

EFFECT OF AQUEOUS EXTRACT OF SPONDIAS MOMBIN ON THE SPERMOGRAM OF WISTAR RATS

EFFET DE L'EXTRAIT AQUEUX DE SPONDIAS MOMBIN SUR LE SPERMOGRAMME DE RATS WISTAR

Oloye AA¹, Oyeyemi M O², Ola-davies O E³ and Innamah O A¹

¹Department Of Veterinary Public Health And Reproduction, College Of Veterinary Medicine, University Of Agriculture Abeokuta.

²Department Of Veterinary Surgery And Reproduction, Faculty Of Veterinary Medicine, University Of Ibadan.

³Department Of Veterinary Physiology, Biochemistry and Pharmacology, Faculty Of Veterinary Medicine, University Of Ibadan.

Abstract

Twenty-five male rats were divided into five groups (A-E) and treated for twenty-one days with aqueous leaf extract of *Spondias mombin* which is a fruit tree whose leaf extract is commonly consumed as local remedies in traditional medical practice. Graded dosages of the extracts were assigned to the groups as follows: 200 mg/ kg, 400 mg/ kg, 600 mg/ kg and 800 mg/ kg for groups B,C,D and E, respectively. Group A rats which served as control were given distilled water. Thereafter, *spermiogram* (mass activity, progressive motility, live-dead and morphology) was evaluated. Rats treated with aqueous extract of *Spondias mombin* at 600 mg/ Kg body weight had the highest ($p < 0.05$) *spermatozoa* concentration of 40.51×10^6 cells/ ml. Compared with other groups A, B, C, and E which had 21.48×10^6 , 29.58×10^6 , 33.56×10^6 , 35.81×10^6 cells / ml, respectively. At dosage of 800 mg/Kg highest ($p < 0.05$) sperm motility of $70 \pm 4.81\%$ was observed. Compared with other groups A,B C and D which had 65.0 ± 0.00 , 65.0 ± 0.00 , 67.5 ± 6.71 and $68.0 \pm 2.89\%$, respectively. The percentage average livability of the control group and of those given 200 mg / kg BW, 70 ± 23.45 and 70 ± 23.09 , respectively, were lower than those for groups C (77.5 ± 15.55), D (87.5 ± 17.68) and E (80.0 ± 17.32) with group D having the highest average percentage livability. Coiled tail was the predominant abnormality in groups A, B and E constituting 40.3%, 24.3% and 30.0% of the total abnormalities, respectively. The work recommended *aqueous* extract of *Spondias mombin* at concentration of 600mg/ml for of fertility enhancement in male animals.

Key words: Effect, Aqueous, Extract, *Spondias mombin*, *Spermiogram*, Wistar rats

Résumé

Vingt-cinq rats mâles ont été divisés en cinq groupes (de A à E) et traités pendant vingt et un jours avec une solution aqueuse de feuilles de *Spondias mombin*, un arbre fruitier dont le jus extrait des feuilles est couramment consommé comme remède dans la médecine traditionnelle. Les dosages proportionnels desdits extraits de *Spondias Mombin* ont été administrés aux différents groupes en fonction des critères ci-après : 200 mg par kg, 400 mg par kg, 600 mg par kg et 800 mg par kg pour les groupes B, C, D et E respectivement. Les rats du groupe A appelés à servir de témoins n'ont reçu que de l'eau distillée. L'équipe de recherche a procédé, par la suite, à l'évaluation du spermogramme (analyse de l'activité en masse, de la motilité progressive, des substances vivantes et mortes et de la morphologie des spermatozoïdes). Les rats auxquels a été administrée la solution aqueuse de *Spondias mombin* à 600 mg / kg de poids corporel présentaient la concentration la plus

élevée ($p < 0,05$) de spermatozoïdes de $40,51 \times 106$ cellules par ml par rapport à d'autres groupes A, B, C et E qui présentaient $21,48 \times 106$, $29,58 \times 106$, $33,56 \times 106$, $35,81 \times 106$ cellules par ml, respectivement. La motilité la plus élevée ($p < 0,05$) des spermatozoïdes de $70 \pm 4,81\%$, a été relevée lors du dosage de 800 mg de solution de *Spondias mombin* par kg, par rapport à d'autres groupes A, C et D qui présentaient des motilités de spermatozoïdes de l'ordre de $65,0 \pm 0,00$, $65,0 \pm 0,00$, $67,5 \pm 6,71$ et $68,0 \pm 2,89\%$ respectivement. Le pourcentage moyen d'espérance de vie du groupe de rats témoins et celui de ceux ayant reçu 200 mg de *Spondias mombin* par kg de poids corporel, $70 \pm 23,45 \pm 23,09$ et 70 respectivement, ont été inférieurs à ceux des groupes C ($77,5 \pm 15,55$), D ($87,5 \pm 17,68$) et E ($80,0 \pm 17,32$). L'on constate par ailleurs que les rats du groupe D présentent le pourcentage moyen le plus élevé d'espérance de vie. L'anomalie prédominante relevée dans les groupes A, B, et E respectivement constituées de 40,3%, 24,3% et 30% de l'ensemble des anomalies est la queue enroulée. Les travaux de recherche ont recommandé l'administration de la solution aqueuse de *Spondias mombin* à une concentration de 600mg par ml comme source d'amélioration de la fertilité chez les mâles.

