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Full Length Research Paper

Analgesic and Anti-Inflammatory effects of Ethanol Extracts of *Buchholzia coriacea* Seeds in Male Rats

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ABSTRACT

The analgesic and anti-inflammatory properties of ethanol extract of Bulchholzia seeds were investigated. Male albino Wistar rats weighing 140 and 200g were used for the study. The animals were randomly divided into five groups: group 1 which served as control; group 2 animals were treated with indomethacin (5mg/kg) only, group 3, 4 and 5 were treated with ethanol extract of *Buchholzia coriacea* seeds at 50mg/kg, 100mg/kg and 200mg/kg respectively. Analgesic effect of the extract was assessed using the formalin-induced paw licking and hot plate latency tests. Anti-inflammatory response of the extract was assessed using the carrageenan-induced paw edema method and cotton pellet granuloma test. *B. coriacea* at 50mg/kg, 100mg/kg and 200mg/kg demonstrated significant ($P<0.05$) analgesic effect by increasing hot plate latency period. This increase in hot plate latency was also significantly different ($P<0.05$) from that obtained in indomethacin treated rats. A dose dependent decrease in the early phase licking time was observed in the formalin test after extract treatment while an increase in nociception in the late phase was observed at 50mg/kg and 100mg/kg *B. coriacea* treatment. Anti-inflammatory studies indicate that the extract at 200mg/kg exerted significant anti-inflammatory effect in the late phase of carrageenan model of anti-inflammatory assessment while at 50mg/kg, 100mg/kg, 200mg/kg significant anti-inflammatory response was observed using the cotton pellet test. This study suggests that *B. coriacea* possess significant analgesic and anti-inflammatory properties.

Keywords: *Buchholzia coriacea*, pain, inflammation, wonder kola.

INTRODUCTION

Pain is an unpleasant sensory and emotional experience with actual or potential tissue damage (IASP, 1994). Pain motivates the individual to withdraw from damaging situations to protect a damaged body part while it heals, and to avoid similar experience in the future (Lynn *et al*, 1984).

According to Woolf (2010), pain is classified into nociceptive pain which is a high threshold pain only activated in the presence of intense stimuli; inflammatory pain which is pain associated with tissue damage and the infiltration of immune cells and neuropathic pain which is pain that is not protective but maladaptive resulting from abnormal functioning of the nervous system. Pain in most cases is resistant to treatment with simple analgesics but may be sensitive to other classes of drugs such as depressants (Woolf and Salter, 2000).

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Buchholzia coriacea is a forest tree with large glossy leathery leaves and conspicuous cream white flowers at the end of its branches. Its leaves and stem bark in various formulations, decoctions and concoction exhibit antihelmintic, antimicrobial and cytotoxic effects on micro-organism (Ajaiyeoba *et al* 2003; Ezekiel and Onyeoziri, 2009). In folklore medicine, the seed of *B. coriacea* is used in the treatment of pain and inflammatory conditions such as asthma, rheumatism and ulcer. The wide range of the acclaimed effects of the seed of this plant earned it the name 'wonder kola'. There is however a dearth of scientific information on the analgesic and anti-inflammatory activity of *B. coriacea*.

This study will therefore investigate the analgesic and anti-inflammatory properties of *B. coriacea*.

MATERIALS AND METHODS

Phytochemical Screening

Phytochemical analysis of *Bulcchozia coriecea* seeds was carried out according to the method described by Trease and Evans (1989).

Plant Material and Extraction

The dried seeds of *B. coriacea* were purchased locally from the market and thereafter identified at the Forestry Research Institute of Nigeria (FRIN). The seeds were air dried for 5 days and subsequently macerated into its powdery form. Ethanol extract of the macerated seed was obtained using the soxhlet apparatus as described by Harbone (1993). The extract obtained was filtered and the filtrate evaporated to dryness in a water bath at 40^{o}C. The extract was stored at 4^{o}C until use. The extract was administered orally to experimental animals at 50,100 and 200mg/kg respectively.

