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Anticonvulsant, anxiolytic and hypnotic effects of Monodora myristica (Gaertn, Dunal.) dried seed essential oil in mice

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ABSTRACT

Background: The seed of *Monodora myristica* (Gaertn, Dunal.) has been reported to be useful in the management of neuralgia, headaches, rheumatism, pain, cough and tonic among others, however, the central nervous system effect of the essential oil has not been investigated. This study was carried out to evaluate the anticonvulsant, anxiolytic and hypnotic effects of *M. myristica* dried seed essential oil in mice.
Method: The essential oil of *M. myristica* (EMM) was obtained from the dried seeds by hydrodistillation, while the median lethal dose (LD_{50}) was determined using Lorke's method. Doses of EMM at 25, 50, 100 mg/kg, per oral were used for the novelty-induced behavior, anticonvulsant and hypnotic activities while lower doses of EMM at 6.25, 12.5, 25 mg/kg, per oral were used for the anxiolytic activities. The novelty-induced behaviours were determined in the open field test (OFT). The essential oil effect on amphetamine-induced hyperlocomotor response in the OFT was determined. The anticonvulsant effect was evaluated using chemoconvulsants such as pentylenetetrazole (PTZ) and strychnine (STR)–induced convulsion models. Potential anxiolytic effect was investigated using hole board (HB) and elevated plus maze (EPM) models. The hypnotic effect was determined using ketamine-induced sleep model where the sleep latency and the total sleeping time were assessed.
Results: Oral LD_{50} of EMM was 283 mg/kg and EMM in the open field test caused significant ($p<0.01$) dose-dependent decrease in locomotion, rearing and grooming activities suggesting that EMM possessed central nervous system depressant activity. It significantly ($p<0.05$) inhibited amphetamine-induced hyperlocomotor behavior therefore suggesting that EMM may be useful in alleviating disorder related to hyperactivity. Oral administration of EMM significant ($p<0.05$) delayed the onset of seizures induced by PTZ and STR but did not protect against seizures. EMM also caused significant ($p<0.05$) reduction in sleep latency and prolongation of total sleep time suggesting that it has hypnotic effect. EMM did not show any significant anxiolytic effects in HB and EPM models.
Conclusion: The study showed that EMM possessed significant central inhibitory and hypnotic effects but has no anxiolytic properties.

INTRODUCTION

Medicinal plants have contributed immensely to modern medicine because of their traditional and folkloric usage. Herbal products made from the diverse medicinal plants are also in use in many parts of the world as complementary and alternative source or therapy[1]. *Monodora myristica* popularly referred to as the "*African Nutmeg*" is of the Annonacea family. Its local names include: *Ariwo* (Yoruba), *Ehuru* or *Ehiri* (Igbo), Jamaica nutmeg, Calabash nutmeg, and *Airama*[2]. The seed (or kernel), when ground to powder, is a popular condiment used to prepare "pepper soup", a popular meal in Africa that acts as a stimulant to relieve constipation, control passive uterine hemorrhage in women immediately after child birth[3]. The seeds are used in treating headache, constipation, hypertension, post-partum haemorrhage, also used as an anti-sickling agent, and as an antimicrobial agent[4,5]. The antimicrobial activity of the seed oil has been reported by Odoh et al.[6] Reports have shown that a number of studies have demonstrated that plant derived essential oils exhibit a variety of biological properties, such as anticonvulsant and central activities among others[7]. *M. myristica* is rich in essential oil and

several other phytoconstituents such as tannin, saponin, flavonoid, resins, steroid, terpenoids alkaloids and phenol. The essential oil from the leaves contains ß-carophyllene, α-humulene and α-pinene, while that from the seed contains α-phillandrene, α-pinene, myrocene, limonene and pinene[8]. Despite the traditional use of various parts of the plant including the seed oil in the treatment of various disorders there are no reports of scientific evaluation of central nervous system activities of the essential oil of the dried seed of *M. myristica* in relation to its possible potential in the treatment of seizure, anxiety disorder, mania and insomnia among others. Hence this study was carried out to investigate the anticonvulsant, anxiolytic and hypnotic effects of the essential oil of the dried seed of *M. myristica* in mice.

MATERIALS AND METHODS

Plant material: The dried seeds of *Monodora myristica* were obtained from new market, Ile-Ife, botanic authentication carried out by Mr. I. I. Ogunlowo, the Herbarium Officer and the voucher specimen deposited at the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. FPI included in the online edition of the Index Herbarium, Obafemi Awolowo University, Ile-Ife, with the number FPI 2111

Drugs: Diazepam (Valium®, Basel, Switzerland), Pentylenetetrazole (PTZ), Strychnine, Amphetamine (Sigma, Buchs, Switzerland), Ketamine (Rotex®, Bleichstraße, Germany).

Animals: Swiss albino mice (18-25 g) obtained from the animal house, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife were used for the study. The mice were maintained under standard environmental conditions had free access to standard diet and water *ad libitum*. The experiment procedures were carried out in accordance with Guide for the Care and Use of Laboratory Animals. 8th ed. Washington (DC)[9].

Preparation of plant extract: The hydro-distillation using Clevenger-like apparatus was done at the Postgraduate Toxicology Laboratory, Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria. Five hundred and sixty-seven gram (567 g) of dried seeds of *M. myristica* was weighed and hydro-distilled using Clavenger-like apparatus for about 4 hours. The oil was collected and dried over sodium sulfate crystals and stored in amber coloured bottle at 4°C. The density of the oil was determined and the percentage yield was also calculated as follows:

$$(\%\ ^{v}/w) = \frac{\text{Volume of oil obtained}}{\text{Weight of dried seed}} \times 100\%$$

Formulation of the essential oil for administration and choice of vehicle

The *M. myristica* oil was readily emulsified with Tween-80; however the emulsified oil was diluted further with distilled water to make the final preparation of Tween-80 to be between 1-5% (v/v) in the final preparation to be administered. Tween-80 at concentration below 32% (v/v) has been found to lack significant locomotion activity, therefore, 5% of Tween-80 is suitable as solvent for the oil and drugs[10]. The density of the oil was initially determined, where equivalent volume corresponding to required weight was taken and emulsified with Tween-80, and then made up to the desired volume and concentration of the essential oil in mg/ml with distilled water, shortly prior to administration to the experimental animals.

