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Neuropharmacological effects of *Alchornea cordifolia* (Schumach. & Thonn.) Mull. Arg. (Euphorbiaceae) in mice

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ABSTRACT

Alchornea cordifolia (Euphorbiaceae) is a common plant, which has featured prominently in traditional medicinal practice. It has been reported that the decoction of the leaves is taken as central nervous system stimulant. This work was therefore undertaken to examine the central nervous system effects. The neuropharmacological profile of the plant was determined in mice to which the plant extract had been orally administered at respective doses of 250, 500 and 1,000 mg/kg. The behavioral models used included novelty-induced behaviors (locomotion, rearing and grooming), holeboard and elevated plus maze (anxiolytic) and forced swimming (antidepressant). The Y-maze was used for the investigation of the plant extract on locomotion, learning and memory. The results obtained showed that both locomotor and rearing activities were significantly decreased at the highest dose of 1000 mg/kg orally, while grooming behavior was significantly decreased at all the doses administered. In the hole board experiment, the frequency of head-dips was decreased significantly at 1000 mg/kg, while there was no significant effect observed in the elevated plus maze. Y-maze model results showed that it had no significant effect on spatial memory. There was no significant difference in the immobility duration due to administration of the extract in the forced swimming test. In conclusion, the present study showed that although the ethanolic leaf extract of *A. cordifolia exhibited* some central inhibitory effect, it is devoid of anxiolytic, antidepressant activities and has no significant effect on learning and memory in mice.

Keyword: *A. cordifolia*, locomotion, grooming, mice, anxiolytic, antidepressant, mice.

INTRODUCTION

Alchornea cordifolia (Schum Thonn) Mull. Ang. (Euphorbiaceae) is a shrub found along the coastal regions of West Africa (Trease and Evans, 1996) and commonly known as Christmas bush. The plant is an

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important crude drug in the indigenous system of medicine for the management of pain, rheumatism, arthritis, pile, toothache, cough, diarrhea and some other inflammatory disease states (Ogungbamila and Samuelsson, 1990; Tona et al., 2000; Neuwinger, 2000). It has been reported that the decoction of the leaves is used as central nervous system stimulant (Defeo, 1992). An extract of the root of *Alchornea floribunda* prepared by macerating the plant material for several days in palm wine is used in Congo as an intoxicant and aphrodisiac (Cousins and Huffman, 2002).

Several different research groups have analyzed the chemical constituents of *A. Cordifolia* leaves and identified tannins, phenolic acids, gallic acid, ellagic acid, protocatechic acid, quercetin, hyperin, guaijavein, terpenes, sterols, carbohydrate, glycosides, saponins triisopent and enylguanidine (Lamikanra et al., 1990; Ogungbamila and Samuelssons, 1990; Ajali, 2000; Banzouzi et al., 2002; Osadebe et al., 2003). Since there are reports showing that the plant is used traditionally as a central nervous system stimulant, and there is no study yet on the general central nervous system (CNS) effects of this plant, we decided to examine the CNS effects of the ethanolic leaf extract of *A. cordifolia* using animal behavioral models: novelty-induced behavioral (Open field) test, hole board and elevated plus maze (anxiolytic/anxiogenic test), Y-maze (learning and memory test) and forced swimming test (antidepressant).

MATERIALS AND METHODS

Plant collection and ethanolic extract preparation

The leaves of *Alchornea cordifolia* were collected in July 2006 on the campus, Obafemi Awolowo University, Ile-Ife, Nigeria. The plant was authenticated by Dr H. I. Illoh, Department of Botany, Obafemi Awolowo University, Ile-Ife and a voucher specimen (Herbarium No FHI 58417) was deposited at the herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The collected plant materials were air dried for 3 weeks and milled using a Christy Milling Machine (Christy and Norris Ltd., Process Engineers, Chelmsford, England to obtain a powder (480 g). The resultant powder of *A. cordifolia* was extracted at room temperature with aqueous ethanol (50%, 4 L) for 48 hours and filtered. The resultant marc was further extracted with aqueous EtOH (50%, 2.4 L) over 48 hours and filtered. The combined filtrate was concentrated *in vacuo* to dryness on a rotary evaporator, yielding a crude extract (63 g, 13.1%) which was stored in the freezer in air tight container until required.Three hundred milligram (300 mg) of ethanolic leaf extract was weighed and dissolved in 5 ml normal saline (vehicle) to obtain a dose of 250 mg/kg administered at maximum volume of 0.5 ml per 30 g body weight. Five hundred milligram (500 mg) of the ethanolic leaf extract was weighed and dissolved in 5 ml normal saline (vehicle) to obtain a dose of 500 mg/kg administered at maximum volume of 0.5 ml per 30 g body weight. Six hundred milligram (600 mg) of the leaf extract was weighed and dissolved in 5 ml normal saline (vehicle) to obtain a dose of 1000 mg/kg administered at maximum volume of 0.5 ml per 30 g body weight. A solution of the plant extract was made fresh at the time of administration with the vehicle (normal saline) and administered at the required dose level in a volume of 0.5 ml/30 g body weight orally.

