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Effects of methanol extract of *Ximenia americana* on sexual behaviour, testicular weight, sperm count and sperm morphology of wister rats

Adeiza A. Abubakar and Minka N. Salka

College of Agriculture and Animal Science, P.M.B. 2134., Division of Agricultural Colleges, Ahmadu Bello University, Mando-Kaduna, Nigeria

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ABSTRACT

The effects of different doses of methanol extracts of leaf, stem bark and root of Ximenia americana (X .a) plant on male reproductive system was investigated in wister male rats. A total of 120 rats were divided into groups (G) 1, 2, 3 and 4 and each group consists of 30 rats. Each group was further divided into three sub-groups consisting of 10 rats per-subgroup. G1, G2 and G3 served as experimental and were administered orally with methanol extracts of leaf, stem bark and root of X .a, respectively at doses of 10, 100 or 1000 mg/kg body weight of each extract, every two days for two weeks of the study period. G4 rats served as control and were given equivalent of 0.2 ml of sterile water per rat. The result showed that the extracts of X.a significantly (P<0.05) decreased the testicular weight, sperm count, sexual behaviour of mounting, sniffing and contact period of a male to a female rat. Furthermore, the extracts increased (P<0.05) incidence of sperm head and tail abnormalities compared to the control. The effect of the extracts was dose and time dependant and the root extract had the highest (P<0.05) deleterious effect. The result suggest that the pro-oxidant activity and inhibitory effect of the extract's flavonoids, saponnins, anthraquinones, alkaloids and terpenoid on neuromuscular tissues may have damage sperm cells, and block olfactory sensitivity, which may have resulted to a "perceptual block" of pheromonal stimuli.

Keywords: Extract, sexual activity, sperm, rat, *Ximenia americana*.

INTRODUCTION

The discovery of healing potential of many plants have been on the increase over the last decade due to increase in poverty, displacement of large populations due to wars or natural disaster,

new emerging diseases, unavailability of modern health facilities and less communication means [5, 15, 17]. Similarly, there is an increasing interest on traditional medicine by many researchers in the developed world in an attempt to discover more potential drugs to combat emerging new diseases or diseases that developed resistances to conventional drugs [5, 17].

In rural tropical countries medicinal plants are indiscriminately used with no proper dosing or method of preparation and preservations [11]. Thus, evaluation of the toxicity, efficacy and proximate mechanism of action of these medicinal plants is of paramount important.

Ximenia americana (X. a) is a tropical plant and it belongs to the Family *Olacaceae*. It is commonly found in the Sudanese to Guinean Savannahs zones. The plant 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 is popularly consumed when it turns yellow and it has a refreshing and acid taste [3]. Traditionally, the extract of the plant is used in the treatment of diseases like skin infections, ulcer, leprosy, malaria and *Trypanosoma congolense* infection in mice [3, 12]. The plant has anti-inflammatory action and it is believed to have antineoplastic and antimicrobial activity [14].

Inspite of the huge acclaimed benefit of *X.a* plant, especially it juicy fruit, there are few studies on the toxicity or otherwise of the plant. It was earlier reported that the plant has a negative effect on neuromuscular behaviour [1]. The aim of the present study was to evaluate the effects of the methanol extracts of the leaf, stem bark and root of *X .a* on the reproductive system of male wister rats.

MATERIALS AND METHODS

Collecting plant materials

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

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 [16] were employed to test for the presence of tannins, phlobatannins and alkaloids. The method of Harbone [8] was used to test for the presence of steroids, saponins, glycosides and flavonoids. Terpenoids were tested using the method of Sofowora [15].

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

A total of one hundred and twenty (120) Wistar male rats and 20 female rats weighing between 260 - 270.6g were used for the study. The rats 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*. The male rats were kept at

different location from the females. The female rats were only used as a probe for measurement of sexual behaviour of males. A standard protocol was observed in accordance with the Good Laboratory Practice (GLP) Regulations of the WHO [17]. The animal laboratory care of CCAC [4] was strictly followed.

The 120 male rats were divided randomly into 4 groups (G1, G2, G3 and G4) of 30 rats each, after the first weighing. On experimental day G1, G2 and G3 were allocated leaf, stem bark and root extracts of *X.a*, respectively, while G4 served as control group. 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 leaf, stem-bark and root 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 (G4) was given equivalent of 0.2 ml of sterile water orally.

The sexual behaviour of the rats was observed in all the groups on day 7 and 15 of the study period, while testicular weight and sperm count and morphology were analyzed on the last day (15) of the study period.

Sexual activity

The sexual activity of mounting, sniffing and contact of male to female was recorded in a cardboard box (50 x 50 x 46 cm high) box. A male rat is brought into a box that is housing a female rat. The number of times the male rat mounts or sniffs the female was recorded within 5 minutes. At the same period the time taken for the male to be in physical body contact with the female within the 5 minutes period was also recorded. The box was cleaned between measurements with soapy water followed by 90% alcohol solution to remove the interfering odours of the previous rats [2, 7, 10].

