



# International Journal of Pharmacology

ISSN 1811-7775

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## Behavioural Effects of Hydroalcoholic Stem Bark Extract of *Randia nilotica* stapf. in Mice

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**Abstract:** The behavioural effects of hydroalcoholic stem bark extract of *Randia nilotica* on the central nervous system were investigated. The tests employed were pentobarbitone sleeping time, amphetamine stereotyped behaviour, exploratory activity, performance on treadmills (rota-rod) and mouse beam walk assay. The results revealed that the hydroalcoholic stem bark extract of *Randia nilotica* has significantly ( $p < 0.001$ ) reduced the onset of pentobarbitone sleeping time in mice and significantly prolonged ( $p < 0.001$ ) the duration. The results were comparable to diazepam that was used as positive control. The extract has a biphasic effect on amphetamine stereotyped behaviour in mice. There was a significant ( $p < 0.001$ ) reduction in jumping/climbing, but reduction in sniffing was observed only at  $5 \text{ mg kg}^{-1}$ . Mean count in limb licking was attenuated only at  $20 \text{ mg kg}^{-1}$  body weight. A paradoxical increase in sniffing was observed with the extract at  $20 \text{ mg kg}^{-1}$  and in licking at 5 and  $10 \text{ mg kg}^{-1}$ . No observable effect on motor coordination (rota rod, beam walk assay) was observed with the extract at the doses tested. The extract also decreased exploratory activity in mice. The results have suggested that the crude hydroalcoholic extract of *Randia nilotica* possesses some biologically active constituents with sedative activities.

**Key words:** *Randia nilotica*, pentobarbitone, motor co-ordination, exploratory activity, ataxia

### INTRODUCTION

Medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in the drug discovery processes (Farnsworth, 1994; Cragg *et al.*, 1997). These herbs/plants are relatively cheap and available and their uses are dependent on ancestral experience (Marin-Bettulo, 1980). The majority of the population in developing countries remain dependent on them for healthcare (Amos *et al.*, 2001).

*Randia nilotica* stapf. (Rubiaceae) is a lowland shrub or tree widespread in Sudan and reported to grow in lowland habitats in Central and East Africa as well as Cameroon and Nigeria (Lemmich *et al.*, 1995).

The plant is commonly known as shagart-el-marfaen in Sudan, while in Northern Nigeria, it is known as barbaji, tsibra, kwanarya or gial goti (Dalziel, 1937). In ethnomedicine, a decoction is used orally to treat mental breakdown and convulsions (Chabra *et al.*, 1991). In Tanzania, dried root is used against epilepsy and for madness (Hedberg *et al.*, 1983). The psychotropic effects of medicinal plants are easily noticed in laboratory

animals. This might encourage natural products scientists in the initial selection of plants with diverse application in traditional medicine for screening and evaluation (Etkin, 1988).

To the best of our knowledge there are no reports on the effect of this plant on the central nervous system. The present study was undertaken to provide scientific basis for the use of this plant in affecting behaviour and consequently the central nervous system.

### MATERIALS AND METHODS

**Animals:** Swiss albino mice (18-25 g each) of either sex maintained at the animal house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria were used. They were housed under standard conditions of temperatures ( $25 \pm 2^\circ\text{C}$ ) and light (12/12 h light/dark cycle) and fed on standard diet (Masters Feeds Plc., Ilorin, Nigeria) and water *ad libitum*. All experiments performed in this study followed the principles of laboratory animal care outlined by the ethical committee of the faculty. The study was conducted in the first quarter of 2007.

**Plant material:** *Randia nilotica* stapf. stem bark was collected in Saye village in the outskirts of Zaria, Kaduna State, Nigeria. These were identified and authenticated by a local taxonomist with the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria by comparing with a voucher specimen (No. 2867) deposited for reference at the herbarium section of the department.

**Preparation of the extract:** The stem bark was carefully removed, washed and cut into pieces. The bark was air dried and grinded into powder form using mortar and pestle and then sieved. The powdered material was macerated in hydroalcoholic solution (ethanol-70% water-30%) in the ratio 1:2.5 with occasional shaking for 24 h and then filtered. The filtrate was then evaporated to dryness over a hot water bath.

