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SOME NEUROPHARMACOLOGICAL EFFECTS OF THE SEED OIL EXTRACT OF *MORINGA OLEIFERA* L. IN MICE AND CHICKS

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ABSTRACT

Moringa oleifera is one of the species of the family: Moringaceae, native to Africa, Asia, South America and Caribbean Island. It has been reportedly used in nervous system disorders (such as muscle spasm, epilepsy and hysteri). This study investigates the anticonvulsant, hypnotic and anxiolytic potentials of the oil of the plant. The anticonvulsant effect of the extract was determined using Maximal electroshock test in chicks, Pentylenetetrazole and strychnine-induced seizure tests in mice. The sleep modulating potential of the extract was investigated using pentobarbitone sleeping time in mice The anxiolytic activity was evaluated using Elevated plus maze, Hole board and Beam walking tests in mice. The extract dose-dependently decreased the recovery time from tonic hind limb extension in the 500 and 1000mg/kg treated groups in the MES induce seizure model. In the PTZ-induced seizure model, the extract at a dose of 500 and 1000 mg/kg afforded 60% protection and significantly ($p < 0.05$) prolonged the onset of seizure in unprotected animals. In the elevated plus maze test, the extract insignificantly increased the frequency of entry and time spent in the open arm. In the hole board test, the extract significantly ($p<0.05$) decreased the number of head dips. The extract did not significantly alter number of foot slips in the beam walking assay. These results suggest that the n-hexane extract of *Moringa oleifera* seed possessed sedative and anti-convulsant activities and lend credence to the ethnomedicinal use of the oil of the plant in the management of epilepsy, anxiety and insomnia.

Key Words: *Moringa oleifera*, Anticonvulsants.Anxiolytic, Epilepsy, Neuropharmacological effects

INTRODUCTION

Epilepsy is a chronic disease of the brain characterized by an enduring predisposition to generate seizures, unprovoked by any immediate central nervous system insult, it affects all ages and sexes with worldwide distribution (Ettore, 2020). The present antiepileptic drugs are unable to control seizures effectively and have several serious adverse effects (Joy *et al.*, 2013). In this context, plant derived phytochemical constituents can play an important role in the treatment of epilepsy as they are known of high therapeutic index and relatively low cost (Joy *et al.*, 2013).

Moringa oleifera is an aboriginal plant of Indian subcontinent and has become naturalized in tropical and subtropical areas around the world. It is a short, slender, deciduous perennial tree and used in the treatment of psychoses, eye diseases, fever and as an aphrodisiac (Chopra, 1993;

Nadakarni, 1973). It has been previously reported that the *Moringa oleifera* leaf possesses nootropic activity and hence can enhance memory (Mohan *et al.,* 2005) probably by altering monoamine level and brain electrical activity (Ganguly and Guha, 2008).

The methanol extract from the leaves has been shown to offer protection against convulsion induced by maximum electroshock seizure test and pentylenetetrazole induced seizures (Amrutia *et al.*, 2011). Mishra *et al.,* (2011) reported the Neuroprotective and anti-inflammatory effects of the of the roots, flowers and seed of Moringa oleifera. The present study is aimed at further evaluation of the oil extracted from the n -hexane fraction of *Moringa oleifera* seed, the findings from this study may further buttress the traditional use of the plants' seed oil extract in the treatment of neurological such as epilepsy. It may also provide a lead for the development of safer and more effective alternatives to the currently available antiepileptic agents.

MATERIALS AND METHODS

Plant Collection and Identification

The dried ripe seeds of *Moringa oleifera* were collected in the month of February, 2018 in the medicinal plant garden of Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The the leaves attached to the stalk and seeds were identified by Dr. Halilu Emmanuel Mshelia and assigned the voucher number PCG/UDUS/Morin/0001.

Preparation of Seed Oil Extract

The seeds were carefully separated from the pericarp with the aid of a sieve. The seeds were then ground to fine powder with the aid of a pestle and mortar.The oil was extracted from the powdered seeds of *Moringa oleifera* by Soxhlet extraction method. The powdered seed of *Moringa oleifera* (308g) was extracted with 1 L of n-hexane. The extraction of the oil was carried out at 60˚C for three hours and allowed to concentrate at room temperature in open air for 5 days and the percentage yield was calculated.

Experimental Animals

Swiss Albino mice of both sexes weighing between 18 and 22g were obtained from the Animal House Facility of the department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The approval for the use of animals was obtained from the Department of Pharmacology and Toxicology, UDUS Animal Ethics Committee and assigned the number (PTAC/Nh (MO)/008-18). The mice were kept at the Animal House Facility of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto under laboratory conditions and fed with laboratory animal feed and water *ad libitum*.