Administration of drugs: Essential oil of *M. myristica* (EMM) was administered to mice through oral route using oral cannula.

Pharmacological Studies

Median lethal dose (LD_{50}) testing: LD_{50} an index of acute toxicity of the oil was determined as described by Lorke[11]. The procedure is consisted of two phases for the rapid determination of LD_{50}. The first phase has three increasing doses; 10, 100 and 1000 mg/kg of essential oil administered orally to three different groups of mice (N=3). There was mortality with all the animals administered 1000 mg/kg. Thereafter, for the phase two, the reduced doses of 100, 200, 400, 600, 800 mg/kg (N = 1) were administered with mortality in 400, 600, 800 and 1000 mg/kg. No mortality with animals administered with 200 and 100 mg/kg after 72 hours of observation.

Mathematically, the LD_{50} = square root of A x B; where

- A is the maximum dose that resulted to 0% death, and
- B is the minimum dose that resulted in 100% death.

Novelty-induced behavioural assessment: The open field model as described by Ajayi and Ukponmwan[12], was used to test the novelty-induced behavioral activities (grooming,

locomotion and rearing). Rearing being the number of times the experimental animal stands on its hind-limbs with fore limbs in the air or against the side walls of the activity cage. Grooming involves nose and face wash and mouth cleaning. Locomotion is estimated by the number of squares crossed with all the limbs in the activity cage[12]. The open field box is a rectangular structure composed of a hardboard floor (36 x 36 cm^2) with a surrounding wall of 30 cm height. The box is made of wood painted white and the floor is divided by permanent marker into squares. The albino mice were divided into five groups (n = 6 per group). Group 1 was administered with vehicle, 5% Tween 80 (10 mg/kg or 0.1 ml/10 kg) as negative control per oral, Group II, III and IV were administered with different doses; 25 mg, 50 mg, 100 mg of Essential oil of *Monodora myristica* (EMM) respectively per oral and Group V received Diazepam 1 mg/kg per oral to serve as positive control. All the mice were pre-treated (60 minutes) with test materials; vehicle, oil (EMM) and standard drugs prior to assessment (20 minutes) for rearing, grooming and locomotion. The mice were placed directly from the home cages into an opaque Plexiglas observation cage (45 x 25 x 25 cm) with only one transparent side for observation. The mice were all observed and assessed one after another, singly and, the observation cage cleaned with 70% alcohol after each assessment to prevent olfactory cue that might promote odour bias from one animal to the other during assessment in the cage. The frequency of rearing, grooming and locomotion was determined for a period of 20 minutes. Mice were assessed in turn for the number of floor units crossed for 20 minutes. A high frequency of locomotion indicates excitation, and low frequency depicts depression[13]. Grooming behavior is expected to be displayed in a novel environment because it is a displacement response. Mice were observed for the frequency of grooming as represented by face washing with the paws, picking of the body and pubis with mouth for 20 minutes. Rearing frequency is the number of times the animal stood on its hind legs or with its forearm against the wall of the observation cage or in the free air[12]. Each mouse was also assessed for 20 minutes in the observation cage.

Amphetamine-induce hyperactivity[14]: The test was done to determine the effect of the essential oil on amphetamine–induced hyperlocomotion in mice. Mice were divided in to eight groups: Group I was administered 5% Tween 80 (10 mg/kg or 0.1 ml/10 g), Group II was treated with amphetamine 1.5 mg/kg, *i.p.*, Group III was treated with EMM (100 mg/kg, p.o) plus vehicle, Group IV was treated with EMM (100 mg/kg, *p.o.*) plus vehicle and amphetamine (1.5 mg/kg, *i.p*); Group V was treated with EMM (50 mg/kg, *p.o.*) plus vehicle, Group VI was treated with EMM (50 mg/kg, *p.o.)* plus vehicle plus Amphetamine (1.5 mg/kg, *i.p.*), Group VII was treated with diazepam (2 mg/kg, *p.o.*) and Group VIII treated with Diazepam (2 mg/kg, *p.o.*) with amphetamine (1.5 mg/kg, *i.p.*). Pre-treatment with the EMM prior to amphetamine administration was one hour per oral. Each animal was placed singly in the observation cage immediately after amphetamine (1.5 mg/kg, *i.p.*) administration and observed for 20 minutes period.

Anticonvulsant test

Pentylenetetrazole-induced seizure: An experimental method for inducing seizures in animals is to utilize the central nervous system modulator pentylenetetrazole (PTZ)[15]. Pentylenetetrazole (PTZ), a GABA receptor antagonist, is a common chemically-induced seizure model that is categorized as a model of generalized seizure. It produces a myoclonic seizure that models absence (petit mal) seizures. It has been reported to be an excellent tool for evaluating anti-seizure characteristics. Compounds that enhance GABAergic transmission are known to be effective in ameliorating seizure activity in the PTZ epilepsy model. In this present study, thirty mice were randomly divided into five groups (n = 6). Group I was treated with vehicle (5% Tween–80) to serve as the negative control, while groups II to IV were treated with essential oil of *Monodora myristica* (25, 50 and 100 mg/kg, *p.o.*) respectively. Group V was administered with diazepam (1 mg/kg, *p.o.*) to serve as the positive control. All the groups were pre-treated one hour before the administration of lethal dose of PTZ (85 mg/kg, *i.p.*)[16]. The treated mice were assessed for onset of convulsion (CL) and time of death (TD) within 30 min period. Animals that survived after 30 minutes of administration of PTZ were assumed to be protected in this model[17]. Mice that did not convulse within 30 minutes after PTZ administration were considered protected[17]. The time of death (TD) for the purpose of analysis was taken as 30 minutes for the animals that survived beyond 30 minutes post administration of PTZ.

