Behavioural Effects of Methanol Stem Bark Extract of *Boswellia dalzielii* Hutch (Burseraceae) in Mice

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**ABSTRACT**

Preparations of *Boswellia dalzielii* stem bark are used traditionally in Nigeria in the treatment of fever, rheumatism, gastrointestinal discomforts and mental derangements. The efficacy of this plant in the treatment of mental disorders is well acclaimed among the Gwandara communities of North-Central part of Nigeria. This study was aimed at evaluating the behavioural effects of the methanol stem bark extract of *Boswellia dalzielii* using diazepam-induced sleep, hole-board, open field and beam walking assay tests in mice at doses of 20, 40 and 80 mg/kg. *B. dalzielii* extract significantly (p< 0.05) and dose dependently prolonged the duration of diazepam-induced sleep in mice. A dose-dependent decrease in the number of head-dips which was significant at p< 0.001 was also produced by the extract. In the open field test, the extract at all doses tested (20, 40 and 80 mg/kg) significantly reduced the number of square crossing and number of rearing in a dose dependent manner. The extract did not produce motor coordination deficit in the beam walking assay. Diazepam (1 mg/kg) did not produce a significant difference in the time taken to reach the goal box either; however, it produced a significant increase (p < 0.05) in the number of foot slips compared to the control group. The results suggest central nervous system depressant action of stem bark extract of *Boswellia dalzielii* which might have contributed to its application in ethnomedicine for the treatment of mental disorders.

Keywords: *Boswellia dalzielii*, Behaviour, Exploration, Motor coordination, Sleeping time

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**INTRODUCTION**

*Boswellia dalzielii* Hutch. (family: Burseraceae) is commonly known as the frankincense tree that grows up to 13 meters high. It has a characteristic ragged pale papery bark and is locally abundant from Northern Ivory Coast to Northern Nigeria, Cameroun and Central African Republic. The common vernacular names of the plant include “Hano” or “Ba-samu” (Hausa), “Andakehi” or “Juguhi” (Fulfulde) (Dalziel, 1937). The plant has various applications in traditional medicine; in Northern Nigeria, the bark is boiled up in large quantity to make a wash for fever and rheumatism, while the fluid is taken for gastro-intestinal troubles and convulsions. The fresh bark is also used as an emetic and to relieve symptoms of giddiness and palpitations (Dalziel, 1937). Decoction of the stem bark is also used in North-Central part of Nigeria in the treatment of mental illness (Ibrahim *et al.*, 2007). The stem bark has been found to contain chemical constituents including phenolic compounds such as protocatechuic acid, gallic acid and ethylgallate as well as a diterpenoid - incensole and a triterpenoid - 3-O-acetyl-11-keto-b-boswellic acid (Alemika *et al.*, 2004; Olukemi *et al.*, 2005).

Previous studies have been reported on the antimicrobial (Adelakun *et al.*, 2001), antiulcer (Nwinyi *et al.*, 2004), antispasmodic (Hassan *et al.*, 2009), hepatoprotective (Odeghe *et al.*, 2012), and hypoglycemic (Balogun *et al.*, 2013) properties of *Boswellia dalzielii* stem bark extract. To our knowledge, there is no report in literature on the behavioural effects of the stem bark extract of the plant. The present study was therefore aimed at providing scientific basis on the use of stem bark extract of *Boswellia dalzielii* in traditional medical practice for the treatment of mental disorder.

**MATERIALS AND METHODS**

**Test drugs and chemicals:** Diazepam 10 mg in 2 ml (Valium® Roche Ltd) was used as the standard drug. Solvents used
include methanol, petroleum ether, chloroform and ethyl acetate (Sigma Chemical Co. St. Louis, USA). Methanol stem bark extract of *Boswellia dalzielii* was weighted and reconstituted with distilled water prior to use.

**Plant material:** The stem bark of *Boswellia dalzielii* was collected at Galadimawa, Giwa Local Government of Kaduna State, Nigeria, in the month of March, 2013. The plant was identified and authenticated in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, by Mallam Umar S. Gillah. Specimen number was obtained (No. 2448) and a voucher specimen was deposited for future reference.

**Preparation of plant extract:** The stem bark was washed and air dried until constant weight was obtained. It was then size reduced to coarse powder using pestle and mortar, after which 400 g was extracted with petroleum ether in a soxhlet apparatus for 24 h. The marc was air dried and then re-extracted to exhaustion with 800 mL of absolute methanol. The extract was then concentrated under reduced pressure to give solid residue and later stored in a dessicator until required in the main study.

