unattractive despite their efficacy. A meaningful percentage of the rural populace in Nigeria resort to traditional herbal remedies for primary health care delivery due to accessibility, affordability and the advantage of having multiple efficacy as a result of presence of many bioactive constituents (Sofowora, 1993). The present study is a scientific approach to re-establish the traditional uses of the plant seed and evaluate its anti-inflammatory activity including its antipyretic and analgesic effects.

### Materials and Methods

**Plant material:** *Buchholzia coriacea* seeds were collected from Nsukka, Enugu state Nigeria and identified by Mr. A.O. Ozioko of the Department of Botany, University of Nigeria, Nsukka. Voucher specimens were retained in the Department of Botany herbarium, University of Nigeria, Nsukka.

**Chemicals:** All chemicals used were of analytical grade and products of May and Baker, England, BDH, England, or Merck, Darmstadt, Germany. The dried yeast extract was obtained from Premier Breweries limited, Onitsha.

**Extraction:** The fresh seeds of *Buchholzia coriacea* were air dried under atmospheric temperature ( $25^{\circ}\text{C} \pm 15^{\circ}\text{C}$ ) for two weeks and milled to a coarse powder with a Crestow milling machine. The pulverized seeds were subjected to cold maceration by methods corresponding to those practiced by Nigerian traditional doctors (Sofowora, 1993). One Kilogramme quantity of the pulverized seeds was soaked in 70% (w/v) ethanol for 24 hours with two changes of solvent. The filtrates (extracts) were combined and concentrated under reduced pressure using a rotary evaporator to yield 3.8% starting material. All doses are expressed in terms of dry weight of crude extract to the body weight of the animals.

**Animals:** All animals were obtained from the Animal house, Faculty of Biological Sciences, University of Nigeria, Nsukka. Albino rats (Wistar strain) weighing 150-210g and Swiss albino mice weighing 25-30g were used. The animals were housed in metal cages for at least one week in the animal room of Biochemistry Department, University of Nigeria, Nsukka prior to testing. Food and water were given *ad libitum* unless otherwise specified.

**Acute toxicity study:** Acute toxicity tests were carried out to define the range of lethal dose and safe range for the extract according to the method

of Lorke (1983). Twenty five inbred albino mice of both sexes (26-35g) were randomly divided into 5 groups of 5 animals each. After overnight (18 hours) fast, groups were treated with extract (200, 500, 1000, 2000 4000 mg/kg) or 4 ml/kg water (control group) by oral intubations using a catheter attached to tuberculin syringe. The animals were observed continuously for the first 2 hours for toxic symptoms and up to 24 hours for mortality. Deaths within a period of 24 hours were recorded and the median lethal dose (LD<sub>50</sub>) of the extract was determined.

**Anti-inflammatory activity:** Acute inflammation was produced by injecting a fresh egg albumin (0.1ml of 50% solution) into the plantar surface of rat hind paw according to a modified method of Winter *et al.*, (1962). The test extract (100, 200, and 400 mg/kg) and indomethacin (5 mg/kg orally) as reference agent were administered (p.o.) 30 minutes before fresh egg albumin injection. The paw volume was measured at 0, 1, 2, 3, and 4 hours using the Archimedes principle and the difference in paw volume from the volume at zero hour was taken as a measure of oedema. The average percentage inhibition was then calculated.

**Antinociceptive activity:** To determine the analgesic activity of the ethanol extract, the writhing test was carried out according to the method described by Koster *et al.*, (1959). Normal saline (3ml/kg), aspirin (200mg/kg), three scalar amounts (200, 400 and 800 mg/kg) of the extracts were respectively administered (p.o.) to 5 groups of 5 rats each. Thirty minutes afterwards, 0.6% acetic acid (10ml/kg) was intraperitoneally injected into each animal. The number of writhings and stretching was counted over a 20 minutes period.

**Antipyretic activity:** The antipyretic activity of ethanol extract of *Buchholzia coriacea* seeds in rats which were made hyperpyretic by injection of 50% suspension of brewer's yeast was investigated by a combination of the methods described by Chattejee *et al.*, (1983) and Kesersky *et al.* (1973). The test was carried out in an air -conditioned room (25-<sup>o</sup>C and 50% humidity). The animals were kept in the room for 18 hours to acclimatize them before starting the experiment. Feed and water were withheld overnight and until the test was completed. Three rectal measurements, the average of which form the basal temperatures were taken in each rat at 30 minutes intervals before the injection of pyrogen.

