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ORIGINAL ARTICAL PAGE

SHORT COMMUNICATION

TESTICULAR MORPHOMETRY AND HISTOLOGY OF MALE WISTAR RATS AND GESTATIONAL PATTERN OF FEMALE WISTAR RATS TREATED WITH GRADED DOSAGES OF THE LEAVES' AQUEOUS EXTRACT OF SPONDIAS MOMBIN

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Abstract

The effects of aqueous extract of Spondias mombin leaves on testicular characteristics and neonatal birth weights after oral treatment of male and female wistar rats with graded dosages were studied. Twenty-five female and male sexually matured wistar rats divided into four treatment groups B,C, D and E and control group(A) were used. Five untreated matured male were also used for mating. The treatment groups were given 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg respectively while distill water was served to the control group for 28 days. After, testicles were harvested and studied grossly and for histology while the females were served by sexually matured untreated male rats introduced at the last half of the oral treatment. The gross and histology studies showed normal conformation of all the testicles. There was no abnormality noticed in all. Weekly vaginal cytology done during treatment revealed that all the female rats cycled normally. Births were recorded in all groups within the average gestation range of 24-28 days. While group A (control group) had the highest average litter size of 13 followed by groups D and E with 9, group C and E had the highest average birth weights of approximately 5g. It is concluded that there was no antifertility consequence of aqueous spondias mombin on the male wistar rat but insipient infertility was noticed with lower dosages for the female but none with dosage as high as 800mg/kg.

Key words: Aqueous extract, Spondias mombin, testicular, neonatal, wistar rat

MORPHOMÉTRIE ET HISTOLOGIE DES TESTICULES DE RATS WISTAR MÂLES ET PROFIL GESTATIONNEL DES RATS WISTAR FEMELLES TRAITÉS AVEC DES DOSES PROGRESSIVES DE L'EXTRAIT AQUEUX DE FEUILLES DESPONDIAS MOMBIN

Resume

Les effets de l'extrait aqueux de feuilles de Spondias mombin sur les caractéristiques des testicules et les poids à la naissance des nouveau-nés après le traitement oral des rats Wistar mâles et femelles avec des doses progressives dudit extrait ont été étudiés. Vingt-cinq rats Wistar femelles et mâles sexuellement matures répartis en quatre groupes de traitement B, C, D et E et un groupe témoin (A) ont été utilisés. Cinq mâles matures non traités ont été utilisés pour l'accouplement. Les groupes traités ont reçu respectivement 200mg/kg, 400mg/kg, 600mg/kg et 800mg/kg, tandis que le groupe témoin a reçu de l'eau distillée pendant 28 jours. Par la suite, les testicules ont été prélevés et soumis à une étude microscopique et histologique. ; Les femelles ont accouplées par des rats mâles sexuellement matures non traités, on été introduites au traitement durant la dernière moitié de l'étude. Les études microscopiques et histologiques ont montré une conformation normale de tous les testicules. Aucun cas d'anomalie n'a été constaté. La

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cytologie vaginale hebdomadaire faite pendant le traitement a révélé que toutes les femelles avaient des cycles normaux. Les naissances ont été enregistrées dans tous les groupes,après une période moyenne de gestation de 24-28 jours. Alors que le groupe A (groupe témoin) avait la plus grande portée moyenne de 13 ratons, suivi par les groupes D et E dont la portée était de 9, les groupes C et E avaient les poids les plus élevés à la naissance, soit environ 5g en moyenne. On a conclu que le spondias mombin aqueux ne causait pas d'infertilité chez le rat Wistar mâle ; un début d'infertilité a été remarqué avec des doses faibles pour la femelle, mais par contre une dose aussi élevée que 800mg/kg n'a pas produit un tel effet.

Mots-clés: Extrait aqueux, Spondias mombin, testiculaire, néonatal, rat Wistar

Introduction

Spondias mombin is a fruitiferous tree that belongs to the family Anacardiacae. It grows in the coastal areas and in the rain forest into a big tree of up to 15-22mm in height. It is readily common in Nigeria and is known as Iyeye in Yoruba language, Ngulungu in Igbo and Isada in Hausa. The plant is widely relied on for various herbal remedies for numerous conditions. It is a common midwife's remedy to help induce labour, reduce bleeding and pain during and after child birth, to bring on the flow of breast milk, and expulsion of placenta. Spondias mombin leaves are among the forages given to domestic animals in SouthEastern Nigeria (Ayoka *et al.,* 2008). The plant is given to expectant ruminant animals and those that delivered without the release of their placenta (Okwu and Ekeke, 2003) and also given for anti-helminthic activities (Ademola *et al.,* 2005). Saponin, found as one of its constituents, has oxytocytic effect (Offiah and Anyanwu, 1989); alkaloids have antispasmodic, analgesic and antibacterial activities (Corthout *et al.,* 1994). Flavanoids and other phenolic compounds also found in the plant have been associated with anti herpes, antioxidative, antiviral and anti ageing properties, (Corthout *et al.,* 1992).