Animals

Young male and female Albino mice (Vom strain) weighing approximately 17.8 ± 0.6 g were obtained from the Animal house of the Nigerian Institute of Medical Research, Yaba, Lagos. The mice were kept in plastic cages in the Animal house of Pharmacology department, Obafemi Awolowo University, Ile-Ife with an *ad lib* access to both food and

water. After 2 weeks of acclimatization, animals with the females caged separately from the males were subjected to the experiments described below. For each of the experiments (as much as possible) an equal number of males and females were used and each animal was tested only once for each of the tests carried out. All tests were carried out between 8.30 a.m and 3.00 p.m and the experimental protocols were approved by the University Research Committee of Obafemi Awolowo University, Ile-Ife (CODE 11-813-AFF) in accordance with the internationally accepted principles for laboratory animal use and care (EEC Directive of 1986; 86/609/EEC).

Novelty-induced behavior

The novelty-induced behavior was monitored for 20 min in the modified open field method, as previously described (Vale et al., 1999; Eckeli et al., 2000; Gazda et al., 2006). The structure consists of a rectangular arena composed of a hardboard floor (36 x 36 cm^2) with a surrounding wall 30 cm height, both made of white painted wood. The floor is divided by permanent red marker into squares at the bottom. Different doses of leaf extract (250, 500 and 1000 mg/kg) as well as 0.5 ml saline/30 g body weight were orally administered to four groups of both male and female rats respectively. One hour after administration, each mouse was introduced into a cage and total locomotion expressed as the number of floor units entered -the floor of the squares crossed with all paws (crossings was counted in a 20 min session.). Episodes of grooming (the number of body cleaning with paws, picking of the body and pubis with mouth and face-washing actions) and rearing frequency (number of times the animal stand on its hind legs or with its hind limbs with its forearm against the wall of the observation cage or in the free air) were counted at intervals of 10 min time interval during for 20 min period. Before introducing each animal, the arena was cleaned with 5% alcohol to eliminate the possibility of any bias due to the odour that could have been left on the board by previous subjects. All behavioural testing was carried out between 08:00 and 15:00 hours each day.

Hole board test

The animals were placed on a board (40 x 40 cm) with 16 holes (symmetrically distributed in four rows) 10 min after being treated as described above and the number of times that the head was dipped into the hole within a 6 minute period was registered. The results were expressed as mean total number of head dips (Goehler et al., 2008).

Elevated Plus-maze

The apparatus is made of wood and has two narrow enclosed arms which are bordered by high walls and has two open arms which are essentially unprotected boards. Naïve mice have been observed to spend much of their allotted time in the enclosed arms. This preference appears to reflect an aversion to the open arms generated by fear and anxiety induced by height and open spaces (Rodgers and Johnson, 1995; Walf and Frye, 2007). The elevated plus-maze is consisting of two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) emanating from a common central platform (5 x 5) with the two pairs of identical arms being opposite each other. The entire apparatus was elevated to a height of 50 cm above floor level throughout the period of the experiment. At the start of each session, the mouse was placed at the centre of the maze, with its head facing an open arm and allowed to explore the maze for 6 min. During the 6 min test period, the following measurements were recorded: the number of entries and the time spent in open and closed arms, and the exploratory behavior (total number of arm entries). An entry with all four feet put into one arm is defined as an arm entry. In this experiment, four groups of mice

were tested as described above. The plus maze was carefully wiped with a wet towel after each animal. The results are expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms (percentage of time spent in open arms), mean ratio of entries into open arms to total entries into both open and closed arm entries (percentage of number of entries) and number of entries of open arms.

Spatial working memory test (Y-maze model)

The Y-maze is used to measure short-term memory and general locomotory activity. It is composed of three equally spaced arms (120°; 41 cm long × 5 cm wide x 15 cm high) of wood. Each group of animals was tested one hour after the administration of vehicle, and different doses of the ethanolic extract of *A. cordifolia* leaf (250, 500 and 1000 mg/kg, p.o.). After administration of the appropriate dose of ethanol extract of the plant, each mouse was placed in one of the arm compartments and was allowed to move freely for 6 min. An arm entry is defined as the body of a mouse except for its tail completely entering into an arm compartment. The sequence of arm entries was manually recorded. An alternation is defined as an entry into all three arms on consecutive choices. For instance, each arm is labelled A, B, or C and then the sequence of entry into each is recorded: ACBCABCACABCA. In this example, the mice entered 14 arms, eight of which were seen to be alternations. The maximum number of spontaneous alternations was then registered as the total number of arms entered minus 2, and the percent alternation calculated as (actual alternations / maximum alternations) x 100 (Conrad et al., 1997; Hiramatsu et al., 1997; Heo et al., 2003; Mamiya et al., 2004). The apparatus was cleaned with 5% ethyl alcohol and allowed to dry between sessions.

