ISSN 0974 – 5211

Journal of Natural Products
Volume 2 (2009)
www.JournalofNaturalProducts.com

Research Paper

Effect of *Ziziphus mauritiania* (L.) seed extracts on spatial recognition memory of rats as measured by the Y-maze test

Yusuf Sadiq[1*], Adelaiye B Alexander[2], Agunu Abdulkarim[3]
[1]Dept. of Physiology, School of Health Sciences, Kampala International University Uganda;[2]Dept. of Human Physiology, Faculty of Medicine, [3]Dept. of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.
[*]Corresponding author
(Received 25 January 2009; Revised 11 February 2009; Accepted 14 February 2009)

ABSTRACT

In this study, the influence of acute administration of ethyl acetate, ethanol and aqueous seed extracts of *Z. mauritiana* on spatial recognition memory in Wistar rats were investigated using the Y-maze. Spatial memory was measured by the total number of entries and duration spent in each arm of the Y-maze following administration of the extracts intraperitoneally (i.p.). Transfer latency (TL) in the Y-maze was expressed as inflexion ratio (IR). The number of arm visits during the first trial and second trial period were measured as an index of locomotor activity. Scopolamine (0.4 mg/kg) was used to induce amnesia in the animals. The results indicated that, after 24 h, IR was slightly increased ($P>0.05$) by ethanol and aqueous extracts while ethyl acetate extract significantly reduced the IR ($P< 0.05$) compared to controls. All doses of ethyl acetate extract impaired spatial recognition memory after a 24 h interval–trial interval, whereas aqueous and ethanol extracts did not showed this impairment. All the extracts significantly decreased locomotor activity of the animals ($P<0.001$). None of the extracts reversed scopolamine induced amnesia in the rats. These data suggests that seed extracts of *Z. mauritiana* impaired spatial recognition memory and decreased locomotor activity of rodents, the activity of which was greatly produced by the portion extracted by ethyl acetate.

Keywords: *Ziziphus mauritiania*, Y-maze, Spatial memory

INTRODUCTION

Emerging evidence suggests that a number of plants are known to be the source of useful drugs in modern medicine (Gowda, 1997) but the validity of their traditional uses to treat various disorders must be scientifically evaluated. *Ziziphus mauritiana* Lam (Family-Rhamnaceae) is an important tropical fruit tree that contains important source of compounds (flavonoids, glycosides, saponins and volatile oil)

with a wide profile of putative therapeutical applications (Dahiru, et al., 2006). It is commonly known as Kurnna or Magarya in Hausa, desert apple or Indian Cherry, Chinese date in English (Morton, 1987).

The roots of *Ziziphus* species are commonly used in folklore medicine for the treatment of diarrhea, digestive disorders, weakness, liver complaints, obesity, urinary troubles, skin infections, loss of appetite, fever, bronchitis, convulsion, epilepsy, insomnia and are known for their anxiolytic and sedative properties (Msonthi, et al, 1983; Jiang, et al., 2007). Substances with sedatives properties have been shown to play crucial role in many types of learning and memory (Spain and Newsom, 1991; Zarrindast and Rezayof, 2004; Alaei, et al., 2006; Wezenberg, et al., 2006; Kaindl, et al., 2008) and may cause cognitive and emotional impairments. Behavioral effects of extracts from *Ziziphus mauritiana* on learning and memory performance have not yet been reported.

The use of Y-maze is based on the innate tendency of rats to explore novel environments (Dellu, et al., 1992, 2000). Y-maze is a simple two-trial recognition neutral exteroceptive model used to assess the normal hippocampus dependent navigation behavior of rodents and this has been shown to be sensitive to disruption by amnestic drugs and manipulations of genes associated with cognition (Martin, et al., 2003; Conrad, et al., 1997, 2003). In this study, we have used the Y-maze to investigate the effect of acute administration of seed extracts of *Z. mauritiana* on acquisition of memory which was unrelated to punishment and reward in the rat.

