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Sociability and sexual motivation in male Wistar rats in response to different doses of extract of *Isoberlinia doka* using "partition" test method

Minka N. Salka*, Adeiza A. Abubakar, Fatima B. Hassan

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

This study investigated the effects of different doses of stem-bark extracts of Isoberlinia doka (I.D) on sociability and sexual motivation of male Wistar rats using partition test method with perforated and non-perforated transparent glass. The results of behavioural activities obtained in rats administered orally with 200 and 600mg/Kg body weight of the extract in response to a female partner a day after the first application showed an increased ($P < 0.05$) in sociability and sexual motivation compared to the control group (CG) and groups treated with 1000, 1400, 1800 and 2200mg of the extract. This was evidenced by a significant ($P < 0.05$) increased in the number of excursions and time spent by the male rats on chemoinvestigative behaviours when a female partner was introduced in the box that was partitioned with a perforated glass. The administration of 1400-2200mg of the extracts to the male rats during the study period induced perceptual block of pheromonal stimuli and increased in anxiety. The overall behavioural results was higher ($P < 0.01$) when the cage was partitioned with a perforated glass than non-perforated. In conclusion, the administration of a single dose of 200 and 600mg of extracts of I.D enhanced sociability and sexual motivation, while higher doses inhibited the sociability and chemoinvestigative behaviour of the male Wistar rats. The partition test method was able to differentiate visual and auditory perceptions from those of sexual motivation. Thus, partition test can be used in pharmacological experiments for study of neurochemical regulation.

Keywords: Extract, *Isoberlinia doka*, male rats, partition test, sexual motivation, sociability.

INTRODUCTION

Plant extracts and materials have been used for medicinal purposes long before the advent of documenting what curative properties plants possess. Plants now play significant roles in our

lives as they are precursors to the synthesis of beneficial drugs. Of recent the WHO estimated that 80 % of people world wide rely on herbal medicines for some aspect of their primary health care [1].

The problem of infertility, especially in men in African and Asian countries have caused untold hardship to millions of families. The problem of infertility is poorly reported in hospitals because it occurrence damages relationships, self-esteem and confidence [2]. Beside, majority of the rural areas lack the basic primary health care system and in urban areas the high cost of medication are not always attainable. The only alternative is to rely on herbal traditional medication.

There exist a large number of plants that are locally used for the treatment of sexual disorder. *Isoberlinia doka* (I.D) is one among many. The plant belongs to the Family Caesalpinioideae and is found in the Sudanese and Guinean Savannahs, on well drained clay and average soils, and distributed from Guinea to Cameroon, as far as Sudan. The roots of I.D are used against nausea, hepatitis, the bark as a vermifuge and healing, medico-religious uses (against curses) while the stem and leaves are used in combating convulsion [13]. The plant has also been shown to alleviate erectile dysfunction [2], traditional treatment of typhoid fever [1], scrotal elephantiasis, infertility and jaundice [4] and as antivenom [5]. Inspite of the general use of I.D as a medicinal plant there are limited or no studies that investigated the effect of the extract on sociability and sexual performance or infertility.

Partition test method has been used widely in assessing the behaviour of individual mice kept in cages separated by a transparent partition which allows the mice to smell, see and hear each other without any physical contact [6, 7]. The test has been successfully used in measuring the level of sociability, aggressive motivation, anxiety, excitability, sexual motivation and olfactory perception in mice ([7, 8, 9].

In the present study phytochemical constituents were screened, the effects of graded doses of water extract of stem bark of I.D on sociability, sexual motivation and olfactory perception were investigated in Wistar rats using partition test method.

MATERIALS AND METHODS

Plant and preparation of aqueous extract of *Isoberlinia doka*

The plant parts used in this study were collected from the rangeland of the College of Agriculture and Animal Science, Ahmadu Bello University, Mando, Kaduna. The plant part was identified as I.D at the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where the voucher specimen is deposited.

The stem bark of I.D were chopped into pieces, air dried at room temperature, and then pulverized using a Kenwood® blender. A sample (600g) of the powdered stem bark was boiled in 3000 ml of distilled water for an hour. It was then filtered hot through a muslin cloth before further filtration using a Whatman No. 1 filter paper. At the end of the extraction, the filtrate was concentrated in a hot air oven at 50^{O}C and subsequently air dried. The extract was pounded into powder using a porcelain pestle and mortar and then stored in an air tight container and kept at 4^{O}C till when required.

Phytochemical screening
The aqueous stem bark extract was screened as described by Harborne [10]. The stem-bark extract of I.D was found to contained secondary metabolites such as flavonoids(++), alkaloids (++), tannins (+), glycosides (++), saponin (+) and volatile oil (+).

