Available online at http://ajol.info/index.php/ijbcs

Int. J. Biol. Chem. Sci. 8(5): 2132-2142, October 2014

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper

http://indexmedicus.afro.who.int

Sedative properties of *Mitracarpus villosus* leaves in mice

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ABSTRACT

The leaves of *Mitracarpus villosus* (Sw.) DC. (Rubiaceae) have several therapeutic applications in the West African folk medicine for the management of a plethora of stress-related diseases including headaches. This study was designed to evaluate the sedative properties of the ethylacetate extract of *Mitracarpus villosus* on open field (OFT), diazepam-induced sleep, staircase climbing, head-dips in the hole-board test and rota rod test in mice. Graded doses (100 – 400 mg/kg) of the extract significantly and dose-dependently prolonged the duration of diazepam-induced sleep ($P<0.05$), decreased the number of squares crossed in the OFT ($P<0.0001$), decreased number of head-dips in the hole-board test ($P<0.05$) and reduced steps climbing ($P<0.05$) in mice. The extract at the doses tested had no effect on motor coordination as observed in the rota-rod treadmill assay in mice. Our results revealed that the ethylacetate extract of *Mitracarpus villosus* leaves may contain psychoactive principles that are sedative in nature, thus supporting further development of the psychoactive components of this plant for management of stress-related diseases.

Keywords: *Mitracarpus villosus*, sedation, diazepam, locomotion.

INTRODUCTION

Mitracarpus vilosus (Sw.) DC. a medicinal plant belonging to the family Rubiaceae is locally known in Nigeria as 'Irawo Ile'-Yoruba, 'Obuobwa' - Ibo and 'Gududal' - Fulani (Jegede et al., 2005). It is an annual herb of about 30 cm high that grows as weeds in old and abandoned farmlands in tropical countries like Senegal, Gambia, Mali, Nigeria and Liberia. The leaves of *Mitracarpus villosus* are widely used in the West African traditional medicine for the management of toothaches, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases, sore throat, skin disease, wound dressing, leprosy as well as neurological disorders such as headaches (Abere et al., 2007). It is also taken as an antidote for arrow poison, diarrhoea, and dysentery (Jegede et al., 2005).

Previous studies revealed that the plant contains biologically active substances such as fatty acids, terpenes, flavonoids and other

DOI : http://dx.doi.org/10.4314/ijbcs.v8i5.18

phenolic compounds with potential hepato-protective, larvicidal, antifungal, antimicrobial, antinociceptive and anti-inflammatory activities (Ekpendu et al., 1994; Germano et al., 1999; Bisignano et al., 2000; Kprou et al., 2010; Makambila-Koebemba et al., 2011; Abdullahi et al., 2011). Furthermore, the pharmacognostic properties of the plant have been studied (Jegede et al., 2005; Abere et al., 2007) and some constituents of the volatile oil isolated and characterized (Ekpendu et al., 1993). The parameters for the standardization of *Mitracarpus villosus* have been reported by Ameh et al. (2014). The use of the plant *Mitracarpus villosus* for the management of neurological and stress-related disorders in the African traditional medicine motivated our interest to investigate the effects of this widely used plant on the central nervous system (CNS) as a step towards the isolation and identification of its biologically active components. The present study was designed to examine the sedative properties of the ethylacetate extract of *Mitracarpus villosus* leaves in order to scientifically describe the potential values of this important medicinal plant in the African traditional medicine for the management of stress related diseases.

MATERIALS AND METHODS

Plant material

The leaves of *Mitracarpus villosus* were collected from Idu, Abuja, Nigeria, in the month of September 2013. The plant was identified and authenticated by Mrs. Grace Ugwabe of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria, where a voucher specimen (NIPRD/H/6606) was prepared and deposited for future reference.

Plant extraction

The leaves were cleaned of debris, air-dried and pulverized to obtain a coarse powder using a pestle and mortar. 250 g of powdered leaves was subjected to Soxhlet extraction using 2 L ethylacetate. The filtrate was concentrated under reduced pressure using a rotary vacuum evaporator and the concentrate was evaporated to dryness on a water bath to give a solid (the extract) with a mean yield of 0.6% w/w.

Animals

Male Swiss albino mice (20 – 29 g), obtained from the Animal Facility Centre (AFC) of the National Institute for Pharmaceutical Research and Development, Abuja, Nigeria were used for the studies. All animals were housed under standard conditions of temperature (22 ± 1 °C), and light approximately (12/12 h light/dark cycle); and fed on standard maintenance diet and water *ad libitum*. Animals were approved for use by the AFC committee after reviewing the protocol for good laboratory practice and animal handling, All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. All efforts were made to minimise the number of mice used and their suffering.

