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Full Length Research Paper

Effects of Ethanol Extract of *Curcuma Longa* Rhizome on Neurobehavioural Activities in Stressed Rats

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ABSTRACT

Human beings are constantly exposed to both emotional and physical stress throughout life and as a way of providing relief for such individuals, this study considered an alternative method of stress management with phytomedicine, hence, we investigated the effects of the ethanol extract of *Curcuma longa* rhizome in experimentally stressed male rats. Stress was induced in the animals by immobilization for six hours in a cylindrical tube with wires at both ends for cross ventilation. The extract was administered orally (p.o) and its effects on spontaneous motor activity (total locomotion, rearing and grooming frequencies) were observed in the open- field test. Putative anxiolytic properties of *Curcuma longa* were studied in the elevated plus-maze (EPM) and the hole board tests. The results revealed that in the open-field test, 100 and 150mg/kg doses of the extract significantly ($p<0.05$) increased the total locomotion and the grooming frequencies. In the EPM test, the 150mg/kg dose of the extract caused a significant increase in the number of open arm entries and the time spent in the open arm .These results suggest that the ethanol extract of *Curcuma longa* may possess some compounds with potential anxiolytic properties which need to be investigated further.

Keywords*: Curcuma longa*, anxyolitic, motor activity, locomotion, neurobehaviour, stress

INTRODUCTION

Curcuma longa(Zingiberaceae) is widely distributed in the tropical and sub-tropical regions especially in India, Thailand and Northern Australia. The powdered rhizomes are used widely as flavours in native dishes and ingredients in many traditional medicines to treat various ailments. For example, it had been used in the management of cancer, dermatitis and hypercholesteronemia (Azuine and Bhide, 1992; Ammon and Wahl, 1991).

Its antifungal (Apisariyakul et al, 1995) and hepato-protective activities (Park et al, 1998) have also been reported. Phytochemical analysis revealed the presence of flavonoids, sterols, proteins and amino acids (Saho and Satena, 2012).

A chinese study (Huang, et al, 2011) investigated the anti-depressant action of the plant. These workers observed that the curcumin therapy significantly reversed the depression-like behaviour induced by

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corticosterone. Based on this and other findings, it is not therefore impossible that it possesses some central effects. We, therefore, looked into the nature of its effects on the nervous system by examining its exploratory activities using some neurobehavioral tests.

MATERIALS AND METHODS

Plant material and preparation of extract: The rhizomes of the plant were **c**ollected from Bodija market, Ibadan, Nigeria in September 2011. The identity was authenticated at the Department of Botany, University of Ibadan, Nigeria where a specimen with voucher no F 24571H was deposited. The rhizome was separated, cleaned and dried under shade. The dried material was powdered using a laboratory blender.
1400g of powdered *Curcuma longa* was transferred into a glass container of 7L capacity and 5L of pure ethanol was added. It was allowed to macerate for 72hrs, and then filtered through Whatmann No 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C and reduced pressure.
The yield of the extract was quantified (6.5%) while the dried material obtained was stored under refrigeration at 0 - 4 °C until ready for use.

Animals: Male albino rats (150g-200g) obtained from the pre-clinical animal house of the College of Medicine, University of Ibadan, Nigeria were used for the study. They were kept under standard laboratory conditions with free access to standard rodent pellet diet and water *ad libitum*. They were maintained under a 12/12h light-dark cycle at room temperature. All experiments were conducted in accordance with international standards of animal welfare recommended by the society for Neuroscience (Handbook for the use of animals in Neuroscience Research, 1972). The experimental protocols were approved by the local Animal Care and Use committee. The minimum number of animals and duration of observation required to obtain consistent data were employed.

Drugs: Diazepam (F. Hoffman-La Roche,Basel, Switzerland)was used as the reference drug (positive control) for anxiolytic and sedative activities(Perez et al, 1998).

Treatment: The extract of Curcuma longa was freshly dissolved in a suitable amount of distilled water and accurately administered per oral (p.o) by an intra-gastric cannula for rats .One hour after the oral administration, the animals were subjected to various behavioural tests. Diazepam(1mg/kg) was dissolved in distilled water 60min before use and then given orally .The control groups received normal saline (10ml/kg) orally.