MATERIALS AND METHODS

***Plant Material*:** Fresh ripen fruits of *Z. mauritiania* were collected from Ahmadu Bello University Farm, Zaria, Nigeria in June, 2004. The ripen fruits were picked directly from the trees and transported to the laboratory in open bags to avoid fermentation. The specimen was identified and authenticated at the Department of Biological Sciences herbarium unit of the same institution. A voucher specimen (No. 7072) is deposited at the herbarium unit of for future reference.

***Preparation of Extract*:** After collection, the fruits were macerated in water to remove the pulp and the seeds were rinsed in clean water. Thereafter the seeds were spread out on a sheet and dried in the sun for one week.

The dried seeds were grounded and sieved through a mesh to obtain a fine powder (100g). This was exhaustively extracted for 24 h at room temperature in a Soxhlet apparatus with 300ml each of water, ethyl acetate and 70% ethanol to give the aqueous, ethanol and ethyl acetate portions of the extract respectively. Extraction was carried out separately with each solvent. After removal of the solvents at reduced pressure and <40 °C, the extracts were stored at -20°C. The yield was 16.7%, 3.3% and 1.7% (w/w) for the aqueous, ethanol and ethyl acetate portions of the extract respectively.

Each extract was dissolved in saline (aqueous and ethanol portion) and polyethylene glycol 400 (ethyl acetate portion) and administered to each rat in doses of 25, 50 and 100mg/kg intraperitoneally (i.p.) at 0.1 ml/100g body weight 30 min before subjecting the animals to the Y-maze.

***Animals*:** Male Wistar rats (120-150g body weight) were obtained from the animal house of the Department of Pharmacology, Ahmadu Bello University, Zaria, Nigeria. Animals were housed five to a cage and maintained at room temperature under a 12 h

light/dark cycle with free access to food and water. They were acclimatized to the laboratory conditions 7 days before behavioral studies and randomly assigned to 3 main experimental groups with 20 rats per group. These include ethanol extract (Group I), ethyl acetate extract (Group II), and aqueous extract (Group III). Each group was further sub-divided into four subgroups with 5 rats each. Each subgroup had a corresponding control. Group IV received 0.4mg/kg scopolamine. The experiments were conducted in accordance with Institutional guidelines for the care and use of animals adapted from the *South Africa MRC Guidelines on Ethics for Medical Research: Use of Animals in Research and Training*.

***Behavioral Apparatus*:** The Y-maze served as the exteroceptive model to evaluate acquisition of spatial memory in rats. The apparatus was constructed of plain wood and consist of identical three arms. The arms were randomly designated: start arm, in which the rat started to explore (always open), novel arm; which was blocked during the first trial, but open during the second trial, and the other arm was always open (Ma, et al., 2007).

Each arm was 35 cm x 6 cm x 15 cm (width x height x length). The maze has an equilateral triangular centre, each arm of the Y beginning from each side of the triangle and extending radially away from the centre at an angle of 120°, forming the letter Y shape of the maze. It was important that the three arms be made similar to prevent preference on the part of the animal when introduced into the maze. The floor of the maze was covered with sawdust, which was mixed after each individual trial in order to eliminate olfactory stimuli.

***Drugs and Chemicals*:** Scopolamine hydrobromide (Sigma, MO, USA) was a gift from National Institute Pharmaceutical of Research and Development (NIPRD) Abuja, Nigeria. The drug was diluted in normal saline and administered intraperitoneally 30 min prior to behavioral testing. Ethanol and ethyl acetate were of laboratory grade.

***Y-maze Test*:** The Y-maze test consisted of two trials separated by an inter-trial interval to assess spatial recognition memory. At the start of each session, each animal was gently placed at the end of the start arm, facing away from the central platform. The time taken by the animal to move from the end of the arm to the centre of the maze was measured and recorded as the Transfer latency (TL). TL was recorded for each animal and if a rat did not get to the centre of the maze within 90-sec, it was gently pushed to the centre and TL was assigned 90sec. The number of entries and time spent in each arm of the maze by each rat was recorded visually over the trial session. Arm entry was defined as the entry of all four paws into one arm.

***Experimental Procedures*:** Thirty minutes before the first trial (training), the animals received different doses (25, 50 or 100mg/kg, i.p.) of ethanol (group I), ethyl acetate (group II) and aqueous (group III) portions of the plant extract in a volume of 0.1 ml/100g body weight. Control groups were injected (i.p.) with saline (groups I, III and V) and polyethylene glycol 400 (group II) in the same volume. Group IV which served as the positive control received 0.4mg/kg of scopolamine i.p.

