Evaluation of the Neurobehavioural Toxicity Potential of Aqueous Ethanol Extracts of Leaf/Seed of *Datura metel*, *Mucuna pruriens*, and *Tapinanthus globiferus* Growing on *Azadirachta indica* Host Tree in Mice

ABSTRACT

Aqueous ethanol extracts of *Mucuna pruriens* seed (AEMPS), *Datura metel* leaf (AEDML) and seed (AEDMS), and *Tapinanthus globiferus* (AETGL) Growing on *Azadirachta indica* host tree are being evaluated for their anxiolytic, antidepressant, anti-parkinsonian, and addictive activities in other studies. The aim of this study was to investigate the liability or otherwise of extracts of the selected medicinal plants for some benzodiazepines-related neurobehavioural toxicities in mice. Actophotometry was used for the evaluation of the locomotory activity related to central nervous system depressant (cns) effect, diazepam-induced sleep potentiation for hypnotic liability, rodent beam (rod)-walking assay for balance and motor co-ordination, and novel object recognition test (NORT) for cognitive deficit evaluation. The results indicate, compared to negative control (distilled water) treatment mean values of 4.69±0.95 % locomotory activity reduction, 430.71±16.80 sec. sleep onset and 168.43±10.56 min. duration, 5.00±0.00 balance/motor co-ordination performance, and 54.41±1.99 novel object recognition, treatments with high oral doses of AETGL and AEMPS (1500 mg/kg each) did not significantly negatively impact these behavioural indices but even enhanced novel object recognition. High oral doses of AEDML and AEDMS (750 mg/kg each), and tramadol (133 mg/kg) caused significant ($p<0.05$) 42.24±2.64, 27.73±2.17, and 36.74±4.44, mean % locomotory activity reductions, 196.86±10.12, 193.88±15.39, and 189.14±18.31 second mean sleep onsets and 319.71±18.85, 309.57±20.27, and 356.00±26.01 minute mean sleep durations, 1.67±0.42, 1.30±0.40, 1.833±0.48 mean balance/motor co-ordination performances, and 40.49±5.45, 31.33±5.23, 19.37±3.96 mean novel object recognitions, respectively. Diazepam (2 mg/kg) treatment caused 33.71±2.19 mean % locomotory activity reduction, 1.33±0.49 mean balance/motor co-ordination performance, and 29.91±2.81 mean novel object recognitions. Additionally, most mouse groups exposed to tramadol, AEDML, and AEDMS extracts displayed unusual (hallucination-like, predator-like) fearful trepidations when in proximity with the novel objects. These findings indicate AETGL and AEMPS extracts may be devoid of neurobehavioural toxicities but tramadol, diazepam, AEDML and AEDMS extracts may be liable to significant sedative, hypnotic, myo-relaxant, and anti-cognitive effects. These findings justify the traditional uses of *Tapinanthus* species and *Mucuna pruriens* extracts for the treatment of memory deficits and related neurological disorders. They also justify the morbid and fatal toxicity risks associated with the use of *Datura metel* extracts, tramadol, and the benzodiazepines.

Keywords: Actophotometer, Evaluation, Mouse, NORT, Tapinanthus spp., Jimson weed, Velvet beans

INTRODUCTION

In drug discovery and development, putative psychoactive agents are often benchmarked against the existing standard drugs not only in efficacy and general toxicity but also in certain special toxicities i.e., neurobehavioural adverse effects – that could limit their anticipated therapeutic usefulness as is the case with the benzodiazepines.

Extracts of the plants selected for this study are being investigated for their anxiolytic, antidepressant, antiparkinsonian, and abuse liability effects in some other studies but scientific reports on their liability to neurobehavioural adverse effects are scarce.

Tapinanthus globiferus (A.Rich.) Thiegh. (Syn: Viscum album) [Plate 1] is one of the several members of the hemiphytic species in the *Loranthaceae* family collectively known as the African mistletoes commonly seen parasitising Neem (Azardirachta indica), Cocoa, Shea butter, Kolanut, and other cash crops [1 - 3]. It is generally believed anecdotally to cure all diseases. It has been reported

to be ethnomedicinally efficacious for hypertensive, cancerous, convulsive, hyperglycemic, and nervous disorders [5 - 8].

Previously, crude aqueous methanol and fractionated leaf extracts of *Tapinanthus globiferus* on *Azadirachta indica* host tree have demonstrated anxiolytic activity comparable to that of diazepam [9-10]. Although the benzodiazepines, the prototype of which is diazepam, are regarded as gold standard in anxiolytic efficacy, their clinical usefulness is limited by such neurobehavioural adverse effects comprising cognitive, locomotor, sedative, hypnotic and abuse liabilities [11, 12]. It is thus necessary to evaluate potential anxiolytic agents for their liability for these toxicities. Scientific literature on neurobehavioural toxicities associated with *Tapinanthus globiferus* extracts is however sparse, apart from the report of Umarudeen and Magaji et al. (2020) on its aqueous methanol leaf extract [13]. Thus, there is a need for further scrutiny of this aspect of the medicinal plant.