Animals

The rats were housed in well ventilated cages and acclimatized for two weeks prior to commencement of experimental procedures. They were fed with standard rat chow and allowed free access to drinking water according to guidelines and regulations of the National Institute of Health (NIH) (NIH publication 85 – 23, 1985) for laboratory animal care and use. 100male albino rats of Wister strain weighing between 140 – 200g were used for the study. They were randomly divided into 4 groups of 25 animals each based on each assessment method. Each of these groups were then subdivided into 5 groups of 5 animals each consisting of the following. Group 1 which served as control were treated orally with 0.2mls of normal saline only; group 2 animals were treated with indomethacin (5mg/kg) only, group 3, 4 and 5 were treated with 50mg/kg, 100mg/kg and 200mg/kg ethanolic extract of *Buchholzia Coriacea* extract. All treatments where orally administered using an oral cannula.

Analgesic studies

Hot plate test

Analgesic responses were assessed in the animals using the hot plate test as described by Eddy (1950). Briefly, the control, indomethacin and extract treated (50,100, 200mg/kg) rats were allowed to habituate on the hot plate apparatus and thereafter the hotplate was set at 55±0.1 with a 30sec cut off time used to prevent tissue damage. The latency measures were taken before extract administration and 60mins after extract administration as the time elapsed between placing the rat on the hot plate and fore paw lick, hind paw lick or jump.

Formalin-induced Paw licking test

Analgesic responses were also assessed using the formalin induced paw licking test as described by Hunkaar and Hole (1987). Briefly 20µl of 2.5% formalin solution was injected into the plantar surface of the left hind paw of the rat 60min after treatment in all the different treatment groups. The time period spent by the rat in licking the injected hind paw was measured as an index of pain and nociception. The initial acute nociceptive response was at 0 – 5min after formalin injection indicated the first phase while nociceptive response at 15 – 30mins indicated the second phase.

Anti-inflammatory studies

Carrageenan Induced Paw oedema

Pedal inflammation was produced in rats according to method described by Winter *et al*., 1962. Three groups (comprising of five animals each) of rats were treated orally with 50, 100 and 200mg/kg of B.coriacea while the control and reference groups received saline (orally) and indomethacin (5mg/kg, orally) respectively. One hour after treatments, 0.1ml of 1% carrageenan was injected into the left hind paw of each animal under the sub plantar aponeurosis. Measurement of paw size was carried out as described previously (Olaleye, *et al*., 2002) by wrapping a piece of cotton thread round the paw and measuring the circumference with a metre rule. Paw sizes were measured immediately before and 1-4hours after carrageenan injection.

Oedema inhibitory activity was calculated according to the following formula:

$$\%\text{inhibition} = \frac{(C_t - C_o)\ \text{control} - (C_t - C_o)\ \text{treated}}{(C_t - C_o)\ \text{control}} \times 100$$

Where C_t = paw circumference at time t, C_o = paw circumference before carrageenan injection and $C_t - C_o$ = Oedema.

The inhibitory values at 3 hours, representing peak oedema are adopted as measure of effect.

Cotton pellet granuloma in rats

This study was carried out as described by Ismail *et al*., (1997). A sterilized cotton pellet weighing 30mg was implanted subcutaneously into the groin region of each rat under light anesthesia. Extract treatment groups thereafter received 50, 100, or 200mg/kg of the *Buchholzia Coriacea* seed extract once daily for seven consecutive. Animals in the control and reference groups received saline and indomethacin (5mg/kg) respectively. The animals were sacrificed on the 8th day with an over dose of ether and the implanted cotton pellets surrounded by granuloma tissue were dissected out carefully and oven dried at 60^0C to a constant weight. The mean weight of the granuloma tissue formed around each pellet was obtained and percentage inhibition was determined.

