International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 10, Issue 4, 2018

Original Article

ANALGESIC EFFECT AND ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS EXTRACT OF *BOSWELLIA DALZIELII* (BURSERACEAE) STEM BARK

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Received: 15 Oct 2017 Revised and Accepted: 08 Mar 2018

ABSTRACT

Objective: To evaluate the analgesic effect and anti-inflammatory properties of *Boswellia dalzielii* (Burseraceae), a medicinal plant commonly used in northern Nigeria as an anti-diarrhoeal, antipyretic, analgesic and anti-inflammatory agent.

Methods: Three doses (25 mg/kg, 50 mg/kg and 100 mg/kg) of the crude aqueous extract of *Boswellia dalzielii* were evaluated for analgesic and anti-inflammatory activities using the acetic acid-induced writhing test, formalin-induced nociception and formalin-induced hind paw oedema in rats. The acute oral toxicity was carried out using the up and down procedure as described by the OECD guidelines.

Results: All doses (25, 50 and 100 mg/kg) of the extract tested were effective against acetic acid induced abdominal constrictions producing a percentage inhibition of (55.43, 69.56 and 71.73%) respectively. A percentage inhibition of the formalin-induced nociception of (7.31, 31.70 and 48.78%-early phase) and (12.82, 21.79 and 48.71%-late phase) respectively was also produced. For the acetic acid writhing test, the percentage inhibition obtained at the dose of 50 and 100 mg/kg (69.56 and 71.73%) were higher than that of the standard drug (Piroxicam, 10 mg/kg) (59.78%). For formalin-induced nociception, the test extract at 100 mg/kg showed a higher percentage inhibition compared to Piroxicam, in early (48.78 and 43.90%) and late phase (48.71 and 39.74 %) respectively. The extract, however, did not show a significant activity against formalin-induced paw oedema at all the doses used.

Conclusion: The present study demonstrated that *Boswellia dalzielii* has significant analgesic properties comparable to that of the standard drug (10% Piroxicam), thus validating the traditional claim of its antinociceptive property.

Keywords: *Boswellia dalzielii,* Analgesic, Anti-inflammatory

DOI: http://dx.doi.org/10.22159/ijpps.2018v10i4.26156

INTRODUCTION

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants [1]. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair [1]. Pain (Algesia) on the other hand is an unpleasant sensation that can negatively affect all areas of a person's life, including comfort, thought, sleep, emotion, and normal daily activity [2]. The side effects of some of the currently available drugs for the management of these conditions pose a major problem in their clinical use. For example, some non-steroidal anti-inflammatory drugs (NSAIDs) may cause gastric ulceration and renal damage [3, 4]. Owing to the safety concerns associated with the use of synthetic anti-inflammatory and analgesic agents, those of natural sources are desired [5].

Boswellia dalzielii (Frankincense) is a tree plant that is abundantly found in north-western Nigeria, where the Hausa speaking people refer to it as Hano or Arrabi [6]. This plant is very popular among the locals as a potent source of ethnomedicine [6]. A previous preliminary survey on the local use of *Boswellia dalzielii* among ten herbalist from Kano and Sokoto states in the northwestern region of Nigeria reveals that, the aqueous stem bark extract of *Boswellia dalzielii* is used in the treatment of gastrointestinal problems (diarrhea and abdominal cramp) and as antipyretic, analgesic and anti-inflammatory agent either alone or in combination with other herbs (unpublished data). Etuk *et al.* has shown that the aqueous stem bark extract of *Boswellia dalzielii* possesses anti-diarrheal activity [7]. The stem bark has also been shown to have an antispasmodic activity [8]. While the aqueous extract of the stem bark produced some anti-ulcer activity [9].

The claimed analgesic, and anti-inflammatory activity of the stem bark extract has however not been found in the literature. This study was therefore carried out to evaluate the analgesic and anti-inflammatory activity of the aqueous stem bark extract of *Boswellia dalzielii* and validate its ethnobotanical use for this purpose.

MATERIALS AND METHODS

Animals

Wistar rats weighing about 110-160 g and mice weighing (20-25 g) of both sexes were used for the experiment. The wistar rats were obtained from the animal house of Ahmadu Bello University Zaria, while the mice were obtained from the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The animals were maintained under standard environmental conditions, fed with standard chow and had access to food and water *ad libitum.* The animals were acclimatized for two weeks before the commencement of the study. The study was approved by the Research Ethics Committee, Usmanu Danfodiyo University, Sokoto. The care of the rats was conducted in accordance with established public health guidelines for handling experimental animals.

Chemicals and drugs

Formalin and acetic acid were obtained from Sigma Chemical Co. (St Loius, MO, USA) while Piroxicam (Feldene® by Pfizer Inc, New York,) was obtained from a local Pharmacy.