Experimental animals
A total of 35 male and 20 female Wistar rats weighing between 113.3 – 192.6g were used for the study. They were purchased from the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were transported to the College laboratory and maintained in clean rat cages in a 12h light/dark cycle with the litter changed every week. They were fed pelletized commercial rat feed (ECWA® Nigeria PLc, Jos, Nigeria) and water was provided *ad libitum* and allowed to acclimatize for a week. The rats were strictly handled and maintained in accordance with the internationally accepted principles for laboratory animal care and use [11, 12].

Administration of aqueous extract of *Isoberlinia doka*
The male rats were randomly divided into 6 groups of 5 rats each (G1-G2) and were administered orally and individually the extract of I.D diluted in sterile water at the dosage rate of 200, 600, 1000, 1400, 1800, 2200mg/Kg body weight, respectively on days 0, 7, 14 and 21 of the study period. The control (CG) was given 1ml of sterile water orally. The female rats were not treated with any extract, but were kept and only used as partners during partition test measurements.

Behavioral Tests
Partition test. The "partition" test method used in the present study has been previously described in detail [7, 8, 9]. A male rat was placed in one side of a cage partitioned by either a perforated or non-perforated transparent glass. After 5 min of acclimatization a male or female partner was placed in the other side of the cage and the behaviour of the rat was recorded as follows: 1. Number of approaches and/or turnings of the head to the partition 2. Total time spent near the partition during the test. This parameter is characteristic of the reaction to the female in the neighboring compartment. Rats move near the partition, smell and touch, clutch and hang on it, and put their noses into or gnaw the holes. Briefly, the accumulated or total time, when males touched the partition with their foreparts (nose, paws) was assessed. The cage was clean between trials with cotton wool soaked in soapy water followed by 90% alcohol solution to remove the odour of the previous rat.

Statistical Analysis
Data were analyzed by 1-way analysis of variance (ANOVA) followed by Tukey's test.

RESULTS

The result of acute toxicity showed no sign of depression, abnormal behaviour or any motor or secretory activities. There was no mortality in all the rats treated with the extracts.

Table 1 showed the behaviour of the male rats in the partition cage in response to a male and female partner before the administration of the extract. The number of excursions and time spent

near the perforated partition was significantly ($P < 0.01$) higher, especially when a female partner was introduced than when a male partner was introduced or when a non-perforated partition was used.

Table 1: Number of excursions and total time (sec) spent by male Wistar rats in response to a male and female partner near a perforated and non-perforated partition cage

Behaviour	*Male alone*	*Male to male partner*	*Male to female partner*
Number of excursions:			
Perforated partition	10.5 ± 1.3^{a}	18.4 ± 2.1^{b}	28.0 ± 2.2^{c}
Non-perforated	9.0 ± 1.1^{a}	10.2 ± 1.0^{a}	8.6 ± 1.1^{a}
Total time (s):			
Perforated partition	5.4 ± 1.5^{a}	40.8 ± 5.7^{b}	135.3 ± 12.5^{c}
Non-perforated	6.1 ± 1.4^{a}	30.2 ± 5.5^{b}	37.0 ± 4.5^{b}

Mean values with the different superscript alphabets along the same row are significantly different at $P < 0.05$.

The number of excursions made by male Wistar rats administered different doses of extract of I.D in a perforated and non-perforated partition cage in response to female partner are shown in Table 2. The result showed that single administration of 200 and 600mg of the extracts significantly ($P < 0.05$) increased the number of excursions made by the male Wistar rats when a perforated partition was introduced compared to non-perforated partition and in rats administered with 1000 to 2200mg of the extract. The overall mean result obtained on the final day of the study in the CG was not significantly ($P > 0.05$) different from G1 and G2 rats, but the result was significantly ($P < 0.01$) higher than those obtained in G3, G4, G5 and G6 rats.

Table 2: Number of excursions made by male Wistar rats administered different doses of extracts of *Isoberlinia doka* in a perforated and non-perforated partition cage in response to female partner

