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Neuromuscular behaviour of wister rats administered methanol extract of *Ximenia americana*

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ABSTRACT

The study investigated the effects of different doses of methanol extract of leaf, stem bark and root of Ximenia americana (X .a) plant on neuromuscular behavior of Wister rats. 120 rats were divided into four groups (control, leaf, stem bark and root) consisting of 30 rats each. Each group was further divided into three sub-groups consisting of 10 rats per sub-group. Each rat was administered orally methanol extracts of either leaf, stem bark or root of X .a at doses of 10, 100 or 1000 mg/kg body weight, respectively, every two days for two weeks of the study period. The control group was given equivalent of 0.2 ml of sterile water per rat. The result of neuromuscular behaviour recorded after the administration of the extracts showed that X .a had an inhibitory effect on locomotor activity and excitability of the rats. The inhibitory effect of the extract was dose dependant and the effect increased significantly ($P<0.05$) with the duration of the study. The methanol extract from the root of X .a had the highest ($P<0.05$) inhibitory effect, compared with the leaf or stem bark. The inhibitory effect of the extract was due to the sedative, spasmolytic and pro-oxidant effects of some substances like tannins, flavonoids, saponnins, anthraquinones, alkaloids and terpenoids found in the methanol extract of X .a. In conclusion, the inhibitory effect of the methanol extract on neuromuscular behaviour suggested that it should be used with great caution in animals and humans, especially in individuals engaged with various machine operations.

Keywords: Excitability, extract, locomotor, neuromuscular behaviour, rat, *Ximena americana*

INTRODUCTION

The increasing interest in the use and study of medicinal plants and their traditional use has widen world wide due to increase in poverty, ignorance, unavailability of modern health

facilities and less communication means [7, 12]. According to WHO, well over 80% of the world's population depends on traditional medicine for their primary health care needs [7].

In spite of the huge benefit driven over the years from the use of these medicinal plants, substantial research have shown the risk involved in the application of some of these plants due to lack of proper dosing, method of preparation and duration of usage [11]. Therefore, scientific evaluation of these medicinal plants is important to the discovery of not only new drugs but also to assess toxicity associated with the use of these herbal preparations.

The plant, *Ximenia americana (X. a)*, belongs to the Family *Olacaceae*. Its habitat is the Sudanese to Guinean Savannahs and cleared forest undergrowth, on gritty soils, bare ground or clay soil near ponds, and distributed from Senegal to Cameroon. It is a bushy and spiny shrub or small tree, 4-5m high with open crown. The fruits are green but turn golden yellow or red when ripe. The fruit when eaten is refreshing and has an acid taste [3]. The plant is used traditionally for the treatment of malaria, *Trypanosoma congolense* infection in mice, leprotic ulcer and skin infections [3, 12]. The plant has anti-inflammatory action and it is believed to have antineoplastic and antimicrobial activity [13]. Considering the ethnomedicinal uses and importance of this plant, the objective of the present study was to study the effect of the methanol extracts of the leaf, stem bark and root of *X .a* on the locomotor activity and tissue excitability in rats, so as to assess its safety or otherwise in humans.

MATERIALS AND METHODS

Collecting plant materials

Fresh parts of *X .a* were collected at Mile Uku village, near the Nigerian Defence Academy, Mando Kaduna State. Taxonomy of the species was determined at the Herbarium of the department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The leaves stem bark and roots of the plant were collected and dried at room temperature in the laboratory for a period of 2 weeks.

Preparation, extraction and phytochemical screening

Extraction from the ground leaves, stem bark and roots of the plant was carried out using a Soxhlet extractor. Methanol was used as the extractant. The method of Trease and Evans [18] were employed to test for the presence of tannins, phlobatannins and alkaloids. The method of Harbone [10] was used to test for the presence of steroids, saponins, glycosides and flavonoids. Terpenoids were tested using the method of Sofowora [16].

