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Boswellia dalzielii Methanol Stem Bark Extract Demonstrates Significant Analgesic Activity in Swiss Albino Mice

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Abstract: Medicinal plants are gaining popularity as safer and more natural alternatives to orthodox medicines for managing health challenges and diseases. One such plant is *Boswellia dalzielii*, a renowned tree in northern Nigeria extensively used due to its ethnomedicinal importance. To determine the analgesic properties of *Boswellia dalzielii* methanol extract (BDME), the extract was screened in mice using thermal and acetic acid-induced pain models. The extract's acute toxicity and phytochemical constituents were determined using standard protocols. The results showed that the acute toxicity of the methanol stem bark extract was greater than 2000 mg/kg. Phytochemical screening of BDME revealed the presence of flavonoids, tannins, saponins, and glycosides. The extract, at the dose of 50 mg/kg significantly ($p < 0.05$) prolonged the pain reaction time at 15 and 30 minutes in the hot plate test, and reduced acetic acid-induced writhing at the 12.5, 25, and 50 mg/kg doses. The analgesic activity of the extract may be attributed to the phytochemical contents. The findings suggest that BDME have analgesic properties and could be beneficial in alleviating painful conditions.

Introduction

Throughout history, plants have been employed as remedies, initially in the form of traditional mixtures and later as isolated active compounds, with knowledge and expertise transmitted from one generation to the next (1). The use of natural plants for human disease management dates back to the earliest human civilizations and to date, the majority of the world population relies on traditional medicine (1). Medicinal plants represent a growing alternative to orthodox drugs in managing health challenges and diseases due to their phytoconstituents. The diverse array of bioactive compounds in medicinal plants makes them valuable resources for the development of new pharmaceuticals and treatment options (2, 3). Commonly known as the frankincense tree, Boswellia dalzielii belongs to the Boswellia genus, which consists of trees in the order Sapindales. Boswellia species are moderately sized flowering plants that grow as trees and shrubs, predominantly distributed across tropical regions of Africa and Asia. *Boswellia dalzielii* stands over 13 meters tall and bears fragrant white flowers. Historically, this tree has been employed in African indigenous medicine to treat diarrhea, ulcers, hypertension, malaria, toothache, abscesses, asthma, yellow fever, mental disorders, vomiting, inflammation, diabetes, and arthritis (4, 5).

Pain is the most common disease symptom and a protective mechanism by which the body responds to harmful stimuli. It is a subjective sensory and emotional experience that alerts individuals to potentially hazardous situations and prevents further injury. Pain can originate from various sources, including mechanical, thermal, or chemical stimuli affecting pain receptors in the skin, joints, and internal organs. Once the noxious stimulus reaches the spinal cord and brain, the individual experiences pain, which can be either acute or chronic (6).

Non-steroidal anti-inflammatory drugs (NSAIDs) are synthetic medications widely prescribed for their effectiveness in alleviating pain and inflammation, and

are included in the World Health Organization's Model List of Essential Medicines. However, numerous placebo-controlled trials and meta-analyses studies have shown that NSAIDs pose risks of gastrointestinal bleeding, increased risk of cardiovascular events, potential interference with platelet function, and hepatic, renal, cerebral, and pulmonary complications (7, 8). On the other hand, opioid analgesics are natural or synthetic compounds that produce analgesic effects by binding to specific opioid receptors in the central nervous system (CNS), mimicking the action of endogenous peptide neurotransmitters. These drugs are used to treat moderate to severe pain, however, tolerance, dependence, and respiratory depression are the major drawbacks of these compounds (9, 10).

Given the limitations of the currently available treatment, investigating alternative options, one possible solution for obtaining analgesic medications is to investigate medicinal herbs and natural compounds. Traditional Chinese Medicine (TCM)-based compounds have shown promising analgesic effects. TCM-derived alkaloids such as tetrahydropalmatine, aloperine, oxysophocarpine, and others exhibit analgesic activity without the same adverse effects seen in conventional therapies (11). Conolidine, an indole alkaloid derived from the bark of Tabernaemontana divaricate has also showed promising results in suppressing both acute and persistent pain (12). Therefore, the present study focuses on evaluating the analgesic potential of the methanol extract of *Boswellia dalzielii* (BDME). This natural product has shown promising antinociceptive, anti-inflammatory, and anti-arthritic effects in preclinical studies (13, 14). Further research on BDME could provide valuable insights into its potential as an alternative analgesic therapy.