Plate 1: *Tapinanthus globiferus* parasitising Azardirachta indica (Neem) tree

Mucuna pruriens [Plate 2], with common names: velvet, devil's beans or cowhage, is a leguminous plant of the Fabaceae family comprising over 100 species [14, 15]. The seed extracts of this medicinal plant have been credited with ethnomedicinal efficacy in male infertility, snake bites, nervous disorders, Parkinson's, and related neurodegenerative diseases [14-16].

Plate 2: Mucuna pruriens dry pods and Mucuna pruriens dry seeds

Reported pharmacological activities of the seed extracts of this plant include anti-inflammatory, antioxidant, anti-hyperglycemic, neuroprotective and antioxidant effects, which are viewed to be due to their rich contents of dopamine precursor L-dopa and other antioxidant factors [16-18]. Despite the highlighted efficacy in neurological and nervous disorders – including Parkinson' s disease – a clinical disorder with profound motor deficits, there has been no scientific evaluation of Mucuna pruriens extracts for their possession or lack of neurobehavioural toxicity liabilities. This study therefore set out to investigate the neurobehavioural deficits, if any, following acute single doses of its seed extract.

Our interest in investigating leaf and seed extracts of *Datura metel* [Plate 3] in this study derives from its largely under-reported widespread abusive use [19] in the northern parts of Nigeria and its well-known ethnomedicinal uses despite severe adverse effects – including fatalities commonly associated with their use - especially when taken in overdose, either intentionally or accidentally. *Datura metel* L. (DM) (Syns.: *D. fastuosa* L., *D. fastuosa* var. alba (Nees) C. B. Cl., D. fruticosa Hornem, D. hummatu Bernh etc.), commonly known as Dhatura, Thorn apple, Devil's or Jimson weed, along with the other well-known related specie *Datura stramonium* L., belong to the Solanaceae family that is well reputed to possess toxic tropane alkaloids [20, 21, 22].

Plate 3: *Datura metel* (Thornapple, Jimson weed) with maturing fruits

Different parts of this plant which grows as a weed plant by the road sides and dumping sites have been reported to be effective for a broad array of ethnomedicinal indications such as psoriasis, dyspepsia, nervous agitation, diarrhea, elephantiasis, rabid dog bites, Parkinson's disease, insanity, heart failure, impotence, bronchitis and asthma, ear discharge, catarrh, rheumatic arthritis, epilepsy, mastalgia, anesthesia and ritual or recreational hallucinogenic effects [21 – 26]. Reported pharmacological activities of DM's extracts include neuro-protective, analgesic, glycosidase-inhibitory, anti-neoplastic, anti-hyperglycemic, antioxidant, antimicrobial, immunomodulatory, vulnerary, anesthetic (due to potent sedative effect), herbicidal, contraceptive, antiviral, antibacterial, and insecticidal [23, 24]. These biological activities are viewed to be due to the presence of several important bioactive compounds in different parts of the plant including withanolides (withafastuosin, withametelins), flavonoids, saponins, lectins, β-sitosterol, fatty acids, tannins, several volatile terpenes, and tropane alkaloids (datumetine, littorine, valtropine, hyoscyamine, fastucine, fastucinine, acetoxytropine, hyoscine, and tropine) [24 – 32]. Apart from beneficial pharmacological effects, extracts from most parts of DM have been shown to be potentially toxic due largely to the presence of tropane alkaloid phytochemicals. Deleterious complications that could result from ingestion or inhalation of high doses of leaf/seed extracts of DM include hepatotoxicity, nephrotoxicity, seizures, and potent anticholinergic effects such as acute dyspnea, acute hyperpyrexia, mydriasis, skin flushing, severe headache, hallucinations, blurred vision, dry mouth, ataxia, agitations, amnesia disorientation, schizophrenic symptoms, coma and even, fatalities [32 -37]. However, despite the above-highlighted ethnomedicinal, psychoactive therapeutic and toxic effects, scientific assessment of the neuro-behavioural fallouts of accidental/intentional ingestions of high doses of DM is sparse. The aim of this study therefore is to evaluate the neuro-behavioural toxicity potential of single high doses of DM seed/leaf extracts in mice.