**Test drugs and chemicals:** Hydroalcoholic (HA) stem bark extract, d-amphetamine sulphate (Sigma Chemical Comp., USA) and pentobarbitone sodium (Sigma Chemical Comp., USA) were prepared by dissolving the powder in deionised water prior to administration. Chlorpromazine was supplied in ampoules and appropriate dilutions were made with deionised water prior to use.

**Hole-board test:** The exploratory activities of the extract in mice following oral administration was determined using the hole-board test (File and Wardil, 1975) as modified by Yemitan *et al.* (2001). The apparatus used consists of a white wooden board (40×40 cm) with four equidistant holes (1 cm diameter×2 cm depth).

Animals were randomly divided into five groups of 6 mice each. Each mouse was placed singly at one corner of the board. It was allowed to move about and dip its head into the holes. Poking the nose into a hole is atypical behaviour of the mouse indicating a certain degree of curiosity. The number of dips in 7.5 min (enough time to exhibit curiosity or otherwise) was recorded. The test was carried out 30 min after intraperitoneal (i.p.) treatment with the extract at doses of 5, 10 and 20 mg kg<sup>-1</sup>. Normal saline (1 mL kg<sup>-1</sup>) and diazepam 1 mg kg<sup>-1</sup> (benzodiazepines tend to suppress nose poking at relatively low doses-Vogel and Vogel (1997) were used as control.

**Rota-rod test:** The method used for the assessment of locomotor (forced motor) activity in mice was described by Ozturk *et al.* (1996). A rota-rod treadmill device (Ugo Basile No. 7600 Varese, Italy) was used for this purpose. Mice were trained to remain on slowly-moving (16 revolutions min<sup>-1</sup>) rods of 5 cm in diameter for 150 sec by walking. The mice were randomly divided into 4 groups of 6 mice each. The first group served as control and

received normal saline (1 mL kg<sup>-1</sup>), while the second, third and fourth groups received extract of *R. nilotica* at doses of 5, 10 and 20 mg kg<sup>-1</sup> body weight i.p., respectively. Thirty minutes after receiving the injection, animals were placed on the rod at intervals of 30 min up to 2 h. If an animal failed more than once to remain on the rod for 3 min, the test was considered positive, meaning that there was a lack of coordination (Ozturk *et al.*, 1996).

**Mouse beam walking assay:** This method offers improved sensitivity over the mouse rota rod in determining motor coordination deficits induced by psychotropic agents (Stanley *et al.*, 2005). Mice were allowed to walk 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 (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 (Stanley *et al.*, 2005).

Once the mice has been tested on the ruler, they were moved immediately to the beam test. The beam was made of wood, 8 mm in diameter, 60 cm long and elevated 30 cm above the bench by metal support. Four groups of 6 mice each were used. Mice in the first, second and third groups received extract at 5, 10 and 20 mg kg<sup>-1</sup>, respectively while the 4th group was injected with diazepam-1.5 mg kg<sup>-1</sup> and served as control. The mice were placed on the beam at one end and allowed to walk to the goal box thirty minutes after treatment with either extract or diazepam. Mice that fell were returned to the position they fell from, with a maximum time of 60 sec. allowed on the beam. The measurements taken were time on beam, the number of foot slips (one or both hind limbs slipped from the beam) and the number of falls.

**Pentobarbital sleeping time:** Adult mice of either sex were divided into 5 groups of 6 mice each. Three groups were administered hydroalcoholic stem bark extract of *Randia nilotica* at doses of 5, 10 and 20 mg kg<sup>-1</sup>. The fourth group received normal saline (1 mL kg<sup>-1</sup>) and the fifth group diazepam (1 mg kg<sup>-1</sup>). Treatment was given via intraperitoneal route. Thirty minutes later, pentobarbitone sodium (20 mg kg<sup>-1</sup>) was then administered to all the mice via similar route as previously mentioned. Each mouse was then observed for the onset and duration of sleep. The criteria for sleep is the loss of righting reflex, in which the mice cannot roll back when turned over (Miya *et al.*, 1973). The interval between loss and recovery of the righting reflex was used as the index of hypnotic effect (Fujimori, 1965; Soulimani *et al.*, 2001).