**Phytochemical screening:** Thin layer chromatographic analysis was carried out on the methanol stem bark extract of *Boswellia dalzielii* according to method described by Wagner and Bladt, (1996). The solution of the extract was spotted on silica gel-coated thin layer chromatographic (TLC) plates (4 cm×8 cm). The plates were developed in a solvent system (Chloroform: Ethylacetate, 5:3). Each plate was sprayed with a different visualizing reagent to screen for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannins.

**Animals:** Swiss Albino mice (18-25 g) of either sex obtained from the Animal House of the Department of Pharmacology and Therapeutics, A.B.U., Zaria were used for the study. The animals were maintained in a well-ventilated room under ambient temperature and fed with standard diet (Feeds Master, Ilorin, Nigeria) and water *ad libitum*. The experimental protocols were approved by the University Animal Ethics Committee with the protocol number DAC/IW-OT/013-13. The animals were handled in accordance with the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health (Publication No. 80-23, revised 1996).

**Acute toxicity study:** The intraperitoneal median lethal dose was estimated using the method described by Lorke, (1983) in mice. The study consisted of two phases, with an initial phase in which three groups each containing three mice received the methanol stem bark extract at doses of 10, 100 and 1000 mg/kg respectively, followed by observations for signs of toxicity and death within 24 h. In the second phase, four mice were treated with specific doses each (200, 400, 800 and 1600 mg/kg, determined from outcome of phase one) of the extract respectively and observed for signs of toxicity and death within 24 hours. The LD₃₀ was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

**Behavioural studies**

**Diazepam-induced sleep test in mice:** The method described by Rakotonirina et al., (2001) was used. Twenty mice were divided into four groups each containing five mice. The first group was pretreated with normal saline 10 mL/kg to serve as control. The second, third and fourth groups were pretreated with 20, 40 and 80 mg/kg of the extract respectively all through the intraperitoneal route. Thirty minutes post treatment, mice in all groups received diazepam at 25 mg/kg *i.p.*. Thereafter, the onset and duration of sleep were determined. Loss of righting reflex was considered as the criterion for sleep (Rolland et al., 1991), while the interval between the loss and recovery of righting reflex was considered as the duration of sleep (Fujimori, 1965).

**Hole-board test for exploratory activity in mice:** The method described by File and Wardill, (1975) was adopted. The apparatus used was a wooden board (60 cm x 30 cm) with sixteen evenly spaced holes (2 cm diameter x 2 cm depth). Thirty mice were randomly divided into five groups of six mice each. The mice in the first group served as negative control and received normal saline 10 mL/kg. The second, third and fourth groups received 20, 40 and 80 mg/kg of the extract respectively, while the fifth group served as positive control and received 0.5 mg/kg diazepam. All administrations were through the intraperitoneal route. Thirty minutes post treatment, each mouse was placed individually at one corner of the board and allowed to move about. The number of head dips into the holes was counted with the aid of a tally counter during a 5 min. period (Wolfman et al., 1994).

**Open field test in mice:** The open field apparatus is made up of white plywood and measuring 72 cm×72 cm×36 cm (length×breadth×height). One of the walls is a clear plexiglas, so that the mice could be visible in the apparatus. Blue lines were drawn on the floor with a marker and were visible through the plexiglas. The lines divided the floor into 16 squares (18 cm×18 cm) with a central square (18 cm×18 cm) drawn in the middle of the open field (Brown et al., 1999). Thirty mice were randomly divided into five different groups of six mice each. The first group was administered normal saline (10 mL/kg). The second, third and fourth groups of mice received 20, 40 and 80 mg/kg of the extract respectively, while mice in the fifth group received 0.05 mg/kg diazepam all through the intraperitoneal route. Mice were carried to the test room in their cages and were handled by the base of their tails at all times. Each mouse was placed individually in a corner of the open field and allowed to explore the apparatus for 5 min. After the 5 mins., the mouse was returned to the home cage and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. The behaviours scored in the experiment include line crossing, center square entry and rearing.

**Beam walking assay in mice:** The method described by Stanley et al., (2005) was used. Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by wooden support to a goal box (enclosed hamster house). Three trials were performed for
each mouse, and were designed such that the mice tested would be aware that there was a goal box that could be reached. A ruler was used because the mouse found this easy to cross and at the same time, it induced minimum anxiety. The mice that successfully walked along the ruler were randomly grouped into five groups each containing five mice. The first group received normal saline (control) at the dose of 10 mL/kg i.p. The second, third and the fourth groups received the methanol extract at doses of 20, 40 and 80 mg/kg respectively, i.p., while the fifth group received diazepam (1 mg/kg, i.p.). The beam was made of wood, 8 mm in diameter, 60 cm long and elevated 30 cm above the bench by a metal support. Thirty minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from, with a maximum time of 60 s allowed on the beam. The number of foot slips (one or both hind limb slipping from the beam), time taken to complete the task and number of falls were observed.