Strychnine (STR) - induced seizure: The procedure described for the PTZ – induced convulsion was employed using strychnine (3 mg/kg, *i.p.*,) as the convulsant agent and diazepam (5 mg/kg, *p.o.*) as the positive control[18]. All the test materials (5% Tween-80, EMM and diazepam 5 mg/kg,

p.o.) were administered 60 min per oral before administration of strychnine 3 mg/kg, *i.p.*). Each mouse was observed for convulsion latency (CL) and the time of death (TD) within 30 min period. Animals that survived after 30 minutes of administration of strychnine were assumed to be protected in this model[17].

Anxiolytic tests

The potential anxiolytic effect of essential oil of *Monodora myristica* was assessed using Hole Board and Elevated Plus Maze (EPM) models.

Hole Board Test: The hole-board apparatus is made of wooden panel (40 x 40 cm, 2.2 cm thick) with sixteen equidistant holes 3 cm in diameters on the floor. The apparatus has no walls and was elevated to a height of 18 cm above floor level. Mice were divided into five groups (n = 6), the first group of mice served as control and received vehicle (5% Tween-80, 0.1ml per 10 g, *p.o.*) while the experimental groups received EMM at doses of 6.25, 12 .5, 25 mg/kg per oral. The standard group received diazepam (1 mg/kg, *p.o.*). Each mouse was placed singly in the center of the board facing away from the observer 1 h after the administration of vehicle or test substances. The number of times that the animal dipped its head into the hole within 5 min period was recorded and the result expressed as mean total number of head dips[19,20]. It has been reported that changes in head-dipping behavior may reflect the anxiogenic and/ or anxiolytic state of animals as it known that typical anxiolytics increase, while anxiogenics decrease, the number of head-dipping[21].

Elevated Plus Maze (EPM) Test: The test used an elevated plus maze model based on the natural distaste of experimental animals especially rodents, for open spaces and heights[22,23]. The model is made of wood and has two narrow enclosed arms which are bordered by high walls and also has two open arms which are essentially not enclosed with high walls. The elevated plus maze consists of two open arms (30 x 5 x 0.25 cm^3) and two closed arms (30 x 5 x 15 cm^3), both of which share a center stand (5 x 5 cm^2) with two pairs of identical arms placed opposite each other. The mice were grouped into five as earlier described for the hole board test. After 1 h of oral administration of vehicle (5% Tween-80, 0.1 ml per 10 g, *p.o.*), diazepam (1 mg/kg, *p.o.*) and EMM at doses of 6.25, 12.5, 25 mg/kg, each mouse was individually placed on the central platform facing towards open arm and the number of entries and time spent in closed and open arms were scored for 5 min. Entry into an arm was defined as the animal placing all four paws on the arm. After each test, the maze was carefully cleaned with tissue paper wet with a 10% ethanol solution. The percentage of time spent (duration) in open arms and frequency of open arm entries were calculated[23]. The Index of Open Arm Avoidance (IOAA) that gives a measure of anxiety was also determined:

$$[100 - \frac{(\%\text{ time spent on open arm} + \%\text{ entries into open arms})}{2}]$$

If the result obtained is at least 10 points less than vehicle's the test substance is adjudged to be anxiolytic; likewise if the result is at least 10 points greater than vehicle's, then it is considered to be anxiogenic[24].

Hypnotic test - Ketamine-induced sleeping time: Ketamine is an antidepressant and hypnotic drug acting as an antagonist at excitatory *N*-methyl-D-aspartate (NMDA) glutamate receptor[25]. In animal models of depression, Ketamine has been reported to also induce antidepressant-like effects at low doses[25,26] and hypnosis/dissociative anesthesia after high-doses in naïve rodents[27]. Therefore, in order to determine the effect of EMM on sleep latency and prolongation of total sleeping time induced by ketamine, thirty mice were randomly allotted into five groups (n = 6). Group I was administered with 5% Tween -80 (negative control, 0.1 ml/10 g, *p.o.*,), Groups II to IV were administered different doses of EMM (25, 50 and 100 mg/kg, *p.o.)* while Group V was administered diazepam (1 mg/kg, *p.o.*) (positive control). After 1 hour pre-treatment of the mice with vehicle, diazepam and EMM, ketamine (100 mg/kg, *i.p.*) was administered[27]. The hypnotic response was determined by assessing loss of the righting reflex (LORR) after ketamine injection. The time difference between ketamine administration and loss of righting reflex was considered as latency to sleep (SL), while the time from the loss to the regaining of righting reflex was the duration or total sleep time (TST)[27].

Statistical Analysis

The results obtained were expressed as mean ± SEM (standard error of mean). The statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Dunnett's posthoc test. Significant differences were set at $p<0.05$. Graph pad prism, version 5.01 (UK) was used.

RESULTS

Distillation and Density: The EMM obtained from the hydro-distillation procedure has a sweet, spicy and

aromatic smell with a pale yellow colour and density of 0.87 mg/ml. The percentage yield was 4.4% ^{v}/w.

Determination of LD_{50}: Acute administration of EMM did not cause significant behavioural changes and the LD_{50} was found to be 283 mg/kg and most of the mice remained calm during the period.

Novelty-induced behavior

Effect of EMM on novelty-induced locomotion in mice

The three dose levels of EMM (25, 50 and 100 mg/kg, *p.o.*) caused significant [$p<0.05$; $F_{(4, 29)} = 23.08$] dose-dependent decrease in locomotion in mice when compared with the vehicle treated group (Figure 1).