Acute Toxicity Study

The acute toxicity of the seed oil extract of *Moringa oleifera* (MOSOE) was determined using Lorke's method (1983). The method is divided into phase 1 and 2 respectively. Briefly describe the method here. Three groups of three mice or chicks were treated with MOSOE at doses of 10, 100, and 1000 mg/kg body weight orally and observed for signs of toxicity and death for 24 hours. In the second phase, 3 groups of one mouse or chick was treated with 1600, 2900 and 5000 mg/kg body weight respectively and also observed for signs of toxicity and death. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1) respectively.

Animal Grouping

For each study (except MEST), thirty (30) mice were randomly divided into five groups each consisting of six mice. The 1st group was treated with distilled water 10ml/kg, the 2nd, 3rd and 4th groups were administered with 250, 500, and 1000mg/kg of *Moringa* seed oil respectively by oral route. The 5th group was treated with the standard drug diazepam10 mg/kg (PTZ and Strychnine models), 0.5 mg/kg hole board and EPM models and 2.5 mg/kg beam walk assay. For the MEST, fifty (50) day old chicks were randomly divided into five groups of ten chicks each. Extract and drug administration was done as described for PTZ except that the fifth group received Phenytoin (20 mg/kg).

Phenobarbitone-induced sleeping time

In the phenobarbitone induced sleeping time test, the method of Magaji *et al*. (2007) was adopted with slight modification. Twenty (20) chicks were divided into four groups of five chicks each. Group 1 received distilled water (10 ml/kg) which served as the control group; group 2-4 received 250,500 and 1000mg/kg of *Moringa* seed oil respectively via oral route of administration. One hour post administration all the groups were administered with phenobarbitone (40 mg/kg *i.p*).The chicks were observed for latent period (time between phenobarbitone administrations to loss of righting reflex) and duration of sleep (time between loss and recovery of righting reflex).

Anticonvulsant Studies

Maximal electroshock seizure test in chicks

One hour after pre-treatment with distilled water, seed oil extract (250-1000 mg/kg) and standard drug (phenytoin 20 mg/kg), maximal electroshock was administered to the chicks using Ugobasile electroconvulsive machine (model 57800-001) with corneal electrodes placed on the upper eyelids of the chicks after being moistened with normal saline. The shock duration, frequency and pulse width were set and maintained at 0.8s, 200 pulse/sec, 0.8m/s respectively. A current of 90 mA was maintained throughout. Seizure was manifested as tonic hind limb extension (THLE) in the chicks (Swinyard, 1969). The ability to prevent this feature or shorten the recovery from THLE was considered an indication of anticonvulsant activity (Swinyard, 1969; Sayyah *et al.,* 2002).

Pentylenetetrazole induced seizure

One hour post treatment with distilled water, *Moringa* seed oil extract, and standard drug, clonic seizure was induced in the mice by administration of 90 mg/kg PTZ *s.c.* The onset of seizure and number of animals protected was recorded following thirty minutes observation. Lack of an episode of clonic spasm lasting for 5 seconds indicates the extract's ability to prevent convulsion PTZ-induced seizure (Swinyard *et al.,* 1989).

Strychnine induced seizure

One hour post treatment, mice in all the groups received strychnine nitrate 2 mg/kg *i.p*. The onset and proportion of mice presenting tonic convulsions was recorded. Abolition of tonic extensor jerks of the hind limbs within 30 minutes after strychnine administration was considered an indicator that the extract could prevent strychnine-induced convulsions. (Porter *et al*., 1984).

Behavioural Studies

Elevated plus maze test

One hour post treatment of distilled water and Moringa seed oil extract; and thirty minutes after intraperitoneal treatment of diazepam, each mouse was placed in turn in the center of the maze facing one of the closed arms and assessed for 5 minutes.

After each mouse assessment, the lingering olfactory cues were cleansed using 70% alcohol and allowed to dry between tests. The behavior scored included; the number of entries into the closed and open arms and the time spent in the closed and open arms (Lister, 1987). The Elevated Plus Maze (EPM) test is used to assess anxiety-related behavior in rodent models of CNS disorders. The EPM apparatus consists of a "+"-shaped maze elevated above the floor with two oppositely positioned closed arms, two oppositely positioned open arms, and a center area. As subjects freely explore the maze, their behavior is recorded and analyzed using a video tracking system or close observation. The preference for being in open arms over closed arms (expressed as either as a percentage of entries and/or a percentage of time spent in the open arms) is calculated to measure anxiety-like behavior. This test can be used to phenotype strains of transgenic mice and to screen for putative anxiolytic compounds.