Tramadol – a synthetic weak opioid analgesic effective for acute/chronic moderate to severe pain, including neuropathic and arthritis pain - was included in this investigation because of its known abuse liability and abusive use despite its potent central nervous system (CNS) depressant effect at high doses. The abuse predisposition of this analgesic agent is thought to derive from its narcotic and anti-fatigue effects resulting from the opioid receptor agonist activity combined with its noradrenaline and serotonin re-uptake inhibitory action – both of which may enhance the emergence of dependence and withdrawal symptoms when its use is suddenly terminated or withdrawn [38 - 40].
In Nigeria and most parts of the globe, reports indicate tramadol abuse is widespread – especially amongst the youths who indulge in it for its narcotic, sedative, 'aphrodisiac,' euphoric, and calming effects. The concerns over tramadol abusive use derive from the propensity to crime and the morbid/mortal complications associated with its use – both of which are increased when taken in high doses or polydrug use [41 - 43]. Reported tramadol overdose toxic complications include anorexia, emesis, depressed sensorium and respiration, hypertension, pruritus, cardiac irregularities, gastro-intestinal upset, hyperreflexia, seizures, severe headache, pinpoint miosis, rhabdomyolysis, multiple organ failures, anxiety, agitation, coma, and even deaths [44-51]. However, additional scientific reports are still needed on the myo-relaxant, cognitive, sedative, and hypnotic liabilities of this widely abused drug. The aim of this study therefore is to further evaluate these neurobehavioural toxicities of tramadol in Swiss albino mice using relevant experimental paradigms.

2. **MATERIALS AND METHODS**

2.1 **Materials**

2.1.1 **Plant parts**

Dry *Mucuna pruriens* seed (MPS) were obtained from Moniya foests, Ibadan, Oyo State, Nigeria. Fresh leaves of T*. globiferus* (TGL) atop an *Azadirachta indica* (Neem) host tree sited in Danboa area of Sokoto metropolis, Sokoto State, Nigeria. *Datura metel* L. leaves (DML) and seeds (DMS) were obtained along the Teaching hospital road Gwagwalada township, Abuja, Nigeria. All plant parts were identified Dr Idirisu Mohammed of the Faculty of Agriculture, University of Abuja, Federal Capital Territory, Abuja, Nigeria.

2.1.2 **Experimental animals**

A pool of about two hundred 12-16 weeks old Swiss Albino mice of both sexes was obtained from the National Institute of Pharmaceutical Research and Drug Development (NIPRID), Idu, Abuja. The mice were kept in a well-ventilated animal housing of the Behavioural laboratory of the Department of Pharmacology & Therapeutics, Faculty of Basic Clinical Sciences, for about three weeks under a 12-hour light/dark environment with free access to food and water before the commencement of the behavioural studies.

2.1.3 **Drugs and Reagents**

Absolute ethanol (analytical grade), diazepam tablets (Swipha, 5 mg) and injection (Swipha, 5 mg/ml), Tween 80 universal solvent, tramadol tablets (50 mg) and injection 50 mg/ml) marketed by Merit HealthCare Limited, Lagos, Nigeria were obtained from pharmaceutical outlets in Abuja City, Nigeria.

2.1.4 **Venue of the study**

All experiments – plant preparations and extractions, acute toxicity, and the neurobehavioral studies took place in the Neuroscience & Behavioural Lab of the Department of pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Abuja, Federal Capital Territory, Nigeria between 10. 00 and 24.00 HRS, the month of July/August 2023.

2.2 Methods

2.2.1 Plant preparation and extraction

Following authentication, all plant parts were subjected to drying under early morning sunlight for hours at the initial phase of the drying process followed by air-drying until constant weights were achieved. They were then kept in non-transparent dry plastic containers until use. *Mucuna pruriens* seeds were roasted at 110ºC for 30 minutes, allowed to cool off and then powdered. All plant parts were each powdered with a mechanical blender. The collected plant parts were briefly rinsed in water, air-dried, then powdered and stored dry in non-transparent plastic containers for subsequent use. One hundred (100) g of fine powder of each plant part was soaked in 200 ml of aqua/ethanol (V/V: 30/70) for 18 hours. They were then each separately Whatman's paper-filtered and the filtrates

subjected to electric fan-assisted air-drying. MPS yielded 23.7 g rich brown, TG, 10. 4 g glittering green, DML, 14.5 g deep green, and DMS, 9.3 g greenish brown dry extracts.

2.2.2 Acute toxicity testing

A preliminary acute toxicity testing to determine the respective lethal doses (LD50s) of each extract and tramadol was carried out using the limit dose tests for TGL and MPS, and toxic class toxicity protocols for DML, DMS, and tramadol. The choice of what protocol for the extracts was made based on previous toxicity reports on the plants and drugs. Oral LD50 value > 5 000 mg/kg/10 ml was found for aqueous ethanol TGL and MPS extracts, oral LD50 > 2, 000 mg/kg/10 ml for aqueous ethanol DML and DMS extracts, and oral LD50 > 400 mg/kg/10 ml for tramadol.