Experimental animals and the administration of extract of *Ximenia americana*

A total of one hundred and twenty (120) Wistar rats of both sexes weighing between 260 - 270.6g used for the study were purchased from the National Institute for Trypanosomiasis Research, Kaduna, Nigeria. They were maintained in clean rat cages in a 12 h light/dark cycle with litter changed every week. They were fed pelleted commercial rat feed (ECWA, Nigeria PLc, Jos, Nigeria), and were watered *ad libitum*. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO [19]. The animal laboratory care of CCAC [6] was strictly followed.

The 120 rats were divided randomly into 4 groups of 30 rats each, after the first weighing. On experimental day the rats were divided into four groups (leaf, stem bark, root and control groups) consisting of 30 rats each. Each group was further sub-divided into three comprising of 10 rats per sub-group and each sub-group was administered orally methanol extracts of root, leaf and stem-bark of *X .a*, all reconstituted with sterile water, at a dose of 10, 100 or 1000 mg/Kg body weight, respectively, on day 1, 3, 5, 7, 9, 11, 13 and 15 of the study period. The control group was given equivalent of 0.2 ml of sterile water orally. The motor activity and excitability of the rats were observed in all the groups before the study period (day 0 before the administration of extract) and subsequently on day 3, 5, 7, 9, 11, 13 and 15 between 8 – 9 am of the study period.

Evaluation of locomotor activity

The locomotor activities of the rats were assessed using the open-field apparatus as earlier described [1, 5, 9]. The apparatus was made up of a cardboard box (50 x 50 x 46 cm high) with clear plexiglass on the inner surface. The floor of the box was divided into 25 equal squares. The motor activity was assessed by placing a rat in the box and then allowing it to walk freely for 3 minutes for the rat to be accustomed with the environment. Thereafter, the number of squares crossed by the rats with all it paws and the direction of movement during the next two minutes was recorded after which the rat was removed from the box. The box was cleaned between trials with soapy water followed by 90% alcohol solution to remove the interfering odours of the previous rat [2, 9].

Assessment of excitability scores

The excitability scores of the rats were assessed as described by Ayo *et al*. [4] and Ambali [2] with little modification. Each rat was held by the tail upside down and was kept in such position for 30 seconds. The degree of wriggling and forepaw movement was graded and scored as follows:

Score 1- Rat did not show any sign of wriggling or paw movement.
Score 2- Rat responded through gentle wriggling and movement of forepaw.
Score 3- Rat responded through a stronger wriggling and movement of forepaw
Score 4- Rat responded by vigorous wriggling, strong movement of fore- and hind-limbs and successfully climbed the tip of its tail.

Statistical analysis

The results obtained were expressed as mean ± SEM. The significance different between groups were analysed using ANOVA, followed by post hoc analysis using the Student *t*-test.

RESULTS

Pyhtochemical screening

The result of phytochemical analysis revealed that the methanol leaf, stem bark and root extracts of *X .a* had very high contents of tannin, saponins, terpenoids/isoprenoids, while flavonoids, anthroquinones and alkanoids were moderately present.

Effect of extract of *Ximenia americana* on motor (locomotor) behavior of rats.

The pre-experimental result of locomotor activities recorded in all the groups of rats showed that each rat was able to cross about 28.6 square boxes per 2 min. There was no significant difference between the groups and sex of the rats.

Figure 1 showed the effect of different doses and duration of administration of the leaf extract of *X .a* on rats. The result showed that the locomotor activity of the rats were dependent on the dosage and duration of administration of the extract. Generally, rats administered with extracts had less motor activity compared with the control group. Cumulatively, rats administered 1000 mg/Kg body weight of the leaf extract had the least locomotor activity (12.5 ± 2.1 square boxes per 2 min) compared with those administered with 10 mg (20.5 ± 2.5 square boxes per 2 min) and 100mg (17.8 ± 1.9 square boxes per 2 min).

