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Histomorphological Study of the Anti-Fertility Effect of *Spondias Mombin* L. In Adult Male Rats

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Abstract: *The effects of ethanol extract of Spondias mombin leaf on male rats' fertility were investigated. The extract was orally administered with 250 and 500mg/kg doses for 8 weeks. There was significant decrease in testicular and epididymal weight in the treated animals compared to the control. Histomorphology of the testis showed distortion in the arrangement of seminiferous tubules, loose germinal epithelium, low number of germ cells and Sertoli cells. Tubular sizes of epididymis were reduced with vacuolation and decreased sperm. The serum level of testosterone was significantly decreased ($p<0.05$) at 500mg/kg compared to control. We conclude that Spondias mombin leaf extract can suppress the process of spermatogenesis which can lead to infertility in laboratory animals.*

Keywords*: Epididymis, Infertility, Reduction, Spermatogenesis, Spondias mombin, Testis.*

I. Introduction

Medicinal plants are distributed worldwide, but they are most abundant in tropical countries [1]. It is estimated that about 25 per cent of all modern medicines are directly or indirectly derived from plants [2]. According Bodeker *et al,*[3], 65 to 80 per cent of the world's population, living in developing countries, depends mostly on plants for health care due to poverty and lack of modern medicine. Traditional medicines are now widely accepted due to its cultural acceptability, compatibility with the human body, effectiveness and less side effects [4]. In Africa, rural communities depend on medicinal plants as a source of primary health care due to the high cost and unavailability of synthetic drugs [5]. Contraception is important to health, development, and quality of life and has allowed couples to plan their families and safely space births. Several methods of contraception for family planning had been used over the years, however, due to the adverse effect associated with synthetic contraceptives, herbal plants have been investigated for their contraceptive potentials[6 -12]. Several plants and plant products are reported to impede various stages of testicular function in many animal species such as dogs, rats, humans and monkeys [13-17]. However, several more plants have been used by indigenous traditional medicines as a form of contraceptive including *Spondias mombin.* It is relied on for various herbal remedies for numerous conditions with almost every part of the tree being used; from its thick corky bark, to its leaves, fruits, and roots to even its flowers. The leaves are locally used for various digestive problems including stomachaches, diarrhea, dyspepsia, gastralgia, colic, and constipation. It has been reported as a medicinal plant with potentials that is valuable and a source of active drugs for treating diseases that has not been fully tapped [18].

Biological activities of the plant include; uterine stimulant actions [19-20]; smooth muscle relaxant actions [21]; uterine antispasmodic [22]; sedative and anticonvulsant actions, and anti-anxiety actions [23,24]; anti-inflammatory[25]. Raji *et al* [26] showed the antifertility action of aqueous Spondias mombin bark extract. However, leaves of *Spondias mombin* is used by traditional birth attendants in Southern Cross River as an infertility agent. The present study was designed to investigate the histological alterations of testicular and epididymal tissues through two doses of oral administration of *Spondias mombin* in male rats' reproductive system.

II. Materials and Methods

2.1 Plant material and extraction

Leaves of *Spondias mombin* were harvested from a community in Yakkur, Cross River. The leaves were washed to remove dirt and dried. An ethanolic extract was prepared using the cold extraction method [27]. The leaves were finely powdered and 500 g of this powder was soaked in 1L of 99.9% ethanol for 48 hours. The contents were filtered and ethanol was evaporated under reduced pressure in a rotary vacuum evaporator. This filtrate was dried at room temperature and dried mass was stored at 4°C. The yield of extract was 4.6% of the

starting raw material. Two different doses of 250 and 500 mg/ kg/ body weight/day were prepared from the stock solution by dissolving 4 g of the extract in 1000 ml of physiological saline.

2.2 Animals

Adult male rats of Wistar strain (n = 30), were used. They were housed in wooden cages with standard laboratory diet and water was given ad libitum. They were kept for two weeks to acclimatize and then weighed. The Institutional Animal Ethical Committee clearance was obtained before the commencement of study.

2.3 Dosage

The animals were randomly divided into three groups (n = 10). Group A served as controls, and were given normal saline. Animals in group B and C received different doses of extract orally (250 and 500 mg/kg respectively) for 8 weeks. The body weight of the rats was recorded weekly throughout the treatment period and also at the end of the treatment.