2.2.3 Behavioural studies

One third of the preliminary LD50s of extracts and drugs were selected as sub-lethal single high oral doses likely to produce neurobehavioural toxic effects in the experimental mice in the anticipated neuro-behavioural studies. Accordingly, 1500 mg/kg for TGL and MPS extracts, 750 mg/kg for DML and DMS extracts, and 133 mg/kg for tramadol, and experimental protocol-relevant diazepam doses were used for the subsequent behavioural assays. Diazepam was used as a positive control since most of the neurobehavioural toxicities to be evaluated are benzodiazepine related and tramadol for the diazepam-induced sleep potentiation assay.

2.2.3.1 *Evaluation of the effect of acute high oral doses of extracts on Locomotory Activities of Mice*

The evaluation of the effect of single high oral doses of extracts and drugs on the locomotory activity (an index of wakefulness/alertness of the mental state) of mice was carried out using a digital actophotometer according to the protocol adopted by Sugumaran et al., 2008 [52] with minor modifications. An actophotometer is a square or circular chamber which consists essentially of 5-6 horizontal light beams from one side of the chamber onto a set of photocells situated on the opposite side, a floor embedded with rod grid lines through which varying degrees of electric (0 – 100 volts) currents are passed, and a glass seal at the top of the device for introducing the experimental animals into the chamber.

The photocells are coupled to a digital counter which records a count when each animal interrupts the horizontal light beams as it moves within the closed chamber either spontaneously or as facilitated by the non-injurious electric currents in the chamber's floor rod grid lines. A current of 20 volts was used throughout this assay.

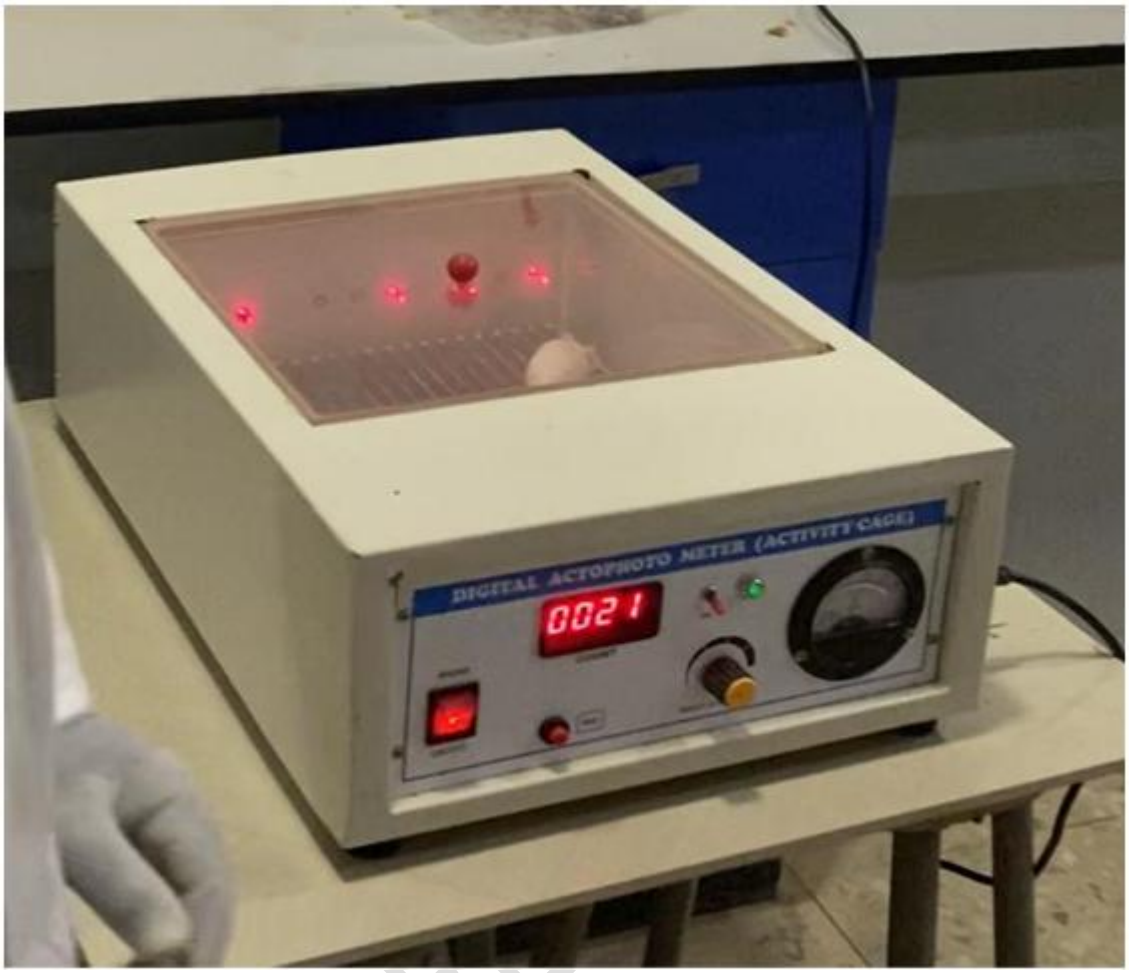

Plate 4: Department of Pharmacology & Therapeutics, U,of A. Actophotometer with a mouse in-situ

