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# Analgesic activity of the methanolic seed extract of *Buchholzia coriacea*

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**ABSTRACT**

Tail flick, hot plate and acetic-acid induced writhing reflex analgesic models in mice were used to investigate the analgesic activities of the methanolic seed extract of Buchholzia corincea. The extract was used at the doses of 250, 500 and 1000mg/kg while acetylsalicylic acid (ASA) (400mg/kg) was used as the standard reference drug in all the models. In the acetic acid induced writhing reflex model all the doses of the extract used and the reference drug showed a potent analgesic activity by significantly ($p < 0.0001$) decreasing the number of abdominal constrictions and also increased the percentage inhibition of writhing in a dose dependent manner. In the tail flick and hot plate models, the extract just like the reference drug showed high analgesic activity by dose dependently and significantly ($p < 0.0001$ and $p < 0.005$ respectively) increasing the pain reaction time (PRT) with the extract at the dose of 1000mg/kg displaying a better analgesic effect than the reference drug (ASA). These results suggest significant analgesic potential of B. coriacea and may be acting through both peripheral and central mechanisms.

**Key Words:** Buchholzia coriacea, hot plate tail flick, writhing reflex, Acetysalicylic acid.

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## INTRODUCTION

Pain is a common and distressing feature of many diseases such as tumour, surgical procedures, physical trauma, noxious chemical stimulation etc [1]. It is mostly a warning signal and primarily protective but excessive pain can lead to other side effects such as sweating, apprehension, nausea and palpitation [2]. Analgesics are drugs used to relieve pain and the existing ones have serious adverse effect such as additive potential, drowsiness, nausea, respiratory depression as seen in opiates [3] and gastrointestinal bleeding and ulceration as seen with Non steroidal anti-inflammatory drugs (NSAIDS) [4]. These make the search for new analgesic drugs a necessity and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity [5]. *Buchholzia coriacea* is one of such plants.

*B. coriacea* commonly known as "wonderful kola" belongs to the family *Capparaceae.* The plant was named after R.W Buchholz who worked with some plants in Cameroon in late $19^{th}$ century [6]. The leaves and seeds of *B. coriacea* have been reported to have anthelmmtic properties [6, 7]. Also the seeds have been shown to have antimicrobial properties. [8] In Nigeria traditional medicine, the seeds have been used for the treatment of cough, irregular menstruation, toothache, waist pain, malaria etc. Also the seeds are grinded and small quantity placed on the fore head for instant relief of headache. This study was therefore undertaken to investigate the analgesic activities of *B. coriacea* and to establish the pharmacological basis for the use of the seeds for treatment of pains.

## MATERIALS AND METHODS

**Collection and extraction of pant materials**.

The fresh fruits of *B. coriacea* were plucked from the tree in Enugu_Ezike, Igbo-Eze North Local Government Area of Enugu State, Nigeria and identified by Mr. A.O. Ozioko of Bioresources Development and conservation programme (B.D.C.P) Nsukka, Enugu state and a voucher specimen deposited in the department of Veterinary Physiology, Pharmacology and Biochemistry, Michael Okpara University of Agriculture, Umudike herbarium. Extraction was done by cold maceration method. The fruits were cut into small pieces, dried under mild sunlight, pulverized into a coarse powder of about 1mm in diameter. 300 g of the plant material was extracted in 80% methanol for 48 hours with intermittent shaking at 2 hours interval.

The extract was then filtered using Whatman No 1 filter papers and later concentrated using oven at $40^0$C. The yield was determined and the extract stored in a refrigerator at $15^0$C until time of use.

**Animals**

Mature albino Wistar male mice (28-36) obtained from the laboratory animal units of the faculty of Veterinary Medicine, University of Nigeria, and Nsukka were used for the experiment. The lighting averaged 12 hours a day and temperature varied between 25-30$^0$C and relative humidity of about 45-55%. The animals were kept in stainless steel cages and clean drinking water provided *ad libitum* while they were fed with standard commercial pelleted feed (Vital feed®, Nigeria). Ethical conditions governing the conducts of experiments with life animals as stipulated by ward and Elsea were strictly observed. [9] Also the study protocol was approved by the institutions ethical committee.

**Acetic Acid Induced Writhing Method**

This study was carried out using acetic acid induced abdominal writhing reflex pain model [10,11]. Thirty five mature mice were randomly divided into 5 groups (1-5) of 6 mice per group, fasted for 12 hours and treated as follows, Group 1 (negative control group) received 10ml/kg normal saline, group 2 (positive control group) received 400mg/kg of Acetylsalicyclic acid (ASA); groups 3, 4 and 5 received 250, 500 and 1000mg/kg of *B. coriacea* extract respectively using gastric gavage. One hour after drug and extract administration, 0.6% glacial acetic acid (10ml/kg) was administered intraperitoneally (I.P) to all the mice to induce abdominal contortions or writhings. The analgesic effect was assessed in each mouse for 30 minutes and recorded. The degree of analgesia was calculated using the following formula [11].

$$\frac{\text{Mean of control group-mean of treated group}}{\text{Mean of control group}} \times \frac{100}{1}$$

This represents the percentage of inhibition of writhing.

