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Responses to Aqueous Leaf Extract of *Luffa aegyptiaca* Mill on Mefloquine-Induced Seizure in Rat Using Open Field Test

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ABSTRACT

Neuro-behavioural responses to *Luffa aergyptiaca mill* on mefloquine-induced seizure was investigated in rats. Twenty five male Wistar rats (190-250 g) were assigned into 5 groups with five rats per group, namely; control group, mefloquine group only (seizure, 4.28 mg/kg), mefloquine and then diazepam (seizure + diazepam, 5 ml/kg), mefloquine and then low dose *Luffa aergyptiaca mill* group (seizure + low dose *Luffa*, 400 mg/kg), and mefloquine and then high dose of extract (seizure + high dose *Luffa*, 1200 mg/kg). Doses of mefloquine and *luffa* extract were administered to the rats according to their body weight. After thirty (30) minutes of administration, each rat was placed subsequently in the centre platform of the open field and then observed for five (5) minutes. Line crossing, central square entry, central square duration, stretch attends posture, number of faecal droppings, urination, grooming, and frequency of rearing were recorded with a video camera. Precaution was taken to ensure that no external stimulus evoked anxiety in the rat. Line crossing and rearing were significantly lower in the MFQ group than those of the control and other treated groups. No significance difference was observed between control and treated groups for centre square entry, centre square duration and grooming. There was significant difference for stretch attend posture, freezing time, urination and defecation between control and other groups. Increased line crossing shows anxiety reduction in the open field. Hence these results do suggest that aqueous extract of leaf of *Luffa aergyptiaca mill* can revoke anxiety in rats.

Keywords: *Luffa aergyptiaca mill, Anxiety, Aqueous extract, Mefloquine, Open field test*

INTRODUCTION

The open field test has been used as a behavioural test for assessing the effect of plant extract and drugs on experimental animals (Adjene and Abudu 2009). The increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and developments of several drugs and chemotherapeutics from plants as well as from traditionally used herbal remedies. This practice has gained more grounds because of the ready availability of plants, the insignificant cost of preparation and the desire to avoid the side effects of chemotherapy (Anwar et al. 2007). Although the use of medicinal plants is sometimes associated with superstition, and therefore rejected by some people in favour of western medicine (Ojo et al. 2006). Conversely, there are millions of Africans who prefer traditional methods of treatment. The valuable medicinal properties contained in certain plants are not, however, in doubt (Ojo et al. 2006). Some plants are valued more than others due to their increased

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usage, less or no toxic effect and as such every part of the plants is use in medicinal practice (Anwar et al. 2007).

Luffa aergyptiaca mill has several medicinal values. The fruit, seed, leaves and root have all been used to treat several diseases. The plant belong to the family *Curcubitaceae*, genus; *Luffa* and specie *aegyptiaca mill*. It is commonly known as sponge gourds.

Sponge-gourds, the fruit of *Luffa aegyptiaca mill*, are widely used throughout the world. It is an annual climbing crop which produces fruit containing fibrous vascular system. It is a summer season vegetable.

Anxiety is an unpleasant state of inner turmoil, often accompanied by nervous behaviour, such as pacing back and forth, somatic complaints, muscular lesion, restlessness, fatigue, problems in concentration and rumination (Davison 2008). Anxiety is not the same as fear, which is a response to a real or perceived immediate threat. Anxiety is a feeling of fear, worry and uneasiness, usually generalized and unfocused as an overreaction to a situation that is only subjectively seen as menacing (Bouras and Holt 2007; American Psychiatric Association 2013). Anxiety related disorders such as panic, phobias, obsessive-compulsion and post-traumatic stress are the most common mental illness and major cause of disability in the world and has become a very important area of research interest in psychopharmacology (Rabbani et al. 2003).

Defensive behaviours are innate and unconditioned reactions of an organism towards actual or potential threats (Yang et al. 2004). Anxiety, fear and stress are behavioural outcome of the activation of CNS defensive system (Blanchard et al. 2003). Rodent becomes defensive when placed in a strange place (Blanchard and Blanchard 1987). Antimalarial drug mefloquine disrupts central autonomic and respiratory control in the brainstem of Wistar rat (Lall et al. 2012). Open field test provides simultaneous measures of locomotion, exploration and anxiety (Carrey et al. 2000). The open field test was used to investigate the involvement of the cholinergic system in restraint stress induced neurobehavioral alterations in rodents and results showed a significant decrease in the frequency of rearing, grooming and locomotion activities in the stressed animal compared with the control (Ibironke and Olley 2014). Open field test was also used to investigate locomotor activities in adult Wistar rats given *Phyllantus amarus* extract (Adjene and Abudu 2009).

