Behavioural studies on crude Ethanol leaf extract of *Cadaba farinosa* Forssk. in mice

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Abstract

Cadaba farinosa Forssk. (*Capparaceae*) is found all over the world, mostly in tropical and subtropical areas. It is used to treat pain, dysentery, rheumatism, cough, and fever, as well as an antidote and in the treatment of neurological disorders. In mice, the median lethal dose (LD_{50}) of Crude Ethanol leaf Extract (CEE) of *Cadaba farinosa* was found to be 2154.1 mg/kg body weight. The presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins, and tannins was discovered during a preliminary phytochemical analysis of the dark gummy mass crude extract. The CEE was then put through pharmacological testing on mice. In mice, diazepam-induced sleep, beam walking, and hole-board assays were used to investigate behavioral activities. CEE of *Cadaba farinosa* had no effect on the onset of sleep in mice when given at the tested doses of diazepam. In contrast, it significantly increased sleep duration at all doses tested (75 mg/kg p 0.001, 150 mg/kg $p \leq 0.05$, and 300 mg/kg $p \leq 0.01$). The CEE significantly ($p \leq 0.01$) increased the number of foot slips in the Beam walking assay at a dose of 150 mg/kg. Similarly, diazepam at 0.25 mg/kg increased the number of foot slips significantly ($p \leq 0.001$). The extract at a dose of 300 mg/kg and diazepam increased the time required to complete the task significantly ($p \leq 0.05$). At 150 mg/kg (p 0.01) and 300 mg/kg ($p \leq 0.05$), the hole-board experiment significantly reduced the number of head dips. In contrast, the standard drug (diazepam) had no discernible effect on the number of head dips. These findings suggest that CEE and Diazepam at the highest dose significantly affected the behaviour of mice in the beam walking assay. Thus, the extract showed no activity on the onset of sleep but significantly prolong duration of sleep at the tested doses. The CEE significantly produced a decrease in the exploratory behavioural pattern, indicative of the fact that CEE possesses sedative property.

Introduction

The Ayurvedic, Chinese, and Egyptian systems, which have been an integral part of natural product for centuries, are among the most renowned ancient traditional systems known to man for centuries.[1] As a result, Indian and Chinese medicinal systems, as well as those used by African tribes, are excellent sources of traditional wisdom. As a result, with the assistance of modern scientific methods, they will undoubtedly continue to serve as the foundation for the discovery and development of new agents with therapeutic value.[2]

In fact, the majority of the world's population still relies on traditional healthcare systems. The World Health Organization also stated that it is the most dependable method of providing healthcare to the

world's population[3] because approximately 88% of people in the developing world rely on traditional medicine.[1]

Cadaba farinosa Forssk. is a plant in the *Capparidaceae* family. It is found all over the world, but is most common in the tropical and subtropical zones. In dry short grass savannahs, the plants are usually herbs, erect or scandent, shrubs, and rarely trees. The leaves are complete, silvery grey, and have simple scales.[4] It is known locally as bagayi or hanza in Hausa, and bultu in Northern Nigeria.[5]

Panic, phobias, obsessive compulsion, and post-traumatic stress disorder are the most common mental illnesses and the leading cause of disability worldwide.[6] It affects roughly one-eighth of the global population and has become a major area of research interest in psychopharmacology.[7] For a long time, Benzodiazepines (BDZs), barbiturates, and Tricyclic Antidepressants (TCAs) have been used to treat anxiety disorders. The serious side effects of these drugs, such as rebound insomnia, sedation, muscle relaxation, anterograde amnesia, withdrawal and tolerance (BZDs, barbiturates, and alcohols), sexual dysfunction, anticholinergic, antihistaminic effects (TCAs), have limited their use in patients. Many of the secondary metabolites of plants are used in traditional medicine practice for the treatment of psychotic disorders, particularly anxiety, the majority of which directly or indirectly affect the Central Nervous System (CNS), noradrenaline, serotonin, Gamma Amino Butyric Acid (GABA), and BDZ neurotransmitter activities.[8-12] As a result, there is a need to look into plants in order to find new bioactive secondary metabolites with few or no side effects.

