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Neuropharmacological Effects of the Methanol Extract of *Leptadenia hastata* Leaves in Rodents

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Abstract

This study investigated the Neuropharmacological effects of the Methanol extract of *Leptadenia hastata* Leaves (MELH) in Rodents. The oral median lethal dose (LD50) of MELH was evaluated using Lorke's method in rats. The effect of MELH (200 and 400 mg/kg p.o.), diazepam (2.5 mg/kg, i.p.), and 10 ml normal saline/kg on anxiety-like behavior and locomotion activity were evaluated in rats on elevated plus maze (EPM), Zero-maze and open field apparatus, respectively. The oral LD50 value of MELH was estimated to be 5000 mg/kg body weight in rats. MELH significantly ($p<0.0001$) increased time spent in the open arm of EPM and significantly ($p<0.0001$) increased time spent in open arms of the Zero maze. MELH also significantly increased total locomotor activity ($p<0.0001$) and rearing in the open field apparatus. It was concluded that *L. hastata* possess potent anxiolytic effects as evidenced in the results obtained from the various used models. Hence, this observation provides rational scientific evidence for its continuous use in folkloric medicine for calming tensed individuals.

Keywords: *Leptadenia hastata*, Neuropharmacological, Anxiety, Rodents

1.0 Introduction

Anxiety and depression are the most common psychiatric disorders. Over 20% of the adult population suffers from these illnesses at some time during their lives (Titov *et al.*, 2010). It has become an important area of research interest in psychopharmacology during this decade (Woode *et al.*, 2011).

Traditional medicine involves the use of herbal medicine, animal parts and minerals. However, herbal medicines are the most widely used of the three. Herbal medicines contain an active ingredient, aerial or underground parts of plants as their petal or seeds materials or combinations thereof, whether in the crude state or as plant preparations. Furthermore, about 80% of the world population is dependent

(wholly or partially) on plant-based drugs (WHO, 1996). In Nigeria and most developing countries of the world, rural and urban dwellers, literate or illiterate rely heavily on herbal preparations for the treatment of various diseases despite the availability of orthodox medicine (Nwabuise, 2002).

Leptadenia hastata belongs to the family asclepiadaceae widely used in Tropical Africa as vegetable (Burkil, 1985). The plant is medicinally important in the treatment of many ailments (Burkil, 1985; Oliver-Boyer, 1986; Aliero *et al*., 2001). Ethnobotanical information obtained from traditional medical practitioners in northern Nigeria revealed that *L. hastata* is used for the treatment of diabetes mellitus. The antibacterial and antimicrobial effects of *L. hastata* have been reported (Aliero and Wara, 2009) and the result of its toxicity studies showed that the plant is relatively safe (Tambuora *et al*., 2005). There is however paucity of information confirming the neuropharmacological potentials of *L. hastata*. Hence, this research evaluated the neuropharmacological potentials of *L. hastata* in rodents.

2.0 Materials and Methods

2.1 Chemicals and drugs

Methanol and all chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA).

2.2 Animals

Adult male Wistar rats weighing 100–150g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

2.3 Plant Collection and Identification

The leaves of *Leptadenia hastata* were collected from a natural habitat in Ejule, Kogi State, Nigeria. The plants were identified at the Herbarium Unit of the Department of Biological Sciences, Federal University, Lokoja, Kogi State.

2.3.1 Preparation of extracts

The leaves of *Leptadenia hastata* were shade- dried for seven (7) days and pulverized using an electric blender. One thousand (1000) gram of the pulverized leaves was soaked in distilled water for 48- hours. A filterate was obtained using Whatmann filter paper (Size No1) and the extract was concentrated using rotary evaporator. The extract shall henceforth be reffered to as MELH.

2.4 Acute Toxicity Study

The oral median lethal dose (LD50) of the extracts was determined in rats according to the method of Lorke *et al.* (1983)

2.5 Behavioral tests

All the behavioral procedures were carried out between 8:00 am and 12:00 pm in a temperature controlled room (23 ± 1 ◦C). The mice were grouped such that each group consisted of equal number of males and females which were separately housed.