For the first trial, rats were placed inside the start arm while the novel arm was blocked with a block. Therefore, rats were able to explore the start and other arms, but not the novel arm during a 20min period (Ma, et al., 2007; Jung, et al., 2008). Memory retrieval (second trial) was evaluated in a test session carried out 24 h after the first trial (Itoh, et al., 1990; Pearl, et al., 2004). For this trial, trained animals were

placed back in the maze in the same starting arm, with free access to all three arms for 20min. TL was recorded for each animal in each trial and expressed as inflexion ratio (IR). IR was calculated by the formula described by Jaiswal and Bhattacharya (1992):

$$\text{Inflexion ratio} = (L_1 - L_0)/L_0$$

Where L_1 is the initial TL (sec) and L_0 is the TL (sec) after 24 h.

To measure spatial recognition memory, the number of entries and time spent in each arm of the maze by each rat was recorded and novelty versus familiarity was analyzed by comparing behavior in all three arms. The number of arms visited was taken as an indicator of locomotor and exploratory activity.

***Statistical analysis*:** Data were expressed as total time duration spent in arms during the second trial as an index of spatial recognition memory and the number of entries into each arm (as an index of locomotor activity). The effects on maze performance were evaluated using student's t-test and one way analysis of variance (ANOVA) whenever appropriate for determining the statistically significant differences among the groups followed by Tukey's multiple comparison (*post hoc*) tests. The values were expressed as means ± standard error of mean (S.E.M). Difference between groups were considered significant at $P<0.05$.

RESULTS

***Effects of acute Z. mauritiania on acquisition of spatial recognition memory in Y-maze*:** As can be seen in Table 1, when given i.p. ethanol and aqueous portions of the extract slightly increased the IR ($P>0.05$) while the ethyl acetate extract significantly decreased IR ($P<0.001$) after 24 h when compared to control. Scopolamine decreased the IR as expected ($P<0.001$). None of the fractions antagonized the amnesic effect of scopolamine [data not shown].

As shown in Figure 1, the total duration of visits in the novel arm was significantly increased in animals administered with aqueous extract (Fig 1a; $F2.12=3.89$, $P=0.001$ [25mg/kg], $P=0.001$[50mg/kg] and $P=0.006$ [100mg/kg]) and ethanol extract (Fig 1b; $F2.12=3.39$, $P=0.005$ [25mg/kg], $P=0.002$ [50mg/kg] and $P=0.002$ [100mg/kg]) when compared with the start and other arms after 24 h. There was no arm difference in animals treated with ethyl acetate extract (Fig 1c; $F2.12 = 3.89$, $P=0.2$) but when compared to the control, animals in this group spent less time in the novel arm ($P < 0.01$) indicating that animals in this group fail to recognize the novel arm after 24 h time interval.

***Effects of acute Z. mauritiania on locomotor activity*:** The effect of the extracts on arm visits is presented in Fig. 2a-c respectively. The results indicate that the extracts dose dependently decreased the number of arm entries when compared to control. Measurement of total number of arm entries during the second trial session revealed a significant difference among the three arms in each group after 24 h.

Table-1: Effect of seed extracts of *Z. mauritiania* on transfer latency of rats in the Y-maze.

Treatment	Dose [mg/kg]	Inflexion Ratio (Mean ± SEM)
Vehicle	-	0.17 ± 0.02
Ethanol fraction	25	0.22 ± 0.01
	50	0.25 ± 0.02
	100	0.20 ± 0.04
Ethyl Acetate fraction	25	-0.33 ± 0.02**
	50	-0.65 ± 0.01**
	100	-0.69 ±0.05**
Aqueous	25	0.21 ± 0.02
	50	0.20 ± 0.04
	100	0.25 ± 0.03
Scopolamine	0.3	- 0.77 ± 0.01**

- Transfer latency is expressed as the inflexion ratio.
- Drugs were administered 30 minutes before the session in the Y maze.
- Data are expressed as mean ± SEM
- $P<0.05$, ** $P<0.01$ compared to control (vehicle) n = 5