Mots clés: Effet, solution aqueuse, extrait aqueux, *Spondias mombin*, spermogramme, rats Wistar

Introduction

Spondia mombin is a fructiferous tree that belongs to the family *Anacardiaceae*. It grows in the coastal areas and in the rain forest into a big tree of up to 15–22mm in height. It is common in Nigeria, Brazil and several other tropical forests of the world with high genetic variability among populations (Ayoka et al., 2008). Phytochemical screening of the plant's leaf extracts using standard methods (Sofowora, 1982) shows presence compositions of saponins, tannins, alkaloids, flavonoids, phytate and cyanogenic glycosides been attributed to compounds (Ayoka et al., 2008). The alkaloids affect glucagon, thyroid stimulating hormone and inhibit certain mammalian enzymatic activities (Okaka et al., 1992). Steroidal saponins and alkaloids such as ergot alkaloids have been reported to elicit uterine muscle activity. These have also been associated with oxytocic and abortifacient activity of the plant's leaf extract (Offiah and Anyanwu 1989).

Reports have revealed the anxiolytic effect (Ayoka et al., 2005), sedative, antiepileptic and antipsychotic effects of the leave extract of *Spondia mombin* in mice and rats (Ayoka et al., 2006). Raji et al. (2006) in their study showed the antifertility action of aqueous *Spondia mombin* bark extract. They reported a marked dose dependent reduction in epididymal sperm progressive

motility, sperm count, viability (alive/dead ratio) and a dose-dependent increase in percentage abnormal spermatozoa. Epididymal α -glucosidase activity was significantly reduced. However, cessation of treatment resulted in full recovery within four weeks. The indiscriminate use of this herbal preparation among the locals is so rampant hence the need to study the likely effect it may have on the reproductive viability of the male. The male animal model (Wistar rat) used reaches puberty at 40 to 60 days of age. Descent of the testes usually occurs between 30 and 60 days. Sperm counts vary by strain (Wilkinson et al., 2000). The male rat has an os penis and the following accessory sexual organs: ampulla, seminal vesicles, prostate, bulbo-urethral glands, coagulating glands, and preputial glands. The coagulating gland and prostatic and vesicular secretions are responsible for the copulation plug, a firm plug deposited in the vagina of the female after copulation. (This plug, when found outside the female rat, is capsule-shaped and approximately 5 mm long.). The work sought to determine the effect of repeated dosing of the leaf extract of *Spondias mombin* would have on the male reproductive parameters.

Materials and Methods

Plant collection and preparation of aqueous extract

Fresh leaves of *Spondias mombin* were collected at the University of Agriculture, Abeokuta, Ogun State of Nigeria in the month of June. The leaves were rinsed in clean water and air-dried (at room temperature until they crumbled to touch) to a constant weight. The dried leaves were ground into a fine powder using a mechanical grinder, packaged in glass jars and stored at 4°C as described by. Aqueous extract was produced using water as extraction medium. The plant was soaked in water for three days. It was filtered using sieve to separate the leave particles. The filtrate was diluted again and filtered. The filtrate was pre-concentrated by heating to evaporate excess water until it turned into paste. The paste was then collected and stored at 4°C, refrigerator.

Animals

Male Wistar rats weighing 180 - 250 g were used in the study. All the experimental procedures were done following the experimental guidelines of Institutional Animal Ethics Committee (IAEC). Twenty-five male rats were used. The rats were housed in standard cages and fed *ad-libitum* with commercially prepared feed. Water was also given *ad-libitum*. Two weeks of stabilization was observed. The male rats were divided into five groups (A to E) and treated for 21 days. Graded dosages of the extracts were assigned to the groups as follows: 200 mg/ kg, 400 mg/

kg, 600 mg/ kg and 800 mg/ kg for groups B,C,D and E respectively. Group A rats which served as control were given distilled water. Thereafter, spermogram (mass activity, progressive motility, live-dead and morphology) was evaluated.

Spermogram

The rats were anaesthetized with ether before sacrificing through cervical dislocation. In performing orchidectomy, a pre-scrotal incision was made and the testicles were milked out of the incision site to expose them. Semen samples were thereafter collected from the caudal epididymis using a method similar to that described by. The samples were analyzed immediately after collection.

A drop of buffered semen was placed on a clean glass slide before applying cover slip. It was viewed at X40 objective for gross motility of spermatozoa. Nigrosin- Eosin stained smear was also prepared by placing a drop of the epididymal spermatozoa on a clean glass slide and stained with Nigrosin-Eosin to observe the live- dead ratio. In like manner, Wells and Awa stain stained smear was prepared to examine the morphology of the spermatozoa.