Experimental Section

Drugs and Chemicals

Acetic acid (Oak Chemicals Allied and Inter Trade Ltd), Methanol (Brenntag Nigeria), Pentazocine (Rambaxy), and Diclofenac sodium (Hovid).

Laboratory Animals

Forty-six mice weighing between 24-28 g were sourced from the animal house facility of the Department of Pharmacology, Bauchi State University Gadau. The mice were maintained under standard laboratory conditions following a protocol approved by the Faculty of Basic Medical Sciences Research and Ethics Committee (FBMSRC) (BASUG/FBMS/REC/VOL.3/0095). They were provided with a standard diet and clean water.

Collection of Plant Material

The stem bark of *Boswellia dalzielii* was collected in Bojinji village, Bauchi State, Nigeria, and was authenticated at the Herbarium Unit within the Department of Biological Sciences at Bauchi State University, Gadau. Dr. U.A. Muhammed verified the plant to be *Boswellia dalzielii* and assigned a voucher number 0020 for future reference.

Extraction of Plant Material

The collected stem bark was thoroughly washed, cut into pieces, air-dried under shade, and ground into a fine powder using a pestle and mortar. The course powder weighing 100 g was subjected to cold maceration with 100% methanol for 72 hours. The resulting mixture was filtered using filter paper and then evaporated at a temperature of 45–50^{o}C until a constant weight of the extract was obtained.

Preliminary Phytochemical Screening

The identification of the phytoconstituents in Boswellia dalzielii methanol extract was carried out using standard conventional protocols for qualitative phytochemical tests (15, 16).

Acute Toxicity Studies

Swiss Albino mice of both sexes (with weights ranging from 25–28 g) were chosen for acute toxicity studies according to the guidelines outlined by OECD423 (17). Both sexes of the experimental animals were included because males and females can respond differently to the same substance due to biological differences such as hormonal levels, metabolism, and body size, thus, it allows for a more comprehensive understanding of the potential toxic effects across a broader demographic (18). The animals were randomly assigned into two groups: Group 1 served as the control group and received only normal saline (5 mL/kg), while Group 2 was administered the extract at a dose of 2000 mg/kg. Over a period of 14 days, the animals were monitored for any toxicity or notable behavioural changes.

Acetic Acid-Induced Pain

The acetic acid-induced pain test was conducted as described by Fontenele et al. (1996) (19). Twenty mice were randomly divided into five groups, comprising four mice per group; Group 1 (negative control) was treated with normal saline (5 mL/kg); Groups 2, 3, and 4 were treated with 12.5, 25, and 50 mg/kg of Boswellia dalzielii extract, respectively; Group 5 acted as the positive control and received Diclofenac sodium at 10 mg/kg. Treatments were administered via intraperitoneal injection (i.p.). Thirty minutes post-treatment, all animals were injected with an aqueous solution of 0.06% acetic acid at 10 mL/kg. Five minutes later, the number of writhes (abdominal constrictions accompanied by backward stretching of hind limbs) was recorded for each mouse during a 10 min observation period. Results were compared with those of the negative control group, and the percentage inhibition was calculated using the Equation 1.

$$\% \, Inhibition = \frac{Num\ of\ writhing_{control} - Num\ of\ writhing_{sample}}{Num\ of\ writhing_{control}} \times 100$$ **Equation 1**

Table 1. Phytochemical constituents of *Boswellia dalzielii* methanol extract (BDME).

Test	Inference
Alkaloids	+
Saponins	+
Tannins	+
Glycosides	+

Note: (+) = Presents

Table 2. Observations from acute toxicity study of *Boswellia Dalzielii* extract in mouse.

Group	Treatment	Dose (mg/kg)	Observation Period	Behavioural Changes	Interpretation
1	Normal Saline (5 mL/kg)	0	14 days	None	Safe
2	BDME	2000	14 days	Decreased locomotion, increased sensitivity to external stimuli	Safe

Table 3. Effect of methanol stem bark extract of *Boswellia dalzielii* on acetic acid-induced writhing.