Statistical analysis
Data were expressed as mean ± standard error of mean (S.E.M.). Difference between means was analyzed by one way analysis of variance (ANOVA). Statistical significance was obtained with ANOVA followed by Dunnett’s post hoc test. Values of p < 0.05 were considered significant.

RESULTS
Methanol stem bark extract of Boswellia dalzielii thus obtained was semi-solid in nature and possessed dark brown colour with honey-like smell. Percentage yield of the extract was 8.66% w/w. Thin layer chromatographic analysis on the extract revealed the presence of cardiac glycosides, flavonoids, saponins and tannins while alkaloids and anthraquinones were absent.

Acute toxicity studies: The intraperitoneal median lethal dose of methanol stem bark extract of Boswellia dalzielii was estimated to be 565.7 mg/kg in mice. The signs of toxicity were decreased locomotive activity and respiratory depression.

Behavioural studies
Effect of methanol stem bark extract of B. dalzielii on diazepam-induced sleep in mice: Boswellia dalzielii extract did not show a significant decrease in the onset of sleep, however, a dose dependent and significant increase in the duration of sleep was produced at 20 mg/kg F [(3, 16) = 18.12, (p< 0.01)] and at 40 and 80 mg/kg F [(3, 16) = 18.12, (p< 0.001)] compared to control group (Fig. 1).

Fig. 1: Effect of methanol stem bark extract of Boswellia dalzielii (MEBD) on onset and duration of diazepam-induced sleep in mice. MEBD significantly prolonged the duration of sleep compared to control group F [(3, 16)= 18.12, MEBD 20 mg/kg **p< 0.01, MEBD 40 and 80 mg/kg ***p< 0.001; n = 5] One way ANOVA followed by Dunnett’s test, DZP = Diazepam (25 mg/kg), N/Saline = Normal saline

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Fig. 2: Effect of methanol stem bark extract of Boswellia dalzielii (MEBD) on exploratory behaviour in mice. MEBD and diazepam significantly decreased the number of head dip compared to normal saline F [(4, 25) = 23.04, ***p< 0.001; n = 6] One way ANOVA followed by Dunnett’s test
Effect of methanol stem bark extract of *Boswellia dalzielii* on performance of mice in open field test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Number of centre square crossing</th>
<th>Number of square crossing</th>
<th>Number of rearing</th>
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<tr>
<td>NS</td>
<td>10 mL/kg</td>
<td>1.00 ± 0.00</td>
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<td>21.67 ± 1.41</td>
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<tr>
<td>MEBD</td>
<td>20</td>
<td>1.00 ± 0.00</td>
<td>37.00 ± 6.17*</td>
<td>09.50 ± 2.84***</td>
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<tr>
<td>MEBD</td>
<td>40</td>
<td>1.00 ± 0.00</td>
<td>24.17 ± 8.64**</td>
<td>06.00 ± 1.34***</td>
</tr>
<tr>
<td>MEBD</td>
<td>80</td>
<td>0.00 ± 0.00</td>
<td>08.83 ± 2.51***</td>
<td>03.00 ± 0.83**</td>
</tr>
<tr>
<td>DZP</td>
<td>0.05</td>
<td>1.00 ± 0.00</td>
<td>35.67 ± 5.61*</td>
<td>08.00 ± 2.22***</td>
</tr>
</tbody>
</table>

Values are presented as Mean ± SEM, MEBD produced a significant increase in number of square crossing F (4, 25) = 6.62, *p< 0.05; **p< 0.01; ***p< 0.001; n=6) and number of rearing F (4, 25) = 3.35, **p< 0.01; n=6) compared to control group - One way ANOVA and Dunnett’s test, MEBD = Methanol stem bark extract of *Boswellia dalzielii*, DZP = Diazepam, NS = Normal saline

Effect of methanol stem bark extract of *B. dalzielii* on exploratory behavior in mice: The exploratory behaviour studies showed that *Boswellia dalzielii* stem bark extract at doses of 20, 40 and 80 mg/kg exhibited a dose-dependent and significant decrease F (4, 25) = 23.04, (p< 0.001) in the number of head dips compared to the control group. Similar decrease in the number of head-dip was obtained with diazepam at 0.05 mg/kg but the effect was less when compared to the extract at 40 and 80 mg/kg (Fig. 2).

