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SOME BEHAVIOURAL STUDIES ON METHANOL ROOT BARK EXTRACT OF *BURKEA AFRICANA* (FABACEAE) IN MICE

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ABSTRACT
Burkea africana is a plant that belongs to then family Fabaceae; it is widely spread in tropical Africa including Nigeria. It is of valuable in ethnomedicine especially in the treatment of antidote for venomous stings and bites, cutaneous and sub cutaneous parasitic infection, convulsion and pulmonary troubles. The research was conducted to evaluate some central nervous system properties of the root bark methanol extractof B. africana in mice. It involved the following animal models: diazepam-induced sleep, hole-board and walking beam assay. Results: The methanol extract showed a significant decrease in the onset of sleep at doses of 40 mg/kg and 80 mg/kg (p<0.05); as well as produced significant increase in the duration of sleep (40 and 80 mg/kg) at p<0.05, p<0.005 respectively. The number of head dips significantly increased at 20 and 80 mg/kg (p<0.05 and 0.005 respectively). From the beam walking test for motor deficits, the result showed a significant increase in the number of foot slips at doses of 20 mg/kg (p<0.05); 40 and 80 mg/kg (p<0.005), where as there was no significant difference in the time taken to cross the two ends of the beam (time taken to complete the task). The median lethal dose (LD_{50}) value of B. africana extract was found to be 288.5 mg/kg (i.p) in mice. The preliminary phytochemical screening revealed the presence of carbohydrates, saponins, flavonoid, aglycones, tannins, anthraquinones, cardiac glycosides, unsaturated steroids and triterpenes. Our results suggest that the B. africana extract contains biologically active compounds with potential sedative and anxiolytic properties.
Key Words: Sedation, B. Africana, Diazepam, ethnomedicine

INTRODUCTION

Drugs that act upon the central nervous system (CNS), which may either cause CNS stimulation or depression, influence the daily lives of everyone (Charles and Stitzel, 2003). Most drugs used as sleep aids are known to increase the function of the inhibitory neurotransmitter gamma amino butyric acid (GABA) in the brain (Wilson and Nutt, 2007). This enhancement of neuronal inhibition by GABA is one of the most powerful therapeutic strategies for treatment of CNS diseases such as sleep disturbances, anxiety disorders, muscle spasms, and seizure disorders (Mohler, 2001). A number of medicinal plants have been found and put into use in ethnomedicine by traditional healers in the management of many ailments for many years (Sofowora, 1993). Many medicinal plants are believed to be important source of new chemical compounds with potential therapeutic effects (Eisner, 1990). The stem bark of *Burkea africana* is used as antidote for venomous stings and bites, cutaneous and sub cutaneous parasitic infection, convulsion, pulmonary troubles. The plant is indigenous to Tropical Africa including Nigeria and commonly known as *Bakin makarho* and *Apasa* among the Hausas and Yoruba, respectively (Burkill, 1985). Previous works revealed that the stem bark extract of the plant possess pharmacological properties which include antioxidant and weak antimicrobial activity (Mathisen *et al*., 2002); antidiarrhoeal activity (Tanko *et al.,* 2011); anticonvulsant activity (Yaro *et al*., 2010; Mahdi *et al*., 2013) and antioxidative stress of the polyphenolic-rich stem bark fractions in cell lines (Cordier *et al*., 2013). Therefore, this study is aimed at evaluating the root bark methanolic extract of *B. africana* for some behavioural activity in mice. To the best of our knowledge, this has not been reported elsewhere.

MATERIALS AND METHODS

Animals

Swiss albino mice of both sexes with body weight range from 18 - 24 g, bred in the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria were used. The Committee on Animal Use and Handling of the Department granted permission for their use in the experiment. They were kept in plastic cages with stainless steel wire mesh covers, floored with wood shavings to absorb urine, faecal matter and spilled water and maintained at a relative humidity and room temperature. The animals were used in compliance with the National Institute of Health Guide for the Care and use of Laboratory Animals (Publication nos. 85-23, revised 1985).

Plant Material

The plant material was collected from Sabon Gari, Zaria, Kaduna State, Nigeria, in the month of July (rainy season).

It was identified by a taxonomist at the Herbarium Section of Department of Biological Sciences, Ahmadu Bello University Zaria. A voucher number was assigned (V/No. 075) and the specimen deposited in the Herbarium for future reference. The root bark of the plant was then allowed to dry under shade until constant weight was obtained.