**Stereotyped behavioural studies:** The method of Randrup and Munkvad (1967) and of Ellinwood *et al.* (1973) was adapted for the stereotyped behavioural studies. The test was performed in five groups of 6 mice each. The first group was administered with saline as control, while animals in the second, third and fourth groups, received the extracts at doses of 5, 10 and 20 mg kg<sup>-1</sup> body weight i.p., respectively. The animals in the fifth group were injected i.p. with chlorpromazine (CPZ) -2 mg kg<sup>-1</sup> body weight. Thirty minutes after treatment all the animals were injected with d-amphetamine (2 mg kg<sup>-1</sup>). The signs of stereotype behaviour recorded included circling, jumping, limb licking, sniffing and general locomotion.

**Statistical analysis:** Results were presented as mean±SEM. Results were analysed using one factor ANOVA. Significant differences were further analysed using student t-test followed by Dunnet's and Scheffe's post hoc tests for multiple comparison. Values that are ≤0.05 will be considered significant in all studies.

## RESULTS

**Effect on exploratory behaviour:** The hydroalcoholic stem bark extract (5, 10 and 20 mg kg<sup>-1</sup> b.wt. i.p.) exhibited a dose dependent decrease in the number of head dips in the hole board experiments. Twenty milligram per kilogram has a significant (p<0.05) decrease in the exploratory behaviour in the mice. Diazepam which acts as a positive control drug, also showed a significant decrease in exploratory activity (Fig. 1).

**Effect on motor coordination:** There was no significant effect on motor coordination as determined by the treadmill device (Table 1) following intraperitoneal administration of the HA stem bark extract (5, 10 and 20 mg kg<sup>-1</sup>).

**Effect on mouse beam walk assay:** The crude HA stem bark extract did not show any significant difference with control in time to reach goal box at all doses tested (Fig. 2). Diazepam used as a control drug did not show any significant difference with normal saline in time to reach goal box. The mean number of foot slips produced by the extract (5, 10, 20 mg kg<sup>-1</sup>) did not differ significantly from that of normal saline (Fig. 3). On the other hand, diazepam showed mean number of foot slips which is significantly different from that of normal saline (p<0.001) and extract (p<0.05) at the doses tested.

**Effect on pentobarbital sleeping time:** The HA stem bark extract of *R. nilotica* decreased onset of sleep which is significant (p<0.001) at 20 mg kg<sup>-1</sup> b.wt. (Fig. 4). The

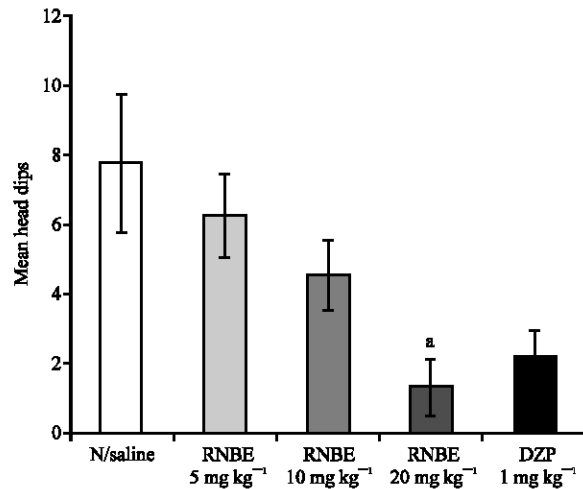


Fig. 1: Effect of normal saline (N/Saline), stem bark extract of *Randia nilotica* (RNBE) and diazepam (DZP) on exploratory behaviour in mice. Significant difference between normal saline and treated groups: p<0.05 (a). N = 5 in each group

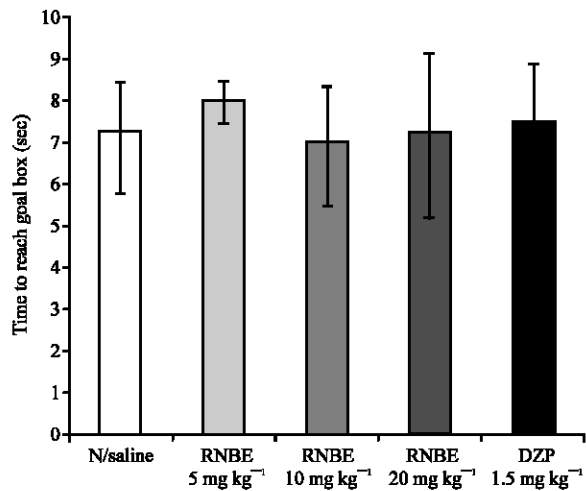


Fig. 2: Effect of normal saline (N/Saline), stem bark extract of *Randia nilotica* (RNBE) and diazepam (DZP) on time to reach goal box in the beam walking assay in mice. No significant difference between normal saline and treated groups. N = 6 in each group