Plant collection and identification

The plant was collected from Bare village, in Gezawa local government, Kano state, on 12th May 2015. It was identified at the herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, and authenticated by Dr. Halilu Mshelia. The plant was given a voucher number PCG/UDUS/BURS/0001 and preserved in the herbarium for future reference.

Preparation of the extract

The bark was air dried for about 10 d and then size reduced using mortar and pestle. Two hundred gram (200 g) of the powdered bark was cold macerated with 1.5 l of distilled water for 72 h. The extract was filtered and transferred into an evaporating dish of known weight. It was evaporated to dryness in a hot air oven at 40 °C. The percentage yield was then determined.

Acute toxicity studies

Limit test at 2000 mg/kg as described by the Organisation for Economic Co-operation and Development (OECD) guidelines [11] for oral acute toxicity testing was used. All experimental animals were maintained under close observation for 14 d.

Anti-inflammatory studies

The formalin (2.5%) induced inflammation as described by Winter *et al.* (1962) [12] was used in this study. Animals were divided into 5 groups of 5 rats each. Thirty minutes before the injection of formalin, the first group received distilled water (1 ml/kg), groups 2-4 received 25, 50 and 100 mg/kg intraperitoneally (i. p) of the extract respectively. The fifth group received 10 mg/kg i. p of piroxicam. The increase in paw diameter was measured using vernier caliper (Digital caliper, Skole®0-150 mm, model number 605035 from Frits Pedersen, Denmark). The difference in the weight of the right hind paw and the left hind paw indicates inflammation. The measurement was done immediately before and after 1-5 h following formalin injection.

Analgesic studies

Acetic acid induced writhing test in mice

This test was conducted employing the method described by Koster *et al.* [13], employing abdominal constriction. Swiss albino mice were divided into five (5) groups each containing five (5) mice. The first group was given 10 ml/kg of distilled water intra-peritoneal and served as the negative control, groups 2,3 and 4 received 25, 50 and 100 mg/kg body weight respectively of the aqueous extract of *Boswellia dalzielii* stem bark intraperitoneally,while the fifth group received piroxicam (10 mg/kg), intraperitoneally. Thirty (30) minutes later, mice in all the groups were treated with acetic acid (1% acetic acid of 1 ml per kg body weight, i. p.). Five minutes after acetic acid injection, mice were placed in individual cages and the number of abdominal contraction was counted for each mouse for a period of 10 min. Percentage inhibition of writhing was calculated using the formula:

$$\text{Inhibitaon (\%)} = \frac{\text{Meannumber of writhing (control)} - \text{Meannumber of writhing (test)}}{\text{Meannumber of writhing (control)}} \times 100$$

Formalin-induced nociception in rats

The formalin-induced pain test in rats as described by Duduisson and Dennis [14] which involves recording the severity of pain as a score was used. Animals were grouped into 5 groups of 5 animals each. Distilled water (10 ml/kg intraperitoneal) was given to group 1 to serve as negative control. The aqueous extract (25, 50 and 100 mg/kg intraperitoneal) was given to groups 2, 3 and 4, respectively. Piroxicam (10 ml/kg intraperitoneal) was given to group 5 to serve as positive control. Thirty (30) minutes post-treatment, 0.05 ml of 2.5% formalin was injected under the planter surface of the left hind paw. The rats were then placed in transparent boxes for observation. The severity of the pain was recorded as scores: (0), rat walked or stood firmly on the injected paw: (1), rat partially elevated or favoured the paw: (2), rat elevated the paw from the floor: or (3), rat licked, bit or shook the paw. The cut off point for the observation were every 2 min for the first 10 min (early phase) and at every 5 min for the period between 10th and 60th minute (late phase). The Percentage inhibition was calculated from the expression

$$Inhibition(\%) = Mean\ (control) - Mean\ (treated)/Mean\ (control) x100$$

Statistical analysis

The results of the experiment are represented as mean±standard error of the mean (SEM). The results were analyzed using GraphPad instant version 3 software and student's *t*-test was employed for comparing the means between groups. Differences were considered to be significant at $p<0.05$.

RESULTS

Percentage yield of the plant extract

The total weight of the extract obtained was 13 g and it was extracted from 200 g of the powdered stem bark of *Boswellia dalzielii.* The percentage yield of the plant extract was calculated to be 6.5%.

Acute toxicity studies

A single oral administration of the extract (2000 mg/kg) caused restlessness, itching of the mouth, and sleep in all the animals and no mortality occurred within 24 h after administration and after fourteen days observation. The LD_{50} of the stem bark extract of *Boswellia dalzielii* is, therefore, more than 2000 mg/kg.