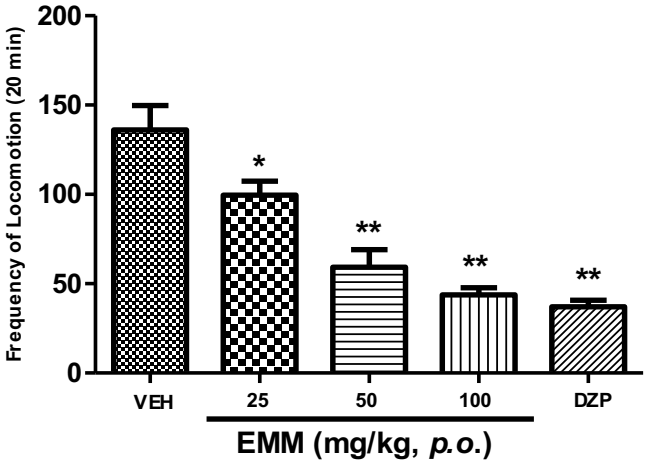

Figure 1: Effect of EMM on novelty-induced locomotion in mice. DZP = Diazepam (2 mg/kg, *p.o.*), VEH = VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried.* P< 0.05; **P< 0.01 when compared with VEH. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Effect of EMM on novelty-induced grooming in mice: EMM (50 and 100 mg/kg, *p.o.*) dose-dependently caused significantly [$p< 0.01$; $F_{(4,29)} = 16.45$] reduction in grooming behavior in mice when compared with the vehicle treated group (Figure 2).

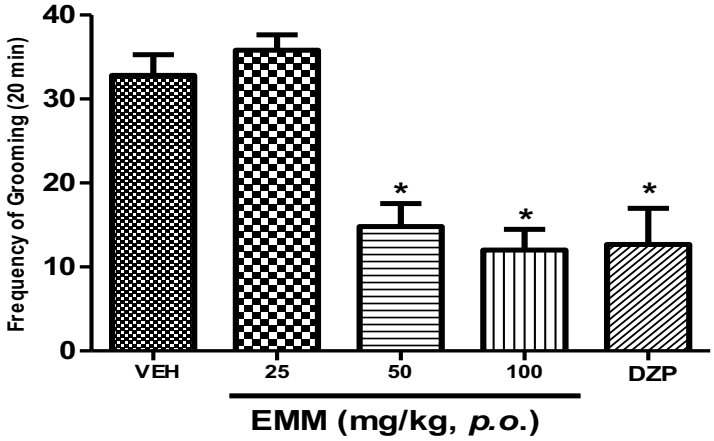

Figure 2: Effect of EMM on novelty-induced grooming in mice. DZP = Diazepam (2 mg/kg, *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. *P< 0.05 when compared with VEH. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Effect of EMM on novelty-induced rearing in mice: The EMM (50 and 100 mg/kg, *p.o.*) significantly [$p<0.01$; $F_{(4, 29)} = 13.52$]) reduced rearing behaviour in mice when compared with the vehicle (Figure 3).

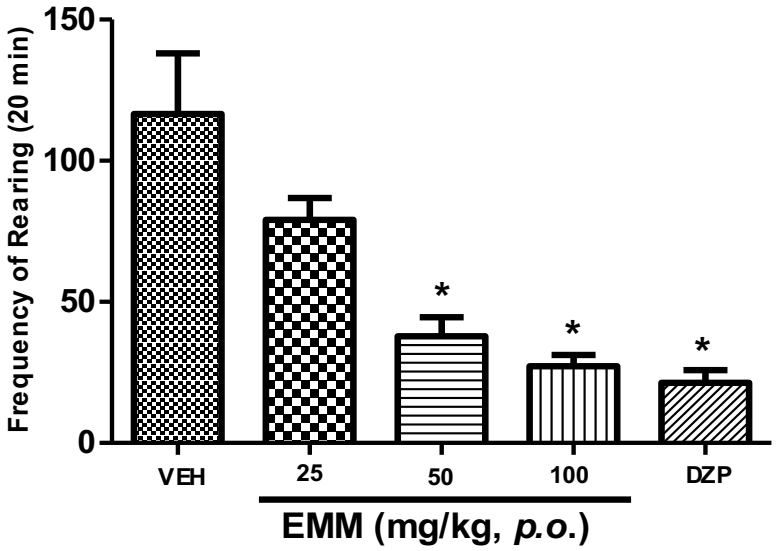

Figure 3: Effect of EMM on novelty-induced rearing in mice. DZP = Diazepam (2 mg/kg, *p.o.*), VEH = VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. *P< 0.01 when compared with VEH. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Effect of EMM on amphetamine-induce hyperlocomotion in mice: Amphetamine, (1.5 mg/kg, *i.p.*) caused a significant increase in frequency of locomotion by increasing the locomotion (square crossing) significantly [$F_{(3, 23)} = 24.078$, $p<0.01$] when compared to the vehicle treated group, EMM and diazepam alone. EMM was observed to significantly inhibited the amphetamine-induced hyperlocomotion in mice (Figure 4).