Antidepressant test (Forced Swimming Test)

The FST was carried out in mice individually forced to swim in an open cylindrical container (diameter 12 cm, height 15 cm) containing 7 cm of water at 22 ± 1 ^{o}C; the duration of immobility in a period of 6 min was scored as described previously (Eckeli et al., 2000). Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. At the end of the swimming exposition the animal was removed from the water and dried gently. Time sampling technique was used to score the two types of active behaviour (immobility, Struggling: swimming and climbing) as described by Detke et al. (1995). At the end of each 60 sec period, the following behavioural actions were recorded using a stopwatch. (1) Immobility- a mouse was judged to be immobile when it remained floating on the water; (2) Struggling: (Swimming-when the mouse made active swimming motions more than necessary to merely maintain its head above water; Climbing- a mouse was judged to be climbing when it was making active movements with its forepaws in and out of the water, usually directed against the walls). A 2-min period was allowed for acclimatization and the last 4 min period scoring was recoded for analysis. Four groups (n=5-8) of mice were randomly selected for these behavioural experiments and each mouse was tested only once. Group 1 was taken as control and was administered the vehicle while the other three groups were orally administered with *A. cordifolia* the ethanolic leaf extract at doses of 250, 500 and 1000 mg/kg, respectively. The extract was administered orally 1 hr prior to the test.

Statistical analysis

All behavioural data was analyzed by analysis of variance (ANOVA) [Primer

statistical software by Stanton A. Glantz, Version 3.01, McGraw-Hill INC], and post hoc tests (Student Newman Keul test) were carried out to determine the source of a significant main effect or interaction. The results are expressed as Mean ± SEM, values of $p<0.05$ were considered statistically significant

RESULTS

Novelty-induced behavior

The results obtained in Figures 1a-c are observations of the novelty induced behavior of *A. cordifolia* ethanolic extract in albino mice at twenty minute time intervals. This showed the effect of the extract at different dose levels on locomotion, rearing and grooming in a novel environment. Statistical analysis showed that there a significant [$F (3, 25) = 16.91$, $p< 0.001$] effect on locomotory activity due to administration of the extracts. Post hoc analysis revealed that only the dose of 1000 mg/kg caused this significant decrease effect on locomotion when compared to the control group. The observed effect of *A. cordifolia* ethanolic extract on rearing behavior showed a bi-phasic effect and is statistically significant [$F (3, 25) = 36.89$, $p < 0.001$] when compared to the control group. The single session of 20 minute showed a bi-phasic effect on the grooming behavior that is statistically significant [$F (3, 25) = 7.82$, $p = <0.001$] when compared to the control group.

Hole board

Exploratory behavioral effect of *A. cordifolia* ethanolic leaf extract in mice is summarized in Figure 2. The vehicle and the 250 mg/kg dose level exhibit almost similar mean values. The result showed a bi-phasic effect with the 500 and 1000 mg/kg dose levels. The increased exploratory activity is observed with the 500 mg/kg dose level. This showed a significant [$F (3, 22) = 3.32$, $P <0.05$] effect when compared to the vehicle treated group (control).

Elevated plus Maze

The effects of orally administered doses of *A. cordifolia* ethanolic extract on the exploratory behavior on the elevated plus maze are summarized in Figures 3a, b and c. The extract, at all the doses administered did not change the open arm entries and the time spent in the open arms of the maze significantly (Figure 3a: $F (3, 23) = 0.09$, $P = 0.967$; Figure 3b; $F (3, 23) = 0.50$, $P = 0.685$) when compared with the vehicle values respectively. The open arm entries also revealed no significant effect in mice (Figure 3c).

Spatial working memory

The effects of the orally administered dose of *A. cordifolia* ethanolic leaf extract on learning, memory and locomotion using the Y – maze paradigm is summarized in Figures 4a and 4b respectively. The results obtained showed that *A. cordifolia* extract caused a dose dependent increase and subsequent decrease in spatial memory in mice showing a bi-phasic effect. Although, this difference relative to the vehicle condition is statistically non-significant ($F (3, 25) = 1.14$; $p = 0.356$). In Figure 4b, *A. cordifolia* ethanolic leaf extract caused an increase of locomotors activity at 250 mg/kg dose level, with a further increase in locomotion of 500 mg/kg dose level, however, pre-treatment with a dose of 1000 mg/kg produced a reduction in the locomotors activity.

Forced swimming test

The antidepressant effect of *A. cordifolia* is summarized in Figure 5. The results denote immobility time in seconds. The extract has no significant [$F (3, 25) = 2.52$, $p = 0.085$] antidepressant effect at all doses administered. The change in the immobility time at the different dose levels in relation to the vehicle is statistically non-significant.