2.6 Elevated plus maze

The elevated plus maze is an anxiety paradigm based on the rodent's natural aversion to a novel and potentially dangerous environment represented by the open and elevated spaces (Lister, 1987). The elevated plus maze apparatus is a plus (+) shaped wooden structure, consisting of two open arms (40×5×10 cm3) and two enclosed arms (40×5×10 cm3) extended from a central platform (10×10 cm2). The maze was elevated 50 cm from the room floor. Rats were habituated to the testing room under dim light for at least 1 h before the test and then randomly divided into four groups. The rats that served as control group received 10 ml normal saline/kg body weight orally, while the treated rats received MELH (200 and 400 mg/kg body weight orally) and diazepam (2.5 mg/kg body weight i.p.). One hour after oral treatment with MELH and thirty minutes after intraperitoneal administration of diazepam, each rat was placed at the center of the maze, facing one of the open arms and allowed to explore the maze freely for a 5-min testing period. The time spent in open and enclosed arms were recorded. The maze was thoroughly cleaned between tests with a tissue paper moistened with 70% ethanol.

2.7 Elevated zero maze

Rats were randomly divided into eleven groups of six rats each. One hour before this test, rats were treated with MELH (200 and 400 mg/kg, orally). The control group received 10 ml normal saline/kg while standard reference drug diazepam (2.5 mg/kg, i.p.) was

administered thirty minutes before the test. Elevated zero maze is a modification of the elevated plus maze model of anxiety in rodents. The novel design consists of an elevated (50 cm above the floor) circular platform (6 cm width and 40 cm inner diameter) that is equally divided into four quadrants. Two quadrants on opposite sides of the platform are enclosed by 12 cm high walls while the other two quadrants are opened and bordered by 0.6 cm high lip. Thus removing any ambiguity in the interpretation of the time spent in the central square of the traditional design (elevated plus maze) and allowing uninterrupted exploration. One hour after drug administration, each rat was placed at the center of the open arm (facing toward the closed chamber). The times spent in both open and closed arms of the maze were manually recorded. The maze was thoroughly cleaned between tests with a tissue paper moistened with 70% ethanol.

2.8 Open-field test (OF)

Locomotor activity and exploratory behavior were assessed in an open field by the method described by Souza *et al.* (2010). The OF apparatus consist of a clear glass box (45×45 cm2). The floor was divided by lines drawn into 9 equally sized squares. Twenty four rats were randomly divided into four groups of six rats each. One hour before test session, rats were treated orally with MELH (200 and 400 mg/kg) while the control received 10 ml normal saline/kg orally. One hour later each rat was placed individually in the center of the apparatus and observed for 5 min to record the locomotor (number of squares crossed with four paws) and exploratory activities (indicated by frequency of rearing) (Walsh and Cummins, 1976; Souza *et al.*, 2010).

2.9 Statistical Analysis

All data were expressed as mean ± SEM. Statistical analysis was carried out using one way analysis of variance (ANOVA). Any significant difference between means was assessed by student's t-test at 95% level of significance.

3.0 Results

3.1 Acute Toxicity

In the acute toxicity study, the extract did not cause any death or produced signs of toxicity in rats up to a dose of 5000 mg/kg of MELH. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg according to Lorke's method.

3.2 Effect of MELH on elevated plus maze (EPM)

The extract significantly ($p<0.0001$) and dose-dependently decreased the time spent in the closed arm of the elevated plus maze (EPM). Diazepam was more potent in reducing the time spent in closed arm of the EPM than both does of MELH (Figure 3). Similarly, MELH significantly ($p<0.0001$) increased the time spent in the closed arm of the elevated plus maze (Figure 1).