Treatment (mg/kg)	Mean Onset of Writhing	Inhibition of Writhing (%)
N/S 5 mL/kg	55.50±5.50	0
Diclofenac Sodium 10	19.00±0.41*	65
BDME 12.5	13.75±.0.75*	75
BDME 25	13.75±.0.75*	75
BDME 50	14.00±1.23*	74

Note: Data were presented as mean ± SEM and analysed using one-way analysis of variance followed by a Dunnett post hoc test; * = represents a significant difference at the $p< 0.05$ level. BDME= *Boswellia dalzielii* Methanol Extract; N/S=Normal saline.

Thermally Induced Pain (Hot Plate Test)

The hot plate test was conducted following the method described by Eddy and Leimbach (1953) (20). Mice were placed on a hot plate maintained at a temperature of 50-55℃, and the time taken for paw licking or jumping by each mouse was recorded. Mice demonstrating an initial nociceptive response within 20 seconds were included in the study. Group 1 received normal saline at 5 mL/kg, while Group 2 was administered Pentazocine at 10 mg/kg. Conversely, Groups 3, 4, and 5 were treated with BDME at 12.5, 25, and 50 mg/kg, respectively. Each mouse was repeatedly placed on the hot plate at time intervals of 0, 15, 30 and 60 min after injection, and the pain reaction time was recorded in seconds. The results were then compared with those of the negative control group.

Statistical Analysis

Statistical analysis was performed for all experimental animal models using one-way ANOVA followed by the Dunnett post hoc test, with a significance level set at $p<0.05$ (SSPS, 2010: Version 22). All test samples and standard drug parameters were compared with the control group at their respective time points.

Result

Extraction and Phytochemical Constituents

Dried stem bark powder of Boswellia dalzielii weighing 100 g gave a brownish residue weighing 10 g, which is a 10% yield of methanol stem bark extract of Boswellia dalzielii. The crude methanol stem bark extract's phytochemical screening reveals alkaloids, saponins, tannins, and glycosides (Table 1).

Acute Oral Toxicity

The methanol stem bark extract of Boswellia Dalzielii appears to be safe at a dose 2000 mg/kg following oral administration (Table 2).

Acetic Acid-induced Writhing

At the dosage of 12.5 mg/kg, BDME exhibited a substantial 75% inhibition rate, which was found to be statistically significant ($p<0.05$) compared to the negative control. Notably, escalating doses of 25 mg/kg and 50 mg/kg yielded even more pronounced inhibitory effects, with both doses showcasing impressive 75% inhibition rates. In contrast, the positive control diclofenac sodium, administered at 10 mg/kg, displayed a 65% inhibition rate, further highlighting the potency of BDME in inhibiting the targeted response (refer to Table 3 for detailed comparison).

Hot Plate-induced Pain

The results of the hot plate-induced pain in mice above indicated that the BDME at doses of 2.5 mg/kg, 25 mg/kg, and 50 mg/kg led to a statistically significant ($p<0.05$) increase in latency at all timepoints, demonstrating its analgesic properties. Similarly, pentazocine, a well-known opioid agonist, exhibited a significant increase in latency at all timepoints, underscoring its robust analgesic efficacy (refer to Table 4 for details).

Table 4. Effect of *Boswellia dalzielii* methanol extract on hot plate-induced pain.

Treatment (mg/kg)	0 min (s)	15 min (s)	30 min (s)	60 min (s)
N/S 5 mL/kg	10.00±1.29	11±4.14	03.50±0.64	02.50±0.50
Pentazocine 10	12.25±3.50	13.81±2.67	19.00±2.55*	16.75±2.14*
BDME 12.5	11.00±1.08	34.00±1.95*	23.00±2.50*	19.00±4.02*
BDME 25	12.75±1.31	29.5±3.3*	26.00±1.08*	17.50±3.66*
BDME 50	16.5±4.29	32.75±2.10*	23.5±0.96*	16.50±1.04*

Data were presented as means ± SEM and analysed using one-way analysis of variance followed by a Dunnett post hoc test; * = represents a significant difference at the $p<0.05$. BDME=Boswellia dalzielii Methanol Extract. N/S=Normal saline.

Discussion

The 10% yield of methanol stem bark extract of *Boswellia dalzielii* suggests that the extraction process is efficient and produces a relatively concentrated sample. The presence of alkaloids, saponins, tannins, and glycosides in the crude extract shows that the extract contains diverse secondary metabolites, which might contribute to its biological activities. Previous phytochemical studies of the stem bark also reported the presence of the discovered phytochemicals (21-23). Natural compound reported to possess analgesic activities include alkaloids, steroids, and saponin-containing substances (24).