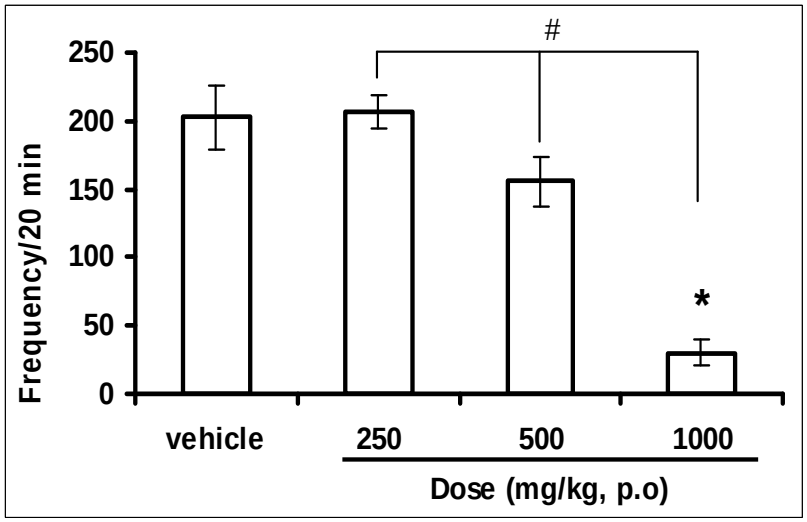

Figure 1a: The effect of ethanolic extract of *A. cordifolia* on locomotory activity in mice in the total session of 20 min. Each column and bar represent Mean ± SEM after an oral administration of either Vehicle or ethanolic extract (250, 500 and 1000 mg/kg (n=5-8); *p<0.01 compared to the control group (Vehicle-treated) and #p<0.01 vs 1000 mg/k group.

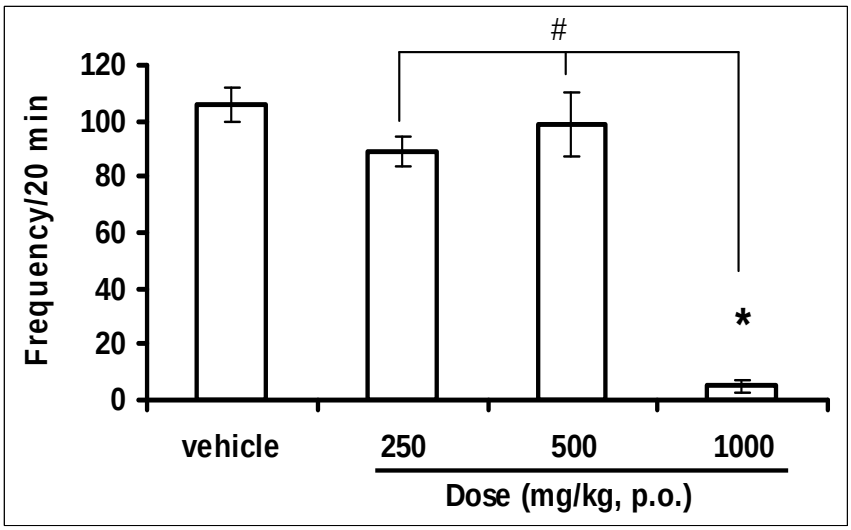

Figure 1b: The effect of ethanolic extract of *A. cordifolia* on rearing behaviour in mice in the total session of 20 min. Each column and bar represent Mean ± SEM after the oral administration of either Vehicle or ethanolic extract (250, 500 and 1000 mg/kg (n=5-8); *p<0.01 compared to the control group (Vehicle-treated) and #p<0.01 vs 1000 mg/k group.

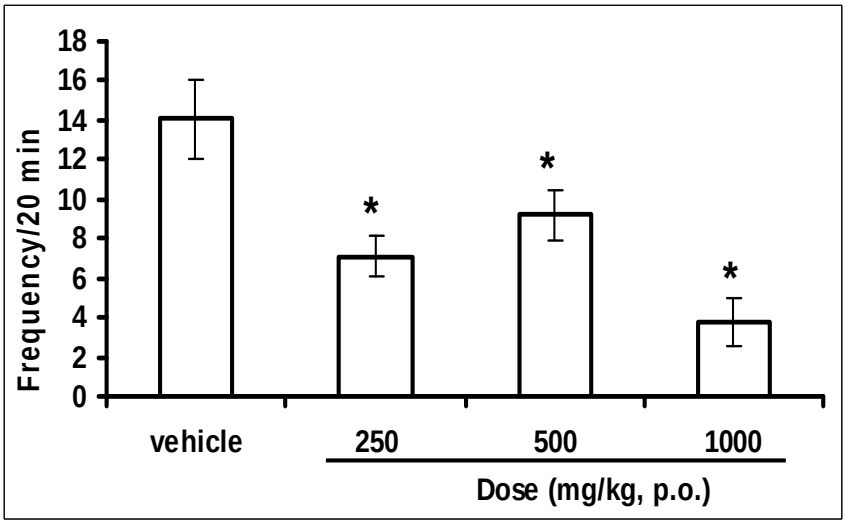

Figure 1c: The effect of ethanolic extract of *A. cordifolia* on grooming behaviour in Mice in the total session of 20 min. Each column and bar represent Mean ± SEM after an oral administration of either Vehicle or ethanolic extract (250, 500 and 1000 mg/kg (n=5-8); *p<0.01 compared to the control group (Vehicle-treated).