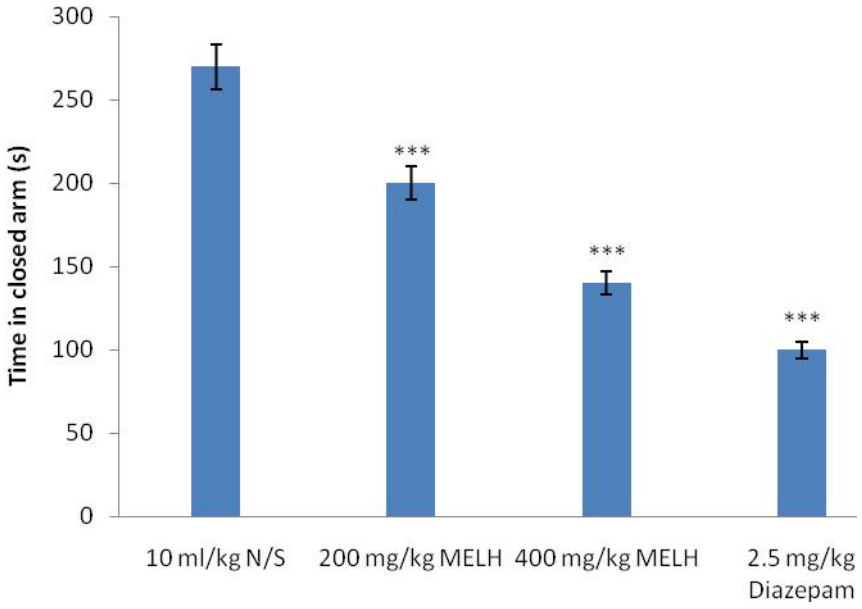

Figure 1: Effect of MELH on time spent in closed arm of the EPM. * Significantly different from the control at p<0.0001**

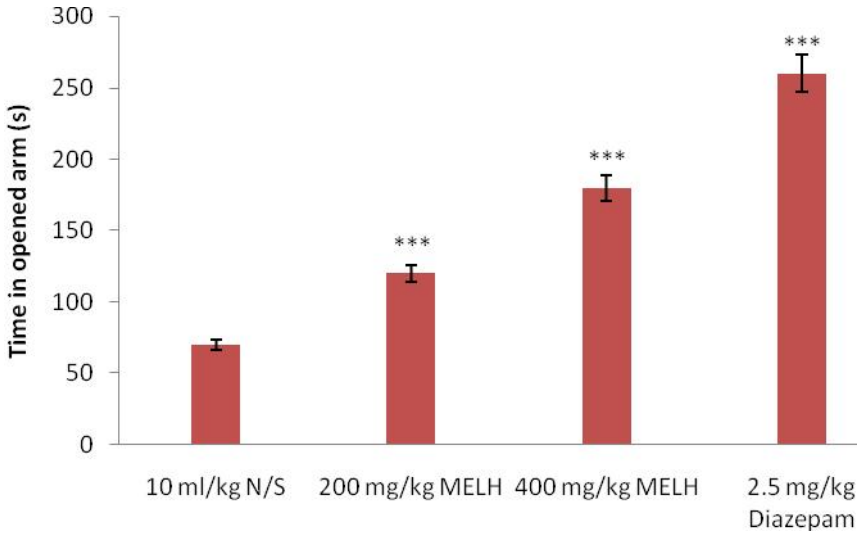

Figure 2: Effect of MELH on time spent in opened arm of the EPM. * Significantly different from the control at p<0.0001.**

3.3 Effect of MELH zero maze

The extract at the both doses significantly (p<0.0001) decreased the time spent in the closed arm of the maze (Figure 3) and significantly (p<0.0001) increased the time spent in the open arm of the elevated zero maze, while diazepam increased time spent on open arm of the maze (Figure 4).