DISCUSSION

The two-trial Y-maze is a specific and sensitive test of spatial recognition memory in rodents (Conrad, et al., 2003). The test relies on an innate tendency of rats to explore a novel environment but not on learning a new behavior or rule. Some studies have also used the number of arm visits as an index of locomotor activity as well (Dellu, et al., 2000; Ma, et al., 2007). The Y- maze used in this study involves no aversive stimuli and was considered suitable for evaluating memory. The experiment described in this communication, to the best of our knowledge, is the first to report that pre-training administration of seed extract of *Z. mauritiana* caused deficit in the rat's acquisition of spatial recognition memory.

In this study, animals treated with ethanol and aqueous portions of the extract were able to distinguish the novel arm from the other two familiar arms after a 24 h ITI. This suggests that these portions of the extracts did not display any effect on acquisition of spatial recognition memory 24 h after administration. However, animals administered with ethyl acetate portion of the extract like the anti-muscarinic drug scopolamine, failed to recognize the novel arm after 24 h interval indicating that, there must have been very little perception of the environment stimuli during the training session. This is reflected by the decreased in IR and duration of time spent in the novel arm of the maze when compared to the controls. These observations imply that, this portion of the extract influence acquisition of memory or impair performance on spatial memory tasks by altering neural systems involved in spatial memory.

Spatial memory as measured by the Y-maze tests is dependent on hippocampal learning and memory function and is related to the NMDA receptor/Ca2+ influx signaling pathway (Dellu, et al., 2000; Conrad, et al., 2003). It is possible that, compounds contained in the ethyl acetate portion of the extract may inhibit this hippocampal NMDA receptor/Ca2+ signaling pathway.

We found that *Z. mauritiania* reduces locomotor activity in the animals. The reduction in locomotor activity in the Y-maze test confirms the sedative or central activity of *Z. mauritiania*, since it is conceded that, this activity is a function of the excitability level of the central nervous system (Mansur, et al., 1971). Although some authors suggest that flavonoids and oils of *Ziziphus* species could be the potential active components in sedation (Cheng, et al., 2000) but the efficacy of the extract is perhaps better explained by the array of chemicals acting in concert rather than a single chemical found in the plant.

Our present study demonstrates that seeds of *Z. mauritiania* extracted with ethyl acetate not only impair the acquisition but also consolidation and retrieval of spatial recognition memory in animals in the Y- maze. The observations indicates that *Z. mauritiania* may contain compounds that maybe useful in the development of therapeutic agent for neuro-protection.

REFERENCES

Alaei, H., Borjeian, L., Azizi, M., Orian, S., Pourshanazari, A., Hanninen, O., (2006): Treadmill running reverses retention deficit induced by morphine. *Eur. J. Pharmacol*., 536:138-141.

Cheng, G., Bai, Y. J., Zhao, Y. Y., Tao, J., Lin, Y., Tu, G. Z., Ma, L. B., Liao, N., Xu, X. J., (2000) Flavonoids from *Ziziphus jujuba* Mill var. spinosa. *Tetrahedron*, 56: 8915-8920

Conrad, C.D., Lupien, S.J., Thanasoulis, L.C., McEwen, B.S., (1997): The effects of type I and type II corticosteroid receptor agonists on exploratory behavior and spatial memory in the Y-maze. *Brain Res*., 759:76–83.

Conrad, C.D., Grote, K.A., Hobbs, R.J., Ferayorni, A., (2003): Sex differences in spatial and non-spatial Y-maze performance after chronic stress. *Neurobio Learn Mem*., 79:32-40.

Dahiru, D., Sini, J.M., John-Africa, L., (2006): Antidiarrhoeal activity of *Ziziphus mauritiana* root extract in rodents. *Afr J Biotechol*., 5(10): 941-945.

Dellu, F., Mayo, W., Cherkaoui, J., Le Moal M., Simon, H., (1992): A two-trial memory task with automated recording: study in young and aged rats. *Brain Res*., 588:132–139.