The results of locomotor activities obtained using stem bark (Figure 2) and root (Figure 3) extracts of *X .a* followed a similar pattern of change as those obtained using leaf extract.

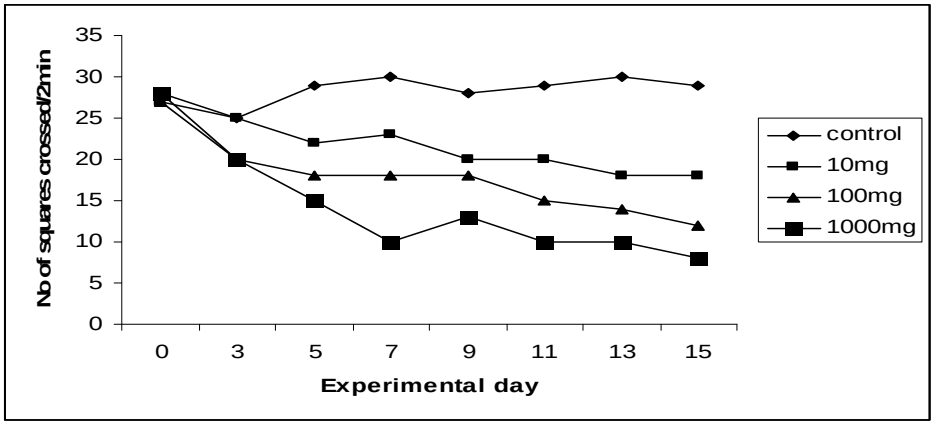

Figure 1: Effects of different doses and duration of administration of methanol leaf extract *Ximenia americana* on locomotor behaviour of rats

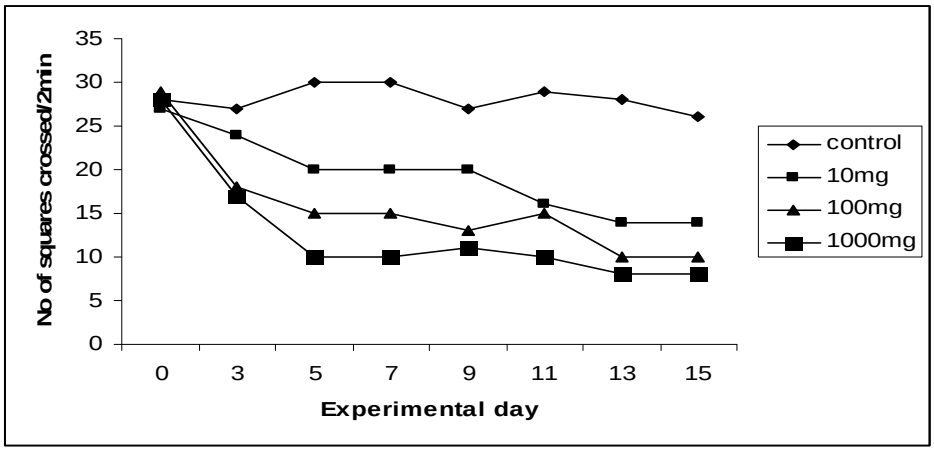

Figure 2: Effects of different doses and duration of administration of methanol stem bark extract *Ximenia americana* on locomotor behaviour of rats

