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Reproductive, Haematologic and Biochemical Profiles of Male Rats Treated with Aqueous Extract of *Spondias mombin* Bark

[1]Y. Raji, [2]M.A Gbadegesin, [3]O.A Osonuga, [2]Rahmat A. Adisa, [4]O.S. Akinsomisoye, [5]F.O Awobajo, [6]Olufadekemi T. Kunle-Alabi, [6]P.R.C. Esegbue Peters, [1]I.O Osonuga and [1]A.F. Lamidi
[1]Departments of Physiology and [2]Biochemistry, University of Ibadan, Nigeria
[3]Department of Pharmacology, Olabisi Onabanjo University, Shagamu, Nigeria
[4]Department of Physiological Sciences, Obafemi Awolowo University, Ile-Ife,
[5]Departments of Physiology, Igbinedon University Okada, Edo State
[6]Olabisi Onabanjo University Ikenne, Nigeria

Abstract: Aqueous extract of *Spondias mombin* in different dilutions was employed to assess its impact on male reproductive, haematologic and biochemical indices of male albino rats. A single daily intragastric administration of 8.4, 16.8 and 33.6 mg kg^{-1} b.w day^{-1} of the extract for four weeks did not cause any adverse effect on body and organ weights except the weight of the liver that showed a slight increase. There was a marked dose-dependent reduction ($p<0.05$) in epididymal sperm progressive motility, sperm count, viability (live/dead ratio) and a dose-dependent increase ($p<0.05$) in percentage abnormal spermatozoa. Abnormalities like double heads, double tails, detached heads and broken tails were frequently observed. Epididymal α-glucosidase activity was significantly reduced ($p<0.05$). However, prostatic acid phosphatase activity and citric acid levels and seminal fructose concentrations remained unchanged following *Spondias mombin* treatment. Blood analysis showed that red cell and white cell counts and haematocrit (Hct) levels were in the normal range. Bilirubin, serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), urea and protein concentrations were slightly altered by the extract of *Spondias mombin.* Discontinuation of the extract resulted in full recovery within four weeks of treatment cessation. The results suggest that aqueous extract of the bark of *Spondias mombin* has reversible antifertility action, the testis and the epididymis probably being the prime sites of action.

Key words: *Spondias mombin*, antifertility, antimicrobial, aqueous extract, male rats, sperm

INTRODUCTION

Spondias mombin is a medicinal plant naturally grown in tropical America and West-African countries. It is reputed to have several medicinal values. A decoction of the bark is known to relief sever cough with inflammatory symptoms while dry powdered bark is applied as a wound dressing in circumcision[1]. The bark is also used in Ivory Coast to treat sores, bronchitis, nausea and as a poison antidote as well as to facilitate parturition[1]. Decoctions of Spondias mombin is sometimes taken for gonorrhea and as an aphrodisiac in Nigeria while the leaves are used on malignant tumours in Cuba for uterine cancer[2]. In Senegal, Mandinka women with a history of miscarriage take young leaves of the plant for maintenance of pregnancy. Different parts of the plant have also been used as antibacterial and antihelminthic agents and have been used to treat diarrhea and related diseases[1]. The plant also has febrifugal actions[2].

Although the medicinal values of *Spondias mombin* bark extract have been heavily exploited, there is a dearth of information on its pharmacological activity. The present study therefore carried out toxicological, biochemical, haematological and reproductive tests to evaluate the action of *Spondias mombin* bark extract in male reproduction.

MATERIALS AND METHODS

Plant material and extract preparation: The stem bark of *Spondias mombin* (320 g) was collected at the Department of Biochemistry, University of Ibadan, Nigeria, and air-dried. About 30 g of the powdered bark was soaked in 300 mL distilled water for three days. The resulting solution was then filtered. The extract in the

Correspondence Author: Dr. Y. Raji, Department of Physiology, College of Medicine University of Ibadan, Nigeria
Tel: +234 8023263626 Fax: +234 2 2411768

filtrate yielded 8.073 g (26.91% yield). This was stored in the refrigerator and used for the study, as it is used locally in traditional medicine.