Statistical Analysis

Data obtained are expressed as the mean ± SEM. Student t-test was used to assess the level of statistical significance at P≤0.05.

RESULTS

Phytochemical screening of Bulcchozia coriecea seeds

The phytochemical screening of Bulcchozia coriecea seeds revealed the presence of alkaloids, anthraquinone, cardenolides, carbohydrates, cardiac glycosides, flavonoids glycosides, resins saponins, steroidal ring, steroidal terpenes, and tannins.

Analgesic studies of ethanolic extract of B.coriacea seeds

Hot plate latency test: The analgesic potential of ethanolic extract of *B.coriacea* seeds in rats using the hot plate test is shown in figure 1. The extract showed significant (P<0.01) analgesic activity at extract concentrations of 50mg/kg (46.4%), 100mg/kg (83.8%) and 200mg/kg (95.8%) when compared with control animals. A significant increase (P<0.05) in analgesic activity was also observed in all extract treated animals when compared with the standard analgesic drug, indomethacin, treated animals.

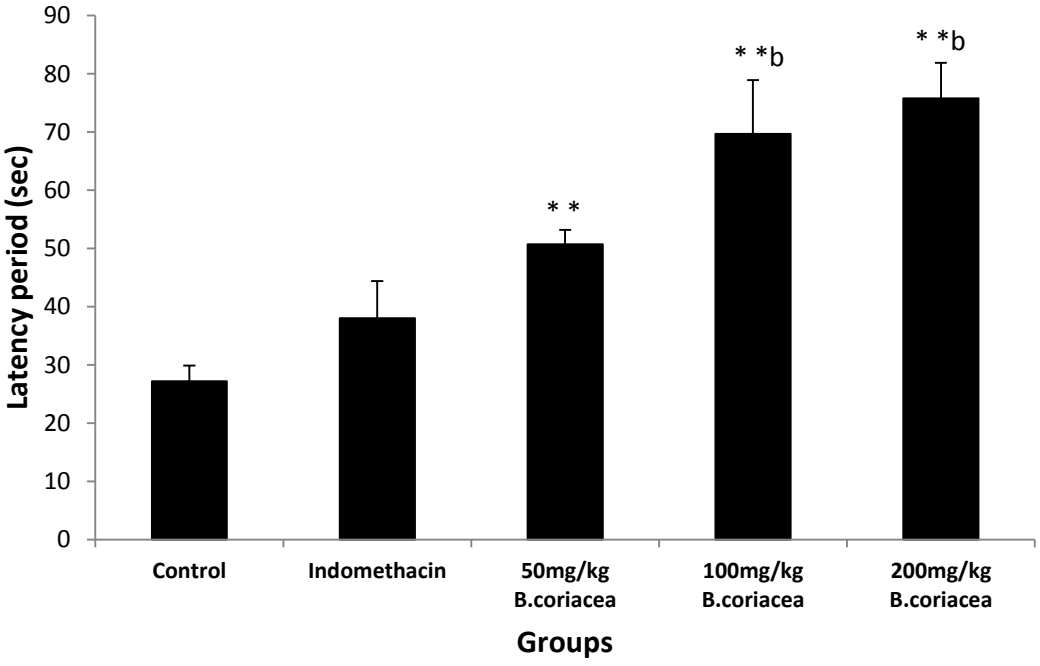

Fig. 1 Effect of ethanolic extract of *B. coriacea* seeds on hot plate latency in rats. Values are represented as Mean ±SEM. *indicate values that are significantly different from corresponding values obtained in control animals. (** = P<0.01) [b] indicate values that are significantly different from corresponding values obtained in indomethacin only treated animals. ([b] = P<0.05)

Table 1:
Anti-nociceptive effects of ethanolic extract of *Buchholzia coriacea* seeds on formalin induced paw licking test in rats. Values are represented as Mean ±SEM. * indicate values that are significantly different from corresponding values obtained in control animals. (* = P<0.05)