Toxicity analysis of any extract is the first step in evaluating medicinal plants' pharmacological activities and potentials. It provides an insight into the effect of a chemical substance upon short-term, usually single-dose exposure. It also helps select appropriate doses for the desired pharmacological activity (25). In this study, a 2000 mg/kg dose of the extract showed no toxicity or mortality in the tested mice, indicating that the oral dose given is practically nontoxic (OECD, 2002). This suggests that the extract could potentially be used in therapeutic applications without causing harmful effects, at least at this dosage. Several previous studies have presented similar conclusions on *Boswellia dalzielii*, including one that used a dose of 3000 mg/kg of the plant and recorded no signs of toxicity (26, 27). The observed behavioural changes, including decreased locomotion and increased sensitivity to external stimuli, suggest that while the extract may not be acutely toxic, it could have some impact on the nervous system or behaviour.

Acetic acid-induced pain is typically analysed through the writhing response test, which measures the number of abdominal muscle contractions resulting from peritoneal inflammation and prostaglandin release (28, 29). Pain induced by acetic acid is via peripheral pathway, and the cyclooxygenase (COX) pathway products such as prostaglandins contribute to the development of inflammation and pain. The number of writhing seen in mice after intraperitoneal injection with acetic acid indicates the presence of pain, and the reduction in the number of writing responses is indicative of analgesic activity (29). In this test, BDME shows a significant analgesic effect. At a dose of 12.5 mg/kg, it shows a 75% inhibition rate, which statistically significant compared to the negative control (Table 3). Higher doses of 25 mg/kg and 50 mg/kg show similar inhibition rates of 75% and 74%, respectively. Interestingly, BDME at these doses shows a higher inhibition rate than Diclofenac Sodium (a positive control), which has a 65% inhibition rate at 10 mg/kg. This indicates that BDME contains constituents capable of reducing inflammatory pain caused by acetic acid in mice. The mechanism of action of *Boswellia dalzielii* in reducing writhing is not fully understood, however, previous studies suggested that the analgesic effect may be due to the activation of nitric oxide (NO)/cyclic guanosine monophosphate (cGMP)/adenosine triphosphate (ATP)-sensitive potassium channels (13). The opening of NO/cGMP/ATP-sensitive potassium channels induces membrane hyperpolarization, reducing the depolarization and action potential transmission abilities of neurons, which ultimately leads to a reduction in pain (13).

The hot plate test is a model of centrally-mediated nociception, where the latency to respond to a painful stimulus is measured. Longer latencies indicate less sensitivity to pain (30). The results (Table 4) showed that BDME at doses of 12.5 mg/kg, 25 mg/kg, and 50 mg/kg produced a significant increase in the withdrawal time of the mice, indicating a reduction in pain. This is comparable to the effects of Pentazocine. The reduction in pain may be due to the inhibition of the cyclooxygenase (COX) pathway of arachidonate metabolism, which produces prostaglandins that have various effects on blood vessels, nerve endings, and cells involved in pain and inflammation (31).

Conclusion

The phytochemical analysis of BFME uncovered the presence of various bioactive compounds, including flavonoids, tannins, saponins, and glycosides, indicating its complex chemical composition. Additionally, the acute toxicity assessment of the extract revealed a favorable safety profile with a lethal dose greater than 2000 mg/kg, suggesting its potential as a safe therapeutic agent. Moreover, in experimental models such as the acetic acid-induced writhing test and the hot plate test, BFME demonstrated notable analgesic properties, underscoring its potential as a natural alternative for pain management. These findings lay a foundation for considering BDME as a promising analgesic candidate. However, despite these

promising results, further comprehensive studies are imperative to elucidate the precise mechanism of action underlying the analgesic effects of BDME.

Declarations

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Conflict of Interest

Authors declare no conflict of interests.

Data Availability

All data supporting the findings of this study are available within the manuscript.

Ethics Statement

Ethical approval for the study was obtained from the Faculty of Basic Medical Sciences Research and Ethics Committee (FBMSRC) (BASUG/FBMS/REC/VOL.3/0095).

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