Preliminary phytochemical screening

The extract was screened for the presence of tannins, saponins, alkaloids, glycosides, flavonoids, carbohydrates, terpenes according to procedures described by Trease and Evans (1989).

Acute toxicity studies

The method described by Lorke (1983) was employed for this study with modifications. Briefly, in the first phase, mice

were randomly placed in three groups of 3 mice per group. Mice from groups I, II, III received 10, 100 and 1000 mg/kg of extract in 0.5% Tween 80 respectively. The animals were observed for physical signs of intoxication for a period of 24 h. The next dose was scheduled based on the number of death recorded in stage one and animals (n=1) received 1600, 2900 and 5000 mg/kg of extract. The median lethal dose (LD_{50}) was calculated as the geometric mean of lowest non-lethal dose multiplied by the highest non-lethal dose from the second stage of dosing.

Pharmacological tests

Open Field Test

This test was conducted using an open field apparatus which consists of a clear glass box with dimensions (45 cm × 45 cm). The floor was divided by lines into 9 equal-sized squares. Five groups of six mice per group were treated intraperitoneally with vehicle, 3 doses of the extract (100, 200 and 400 mg/kg) and diazepam (1 mg/kg) respectively. After 30 min of treatment, each mouse was placed in the proximal right-hand corner of the maze and allowed to explore the apparatus unobstructed for five minutes. The total numbers of horizontal line crossed (number of squares crossed) and vertical activity (number of rearing) were counted for 5 min. This was recorded as the total locomotive activity (Tijani et al., 2012). After each five minute test, the maze was cleaned with 70% ethanol and allowed to dry before introduction of the next animal.

Diazepam induced sleep in mice

Mice were randomly divided into four groups of 8 mice per group. The first group received vehicle (0.5% Tween 80 in normal saline), which served as control while the other three groups of mice receive graded doses of the extract (100, 200 and 400 mg/kg) in 0.5% Tween 80. Thirty minutes later, diazepam 25 mg/kg was administered intraperitoneally to each mouse. The time taken to lose a righting reflex indicated the onset of sleep, while the time between the loss and recovery of the righting reflex for each mouse was taken as the duration of sleep (Rakotonirina et al., 2001).

Hole-board exploratory tests

The apparatus used is the Letica hole board instrument with 16 equidistant holes (1 cm diameter x 2 cm depth). The animals were treated with the vehicle, graded doses (100, 200 and 400 mg/kg) of the extract or diazepam 1 mg/kg, *i.p*. 30 minutes later, each mouse was placed at the middle of the board and the number of head dips over a period of 5 min was recorded (Perez, 1998).

Stair case assay

This method consists of placing an experimentally naïve mouse in an enclosed staircase with five steps (2.5 cm × 10 cm × 7.5 cm). The apparatus of 45 cm in length, with one end 12 cm and the other 25 cm in height was used. Five groups of six mice per group were treated intraperitoneally with vehicle, 3 doses of the extract (100, 200 and 400 mg/kg) and diazepam (1 mg/kg) respectively. After 30 min of treatment, each mouse was placed individually on the floor of the box with its back to the staircase and then the behaviour observed. The numbers of steps climbed and rearing were recorded for 5 min. A step was considered to be climbed only if the mouse had placed all four paws on the step (Y'au et al., 2011). Rearing was noted as when the mouse rose on its hind legs either on the step or against the wall to sniff the air. The number of steps descended was not counted. After each test, the box and staircase was cleaned with 70% ethanol in order to eliminate any olfactory clues that may modify the behaviour of the next animal (Lanhers et al., 1990).

Rota rod test

A rota-rod treadmill device (Ugo Basile, Italy) was used for this study. Mice were pre-trained to remain on slowly-moving (16 revolutions/min) rods of 5 cm diameter for 180 seconds. Only those animals, which demonstrated their ability to remain on the revolving rod for 3 min, were used for the test. Subsequently, the animals were grouped into four groups (n = 6) and treated with 0.5% Tween 80, extract (100, 200, 400 mg/kg) or Diazepam 1 mg/kg (i.p). 30 minutes post-treatment, animals were placed on the rod at intervals of 30 minutes, up to 2 hours. If an animal failed more than once to remain on the rod for 3 minutes, it was considered to lack motor coordination (Amos et al., 2005).