Dellu, F., Contarino, A., Simon, H., Koob, G.F., Gold, L.H., (2000): Genetic differences in response to novelty and spatial memory using a two-trial recognition task in mice. *Neurobiol Learn Mem*., 73:31–48.

Gowda, T.V., (1997): Interaction of snake venom phospholipases A2 with plant isolates. In: Venom Phospholipase A2 Enzymes, Chichester (Edited by Kini R.M.,), John Wiley & Sons, pp. 205–221.

Itoh, J., Nabaeshima, T., Kameyama, T., (1990): Utility of an elevated plus maze for the evaluation of nootropics, scopolamine and electro convulsion shock. *Psychopharmacology*., 101: 27-33

Jaiswal, A.K., Bhattacharya, S.K., (1992): Effects of Shilajit on memory, anxiety and brain monoamines in rats. *Indian J Pharmacol*., 24: 12-17

Jiang, J.G., Huang, X.J., Chen, J., Lin, Q.S., (2007): Comparison of the sedative and hypnotic effects of flavonoids, saponins, and polysaccharides extracted from Semen *Ziziphus jujube*. *Nat Prod Res*., 21(4):310-20

Jung, W.R., Kim, H.G., Kim, K.L., (2008). Ganglioside GQ1b improves spatial learning and memory of rats as measured by the Y-maze and the Morris water maze tests. *Neurosci Lett*., 439: 220–225

Kaindl, A.M., Koppelstaetter, A., Nebrich, G., Stuwe, J., Sifringer, M., Zabel, C., Klose, J., Ikonomidou, C., (2008): Brief Alteration of NMDA or GABAA Receptor-mediated

Neurotransmission Has Long Term Effects on the Developing Cerebral Cortex. *Mol Cell Proteomics*, 7:2293-2310.

Ma, M.X., Chen, Y.M., He, J., Zeng, T., Wang, J.H., (2007): Effects of morphine and its withdrawal on y-maze spatial recognition memory in mice. *Neuroscience*., 147**:** 1059–1065

Masur, J., Martz, R.M.W., Carlini, E.A., (1971): Effects of acute and chronic administration of *Cannabis sativa* and (−) α9-trans-tetrahydrocannabinol on the behavior of rats in an open field arena. *Psychopharmacology*, 19: 338–97.

Martin, S., Jones, M., Simpson, E., Van den, B.M., (2003): Impaired spatial reference memory in aromatase-deficient (ArKO) mice. *Neuroreport.,* 14:1979–1982.

Morton, J., (1987): Indian Jujube. In: Fruits of Warm Climates (Edited by Morton, J.F.), Miami, Florida. pp 272-275

Msonthi, J.D., Magombo, D., Hamdard, (1983): Medicinal Herbs in Malawi and their Uses. *Hamdard Medicus*., 26 (2): 94-100.

Parle, M., Dhingra, D., Kulkarni, S.K., (2004): Improvement of mouse memory by *Myristica fragrans* seeds. *J Med Food.,* 7: 157-161

Spain, J.W., Newsom, G.C., (1991): Chronic opioids impair acquisition of both radial maze and Y-maze choice escape. *Psychopharmacology* (Berl), 105:101–106.

Wezenberg, E., Sabbe, B.G., Hulstjn, W., Ruigt, G.S., Verkes, R.J., (2007): The role of sedation tests in identifying sedative drug effects in healthy volunteers and their power to dissociate sedative-related impairments from memory dysfunctions. *J Psychopharmacol*., 21:579.

Zarrindast, M.R., Rezayof, A., (2004): Morphine state-dependent learning: sensitization and interactions with dopamine receptors. *Eur J Pharmacol*., 497:197–204.