Testicular weight, sperm count and sperm morphology

On the last day of the study the epididymis of the male rats were separated from the testis after a careful laparatomy. The testis were cleaned of all accessory tissue debris and then weighed.

Filtrate of the right epididymis was obtained by mincing the epididymis in 1ml of phosphate buffered saline, pH 7.4. 0.5ml of the aliquot was diluted 1:200. Neubaeur counting chamber (Hawksley, England) and microscope (HM-Lux, Germany) at a magnification of x40 were employed for counting the sperms. Classifications of normal and abnormal sperm, sperm morphology were done as described by [9, 17]. Sperm morphology test was done from a smear of the epididymal filtrate prepared on a clean glass slides by addition of a drop of 1% eosin. The glass slides were dried and observation done under a microscope using x 40 objective lens and abnormalities of either head or tail were noted. For each rat 200 sperms were screen [9, 17].

Statistical analysis

The results obtained were analyzed using the Student's *t*-test and expressed as mean ± SEM. The significance different between groups were analyzed using ANOVA and Tukey's test was used to test the interaction of sexual behaviour between sampling periods. $P < 0.05$ was considered significant.

RESULTS

Table 1 showed that 100-1000mg/kg body weight of extracts of *X.a* reduced (P<0.05) the numbers and time the male rats spent mounting, sniffing and in contact with a female partner compared with the control (G4) rats. The effect was dose and time dependant.

Table 1: Effects of extract of *Ximenia americana* on sexual behaviour of male rats on day 7 and 15 of the experimental period

(Mean ±SEM, n = 10 in each sub-group).

	Number of mounting/5min		Number of sniffing/5min		Contact period/5min	
Group/dose	Day 7	Day 15	Day 7	Day 15	Day 7	Day 15
G4 (control)	7.5±1.2^{a}	8.9±0.5^{a}	10.2±1.1^{a}	9.2±1.5^{a}	4.5±0.7^{a}	4.3±0.5^{a}
G1 (leaf, mg)						
10	5.5±0.5^{ax}	2.4±0.1^{by}	4.2±0.3^{bx}	2.5±0.2^{by}	2.5±0.2^{bx}	2.0±0.2^{bx}
100	2.1±0.5^{bx}	1.2±0.2^{cy}	1.1±0.2^{cx}	0.8±0.1^{cx}	2.2±0.2^{bx}	0.7±0.1^{cy}
1000	0.10±0.00^{c}	0.10±0.00^{d}	1.4±0.4^{cx}	1.5±0.1^{cx}	0.10±0.00^{c}	0.10±0.00^{d}
G2 (stem, mg)						
10	5.3±0.5^{ax}	3.4±0.2^{by}	5.2±0.9^{bx}	3.0±0.5^{by}	2.1±0.5^{bx}	2.2±0.5^{bx}
100	2.0±0.5^{bx}	1.4±0.1^{cy}	2.1±0.2^{cx}	1.8±0.1^{cx}	1.2±0.4^{dx}	1.0±0.1^{cx}
1000	0.01±0.00^{c}	0.10±0.00^{d}	0.8±0.01^{dx}	0.5±0.01^{dx}	0.01±0.00^{c}	0.10±0.01^{d}
G3 (root, mg)						
10	4.2±0.2^{ax}	2.1±0.1^{by}	5.5±0.5^{bx}	2.0±0.1^{by}	1.1±0.01^{dx}	0.8±0.02^{cx}
100	2.2±0.1^{bx}	1.1±0.1^{cy}	0.5±0.1^{dx}	0.00±0.00^{dx}	0.5±0.01^{cx}	0.00±0.00^{dx}
1000	0.10±0.00^{c}	0.01±0.00^{d}	0.01±0.01^{d}	0.01±0.00^{d}	0.10±0.00^{c}	0.01±0.01^{d}

^{ab} = Values with different superscript alphabets under the same heading along the same column are significant different at P<0.05; ^{xy} = under the same heading along the same row are significantly different at P<0.05.

Table 2: Effect of methanol extract of *Ximenia americana* on testicular weight (g) and sperm count (x10^{6}) in rats

(Mean ±SEM, n = 10 in each sub-group).

Group/dose	Testicular weight (g)	Sperm count (x10^{6})
G4 (control)	1.456±0.06^{a}	840.7±40.5^{a}
G1 (leaf, mg)		
10	1.400± 0.01^{a}	812.4±34.5^{a}
100	1.105±0.04^{b}	410.7±40.1^{b}
1000	0.857± 0.04^{c}	50.8±12.6^{c}
G2 (stem, mg)		
10	1.108±0.08^{b}	720.4±45.2^{a}
100	0.987±0.03^{b}	210.3±27.4^{d}
1000	0.764±0.01^{c}	10.9±5.1^{e}
G3 (root, mg)		
10	0.845±0.07^{c}	400.5±12.8^{b}
100	0.745±0.05^{c}	40.7±3.7^{c}
1000	0.564±0.01^{d}	5.3±1.4^{e}

Values with different superscript alphabets under the same heading along the same column are significant at P<0.05.