	Experimental days				
Extract/group	1	7	14	21	Mean ± SEM
200mg: Perforated	30 ± 8.8^{axe}	28 ± 11.4^{aye}	21 ± 7.5^{aze}	22 ± 5.5^{aze}	22.3 ± 4.6^{aze}
Non-perforated	5 ± 1.5^{bxf}	10 ± 2.1^{bxf}	5 ± 1.0^{bxf}	7 ± 0.5^{bxf}	6.8 ± 1.2^{bxf}
600mg: Perforated	30 ± 6.2^{axe}	24 ± 6.5^{aye}	$28\pm11.4xe^{a}$	25 ± 1.7^{aye}	26.8 ± 7.3^{aye}
Non-perforated	5 ± 2.1^{bxf}	6 ± 1.1^{bxf}	4 ± 2.5^{bxf}	5 ± 1.2^{bxf}	5.0 ± 1.1^{bxf}
1000mg: Perforated	28 ± 3.1^{axe}	4 ± 8.2^{ayf}	6 ± 1^{ayf}	0xf	8.5 ± 2.2^{ayf}
Non-perforated	6 ± 1.6^{bxf}	4 ± 2.1^{bxf}	$2\pm1.1xf^{b}$	1.5 ± 0.8^{a}	3.3 ± 1.3^{bxf}
1400mg: Perforated	5 ± 0.9^{axf}	3 ± 2.2^{axf}	0	0	2 ± 0.4^{axf}
Non-perforated	4 ± 1.1^{axf}	3 ± 0.8^{axf}	0	0	1.8 ± 0.5^{axf}
1800mg: Perforated	2 ± 1.1^{axf}	3 ± 1.6^{axf}	$1.1\pm.0^{axf}$	1.3 ± 2.1^{axf}	2.5 ± 1.0^{axf}
Non-perforated	0	2 ± 1.0^{axf}	4 ± 1.1^{axf}	0	1.5 ± 0.9^{axf}
2200mg: Perforated	4 ± 1.8^{axf}	2 ± 0.3^{axf}	0	1.0 ± 0.9^{axf}	2.5 ± 1.5^{axf}
Non-perforated	0	2 ± 0.7^{axf}	1.1 ± 0.7^{axf}	2.0 ± 0.8^{axf}	1.3 ± 1.1^{axf}
Control: Perforated	24 ± 2.4^{axe}	27 ± 4.6^{axe}	25 ± 12.5^{axe}	15 ± 3.4^{aye}	24.5 ± 10.2^{axe}
Non-perforated	15 ± 3.7^{bxg}	22 ± 5.5^{bxg}	15 ± 2.7^{bxf}	7 ± 1.6^{bxf}	18.3 ± 3.3^{bxf}

^{ab} *= Mean values under each dose with the different superscript alphabets along the same column are significantly different at $P < 0.05$.*

^{xy} *= Significant ($P<0.05$) difference between days of administration of the extracts.*

^{efg} *= Significant ($P<0.05$) difference between doses of the extracts.*

Similarly, the total time spent by the rats in G1, G2 and CG near the partition was significantly higher ($P < 0.01$) than the result obtained in G3, G4, G5 and G6 rats (Table 3).

Table 3: Total time (sec) spent by male Wistar rats administered different doses of extract of *Isoberlinia doka* in response to a female partner near a perforated and non-perforated partition cage

	Experimental days				
Extract/group	1	7	14	21	Mean ± SEM
200mg: Perforated	170.6±10.4^{axe}	120.5±11.4^{aye}	140.2±9.4^{aze}	135.4±9.7^{aze}	141.3±10.5^{aze}
Non-perforated	61.2±7.3^{bxf}	75.5±10.2^{bxf}	100.3±9.5^{byf}	106.2±10.1^{byf}	85.5±10.2^{bxf}
600mg: Perforated	161.4±11.4^{axe}	140.2±10.3^{aye}	109.4±10.5^{azf}	140.4±10.0^{aze}	142.5±11.4^{aze}
Non-perforated	51.4±10.4^{bxf}	40.3±5.6^{bxf}	65.4±9.5^{byg}	40.2±4.6^{bxg}	49.0±5.5^{bxg}
1000mg: Perforated	90.0±3.6^{axg}	32.4±7.5^{ayf}	40.2±9.5^{ayg}	7.1±2.5^{azk}	42.3±9.5^{ayg}
Non-perforated	56.0±8.9^{bxf}	0	20.1±7.5^{byk}	7.3±1.5^{azk}	20.8±3.5^{byk}
1400mg: Perforated	50.3±9.7^{axg}	15.6±4.7^{ayg}	20.5±4.6^{ayk}	0	21.3±2.7^{ayk}
Non-perforated	20.5±4.8^{bxk}	3.2±1.3^{byg}	10.5±3.6^{byk}	0	8.3±6.4^{byk}
1800mg: Perforated	40.6±7.5^{axf}	66.5±10.1^{ayf}	10.5±5.2^{ak}	0	29.0±3.7^{azk}
Non-perforated	10.1±10.4^{bxk}	20.5±7.5^{byg}	10.4±5.1^{axk}	0	22.5±8.5^{byk}
2200mg: Perforated	30.7±9.1^{axg}	10.1±2.7^{ayg}	40.7±8.9^{axg}	2.5±1.1^{azk}	20.0±8.5^{ayk}
Non-perforated	0	5.1±2.1^{axg}	4.8±1.7^{bxk}	2.5±1.0^{axk}	2.8±1.1^{bxk}
Control: Perforated	150.4±15.3^{axe}	130.2±12.3^{axe}	140.3±11.5^{axe}	154.5±11.7^{axe}	143.5±9.5^{axe}
Non-perforated	92.4±7.7^{bxf}	70.5±9.1^{bxf}	70.1±10.5^{bxf}	90.3±10.5^{bxf}	95.5±10.4^{bxf}