Chemicals: All chemicals were of analytical grade. Kits used in the measurements of serum testosterone and other profiles were products of Span Diagnostics, India. Enzyme substrates were purchased from Sigma.

Acute toxicity test in mice: This was performed essentially as earlier described[3]. Thirty male mice weighing 10-15 g were divided into six groups and treated with increasing doses of the extract ranging from 5, 10, 20, 40 to 80 mg kg^{-1} given orally as a single dose. The control group received 0.5 mL of distilled water (vehicle for the extract). The mortality rate within 24 h period was determined and LD_{50} calculated from probit analysis. All animals were observed for general behaviour over a period of 1 week.

Animals: Wistar strain albino rats (150-200 g) obtained from the central animal house, College of Medicine, University of Ibadan were used. The animals were kept in wire mesh cages, acclimated to laboratory conditions (12D: 12L cycles; 24±1°C) and had free access to food and water *ad libitum*. Each animal was certified fertile by isolated mating technique before inclusion in the study. Generally the study was conducted in accordance with the recommendations of Helsinki on guiding principle in care and use of animals.

Experimental design: A total of 40 male rats divided into eight equal groups were used and treated as follows: groups 1, 2, 3 and 4 were administered with distilled water, 8.4, 16.8 and 33.6 mg kg^{-1} b.w. day^{-1} of the Spondias extract respectively for four weeks. Each group had a corresponding recovery group with five rats each.

At the end of the treatment period, rats were sacrificed under urethane anaesthesia (0.6 mL per 100 g b.w.). The testis, epididymis, seminal vesicle, prostate gland, kidneys, heart and liver were excised from the animals, cleared of adherent tissues and weighed. All organs except the cauda epididymis were stored at -20°C until used for the biochemical investigations. An aliquot of blood collected was immediately used for the haematologic testing. Serum was separated from the remaining blood and stored at -20°C until serologic testing.

Haematological analysis: Cell count determinations were carried out using the improved Neuber type haemocytometer. Haematocrit (Hct) was done using the microhaematocrit centrifuge and reader.

Enzyme assays: Serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxoloacetate (SGOT) activities, urea, serum proteins and bilirubin were determined using kits according to the manufacturer's instructions. Pre-weighed tissue of each of the accessory sex organs was homogenized in distilled water. Neutral α-glucosidase activity was measured in the epididymal extract using p-nitrophenyl α-D-glucopyranoside as substrate at pH 6.8 according to Mortimer[4]. Total and tartarate resistant acid phosphatase was assayed using p-nitrophenyl phosphate as substrate at pH 4.8[5]. The enzyme activity is expressed in units such that one unit denotes μmoles of product formed per hour at 37°C.

Critic acid and fructose determination: Tissue extracts were deproteinized and critic acid was measured colorimetrically after addition of acetic anhydride and pyridine[6] while fructose levels were quantified colorimetrically by using the resorcinol reagents[7].

Sperm analysis: The semen analysis consisted in the determination of sample volume, sperm density, total sperm count, progressive motility, viability and morphology as described by WHO[8]. In this study, epididymal sperm counts, forward progressive motility, morphology and viability (live/dead ratio) were determined as earlier described[9-11]. Progressive sperm motility was done immediately after semen collection. Two drops of the semen were placed on the microscope slide and two drops of warm 2.9% sodium citrate were added. This was then covered with the cover slip and examined under the microscope using X 40 objective with reduced light. Sperm viability was done using the eosin/nigrosin stain. The dead sperm took up the stain. Sperm morphology was carried out by means of the Walls and Ewas stain. Sperm count was carried out using the new improved Neubeur's haemocytometer counting chamber.

Statistical analysis: Statistical analysis was carried out using the Student's t-test. The significance of difference was accepted at $p<0.05$. Data are presented as mean±standard error of mean (M±SEM).

RESULTS AND DISCUSSION

Acute toxicity: The results of the acute toxicity study showed the LD_{50} to be 55.9 mg kg^{-1} while the sub-lethal dose of LD_{20} and LD_{10} to be 40.1 and 36.5 mg kg^{-1}, respectively.