Hole board test

The apparatus used was a white painted wooden board (60 cm x 30 cm) with 16 evenly spaced holes (1cm diameter x 2 cm depth) (Magaji *et al.*, 2017). One hour post oral treatment of distilled water and extract and thirty minutes post intraperitoneal treatment of diazepam, each mouse was placed in turn at the center of the board and the number of head dips into the holes was scored over a 5 minutes period. After each trial the hole board apparatus was wiped with 70 % alcohol and allowed to dry to remove olfactory cues Number of head dips into the hole was used to assess the behavior (Lister, 1987). A head dip was considered as dipping the head into the hole to the level of the eyes (Magaji *et al.*, 2017).

Beam walking assay

The beam apparatus consists of 1-meter beams with a flat surface of 12 mm or 6 mm width resting 50 cm above the table top on two poles. A goal box is placed at the end of the beam as the finish point. Nesting material for home cages is placed in the goal box to attract the mouse to the finish point. A lamp (with 60-watt light bulb) is used to illuminate the starting point and serves as an aversive stimulus (Carter *et al.,* 2001; Southwell *et al.,* 2009). Prior to the actual testing each mouse was individually trained to cross the wider beam (8 0cm×3 cm). One hour post oral treatment of distilled water and extract and thirty minutes post intraperitoneal treatment of diazepam, each mouse was placed in turn at one end of the narrower beam (60cm long and 8cm in diameter) and assessed for a period of one minute. After each observation the beam and goal box were cleaned with 70 % Alcohol to remove droppings left by preceding subject. The number of foot slips (one or both hind limbs slipping from the beam) was recorded with the aid of a tally counter (Carter *et al.,* 2001).

RESULTS

Effect *Moringa oleifera* seed oil Extract in Phenobarbitone-induced sleep-in chicks

MOSOE significantly (p <0.05) decreased the onset of sleep at a dose of 500 mg/kg and 1000 mg/kg. The effect of the extract on the duration of sleep was not consistent and therefore insignificant (p>0.05) (Figure 1).