Cadaba farinosa, on the other hand, has been found to contain a high concentration of active secondary metabolites, such as alkaloids, sugar, carbohydrates, glycoside, protein, amino acid, flavonoid, saponin, tannins, phenolic compounds, gums, mucilage, and steroids.[13] The plant has been found to contain alkaloids such as L-Stachydrine and 2-hydroxystachydrine.[14] Burkill[5] isolated some alkaloids (Cadabicine, Cadabicine methyl ether, Cadabicine diacetate) from the plant, and the pharmacological effects of alkaloids include analgesics and narcotics, CNS stimulants, anticancer, anti-asthmatics, anti-hypertensives, smooth muscle relaxants, and antiparasitic, among others. Flavonoids have anti-allergic, anti-cancer, antioxidant, anti-inflammatory, and anti-viral properties in addition to being potent antioxidant compounds.[1] However, studies in goats using *Cadaba rotundifolia* from the genera revealed ataxia, diarrhoea, depression, dyspnea, and other symptoms.[15]

The purpose of this study is to evaluate the anxiolytic activity of a crude ethanol leaf extract of *Cadaba farinosa* Forssk. in laboratory animals.

Materials and Methods

Plant material collection, identification, and preparation

Cadaba farinosa fresh leaf samples were collected from the Maiduguri Metropolitan Council Area of Borno State, Nigeria. The plant specimen was identified and authenticated at the Herbarium section of Ahmadu Bello University's Department of Biological Sciences in Zaria, Nigeria, which corresponded to voucher specimen number V/No: 2744. The leaf was air-dried in the shade for several days before being ground into a fine powder for extraction.

Plant material extraction

The air-dried ground powdered leaf material (1,500 g) was extracted thoroughly with 70% ethanol over several days using the cold maceration method with occasional shaking. Crude Ethanol leaf Extract (CEE) of *Cadaba farinosa* was coded after being concentrated to dryness on a water bath at 50° C. The coded extract was used as a working sample for the plant's chemical investigations, acute toxicity determination, and pharmacological studies.

Animals

Adult Swiss albino mice (16-30 g body weight) of either sex were obtained from the Animal House facility of Ahmadu Bello University Zaria, Nigeria's Department of Pharmacology and Therapeutics. The animals were fed a laboratory diet and given free access to water, and they were kept in propylene cages at room temperature under standard conditions. Animal handling ethical clearance was sought from the Department of Pharmacology and Therapeutics, Faculty of Pharmacy, Ahmadu Bello University in Zaria, Nigeria.

Drugs and equipment

Beam walking assay in mice

Diazepam (F. Hoffmann-La Roche Basel, Switzerland). 0.25 mg/kg dose

Diazepam-induced sleep in mice

Diazepam (F. Hoffmann-La Roche Basel, Switzerland). The dose is 20 mg/kg. For the Beam walk assay, a locally constructed wooden Hole board (60 cm x 30 cm) with sixteen evenly spaced holes, an aluminum cage, and a wooden stick were used.

Phytochemical screening

A small amount of the CEE was tested for the presence of alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins, and tannins as described in other studies.[16-20]

Acute toxicity studies

Lorke's method was used to calculate the median lethal dose (LD_{50}). In brief, three mice of each sex were divided into three groups in the first phase. *Cadaba farinosa* CEE was administered intraperitoneally (ip) at doses of 10 mg/kg, 100 mg/kg, and 1,000 mg/kg per body weight. Within 24 hours, the animals were examined for signs of toxicity and death (none of the animals died). In the second phase, a mouse from each of the three groups was administered ip with more specific doses of the crude extract of 1,600 mg/kg, 2,900 mg/kg, and 5,000 mg/kg body weight based on the results of the first phase, and was observed for signs of toxicity and death within 24 hours. The geometric mean of the lowest dose that caused death and the highest dose of survival (*i.e.,* square root of the product of the lowest lethal dose and the highest non-lethal dose for which the animal survived)[21] was used to calculate the median lethal dose (LD_{50}).