Statistical analysis

Results were expressed as mean ± SEM. Data were analysed using One-way ANOVA follow by Dunett's post hoc test for multiple comparisons. Results were regarded as significant at $P < 0.05$.

RESULTS

Acute toxicity studies

The intraperitoneal LD_{50} of the extract was estimated to be 1264.91 mg/kg in mice. The animals treated with extract showed signs of sedation, lethargy and general decreased physical activity. However, no signs of abdominal constriction, salivation, hyperactivity, convulsion and tremors were observed.

Phytochemical analysis

The extract tested positive for the presence of tannins, terpenes, flavonoids, phenols, resins, and carbohydrates.

Open field test

Mice treated acutely with *M. villosus* leaf extract showed a general decrease in locomotor activity. Compared with the control group, extract treated mice exhibited significant ($P<0.0001$) dose-dependent decreased locomotor activity, and a decrease in number of rearings however this effect is not dose dependent (Figure 1).

Diazepam induced sleep

The extract (100–400 mg/kg, i.p.) significantly ($P < 0.05$) prolonged the duration of diazepam-induced sleep in mice dose-dependently. There was no significant difference in the time of onset of sleep in mice treated with the extract from the control mice. The duration of sleep was increased from 110.43 ± 21.93 min in the control group to 274.86 ± 28.49 min at 400 mg/kg of extract. The effect of the extract on duration of sleep was significant ($P < 0.05$) when compared to that of control (Figure 2).

Exploratory behaviour (hole-board)

The extract at the doses tested caused a significant ($P<0.0001$) and dose-dependent reduction in the number of head-dips when compared to control. Diazepam 1 mg/kg also produced a decrease in the frequency of head-dips as shown in Figure 3.

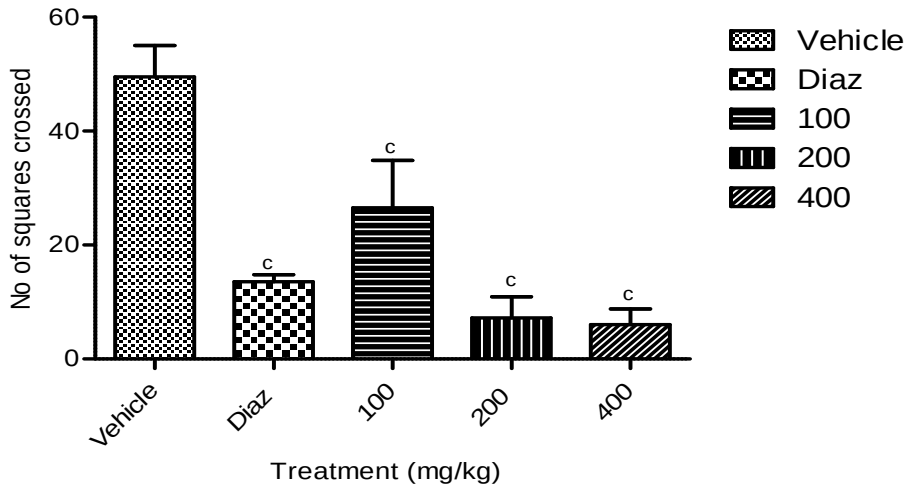
Staircase assay in mice

The extract decreased the steps climbed and rearing on the hind legs of the mice. These were dose-dependent and significant ($P<0.05$) when compared to the control group. Similarly Diazepam 1 mg/kg reduced both the number of steps and rearings (Figure 4).

Rota-rod test in mice

The extract (100–400 mg/kg i.p.) did not reveal significant effect on the rota-rod performance of the mice as all the animals stayed on the rod for 180 sec without falling off the rods (Table 1).