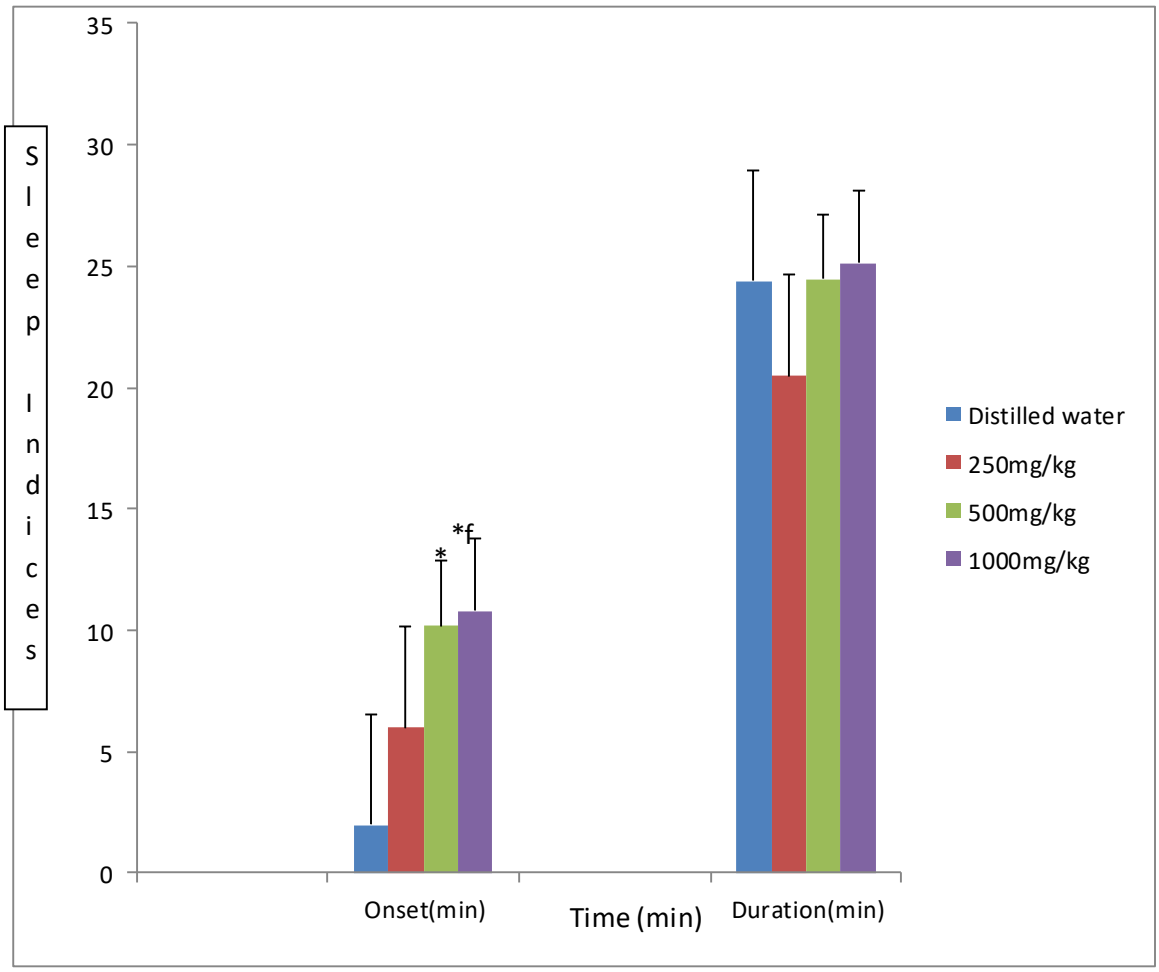

Figure 1: Effect of *Moringa oleifera* seed oil extract on the onset and duration of sleep-in chicks. Data are presented as mean ± SEM; n=5, *=p<0.05; compared to control. One way ANOVA followed by Dunnett's post hoc test.

Anticonvulsant activity of MOSOE on maximal electroshock-induced seizure (MES) in chicks

MOSOE did not protect the animals from THLE, but the extract at 500 mg/kg, and 1000 mg/kg significantly (p < 0.05) decreased the recovery time after hind limb tonic extension (HLTE). The positive control group treated with phenytoin 20mg/kg showed a significant (p<0.001) decrease in the recovery time, and provided 80% protection against hind limb tonic extension (Figure 2).

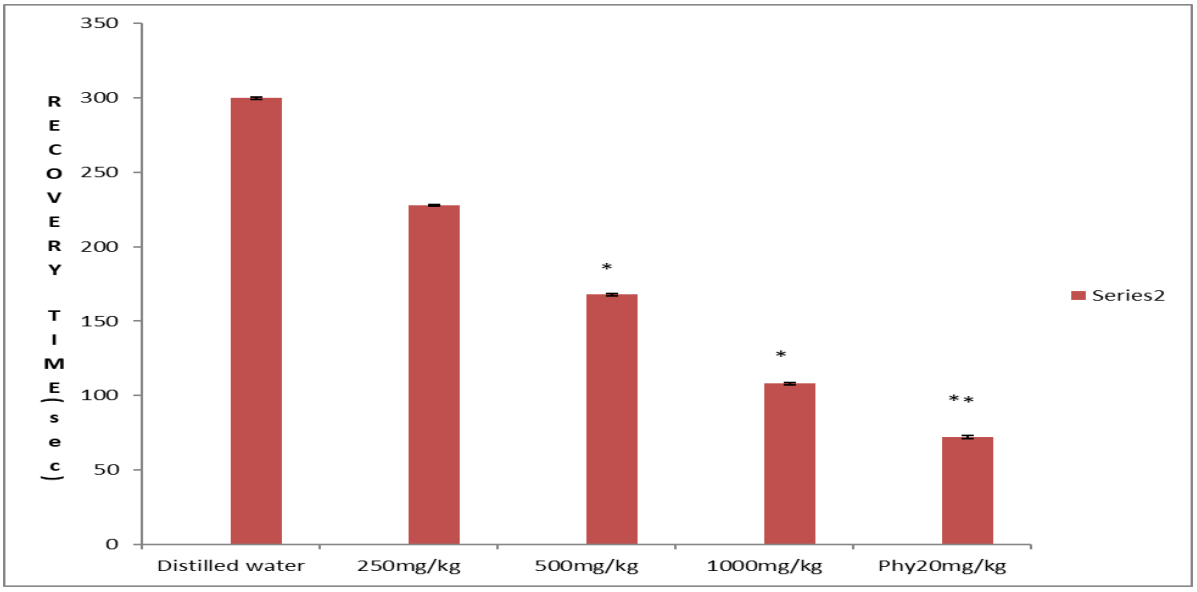

Figure 2: Effect of *Moringa oleifera* seed oil extract on Maximal electroshock-induced seizure in chicks
Data presented as Mean ± SEM; n=5, *=p<0.05; **=p<0.00; Compared to control. One way ANOVA followed by Dunnett's post hoc test, Phy= phenytoin

Effect of *Moringa oleifera* seed oil extract on Pentylenetetrazole induced seizure in mice

MOSOE did not protect the animals against PTZ-induced seizure but it prolonged the onset of the seizure at a dose of 500 mg/kg. The extract afforded 66.67% protection against PTZ-induced mortality at the highest dose tested. Diazepam (5mg/kg) afforded 100% protection against PTZ-induced seizure (Table 1).