Beam walking assay in mice

The Stanley *et al.* method was used. Mice were taught to freely walk (at least three times) from a start platform along an 80 cm long and 3 cm wide ruler elevated 30 cm above the ground by a metal support into a goal box (hamster house). The successful mice were divided into five groups of six mice each. Mice in groups one, two, three, and four were given 10 ml/kg normal saline, 75 mg/kg, 150 mg/kg, and 300 mg/kg plant CEE ip, respectively. Thirty minutes after pre-treatment, the fifth group received 20 mg/kg diazepam ip. Each mouse from each group was placed at one end of a beam (60 cm long, 8 mm in diameter, and 30 cm above the ground) and allowed to walk into the hamster house. Mice that fell were returned to their original position within the maximum time of 60 seconds allowed on beam. The time it took to complete the task, as well as the number of foot slips (one or both hind limbs slipping from the beam), were recorded using a tally counter.[22]

Diazepam-induced sleep in mice

The previously described method by Rakotonirina *et al.* was used.[23] Each group received diazepam (20 mg/kg) 30 minutes after pre-treatment. For each mouse, the onset and duration of sleep were recorded. The loss of rightening reflex after diazepam administration was considered the onset of sleep,[24] and the time between loss and recovery of rightening reflex was considered the duration of sleep.[25]

Hole-board test in mice/exploratory behaviour in mice

The method used was similar to that described previously by File and Wardill. A white-painted (60 cm X 30 cm) board with 16 evenly distributed holes (1 cm diameter X 2 cm thick/depth)[26] was used as the hole-board. Mice in groups one, two, three, and four were given 10 ml/kg normal saline, 75 mg/kg, 150 mg/kg, and 300 mg/kg plant CEE ip, respectively. The final five groups were given 0.25 mg/kg diazepam intravenously. Thirty minutes after pre-treatment, each mouse in each group was placed in a corner of the hole-board, and the number of head dips (to eye level) were recorded using a tally counter[27] within a five-minute period.

Statistical analysis

The results were expressed as the mean ± standard error of mean (mean ± SEM). The information was then subjected to one-way Analysis of Variance (ANOVA). A post hoc Dunnett's t-test for multiple comparisons was used when a statistically significant difference was found. Differences were considered significant at $p < 0.05$.

Discussion

Cadaba farinosa leaves contain compounds that may be useful in the treatment of general body pains, dysentery, rheumatism, cough, fever, poisoning, amenorrhea, dysmenorrhoea, liver damage, cancer, and uterine obstruction, among other things. The extract's ability to increase the number of foot slips suggests a motor co-ordination deficit, which is similar to clinical sedation.[22]

Only diazepam and CEE at the highest dose significantly affected mouse behavior in the beam walking assay, indicating motor coordination impairment. As a result, the standard drug and extract showed activity in the peripheral nervous system at the highest dose. Sedatives, hypnotics, tranquilizers, neuroleptics, and antidepressants have all been shown to prolong diazepam-induced sleep time.[23, 28-30] However, analeptics and stimulants have been shown to reduce sleep time.[28,30] Sedative-hypnotic agents either directly activate $GABA_A$ receptors or, more commonly, enhance GABA's action on its receptors to increase GABA-mediated synaptic inhibition. Barbiturates and benzodiazepines are two commonly used therapeutic agents that act as positive allosteric modulators at $GABA_A$ receptors or by enhancing GABA's action on $GABA_A$ receptors.[31] The results showed that CEE of *Cadaba farinosa* could enhance diazepam-induced sleep. This implies that it may have sleep-inducing properties.[32-33] As a result, the extract showed no activity on sleep onset but significantly prolonged sleep duration at all doses tested; thus, there is a strong possibility that the crude ethanol leaf extract of *Cadaba farinosa* contains substance(s) with the ability to interact with GABA-mediated channels. As a result, it may be beneficial in the maintenance of sleep.