2.4 Termination of experiment

At the end of treatment, the animals were sacrificed under chloroform anesthesia. Blood was drawn through cardiac puncture and was centrifuged; plasma was separated and stored at -20°C for testosterone analysis. Serum testosterone concentration was measured by Enzyme immunoassay (EIA) kit (Microwell method of DIALAB). Testis and epididymis were dissected out, blotted free of blood and weighed. Vernier callipers were used to measure the length and width of testes and epididymis.

2.5 Histopathology

Testes and epididymis were fixed in Bouin's fluid and embedded in paraffin. Sections were cut at 5µm and stained with haematoxylin and eosin. Testis and epididymis were studied under light microscope, photomicrographs were taken.

2.6 Statistical analysis

Mean ± SEM of data of control and *Spondias mombin* treated groups were determined. The data was analyzed and compared by one way ANOVA. Level of significance was considered at $P < 0.05$.

III. Results

The present study investigated the influence of ethanolic extract of SpM leaves on testes and epididymis of Wistar rats for 8 weeks. Significant ($p<0.05$) decrease in the weight of testes and epididymis in treated rats were observed compared to control, and also a significant ($p<0.05$) difference within the treatment groups (Table 1). Serum testosterone level in rats treated with 250mg/kg and 500mg/kg of SpM were significantly ($p<0.05$) reduced compared to control, with the value of the high dose being most significantly lower than that of the low dose (Table 2).

Tunical thickness, Leydig cell diameter, seminiferous tubule diameter, epithelial height and Sertoli cell diameters were measured histometrically (Tables 3). Cross sections of control rat's testis revealed compactly arranged seminiferous tubules, with Sertoli cells found between spermatogenic cells. Irregularly shaped Leydig cells were also seen. The seminiferous tubules were observed to be undergoing spermatogenesis (Fig 1). Marked degenerative changes were observed in the testis of the experimental rats. Alterations were observed in the histological appearance of the seminiferous tubules, with damage to the basement membrane and scanty cytoplasm was observed in the treated rats that received 250mg/kg extract (Fig 2). Treatment with 500mg/kg extract resulted in necrotic changes in the seminiferous tubules, shrunken nuclei of germinal epithelium, and total arrest of spermatogenesis (Fig 3).

Decrease in tubular diameter of epididymis was recorded in the treatment groups compared to control. The decrease was significant ($P<0.05$) in the high dose. A non-significant ($P>0.05$) decrease in epithelial height was recorded in the treated rats' epididymis compared to control (Table 4). Cross section of control epididymis showed compactly packed tubules, lined with well defined pseudostratified epithelium (Fig 4). A dose-dependent impairment of epididymis was caused by extract treatment compared to control. The epididymal tubules in the low dose group showed hypertrophy of lumen and reduction of viable sperm cells (Fig 5). Epididymis of 500mg/kg ethanol extract showed thinning of epithelial lining and loss of spermatic elements (Fig 6).

Table 1: Effect of *Spondias mombin* extract doses on the mean testicular and epididymal weight and size in control and treated rats.

	Testes			Epididymis	
Group	Total weight	Total length	Total width	Total weight	Total length
Control	26.54 ± 2.48	16.26± 0.92	8.68±0.32	0.084±0.05	42.64±3.28
250mg/kg	18.98 ± 1.57[a]**	14.62±o.36[a]**	7.24±0.85 [a]*	0.062±0.07 [a]*	38.43±2.02 [a]**
500mg/kg	14.56 ±0.85 [a]**	10. 98±0.66 [a]** [b]**	5.68 ± 0.67 [a]* [b]**	0.058±0.09 [a]*	36.76±1.53 [a]** [b]**

Value (mean ± SEM) a=control vs 250mg/kg and 500mg/kg, b=250mg/kg vs 500mg/kg, * P<0.05, ** P<0.01, *** P<0.001.

Table 2: Serum testosterone levels in control and experimental rats.

Group	Testosterone concentration (ng/ml)
Control	8.20 ±0.91
250mg/kg	5.78±2.24 [a]**
500mg/kg	3.92±1.01 [a]*** [b]***

Value (mean±SEM) a=control vs 250mg/kg and 500mg/kg, b= 250mg/kg vs 500mg/kg, * P <0.05, ** P<0.01, *** P<0.001.