**Tail Flick Method**

The experiment was carried out by measuring tail withdrawal time from hot water [12]. 30 mice were randomly divided into five groups (1-5) of 6 mice per group and fasted for 12 hours. The mice were pretreated 1 hour before the experiment with 10ml/kg normal saline ; group 2, (positive control) was given 400 mg/kg and 250, 500 and 100mg/kg of *B. coriacea* extract for groups 3,4 and 5 respectively (treatment groups) using gastric gavage. About 3-5cm of the tail of each mouse was dipped into a water bath containing warm water maintained at the temperature of 50±1$^0$C and the time taken for the mouse to flick the tail known as the pain reaction time (PRT) was recorded for all the mice.

**Hot Plate Method**

The study was done using the effect of hot plate induced pain in mice [13, 14] mature mice mere randomly divided into 5 groups (1-5) of 6 mice per group, fasted for 12 hours with

clean drinking water provided *ad libitum*. The pre drug PRT was assessed by placing each mouse upon a heated metal plate (Hot plate) maintained at the temperature of about 55-60$^0$C within a restraining cylinder. The PRT for each mouse was determined using a stop watch to measure the time it took the mouse to flick or lick the hind paw or jump about. The cut off time was put at 20 seconds. This served as the control reaction time. The mice were then treated with 10ml/kg normal saline for the negative control group (group 1); 400mg/kg ASA for the positive control group (group 2) and 250, 500 and 1000mg/kg *B. coriacea* extract for groups 3, 4 and 5 respectively (treatment groups), all by gastric gavage. 30 minutes after drug and extract administration the PRT for each mouse was again determined using the same method as above.

**Data Analysis**

The results were presented as mean ±SEM. The analysis was done using one way analysis of variance (ANOVA). And the difference between the means tested using Post Hoc LSD and T- test... The value of $p<0.05$) were considered statistically significant.

## RESULTS

**Extraction**

The extract was brownish in colour and had an oily smell. The yield was 18.4% w/w dry matter.

**Acetic acid- induced writhing reflex**

The results of the analgesic effect of *B. coriacea* extract an acetic acid induced writing reflex method is presented in Table 1. The results showed that the extract at the doses used just like the reference drug ASA significantly ($p< 0.0001$) reduced the mean number of abdominal constrictions or writhing in a dose dependent manner when compared to the negative control group. The percentage inhibition of writhing was also dose dependently increased from zero in the negative control group (normal saline) to 89% in the group that received 1000mg/kg of the extract. There was no significant difference between the group that were given 500 mg/kg and those treated with the reference drug ASA but the extract at the dose of 1000 mg/kg had a better analgesic activities than the reference drug.

**Tail Flick Response in mice**

The tail withdrawal response or tail flick time was significantly ($p < 0.0001$) increased from 2.4 ± 0.07 seconds in the negative control group (10ml/kg normal saline) to 5.7± 0.39 in the ASA (400mg/kg) treated group and 6,5 ± o0.32 seconds in the highest dose of the extract (1000 mg/kg). Table 2.

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**Hot Plate Method**

The result of the effect of *B. coriacea* on hot plate induced pain in mice is presented in Table 3.

The result showed that there was no significant difference in mean pre drug PRT among the groups but after administration, the extract generally significantly ($p < 0.005$) increased the post drug PRT. In the individual group there was no significant difference between the pre and post drug PRT in group 1 (normal saline treated group) and group 3 (250mg/kg of the extract). The extract at 500mg significantly ($p < 0.001$) increased the PRT when the pre post drug PRT are compared while the reference drug ASA (400mg/kg) and the extract at the dose of 1000mg/kg significantly ($p < 0.0001$) increased the PRT when the pre and post drug PRT are compared. Also the extract at 1000mg/kg had a better analgesic effect then the reference dry ASA in this study.