A



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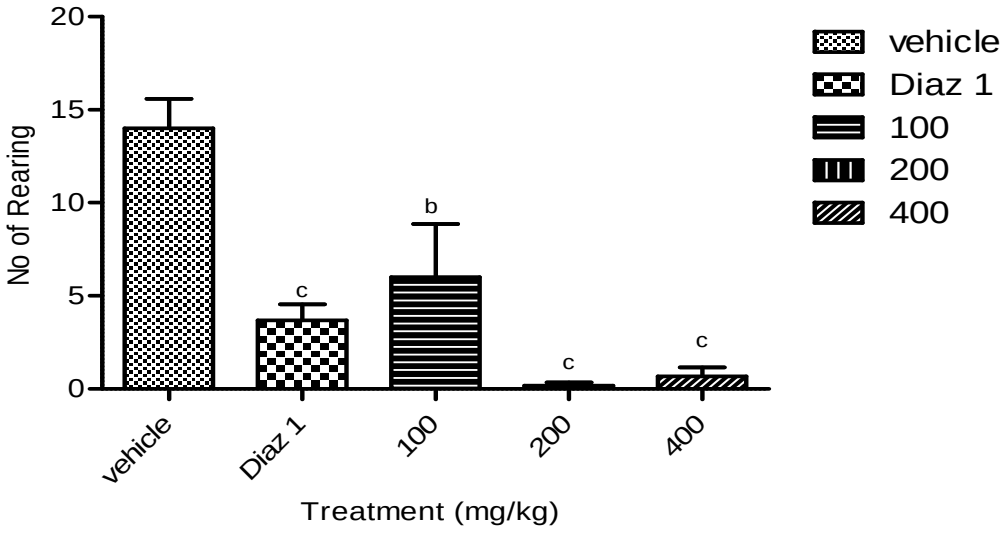


Figure 1: Effect of ethylacetate extract of *Mitracarpus villosus* on Open Field Test. Effect of *Mitracarpus villosus* leaves extract on Open Field Test represented as (A) effect on locomotion; (B) effect on rearing. Values are presented as mean ± SEM (n = 6); $^{b}P< 0.001$, $^{c}P<0.0001$, significant when compared to control.

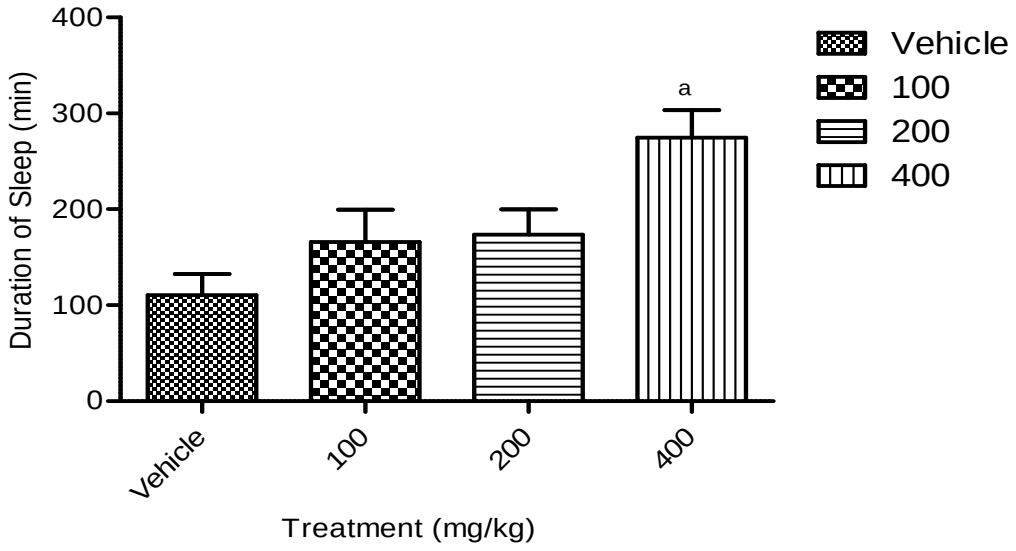


Figure 2: Effect of ethylacetate extract of *Mitracarpus villosus* on diazepam-induced sleeping time.
Effect of *Mitracarpus villosus* leaf extract on diazepam-induced sleeping time. Values are presented as mean ± SEM (n = 8); [a] P<0.05, significant when compared to control.