Pyrexia was induced in albino rats each by subcutaneous injection of 50% dried brewer's yeast suspension in 0.9% NaCl (1ml/100g bodyweight). Initial rectal temperature was recorded. After 18 hours, animals that showed an increase of 0.3-0.5<sup>o</sup>C in rectal temperature were selected. The extract (200, and 400 mg/kg) was administered to two groups. The control group received 0.3ml normal saline. Paracetamol (administered orally, 100mg/kg by gastric intubations) was used as reference drug. Rectal temperature was measured by Ellab themistor thermometer 1 and 2 hours after extract/ reference drug administration.

**Statistical analysis:** The results and data obtained in this study were evaluated using the one- way analysis of variance (ANOVA) test between two mean groups; control and test groups, followed by student's t- test. Significant levels were at  $p < 0.05$ .

## Results

**Acute toxicity:** The LD<sub>50</sub> of the extract was found to be 1445.4 mg/kg body weight. All doses used in this study were chosen to exclude the lethal dose and were adjudged to be within the safe range.

Table 1 shows that when compared to the control group, the standard non-steroidal anti-inflammatory drug, indomethacin reduced the oedemagenic effect of the egg albumin slightly (from 0.73 ± 0.05 ml to 0.53 ± 0.05 ml) after 1 hour but reduced greatly and significantly ( $P < 0.05$ ) after 4 hours from 0.43 ± 0.12ml to 0.20 ± 0.07ml. The table shows further that the extract inhibited the paw volume increases in concentration related manner. Like indomethacin, the extract was more active against the later stage of the increase (after 4 hours) than the earlier stage. The effects of the extract at 400mg/kg compare well with those of indomethacin at 5mg/kg.

Using writhing induced by acetic acid treatment as an index of nociceptive response, it is evident in Table 2 that both aspirin (at 200mg/kg) and the extract (at 800mg/kg) exerted comparable levels of inhibition of this response. Relative to controls that received normal saline treatment, the effect of the extract was dose related and statistically significant ( $P < 0.05$ ).

Pyrexia was induced experimentally in all the rats with yeast. In control animals treated with normal saline, the temperature increased and peaked after 19 hours (1 hour after treatment) and decreased by only 0.5<sup>o</sup>C after 20 hours (2 hours after treatment). In paracetamol treated animals, the temperature began reducing by 4<sup>o</sup>C at the 19<sup>th</sup> hour (1 hour after treatment) and at the 20th hour (2 hours after treatment) the reduction was remarkable (1.4<sup>o</sup>C). Two hours after treatment the extract reduced the temperature increases observed at the 18th hour by 0.6<sup>o</sup>C for 200mg/kg treated group and by 0.9<sup>o</sup>C for 400mg/kg treated group.

## Discussion

The LD<sub>50</sub> value of 1445.4 reveals that all treatments given below 100mg/kg are within the safety range. The does 100 – 800 mg/kg were therefore considered sub-acute.

The observation in Table 1 showing that *Buchholzia* extract inhibited oedema formation induced with egg album in rat paws in a concentration related manner and significantly indicated that the extract exhibited anti-inflammatory effect. Like the standard anti-inflammatory drug, indomethacin, it suppressed the later phase (after 4 hours) of paw volume increase significantly ( $P < 0.05$ ). The extract at 400 mg/kg produced an inhibition (58.84%) close to that of indomethacin (53.49%) at 5mg/kg. The paw edema model is a standard method used for evaluation of anti-inflammatory activity of anti-inflammatory,

**Table 1: Effect of *Buchholzia* seed extract on egg albumin- induced rat paw oedema**

Treatment groups	Mean change in paw volume after 1 hour (ml).	Mean change in paw volume after 2 hours (ml).	Mean change in paw volume after 3 hours (ml).	Mean change in paw volume after 4 hours (ml).	% oedema inhibition relative to the control after 4 hours.
1 Control (tween 80)	0.73±0.05	0.63 ± 0.05	0.53 ± 0.05	0.43 ± 0.12	
2 Indomethacin (5mg/kg)	0.53 ± 0.05	0.32 ± 0.05	0.25 ± 0.05	0.20 ± 0.07	53.49%
3 Extract (100mg/kg)	0.62 ± 0.05	0.53 ± 0.05	0.35 ± 0.17	0.27 ± 0.05	37.72%
4 Extract (200mg/kg)	0.58 ± 0.08	0.50 ± 0.10	0.34 ± 0.05	0.24 ± 0.05	44.19%
5 Extract (400mg/kg)	0.53 ± 0.05	0.46 ± 0.05	0.31 ± 0.10	0.22 ± 0.04	58.84%

Each value represents the mean  $\pm$  S.D. (n=5) \*P<0.05 compared with the control group (student's t- test).