Effect of methanol stem bark extract of *B. dalzielii* on performance of mice in open field test: *Boswellia dalzielii* stem bark extract (20, 40 and 80 mg/kg) significantly reduced the number of square crossings F (4, 25) = 6.62, (p< 0.05) in a dose dependent manner when compared to control group. The extract also dose dependently reduced the number of rearing with significant difference F (4, 25) = 3.35, (p< 0.001) at all the doses tested. However, there was no significant difference in the number of crossings of center square (Table 1).

Effect of methanol stem bark extract of *B. dalzielii* on motor coordination in mice: The methanol extract of *Boswellia dalzielii* at doses of 20, 40 and 80 mg/kg did not show any significant difference in time to reach the goal box and number of foot slips compared to the negative control. Diazepam (1 mg/kg) did not produce a significant difference (p > 0.05) in the time taken to reach the goal box whereas, for the high dose of 80 mg/kg, a significant increase F (4, 17) = 3.18, (p< 0.05) in the mean number of foot slips when compared to the control group (Table 2).

DISCUSSION

The estimated LD₅₀ on *Boswellia dalzielii* stem bark extract showed that the extract is slightly toxic in mice following intraperitoneal administration according to Lu, (1996) classification of LD₅₀ values. Nevertheless, the doses of the extract used in this study were lower than 30% of the LD₅₀ and have been shown to be relatively safe for ethnopharmacological research (Vongtau et al., 2004).

Sedatives, hypnotics and tranquilizers as well as antidepressants at high doses are known to prolong diazepam induced sleeping time (Rakotonirina et al., 2001; Vogel, 2008; Musa et al., 2016). Neuroleptics also prolong diazepam induced sleeping time while analeptics and stimulants shorten sleeping time (Vogel, 2008). Potentiation of diazepam-induced sleep by *Boswellia dalzielii* stem bark extract suggests that it possesses central nervous system depressant activity and sedative-like property (Perez et al., 1998; Rakotonirina et al., 2001). Sedative-hypnotic agents act to increase GABA-mediated synaptic inhibition either by directly activating GABAA receptors or by enhancing the action of GABA on GABAA receptors. Benzodiazepines and barbiturates are examples of therapeutic agents that act at possible allosteric modulators at GABAA receptors (Johnston, 2005). The ability of *Boswellia dalzielii* stem bark extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission.

The hole-board test is an accepted experimental model for the evaluation of psychotic, sedative and anxiety condition in animals (Crawley, 1985). A decrease in the number of head dips reveals a sedative behaviour (File and Pellow, 1985), a high propensity for antipsychotic action (Feilding and Lal, 1978) and a measure of central nervous system (CNS) depressant activity (Adzu, 2002). The decrease in exploratory behaviour as observed with methanol stem bark extract of *Boswellia dalzielii* further supports its sedative property.

Exposure to a novel environment is associated with emotional disturbance and anxiety and therefore, preference of the animal to stay close to the walls is a natural tendency of rodents when anxious. The number of entries into and the time spent in the central arena as well as the number of rearing are thus measures of anxiety in the open field test (Sethi et al., 2005). The methanol stem bark extract of *B. dalzielii* produced a dose-dependent and significant decrease in the number of square crossings and the number of rearing. This tendency could also be attributed to the sedative effect of the extract at tested doses.
The beam-walking assay tests the effect of novel compounds on motor coordination in laboratory animals. The number of foot slips has been found to be a sensitive measure in determining benzodiazepine-induced motor coordination deficits and is a good predictor of doses producing clinical sedation (Stanley et al., 2005). Boswellia dalzielii extract did not produce a significant effect on motor coordination in mice and this confirms that its observed sedative activity might be through central mechanisms and not peripheral neuromuscular blockade.

The biological actions produced by plant extracts are usually attributed to the presence of secondary metabolites (Kens and Yasmin, 2011). Phytochemical constituents like flavonoids, saponins and tannins have also been reported by to be useful in many CNS disorders (Verna et al., 2010). Saponins for example have been reported to possess potent sedative effects (Jiang et al., 2007) while flavonoids were found to be ligands for the GABA receptors in the CNS which led to the assumption that they can act as benzodiazepine-like molecules (Jäger and Saaby, 2011). The CNS depressant effect of Boswellia dalzielii may thus be due to the presence of its phytochemical constituent(s). However, further studies are needed to isolate and characterize the bioactive constituents responsible for the observed effect.

In conclusion, the results obtained from this study provided scientific evidence that suggest that stem bark extract of Boswellia dalzielii contains bioactive constituents with neurosedative effects and lend credence to the ethnomedicinal use of the plant in treatment of mental disorders.

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Conflict of interest
The authors declared no conflicts of interest.

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