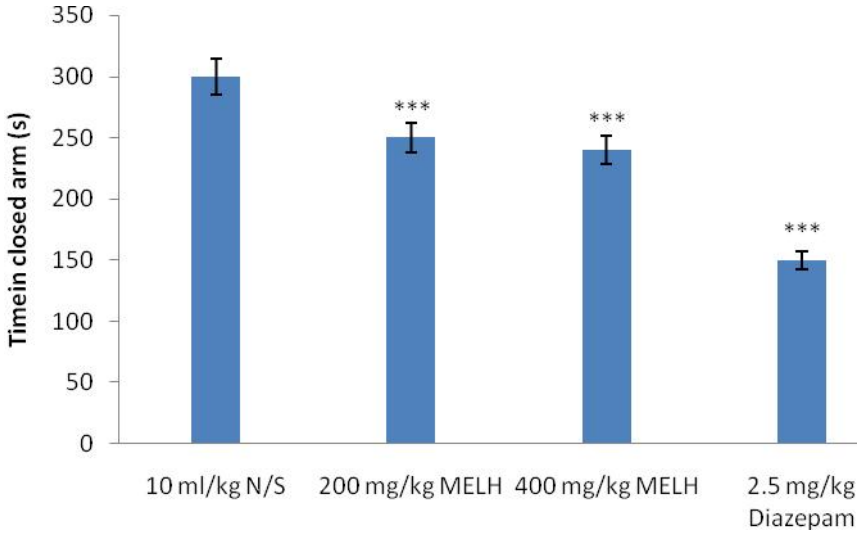

Figure 3: Effect of MELH on time spent in closed arm of zero maze. * Significantly different from the control at p<0.0001.**

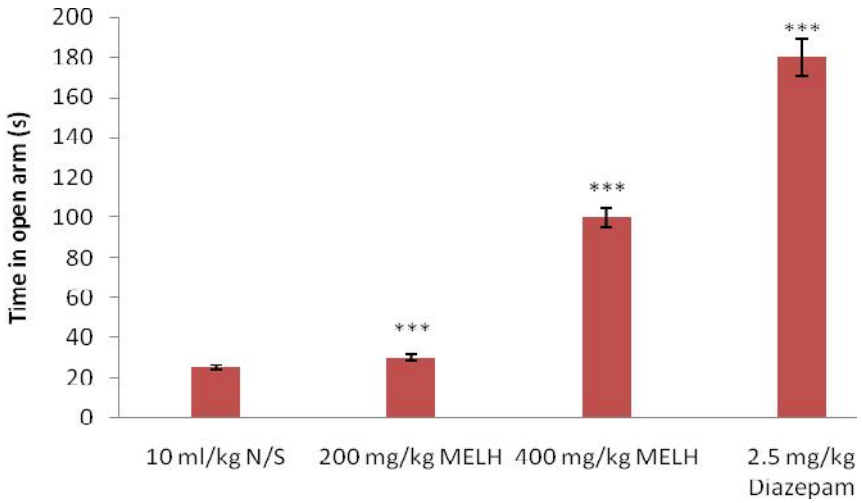

Figure 4: Effect of MELH on time spent in open arm of zero maze. ***Significantly different from the control at p<0.0001.

3.4 Effect of MELH on total locomotive activity

The extract significantly (p<0.0001) and dose-dependently increased the total locomotive activity of rats on open field apparatus (Figure 5). Also, the extract significantly (p<0.0001) increased the frequency of rearing (Figure 6).

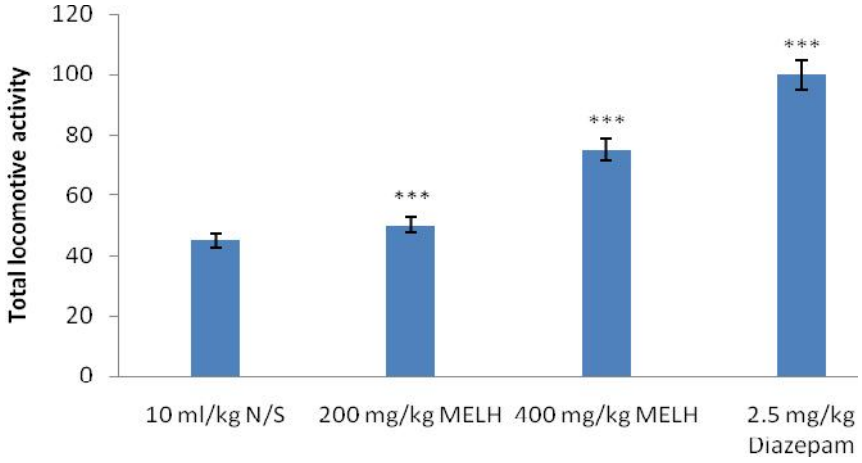

Figure 5: Effect of MELH on total locomotive activity on open field apparatus. ***Significantly different from the control at p<0.0001