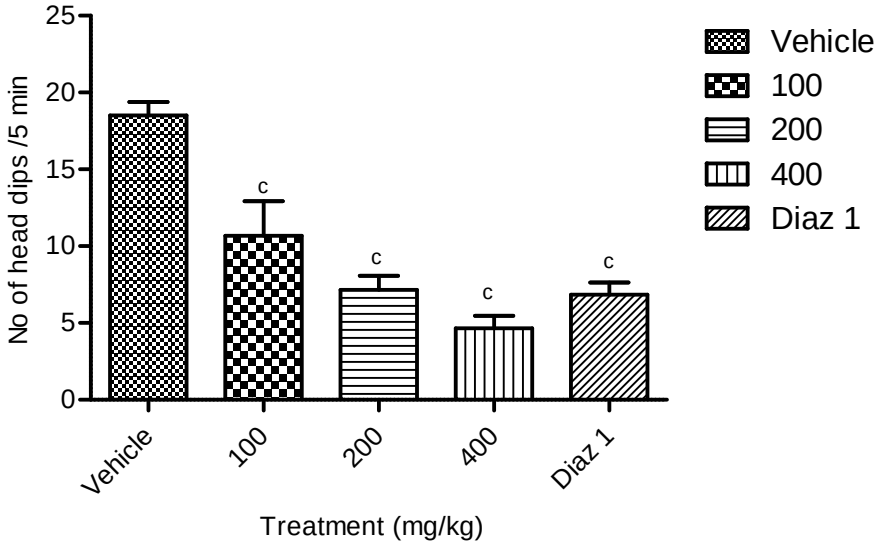
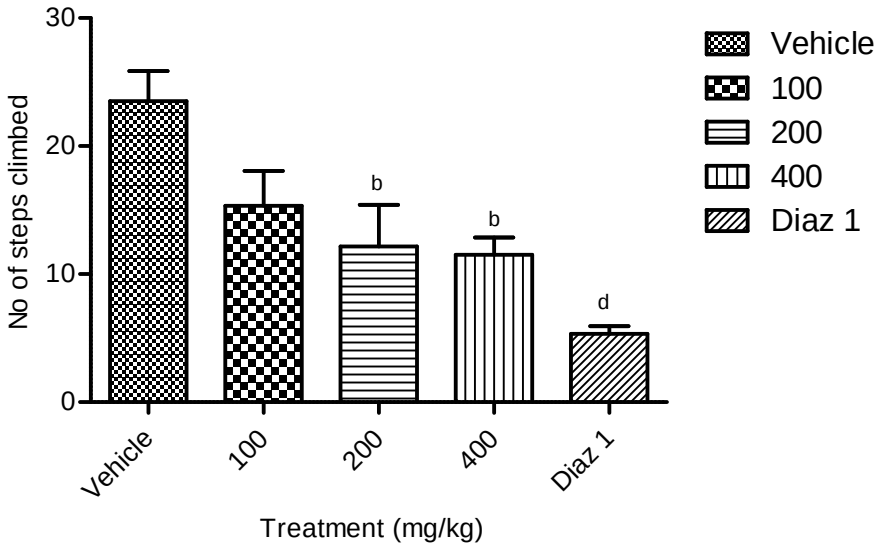


Figure 3: Effect of ethylacetate extract of *Mitracarpus villosus* on exploratory behaviour using Hole Board test. Effect of *Mitracarpus villosus* leaf extract on Hole board exploratory behaviour in mice. Values are presented as mean ± SEM (n = 6); [c]P<0.0001, significant when compared to control.

A



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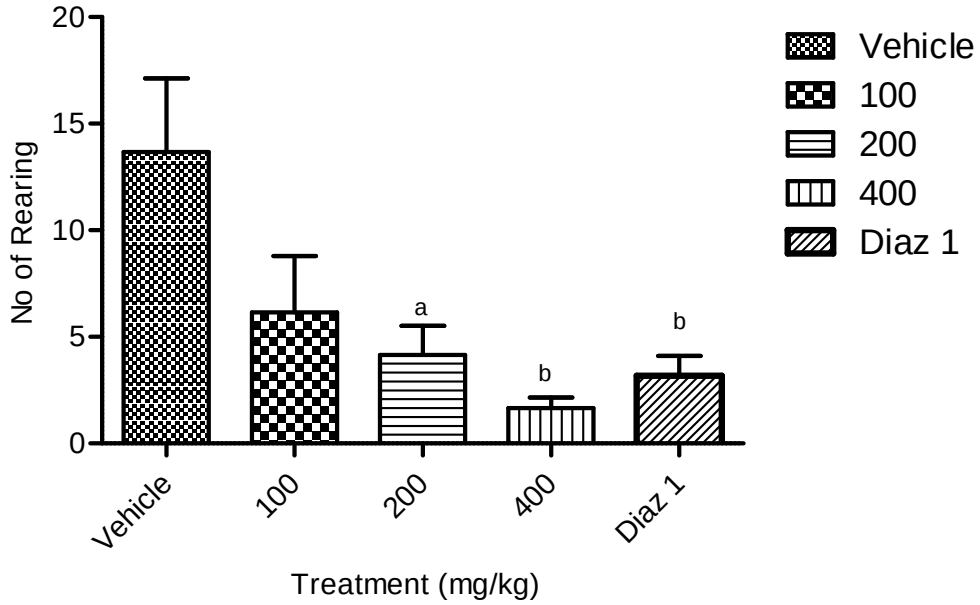

Figure 4: Effect of ethylacetate extract of *Mitracarpus villosus* on Staircase assay. Effect of *Mitracarpus villosus* leaves extract on Staircase assay (A) effect on climbing (B) effect on rearing. Values are presented as mean ± SEM (n = 6); [a]P< 0.05, [b]P<0.001, [d]P<0.0002 significant when compared to control.