extract significantly (p<0.01) prolonged the duration of pentobarbitone sleep at 5, 10 mg kg<sup>-1</sup> from 6 min to 39 and 37 min, respectively. A significant (p<0.001) increase in duration was also observed with the extract at 20 mg kg<sup>-1</sup> i.e., from 6 min to 162 min (Fig. 4).

**Effect on amphetamine-induced stereotype behaviour:** The HA stem bark extract had a biphasic effect on amphetamine-induced stereotype behaviour in mice.

Table 1: Effect of normal saline (control) and stem bark extract of *Randia nilotica* (RNBE) on motor coordination in mice

| Treatments                  | Time on rod (sec) | 30 Fail. % | Time on rod (sec) | 60 Fail. % | Time on rod (sec) | 90 Fail. % | Time on rod (sec) | 120 Fail. % |
|-----------------------------|-------------------|------------|-------------------|------------|-------------------|------------|-------------------|-------------|
| Control                     | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0         |
| RNBE 5 mg kg <sup>-1</sup>  | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0         |
| RNBE 10 mg kg <sup>-1</sup> | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0         |
| RNBE 20 mg kg <sup>-1</sup> | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0        | >180.0±0.0        | 0.0         |

Results are presented as mean±SEM, N = 6 in each group

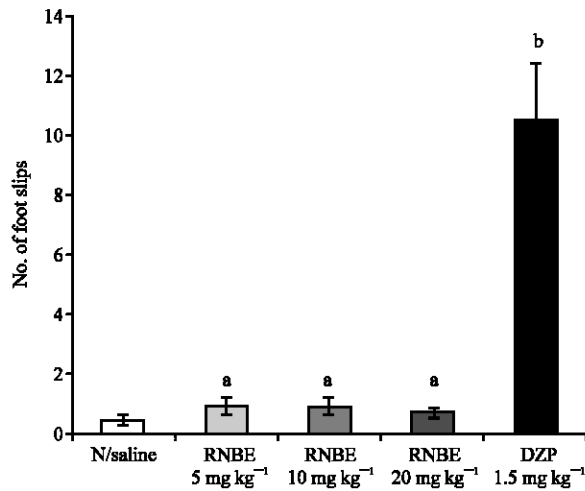


Fig. 3: Effect of normal saline (N/Saline), stem bark extract of *Randia nilotica* (RNBE) and diazepam (DZP) on foot slips in the beam walking assay in mice. Significant difference exists between normal saline and diazepam ( $p \leq 0.001$ ) (b) and between diazepam and extracts ( $p \leq 0.05$ ) (a). N = 6 in each group

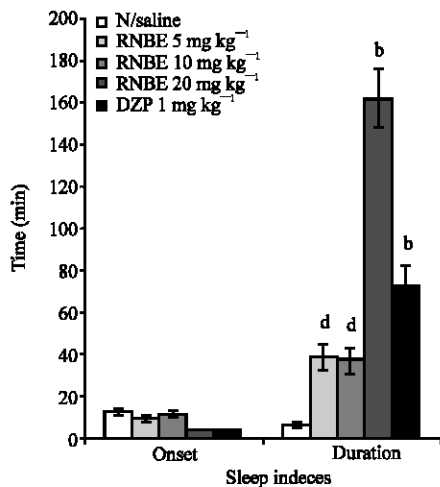


Fig. 4: Effect of normal saline (N/Saline), stem bark extract of *Randia nilotica* (RNBE) and diazepam (DZP) on onset and duration of sleep in pentobarbitone induced sleep in mice. Significant difference exist between normal saline and treated groups:  $p \leq 0.01$  (d) and  $p < 0.001$  (b). N = 5 in each group