Acetic acid induced writhing test

The results of the inhibition of acetic acid-induced abdominal writhing in rats are presented as mean±SEM. *Boswellia dalzielii* at the dose of 25, 50 and 100 mg/kg exhibited a significant inhibition of the writhing at the rate of 55.43, 69.56 and 71.73% respectively. The standard drug piroxicam (10 mg/kg) also produce a significant inhibition of the acetic acid writhing with a percentage inhibition of 59.78%. The percentage inhibition obtained at the dose of 50 and 100 mg/kg (69.56 and 71.73%) were higher than that of the standard drug piroxicam (59.78%). This is shown in table 1.

Table 1: Effect of *Boswellia Dalzielii* on acetic acid induced writhing response

Treatments	Dose (mg/kg) i. p.	No. of abdominal writhing	% Inhibition
Distilled water	10 (ml/kg)	18.4±1.97	0.00
Boswellia dalzielii	25	8.2±2.01*	55.43
Boswellia dalzielii	50	5.6±1.17*	69.56
Boswellia dalzielii	100	5.2±0.58*	71.73
Piroxicam	10	7.4±3.12*	59.78

Data presented as mean±SEM, n= 5 for all groups, *Significantly different from the control at $p<0.05$

Formalin induced nociception

The aqueous extract of *Boswellia dalzielii* stem bark significantly inhibited the first phase (neurogenic pain) of the formalin-induced pain in rats at the dose 50 and 100 mg/kg in a dose-dependent manner. The percentage inhibitions obtained were 7.31, 31.70 and 48.78% for 25, 50, and 100 mg of the extract respectively. The extract also produced a significant inhibition of the late phase at the dose of 50 and 100 mg/kg. The percentage inhibitions of the extract in the late phase were 12.82, 21.79 and 39.74% for 25, 50 and 100 mg/kg respectively. When compared to the positive control (piroxicam 10 mg/kg), the test extract at 100 mg/kg showed a higher percentage inhibition in early (43.90 and 48.78%) and late phase (39.74 and 48.71), respectively (table 2).

Table 2: Effect of *Boswellia dalzielii* on formalin induced pain in rats

Treatment (mg/kg)	Early phase pain score	% inhibition	Late phase pain score	% inhibition
Distilled water 10 ml/kg	1.64±0.19	-	1.56±0.09	-
Boswellia dalzielii 25	1.52±0.17	7.31	1.36±0.09	12.82
Boswellia dalzielii 50	1.12±0.07*	31.70	1.22±0.09*	21.79
Boswellia dalzielii 100	0.84±0.11*	48.78	0.8±0.11*	48.71
Piroxicam 10	0.92±0.14*	43.90	0.94±0.07*	39.74

Data presented as mean±SEM, n= 5 for all groups, *Significantly different from the control at p<0.05Anti-inflammatory studies, the aqueous extract of *Boswellia dalzielii* did not produce a significant inhibition of the formalin induced inflammation at all doses used throughout the period of the study (table 3).

Table 3: Anti-Inflammatory activity of *Boswellia Dalzielii* in rat

Treatment (mg/kg)	1st h	2nd h	3rd h	4th h	5th h
Distilled water (10 ml/kg)	0.65±0.36	1.37±0.41	1.62±0.25	1.37±0.45	1.38±0.38
Boswellia dalzielii 25	0.73±0.21	1.92±0.45	2.10±0.54	1.88±0.33	1.53±0.16
Boswellia dalzielii 50	0.74±0.24	1.32±0.14	1.39±0.37	1.14±0.14	0.74±0.23
Boswellia dalzielii 100	0.80±0.30	1.52±0.27	1.68±0.28	1.53±0.34	1.14±0.19
Piroxicam 10	1.41±0.38	1.37±0.23	1.44±0.43	1.18±0.30	0.88±0.23

Data presented as mean±SEM, n= 5 for all groups, *Significantly different from the control at p<0.05

DISCUSSION

The percentage yield obtained after aqueous extraction of powdered *Boswellia dalzielii* bark was found to be 6.5%. This result is similar to previous studies where it was shown to have a percentage yield of 6.8% [9].

The oral median lethal dose value of the extract in rats using the up and down procedure was found to be>2000 mg/kg. A previous study showed that at doses ≤ 2000 mg/kg per oral administration of aqueous extract of *Boswellia dalzielii*, no obvious behavioural changes or toxicity signs or even death were seen within the observation period of 72 h and the LD_{50} was>2000 mg/kg [7]. The LD_{50} value obtained shows that the bark extract of *Boswellia dalzielii* is relatively non-toxic and therefore safe for use. The LD_{50} value also justifies its use traditionally without any observed toxic effect.