Preparation of the Extract

The dried root bark was crushed and size-reduced to fine powder using mortar and pestle. Ground powder (500g) was cold macerated with 1000 ml of methanol for one week with occasional shaking. The extract was filtered and concentrated under reduced pressure, at a temperature of about 40^0C, and this gave a brown mass yield.

Drugs, Chemicals and Equipments

Diazepam (Roche Product Ltd. Switzerland) was the drug used in this study**;** Metler balance (P162 Gallen Kamp, UK), Weighing balance (Ohio, New York, USA), hole board 40 × 40 cm with equidistant holes, wooden beam rod (8 mm × 60 cm) and wooden beam rod (8 mm × 60 cm) were the equipments used.

Diazepam-induced Sleeping Time in Mice

Mice of either sex were divided into four groups of six each. Group 1 received normal saline (10 ml/kg) and served as control, while those in Groups 2, 3 and 4 received the extract at doses of 20, 40 and 80 mg/kg (*i.p.*) respectively. Thirty minutes post-treatment, all the animals were administered diazepam (30 mg/kg, *i.p.*). Each mouse was observed for the onset and duration of sleep, with the criterion of sleep being loss of righting reflex (Wambebe, 1985; Amos *et al*., 2001). The time from the loss of righting reflex to recovery was recorded as sleeping time (Soulimani *et al*., 2001).

Exploratory Behavior in Mice

This study was done using the head-dip test on the hole-board (Ramirez *et al*., 1998). It was carried out using wooden board (40 × 40 cm) with four equidistant holes (1 cm diameter, 2 cm depth). Mice of either sex were divided into five groups of six mice each. Animals in Group 1 received normal saline (10 ml/kg, *i.p.*) and served as control, while those in Groups 2, 3 and 4 received the extract at doses of 20, 40 and 80 mg/kg *i.p.* respectively. The animals in Group 5 received diazepam (0.5 mg/kg, *i.p.*). Thirty minutes after treatment, each mouse was placed at one corner of the board and allowed to move about and dipped its head into the holes indicating exploratory behaviour. The number of times the mice dipped their heads into the holes during the 5-minutes period was counted and recorded.

Beam Walking Test for Motor Coordination Deficits in Mice

The study was done according to the method described by Stanley *et al* (2005). Mice were trained to travel from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by metal supports, to a goal box. 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. Mice of either sex were divided into five groups of six mice each. Group 1 received normal saline (10 ml/kg, *i.p.*) and served as control, while Groups 2, 3 and 4 received 20, 40 and 80 mg/kg i.p. doses of extract, respectively. Group 5 received diazepam at a dose of 0.5 mg/kg. *i.p.* Thirty minutes later, each mouse was placed at one end of wooden beam (8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal supports), and allowed to walk to the box within a maximum of 60 s. The time taken on the beam, number of falls and the number of foot slips were counted and recorded.

Statistical Analysis

Results were presented in tables and expressed as Mean ± SEM. The level of significance between means was tested by one-way ANOVA followed by Dunnett's Posthoc multiple test for comparison and results were regarded as statistically significant from $P<0.05$.

RESULTS

The root bark extract of *B. africana* gave a brown mass yield weighing 68.47 g (13.70%). The extract produced significant ($p<0.05$) activity at 20, 40 and 80 mg/kg; a decreased onset and an increased duration of sleep. The activity was more on the duration of sleep ($p<0.005$) at 80 mg/kg (Table 1).

Also, there was significant increase in the number of head dips as a measure for exploratory behaviour; diazepam (0.5 mg/kg) was at $p<0.005$ and that of the extract (20, 40 and 80 mg/kg) was at $p<0.05$ (Table 2).

Again, the extract gave an array of significant activity in the test for motor coordination. There was significant increase in the number of hind limb slips for diazepam (0.5 mg/kg) and the extract treated (20, 40 and 80 mg/kg) groups, at $p<0.001$. Whereas the time taken for the mice to move along the beam was significantly ($p<0.05$) increased at 20, 40 and 80 mg/kg (Table 3).

The preliminary phytochemical screening revealed the presence of carbohydrates, saponins, flavonoid aglycones, tannins, anthraquinones, cardiac glycosides, unsaturated steroids and triterpenes (Table 4).

Table 1: Effect of Methanol Leaf Extract of *Burkea africana* (BA) on Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Mean Onset of sleep (min)	Mean Duration of Sleep (min)
N/saline (10 ml/kg)	3.50 ± 0.8	94.30 ± 13.30
BA 20	4.80 ± 2.2	76.00 ± 40.40
BA 40	2.00 ± 0.3 [b]	219.80 ± 67.8 [b]
BA 80	2.00 ± 0.3 [b]	377.2 ± 34.80[a]

Values are presented as Mean ± SEM, n = 6 per group, BA = *Burkea africana,*
Significant difference from control (Saline) group at [a]$p<0.005$ [b]$p<0.05$, (ANOVA test).