**Table1: Effect of *B. Coriacea* on Acetic Acid Induced Writhing Reflex in Mice**

| Group | Treatment (mg/kg, P.O) | Mean number of writhing in 30mins ± SEM | % Inhibition |
|---|---|---|---|
| 1 | Normal saline 10ml/kg | 34.5 ±1.11 | 0 |
| 2 | Acetylsalicylic acid 400 | 16.0 ± 1.60* | 33 |
| 3 | *B. coriacea* 250 | 23.1 ± 1.93* | 54 |
| 4 | *B. coriacea* 500 | 14.0 ± 1.00* | 60 |
| 5 | *B. coriacea* 1000 | 3.7 ± 0.91* | 89 |

P < 0.0001 when compared to negative control group

**Table 2: Effect of *B. Coriacea* on Tail Flick Response in Mice**

| Group | Treatment (mg/kg, P.O) | Mean PRT± SEM (secs) |
|---|---|---|
| 1 | Normal saline 10ml/kg | 2.4 ±0.07 |
| 2 | Acetylsalicylic acid 400 | 5.7 ± 0.39* |
| 3 | *B. coriacea* 250 | 4.1 ± 0.41* |
| 4 | *B. coriacea* 500 | 5.4 ± 0.55* |
| 5 | *B. coriacea* 1000 | 6.5 ± 0.32* |

P< 0.001 when compared to negative control group.

**TABLE 3: Effect of *B. Coriacea* on Hot Plate induced Pain in Mice**

| Group | Treatment (mg/kg, P.O) | Mean pre drug PRT ± SEM (sec) | Mean post drug PRT ± SEM (sec) |
|---|---|---|---|
| 1 | Normal saline 10ml/kg | 4.3 ±0.85 | 4.9 ±0.24 |
| 2 | Acetylsalicylic acid 400 | 2.0± 0.17* | 8.0 ± 0.53** |
| 3 | *B. coriacea* 250 | 4.9 ± 0.47* | 5.1 ± 0.67 |
| 4 | *B. coriacea* 500 | 3.4 ± 0.49* | 6.6 ± 1.15* |
| 5 | *B. coriacea* 1000 | 3.6 ± 0.28* | 10.0 ± 0.57** |

* $p < 0.001$ and

** $p < 0.0001$ when the group pre drug and post drug PRT are compared

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## DISCUSSION

Certain noxious stimuli are painful and reflex movements or behaviors resulting from such stimuli are indicative of a pain threshold [14]. The stimulus may be thermal, electrical, mechanical or chemical [15] and this informed the adoption of the three analgesic models viz. Acetic acid-induced writhing, tail flick and hot plate methods.

The acetic acid-induced abdominal contortions or writhing reflex model is a sensitive method for screening analgesic effects of compounds [16]. Some chemicals such as acetic acid could induce abdominal contortions in laboratory animals [17]. The writhing reflex seen in this experiment was produced by injection of 0.6% glacial acetic acid. Intraperitoneal injection of acetic acid produces writhing reflex in the animals by activation of the chemo-sensitive nociceptors [18]. The percent reduction in the number of abdominal contortions indicates the level of analgesia in the acetic acid writhing reflex model [19]. The acetic acid induced abdominal constrictions or writhing was significantly ($P<0.0001$) reduced by *B. coriacea* extract in all the doses used in a dose dependent manner when compared to the negative group. Also the percentage inhibition of abdominal writhing was increased from zero up to 89%. The analgesic effect produced by the extract at the dose of 1000mg/kg was better than the reference drug ASA (400mg/kg). Acetic acid induced writhing reflex is sensitive method for screening peripherally acting analgesics and the response in thought to involve local peritoneal cells and mediated through the prostaglandin pathway [20]. This suggests that the analgesic effect of *B. coriacea* seen in this study may be mediated through peripheral pain mechanism and or may be through inhibition of the activities or synthesis of prostaglandins.

In tail flick method the procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex of mice [21] while in hot plate the paws of mice are very sensitive to temperatures of between 55-60$^0$C [2]. In tail flick and hot plate models, increase in pain reaction time (PRT) indicates the level of analgesia of the drug or extract [21]. From the results, *B. coriacea* extract significantly ($p<0.0001$) increased the pain tolerance level exhibited by increased PRT dose dependently when compared with the negative control group. Also generally the extract significantly ($p<0.005$) increased the mean PRT when the post drug PRT were compared in the hot plate model but had different levels of increase in PRT when individual group pre drug and post drug PRT were compared as shown in table 3. In both experiments the extract at the dose of 1000mg/kg had better analgesic activities than the reference drug ASA (400mg/kg).

Hot plate and tail flick models are used to test pains mediated by central activity [2]. In these models the sensory nerves sensitize the nociceptors and there is minimized involvement of endogenous substances such as prostaglandins [23]. These assertions suggest that the analgesic activities of *B. coriacea* may include central nervous system involvement.

In conclusion, the methanolic seed extract of *Buchholzia coriacea* showed a significant level of analgesic activities in all the models used, thus establishing a pharmacological basis for

the its use in pain treatment in folk medicine and the action may be mediated through both peripheral and central mechanisms, however, more work is required to establish the exact mechanism of action and for the isolation and characterization of the active principle.

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