Ekong et al. (2010) demonstrated the behavioural patterns of rats in an open field following treatment with artesunate and amodiaquine combination. Ebeye and Onduku (2014) also carried out a research on the effect of *Garcina kola* on the open field which showed anxiolytic effect.

The leaf of *Luffa aergyptiaca mill* has been used traditionally in Ogoni, Rivers State, Nigeria to treat anxiety and convulsion but no scientific study has been carried out. There have been claims that *Luffa aergyptiaca* leaf extract influences behavioural patterns, but this has not been fully evaluated. However this research and experiment is focused on investigating and evaluating the effects of *luffa* on neuro-behavioural patterns in albino Wistar rat. Mefloquine was used to create the animal model of seizure due to its use in the treatment of malaria which can be easily abused by human.

MATERIALS AND METHODS

Plant Material

The leaves of *Luffa aergyptiaca mill* was collected from several plants in Khana Local Government Area of Rivers State in the month of June 2014. This plant *Luffa aergyptiaca mill* was identified by the Dr. Edwin, Department of Plant Science, University of Port Harcourt, Choba Nigeria.

Ethical clearance was obtained from the School of Post Graduate Studies, University of Port Harcourt, Choba, Rivers State, Nigeria. All procedures involving the use of animals in this study were done in accordance with the guiding principles for research involving animals as recommended by The Research Ethics Committee of the University of Port Harcourt.

The crude extract (1 g) was completely dissolved in 100 ml of distilled water forming the stock solution.

Experimental protocol

Twenty five male albino Wistar rats were kept in the propylene cages and maintained at a temperature of about 25 degree Celsius and were allowed free access to food and water *ad libitum*. The rats were randomly assigned into five groups with five rats per group, namely: control group, mefloquine group only (seizure, 4.28 mg/kg), mefloquine and then diazepam (seizure + diazepam, 5 ml/kg), mefloquine and then low dose *Luffa aergyptiaca mill* group (seizure + low dose *Luffa*, 400 mg/kg), and mefloquine and then high dose of extract (seizure + high dose *Luffa*, 1200 mg/kg). The administration was orally, and thirty (30) minutes after administration, each rat was placed subsequently in the centre platform of the open field and then observed for five (5) minutes. Thereafter, rat was returned in their cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. The experiment was repeated for all the groups, lasted for 14 days.

Open Field Test Apparatus

Developed by Hall (1934) to test emotionality of rodents, the open field test (OFT) is a commonly used qualitative and quantitative measure of general locomotor activity and willingness to explore in rodents. The open field is an arena with walls to prevent escape. Commonly, the field is marked with a grid and square crossings. Rearing and line crossing were used to determine the activity of the rats. In the

modern open field apparatus, infrared beams or video cameras with associated software is use to automate the assessment process. The OFT was used to assess anxiety by including additional measures of defecation, time spent in the centre of the field, and the first few minutes of activity. The open field apparatus (72 x 72 cm) for measuring anxiety and exploration, as well as locomotion was constructed using white plywood. One of the walls was clear Plexiglas, so rat could be visible in the apparatus. Blue lines were drawn on the floor with a marker and were visible through the clear Plexiglas floor. The lines divided the floor into sixteen 18 x 18 cm squares. A central square (18 cm x 18 cm) was drawn in the middle of the open field. The central square was used because some rat strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Also, the central square creates sufficient space surrounding it to give meaning to the central location as being distinct from the outer locations (Carrey et al. 2000). The maze was located in a 1.8 x 4.6 m test room and lit by a 60-watt red lamp for background lighting. The open field maze was cleaned between each rat using 70% ethyl alcohol. Behaviour was scored and each trial was recorded for latter analysis, using a video camcorder that was positioned above the apparatus. Measures of line crosses was obtained with the camera fixed to the ceiling, 2.1 m above the apparatus.