Table 2 showed the effect of extracts of *X.a* on testicular weight and sperm count. Rats administered with 1000mg/kg body weight of extract of *X.a* had the highest (P<0.05) testicular weight loss and lowest (P<0.05) sperm count. The effect was significantly (P<0.05) higher in the

rats administered with the root extract (G3) compared to those administered with leaf (G1) or stem bark (G2) extracts.

The effect of *X.a* extract on sperm morphology showed that the extract at a dose of 100-1000mg/kg body weight increased (P<0.05) sperm head and tail abnormalities compared with the G1 and rats administered with 10mg of the extracts (Table 3).

Table 3: Effect of extract of *Ximenia americana* on sperm morphology in rats
(Mean ±SEM, n = 10 in each sub-group)

Group/dose	Normal	Head abnormality	Tail abnormality
G4 (control)	164.5±6.4^{a}	11.5±4.5^{a}	9.6±2.5^{a}
G1 (leaf, mg)			
10	154.3±5.2^{a}	17.5±5.5^{a}	20.4±4.5^{b}
100	130.7±7.8^{b}	24.6±5.9^{b}	26.4±2.5^{b}
1000	100.0±5.5^{c}	30.4±7.3^{b}	40.6±5.5^{c}
G2 (stem, mg)			
10	160.7±9.2^{a}	20.4±2.1^{b}	18.7±3.5^{b}
100	120.5±7.8^{b}	30.4±4.5^{b}	34.6±6.5^{c}
1000	90.7±12^{c}	55.5±4.3^{b}	48.7±7.2^{c}
G3 (root, mg)			
10	140.7±9.5^{b}	25.6±6.2^{b}	20.4±5.0^{b}
100	72.8±7.4^{c}	60.7±6.8^{c}	55.7±5.5^{c}
1000	40.4±5.5^{d}	70.8±8.9^{d}	80.4±4.5^{d}

Values with different superscript alphabets under the same heading along the same column are significant at P<0.05.

DISSCUSION

Our previous study implicated extract of *X.a* in oxidative damage and also demonstrated it inhibitory effect on neuromuscular behaviour in rats [1]. The result obtained from the present study further revealed that extract of *X.a* decreased sexual behaviour, sperm count, testicular weight and increased defects in sperm morphology. The effect was dose dependant, that is the higher the dose the greater the effect. Root extract produced the greatest effect on sexual behaviour compared to extract from leaf and stem bark. Thus, the therapeutic usage of this plant by both animals and humans and the consumption of it fruits need to be seriously re-addressed.

The significant decrease in sexual behaviour of mounting and sniffing may be attributed to either the effect of the extract on locomotor activity due to the presences of tannin, saponin and terpanoids in the extracts, which are shown to induce spasmolytic effect on neuromuscular behaviour as earlier reported by Adeiza and Minka [1] or the effect of the extract on olfactory, which is responsible for detecting pheromonal signals coming from the female partner in the box [10].

The fact that the extract contained some pro-oxidants like flavonoids, saponnins, anthraquinones, alkaloids and terpenoid suggests that the administration of the extract at higher doses of 100-1000mg/kg body weight for two weeks may lead to oxidative damage due to free radical (FR) and reactive oxygen species (ROS) generation. Intense and prolong stress, especially oxidative stress brought about by the generation of ROS and FR has been implicated in the pathological

changes in olfactory sensitivity and forms a "perceptual block" of pheromonal stimuli [10]. Consequently, rats under such conditions in this present study i.e. rats administered with extracts were unable to detect new odour and failed to mount or sniff a female partner in the box. The effect increased ($p<0.05$) with the period of administration. Thus, the rats' sexual ability was considerably reduced or eliminated completely at the end of the experiment.

The low sperm counts and pathological defect in sperm morphology detected in rats administered with the extract at higher doses demonstrated that the extract may have direct effect on sexual glands and sperm cells beside it pro-oxidant effect. Presumably, the ROS and FR generated in rats administered with the extracts may have destroyed testicular germ cells either to the membrane damage or macromolecule degradation, which resulted to significant decrease in the sperm count, testicular weight and sperm abnormality. Furthermore, the ROS and FR may have oxidized the fatty acids found in phospholipids which are a component of mitochondrial sheaths in which motility of sperm depends. Heavy loss of testicular sperm cells in the testis is reported to be a major cause of testicular weight loss in rats [13]. Similar results that ROS decreases sperm motility due to increase in lipid peroxidation and loss of membrane polyunsaturated fatty acid has been reported [6, 9, 13, 18].

The level of testosterone was not evaluated in the present study, however several studies have shown that a decrease in sexual activity as observed in the present study can be interpreted as a decrease in sexual motivation if there is also a decrease in the blood testosterone level [10]

The overall result showed that exposure of rats to extract of *X.a* at a dose of 100-1000mg/kg body weight for two weeks is toxic and resulted into decreased sexual behaviour, sperm damage and testicular weight loss. Thus, the extract should be administered with great caution. Further study is required to elucidate the proximal mechanism of action of the extract of *X.a* on reproductive system of both males and females.

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