The hole-board test is one of the most widely accepted models for assessing psychotic, anxiety, and sedative conditions in experimental animals;[29,34] a decrease in this parameter reveals sedative behavior[33] and CNS depressant activity.[35] The dose(s) tested of *Cadaba farinosa* CEE that significantly reduced exploratory behavior as measured by the number of head dips in the hole-board experiment indicated that it possessed sedative properties but did not have anxiolytic activity. As a result, the decrease in the number of head dips in the hole-board test suggests that it has sedative properties.

So far, this is one of the most comprehensive reports on studies of this plant-based pharmacological model. As a result, more detailed phyto-pharmacological research on this part of the plant was required to understand the structure of the phytochemicals as well as their mechanism of action.

Results

Phytochemical screening

The extractive value of *Cadaba farinosa* CEE extracted from 1,500 g plant material was found to be 12.63% w/w (189.37 g; dark gummy mass). The crude extract contained alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, glycosides, saponins, and tannins, according to preliminary phytochemical analyses.

Acute toxicity studies

In mice, the median lethal dose (LD_{50}) of CEE of Cadaba farinosa was found to be 2154.1 mg/kg body weight when administered ip.

Beam walking assay in mice

At a dose of 150 mg/kg, the CEE of Cadaba farinosa significantly ($p \leq 0.01$) increased the number of foot slips. Similarly, diazepam at 0.25 mg/kg increased the number of foot slips significantly ($p \leq 0.001$). The extract (300 mg/kg) and diazepam significantly ($p \leq 0.05$) increased the time required to complete the task (Table 1 and Figure 1).

Diazepam-induced sleep in mice

At the tested doses, *Cadaba farinosa* CEE had no effect on the onset of sleep. In contrast, it significantly increased sleep duration at all doses tested (i.e. 75 mg/kg, $p \leq 0.001$, 150 mg/kg, $p \leq 0.05$ and 300 mg/kg, $p \leq 0.01$; Table 2 and Figure 2). As a result, the effect was not dose-dependent.

Hole-board test in mice/exploratory behaviour in mice

Cadaba farinosa CEE reduced the number of head dips significantly at 150 mg/kg ($p \leq 0.01$) and 300 mg/kg ($p \leq 0.05$). In con-

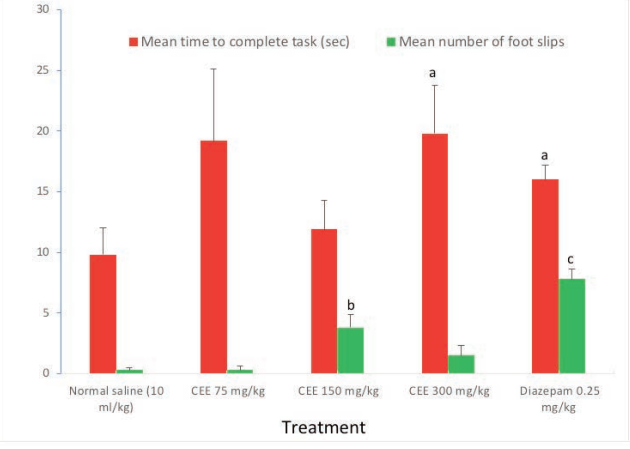

Figure 1. Graphical presentation of the effect of CEE of *Cadaba farinosa* on beam walk assay in mice. CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; [a]p ≤ 0.05, [b]p ≤ 0.01 and [c]p ≤ 0.001 (compared with control. Dunnett's test for multiple comparison); n = 6

