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Studies on neuropharmacological profile of ethanol extract of *Moringa oleifera* leaves in mice

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ABSTRACT

Ethnopharmacological relevance: *Moringa oleifera* (family Moringaceae), commonly called Horseradish or tree of life, is traditionally used for the treatment of epilepsy and neurologic conditions.
Aim of the study: The objective of this study is to investigate the neurobehavioural and anticonvulsant properties of the ethanol extract from the leaves of *Moringa oleifera*.
Materials and methods: Neurobehavioural properties were evaluated using the open field, hole board, Y-maze, elevated plus maze (EPM) and pentobarbitone-induced hypnosis. Pentylenetetrazole (leptazol), picrotoxin and strychnine induced convulsion tests were used to investigate the anti-convulsive actions of *Moringa oleifera*.
Results: The result showed that the extract (250–2000 mg/kg) caused a significant dose-dependent decrease in rearing, grooming, head dips and locomotion ($P<0.001$). It also enhanced learning and memory and increased anxiogenic effect. In addition, the extract (2000 mg/kg) protected mice against pentylenetetrazol induced convulsion, but has no effect on picrotoxin and strychnine induced convulsion. The effects of the extract in the various models were comparable to those of the standard drugs used except in Y-maze, EPM and picrotoxin and strychnine induced convulsion. The LD_{50} obtained for the acute toxicity studied using oral route of administration was >6.4 g/kg.
Conclusion: The findings from this study suggest that the ethanol extract of *Moringa oleifera* leaves possesses CNS depressant and anticonvulsant activities possibly mediated through the enhancement of central inhibitory mechanism involving release γ-amino butyric acid (GABA). The results partially justified the traditional use of the extract for the treatment of epilepsy.
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1. Introduction

Moringa oleifera (Moringaceae) is a fast growing tree, originally found in sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan where it is used in folk medicine (Fahey, 2005). However, it is at the moment distributed all over the world (Lockelt and Grivetti, 2000).

All over the world, the nutritional value of the plant is well known and it is also used for the treatment of different and unrelated ailments. Different parts and preparations of *Moringa oleifera* have been reported to be used in traditional medicine and in the treatment of various conditions (Fahey, 2005). They are also used as nutritional supplement (Andoh, 2008; Maponga and Monera, 2010). Some of the conditions for which the plants is used to treat include nervous disorders (such as muscle spasmodic, epilepsy, headache, hysteria [Fahey, 2005]); hypertension and diabetes (Mishra et al., 2011). Its bark, sap, root, leaves, seed, oil and flower are used as folk remedy for stomach complaints, catarrh, cancer, gastric ulcers, skin diseases, lowering blood sugar, increasing bone density, nervous conditions, diabetes, fatigue, increase lactation, hayfever, impotence, edema, cramps, hemorrhoids, headaches, sore gums. They are also used to improve sight, cause better brain development, enhance better functioning of the liver, the gall, digestive respiratory and immune system, and as blood cleanser and blood builder (Patel et al., 2010). It has previously been reported that *Moringa oleifera* leaf possesses nootropic activity and hence can enhance memory (Mohan et al., 2005) probably by altering brain monoamine levels and electrical activity (Ganguly and Guha, 2008). Also, the methanol extract from the leaves has been shown to provide protection against convulsion induced by maximum electroshock seizure test and pentylenetetrazole—induced seizures (Amrutia et al., 2011).

The present study was undertaken to investigate the effect of the ethanol extract of *Moringa oleifera* leaves (EEMOL) on the central nervous system based on its traditional use in the treatment of epilepsy and nervous conditions.

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2. Materials and methods

2.1. Plant collection and authentification

Moringa oleifera leaves were collected at the Botanical garden of the Obafemi Awolowo University, Ile- Ife in August, 2010. The leaves were identified and authenticated by Mr. Oshinyemi, O.A., a plant taxonomist at the Forestry Research Institute of Nigeria (FRIN) Ibadan, where a voucher specimen with the number (FHI 109601) was deposited.

2.2. Extraction

The leaves were air-dried, pulverized and 500 g was macerated for 72 h in 1 l of 50% ethanol. It (the ethanol) was decanted, filtered several times using cotton wool and Whatman's No.1 filter paper and concentrated using rotary evaporator (BUCHI Rota vapor R-205) at the Central Laboratory of the University of Ibadan, Ibadan, Nigeria. The percentage yield was 15.61% representing 78.05 g extraction from the 500 g of dried pulverized leaves. On each day of experiment, the dark tan coloured extract obtained was freshly dissolved in 5% Tween 80 (vehicle) which served as vehicle.