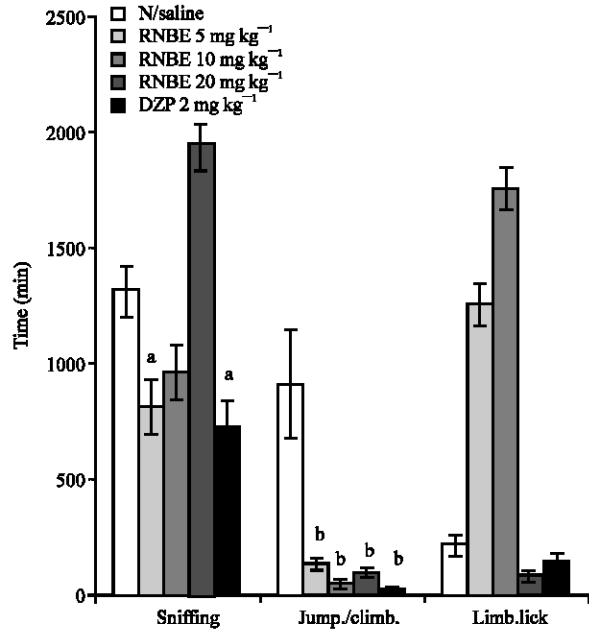


Fig. 5: Effect of normal saline (N/Saline), hydroalcoholic stem bark extract of *Randia nilotica* (RNBE) and chlorpromazine (CPZ) on amphetamine induced stereotyped behaviour in mice. Significant difference exist between normal saline and treated groups:  $p \leq 0.05$  (a) and  $p \leq 0.001$  (b)

There was a significant reduction in jumping/climbing episodes ( $p < 0.001$ ) produced by extract (5, 10 and 20 mg kg<sup>-1</sup>) compared to control. Reduction in sniffing was observed with extract only at 5 mg kg<sup>-1</sup> b.wt. which is significant ( $p < 0.05$ ) compared with control. Higher doses paradoxically produced increases rather than decrease in sniffing. Similarly, the HA stem bark extract of *R. nilotica* attenuated mean count in limb licking at 20 mg kg<sup>-1</sup> dose only. A significant ( $p < 0.05$ ) increase in limb licking was observed with extract at 5 and 10 mg kg<sup>-1</sup> compared with normal. However, chlorpromazine (2 mg kg<sup>-1</sup>) reduced all episodes of amphetamine-induced stereotyped behaviour (Fig. 5).

### DISCUSSION

The data obtained in this study demonstrated that, the HA stem bark extract of *Randia nilotica* possess substances that may be sedative in nature.

The extract dose dependently decreased number of head dips in the hole board experiment (Fig. 1). The hole board test is a measure of exploratory behaviour in animals (File and Wardil, 1975). It has been accepted as an experimental model for the evaluation of psychotic, sedative and anxiety condition in animals (Crawley, 1985; Baldessarini, 1996). A decrease in number of head dips suggests a sedative behaviour (File and Pellow, 1985) and a high propensity for antipsychotic action (Feilding and Lal, 1978).

Absence of observable effects on motor coordination by the extract in the treadmill device (Table 1) suggests that, the inhibitory effects seen in other studies might be elicited centrally and not due to a peripheral neuromuscular blockade (Perez *et al.*, 1998). It is possible that, the sedative actions of the extract were produced centrally. The test also demonstrated lack of neurological impairment caused by this extract (Stables and Kupferberg, 1997). The findings in this study signifies lack of motor deficit with extract of *Randia nilotica* even at a relatively lower dose. Absence of ataxia observed in this test also signifies that, the extract is not likely to cause clinical sedation at the doses tested (Stanley *et al.*, 2005). Sedation has been a major limiting side effect in the use of centrally acting drugs like benzodiazepines (Buffett-Jerrott and Stewart, 2002).

Furthermore, potentiation of pentobarbitone-induced sleep strongly suggests central depressant activity of this extract. The hypothesis of the central activity of this extract was further supported with the extract attenuating certain components of amphetamine-induced stereotyped behaviour in mice, which might be related to antidopaminergic actions on the limbic system as suggested by Anca *et al.* (1993). The increase at certain doses of certain stereotypical behaviours (sniffing, limb licking) may be due to potentiating effect of the extract on amphetamine (causes release of dopamine) in certain areas of the brain particularly in the mesolimbic system (Anca *et al.*, 1993).

The overall results of these studies indicate active sedative properties of the extract of *R. nilotica* that might be relevant to its application in traditional medicine.

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