Table 1: Effect of ethylacetate extract of *Mitracarpus villosus* leaves on rota-rod test in mice.

Treatment	Post Treatment (Min)							
	30		60		90		120	
	Time on rod	Fail. %	Time on rod	Fail. %	Time on rod	Fail. %	Time on rod	Fail. %
Vehicle	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
100 mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
200 mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
400 mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0

Results are presented as mean ± SEM (n=6). No significant difference between saline and treated groups.

DISCUSSION

The sedative effects of the ethylacetate extract of *Mitracarpus villosus* were investigated on open field, diazepam-induced sleep, hole-board, staircase, and rota-rod tests. The data obtained indicates that the plant may contain psychoactive substances that are sedative in nature. The intraperitoneal LD_{50} of the plant of 1264.91 mg/kg suggests that the plant could be considered of low-toxicity when administered intraperitoneally (Hosseinzadeh et al., 2013). Preliminary phytochemical analysis revealed the presence of flavonoids, tannins, terpenoids, phenols, resins and carbohydrates in the extract. These secondary metabolites may be responsible for the observed pharmacological effects (Jiang et al., 2007; Akindele and Adeyemi, 2010; Ajao and Akindele, 2013).

Assessment of general behavioural and locomotor activity of rodents can be achieved using the open field test and mouse staircase. These activities have been used to determine sedative (predominantly locomotion) or stimulant effects of a pharmacological agent (Steru et al., 1984; Martinez-Vazquez et al., 2012). The numbers of squares crossed and rearings are indicative of locomotor activity (Asuquo et al., 2013). Locomotion connotes alertness and decreased locomotor activity is suggestive of sedation; Increase in the number of rearing denotes anxiety while a reduction in the number of steps climbed is a suggestion of sedative activity (Gahlot et al., 2011; Yau et al., 2011; Rout and Kar, 2013). The reduction in both the number of steps climbed and number of rearings observed is indicative of possession of sedative effects by the extract.

The hole-board experiment is a measure of exploratory behaviour. Agents that decrease this parameter are generally regarded as possessing sedative properties (File and Fernandez, 1994; Amos et al., 2003) while anxiolytics increase the head-dip counts. The reduction in the number of head dips shown by the extract is therefore an indication of the presence of psychoactive constituents that are sedative in nature.

The sedative property of the plant was confirmed by its ability to potentiate the duration of diazepam induced sleep (Ngoupaye et al., 2013). Hypnotic/sedative agents like the benzodiazepine act by enhancing the action of Gamma Amino Butyric Acid (GABA) on GABA receptor channel complex by binding on specific allosteric sites on GABA receptor type-A (Ritcher et al., 2012) to enhance GABA binding to the GABA receptor resulting to the potentiation of GABA responses (Mellor and Randall, 2004; Ezekiel et al., 2010). In this experiment, the ethylacetate extract of *Mitracarpus villosus* prolonged the duration of diazepam-induced sleep possibly by modulating the action of GABA at the BDZ binding site of $GABA_A$ receptors. The extract may also be mediating the observed sedative activity by modulating the expression level of

glutamic acid decarboxylase (GAD) the rate-limiting enzyme in GABA biosynthesis; the enzyme also plays a role in maintaining GABA levels in the mammalian brain (Kim et al., 2011).

The rotarod test provides an index of skeletal muscle relaxation. The ability of mice to stay on the rotating rod during the study suggests that the sedation at the doses tested did not interfere with activity on the treadmill or the extract may be devoid of skeletal muscle relaxant activity (Woode et al., 2011).

Conclusion

The results from this study provide scientific evidence that *Mitracarpus villosus* leaves may contain psychoactive principles that are sedative in nature. Studies are in progress in our laboratories to isolate and mechanistically characterize the biologically active components from this important medicinal plant that is already in common use.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the technical assistance of Sunday Dzarma, Glory Wangs for donation of diazepam and NIPRD for funding this study.

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