Effects of *Spondias mombin* on body and organ weights of male rats: There were no changes in animal behaviour or body weight during treatment with any of the doses of *Spondias mombin* extract (Table 1). Similarly there was no significant difference in the weight of the reproductive and other organs (except the liver whose weight slightly increased) of the extract treated rats when compared with the control (Table 2). Serum SGOT, SGPT, protein and urea levels were non significantly different from their respective control in all groups (Table 3).

Table 1: Effect of *Spondias mombin* on body weight

Dose (mg kg^{-1} b.w.)	Weeks 1	2	3	4
Control	166.10±10.75	174.80±11.16	180.60±10.72	186.02±10.75
	(180.00±10.23)	(184.11±5.52)	(185.12±6.10)	(188.21±5.71)
8.4	166.11±6.77	167.80±6.82	170.00±6.70	171.20±6.95
	(179.00±6.19)	(188.00±6.43)	(180.60±10.72)	(187.60±8.72)
16.8	176.00±8.11	177.60±8.59	178.00±8.62	178.20±8.73
	(186.02±8.11)	(194.20±7.52)	(182.70±9.72)	(189.60±9.71)
33.6	174.11±16.91	176.00±16.91	172.20±17.05	178.00±16.59
	(187.13±16.20)	(196.12±16.20)	(185.60±10.72)	(188.60±7.70)

Values presented as Mean±SEM (n=5). Recovery values are in parenthesis

Haematological indices of rats treated with *Spondias mombin*: Blood counts and haematocrit values remain normal in *Spondias mombin* treated rats when compared with the control (Table 4).

Biochemical and blood profiles of rats treated with *Spondias mombin*: Biochemical parameters used to assess the function of the seminal vesicles and prostate includes, total acid phosphatase, fructose concentration in seminal vesicle, citric acid in prostate and tartarate resistant acid phosphatase in prostate. The values of these parameters were not affected by the Spondias treatment when compared with the control (Table 5). However there was a dose dependent decrease in epididymal α-glucosidase activity in the treated rats (Table 5).

Effects of *Spondias mombin* on reproductive indices of male rats: The progressive sperm motility of the treated rats showed a dose-dependent decrease when compared with the control group (Table 6). This decrease was significant in the groups of rats that received 16.8 mg kg^{-1} b.w. and 33.6 mg kg^{-1} b.w., respectively for 4 weeks. Percentage live spermatozoa also decreased in a dose-dependent manner. Similarly, there was a dose-dependent decrease ($p<0.05$) in sperm count of extract treated rats when compared with the control. In almost all the test groups, detached head and simple bent tail accounted for over 60% of the abnormalities observed. These abnormalities were dose-dependent with significant increase ($p<0.01$) in the rats that received the highest dose. Discontinuation of extract treatment restored these parameters when compared with control values (Table 6).

Several beneficial effects of *Spondias mombin* have been described in folkloric medicine. However, the impact of this medicinal plant on male reproduction has not been previously investigated. The results obtained from this study demonstrate for the first time that aqueous extract of *Spondias mombin* could impair sperm functions in rats. The extract did not however have any considerable effect on body and reproductive organ weights of the treated rats.

Table 2: Effect of *Spondias mombin* or organ weight in albino rats treated for four weeks