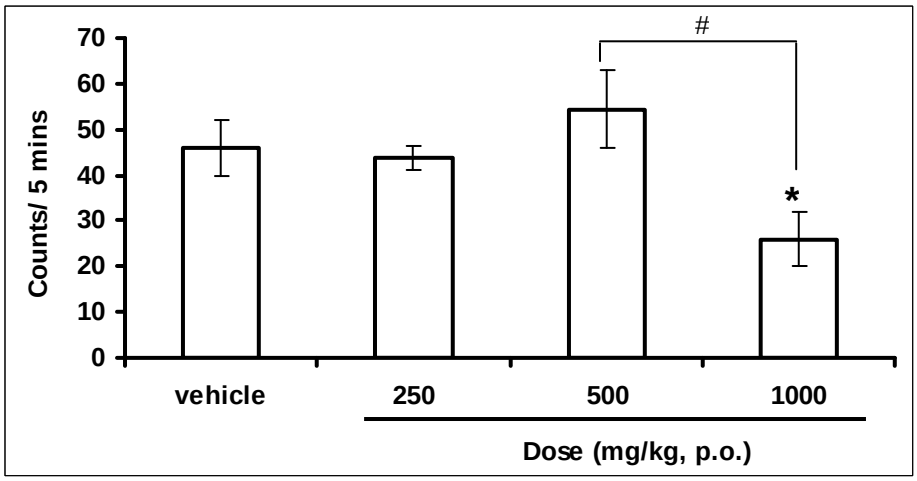

Figure 2: The effect of ethanolic extract of *A. cordifolia* on exploratory behaviour in mice (n=5-8 per group). Each column and bar represents Mean ± SEM after an oral administration of either Vehicle or different doses of ethanolic extract (250, 500 and 1000 mg/kg), *p<0.05 compared to the control group (Vehicle-treated) and #p<0.05 vs. 1000 mg/kg group.

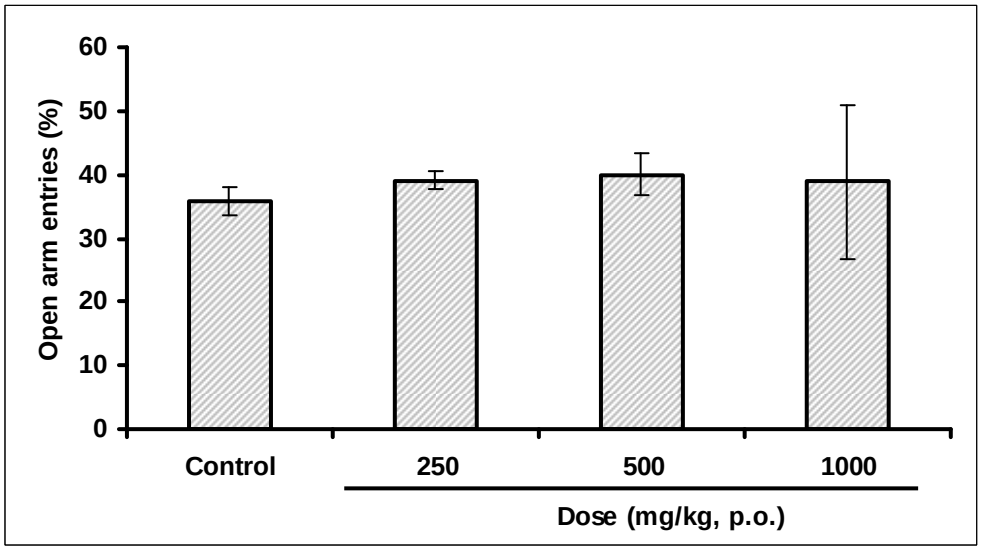

Figure 3a: The effect of ethanolic extract of *A. cordifolia* on exploratory behaviour of mice in elevated plus maze open arms (n=5-7 per group). Each column and bar represent Mean ± SEM of an oral administration of either Vehicle, different doses of ethanolic extract (250, 500 and 1000 mg/kg).