Table 3: Histometrical analysis of testes of control and male rats treated with extracts for 8 weeks.

Group	Tunical thickness (µm)	Epithelial height (µm)	Seminiferous tubule diameter (µm)	Leydig cell nuclear diameter (µm)	Sertoli cell nuclear diameter(µm)
Control	20.26±0.88	52.97±0.87	176.82±6.36	9.02±0.16	9.64±0.15
250mg/kg	16.31±0.23 [a]*	34.83±0.70 [a]***	162.50±4.34 [a]**	5.80±0.21 [a]**	7.83±0.29 [a]**
500mg/kg	12.48±0.57 [a]** [b]***	30.50±1.06 [a]*** [b]***	144.68±2.38 [a]*** [b]***	4.26±0.16 [a]*** [b]***	7.36±0.24 [a]*** [b]***

Value (mean±SEM) a=control vs 250mg/kg and 500mg/kg, b= 250mg/kg vs 500mg/kg, * P <0.05, ** P<0.01, *** P<0.001.

Table 4.Histometrical analysis of epididymis of control and male rats treated with extract for 8 weeks.

Group	Caput epithelial height (µm)	Caput tubule diameter(µm)	Corpus epithelial height (µm)	Corpus tubule diameter(µm)	Cauda epithelial height(µm)	Cauda tubule diameter(µm)
Control	28.63±o.50	120.06±2.68	26.44±0.95	148.68±1.50	20.26±0.64	160.02±4.62
250mg/kg	28.32±0.62	108.66±1.50[a]*	22.75±0.32 [a]*	146.80±4.63	19.32±0.53	158.60±4.24
500mg/kg	26.42±0.54	96.20±0.85 [a]*	20.16±0.48 [a]*	144.50±2.68 [a]*	18.26±0.68	155.71±3.75 [a]*

Value (mean±SEM) a=control vs 250mg/kg and 500mg/kg, * P < 0.05.

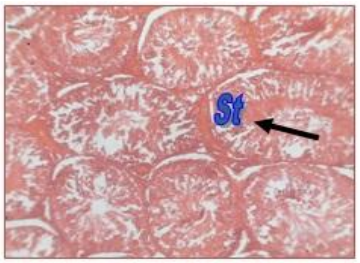

Fig 1: Testis of control animal showing well arranged seminiferous tubules showing alteration (St) and normal process of Spermatogenesis. (H & E X 400). (St), damaged basement membrane, loss of interstitial tissue (Is) and Leydig cells (L). (H & E X 400).

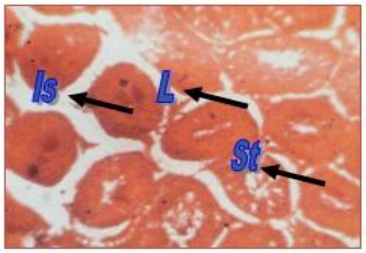

Fig 2: Testis of 250mg/kg of seminiferous tubules

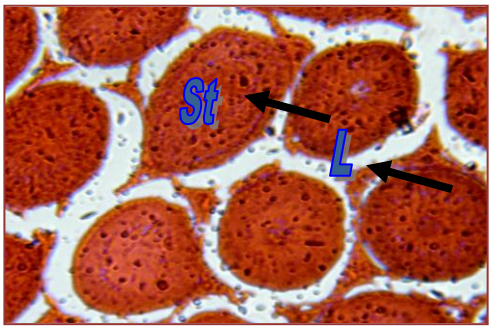

Fig 3: Testis of 500mg/kg showing necrotic seminiferous tubules compactly packed (St), shrunken nuclei , total arrest of spermatogenesis and total loss of Leydig cells (L), (H & E X 400).

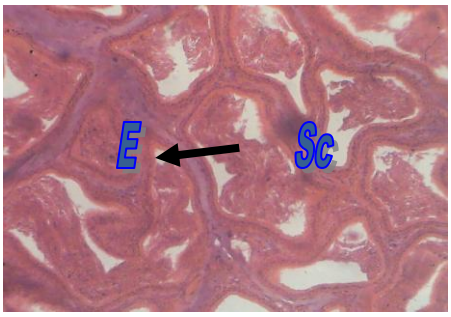

Fig 4: Epididymis of control showing tubules lined with well defined epithelium (E) and filled with sperm cells (Sc). (H & E X 400).