Raji *et al.,* (2006) reported antifertility action of aqueous Spondia mombin bark extract in male wistar rat.

This study was carried out, using the wistar rat as a model, to investigate the effect the aqueous extract of the leaves can have on some reproductive potentials as the leaves are served animals for different medicinal purposes

Materials and Methods

Plant was collected identified and prepared into an extract at the University of Ibadan using standard method of extract preparation. Twenty-five female and twenty five male pubertal wistar rats divided into four treatment groups (five rats per group) B, C, D and E and control group(A) were used. They weighed 120g. The treatment groups were given 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg respectively while distill water was served to the control group for 28 days. The male were harvested of their testicles at the end of oral treatment and testicles were subjected to morphometry and histology. Vagina cytology of the female rats was done for two consecutive weeks to establish an estous cycle pattern. The female rats were thereafter served with untreated sexually matured male rats at the ratio of five female to one male. Identification of sperm plug in the vagina was taken as the day 0 of gestation. Gestation length, litter size, and live birth weight were measured. Student's t-test was used to analyse the data (Steele and Torrie 1996). The difference of means were considered significant at $p<0.05$. SPSS statistical package (version 16.0) was used.

Results

tThe testicular weights of the treated groups were not significantly different from the control group (Table 1). The gross and histology studies showed normal conformation of all the testicles (Figure 2). Average testicular length, width and epididymal length were not significantly different comparing all the groups and between right and left testes within each group (Table 2). There was no abnormality noticed in all. Weekly vaginal cytology done during treatment revealed that all the female rats cycled normally. Specific epithelial cells predominant and consistent with each stage

Table 1: Effect of aqueous extract of Spondias mombin on the testicular weight of treated wistar rats (±SD)

Group	Dosage (mg/kg)	Right testis (g)	Left testis (g)
A (Control)	Distilled water	0.9±0.15	0.9±0.20
B (200mg/kg)	200	1.1±0.10	1.0±0.06
C (400mg/kg)	400	1.1±0.10	1.0±010
D (600mg/kg)	600	0.9±0.06	1.0±0.12
E (800mg/kg)	800	1.2±0.10	1.1±0.00

Table 2: Effect of aqueous extract of Spondias mombin on the testicular length and width and epididymal length of treated wistar rats (±SD)

Group	Average Teticular length (cm)		Average Testicular Width(cm)		Average Epididymal Length (cm)	
	R	L	R	L	R	L
A	1.9±0.30	1.9±0.30	1.1±0.13	1.1±0.13	4.0±0.22	4.0±0.61
B	1.7±0.32	1.7±0.30	1.1±0.9	1.1±0.51	4.3 ±0.33	4.3±0.19
C	1.9±0.21	1.9±0.26	1.1±0.15	1.0±0.06	4.1±0.50	4.0±0.71
D	1.7± 0.06	1.7±0.06	1.1±0.13	1.1±0.13	3.5 ±0.56	3.7±0.45
E	1.7±0.10	1.8±0.10	1.2 ±0.22	1.1±0.10	4.1±0.10	4.2±0.20

Table 3: Effect of aqueous extract of Spondias mombin on the average gestation length, litter size and live birth weight of treated wistar rats (±SD)

Group	Number in group	Average Gestation Length	Average litter size	Average live birth weight (g)
A	5	24.7±2.52	12.7±4.73	4.6±0.57
B	5	24.7±4.16	4.7±0.577	4.9±0.94
C	5	28.5±7.05	6.5±2.08	4.8±0.57
D	5	22.3±1.53	8.0±4.50	5.7±1.01
E	5	24.7±7.23	8.7±0.577	4.74±0.48

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Rt test L	5	1.7800	.10954	.04899
Lft test L	5	1.8000	.10000	.04472

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Differencep	
					Lower	Upper
Rt test L	36.334	4	.000	1.78000	1.6440	1.9160
Lft test L	40.249	4	.000	1.80000	1.6758	1.9242

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Rt Test Width	5	1.1200	.04472	.02000
Lft Test Width	5	1.0800	.04472	.02000
Rt Epid Lgth	5	4.0000	.30000	.13416
Lft Epid Lgth	5	4.0400	.23022	.10296

One-Sample Test

	Test Value = 0					
	t	Df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Rt Test Width	56.000	4	.000	1.12000	1.0645	1.1755
Lft Test Width	54.000	4	.000	1.08000	1.0245	1.1355
Rt Epid Lgth	29.814	4	.000	4.00000	3.6275	4.3725
Lft Epid Lgth	39.240	4	.000	4.04000	3.7541	4.3259

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
Gest Lgth	5	24.9800	2.22531	.99519
Litter Size	5	8.1200	2.98530	1.33507
Live BW	5	4.9400	.43932	.19647