Restraint procedure: This was carried out using a transparent perspex tube measuring 18cm long and 5cm radius with a wire mesh at both ends for adequate ventilation as previously described (Ibironke and Mordi, 2011).

Behavioural evaluation

The hole- board test: This test was carried out on the stressed rats using a wooden board measuring 40 × 40cm with 16 evenly spaced holes (Perex et al ,1998). The stressed rats were grouped (n=6) and treated with the extract (50-150mg/kg), diazepam (1mg/kg) or normal saline (10ml/kg), all per oral. One hour later the stressed animals were placed on the hole-board apparatus and the number of head dips into the holes in a 5min trial was counted. The result was expressed as means for the various trial groups. At the end of each test the board was cleaned with 70% alcohol to eliminate olfactory bias.

Elevated plus-maze test: This test has been widely validated to measure anxiety in rodents (Pellow et al, 1985; Lister,1987). The apparatus, constructed from a wooden material consisted of two open arms (50cm× 10cm each), two enclosed arms (50cm×10cm×40cm) each and a central platform (10cm×10cm) arranged in such a way that two arms of each type were opposite each other .The maze was elevated 70cm above the floor. Sixty minutes after treatment with the extract (50-150mg/kg), diazepam (1mg/kg) or normal saline (10ml/kg/kg), each stressed animal was placed at the centre of the maze facing one of the enclosed arms. During the 5 min test period, the number of open and closed arm entries, plus the time spent in the open and closed arms were recorded (Pellow and File, 1986). Entry into an arm was defined as the point when the animal places all the four paws into the arm. After the test, the maze was cleaned with 70% ethanol solution to eliminate olfactory bias.

Open-field test: The open field box is a rectangular arena composed of a hard floor measuring 36cm×36cm×26cm and made up of a white painted wood. The floor was divided by permanent red markings into 16 equal squares at the bottom. Generally, spontaneous motor activity was monitored for 30min in the open field as described by Ajayi and Ukponmwan (1994). One hour after oral administration of the extract (50-150mg/kg), diazepam (1mg/kg) or normal saline

(10ml/kg) , the stressed animals were singly placed in one of the corners of the box and the total locomotion (number of floor units entered with all paws), rearing frequency (number of times the animal stood on its hind limbs or with the fore limbs against the wall observation box or free in the air) and grooming frequency (number of body cleaning with paws ,picking of the body and pubis with mouth and face washing actions) within each 10min interval were recorded .The arena was cleaned with 70% ethanol to eliminate olfactory bias and allowed to dry before introducing a fresh animal.

Data analysis

Values were expressed as mean ± SEM. The data were analyzed using student's t-test. A value of $p < 0.05$ was considered significant.

RESULTS

Open-field test: In this test, the extract (100 and 150mg/kg) and the reference drug significantly ($p<0.05$) increased the total locomotion (fig 1) and the rearing frequency (fig2).

Hole board test: The result showed both 150mg/kg dose of the extract and the reference drug caused a significant ($p<0.05$) increase in the number of head dips (fig 3).

Elevated plus-maze test: The extract (150mg/kg) and the reference drug (diazepam, 1mg/kg) significantly ($p<0.05$) increased the number of entries into the open arm (fig 4) .The 100 and 150mg/kg doses of the extract and diazepam (1mg/kg) significantly ($p<0.05$) increased the time spent in the in the open arm (fig 5) .