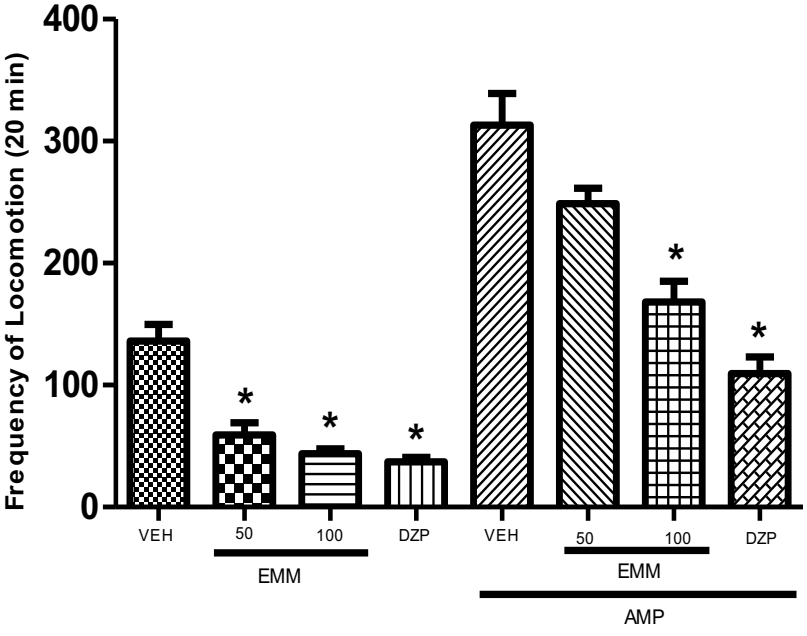

Figure 4: Effect of EMM on amphetamine-induced hyperlocomotion in mice (Locomotion is enhanced following AMP, reflecting mania-like elevated drive. Amphetamine-induced hyperactivity is blocked by EMM and DZP. DZP = Diazepam (2 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), AMP = Amphetamine (1.5 mg/kg, *i.p.*), EMM = Essential oil of *Monodora myristica* dried. *P< 0.01 when compared with VEH and VEH +AMP. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Effect of EMM on chemically– induced convulsions in mice

Effect of EMM on Pentylenetetrazole (PTZ)– induced convulsion in mice: EMM offered 16.67% protection at 25 and 100 mg/kg, *p.o.* dose levels while it had 0% at 50 mg/kg, *p.o*. The standard drug (diazepam, 1 mg/kg, *p.o*.) offered 100% protection. It was observed that EMM (25, 50 and 100 mg/kg, *p.o*.) dose-dependent caused delay in the onset of seizure significant ($p < 0.01$] when compared with the vehicle that had no effect on PTZ –induced convulsion (Table 2). EMM did not offer protection against death while the standard drug (diazepam 1 mg/kg, *p.o*.), significantly ($p < 0.05$) inhibited convulsion and death (Table 1).

Table 1: Effect of EMM on the pentylenetetrazole (PTZ) – induced convulsion

Treatment (mg/kg, p.o.)	Seizure Latency (s)	Seizure duration before death (s) (Seconds)	Quantal protection against mortality	Percentage Protection
VEH	49.67 ± 2.50	480.00 ± 21.91	0/6	0.00
EMM 25	75.00 ± 7.81*	230.00 ± 54.59	1/6	16.67
50	87.83 ± 5.76*	310.00 ± 44.94	0/6	0.00
100	121.30 ± 4.95*	610.00 ± 222.58	1/6	16.67
DZP (1 mg/kg, p.o.)	No convulsion	No convulsion	6/6	100.00

Values are mean ± SEM, n = 6; EMM: -Essential oil of *Monodora myristica,* DZP: diazepam (1 mg/kg, *p.o);* VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g). *p<0.05 when compared with vehicle.

Effect of EMM on Strychnine–induced convulsion in mice: EMM (25, 50 and 100 mg/kg) dose-dependently caused significant [$F_{(4, 29)} = 100.37$, $p<0.01$] increase in seizure latency. It was similarly observed that EMM also significantly ($p<0.01$) delayed death latency in strychnine–induced but did not offer any protection against death. Diazepam (1 mg/kg, p.o.) also delayed the seizure latency and death latency when compared with the vehicle treated mice (Table 2).

Table 2: Effect of Oral administration of EMM on Strychnine– induced convulsion in mice

Treatment (mg/kg, *p.o* .)		Seizure Latency (s)	Seizure duration before death (min)	Quantal protection against mortality	Percentage protection
VEH		45.85 ± 3.63	1.00 ± 0.00	0/6	0
EMM	**25**	97.83 ± 3.65*	2.17 ± 0.17*	0/6	0
	50	108.30 ± 2.50*	2.83 ± 0.17*	0/6	0
	100	124.50 ± 1.48*	3.33 ± 0.21*	0/6	0
DZP (1 mg/kg, p.o.)		142.50 ± 5.63*	3.50 ± 0.34*	0/6	0

Values are mean ± SEM, n = 6; EMM; Essential oil of *Monodora myristica,* DZP; diazepam (1 mg/kg, *p.o*.), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g). *p< 0.05, significantly prolonged than vehicle.

Anxiolytic test

Effect of EMM on head dip (Hole board model): EMM significantly [$F_{(4,29)}$= 9.736, p< 0.01] decreased the head dips when compared to the vehicle treated mice. The reference drug, diazepam (1 mg/kg, *p.o*.), however, increased significantly (p<0.05) the head dips, when compared to vehicle treated mice (Figure 5).

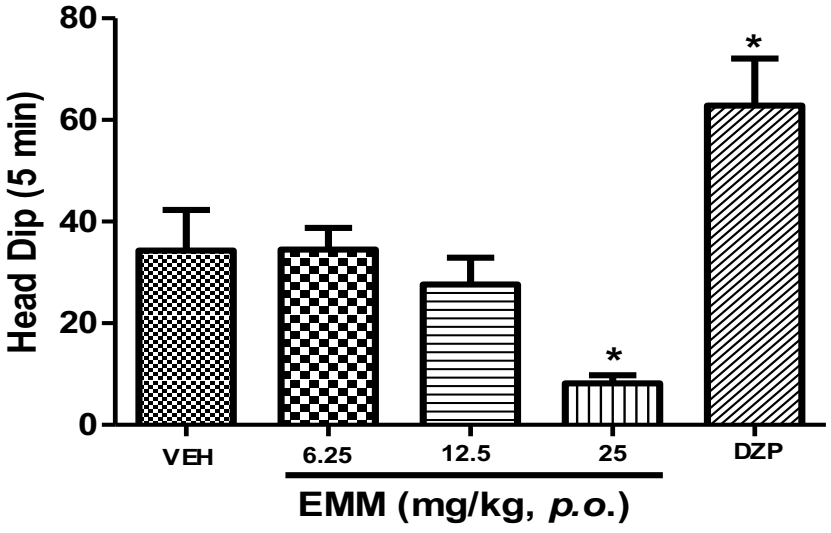

Figure 5: Effect of EMM and Diazepam on head dip in Hole Board model. DZP = Diazepam (1 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. *P< 0.05 when compared with VEH. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Effect of EMM on Elevated plus maze model (EPM)

Effect of EMM on percentage time spent in open arms on the EPM: EMM (6.25, 12.5 and 25 mg/kg, *p.o.*) caused a non-significant increase in the time spent in the open arm (Figure 6).