**Table 2: Effect of ethanol extract of *Buchholzia coriacea* seed and aspirin on nociceptive response induced by acetic acid in mice**

Drug Treatment	No of Writhings (Counts/20mins)	% Analgesic activity
Normal saline	61.40± 5.40	
Extract (400mg/kg)	54.20±4.90	11.73%
Extract (800mg/kg)	24.60±4.33	59.94%
Aspirin (200mg/kg)	24.80± 3.96	59.70%

Each value (no of writhings) represents mean  $\pm$  S.D. (n =5); \* P <0.05 compared to the control group (student's t- test).

**Table 3: Effect of ethanol extract of *Buchholzia coriacea* seed extract and paracetamol (100mg/kg) on brewer's yeast-induced pyrexia in rats**

Drug treatment	Dose	Rectal temperature ( $^{\circ}$ C)			
		Before Treatment		After Treatment	
		0h	18h	1h	2h
Control (normal saline)	5ml/kg	36.6±0.2	37.5±0.3	37.9±0.5	37.4±0.1
Extract	200mg/kg	36.3±0.7	37.2±0.1	37.0±0.1	36.6±0.2
Extract	400mg/kg	36.6±0.1	37. ±0.2	37.3±0.1	36.8±0.8
Paracetamol	100mg/kg	36.2±0.7	37.7±0.9	37.1±0.1	36.3±0.5

Each value (rectal temperature) represents the mean rectal temperature ( $^{\circ}$ C)  $\pm$  S.D. (n=5) \*P< 0.05, compared with the control (student's t-test).

agents including several chemical mediators of inflammation such as prostaglandins, serotonin histamine and bradykinin (Vinegar *et al.*, 1987; Winter *et al.*, 1962; Dananukar *et al.*, 2000).

In Table 2 where the plant extract (at 800mg/kg b.w) caused a significant (P< 0.05) suppression of nociceptive response (59.94% inhibition of acetic acid induced writhing response) in mice when compared to the control, the result showed that the extract possessed analgesic property. The result compared well with that of the reference analgesic drug, aspirin (at 200 mg/kg b.w.), which produced similar protective effects (59.70% inhibition) towards the acetic acid induced pain in mice. The writhing test is generally used for screening of antinociceptive effects (Koster *et al.*, 1959). Antinociceptive action could be due to inhibition of sensory receptor stimulation or due to anti-inflammatory action (Dubuission and Dennis, 1977). The analgesic action of aspirin (a reference drug in this study) on inflammation is due to the inhibition of prostaglandin synthesis via cyclo-oxygenase activity (Vane, 1987). In the present study, the ethanol extract of *Buchholzia coriacea* may have exerted its analgesic activity by inhibition of some inflammatory mechanisms.

In evaluating the antipyretic effect of *Buchholzia coriacea* seed extract at (200 and 400 mg/kg), it was found that the extract (at 400 mg/kg)

decreased significantly (P< 0.05) the temperature elevations induced experimentally with yeast. The result obtained with the extract was comparable to that of the reference antipyretic drug, paracetamol (100mg/kg) and at these concentrations; both agents nearly normalized the temperature.

The yeast-induced fever in experimental animals is a well-established model for assessing antipyretic effect and it has been used in a number of studies (Kesersky *et al.*, 1973; Chatterjee *et al.*, 1983; Lakshman *et al.*, 2006.). The antipyretic effects obtained here with paracetamol were comparable to those obtained in previous studies (Lakshman *et al.*, 2006) it is interesting to note that while the groups of animals treated with the drugs showed marked reduction in fever, the control did not show any observable reduction in pyrexia. The effect the antipyretic agents had was a lowering of the raised body temperature. In fever, the hypothalamus temperature controlling mechanism is set at a higher level than that at which the body temperature is usually maintained, and antipyretic drugs in general reset the central mechanism at a lower temperature. Calcium ions have been implicated in this mechanism (Cooper, 1987). Generally, non-steroidal anti-inflammatory drugs exert analgesic, antipyretic and anti-inflammatory activities by inhibition of prostaglandin synthesis via cyclo-oxygenase activity (Vane, 1987). The present

study shows that the seed extract of *Buchholzia coriacea* has fairly good antipyretic effect, which is comparable to that of paracetamol though at different dose ranges. The mechanism of the antipyretic effect of the plant extract could be by indirect inhibition of the prostaglandin pathway (as in the case of paracetamol).

The results reported here showed that the seed extract of *Buchholzia coriacea* exerted anti-inflammatory, analgesic and antipyretic activities (comparable to reference anti-inflammatory drugs) in experimental animal models. This finding showed justification for the use of the plant extract in the management of inflammatory conditions in Nigerian ethnomedicine. More investigations are in progress to elucidate the mechanisms of action of this drug and possibly to isolate and identify the active compounds.

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