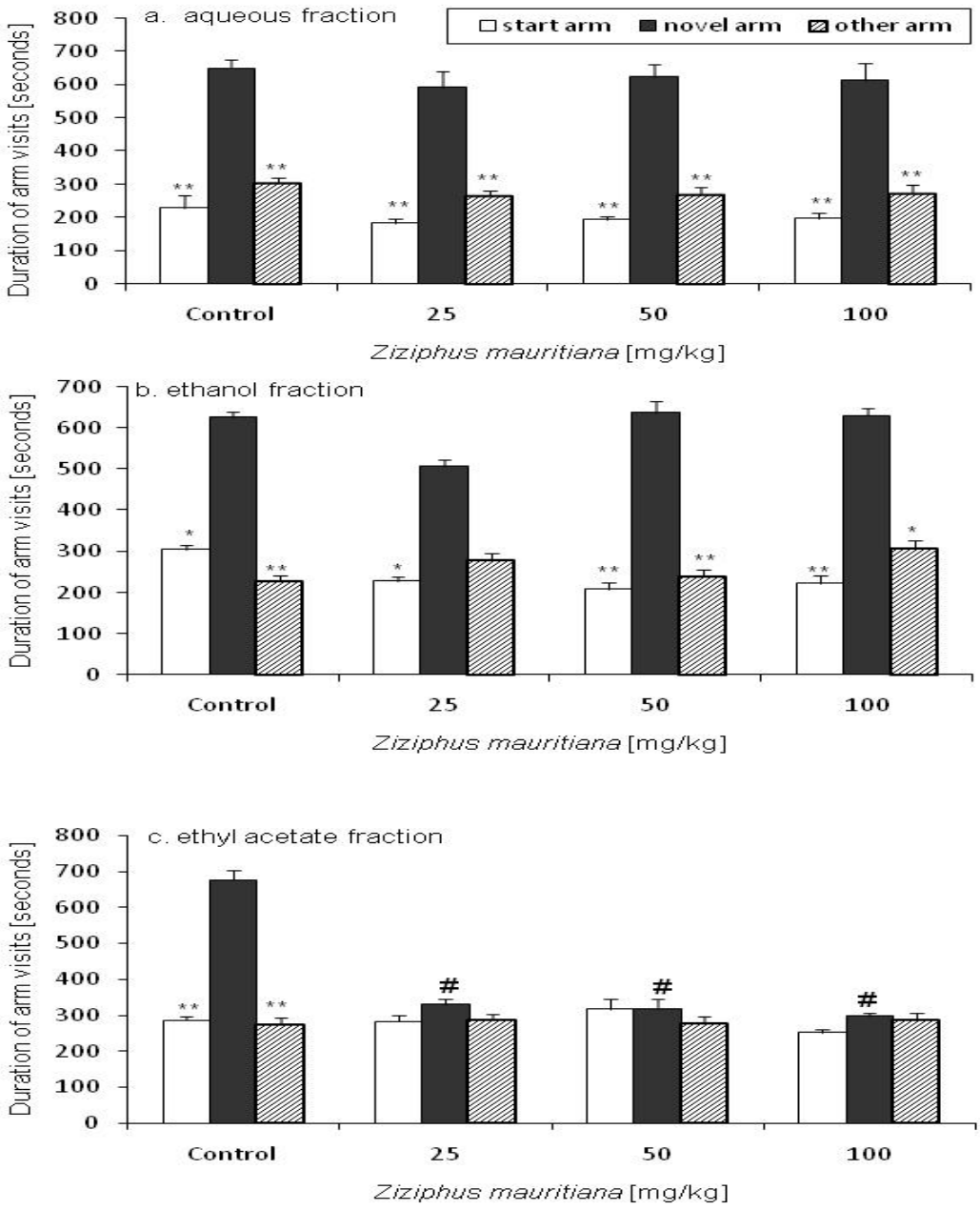

Figure-1: Effects of acute administration seed extracts of *Z. mauritiana* on the spatial memory of rats in Y-maze after 24 h inter training interval.

- This was assessed by determining the effect of aqueous (a), ethanol (b) and ethyl acetate (c) extracts on mean total duration (seconds) of arm visits for rats visiting the novel, start and other arms 24 h after drug administration.
- Data are expressed as mean ± SEM
- * $P<0.05$, ** $P<0.001$ for difference in performance of rat in the novel arm compared to the start and other arms. # $P<0.05$ for difference in performance in the novel arm between rats treated with the extract and controls. n = 5 per group.