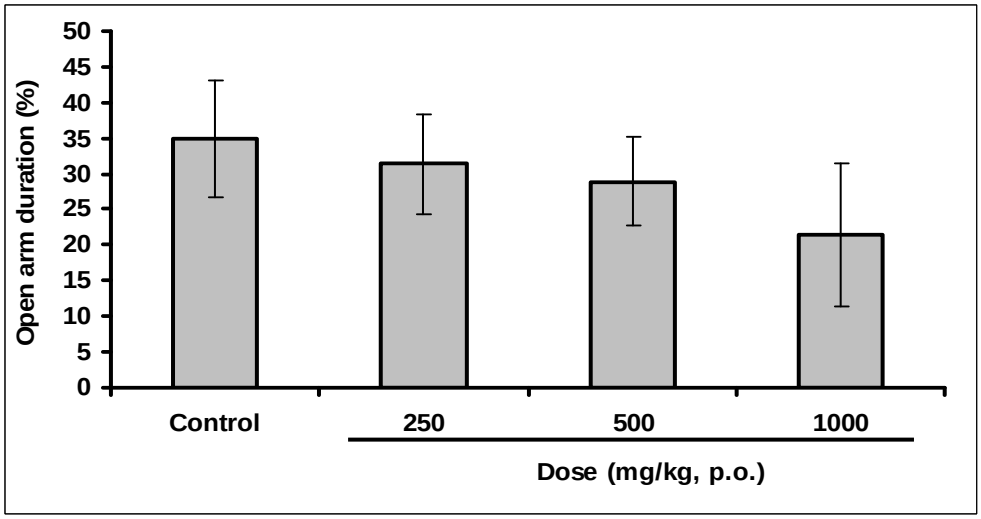

Figure 3b: The effect of ethanolic extract of *A. cordifolia* on exploratory behaviour of mice in elevated plus maze open arms (n=5-7 per group). Each column and bar represent Mean ± SEM of an oral administration of either Vehicle, different doses of ethanolic extract (250, 500 and 1000 mg/kg).

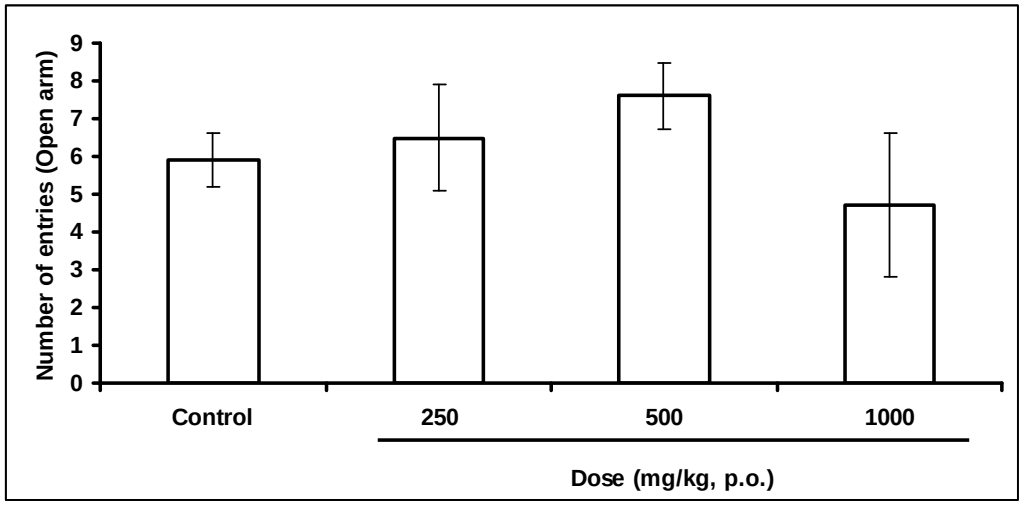

Figure 3c: The effect of ethanolic extract of *A. cordifolia* on exploratory behaviour of mice in elevated plus maze open arms (n=5-7 per group). Each column and bar represent Mean ± SEM of an oral administration of either Vehicle, different doses of ethanolic extract (250, 500 and 1000 mg/kg).