Table 2: Effect of Methanol Leaf Extract of *Burkea africana* (BA) and Diazepam (DZ) on Exploratory Behaviour in Mice

Treatment (mg/kg)	Number of Head Dips in 5 min
N/saline (10 ml/kg)	7.2 ± 1.1
BA 20	10.4 ± 0.5[b]
BA 40	11.4 ± 2.6[b]
BA 80	11.6 ± 2.2[b]
DZ 0.5	20.0 ± 2.3[a]

Values are presented as Mean ± SEM, n = 6 per group, BA = *Burkea africana*, DZ = diazepam, significantly different from control (Saline) group at [a]p<0.005 and [b]p<0.05 (ANOVA test).

Table 3: Effect of Methanolic Leaf Extract of *Burkea africana* (FV) and Diazepam (DZ) on Motor Coordination Deficit in Mice

Treatment (mg/kg)	Number of Hind Limb Slips	Time taken for the Task (s)	Number of Falls
N/saline	0.00	6.0 ± 0.4	0/6
BA 20	3.7 ± 0.8 [a]	9.8 ± 1.0 [b]	0/6
BA 40	2.8 ± 0.7 [a]	9.4 ± 1.2 [b]	0/6
BA 80	3.5 ± 0.9 [a]	10.5 ± 1.6 [b]	0/6
DZ 0.5	5.3 ± 0.8 [a]	8.3 ± 0.7[b]	0/6

Values are presented as Mean ± SEM, n = 6 per group, BA = *Burkea africana*, DZ = Diazepam, Significant difference between control (saline) group at [a]p<0.001, [b]p<0.05, (ANOVA test).

Table 4: Preliminary Phytochemical Screening of the Methanol Root Bark Extract *Burkea africana*

Constituents	**Inference**
Carbohydrates	+
Flavonoids	+
Tannins	+
Alkaloids	-
Cardiac glycosides	+
Saponins	+
Triterpenes	+
Antraquinones	+
Unsaturated steroids	+

Key: + (present); - (absent)

DISCUSSION

The extract of *B. Africana* significantly decreased the onset of sleep, and increased the duration of sleep (Table 1). This effect may be due to inhibition of diazepam metabolism (Kaul and Kulkarni, 1978) or an action on the central mechanisms involved in the regulation of sleep (N'Gouemo *et al*., 1994; Amos *et al*., 2001). It has also been reported that activation of $GABA_A$ receptor in the CNS is known to favour sleep (Gottesmann, 2002). Hypnotic effects are classically considered to involve more pronounced depression of the central nervous system than sedation, and this can typically be achieved by increasing the dose of sedative-hypnotic drugs and therefore bring about shortening of sleep latency, increase of total sleep time and sleep efficiency (Tobler *et al*., 2001). Standard drugs such as diazepam and phenobarbitone are thought to produce their effects by enhancing GABA-mediated inhibition in the brain (Rogawski and Porter, 1990). Thus, the shortening of sleep latency and an increased sleep duration observed in this study as well as its anticonvulsant activity previously reported by Yaro *et al*., (2010) could be linked to GABA-mediated CNS inhibition.

The hole-board test is a measure of exploratory behaviour in mice; to evaluate psychotic, sedative and anxiety condition in animals (Crawley, 1985; Danjuma *et al*., 2008). Agents that decrease this parameter reveal a sedative behaviour (File and Pellow, 1985). According to Takeda *et al* (1998), anxiolytic agents have been shown to increase the number of head dips in the hole-board test. Therefore, the increase in exploratory behaviour, characterized by an increase in the number of head dips may serve as a basis to predict possible anxiolytic effect of the extract in comparism to low dose of diazepam (Table 2).

Beam walking test assess benzodiazepine-induced ataxia as a predictor of sedative effects by measuring the extent of motor deficits caused by damage to the motor cortex, and it is highly defined by the extent of foot slips (Stanley *et al*., 2005). The extract significantly increased the number of foot slips and time taken to complete the task on the beam (Table 3). This may also be an indicator that the extract might have its effect via peripheral neuromuscular blockade (Perez *et al*., 1998) and mimicked the effect of benzodiazepine in producing ataxia, thus, possess sedative property.

CONCLUSION

It can be said that the extract contains biologically active compounds which were responsible for the observed pharmacological effects. Thus, the results suggest possible central depressant and/or anxiolytic effects of the root bark extract of the plant.

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