2.3. Animals

Young male albino Swiss mice (18–25 g) were obtained from the Animal Centre, College of Medicine, University of Ibadan, Nigeria, and were housed in plastic cages at room temperature with a 12:12 h light–dark cycle. They were fed with balanced rodent pellet diet and water ad libitum. The animals were acclimatized for at least 1 week before being used for experiments. The experimental procedures were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.4. Drugs and chemicals

Tween 80 (Sigma-Aldrich, Denmark), diazepam (Hoffman-La Roche, Switzerland), pentobarbitone (Sigma, USA), pentylenetetrazole (Sigma, USA), picrotoxin (Sigma, USA), and strychnine (Shaanxi Xin Sheng Long Industrial Co., Ltd. China).

2.5. Experimental design

For each of the model studied, thirty mice were randomly divided into six groups ($n=5$). The groups include two controls (vehicle and standard drug) and four treatment groups for doses 250, 500, 1000 and 2000 mg/kg. Similar grouping was used for anticonvulsant test but there were 10 animals per group ($n=10$).

2.6. Acute toxicity testing

Acute toxicity of ethanol extract of *Moringa oleifera* leaves (EEMOL) was evaluated according to the method described by Miller and Tainter (1944), Amida et al. (2007). The mice were left unfed for 12 h and divided into seven groups of five each. Six groups were administered with different doses of the extract at 200, 400, 800, 1600, 3200 and 6400 mg/kg orally. The control mice were given 10 ml/kg of the vehicle (Oyemitan et al., 2008). The mice were observed for behavioural changes and mortality within 24 h.

2.7. Novelty induced behavior (NIB)

NIB was assessed by the method described by Ajayi and Ukponmwan (1994) with some modifications. The mice were allowed 6–10 min epochs during which locomotion, rearing and grooming were observed and scored to allow for characterization of drug-induced alterations. The mice were then returned to their home cages. Each test session involved allowing the mice to acclimatize to the testing environment (a quiet well ventilated room) for 30 min. All behavioural testing was carried out between 9 am and 2 pm. Six groups of five mice each were given 10 ml/kg vehicle; 250, 500, 1000, and 2000 mg/kg EEMOL; and 3 mg/kg diazepam orally, before placement in the open field arena. The extract dosages were chosen based by using half of highest value in the toxicity test, which was further scaled down geometrically. (Similar doses were used in other protocols in this study.) The open field is a rectangular arena composed of a hardboard floor (36×36 cm^2) with a surrounding wall (30 cm high) made of white painted wood. The floor was divided into squares of 9 cm^2. One hour after administration, each mouse was introduced into the arena and frequency of grooming (the number of body cleaning with paws picking of the body and pubis with mouth and face washing actions) and rearing frequency (number of times each animal stands on its hind legs or with its forearm against the wall of cage or in free air) were scored for 30 min. The procedure was repeated for all the mice in the different groups. After each session, the floor of the apparatus was wiped with 70% ethanol and dried thoroughly to remove traces of previous path.

2.8. Exploratory activity

The hole-board test was used to determine potential sedative effects. The hole-board is a wooden board (40×40 cm) with 16 holes (diameter 3 cm) evenly spaced on the floor (Hui et al., 2001). One hour after oral administration, each mouse was placed at the centre of the board and the number of head dips into the holes was scored over a 5 min period. Results obtained were expressed as mean total number of head dips (Lister, 1987). The procedure was repeated for all the mice in the different groups. There were six groups of five mice each. The groups were treated with 10 ml/kg vehicle; 250, 500, 1000, and 2000 mg/kg EEMOL; and 3 mg/kg diazepam. After each trial, the floor of the apparatus was wiped with 70% ethanol and dried thoroughly to remove traces of previous path.

2.9. Learning and memory

Y-maze was used to assess the effect of the extract on short term memory. The Y-maze is composed of three equally spaced arms (120°; 41 cm long × 15 cm high × 5 cm wide). The parameters assessed were arm entries (locomotor activity) and spontaneous alternation performance (memory). One hour after oral administration, each mouse was placed in one of the arm compartments and allowed to move freely for 5 min. Entry was defined as when the body (excepting the tail) of a mouse completely enters into an arm compartment. The sequence of entry was manually recorded. Alternation is defined as an entry into all three arms consecutively. The arms were labelled A–C, thus consecutive entries is ABC, BCA, and CAB. The percentage alternation for each mouse was determined as the ratio of actual to possible alternations (defined as the total number of arm entries minus 2), multiplied by 100 as shown by the following equation: % Alternation=[(Number of alternations)/(Total arm entries − 2)] × 100 (Heo et al., 2009). The procedure was repeated for all the mice in the different groups. There were six groups of five mice each. The groups were 10 ml/kg vehicle; 250, 500, 1000, and 2000 mg/kg EEMOL; and 3 mg/kg

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