Statistical Analysis

Student t-test was used to analyze the data (Steel and Torrie,1996). The difference of the means were considered significant at $p < 0.05$. SPSS 16.0 (©2007 SPSS

Table 1: Effect of aqueous extract of *S. mombin* on the concentration of spermatozoa of groups A, B, C, D and E (\pm SD)

Group	Dosage (mg/Kg)	Average Concentration 1	Average Concentration 2
A	Distilled water	21.48 \pm 8.26	18.6
B	200	31.0 \pm 17.01	29.58
C	400	35.0 \pm 21.87	33.56
D	600	41.4 \pm 24.39	40.51
E	800	39.8 \pm 25.40	35.81
Mean \pm SD		31.61 \pm 8.28	

1 = ($\times 10^6$ sperm cells/ml)

2 = adjusted by Testicular weight ($\times 10^6$ sperm cells/gml)

Table 2: Effects of Aqueous Extract of *S. mombin* on the average Motility, Concentration and Livability of spermatozoa of Rats in Groups A (control), B, C, D and E (\pm SD).

Group / Dosage (mg/ Kg)	Average Motility (%)	Average Concentration ($\times 10^6$ Cells/ml)	Average Liveability (%)
A (Distilled water)	65.0 \pm 0.00	18.6 \pm 8.26	70.0 \pm 23.45
B (200 mg/Kg)	65.0 \pm 0.00	29.58 \pm 17.01	70.0 \pm 23.09
C (400 mg/Kg)	67.5 \pm 6.71	33.56 \pm 21.87	77.5 \pm 15.55
D (600 mg/Kg)	68.0 \pm 2.89	40.51 \pm 24.39	87.5 \pm 17.68
E (800 mg/Kg)	70.0 \pm 4.81	35.81 \pm 25.40	80.0 \pm 17.32

Table 3 : One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
conc	5	31.6120	8.27698	3.70158
mot	5	67.1000	2.13307	.95394
live	5	77.0000	7.37394	3.29773

Table 4 : Mean Difference

Test Value = 0						
95% Confidence Interval of the Difference						
	t	df	Sig. (2-tailed)	Mean Difference	Lower	Upper
conc	8.540	4	.001	31.61200	21.3348	41.8892
mot	70.340	4	.000	67.10000	64.4514	69.7486
live	23.349	4	.000	77.00000	67.8440	86.1560

incs licensed to Team EQX 6th birthday 1337) was used.

Results

It was observed that rats treated with aqueous extract of *S. mombin* at 600 mg/Kg body weight had the highest spermatozoa concentration of 40.51×10^6 cells/ ml. This was highly significant ($p < 0.05$) compared with other groups A, B, C, and E which had 21.48×10^6 , 29.58×10^6 , 33.56×10^6 , 35.81×10^6 cells / ml, respectively (Table 1, 3 and 4).

It was also observed that rats given the highest dosage 800 mg/Kg had highest sperm motility of $70 \pm 4.81\%$ which was significant ($p < 0.05$) compared to other groups A, B C and D which had 65.0 ± 0.00 , 65.0 ± 0.00 , 67.5 ± 6.71 and $68.0 \pm 2.89\%$, respectively (Table 2, 3 and 4).

At $p < 0.05$, significantly the percentage average liveability of the control group and those given 200 mg / Kg (body weight) 70 ± 23.45 and 70 ± 23.09 were lower than those for groups C (77.5 ± 15.55), D (87.5 ± 17.68) and E (80.0 ± 17.32) with group D having the highest average percentage liveability (Table 2, 3 and 4).

Coiled tail was the predominant abnormality in groups A, B and E constituting 40.3%, 24.3% and 30.0% of the total abnormalities, respectively.

Discussion and Conclusion

The study showed that the percentage motility of the control experiment (group A), $65 \pm 0.00\%$, was lower than that reported by Oyeyemi et al (2006) for rats. The concentration 21.48

$\times 10^6$ cells / ml and percentage liveability 70.0 ± 23.44 % of the control group was lower than 67.40×10^6 and 76.0 ± 2.40 % reported for concentration and percentage liveability, respectively, of rats by (Oyeyemi *et al.*, 2006).

The study also showed that group D given 600 mg /Kg of aqueous *S. mombin* extract had a higher percentage liveability of 87.5 ± 17.68 % than the control group 65.0 ± 0.00 % but lower than 88.0 ± 3.39 % reported by (Oyeyemi *et al.*, 2006). The study's report is at variance with (Raji *et al.*, 2006) submission (using bark extract) that the plant reduces the reproductive potential of the Wistar rat. Coiled tail was predominant in the control group (A). This secondary sperm cell morphological abnormality was predominant compared with the treated group. In conclusion, aqueous extract of *Spondia mombin* supported fertility in the male Wistar rat at concentration of 600mg/ml hence can be a good source of fertility enhancement in male animals.

Impact

There are a lot of reproductive problems currently. Some of these problems can be traced to habits in different societies especially the Third World countries. There are some other practices that are beneficial to reproduction but not discovered and documented. One such is the consumption of concoctions having large doses of *Spondias mombin*. This work was able to reveal a beneficial impact of this medicinal plant to the male reproductive indices.

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