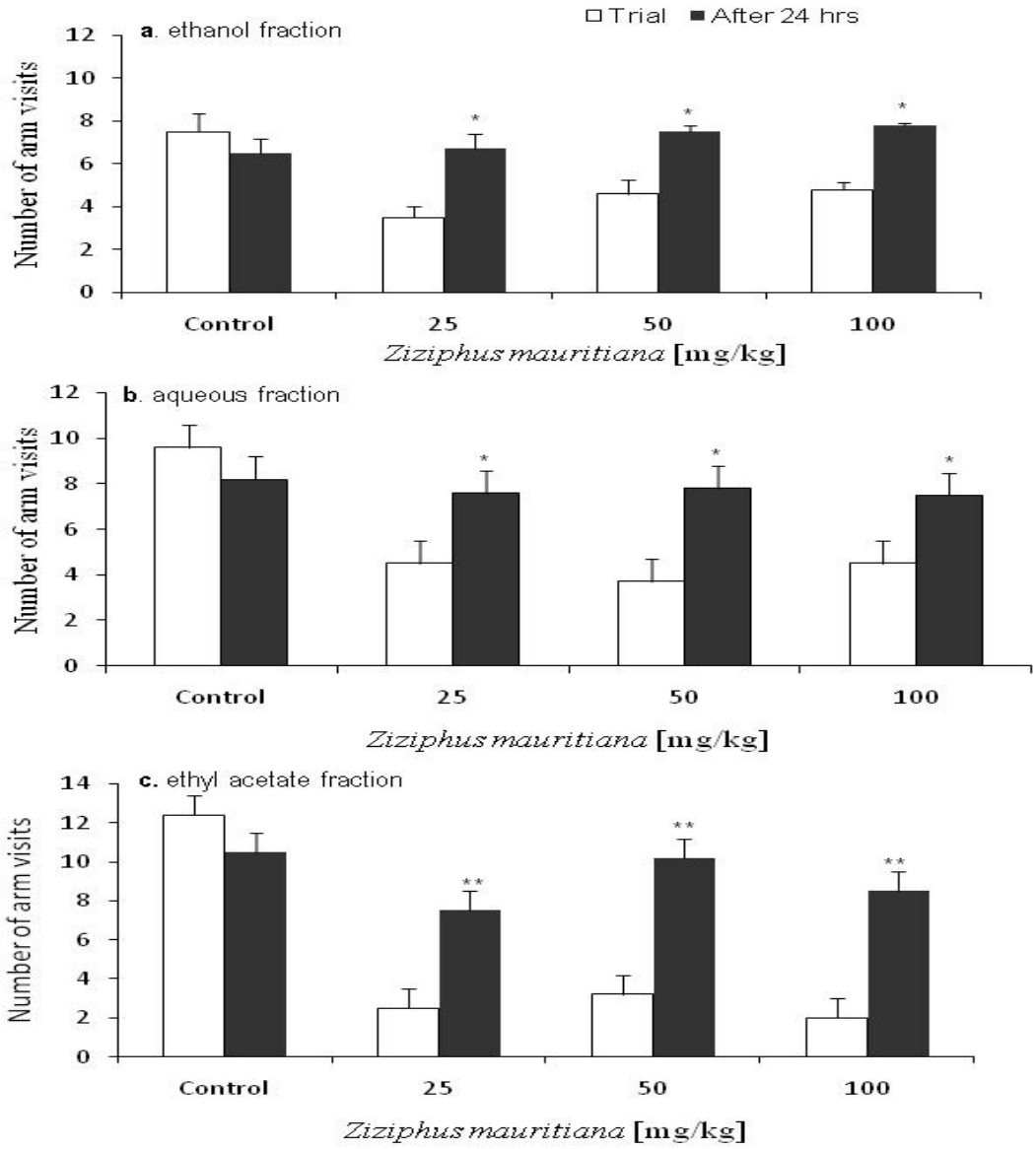

Figure-2: Effect of seed extracts of *Z. mauritiania* on locomotor activity.

- Panel a-c indicates effect of aqueous, ethanol and ethyl acetate extract respectively. All fractions of the extract decreased the locomotor activity during the trial period but not 24 h later as reflected by higher number of arm visits.
- Data are expressed as absolute number of arm visits (mean ± SEM).
- $P< 0.05$ ** $P< 0.01$ for difference in number of arm visits during trial compared to 24 h later.