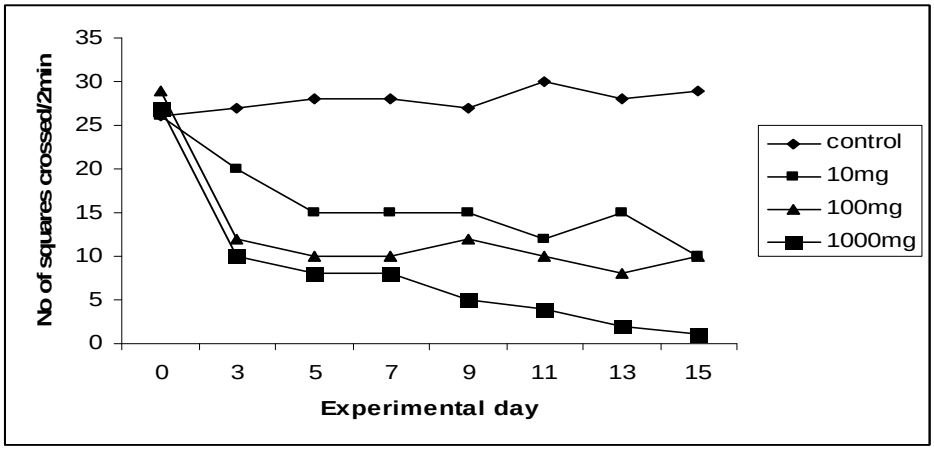

Figure 3: Effects of different doses and duration of administration of methanol root extract *Ximenia americana* on locomotor behaviour of rats

Cumulatively, the locomotor activity recorded in all the groups of rats during the study period was significantly ($p<0.05$) affected by the type of extract and duration of administration throughout the study period (Figure 4).

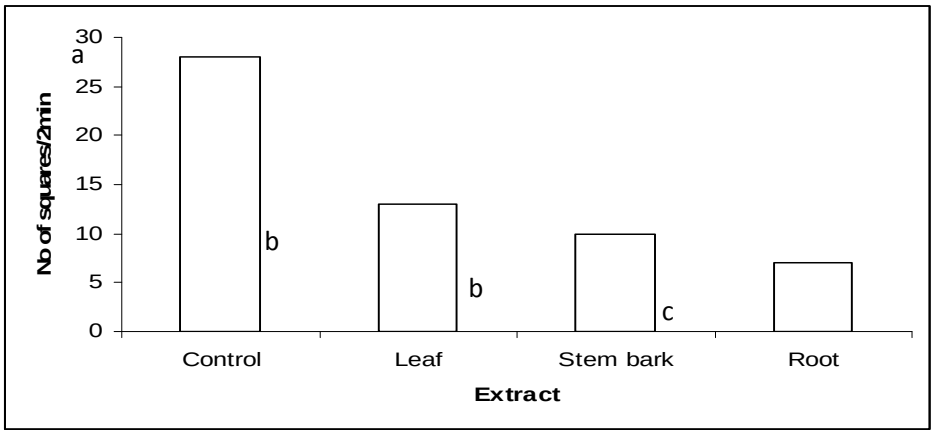

Figure 4: Cumulative effects of methanol extract of *Ximenia americana* on locomotor behaviour of rats

The direction of movement of the rat before the administration of the extract, and in the control and in rats administered with 10 mg/Kg body weight of the extract was in contact and along the walls of the box in a straight line, either clockwise or anticlockwise directions (Figure 5a). In rats administered 100 and 1000 mg/Kg body weight of the methanol leaf, stem bark and root extracts of *X .a*, the direction of movement was affected. Majority (89%) of the rats administered the extract lost the pattern of directional movement; the rats walked sluggishly to the center of the box and returned bark or moved to another direction, or did not walk at all (Figure 5b).