Group	Dose	Early phase (paw licking time) secs.	Late phase (paw licking time) secs.
Control	0.2ml	32.4±0.2	67.8±0.3
IND	5mg/kg	26.6±0.4*	54.0±0.2*
B. coriacea	50mg/kg	32.8±0.5	73.4±0.3*
B. coriacea	100mg/kg	29.8±0.3*	71.4±0.1*
B. coriacea	200mg/kg	28.2±0.2*	61.4±0.2*

IND = Indomethacin

Table 2:
Anti-inflammatory effects of ethanolic extract of B.Coriacea seeds on carrageenan induced granuloma in rats. Values are represented as Mean ±SEM. * indicate values that are significantly different from corresponding values obtained in control animals. (* = P<0.05)

Group	Dose	Paw size (cm) after 1hr	Paw size (cm) after 3hr
Control	NS	2.5±0.2	2.4±0.1
IIND	5 (mg/kg)	2.4±0.1	2.2±0.1
B. coriacea	50 (mg/kg)	2.9±0.1	2.2±0.1
B. coriacea	100 (mg/kg)	2.8±0.1	2.3±0.1
B. coriacea	200 (mg/kg)	2.6±0.1	1.8±0.1 *

IND = Indomethacin; NS = Normal saline

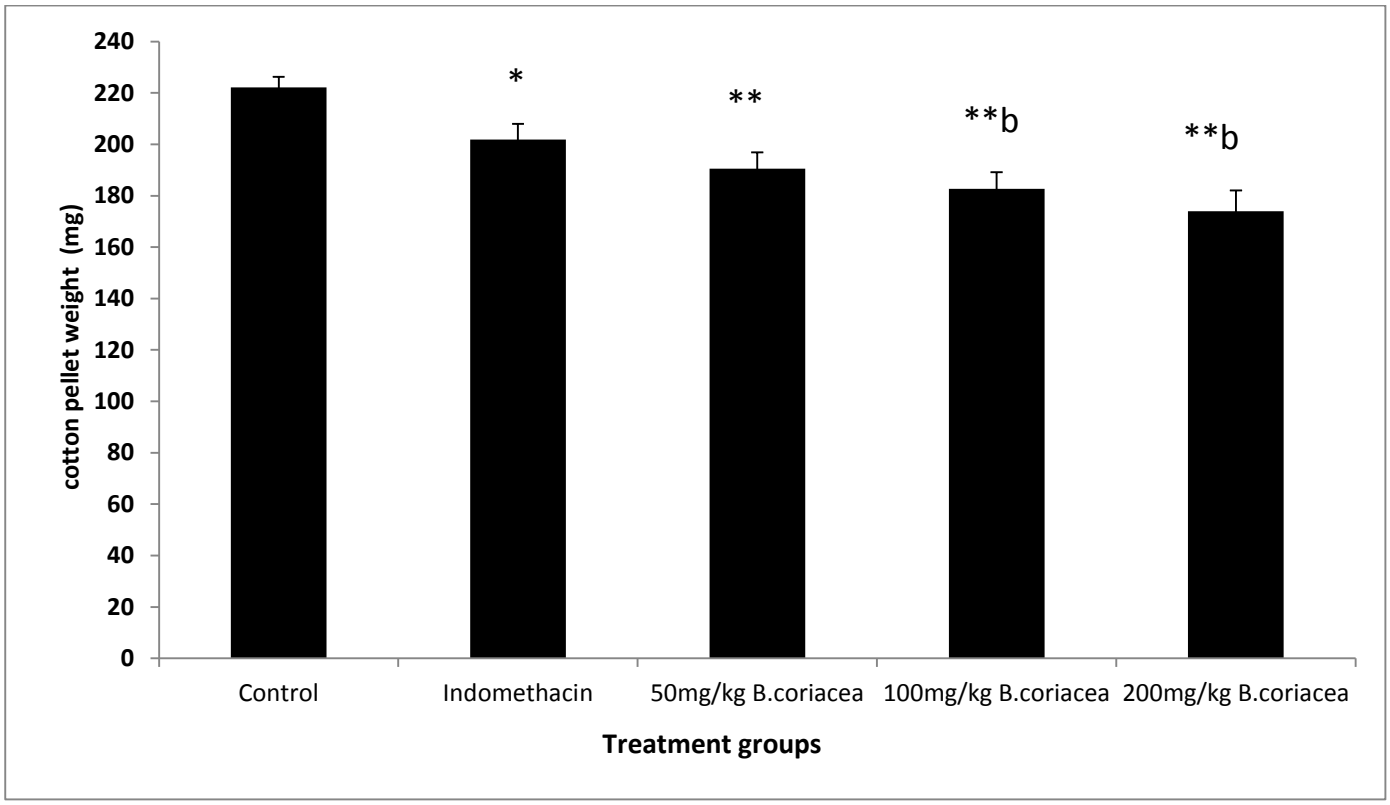

Fig. 2
Anti-inflammatory effects of ethanolic extract of *B. Coriacea* seeds on Cotton Pellet-induced Granuloma in rats Values are represented as Mean ±SEM. * indicate values that are significantly different from corresponding values obtained in control animals. (* = P<0.05; ** = P<0.01). [b] indicate values that are significantly different from corresponding values obtained in indomethacin only treated animals. ([b] = P<0.05)

Formalin induced paw licking test in rats: Table 1 shows the analgesic effects of ethanolic extract of B.coriacea using the formalin induced paw licking test. Results indicate that the extract had a dose dependent analgesic effect in both the early phase and late phase of the formalin induced paw licking test. In the early phase a significant difference ($P<0.05$) in analgesic