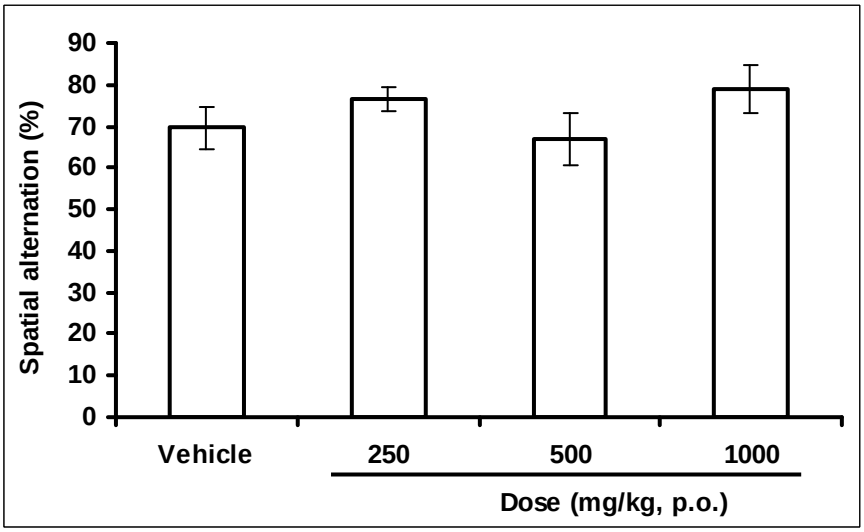

Figure 4a: The effect of ethanolic extract of *A. cordifolia* on learning and memory of mice in Y-Maze (n=5-8). Each column and bar represent Mean ± SEM of an oral administration of either Vehicle, different doses of ethanolic extract (250,500 and 1000 mg/kg).

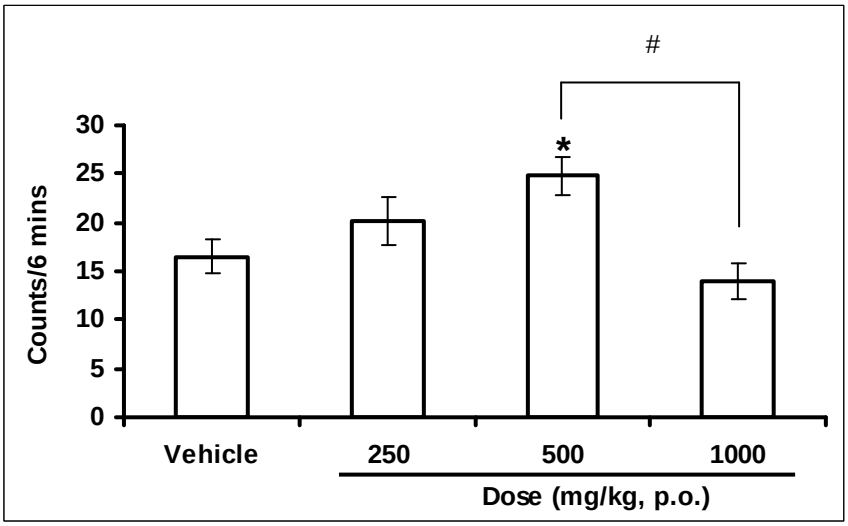

Figure 4b: The effect of ethanolic extract of *A. cordifolia* on locomotory activity of mice in Y-Maze (n=5-8). Each column and bar represent Mean ± SEM of an oral administration of either Vehicle, different doses of ethanolic extract (250, 500 and 1000 mg/kg).

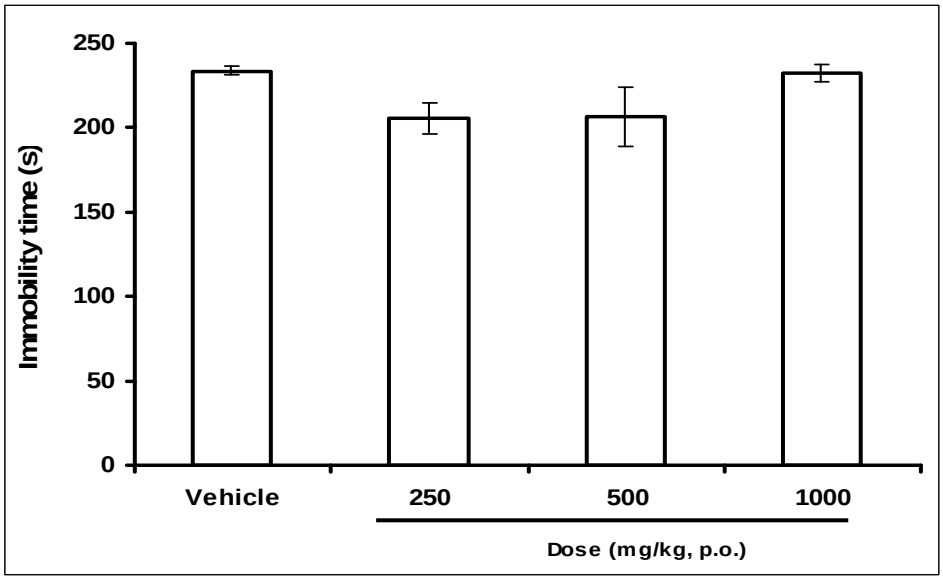

Figure 5: The effect of ethanolic extract of *A. cordifolia* on immobility time in forced swimming test of mice (n=5-8). Each column and bar represent Mean ± SEM of an oral administration of either Vehicle, different doses of ethanolic extract (250, 500 and 1000 mg/kg).