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
GestL	25.101	4	.000	24.98000	22.2169	27.7431
LittS	6.082	4	.004	8.12000	4.4133	11.8267
LiveBW	25.144	4	.000	4.94000	4.3945	5.4855

One-Sample Statistics

	N	Mean	Std. Deviation	Std. Error Mean
WeightR	5	1.0400	.13416	.06000
WeightL	4	1.0250	.05000	.02500

One-Sample Test

	Test Value = 0					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
WeightR	17.333	4	.000	1.04000	.8734	1.2066
WeightL	41.000	3	.000	1.02500	.9454	1.1046

ANOVA

WeightR	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.045	2	.023	1.700	.370
Within Groups	.027	2	.013		
Total	**.072**	**4**			

ANOVA

WeightR	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	2	.008	3.000	.250
Within Groups	.005	2	.002		
Total	**.020**	**4**			

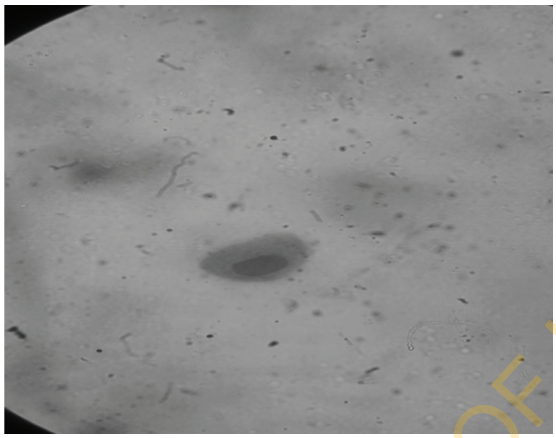

Figure 1: Parabasal epithelial cell indicative of active cycling

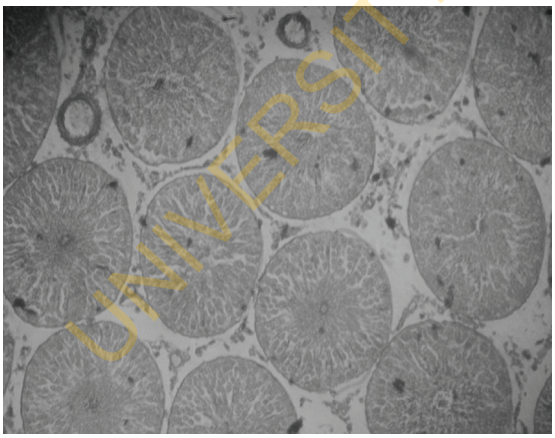

Figure 2: Normal Histological section of the testis of treated wistar rat

of estrous cycle were prominent (Figure 1). Births were recorded in all groups within the average gestation range of 24-28 days with Control group and Group B falling within the lower and upper extremes respectively (Table 3). While Group A (control group) had the highest average litter size of 13 followed by groups D and E with 9. Group C and E had the highest average birth weights of approximately 5g (Table 3). Group B animals appeared disadvantaged having the lowest Percentage conception and litter size.

Results

The testicular weights of the treated groups were not significantly different from the control group (Table 1). The gross and histology studies showed normal conformation of all the testicles (Figure 2). Average testicular length, width and epididymal length were not significantly different comparing all the groups and between right and left testes within each group (Table 2). There was no abnormality noticed in all. Weekly vaginal cytology done during treatment revealed that all the female rats cycled normally. Specific epithelial cells predominant and consistent with each stage of estrous cycle were prominent (Figure 1). Births were recorded in all groups within the average gestation range of 24-28 days with Control group and Group B falling within the lower and upper extremes respectively (Table 3). While Group A (control group) had the highest average litter size of 13 followed by groups D and E with 9. Group C and E had the

highest average birth weights of approximately 5g (Table 3). Group B animals appeared disadvantaged having the lowest Percentage conception and litter size.

Discussion and Conclusion

The undistrupted estous cycle give credence to the report of Nwude *et al.,* (2006) that the extract of Spondia mombin has no effect on the oestrogenic activity and by extension the oestrous cycle of the wistar rat. The unaffected male testicular characteristics after treatment is in consonance with Oloye *et al's* (2011) finding on the positive impact of the extract on the male spermiogram .The leaves served to animals for antihelminthic purpose is therefore safe in the male as it does not interfere with fertility. It is concluded that there is no antifertility consequence of aqueous spondias mombin on the male wistar rat but insipient infertility was noticed with dosages as low as 200mg for the non gravid female but none with dosage as high as 800mg/kg.

Impact

The use of natural products for enhancement of animal health can not be overemphasized. However it is needful also to ensure the preservation of the reproductive life of these animals in the course of using the natural products. This work has helped to ascertain the need for care in using Spondias mombin in pregravida animals.

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