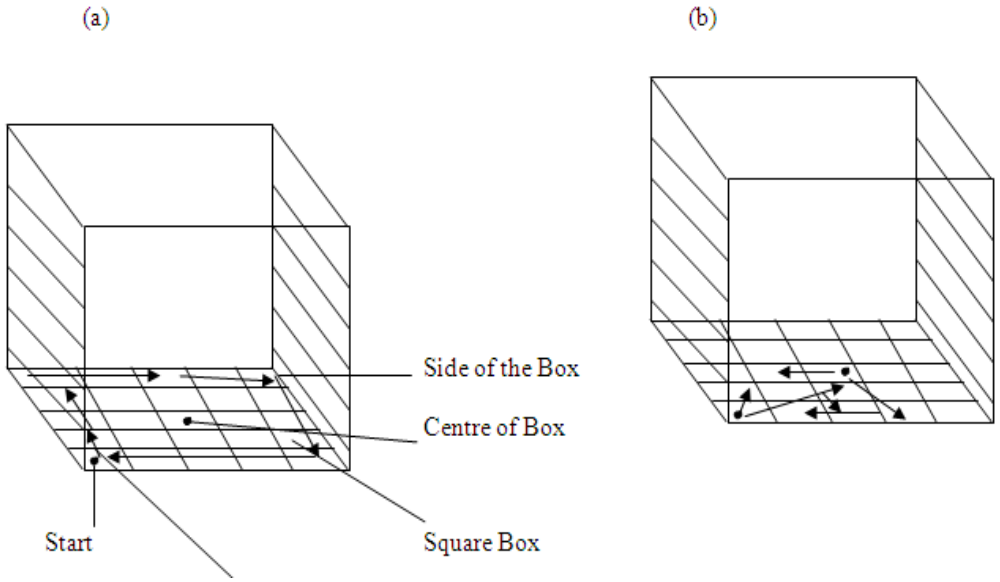

Figure 5: (a) Normal direction of movement of control rats administered sterile water and (b) incoordinated direction of movement of rats administered with methanol extract of *Ximenia americana*

Effect of methanol extract of *Ximenia americana* on excitability score of rats.
The excitability scores recorded in rats before the administered of the extract showed that 80% of the rats had excitability score of 4, while 20% had score of 3. None of the rats had score of 1 or 2 before the experiment and administration of the extract.

Tables 1 and 2 depict the effect of leaf, stem bark and root extracts and dosage on excitability scores and percentage excitability of the rats, respectively, during the study period. The results showed that 80% of all the control rats had excitability score of 4, while 20% had score of 3.

However, the excitability scores of all the groups of rats decreased ($P<0.05$) with increase in the dosage of the extracts and the duration of the study.

In all the period of the study, rats administered with the root extract had the least ($P<0.05$) excitability score (scores 1 and 2) compared with those of the control, leaf or stem bark extract groups. The cumulative effect of the methanol extract on excitability showed that rats administered with 1000 mg/Kg body weight of the extract had the lowest ($P<0.05$) excitability scores.

Table 1: Effects of methanol extract and duration of administration of *Ximenia americana* on excitability scores of rats.

Mean values with different superscript alphabets are significantly different at p<0.05.

	Experimental days							
Extract/dose	3	5	7	9	11	13	15	Mean ± SEM
Control	3	4	4	3	4	5	4	3.7±0.4[a]
Leaf (mg)								
10	4	4	3	4	3	2	2	3.1±0.2[a]
100	4	3	3	3	2	2	2	2.7±0.1[b]
1000	3	3	2	2	1	1	1	1.8±0.5[c]
Stem (mg)								
10	4	4	3	3	2	3	2	3.0±0.5[a]
100	3	3	3	2	2	1	1	2.1±0.5[b]
1000	3	2	2	2	2	1	1	1.8±0.6[c]
Root (mg)								
10	3	3	3	2	2	2	2	2.4±0.1[b]
100	3	3	1	1	1	1	1	1.6±0.5[c]
1000	2	1	1	1	1	1	1	1.1±0.1[d]

Table 2: Cumulative percent excitability scores of rats administered methanol extract of *Ximenia americana*

	Excitability scores			
Extract	1	2	3	4
Control	0	0	20	80
Leaf	40	50	10	0
Stem bark	45	45	10	0
Root	80	20	0	0