^{ab} = *Mean values with the different superscript alphabets along the same column under the same group are significantly different at $P < 0.05$.*

^{xy} = *Significant ($P<0.05$) difference between days of administration of the extracts.*

^{efg} = *Significant ($P<0.05$) difference between doses of the extracts.*

DISCUSSION

The result obtained in the male rats before the administration of the extract showed that the rats spent less time and made few excursions to the partition when a single rat was in the cage. This suggested no particular activity at the partition and there was no difference in the reaction of the rats when transparent perforated and non-perforated partitions were introduced. However, when another male rat was introduced in the other side of the cage divided by a transparent non-perforated partition, the activity of the rat in the other side increased. The result suggested that the increased in behaviours were triggered by visual and auditory interactions. Furthermore, the significant increased in the number of excursions and time spent by the male rats at the perforated partition compared to non-perforated demonstrated that apart from the visual and auditory interactions, other chemical factors like pheromones probably released by the partner in the other side of the cage may have increased the level of social interaction. This was evidenced by the significant increased in the time spent by the male partner at the perforated partition when a female rat was introduced as a partner in the other side of the cage. The results of this study concurred with the results of several researchers [6, 7, 8, 9, 13]. The behaviour of the rats in the present study demonstrates the tendency of the rats to interact with each other, which is often used as a measure of the level of sociability or communicativety [7]. The use of the partition method showed that the test may be used effectively to measure and distinguished olfactory perception from visual or auditory interactions.

The behaviour of the male rats recorded at the partition a day after the administration of the extracts showed that the extracts at various doses had effect on the level of sociability and sexual interaction. The non-significant ($P < 0.01$) difference in the time spent and number of excursions made by a male rat in the other side of the box in response to another male or female rat partitioned by a non-perforated partition suggested that the rat was unable to differentiate between a male or female partner. Thus, the behaviours recorded were basically due to visual and auditory perceptions. However, the significant ($P < 0.01$) increased in the time spent at the partition and in the number of excursions made by the rats administered 200-600mg/kg body weight of the extract compared with the CG when a female partner was introduced in a cage with perforated partition demonstrated that the perforated partition and the extract had significantly ($P<0.01$) increased the rat's ability to perceived the odour of pheromones released by the female partner in the other side of the perforated partition. The result demonstrated an increased in sexual motivation. The present result supports the traditional believe that extract of I.D had some potentials to enhance sexuality. The exact mechanism by which the extract enhances sexuality requires further investigation. The insignificant changes in the behaviours observed when 200-600mg of the extract was administered to the rats on days 7, 14, and 21 suggested that weekly administration of the extract at doses of 200 and 600mg did not elicit greater effect on the sociability and sexual motivation of the rats.

The significant ($P < 0.01$) decreased in the time spent and number of excursions made by G4-G6 rats to the perforated partition compared to CG and G1-G3 rats showed that the extract at higher doses inhibited not only visual and auditory perceptions, but also sexual motivation. This was evidenced by a significant ($P < 0.01$) decreased in chemoinvestigatory behaviour towards a female partner. The result showed that the extracts may have contained some metabolites that decreased either neuromuscular activity or perception of the female pheromones. Although the proximate mechanism of action of the extract was not investigated in the present study, however, the changes observed in the sociability and sexual behaviours may be due to the presence of different secondary metabolites in the extract as confirmed by the phytochemical results. Such metabolites, especially flavonoids, alkaloids and glycosides when administered in higher doses have been reported to exert neurotoxicity due to their pro- and anti-oxidant activities which have been shown to induce oxidative damage in the brain and other nerve cells by causing necrosis of nerve cells, which may reduce transmission of nerve impulse [14, 15, 16, 17].

Thus, the extract may inhibit neuromuscular activities as observed in the present study. Although, antioxidants levels were not measured in the present study, however oxidative stress has also been implicated in the pathology of male and female infertility [18].

The significant decreased in social behaviour in the group of rats administered higher doses of I.D further indicated an increased in anxiety. Lower social behaviour in rats has been associated to an increase in anxiety [19, 20].

Although I.D is popularly used by many local people in the treatment of sexual disorder and many other ailments such as nausea, elephantiasis, hepatitis, typhoid fever and jaundice [1, 2, 4] it application should be very careful.

CONCLUSION

The present result, for the first time, showed scientific evidence that extract of I.D at a dose of 200-600mg enhanced sexuality in male Wistar rats. The administration of the extract above 600mg/kg body weight may result to anxiety and a decrease in neuromuscular behaviour and sexual motivation.

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