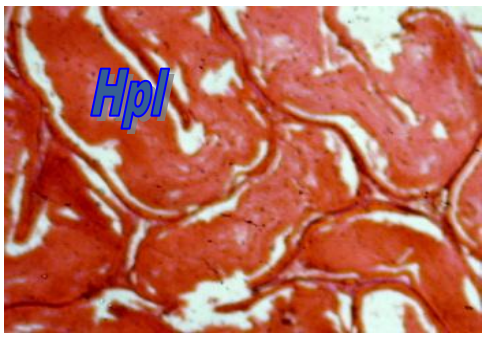

Fig 5: Epididymis of 250mg/kg showing hypertrophy of lumen (Hpl). (H & E X 400)

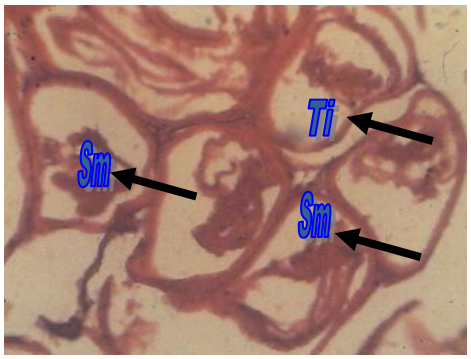

Fig 6: Epididymis of 500mg/kg showing thin epithelial lining (Tl)and loss of spermatic elements (Sm). (H & E 400).

IV. Discussion

Medicinal plants play a major role in health care irrespective of advances in modern medicine. These plants are distributed worldwide, although more abundant in tropical regions. Pharmaceutical companies have demonstrated interest in the investigation of higher plants as sources for new lead structures and for development of phytotherapeutic agents with proven efficacy, safety and quality [28-31]. Population explosion is one of the biggest challenges prevalent in third world countries, Nigeria not excluded, having severe consequences on every aspect of development such as employment, sanitation and environment, education, housing and health care. Majority of the population dwell in rural areas without any approach to modern methods of family planning thereby relying on herbal medicines to control population growth rate, such as inducing abortion, prevent conception and sterilization of either the couple. Literature abounds on research carried out on medicinal plants with antifertility effects [32-40]. The findings of the present study showed that ethanolic extract of *Spondias mombin* significantly altered the fertility potential of male rats. The significant decrease in the organ weights of the treated animals is indicative of the toxic effect of the extract. Decrease in organ weight after administration of a chemical agent has been reported by Simons *et al* [41] to be an indicator of toxicity. The high significant decrease observed in the weights of testis and epididymis of treated animals at 500mg/kg may be due to loss of spermatogenic elements in the testis and the absence of sperm in the epididymis. Several reports have shown degenerative changes in seminiferous tubules without a significant change in organ weight [42]. This contradicts our findings and may be due to the duration of extract administration in this present study.

Alterations of histological features were more pronounced at high dose of 500mg/kg; disruption in seminiferous tubular arrangement was observed with fewer Leydig cells present. Purohit and Dixit [43] had earlier reported alteration of Leydig cell function in rats treated with aqueous extract of *azadirachta indica*. Necrotic germ cells were found in the seminiferous tubules of high dose treatment which may indicate that treatment caused severe impediment in the spermatogenetic process.

Significant decrease was recorded in serum testosterone level in the treatment groups especially at high dose compared to control. This may be due to the deleterious effect on Leydig cell that may consequently be responsible for testicular and epididymal dysfunction as a result of androgen deprivation. This may in

essence affect the process of sperm production and maturation in both organs leading to loss of fertility in treated rats. Similarly, Dixit and Joshi [44] reported the effect of allium sativum on testicular function that led to sterility.

V. Conclusion

The present results suggest that administration of ethanolic leaf extract of Spondias mombin at 250mg/kg and 500mg/kg body weights caused impairment of testicular and epididymal structures, which led to significant decrease in spermatogenic activity in seminiferous tubules. Depletion of Leydig cells in tubular interstitial also caused reduction in serum testosterone level. Therefore, the process of maturation of spermatogenic cells and sperm production in the organs was affected by the extract administration which may lead to infertility in treated rats.

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