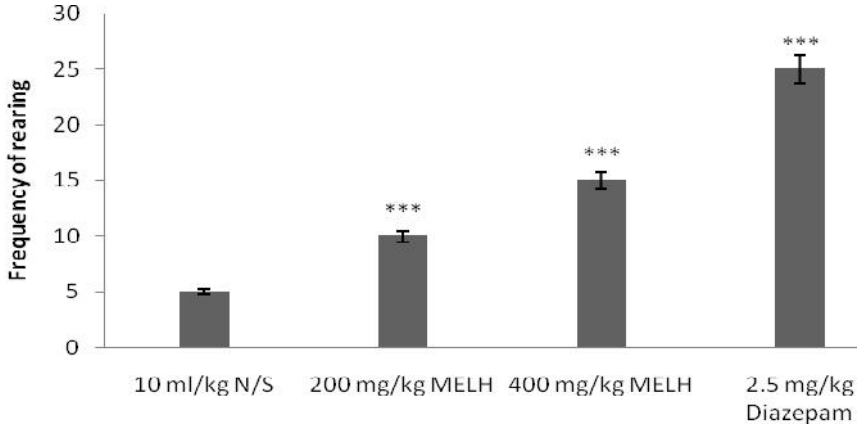

Figure 6: Effect of MELH on rearing. * Significantly different from the control at p<0.0001.**

4.0 Discussion

The oral median lethal dose (LD50) of the extract in rats was estimated to be greater than 5000 mg/kg, indicating that the extract is practically non-toxic acutely (Matsumura, 1975; Corbett *et al.*, 1984).

The models of anxiety used in this study are widely used to screen the new anxiolytic drugs. These models are quite sensitive and relatively specific to all major classes of anxiolytic drugs and are therefore appropriate for the studies. *L. hastata* methanol leave extract at 200 and 400 mg/kg significantly increased the time spent in the open arm of the elevated plus maze. The extract however produced a significant increase in time spent in the enclosed arm of the maze. This observation is not consistent with standard anxiolytic behaving similar to benzodiazepines with anxiolytic effect at low doses and anxiogenic or sedative effect at higher doses (Madara *et al.,* 2013). The indices of anxiety (percentage of open-arm entries, and percentage of time spent in the open arm) are sensitive to agents and are thought to act via the GABA receptor complex, justifying the use of diazepam (DZP) as a positive control in this study. It increased the frequency of open-arm entries and the time spent in the open arms (Crawley & Goodwin, 1980), confirming its anxiolytic effects.

The anxiolytic effect of the extract was further confirmed by the results obtained from the use of the elevated zero maze. The zero-maze has two advantages over the elevated plus maze: no ambiguity associated with the interpretation of the time spent in the central square of the elevated plus maze and allowance of uninterrupted exploration. The extract at 200 and 400 mg/kg produced anxiolytic-like effect which is clearly defined by the increased time spent in the open quadrant of the zero -maze. This observation may be due to the extreme spectrum of anxiolytic sedative effects characterized by sedation-like behavior. This is consistent with the effect of sedative anxiolytics.

The open-field apparatus provides information on anxiety-related behaviour characterized by natural aversion of rodents to an open brightly lit area (Choleris *et al.,* 2001). Animals are thus afraid of the centre and spend more time in the protective corners and in freezing state. Anxiolytics increase total locomotive activity resulting in a reduction of time spent in corners, an increased time spent in the center and a decreased time spent in freezing state. The extract at 200 and 400 mg/kg increased total locomotive activity and increased rearing of treated rats. This observation further confirmed the anxiolytic potential of MELH. Natural products of plant origin may elicit anxiolytic effects via interaction with some endogenous mediators such as GABAergic and serotonergic pathways in the body (Tijani *et al.,* 2012; Kadaba 1994). Most anxiolytics enhances response to GABA through facilitation of the opening of GABA activated chloride ion channels.

5.0 Conclusion

The present study showed that *L. hastata* possessed potent anxiolytic effects as evidenced in the results obtained from the various used models. This observation provides rational scientific evidence for its continuous use in folkloric medicine for calming tensed individuals.

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