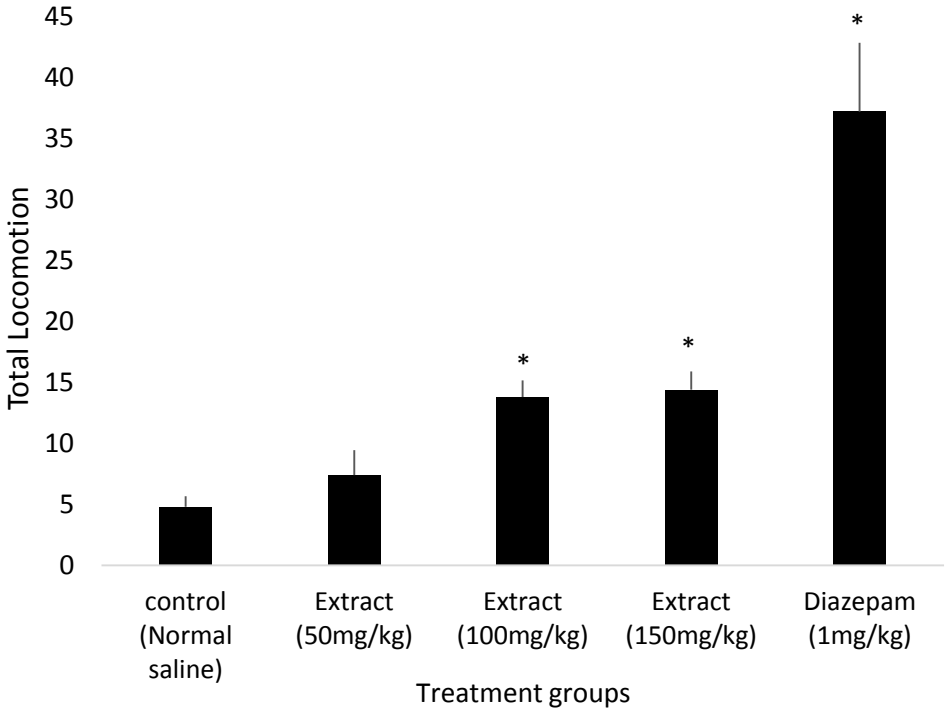

Figure1:
A chart showing the effect of pre-treatment with various doses of ethanol extract of *Curcuma longa* on total locomotion in an open field test in rats. Values are represented as Mean ±SME. *$p<0.05$ compared with control. (n=5)

DISCUSSION

The present study investigated the putative neurobehavioural effects of the ethanol extract from the rhizome of *Curcuma longa* in stressed rats. Results from the open-field test showed marked increases in the total locomotion and rearing frequencies in the stressed animals compared with the normal saline treated control. Locomotion, rearing and grooming are widely accepted as an indication of exploration (Barnett,1963; Kelly,1993). These results suggest a decrease in anxiety, since it has been suggested that exploration can actually be inhibited by anxiety and that the open field test also examines anxiety related behaviours, therefore, increased exploratory activity translates to a decrease in anxiety (Whimbey and Deremberg, 1967; Asano, 1986).

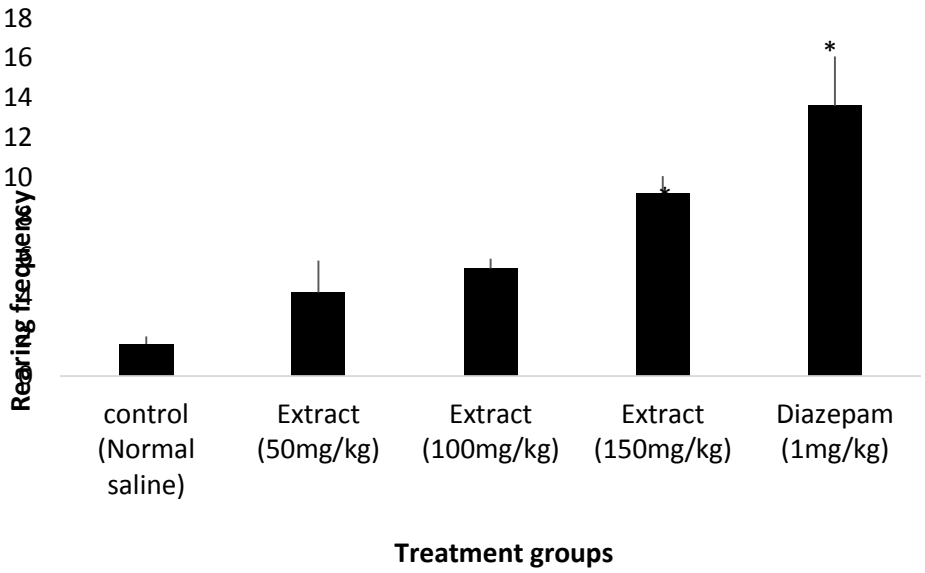

Figure 2:
A chart showing the effect of graded doses of ethanol extract of *Curcuma longa* on rearing frequency observed in an open field test in rats. Values are represented as Mean ±SEM.
*p<0.05 compared with control. (n=5)