Effect of *Moringa oleifera* Seed oil Extract on Strychnine-induced Seizure

MOSOE did not afford any protection against strychnine-induced seizure at all the doses tested and did not significantly alter the onset of seizure and latency to death. At the highest dose tested, the extract protected 50% of the mice against mortality the positive control group treated with Diazepam (5mg/kg) provided 100% protection against STC-induced mortality (Table 2).

Table 1: **Effect of *Moringa oleifera* seed Oil Extract in Pentylenetetrazole-induced seizure in Mice**

Treatment	Dose	Onset of seizure (min)	Time of death (min)	% protection against Mortality
Distilled water	10 ml/kg	5.5±1.18	10.±1.01	33.3
MOSOE 1	250 mg/kg	2.76±1.53	3.8±1.46	66.7
MOSOE 2	500 mg/kg	6.5±1.61	8.3±2.94	50
MOSOE 3	1000 mg/kg	2.2±2.43	3.7±1.36	66.7
Diazepam	5 mg/kg	5.8±1.25	1.8±3.56	100

Results were expressed as Mean ± SEM., n=6, Data were analysed using One way ANOVA

Table 2: **Effect of *Moringa oleifera* seed oil extract on strychnine-induce seizure in mice**

Treatment	Dose	Onset (min)	Time of death (min)	% protection against mortality
Distilled water	10 ml/kg	1.63±2.93	1.71±3.24	0
MOSOE 1	250 mg/kg	2.05±1.5	1.69±6.56	0
MOSOE 2	500 mg/kg	1.23±3.38	2.62±1.50	0
MOSOE 3	1000 mg/kg	1.09±3.59	1.71±3.06	50
Diazepam	5 mg/kg	3.68±1.54	0.98±3.68	100

Results were expressed as Mean ± SEM., n=6, Data were analysed using One way ANOVA.

Effects of *Moringa oleifera* Seed Oil extract in the Elevated plus Maze Test

The oil extract of the seeds of *Moringa oleifera* did not significantly change the number of entries in the open arm. However, at a dose of 500 mg/kg, the extract significantly ($p < 0.05$) decreased the number of entries into the closed arm. Diazepam at a dose of 1 mg/kg significantly ($p < 0.05$) increased the number of entries into the open arm as seen in (Fig. 3). Similarly, the *Moringa oleifera* seed oil extract did not significantly ($p < 0.05$) change the total time spent in both the open and closed arms. Diazepam at a dose of 1 mg/kg significantly ($p < 0.05$) increased the total time spent in the open arm and significantly ($p < 0.05$) decreased the total time spent in the closed arm as seen in (Fig. 4).

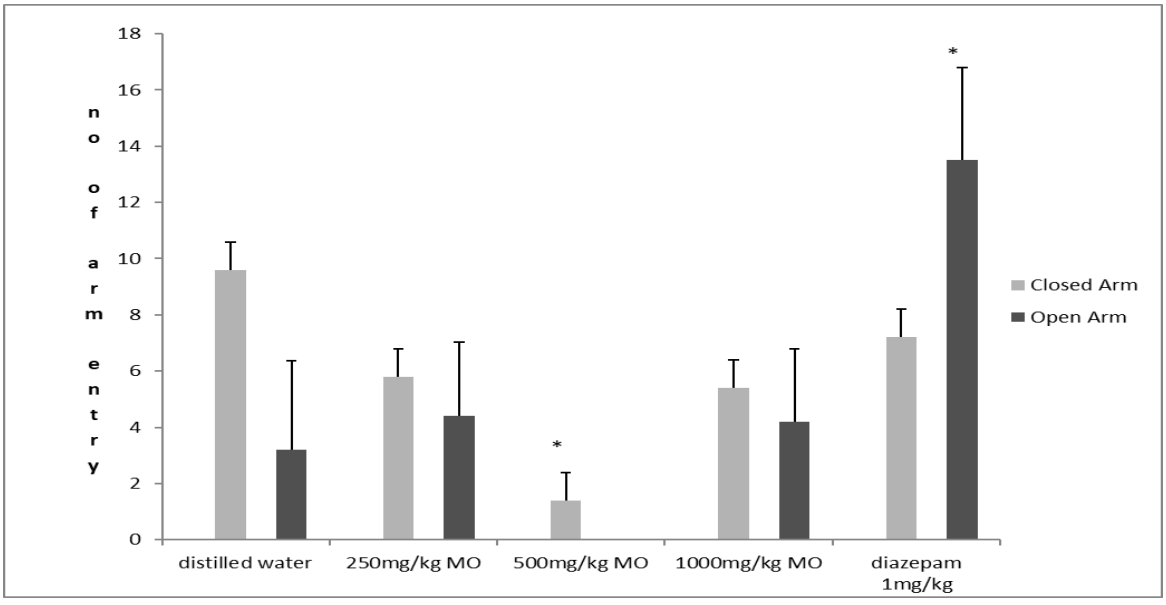

Figure 3: Effect of *Moringa oleifera* seed oil extract on elevated plus maze (number of entries in open and closed arm) in mice

Data are presented as mean ± SEM. MO-*Moringa oleifera*.n=5. *p<0.05; indicate significant difference compared to control. Analysis was carried out using One way ANOVA followed by Dunnet's *post hoc* test.