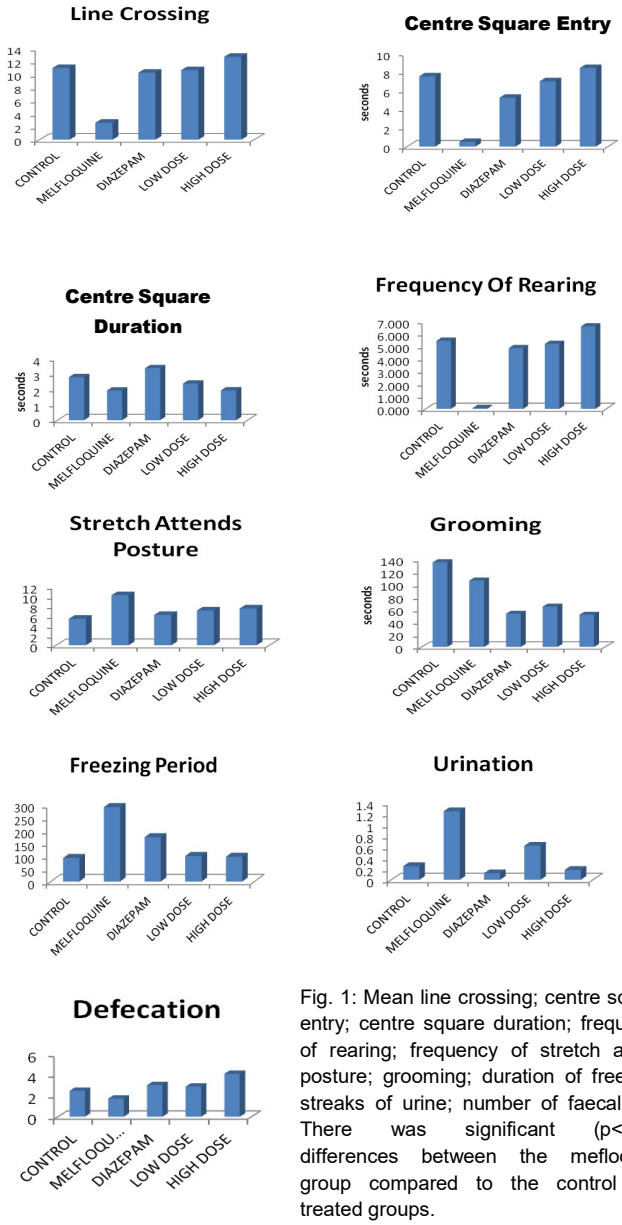

Fig. 1: Mean line crossing; centre square entry; centre square duration; frequency of rearing; frequency of stretch attend posture; grooming; duration of freezing; streaks of urine; number of faecal boli. There was significant ($p<0.05$) differences between the mefloquine group compared to the control and treated groups.

Procedure

Each rat was placed in the centre of the open field and allowed to explore the apparatus for 5 minutes. Thereafter, rat was returned in their cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests.

The following parameters were tested:

1. Line crossing: Frequency with which the rat crossed one of the grid lines with all four paws.
2. Centre square entry: Frequency with which the rat crossed one of the red lines with all four paws into the central square.
3. Centre square duration: Duration of time the rat spent in the central square.
4. Rearing: Frequency with which the rat stood on their hind legs in the field.
5. Stretch attends posture: Frequency with which the rat demonstrated forward elongation of the head and shoulders followed by retraction to the original position.
6. Grooming: Duration of time the animal spent licking or scratching itself while stationary.
7. Freezing: Duration with which the rat was completely stationary.
8. Urination: Number of puddles or streaks of urine.
9. Defecation: Number of faecal boli produced.

Each rat was then given a score for total locomotor activity that was calculated as the sum of line crosses and number of rears. To assess the process of habituation to the novelty of the arena, rats were exposed to the apparatus for 5 minutes on 2 consecutive days.

Data Analysis

The statistical analysis was completed using Statview v5.0.1 (SAS instruments) for windows. Data collected from the open field was analysed using a repeated measures ANOVA, Post hoc comparisons were made using the Student - Newman - Keuls design during both days of exploration.

RESULTS

Open Field Test

The results of the open field test are all expressed as Mean ± standard error. The mean differences between the control and treatment groups were tested for significance using one-way analysis of variance (ANOVA). The result is as shown below.