effect was observed in the 100mg/kg and 200mg/kg treated animals when compared with control untreated animals. The analgesic effect caused by the extract in the early phase at 100mg/kg and 200mg/kg was comparable to the analgesic effect observed in the indomethacin (standard analgesic drug) treated animals. In the late phase of the formalin induced paw licking test, the extract at 50mg/kg and 100mg/kg caused a significant increase in nociception with an increase in paw licking time observed when compared with control untreated animals. However a significant decrease ($P<0.05$) in nociception was observed in the late phase in the 200mg/kg extract treated and indomethacin treated animals when compared with control animals.

Carrageenan induced granuloma in rats: Anti-inflammatory assessment using the Carragenaan induced granuloma model indicate that the extract at 50mg/kg, 100mg/kg and 200mg/kg had no significant anti-inflammatory effect in the first 1hour of assessment while at 3 hours post treatment the extract at 200mg/kg had significant ($P<0.05$) anti-inflammatory effect when compared with control animals.

Cotton pellet induced granuloma in rats: The results of the granuloma test (Fig. 2) showed that the extract of *B. coriacea* at doses of 50mg/kg, 100mg/kg and 200mg/kg had significant ($P<0.01$) anti-inflammatory effects when compared with untreated control animals. Treatment with indomethacin also had a significant ($P<0.05$) anti-inflammatory effect when compared with control animals. The anti-nociception observed in the 100mg/kg and 200mg/kg extract treated animals was significantly different ($P<0.05$) from that observed in the indomethacin treated animals.