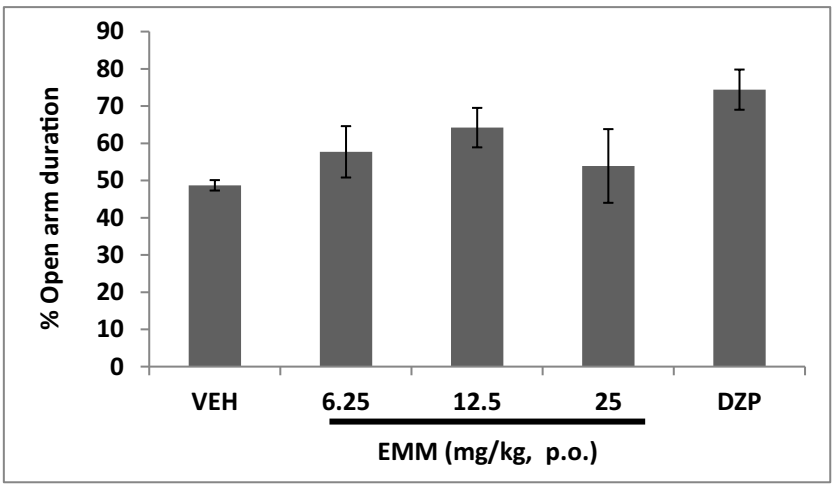

Figure 6: Effects of EMM on the percentage of Open arm duration of the elevated plus maze. DZP = Diazepam (1 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Percentage open arm entries of Effect of EMM on the Elevated plus maze model: EMM (6.25, 12.5 and 25 mg/kg, *p.o.)* has no significant [$F_{(4,29)} = 0.8020$, $p > 0.05$] effect on the percentage of open arm entries (Figure 7).

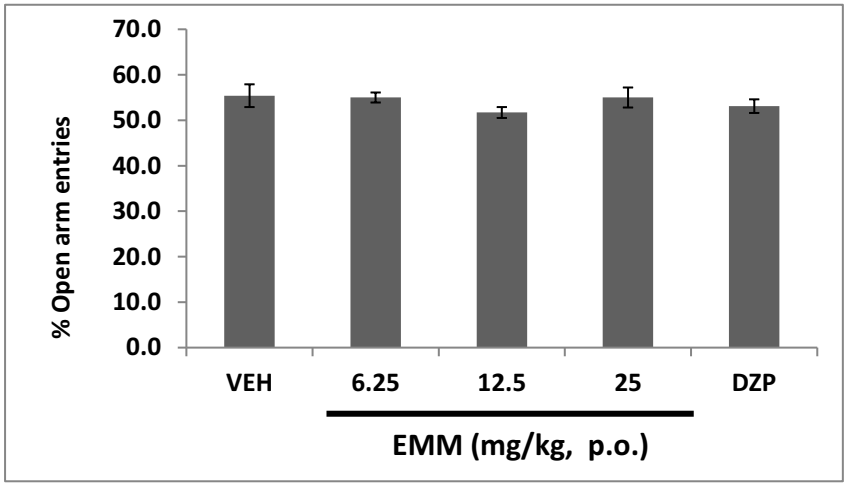

Figure 7: Effects of EMM on the percentage of Open arm entries of the elevated plus maze. Values are Mean ± SEM (n=6). DZP = Diazepam (1 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried.

Index of open arm avoidance (IOAA)

The index of open arm avoidance (IOAA) was found to be 47.90 ± 1.90 for the vehicle (5% Tween 80; 0.1 ml per 10 g); 43.60 ± 3.90, 42.10 ± 2.50 and 45.60 ± 4.30 for the EMM (6.25. 12.5 and 25 mg/kg, *p.o.*) respectively and 36.2 ± 2.9 for diazepam (1 mg/kg, p.o.). The results showed that diazepam has anxiolytic effect (Figure 8).

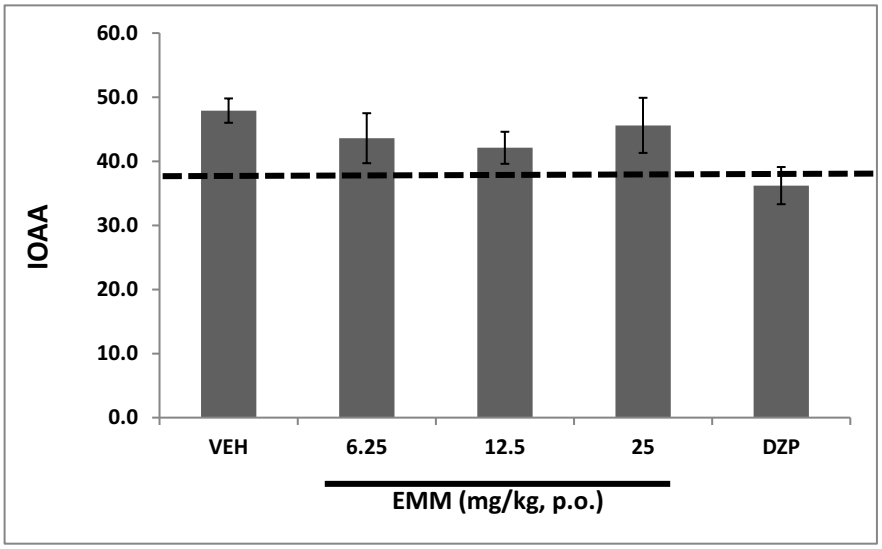

Figure 8: Index of Open arm avoidance (Mean ± SEM, n=6) in the elevated plus maze. DZP = Diazepam (1 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. Broken lines (……..) indicate the cut-off that is 10 points below the vehicle-treated control.