Line crossing was significantly lower in the group induced with mefloquine only than those of the control, those induced with mefloquine and treated with diazepam, those induced with mefloquine and treated with low dose of *luffa aergyptiaca* extract and those induced with mefloquine and treated with high dose of *luffa aergyptiaca* extract. The score for the high dose of *luffa aergyptiaca mill* was higher than the low dose with no significance (Table 1, Figure 1). No significance difference was observed in all the groups for central square entries, centre square duration and grooming. There was significant difference for the group induced with mefloquine only. (Table 1, Figure 1). For rearing frequency, mean difference of control and the group given high dose of *luffa aergyptiaca mill* was significantly higher than the group induced with mefloquine only, treated group with low dose of *luffa aergyptiaca mill* and the group treated with diazepam. These high values recorded indicate increased locomotive and exploratory activities, and reduced anxiety (Table 1, Figure 1). It was also observed that stretch attends posture for those treated with diazepam was significantly lower than those treated with low dose *luffa aergyptiaca mill* and high dose *luffa* while there was no significant difference when compared with the group treated with low dose and high dose *luffa aergyptiaca mill*. There was no significant difference when compare the control and the group treated with diazepam while the group treated with mefloquine only were significantly higher than all the other groups. For freezing time, mean difference of the group induced with mefloquine only was significantly higher when compared to the treated groups and control. Those induced with mefloquine only and those treated with low dose of *luffa aergyptiaca mill* was significantly higher than those treated with diazepam and treated with high dose of *aergyptiaca* mill for urination while defecation was observed to be significantly higher in the group treated with high dose of *luffa aergyptiaca mill* when compared with group the group treated with diazepam, those treated with low dose of *luffa* and the control group (Table 1, Figure 1).

Table 1: Result of the open field test parameters

Parameters	Control	Mefloquine	Mefloquine +Diazepam	Mefloquin + Low Dose Extract	Mefloquine + High Dose Extract
Line Crossing	11.00 ± 0.61	2.60 ± 0.16*	10.28 ± 1.07[b]	10.68 ± 1.21[b]	12.72 ± 1.93[b]
Centre Square Entry	7.55 ± 0.45	0.50 ± 0.00*	5.25 ± 1.00[b]	7.02 ± 1.20[b]	8.45 ± 1.32[b]
Centre Square Duration	2.80 ± 0.28	1.93 ± 1.12[NS]	3.38 ± 1.70[NS]	2.38 ± 1.49[NS]	1.94 ± 1.39[NS]
Rearing	5.47 ± 0.27	0.07 ± 0.07*	4.88 ± 0.64 [b]	5.22 ± 1.50 [b]	6.62 ± 2.33[b]
Stretch Attend Posture	5.42 ± 6.84	10.25 ± 3.52*	6.20 ± 4.38 [b]	7.10 ± 7.20 [NS]	7.52 ± 6.23[NS]
Grooming	135.75 ± 38.42	106.50 ± 28.22[NS]	53.32 ± 37.47[NS]	64.80 ± 48.87[NS]	51.68 ± 39.99[NS]
Freezing	119.25 ± 87.68	292.00 ± 54.68*	173.68 ± 154.08[b]	100.02 ± 78.56[b]	97.18 ± 75.78[b]
Urination	0.25 ± 0.62	1.25 ± 4.40[c]	0.12 ± 0.68[NS]	0.62 ± 0.33[c]	0.18 ± 0.70[b,d]
Defecation	2.50 ± 2.18	1.75 ± 1.76[e]	3.04 ± 2.47[NS]	2.90 ± 2.42[e]	4.14 ± 3.51*

Data are presented as Mean ± Standard Error of Mean. *Significant at p<0.05 compared with the control group. [b]Significant at p<0.05 compared with the Mefloquine group. [c]Significant at p<0.05 compared with the Mefloquine+Diazepam group. [d]Significant at p<0.05 compared with the Mefloquine+Low Dose Extract group. [e]Significant at p<0.05 compared with the Mefloquine+High Dose Extract. [NS]Not significant at p<0.05 compared with the control group. n=5