Briefly, healthy mice (20 – 25 g; both sexes) were randomized into 7 experimental groups I – VII (n = 6) and were each individually introduced into the test chamber of the actophotometer for 10 minutes and a basal activity was recorded for each mouse. An experimental group was subsequently given oral treatment of distilled water 10 ml/kg, aqueous methanol TGL 1500 mg/kg, aqueous methanol MPS 1500 mg/kg, aqueous methanol DML 750 mg/kg, aqueous methanol DMS 750 mg/kg, aqueous tramadol 133 mg/kg, or aqueous diazepam 2 mg/kg. 1 hour following their respective treatments mice were each re-exposed to the test for 10 minutes and their activities were recorded by the device's digital counter. The counter was stopped at the end of each trial for tested mice and was re-set before the next trial. The basal and post-treatment activities for each mouse were compared and the percentage reduction or increase in activity was recorded in a way that each animal acted as its own control.

2.2.3.2 Evaluation of the hypnotic effect of acute high oral doses of extracts in mice

This evaluation was done using a diazepam-induced sleep test according to the procedure of Rakotonirina et al. (2001) [53] adopted in [54]. In brief, experimental mice (22±0.5 g; both sexes) were randomized into 6 groups (n=7) with a group given an oral treatment by gavage with distilled water (DW) 10 ml/kg, AETGL1500 mg/kg, AEMPS 1500 mg/kg, AEDML 750 mg/kg, AEDMS 750 mg/kg, or tramadol 133 mg/kg followed 30 minutes later with oral treatment of diazepam (DZ) 40 mg/kg. Sleep onset was taken as the time gap between last oral treatment and total loss of righting reflex, while sleep duration was taken as time gap between the total loss and full return of the righting reflex in the experimental mice (Plate 4).

Plate 5: Experimental mice at different phases of hypnosis

2.2.3.3 Evaluation of the effect of acute high oral doses of extracts on motor balance and coordination in

mice

Degree of loss of motor balance and coordination is directly proportional to level of CNS depression any myo-relaxation – two of the well-established toxicities of the benzodiazepine anxiolytics. This evaluation was accomplished by using a single horizontal bar variant of the beam (rod)-walking paradigm of Stanley, 2005 (Plate 6) [55] as described in Deacon, 2013 [56], albeit with minor modifications.

Plate 6: Two units of Beam (rod) walking test apparatuses with 2-mm and 4-mm iron rods

The principle underpinning this test is the instinct of rodents to grip/grab objects in their proximity when suspended/floated loosely in space. On day one, mice for the main test the next day were each subjected to a screening trial by being exposed to a 2 mm-rod on the beam balance. Mice that were able to stay or hold onto the rod without falling off for at least 5 seconds were instantly selected for the main test. Mice falling off on their first attempts were given 2 additional trials. Mice that passed the test on their second attempts were not tried the 3rd time. All mice which passed the trial on 1st, 2nd, and 3rd attempts were deemed to have qualified for inclusion in the main test the following day.
The beam balance essentially comprises of a 60 cm-long iron rod horizontally suspended (balanced) on two 50 cm-high vertical wooden beams (poles) set 50 cm apart (Plate 5).
Briefly, on the screening day – with no treatments given, and on the main test day 1 hour following oral treatments of randomized groups (n = 7, both sexes) of mice (23±0.7 g) with DW 10 ml/kg, AETGL1500 mg/kg, AEMPS 1500 mg/kg, AEDML 750 mg/kg, AEDMS 750 mg/kg, tramadol 133 mg/kg, or diazepam 2 mg/kg were each subjected to the test but this time on the 4-mm iron rod. Time spent on the beam balance by each mouse was determined using a watch timer.
Scoring for the times spent by the mice staying on/holding onto the rod was done as described by Deacon, 2013 [56] with modifications, as follows:
Falling off between within 5 seconds = 1, within 6-10 seconds = 2, within 11-20 seconds = 3 and within 21-
30 seconds = 4. Mice that stayed on top of the rod for/longer than 30 seconds = 5; holding onto the rod by placing one or both forepaws on it without falling for 30 sec = 5, and climbing onto the top of the rod with
all 4 paws on it at any time within 30 seconds = 5. Reaching any of the vertical support beams at any time within test duration = 5.
The scores were collated and recorded for each mouse group.