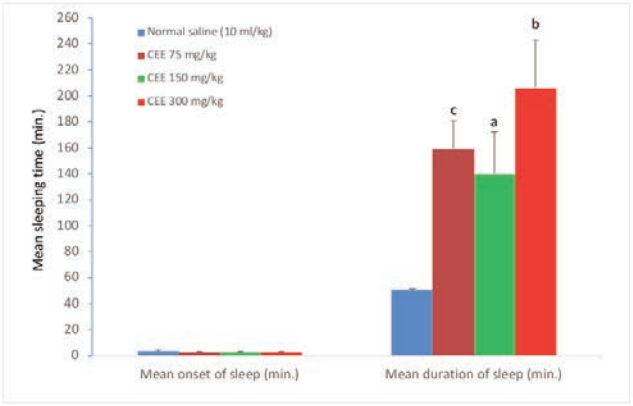

Figure 2. Graphical presentation of the effect of CEE of *Cadaba farinosa* on diazepam-induced sleep in mice. CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ (compared with control. Dunnett's test for multiple comparison); n = 6.

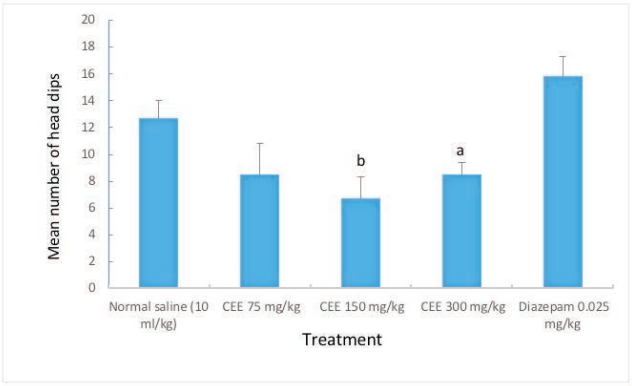

Figure 3. Graphical presentation of the effect of CEE of *Cadaba farinosa* on exploratory behaviour in mice. CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; $^{a}p \leq 0.05$ and $^{b}p \leq 0.01$ (compared with control. Dunnett's test for multiple comparison); n = 6.

Table 1. Effect of CEE of *Cadaba farinosa* on beam walk assay in mice.

Treatment	Dose (mg/kg)	Mean time to complete task (sec.)	Mean number of foot slips
N/Saline	10 (mL/kg)	9.8±2.2	0.3±0.2
CEE	75	19.2±5.9	0.3±0.3
CEE	150	11.9±2.4	3.8±1.1[b]
CEE	300	19.8±4.0[a]	1.5±0.8
Diazepam	0.25	16.0±1.2[a]	7.8±0.8[c]
One way ANOVA			
Df		4.25	4.25
F		1.536	18.447
α		0.222	0.000

N/Saline = normal saline; CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ (compared with control. Dunnett's test for multiple comparison); n = 6.

Table 2. Effect of CEE of *Cadaba farinosa* on diazepam-induced sleep in mice.

Treatment	Dose (mg/kg)	Mean Onset of sleep (Min.)	Mean Duration of sleep (Min.)
N/Saline	10 (mL/kg)	3.4±0.4	50±12.5
CEE	75	2.7±0.5	159.9±21.8[c]
CEE	150	2.8±0.3	140.8±32.5[a]
CEE	300	2.8±0.3	206.2±36.6[b]
One-way ANOVA			
Df		3.18	3.18
F		0.834	5.267
α		0.493	0.009

N/Saline = normal saline; CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; $^{a}p \leq 0.05$, $^{b}p \leq 0.01$ and $^{c}p \leq 0.001$ (compared with control. Dunnett's test for multiple comparison); n = 6.

Table 3. Effect of CEE of *Cadaba farinosa* on exploratory behaviour in mice.