Hypnotic tests with EMM and Ketamine

Effect of EMM on ketamine-induced sleep latency (SL) in mice: EMM (25, 50 and 100 mg/kg. *p.o.*), reduced the sleep latency in a dose-dependent manner with the highest significant [$F_{(4, 29)} = 12.187$, $p<0.01$] effect at 100 mg/kg dose. Diazepam (1 mg/kg, *p.o.)* also caused a significant ($p<0.01$) reduction in sleep latency (SL) in ketamine-induced hypnosis (100 mg/kg, *i.p.*) when compared to vehicle (5% Tween-80) (Figure 9).

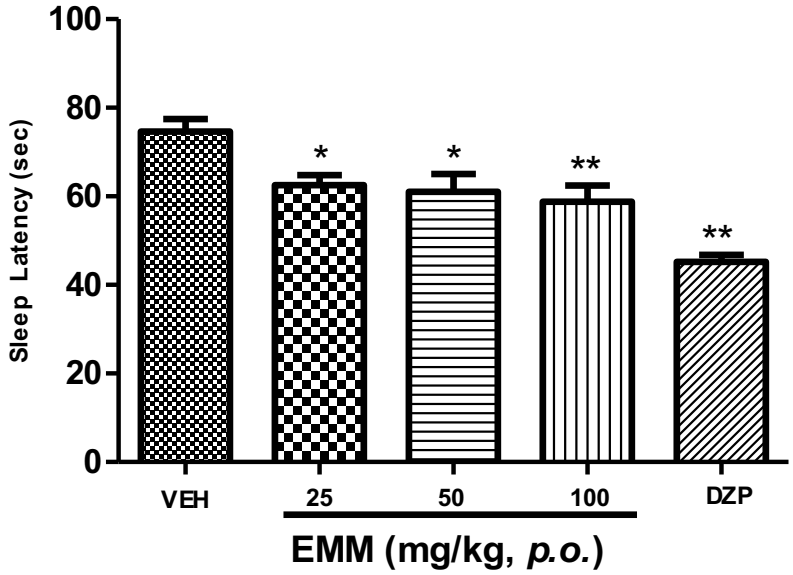

Figure 9: Effect of EMM and Diazepam on sleep latency in ketamine-induced hypnotic test in mice. DZP = Diazepam (1 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. *p< 0.05; *p<0.01 when compared with VEH. **(**Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Effect of EMM on ketamine-induced total sleeping time (TST) in mice: EMM (25, 50 and 100 mg/kg, *p.o.*) increased the total sleeping time that was significant [$F_{(4, 29)} = 21.086$, $p<0.01$] at the dose of 100 mg/kg, *p.o*. Similarly, diazepam (1 mg/kg, *p.o.*) significantly ($p<0.01$) increased the total sleeping time (TST) induced by ketamine when compared to the vehicle (5% Tween 80, 0.1 ml/ 10 g, *p.o.*) (Figure 10).

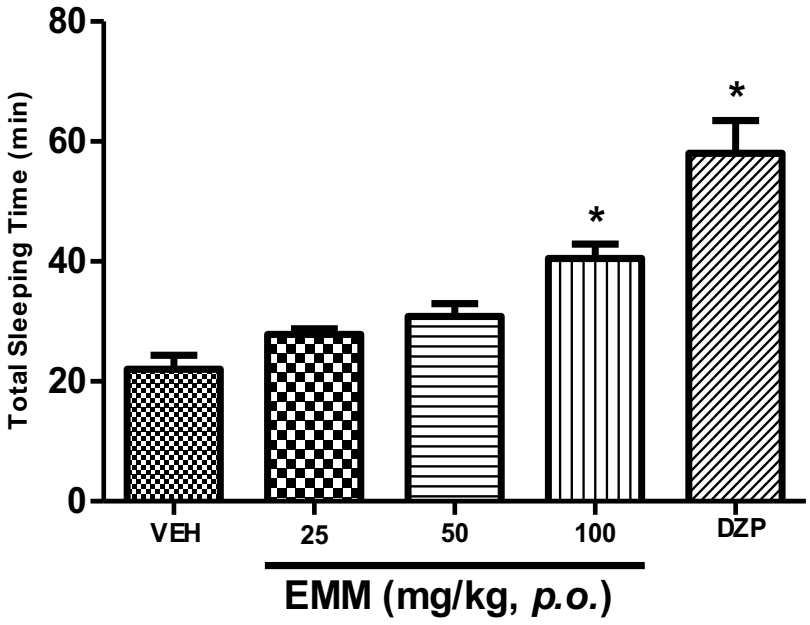

Figure 10: Effect of EMM and Diazepam on total sleeping time in ketamine-induced hypnotic effect in mice. DZP = Diazepam (1 mg/kg. *p.o.*), VEH: Vehicle (5% Tween 80, 0.1 ml/ 10 g), EMM = Essential oil of *Monodora myristica* dried. *p<0.01 when compared with VEH. Each bar represents the **Mean (± SEM)** value obtained from 6 animals.

Discussion

In this study, the median lethal dose (LD_{50}) of essential oil obtained from *Monodora myristica* dried seeds (EMM) was found to be 283 mg/kg per oral. It was observed that EMM showed central inhibitory effect. This study investigated the potential central effects, hypnotic, anxiolytic, and anticonvulsant effects in mice. The novelty-induced behavioural responses are known to be regulated by multiple neurotransmitter systems such as acetylcholine, dopamine, serotonin, histamine, orexins, gamma-amino butyric acid and noradrenaline[28,29]. Locomotion, rearing and grooming are different forms of behaviour that are scored in the open field test model. All these behavioural activities are always induced in a novel environment. In this study, it was observed that all the novelty-induced behaviours were decreased significantly due to oral administration of EMM thus suggesting that it has central inhibitory effects on the central nervous system.