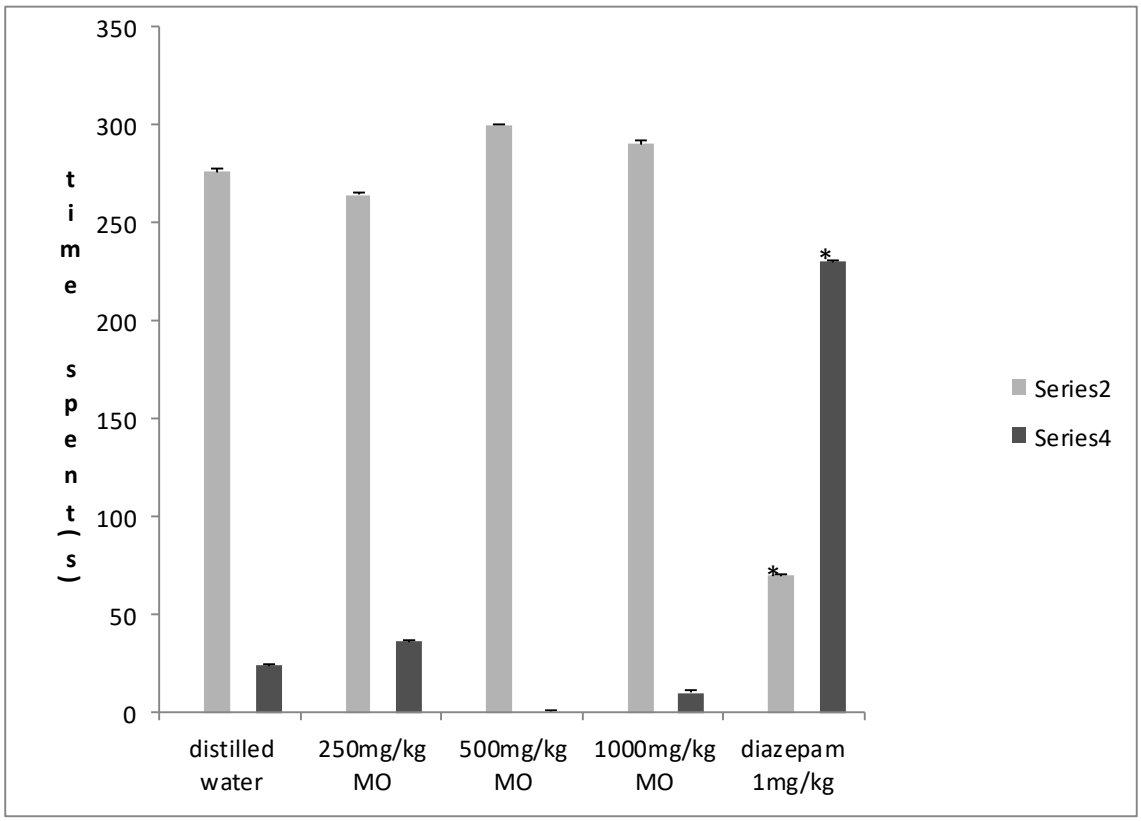

Figure 4: Effect of *Moringa oleifera* seed oil extract on elevated plus maze (time spent in open and closed arm) in mice

Data are presented as mean ± SEM. MO-*Moringa oleifera*. n=5. *p<0.05; indicate significant difference compared to control. Analysis was carried out using One way ANOVA followed by Dunnett's *post hoc* test.

Effect of *Moringa oleifera* seed Oil extract on the Hole Board Test in Mice

The *Moringa oleifera* seed oil extract significantly *(p<0.*05) and dose-dependently decreased the number of head dips in the hole board test. Conversely, Diazepam at a dose of 0.5 mg/kg significantly (p<0.05) increased the number of head dips as seen in (Fig .3).

Effect of *Moringa oleifera* Seed Oil extract on Beam Walking Assay in Mice

Moringa oleifera seed oil extract did not significantly affect the number of foot slips, though there was a dose-dependent increase in number of foot slips. Conversely, diazepam at a dose of 1.5 mg/kg significantly (p<0.05) increased the number of foot slips as seen in (Fig .4).