2.2.3.4 Evaluation of the cognitive effect of acute high oral doses of extracts in mice
The novel object recognition test (NORT) was adopted to assess acute high oral doses of aqueous methanol TGL, MPS, DML, DMS extracts, and Tramadol on short-term memory in mice in accordance with a method previously used by [57]. Our NORT apparatus consists of a walled square open field containing no objects on the training day, but with similar or dissimilar objects placed at opposite corners of the test apparatus on test day, day 2 (Plate 7).

Day 1: Plain open field maze Day 2: Open field with similar objects Day 2: Open field with a novel object

Plate 7: Novel object recognition test experimental set-up

The test relies heavily on the natural proclivity of rodents for novelty – which is created when one of the initial similar objects at the first mouse re-exposure is replaced with a comparatively dissimilar object at the second and final re-exposure.
Briefly, on the first day of the 2-day protocol, experimental mice were each trained to familiarize with the test environment and device by being gently dropped in the middle of the apparatus and allowed to freely explore the test environment for 10 minutes, and then returned to the home cage. The second day i.e., the test day mice were each individually re-exposed to the apparatus, but
this time around with two similar colourless plastic containers cut in half - with the cut ends plastered to the floor at opposite corners within the test apparatus. The mice were allowed to freely explore the

environment for 10 minutes - with only those which explored each of the plastic objects for a minimum of 20 seconds of the test period deemed to have met inclusion criteria for the main cognitive test. Subsequently, mice (21.0±0.3 g) that qualified for the definitive cognitive test were randomized into groups (n = 12) and a group receiving oral treatments of DW 10 ml/kg, AETGL1500 mg/kg, AEMPS 1500 mg/kg, AEDML 750 mg/kg, AEDMS 750 mg/kg, tramadol 133 mg/kg, or diazepam 1 mg/kg. One hour following treatments and 4 hours after the first re-exposure, mice were again exposed to the test for a 5-minute period during which experimental subjects were allowed to freely explore the test environment, but this time around with one of the plastic containers replaced with similarly shaped but red-coloured plastic container.
Times spent by each mouse exploring the objects were recorded.
Recognition or preference index (d3) = [b/e2] X 100. Where b = time spent by the mice exploring the new object, and e2 = time spent by the mice exploring both novel and old objects.

2.3 Statistical Analysis
All data from the experiments were expressed as means ± S.E.M. IBM SPSS version 2.0 was used for data analysis using analysis of variance (ANOVA) followed by Turkey post hoc test. *P*-values less than 0.05 were taken as significant.

3. RESULTS
3.1 The effect of acute high oral doses of aqueous methanol TGL, MPS, DML, DMS extracts, and Tramadol on locomotion in mice

Compared to only 4.69±0.95 mean % locomotory activity reduction in mice exposed to distilled water treatment (Table 1), AETGL or AEMPS (each at 1500 mg/kg) did not significantly ($p>0.005$) alter, but AEDML (750 mg/kg), AEDMS (750 mg/kg), tramadol (133 mg/kg), and diazepam (2 mg/kg) treatments caused significant ($p<0.05$) 42.24±2.64, 27.73±2.17, 36.74±4.44, and 33.71±2.19 reductions, respectively, in the mean percent locomotory activity of experimental mice.

3.2 The hypnotic effect of acute high oral doses of extracts and drugs in mice

Compared to the mean sleep onset (430.71±16.80 sec.) and duration (168.43±10.56 min.) of distilled water-treated mice (Table 2), high oral AETGL and AEMPS treatments at 1500 mg/kg neither significantly ($p>0.05$) shortened diazepam-induced mean sleep onset (398.00±23.57 sec. and 400.71±41.07 sec.) nor increased mean sleep duration (174.00±10.28 min. and 162.57±8.44 min.), respectively, whereas AEDML and AEDMS (each at 750 mg/kg), and tramadol at 133 mg/kg significantly ($P<0.05$) shortened mean sleep onset to 196.86±10.12, 193.88±15.39, and 189.14±18.31 sec., respectively, as well as increased mean sleep durations to 319.71±18.85, 309.57±20.27, and 356.00±26.01 min., respectively, in mice.

3.3 The effect of acute high oral doses of extracts and drugs on balance and motor co-ordination in mice

Our results show (Table 3), compared with distilled water treatment mean performance of 5.00±0.00, AETGL (4.83±0.17) and AEMPS (5.00±0.00) extract treatments did not ($p>0.05$) alter but AEDML (1.67±0.42) and AEDMS (1.30±0.40), tramadol (1.833±0.48), and diazepam (1.33±0.49) did significantly ($p<0.05$) reduce the mean performances of the mice exposed to them on this test.