DISCUSSION

In this study, the analgesic and anti-inflammatory properties of the ethanolic extract of Bulcchozia coriacea (wonderful kola) seeds was investigated in rats. It has been reported that there is no generally accepted paradigm for pain assessment in either human or animal experiments, hence it has become necessary to employ two or more methods in a single study before a definitive conclusion can be made on the action of any agent affecting pain responses (Olaleye *et al*, 2000). Using standard analgesic study models (hot plate latency and formalin induced paw licking) this study has shown that the extract has strong analgesic effects when compared with control untreated animals. The observed analgesic effect was comparable to that of the standard analgesic drug indomethacin. The hot plate test for analgesia is a thermal model for assessing pain and it is has been reported to be appropriate for the detection of anti-nociception that is mediated predominantly by supraspinal structures (Soul and Smith, 1998). It is therefore possible that the antinociceptive activity observed using the hot plate test in the extract treated animals (fig. 1) is being mediated by supraspinal structures. Intra plantar injection of formalin into the rat hind paw is a chemical assay of nociception and elicits a biphasic pain response that may be categorized as acute pain and tonic pain (Carter and Shieh, 2010). These two phases represent the direct effect on nociceptors and anti-inflammatory nociceptive responses respectively (Hunskaar and Hole, 1997). It is therefore possible that the observed antinociceptive response observed in the acute phase by the 100mg/kg and 200mg/kg extract treated animals (table 1) may be as a result of a direct effect on nociceptors in the experimental animals. In the late phase of experiment which coincides with tonic pain, an increase in nociception was observed in the 50mg/kg and 100mg/kg extract treated animals (table 1) which might suggest inflammation of the paws occurring in the late phase of assessment. However extract treatment at 200mg/kg elicited significant anti-nociceptive activity suggesting that at this dose the extract may possess anti-inflammatory anti-noceptive activity.

The carrageenan – induced paw oedema model and cotton pellet granuloma model were used in the assessment of anti-inflammation. The carrageenan paw oedema test was used in this study based on its several advantages which include the ability to detect orally acting anti-inflammatory agents especially in the acute phase (Owoyele, *et al*., 2000). Furthermore, the carrageenan oedema model has two distinct phases (Olajide, *et al*., 2000; Gupta, *et al*., 2003). The first phase starts immediately after carrageenan injection and lasts for about two and half hours while the second phase starts after the first phase and ends at about six hours after carrageenan injection (Willis and Cornelson, 1973). Serotonin, histamine and kinins have been linked with inflammatory processes in the early phase while prostaglandins have been reported to be mainly involved in the second phase of the oedema (Vinegar, *et al*., 1969; Crunkhon and Meacock, 1971). It has been observed that in the carrageenan model of inflammation assessment

maximal response is seen at 3hours post administration. Therefore, the results of the carrageenan test shows that ethanol extract of B.coriacea had a dose dependent effect on oedema formation with the extract at 200mg/kg showing significant anti-inflammatory response. This suggests that the extract at 200mg/kg inhibited prostaglandins mediated inflammation because the extract at this concentration caused a marked reduction in the carrageenan induced oedema after 3h of carrageenan injection (Table 2).

Cotton pellet granuloma is the most suitable method for studying the drug efficacy against proliferative phase of inflammation. The dry weight of the pellets correlates well with the amount of granulomatous tissue (Swingle and Shideman, 1972). Treatment with the extract of B.coriacea at 50mg/kg, 100mg/kg and 200mg/kg caused significant reduction in granulomatous tissue formation (fig. 2). This indicates that the extract may inhibit sub-chronic inflammation in which various types of cellular migration (e.g. fibroblast) take place (Olajide, *et al*., 2003).

Phytochemical analysis of B.coriacea shows that plant contains saponins, cardiac glycosides, anthraquinones, reducing sugars, flavonoids, alkaloids and tannins. Alkaloids, flavonoids, saponins, tannins and glycosides have all been associated with various degrees of analgesic and anti-inflammatory activities (Iwu, 1986; Olaleye, *et al*., 2002).

Therefore it is not unlikely that the analgesic and anti-inflammatory effects observed in the extract treated animals may be due to the activity of one or combination of some of the identified constituents present in the plant extract.

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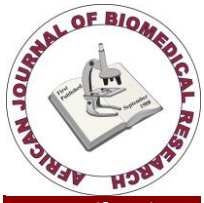

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