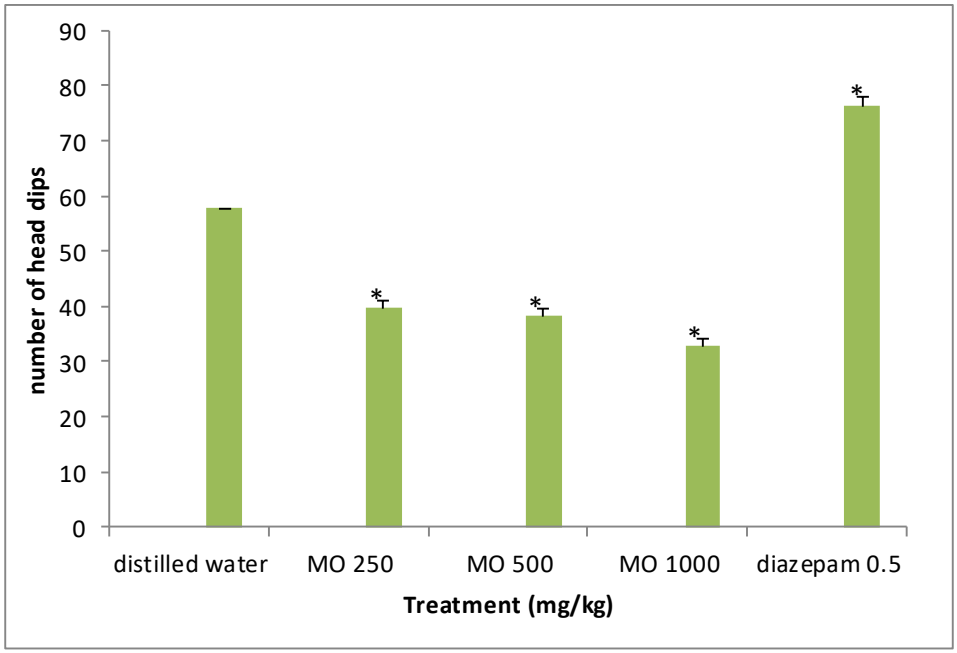

Figure 3: The Effect of *Moringa Oleifera* Seed Oil Extract on Exploratory Behavior in the Hole Board Test in Mice

Data are presented as mean ± SEM. MO-*Moringa oleifera*. n=5. *p<0.05; indicate significant difference compared to control. Analysis was carried out using One way ANOVA followed by Dunnett's post hoc t-test

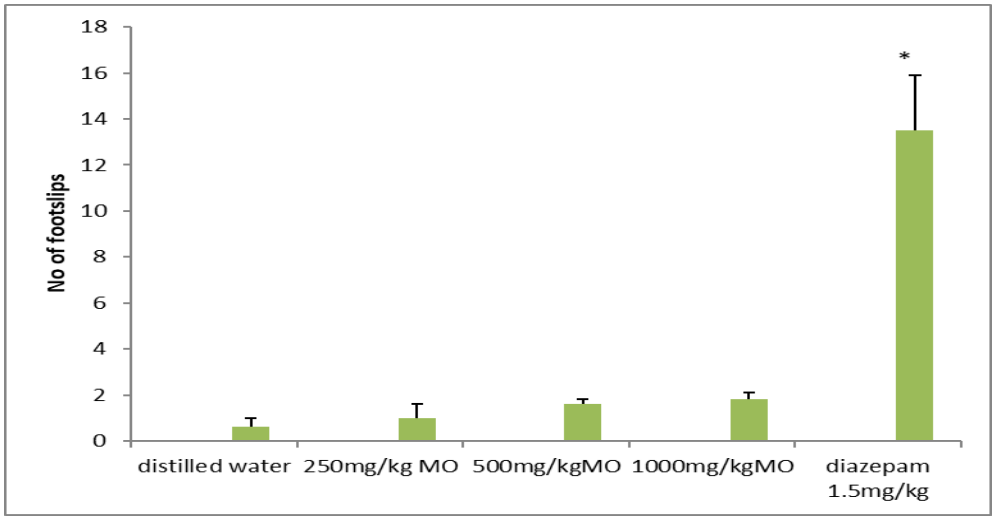

Figure 4: The Effect of *Moringa Oleifera* Seed Oil Extract on Motor Coordination in Beam Walking Assay in Mice

Data are presented as mean ± SEM. MO-*Moringa oleifera*.n=5. *p<0.05; indicate significant difference compared to control. Analysis was carried out using One way ANOVA followed by Dunnett's *post hoc* test.