3.4 Effect of acute high oral doses of extracts and drugs on short-term memory in mice

Compared to the mean value of 54.41±1.99 of mice treated with the negative control (Table 4), single high oral doses of both AETGL (60.191±1.81) and AEMPS (59.19±2.18) did not reduce but instead caused insignificant improvements in the mean recognition indices exposed to them. In contrast, compared to the control mouse group, high oral doses of AEDML (40.49±5.45), AEDMS (31.33±5.23), tramadol (19.37±3.96), and diazepam (29.91±2.81) all significantly ($p<0.05$) caused reductions in the recognition performance in mice exposed to them. In addition, most of the mice treated with tramadol, AEDML, and AEDMS extracts displayed unusual fearful trepidations (? hallucinatory), like when faced with a predator, when in proximity with the novel objects.

Table 1: Effect of acute high oral doses of extracts and drugs on locomotory activity in mice

Treatments	Mean % reduction
Distilled water	4.69±0.95

Aq. ethanol *T. globiferus* leaf	2.98±0.57
Aq. ethanol *M. pruriens* seed	7.45±1.83
Aq. ethanol *D. metel* leaf	42.24±2.64*
Aq. ethanol *D. metel* seed	27.73±2.17*
Tramadol	36.74±4.44*
Diazepam	33.71±2.19*

*Data expressed as mean ± S.E.M. * Statistically significant (p < 0.05)*

Table 2: The hypnotic effect of acute high oral doses of extracts and drugs in mice

Extracts/Drugs	Mean sleep onset (Seconds)	Mean sleep duration (Minutes)
Distilled water+Diazepam	430.71±16.80	168.43±10.56
AETGL+Diazepam	398.00±23.57	174.00±10.28
AEMPS+Diazepam	400.71±41.07	162.57±8.44
AEDML+Diazepam	196.86±10.12*	319.71±18.85*
AEDMS+Diazepam	193.88±15.39*	309.57±20.27*
Tramadol+Diazepam	189.14±18.31*	356.00±26.01*

*Data expressed as mean ± S.E.M. * Statistically significant (p < 0.05)*

Table 3: Effect of acute high oral doses of extracts and drugs on balance and motor co-ordination in mice

Treatments	Mean performaces
Distilled water	5.00±0.00
Aq. ethanol *Tapinanthus globiferus* leaf	4.83±0.17
Aq. ethanol *Mucuna pruriens* seed	5.00±0.00
Aq. ethanol *Datura metel* leaf	1.67±0.42*
Aq. ethanol *Datura metel* seed	1.30±0.40*
Tramadol	1.833±0.48*
Diazepam	1.33±0.49*

*Data expressed as mean ± S.E.M. * Statistically significant (p < 0.05)*

Table 4: Effect of acute high oral doses of extracts and drugs on short-term memory in mice on the novel object recognition test

Treatments	Mean recognition indices (d3)
Distilled water	54.41±1.99
Aq. ethanol *Tapinanthus globiferus* leaf	60.191±1.81
Aq. ethanol *Mucuna pruriens* seed	59.19±2.18
Aq. ethanol *Datura metel* leaf	40.49±5.45*
Aq. ethanol *Datura metel* seed	31.33±5.23*
Tramadol	19.37±3.96*
Diazepam	29.91±2.81*

*Data expressed as mean ± S.E.M. * Statistically significant (p < 0.05)*

4. DISCUSION

This study is essentially a demonstration of alternative ways of evaluating the potential of putative and existing psychoactive agents to exhibit some of the neurobehavioural drawbacks associated with the benzodiazepines. Level of locomotory activity is an index of mental alertness or wakefulness in human and animals – and is often impacted by CNS depressant or stimulants drugs and agents. Actophotometric evaluation of this property in this study, as opposed to the more frequently deployed open-field based evaluation, has some advantages – including semi-automation in recording the activity by a digital counter, and avoidance of the one-trial tolerance liability [58] that is often associated with the latter test since in this assay the experimental animals are their own controls and at least, two trials are needed for a complete locomotory activity profiling. Previously,

actophotometer has been deployed in the evaluation of the locomotion-related CNS depressant potential of medicinal plant extracts [59, 60]. Using this locomotory activity index our findings indicate aqueous ethanol extracts of *Tapinanthus globiferus* leaf and Mucuna pruriens seed (MPS) extracts even high oral doses do not exert any significant CNS depressant or stimulant impact in mice. These findings may the first indicating that MPS extracts may not have cns depressant effect even though several studies have shown its extracts to possess anxiolytic activity [61, 62]. This study may be the second report on extracts of *Tapinanthus globiferus* (TG) not exhibiting negative locomotory effect [13] in mice despite their reported anxiolytic activity [9, 10]. The fact that extracts of these medicinal plants have been reported to exert significant anxiolytic activity with no significant sedative effect that may impact locomotion may suggest that the anxiolytic pharmacophores in these plants may be acting on a neural pathway quite distinct from the benzodiazepine GABAergic mechanism(s). The same findings of ours also show *Datura metel* leaf (DML)/seed (DMS) extracts, and tramadol negatively impacted mouse locomotion in a fashion comparable to diazepam treatment. Sedative sensorium alteration coupled with negative gait impact in humans has been a characteristic aftermath of indulgence in the various forms of DML/DMS preparations. This psychoactive property which is viewed to be due to the presence of certain tropane alkaloids [20 – 22] has been previously reported [63, 64]. This same property was listed as a strong attraction to abusive use of various DML/DMS preparations in a substance abuse survey [19].