Treatment	Dose (mg/kg)	Mean Number of Head dips
N/Saline	10 (mL/kg)	12.7±1.3
CEE	75	8.5±2.3
CEE	150	6.7±1.6[b]
CEE	300	8.5±0.9[a]
Diazepam	0.025	15.8±1.5
One way ANOVA		
Df	4.25	
F	5.559	
α	0.002	

N/Saline = normal saline; CEE = crude ethanol leaf extract; df = degree of freedom, F = F-factor; α = confidence level; $^{a}p \leq 0.05$ and $^{b}p \leq 0.01$ (compared with control. Dunnett's test for multiple comparison); n = 6

trast, the standard drug (diazepam) had no discernible effect on the number of head dips (Table 3 and Figure 3).

Conclusions

At high doses, the extract has a general CNS depressant potential and may cause motor coordination deficit. Despite the fact that a number of secondary metabolites (alkaloids, flavonoids, terpenes, etc.) have been reported from this plant that may be responsible for the plant's pharmacological activities, research on its pharmacological activities is still limited.

Recommendations

As a result, there is a large scope for isolating valuable compounds (*e.g.* anthraquinones, cardiac glycosides, steroids, saponins, tannins, etc.) from the plant and studying their potency, because the plant's use for improving health status and benefits to humans has not yet been thoroughly studied, despite being used in traditional medicines since ancient times.