Dose (mg kg^{-1} b.w.)	Testis (g)	Epididymis(g)	Seminal vesicle (g)	Prostate gland (g)	Kidneys (g)	Heart (g)	Liver (g)
Control	0.97±0.05	0.41±0.04	0.42±0.06	0.51±0.02	0.75±0.04	0.52±0.03	5.34±0.25
	(0.98±0.04)	(0.42±0.03)	(0.43±0.04)	(0.53±0.03)	(0.74±0.03)	(0.53±0.03)	(5.35±0.26)
8.4	0.93±0.09	0.50±0.02	0.43±0.05	0.54±0.02	0.71±0.04	0.51±0.06	5.58±0.28
	(1.15±0.07)	(0.38±0.02)	(0.56±0.03)	(0.52±0.01)	(0.72±0.03)	(0.75±0.02)	(5.78±0.14)
16.8	0.97±0.03	0.42±0.03	0.38±0.04	0.52±0.02	0.79±0.03	0.52±0.05	5.92±0.25
	(1.05±0.09)	(0.34±0.02)	(0.59±0.05)	(0.50±0.02)	(0.68±0.01)	(0.68±0.03)	(5.85±0.30)
33.6	0.94±0.04	0.46±0.03	0.52±0.04	0.54±0.02	0.70±0.04	0.58±0.07	6.16±0.21
	(1.02±0.07)	(0.37±0.01)	(0.38±0.01)	(0.53±0.02)	(0.64±0.02)	(0.66±0.02)	(5.57±0.19)

Values presented as Mean±SEM (n=5). Recovery values are in parenthesis

Table 3: Blood profiles of male rats treated with *Spondias mombin* for four weeks

Dose (mg kg^{-1} b.w.)	SGPT (U mL^{-1})	SGOT (U mL^{-1})	Bilirubin (mg mL^{-1})	Blood urea (mg dL^{-1})	Serum protein (mg dL^{-1})
Control	20.1±1.8	28.1±2.8	0.63±0.10	26.4±2.9	3.80±0.41
	(21.2±1.5)	(27.0±2.5)	(0.62±0.11)	(25.8±2.6)	(3.73±0.36)
8.4	22.3±2.0	24.6±3.1	0.62±0.11	26.6±2.4	3.71±0.38
	(21.4±1.9)	(25.4±2.9)	(0.64±0.10)	(26.8±2.4)	(3.81±0.40)
16.8	24.6±2.6	27.3±2.1	0.64±0.12	27.0±2.0	3.40±0.51
	(23.9±2.3)	(28.1±1.8)	(0.66±0.11)	(28.1±1.6)	(3.75±0.39)
33.6	23.4±2.4	28.3±2.0	0.61±0.11	27.1±2.1	3.70±0.48
	(23.1±2.1)	(27.6±1.9)	(0.60±0.10)	(27.3±2.0)	(3.72±0.42)

Values presented as Mean±SEM (n=5). Recovery values are in parenthesis

Table 4: Haematologic values of rats treated with *Spondias mombin* for four weeks

Dose (mg kg^{-1} b.w.)	Total WBC (10^3 mm^{-3})	Total RBC (10^3 mm^{-3})	Hct (%)
Control	4.00±0.16	4.11±0.11	40.1±1.3
	(4.16±0.17)	(4.01±0.12)	(40.0±1.2)
8.4	3.94±0.15	4.10±0.12	39.4±1.2
	(3.95±0.17)	(4.08±0.11)	(40.1±1.1)
16.8	3.93±0.18	4.14±0.13	40.2±1.3
	(3.94±0.16)	(4.10±0.12)	(39.9±1.3)
33.6	3.95±0.17	4.09±0.11	40.1±1.2
	(3.94±0.16)	(4.11±0.12)	(40.0±1.1)

Values presented as Mean±SEM (n=5). Recovery values are in parenthesis.

Table 5: Biochemical profiles used to assess the functional status of assessory sex organs of male rats treated with *Spondias mombin* for four weeks

Dose (mg kg^{-1} b.w.)	1	2	3	4	5
Control	4.54±13	321.0±11	360±12	101.0±13	141.0±12
	(4.52±14)	(313.0±13)	(358±11)	(103.0±13)	(139.0±13)
8.4	4.25±13	328.0±12	341±13*	100.0±12	141.0±13
	(4.20±12)	(330.0±14)	(356±14)	(99.0±13)	(140.0±14)
16.8	4.60±14	320.0±10	310±12*	100.0±11	145.0±14
	(4.62±13)	(323.0±12)	(352±13)	(101.0±12)	(142.0±13)
33.6	4.28±12	318.0±11	286±11*	98.0±12	138.0±12
	(4.50±14)	(319.0±13)	(342±13)	(100.0±13)	(140.0±13)