DISCUSSION

Open field test provides simultaneous measures of locomotion, exploration and anxiety (Carrey et. al., 2000). The findings from this research shows that oral administration of aqueous leaf extract of *luffa aergyptiaca mill* caused significant (p<0.05) changes in the neurobehavioral scores of the some parameters measured across groups. Bindra and Thompson (1953) agree that defecation and urination in a novel environment are signs of emotionality which is not fearfulness or timidity. Since defecation is a sign of emotionality as suggested by Bindra and Thompson (1953), our results show that the emotional state of the rats was affected as there were significant differences in the groups. Anxiety reduction in the open field is indicated by an increase in the proportion of line crossing and rearing, hence, the results suggest that aqueous

extract of leaf of *Luffa aergyptiaca mill* has anxiolytic-like properties.

Earlier reports stated that flavonoids present in this plant (Carnet et al. 1999; Oh et al. 2004) have high affinity with central benzodiazepine site of the GABAA receptor (Huang et al. 2001; Adeyemi et al. 2006). Therefore the anxiolytic activities of *Luffa aergyptiaca mill* could be readily associated with flavonoid.

Several ethnomedicinal and traditional medicinal plants have been documented for the treatment of central nervous system (CNS). Ethnomedicinal plants such as *Luffa aergyptiaca* plant on which this study is focused could serve as source of effective medication that maybe more readily accessible and inexpensive and thus would be helpful in improving the present status as regards to anxiety. Phenobarbitone and diazepam effectively protect mice against mefloquine-induced seizures (Amabeoku and Farmer 2005) which are similar to the results obtained in this study for the diazepam treated group. Barua et al. (2013) conducted a research on anxiolytic and anticonvulsant activity of methanol extract of leaves of *Alternanthera brasiliana (L.)* kuntze (Amaranthaceae) in laboratory animals. Their report showed significantly increased number and duration of head poking in the hole board test, rearing, assisted rearing and number of square travelled in the open field test; entries and time spent in open arm in the elevated plus maze test (Hogg 1996; Lin et al. 1999); time spent in lighted box, and numbers of crossings and transfer latency time in the light/dark exploratory test. All the three doses of the extract significantly reduced the duration of seizures induced by pentylenetetrazole (chemoshock convulsion). Although they used several apparatus for their investigation, our open field test also shows similar results of increase in rearing and central square entry in the group treated with *Luffa aergyptiaca mill*. Though seizure was induced with mefloquine in our study, similar reduction in the duration of seizure and increase locomotive activity was recorded in this present study as compare to the above study. Electric shock model was not created in our study.

Nevin (2009) demonstrated epileptogenic potential of mefloquine chemoprophylaxis. Ekong et al. (2010) reported the behavioural patterns of rats in an open field following treatment with artesunate plus amodiaquine combination and the results proved there was no significant difference between the experimental groups and the control in total locomotor activity, central square frequencies and duration, and stretch attend. Although no significant difference was also observed in our study between treated groups and control, there was significant difference between treated and induced groups only.

Ebeye and Onduku (2014) also carried out a research on the effect of *Garcina kola* on the open field test and observed that it causes decrease in locomotor and exploratory activities on the open field. Their findings are not in line with our result that shows increase in locomotive and exploratory activities when treated with *Luffa aergyptiaca mill*. Adjene and Abudu (2009) investigated the effects of *Phyllantus amarus* on the open field locomotor activities in adult Wistar rats and their result was similar when compared to our findings. There were significant differences in the frequencies of line crossing, walling and defecation between the treated and control groups in their experiment. Our findings also showed significant difference in the frequencies of line crossing and defecation.

CONCLUSION

From the data analysis, it is clear that mefloquine, diazepam and aqueous *luffa* extract seem to have induced neurological effects, resulting in neuro-behavioural responses in rats. The results also show that aqueous leaf extract of *Luffa aergyptiaca mill* have anxiolytic properties. This effect may be due to the interaction of the extract with chemical mediators in the brain such as gamma aminobutyric acid (GABA) which are responsible for the anxiety-like behaviour. The luffa extract has shown an indication of a good anxiolytic agent in treating anxiety. Further pharmacological researches are ongoing to identify and isolate the active constituents of the aqueous leaf extract of *Luffa aegyptiaca mill* plant responsible for these anxiolytic activities.

Conflict of Interest

None declared.

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