Mania-elevated drive is induced by the administration of psychostimulants, most notably d-amphetamine. D-amphetamine-induced hyperactivity is the most frequently applied rodent model for mania[30]. In the vast majority of rodent models, increased locomotor activity serves as the main and often only preclinical outcome measure[30,31]. Furthermore, hyperactivity is also the major preclinical outcome measure in rodent models for attention deficit hyperactivity disorder. The administration of d-amphetamine has been reported to cause significant increase in extracellular levels of dopamine and other monoamines by increasing dopamine efflux, inhibition of

dopamine reuptake, reduction of dopamine degradation by the enzyme monoamine oxidase[31,32]. Acute administration of amphetamine produces an increase in locomotor and stereotypic activities in rodent models[31]. Amphetamine-induced hyperactivity is a frequently used screening model for antimanic effects[33,34]. Therefore, in this present study, we investigated the effect of EMM on amphetamine-induced hyperactivity in mice. The results showed that amphetamine caused significant ($p<0.05$) increase in locomotor activity suggesting hyperactivity and EMM pretreatment inhibited the amphetamine-induced hyperactivity at doses of 50 and 100 mg/kg per oral suggesting that EMM may be useful in alleviating disorder related to hyperactivity such as mania and other neuropsychiatric disorders[35]. However, there is need for further pharmacological studies on this.

Acute pentylenetetrazole-induced myoclonic seizure model is one of the most widely used model to assess anticonvulsant effect of prospective anticonvulsant drugs[36]. Pentylenetetrazole (PTZ) is a strong GABA receptor antagonist. Therefore, test substances or drugs that enhance GABA levels and/or $GABA_A$ receptor density, and/or $GABA_A$ receptor agonists (like diazepam), and/or agents that mimic GABA are believed to be useful in abolishing PTZ-induced convulsions[36]. Therefore, in this study, Pentylenetetrazole (85 mg/kg, i.p.) was used to induce tonic-clonic convulsion[15,16]. The results obtained showed that in PTZ-induced seizures, EMM only dose-dependently caused significant delay on seizures onset without affecting the duration of seizure significantly (Table 1). However, it was observed that the standard drug, diazepam ($GABA_A$ receptor agonists) gave 100% protection diazepam as it prevented the occurrence of seizures. It was therefore inferred from the study that EMM did not protect the animals from seizures but increased the seizure onset significantly with 16.7% protection at 25 and 100 mg/kg per oral (Table 1). The ability of a test substance or drug to act as an anticonvulsant is determined by its capacity to prevent convulsion or delay the onset of seizures or reduce death rate and/or decrease the frequency of the seizure episodes[38,39]. Therefore, it can be inferred from the results obtained that EMM is only effective in delaying seizures and does not possess anticonvulsant activity. Strychnine is a highly poisonous plant alkaloid of indole type that acts as a blocker or antagonist at the strychnine-sensitive glycine receptor, a ligand-gated chloride channel in the spinal cord and brain[40]. In strychnine-induced seizure, EMM (25, 50 and 100 mg/kg) did not protect the mice used from seizure or death but it significantly ($p<0.05$) delayed the seizure onset and duration dose-dependently (Table 2). Similarly, it was observed that the standard drug used (Diazepam) did not prevent seizure or death but it also caused delay in the onset and duration of seizures significantly ($p<0.05$) when compared to control (Vehicle-treated) group. The peak increase in onset of seizure by EMM was obtained at the dose of 100 mg/kg, per oral. The results obtained showed that EMM may only have minimal effects against seizures. It can be inferred from the anticonvulsant study that EMM can delay the onset of seizure.

The hole board and elevated plus maze test models are among the most commonly used models in the assessment of anxiolytic properties of test substances in rodents[19,20]. In general, animals are exposed to anxiogenic conditions, i.e., either a novel environment (elevated plus-maze test and hole-board test among others). The hole-board test offers a simple method for measuring the response of an animal to an unfamiliar environment[19]. In this study, it was observed that EMM dose-dependently decreased the number of head-dips in the hole-board experiment suggesting anxiogenic effect since it was established that changes in head-dipping behavior in the hole-board test may reflect the anxiogenic and/or anxiolytic state of animals[41]. However, it was observed that the typical benzodiazepine anxiolytic, diazepam, significantly increased the number of head-dips. This observation is consistent with previous report stating that anxiolytic agents usually cause an increase in the frequency of exploratory head-dips on a hole-board model[41]. The doses of EMM and diazepam used in this study did not produce sedation. The EPM is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans[42]. An anxiolytic agent usually increase the frequency of entries into the open arms and the time spent in open arms of EPM[42]. In the present study, EMM did not significantly increase the time spent in the open arm and frequency of entries into open arm. Furthermore, the results obtained from the index of open arm avoidance also confirmed that EMM has no anxiolytic effect[24]. Ketamine is a sedative-hypnotic agent that acts primarily via antagonizing glutamatergic N-methyl-D-aspartate (NMDA) receptors. In this study, ketamine (100 mg/kg, i.p.) was used to induce sleep in mice[27]. EMM (25, 50 and 100 mg/kg, p.o) produced a significant ($p<0.05$) reduction in the onset of sleep induced by ketamine dose-dependently and significantly ($p<0.005$) prolonged the duration of sleep at dose 100 mg/kg that was comparable to that of diazepam (1 mg/kg, p.o.). Therefore, the observed

reduction of sleep latency and prolongation of total sleeping time have shown that EMM has central nervous system inhibitory effects and may be useful as sedative-hypnotic agent.

Conclusion

It can be concluded from the study that the essential oil of *Monodora myristica* has central inhibitory effects, sedative-hypnotic activity, attenuated amphetamine-induced hyperactivity, but has no anticonvulsant and anxiolytic effects. Therefore, it may be useful in alleviating disorder related to hyperactivity such as mania and other neuropsychiatric disorders.

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