Values presented as Mean±SEM (n=5). Recovery values are in parenthesis. Enzyme activity is expressed as U mg^{-1} protein where one unit is defined as micromoles of PNP formed per hour under specified assay conditions. *p<0.01, compared to control.
1: Total Acid phosphatase (prostate), (U mg^{-1}) protein
2: Tartarate resistant acid phosphatase (Prostate) (U mg^{-1}) protein
3: Alpha glucosidase (epididymis), (U mg^{-1}) protein 4: Fructose (Sem.V) (mg g^{-1}) tissue, 5: Citric acid (prostate), (mg g^{-1}) tissue

Table 6: Effects of *Spondias mombin* on semen parameters of rats treated for four weeks

Dose (mg kg^{-1} b.w.)	Sperm motility (%)	Viability (%)	Sperm count (10^6 mL^{-1})	Morphology (%)
Control	94.00±1.00	97.60±0.81	13.00±16.55	1.09±0.79
	(94.00±1.00)	(97.60±0.81)	(13.00±16.55)	(1.09±0.79)
8.4	94.20±3.55	167.80±6.82	43.70±19.82	4.25±0.97
	(95.60±0.68)	(97.20±0.58)	(80.93±15.32)	(1.04±0.12)
16.8	87.00±5.14*	93.20±3.71	15.50±50.00	3.96±0.54
	(96.20±0.58)	(96.60±0.68)	(24.58±18.13)	(1.27±0.05)
33.6	82.00±4.35**	93.00±0.95	10.21±13.79*	8.50±3.03**
	(94.12±1.70)	(95.00±2.42)	(24.63±41.32)	(1.77±0.10)

Values presented as Mean±SEM (n=5). Recovery values are in parenthesis. (*= p<0.05), (** = p<0.01)

Decreases in progressive motility, viability, count and morphology occurred in these rats within four weeks of treatment with the extract. Sperm count being one of the most sensitive tests for spermatogenesis is highly correlated with fertility[12]. Reduction in the viability of the sperm, could be connected with the reduction in progressive sperm motility, as immotile sperms are usually considered dead. This was supported by the fact that the dead sperms took up the eosin/nigrosin stain. The decrease in sperm counts and the high number of morphologically abnormal sperm indicates possible interference with testicular spermatogenesis. The presence of detached heads, curled tails and decrease in sperm motility and tissue α-glucosidase suggest that the epididymis also may be a target for *Spondias mombin* toxicity since this enzyme is involved in epididymal modification of the sperm cell membrane[13]. The extract might therefore be acting on the epididymal site acting as a spermatoxic agent on maturing or mature spermatozoa[14]. There was also no alteration in haematologic and biochemical parameters. The reproductive organ weights also remained unchanged when compared with the control. These effects show a specific action of *Spondias mombin* on spermatozoa, without untoward systemic activity. There was no mortality at the doses employed indicating that the extract has a wide margin of safety. *Spondias mombin* thus has a potential for development into an ideal male contraceptive agent.

It should be noted that different parts of *Spondias mombin* have been used as antibacterial and antihelminthic agents. There is a growing body of evidence implicating antibacterial agents in male infertility. For example, erythrosine[15], tylosine, a macrolide antibiotic agent[16,17] and penicillins[18] have been reported to cause infertility in man and animal species. These antibiotics were reported to impair spermatogenesis thereby inhibiting fertility. Moreover, many antimicrobial and antimalarial medicinal plants have been reported to possess antifertility actions. We have also reported the antifertility actions of *Quassia amara*[19,20], *Azadirachta indica*[10,21], *Morinda lucida*[22] and *Ricinus communis*[23]. It is therefore possible that there is a link between antimicrobial agents and male reproductive functions, which needs to be elucidated. Furthermore, the active principle in *Spondias mombin* extract with the male antifertility activities is not known. Further studies will attempt to identify this principle(s) and possibly explore the mechanisms by which the stem bark of this extract affects spermatogenesis and infertility in rats.

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