DISCUSSION

This current study investigated the anticonvulsant, anxiolytic and CNS depressant effects of *Moringa oleifera* seed oil extract using standard animal models. The phytochemical screening of *Moringa oleifera* seed oil extract shows the presence

of constituents such as steroids, and fatty acids (Sofowora, 2008). The oral median lethal dose of the extract found to be >5000mg/kg suggested that it is non-toxic. According to Lorke (1983) substances with LD_{50} greater than 5000mg/kg are almost practically non-toxic.

The action of *Moringa oleifera* seed oil in this study indicates that the oil may contain bioactive principles with CNS depressant activity. Steroids have been reported to have central nervous depressant effect such as anxiolysis and central antinociception (Bhosale *et al*., 2011). This finding corroborates that of Chindo *et al*. (2003) who reported that the ethanolic extract of *Moringa oleifera* leaves has the ability to potentiate pentobarbitone-induced hypnosis, an effect that may be attributed to an action on the central mechanism involved in the regulation of sleep or an inhibition of pentobarbital metabolism (Kaul and Kulkarmi, 1978). The CNS properties of *Moringa oleifera* seed oil could be as a result of the secondary metabolites presents, which may have synergistic effect at a single/multiple target site associated with a physiological function. According to Kaufman *et al*., (1999), some plants exert their action by having a similar metabolic mechanism as the endogenous metabolites, ligands, hormones, signal transductions, or neurotransmitters. Similarly, Shahrear (2020) reported central nervous system depressant effects of Cardiac glycosides and steroids from *Flemingia stricta,* this also corroborate findings from this study.

Maximal electroshock-induced seizure-test (MES-T) is the most frequently used model for detection of anti-seizure activity of a new drug or plant extract (Loscher and Schmidt, 1988; The action of MOSOE in the MEST test indicates that the oil may contain bioactive constituents with possible anti-convulsant activity. According to Rogawski and Porter (1990), drugs such as phenytoin have the ability to block the MES induced tonic hind limb extension by decreasing the spread of seizure.

PTZ model is widely used to induce convulsion in experimental animals, it also serves as a useful animal model for the development of potential anti-convulsant drugs as well as in exploring the underlying mechanism(s) for their actions. PTZ produced convulsion by blocking GABA receptors thereby impairing GABA-mediated inhibitory neurotransmission (Magaji *et al.,* 2017). Thus, the effectiveness of diazepam against PTZ-induced seizure may be related to its well-known action of potentiating GABA-mediated inhibitory neurotransmission (De sarro *et al.,* 1999). The inability of MOSOE to protect against strychnine-induced seizure indicates that it might not be acting by antagonizing glycine receptors.

To evaluate the anxiolytic potential of the extract, Elevated Plus Maze Test (EPM) was conducted. The EPM test is one of the most widely validated tests for identifying new anxiolytic agents. In the EPM test, it is assumed that animals feel safe in the closed arms but exhibit fear and anxiety during exploration of the open arm (Saiyudthong and Marsden, 2010). An anxiolytic agent increases both the frequency of entries into the open arms and the time spent in the open arms of the EPM (Grundmanna *et al.,* 2007). The present study shows that *Moringa oleifera* seed oil extract did not significantly increase the frequency of entries and time spent in the open arm. Hence it was unable to modify the behavior of the mice subjected to the elevated plus maze test which may indicate absence of anxiolytic activity. This finding is in accordance with a previous study conducted by Bakre *et al*. (2013).

To further evaluate the anxiolytic potential, the ho board test was conducted. The hole board test is a simple model for measuring exploratory activity in rodents (File and Wardill, 1975; Durcan and Lister, 1989). The behavioral head dipping in hole board test is sensitive to changes in the emotional states of the animals; and an increase in head dipping behavior is a reflection of anxiolytic activity (Takeda *et al*.,1998) whereas, a decrease in the parameter reveals a sedative behavior (File and Pellow, 1985). In this test, the effect observed with MOSOE indicates absence of anxiolytic activity and a possible sedative potential.

The mouse beam walking assay used to evaluate the effect of the extract on motor coordination is a more sensitive model than rota rod in predicting clinical sedation in humans caused by novel drugs (Stanley *et al.*, 2005; Magaji *et al*., 2012). The extract did not significantly increase the number of foot slips, an index of motor coordination deficit, thus suggesting that the sedative effect of the extract might possibly be centrally mediated and not due to peripheral muscular blockade (Perez *et al.,* 1998). This finding is in accordance with a previous study conducted by Musa *et al.* (2008).

CONCLUSION

The results obtained in this study suggest that the *Moringa oleifera* seed oil extract contains bioactive constituents with CNS depressant effect and a possible anti-convulsant activity. This may justify the traditional use of *Moringa oleifera* seed oil extract in the management of epilepsy and other CNS disorders

Conflict of Interest: Authors declare no conflict of interest

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