Potentiation of diazepam-induced sleep procedure was used to investigate the hypnotic effects of the extracts and drugs in this report. AETGL and AEMPS at the dose of 1500 mg/kg neither decreased onset nor increased length of sleep whereas AEDML and AEDMS extracts, and tramadol both significantly fast-tracked onset and elongated duration of sleep. Similar non-hypnotic effect in mice has been previously reported for an aqueous methanol TG extract. This finding of non-hypnotic effect of both AETGL and AEMPS despite their anxiolytic activity may suggest their anxiolytic action may be dependent on a non-benzodiazepine mechanism(s). Tramadol hypnotic effect finding in this study is not unexpected since it has been known as a potent CNS depressant agent. This sensorium and mood altering effects of tramadol are also thought to be related to its abuse liability despite its well-known general and neurobehavioural toxicity [38-40]. The findings of concerns to us are the hypnotic effect coupled with the above-highlighted sedative effect of AEDML and AEDMS extracts. Both psychoactive effects are believed to be related to the abuse liability, deep prolonged sleep and associated fatalities often experienced by those exposed to accidental or intentional overdoses (like those used in this study) of the different parts of *Datural metel*. The finding of a significant hypnotic effect for AEDML and AEDMS extracts agrees with previous reports of cases of protracted deep sleep, coma and even deaths following intentional or accidental ingestion of Datura sp. extracts have been reported [33, 35, 65, 66].

Intact balance and motor coordination in man and animals requires the CNS, the peripheral musculo-nervous system, and the bi-directional neural modulatory interplay between these two systems are intact. Deficits in balance and motor coordination therefore can result from CNS depressant and myorelaxant effects of putative and existing psychoactive agents as undertaken in this investigation using the Beam (rod) walking assay. This assay is said to have comparative advantages over the hitherto standard test for elucidating balance and motor coordination deficits in experimental animals – including operational simplicity, cost-effectiveness, improved sensitivity, and predictive capability to clinically relevant sedative benzodiazepine doses [55, 56].

Again, on this assay AETGL and AEMPS extracts displayed no significant negative impact on balance and motor coordination related to CNS depression or myo-relaxation. This finding of nil CNS depressant or myo-relaxant effect on AETGL is agreement with an earlier report [13], but the same finding on AEMPS may be the first of such showing this extract does not have CNS depressant and myo-relaxant liability even at high doses.

The significant deficits in this behavioural parameter observed in mice treated with AEDTL, AEDTS, tramadol, and diazepam are all related to their potent CNS depression, myo-relaxation, and neurotoxicity of these substances [66, 67, 68, 69, 70].

The novel object recognition test (NORT) adopted in this investigation, when compared with other cognition assessing protocols e.g., Morri's water maze and Barnes tests, is viewed to be simpler, more sensitive, less time-consuming, less stressful on the experimental subjects, and has greater face validity for human memory [71]. On the NORT, cognitive performances of the AETGL and AEMPS extracts-treated mice were comparable to and even surpassed that of distilled water-treated mouse group. This suggests both extracts are devoid of any acute negative cognitive deficits. This finding agrees with previous reports of positive cognitive impact of administration of extracts of both *Tapinanthus* and *Mucuna* spp. on experimental rodents [13, 72, 73, 74]. On the other hand, mice treated with AEDML, AEDMS, tramadol exhibited significantly low recognition indices compared to

water-treated mice. This finding corroborates with previous studies which show the negative cognitive effect of AEDML and AEDMS may be related to the potent anticholinergic and neurotoxic effects of compounds – including tropane alkaloids isolated in Datura species [75, 76] in experimental subjects. Diazepam- and tramadol-treated mice performed poorly compared with water-treated mice. Our findings also agree with previous findings in which diazepam and tramadol acute administrations in human and experimental subjects induced acute cognitive deficits [77 - 80].

5. CONCLUSION

This study has shown aqueous ethanol extracts of *Tapinanthus globiferus* leaf and Mucuna pruriens seed did not, those of *Datura metel* leaf and seed as well as tramadol, did exhibit sedative, locomotory, cognitive, and hypnotic effects associated with the benzodiazepines.

CONFERENCE DISCLAIMER:

Some part of this manuscript was previously presented and published in the conference: 44th Global summit on Neurology, Psychiatry & Mental Health dated from August 23-24, 2023 in Durban, South Africa., Web Link of the proceeding: https://www.scitcentralconferences.com/accepteddetails/global-summit-on-neurology-psychiatry-mental-health/2392

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

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