In the analgesic studies, acetic acid induced writhing test and formalin-induced pain in rats were used. The acetic acid-induced writhing test is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test [15]. However, the test is not specific as it does not indicate whether the activity was central and/or peripheral [16]. The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization of chemosensitive nociceptors by prostaglandins [17]. Deraedt *et al.* [18] described the quantification of prostaglandins in the peritoneal exudates of rats by radioimmunoassay, obtained after intraperitoneal injection of acetic acid. They found high levels of prostaglandins PGE2α e PGF2α during the first 30 min after acetic acid injection. Nevertheless, it was found that intraperitoneal administration of acetic acid induces not only the liberation of prostaglandins but also the liberation of sympathetic nervous system mediators [19 and 20]. The extract (25, 50 and 100 mg/kg) significantly inhibited acetic acid-induced writhing in mice in a dose-dependent manner,with the extract at the highest dose (100 mg/kg) showing a higher percentage inhibition compared with piroxicam (10 mg/kg) which is the standard analgesic used in the study. Similar studies conducted on *Caralluma dalzielii* [21], *Schwenckia Americana* [22] and *Mimosa pudica* [23] showed that they all dose-dependently reduced the number of writhing episodes of mice in comparison with that of vehicle-treated animals. This is, however, in contrast with the result of Okechukwu *et al.* [24] where cassava leaves extract significantly inhibited abdominal writhing in mice, in a non-dose dependent manner. The analgesic effect of the extract may, therefore, be due either to its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful messages [22].

The formalin-induced model is very useful for elucidating the mechanism of pain and analgesia [25]. The formalin test has a distinctive biphasic nociceptive response termed early and late phases. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase [16]. The first phase (neurogenic phase) is probably a direct result of stimulation of nociceptors in the paw. Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain [26]. This phase, therefore, reflects centrally mediated pain while the late phase (inflammatory phase) is due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons [25]. The crude aqueous extract of *Boswellia dalzielii* exerted a significant, dose-dependent suppression of both phases as observed in the dose tested (50 and 100 mg/kg) suggesting the presence of both central and peripheral effects. However, the extract has a greater inhibitory effect on the neurogenic pain (early phase). A previous study carried out on *Caralluma dalzielii* showed that the extract inhibited the two phases of formalin-induced pain, suggesting suppression of both neurogenic and inflammatory nociception, and thus possess both central and peripheral mechanism [21]. The result obtained in *Caralluma dalzielii* is similar to that obtained with *Boswellia dalzielii* in terms of its analgesic properties. Also in a study using the methanolic extract of *Schwenckia americana,* a significant inhibition of the early phase and late phase of the formalin-induced pain model in rats was observed [22]. The result is similar to that obtained in this studies except that, *Boswellia dalzielii* produced a significant inhibition at 50 and 100 mg only while *Schwenckia americana* produced a significant inhibition at all the doses (25, 50 and 100 mg/kg).

The formalin-induced edema model was used in the anti-inflammatory studies. Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins and bradykinin [27]. This acute inflammatory response is usually biphasic comprising of an early neurogenic phase mediated by substance P and bradykinin followed by a tissue mediated response where histamine, 5-HT, prostaglandins and bradykinin are known to be involved [28]. It is well known that inhibition of edema induced by formaldehyde in rats is one of the best suitable test procedures for sub-acute anti-inflammatory agents as it closely resembles human inflammation [29]. Inflammation induced by formaldehyde is also a model used for the evaluation of an agent with probable anti-proliferative activities [30]. In the current study, the aqueous extract of *Boswellia dalzielii* did not produce a significant reduction in the inflammatory growth in the hind paw of the rats at all the three doses used (25, 50 and 100 mg/kg). In contrast, a study on the anti-inflammatory properties of *Terminalia brownii* demonstrated a dose-dependent response to carrageenan-induced paw edema induced inflammation [31]. Also, *Boswellia serrata* a related plant

has been evaluated for analgesic and anti-inflammatory activity [32]. The different fractions of *Boswellia serrata*, essential oil (10 ml/kg), gum (100 mg/kg, resin (100 mg/kg) oleo-resin (100 mg/kg) and oleo-gum-resin (100 mg/kg) significantly reduced carrageenan-induced inflammation in rats and showed analgesic activity, as determined by acetic acid induced writhing response, formalin-induced pain, hot plate and tail flick method [32].

CONCLUSION

These results demonstrate that the crude aqueous extract of *Boswellia dalzielii* possesses analgesic but does not show anti-inflammatory activity. The research also supports the ethnomedical claim of the use of macerated *Bosellia dalzielii* bark as a pain reliever. The study also suggests the presence of ingredients with the potentials of being developed into analgesic agents. Further studies are needed for the confirmation of the mechanism of action of analgesia and to find out the active constituents responsible for this property.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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