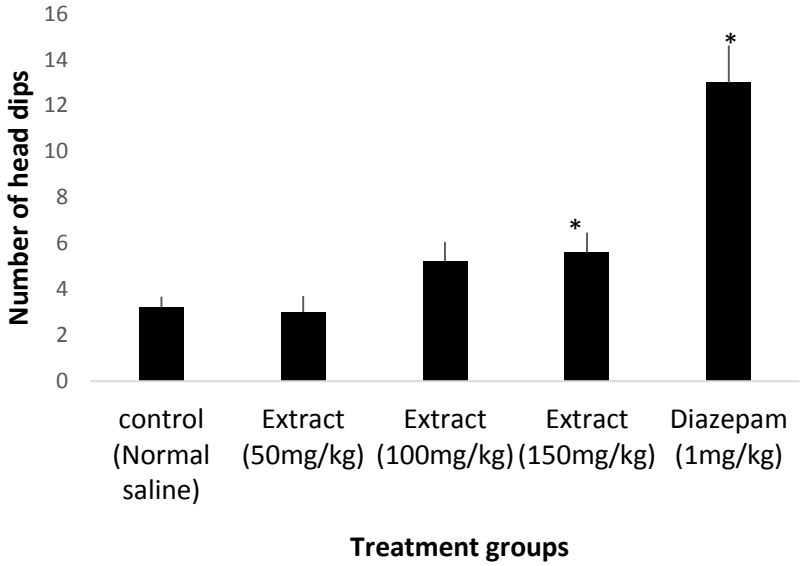

Figure 3:
A chart showing the effect of graded doses of ethanol extract of *Curcuma longa* on the number of head dips in a hole board test in rats. Values are represented as mean ±SME. *p<0.05 compared with control. (n=5)

In the hole-board test, pre-treatment with the extract significantly increased the exploratory activity as shown by an increase in the number of head dips compared with the control .It has been reported that an increase in the number of head dips is a measure of anti-depressant activity (Adzo et al, 2002; Lister 1987). A similar report of an increase in the number of head dips as a measure of anxiolytic activity was made by Mohd et al (2011) with Ipomoea aquatic. The hole board test is a measure of exploratory activities in animals (File and Wadil, 1975). The elevated plus-maze test represents one of the most widely used animal models for screening and accessing anxiolytic properties (Lister 1987). In this study, graded doses of the extract resulted in dose dependent increase in both the number of entry and time spent in the open arms. It is a widely used test based on the natural aversion of rodents to height and open spaces The test is sensitive to both anxiolytics and anxiogenics. It has been validated for both rats and mice (Lister, 1987)

In conclusion, our results showed that the ethanol extract of the *Curcuma longa* rhizome possesses anxiolytic properties as evidenced by increased exploratory activities in the neurobehavioural tests employed, this therefore may explain its use as herbal remedy in the treatment of stress-related disorders.