DISCUSSION

In the present study various tests were carried out to evaluate the central nervous system effects of the ethanolic leaf extract of *A. cordifolia* using novelty-induced behavioral (Open field) test, anxiolytic/anxiogenic test models (Hole board and EPM), learning and memory test (Y-maze) and antidepressant test (FST). The results obtained after the oral administration of 250, 500 and 1000 mg/kg doses in the open field test showed a significant difference between the test doses and the vehicle on locomotory activity. There was a significant ($p<0.05$) decrease in locomotory activity at the dose of 1000 mg/kg. Similarly, the ethanolic extract of *A. cordifolia* caused a significant decrease effect at the dose of 1000 mg/kg when compared with the vehicle–treated group. With reference to the results obtained on locomotion and rearing it is clear that the ethanolic extract of *A. cordifolia* (ACE) has a significant effect centrally at the highest dose administered especially on exploratory behavior. In rodents, both locomotion and rearing behavior are considered excitatory behavior. Furthermore, it has been reported that open field behaviour is thought to reflect emotional reactivity and exploratory behavior (Mathangi and Namsivayam, 2000; Gould et al., 2010). Grooming behavior is known to generally increase with fear or anxiety in rodents and is an index of behavioral adaptation to a stressful situation (Shaw et al., 2007). Grooming has also been primarily regarded as a behaviour, the purpose of which is care of the body surface in animals. However, mild stress such as exposure to a novel environment is known to induce grooming behaviour and central dopaminergic activation has been reported to be involved in grooming behaviour via D_1 receptor (Scalzitti et al., 1999). During the total 20 minute session, grooming showed a significant decrease effect. This showed that the plant ethanolic extract has central inhibitory activity. Thus, it can be inferred from the results that the ethanolic extract of *A. cordifolia* leaf is suggested to induce an appreciable level of central inhibitory activity that is dose dependent as revealed from its effect on locomotion, rearing and grooming behavior in mice.

The essential property of anxiolytic agents is the alleviation of anxiety. Anxiolytic agents are used for the alleviation of relatively minor disorders of both non-psychotic and neurotic origin. Pathological anxiety in man has been defined by its interference with normal functions, by the manifestation of somatic disorders, emotional discomfort, and interference with productivity at work. Present study was conducted to confirm and extend the anxiolytic-like effects of *A. cordifolia* in animal models using the holeboard and elevated plus maze methods. Anxiolytics are known to increase open arms exploration in the EPM (Walf and Frye, 2007). In the holeboard, the results obtained from this experiment showed that there was significant decrease in the frequency of head dips resulting in decrease in exploratory behavior suggesting possible anxiogenic effect at the dose of 1000 mg/kg when compared with the vehicle-treated group. In the EPM, the present study showed that there was no change in the percentage in time spent and percentage of number of entries in the open arms of the maze with the administration of different doses of the extracts in relation to the control group. From the results obtained from holeboard and EPM, it could be said that the plant possesses neither anxiolytic nor anxiogenic effects in mice.

It is well known that that spontaneous alternation is a measure of spatial working memory and to alternate among spatial locations, a rodent (rat or mouse) must remember its previous location. The Y-maze model is used to measure short-term memory, general locomotory activity and stereotypic behaviour. Therefore, spontaneous alternation performance was assessed using a Y-maze. The results from the learning and memory test using the Y-maze showed an increase in the spatial working memory at 250 and 1000 mg/kg when compared with the control group while the 500 mg/kg dose level showed a

decrease effect on spatial working memory. However, analysis showed that this observation was not statistically significant. Thus, it can be inferred that the extract has no significant effect on short-term memory in mice. The locomotory activity in Y-maze showed a significant difference when the test groups are compared with the control group at 500 mg/kg dose level while the highest dose of 1000 mg/kg showed a significant decrease in locomotory activity when compared with the dose of 500 mg/kg. This observation is similar to what was obtained in open field test.

Depression is a collection of psychological symptoms, which includes depressive thoughts, poor self-image, self-blame, feeling of hopelessness. The forced swimming test is widely used to screen for antidepressant activity in mice. During the tests, the mice exhibited some movements indicative of struggle and antidepression (i.e. swimming, struggling and climbing), while some movements were indicative of hopelessness or depression (i.e. floating or not struggling). The forced swimming test (FST) is a behavioural test widely used to screen antidepressant candidates in rats and mice (Petit-Demouliere et al., 2005). This test is known to be very sensitive and specific to all major classes of antidepressant drugs and therefore, the behavioural changes in FST were used as parameters for evaluating antidepressant activity of ethanolic extract of *A. cordifolia* leaf in mice. The results obtained in FST showed that the immobility time did not differ significantly between the test groups and the control group. Thus, it could be inferred that the extract did not exhibit any signs of antidepressant activity at all the three dose levels administered.

In conclusion, the present study showed that the ethanolic leaf extract of *A. cordifolia* possesses central inhibitory effect in mice; however, it is devoid of anxiolytic, antidepressant activities as well as effect on learning and memory.

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