References

1. Sarker SD, Nahar L. Chemistry for pharmacy students: General, organic and natural product chemistry. John Wiley and Sons Ltd., England: 2007; pp. 283-369.
2. Taneja SC, Qazi GN. Bioactive molecules in medicinal Plants: A perspective on their therapeutic action. In: Drug Discovery and Development (Vol. II). Hoboken, New Jersey: John Wiley and Sons, Inc., 2007; pp. 2-23
3. Rukangira, E. The African herbal industry: Constraints and challenges. A paper presented at "The natural products and cosmeceuticals 2001 conference". *Erboristeria Domani* 2001;1:1-23.
4. Kirtikar KR, Basu BD. Indian medicinal plants. Dehradun: International Book Distributors. 1999.
5. Burkill HM (ed.). The useful plants of west tropical Africa, vol. I, families A-D, Great Britain: White friars; 1985, pp 319-20.
6. Ernst E. Herbal remedies for anxiety-A systematic review of controlled clinical trials. Phytomed 2006;4:205-8.
7. Rabbani M, Sajjadi S, Ezarei HR. Anxiolytic effects of *Stachys lavandulifolia* on the elevated plus maze model of anxiety in mice. J Ethnopharmacol 2003;89:271-6.
8. Viola H, Wasowski C, Levi de Stein M, et al. Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepines receptor-ligand with anxiolytic effects. Planta Med 1995;61:213-6.
9. Salgueiro JB, Ardenghi P, Dias M, et al. Anxiolytic natural and synthetic flavonoid ligands of the central benzodiazepine receptor have no effect on memory tasks in rats. Pharmacol Biochem Behav 1997;58:587-91.
10. Paladini AC, Marder M, Viola H, et al. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J Pharm& Pharmacol 1999;519-26.
11. Dhawan K, Dhawan S, Chhabra S, Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Passiflora incarnate* Linneaus: A non-habit-forming anxiolytic. J Pharmacol & Pharm Sci 2003;6:215-22.
12. Wolfman C, Viola H, Paladini A, et al. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coeurolea*. Pharmacol Biochem Behav 2005;47:1-4.
13. Telrandhe UB, Uplanchiwar V. Phyto-Pharmacological Perspective of *Cadaba farinosa* Forsk. Am J Phytomed & Clin Therap 2013;1:11-22.
14. Ahmad V, Rahman UA, Chen A, et al. Cadabicine, an alkaloid from *Cadaba farinosa*. Phytochemistry 1985;24:2709-19.
15. El-Dirdiri NI, Barakat SE, Adam SE, The combined toxicity of *Arastolochia bracteate* and *Cadaba rotundifolia* to goats. Vet Hum Toxicol 1987;29:133-7.
16. BrainK. R, Turner TD. The practical evaluation of phytopharmaceuticals. Wright Science Technica. Bristol, 1975.
17. Silva LG, Lee IS, Kinghorn DA. Special problem with the extraction of plants. In: Cannell RJP. ed. Natural products isolation. New Jersey, Humana Press Inc. 999, Riverview Drive, Suite 208, Totowa: USA. 1998. pp 343-64.
18. Trease, GE, Evans MC. Textbook of Pharmacognosy. 12th ed. Balliere Tindall. London: 1983; pp. 322-83.
19. Trease, GE, Evans MC. Textbook of Pharmacognosy. 15th ed. Balliere Tindall. London: 1996; pp. 247-762.
20. Trease, GE, Evans WC. Textbook of Pharmacognosy. 14th ed. Singapore: Harcourt Brace and Company Asia Pte. Ltd. 2002 pp. 13-53, 117-139, 227, 293-334, 471-511.
21. Lorke D. A new approach to practical acute toxicity testing, Arch Toxicol 1983;54:275-87.
22. Stanley JL, Lincoln RJ, Brown TA, et al. The mouse beam walking assay offers more sensitivity over the rotarod in determining more co-ordination deficits induced by benzodiazepines. J Psychopharmacol 2005;19:221-7.
23. Rakotonirina, SV, Ngo-Bum E, Rakotonirina A, et al. Sedative properties of the decoction of the rhizome of *Cyperus articularis*. Fitoter 2001;72:22-9.
24. Vogel GH. Psychotrophic and neurotrophic activity In: Vogel GH, ed. Drug discovery and evaluation: Pharmacological assays. Springer-Verlag Berlin Heidelberg New York. 2008, pp. 566-874.
25. Musa A, Nazifi AB, Usman AI, et al. Evaluation of analgesic and behavioural potentials of ethanol root bark extract of *Erythrina senegalensis* DC (Fabaceae). Afr J Pharmacol Ther 2016;5:81-6.
26. Nazifi AB, Danjuma NM, Olurishe TO, et al. Behavioural effects of methanol stem bark extract of *Boswellia dalzielii* Hutch (Burseraceae) in mice. Afr J Biomed Res 2017;20:103-8.
27. Rolland A, Fleurentain J, Lanhers M, et al. Behavioural effects of American traditional plant *Eschscholzia california*: Sedative and anxiolytic properties. Planta Med 1991;57:212-6.
28. Fujimori H. Potentiation of barbital hypnosis as an evaluation method of central nervous system depressant. J Psychopharmacol 1965;7:374-97.
29. File SE, Wardill AG. Validity of head dipping as a measure of exploration of modified hole-board. J Psychopharmacol 1975;88:1-11,53-9.
30. Wolfman C, Viola H, Paladini AC, et al. Possible anxiolytic effects of chrysin, a central benzodiazepine receptor ligand isolated from *Passiflora coerviea*. Pharmacol & Biochem Behav 1994;47:1-4.
31. Johnson GAR. $GABA_A$ receptor channel pharmacology. Clin Pharm Design, 2005;11:1867-85.
32. Parez GRM, Perez IJA, Garcia D, et al. Neuropharmacological activity of *Solanum nigrum* fruit. J Ethnopharmacol 1998;62:43-8.
33. File S, Pellow S. The effect of Triazolobenzodiazepines in two animals tests of anxiety and on Hole-board. Bri J Pharmacol 1985;86:729-35.
34. Crawley JN. Exploratory behavior models of anxiety in mice. Neurosci Behav Rev 1985;9:37-44.
35. Adzu S, Amos S, Dzarma CW, et al. Effect of *Zizypus spina christi* mild aqueous extract on the central nervous system in mice. J Ethnopharmacol 2002;79:13-6.