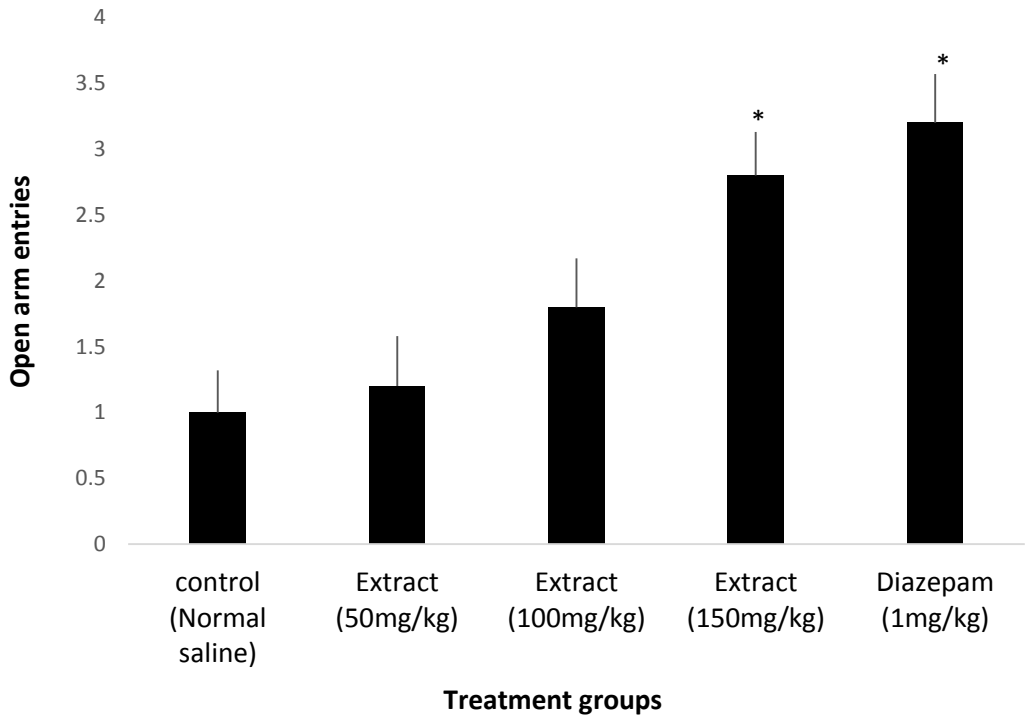

Figure 4:
A chart showing the effect of graded doses of ethanol extract of *Curcuma longa* on open arm entries in the elevated plus maze. Values are represented as Mean ±SME.
*p<0.05 compared with control. (n=5)

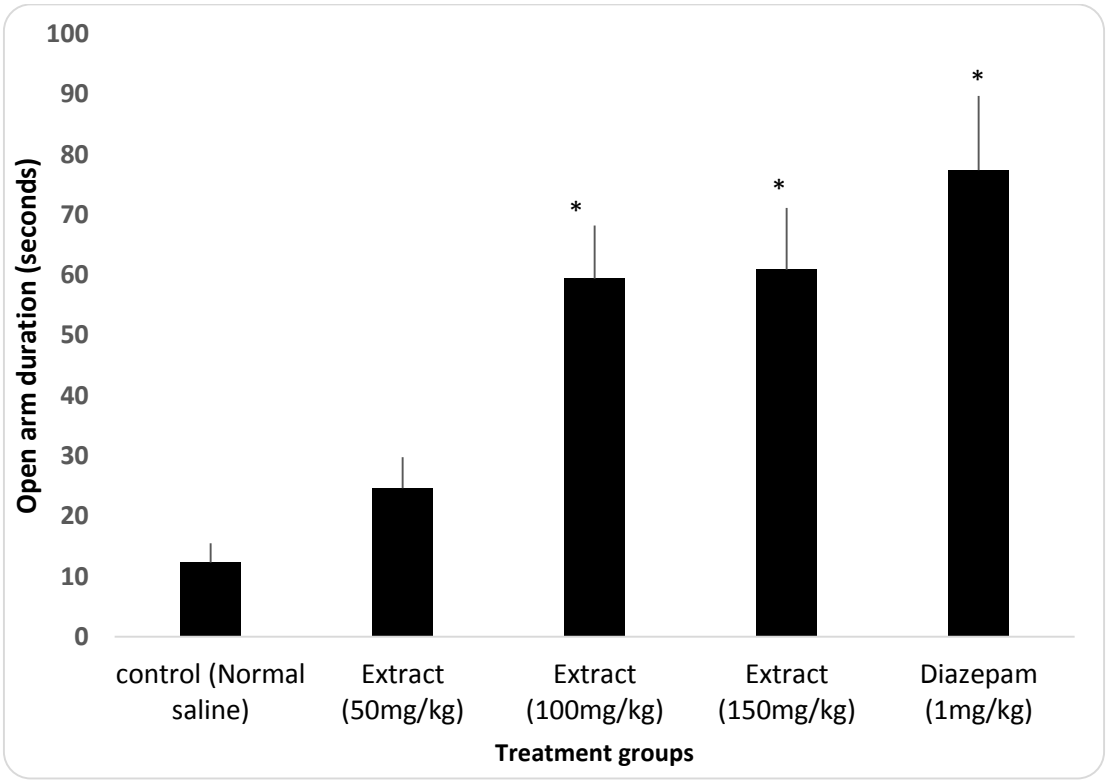

Figure 5:
A chart showing the effect of graded doses of ethanol extract of *Curcuma longa* on time spent in open arms of the elevated plus maze. Values are represented as Mean ±SME. *p<0.05 compared with control. (n=5)

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