DISCUSSION

Plant extracts are some of the most attractive sources of new drugs, and have been shown to produce promising results for treatment of many ailments in humans and animals. The safety usage of most of these plants extracts has generated much controversy across the world due to lack of proper dosage, duration of administration and side effects. Few studies on the acute toxicity, antibacterial and antiparasitic activities of *X .a* showed that the aqueous extracts of stem bark at a dose of 1600 mg/kg body weight was not toxic based on the hematological and histopathological results [12]. However, the result of our study suggested that the methanol extract from the leaf, stem bark and root of *X .a* from a dose of 100 - 1000 mg/Kg body weight has significantly reduced the locomotor activities of the rats as adjudged by the number of squares boxes crossed per two minutes and the percent excitability scores. Similarly, the extract affected all the normal pattern of movements characterized by incoordination due to errors in the rate, range, force and direction of movement. This clearly demonstrated that the cerebellar hemisphere was affected by the extracts. Cerebellar hemisphere is known to interact with the motor cortex in planning and programming movements. Locomotor activity has been used to represent a broad class of sensory, motor and integrative processes [1, 2]. Rang *et al.*, [15] reported that locomotor activity is mediated through dopamine and other neurochemical

pathways. In addition, depressants of the central nervous system (CNS) are known to inhibit locomotor activity of animals [9]. Furthermore, inhibition of acetylcholinesterase (AchE), CNS activation of cholinergic motor inhibitory system and damage to the peripheral muscle due to necrosis of skeletal muscle fibres has been reported to reduce locomotor activity in animals [9].

The result of motor activity obtained in the present study suggests that the methanol extract had some CNS depressant activities and may inhibit AchE. This is true because phytochemical result of the extract of *X .a* showed that the plant contained high level of tannins, saponins, terpenoids/isoprenoid, while alkaloids, flavonoids, and anthraquinones are moderately present. Some of these substances like the tannins and flavonoids are known to induce spasmolytic activity. Besides, both tannins and flavonoids inhibit calcium channels leading to muscular relaxation [9, 14]. In addition, saponins and alkaloids have been reported to have potent sedative activities and anxiolytic action [14, 17].

In spite of some positive usage of the extract of *X .a* in the treatment of animal or human ailments [12, 13], the present study demonstrated that it usage at a dose of 100 – 1000 mg/Kg body weight for a week or two as popularly practiced traditionally may result into a loss in locomotor activity, excitability and uncoordinated movement in humans.

The fact that some of the phytochemical substances of the extracts like isoprenoid, saponnins and anthraquinones are pro oxidants suggests that their effects on the organism may lead to the generation of free radicals (FR) or reactive oxygen species (ROS), which causes lipid peroxidation, neuromuscular inhibition, necrosis of cell membranes and hemolysis of RBC and WBC [8]. Thus, ingestion of the extract for one to two weeks may result into tissue damage and reduced muscular activity.

The results of excitability score showed that the extract progressively decreases tissue excitability of the rats in a dose dependent manner. Excitability scores reflect the state of alertness of the animal showing the state of sensorimotor reflex and neuromuscular coordination of animals [4]. Therefore, the low excitability scores and percent excitability scores obtained in rats administered with methanol extract, especially, 1000 mg/Kg body weight of the root extract suggested that the extract caused a deficit in sensorimotor reflex and inability of the rats to maintain neuromuscular coordination which may be due to impaired neuronal activity in certain region of the brains particularly the cerebral cortex.

In general, the effect of the extract of *X .a* on locomotor activity and excitability scores of rats obtained in the present study suggests for the first time that the extracts of *X .a* may impair neuromuscular activity in animals and possibly humans, as such the extract should be used with caution, especially in individuals engaged in various machine operations.

It is concluded that methanol leaf, stem bark or root extracts of *X .a* inhibits neuromuscular behavior at doses 100-1000 mg/Kg body weight and that the root extract had the most deleterious effect on locomotor and excitability of rats.

More studies should be channeled towards the identification of a single or combination of active substance(s) and the proximate mechanism involved by the extract of *X .a* in inhibition of neuromuscular activity.

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