Original Research Article

Influence of Ethanolic Extracts of *Spondias Mombin* (*Anacardiaceae*) Leaves on Pituitary- Gonadal Axis of Male Wistar Rats.

ABSTRACT

Background: *Spondias mombin* is one of the tropical plants used locally to treat various kinds of ailment, its use as an anti-conceptive remedy in our locality had been reported.

Objective: The aim of this study was to establish a dose-dependent or duration effect of ethanolic leaf extract of *Spondias mombin* on the anterior pituitary cells, testes and epididymides of Wistar rats of Wistar rats.

Materials and methods: A total of thirty (30) matured male Wistar rats were randomly divided into five groups (n=5). Group 1 animals served as control and received vehicle (distilled water). Groups 2 and 3 were administered with 250mgkg^{-1} body weight of extract for 4 and 6 weeks respectively, while groups 4 and 5 received 500mgkg^{-1} body weight of extract for 4 and 6 weeks also. Animals were anaesthetized with chloroform and sacrificed at the end of administration. Body weight, weights of reproductive organs and vital organs were evaluated. Blood was taken from the animals for haematological and biochemical analysis. The pituitary gland, male reproductive and accessory glands were excised and fixed in 10% formalin for routine histological examination.

Results: The influence of ethanolic extract of *Spondias mombin* leaves on the pituitary cells and reproductive organs of male Wistar rats given 250 and 500mgkg^{-1} body weight for 4 and 6 weeks showed loss of cytoplasmic contents and free spaces of pituitary cells, desquamation of seminiferous epithelial cells, degradation of seminiferous tubules and reduction in cells. The epididymis of the test groups showed abundant immature cells and cell debris in their lumen. The accessory glands showed homogenous pinkish fluid, glandular degeneration of the prostate and seminal vesicles with decreased structural integrity. The organ weights of the experimental animals were not significantly affected,

however, a significant (P<0.05) decrease in reproductive organ weights was recorded. Ethanolic extract of *Spondias mombin* on liver enzymes showed a significant protection against hepatobiliary damage.

Conclusion: These results suggest that *Spondias mombin* has a dose dependent and duration deleterious effect on the pituitary and reproductive organs at their cellular levels rather than on the tissue as a whole.

Keywords: Accessory glands, Cells, Degradation, Epithelium, Pituitary, Reproduction

1. INTRODUCTION

Medicinal plants still play major roles in health worldwide irrespective of the advances recorded in orthodox medicine. Interest in drugs derived from higher plants has increased considerably in the past few years with about 20-25% of modern drugs being derived from plants [1-2]. An expression in the biodiversity in number had been carried out with an estimate of 10-100 million species, out of which 2.5-7.5 million to be attributed to plants [3]; 5-105 of these plants have been scientifically evaluated for their therapeutic medicinal properties [4]. Plants have been used and are still in use in most developing countries as their main source of health care [5-6]. These plants are assumed to be safe and free from side effects since they are naturally occurring [7-8]. However, studies have shown that medicinal plants may be toxic [9-12]. These adverse effects are however less frequent when used properly in comparison to synthetic medicines [13]. A lot of these herbal plants used to treat or cure diseases locally have been found to be anticancer [14-16], anti-malarial [17-18], anti-diabetic [19-20]' antihypertensive [21-22], antibacterial [23], antimicrobial [24-25], antifertility [26-27], abortifacient [28-30] etc. These claims were first made by rural users which have been authenticated by biological research. *Spondias mombin* (SpM) is one of such plants used locally to treat various kinds of ailment which biological research has supported [31-37]. We had earlier reported on scientific findings on *Spondias mombin* [38-40]. The present study was carried out to further investigate the effect of *Spondias mombin* on pituitary cells and reproductive organs of male Wistar rats based on duration of administration.

2. MATERIAL AND METHODS

The harvesting and extraction of plant material had earlier been reported in our previous work [40]. A total of thirty (30) mature male Wistar rats (6 weeks old) were randomly divided into five groups (n=5). Rats were kept in temperature controlled room of 25 ± 2°C with a 12-hour light/dark cycle under hygienic conditions and had free access to water & rat chow. The animals were acclimatized for seven days before experimental use. Ethics on the use of laboratory animals was applied and care of the animals was in accordance to the International guidelines for animal research. The methodology was approved by the Department of Human Anatomy ethical committee. Group 1 animals served as control and received vehicle (distilled water). Groups 2 and 3 were administered 250mgkg^{-1} body weight of extract for 4 and 6 weeks respectively, while groups 4 and 5 received 500mgkg^{-1} body weight of extract for 4 and 6 weeks also. The administration of extract was through oral route with the aid of an orogastric tube. Animals were anaesthetized with chloroform and sacrificed at the end of administration. Body weight, weights of reproductive organs and vital organs were evaluated. Blood was taken from the animals for haematological and biochemical analysis. The pituitary gland, male reproductive and accessory glands were excised and fixed in 10% formalin and later processed for histological examination. The pituitary gland was double stained using the bromine alcian blue-orange fuschin green (Br.AB-OFG) method of Slidders [41] to demonstrate anterior pituitary cells.

2:1 Statistical analysis

Data were expressed as Mean ± S.E.M. Statistical analysis was carried out by one-way analysis of variance (ANOVA) with significance expressed as $P< 0.05$.

3. RESULTS AND DISCUSSION

The mean vital organs weight was not affected by the administration of leaf extract of SpM for 4 and 6 weeks (Table 1). However, the reproductive organ weights were significantly ($P<0.05$) reduced at 6 weeks in animals administered with 250 and 500 mgkg^{-1}, irrespective of a non- significant change in body weights across the five groups (Table 2). The identification of the possible harmful effects of chemical and drugs is the analysis of organ weight [42]. In this study, the organ weights and body weights of the experimental groups were not different from that of the control which points to earlier findings of the safety use of SpM extracts [43].

However, reproductive organ weights were affected by extract of SpM which is indicative of the shrunken characteristics observed on histopathological examination of the tissues. The red blood cell counts were increased significantly ($P<0.05$) in groups 3 and 5 which received 250 and 500 $mgkg^{-1}$ for 6 weeks. Similarly, values for haemoglobin also significantly increased ($P<0.05$) in these groups. Values of ALP reduced in groups 3 and 5 (Table 3). Enzymes have been reported to be found in tissues and blood as a result of insult to the cell or from degraded cells [44]. Activities of liver enzymes are determined in serum as indicators of biochemical changes which occur in response to treatment [45]. It had been stated that aminotransferases (ALT and AST) are indicators of hepatotoxicity and hepatocellular damage, while ALP is used in diagnosing hepatobiliary or cholestatic obstruction [46]. ALP is cardinally involved in the transport of metabolites across cell membranes, synthesis of proteins, secretory activities and glycogen metabolism [47]. The significant ($P<0.05$) decrease observed in ALP activity may imply protection against hepatobiliary damage, since most enzymes measured as indices of drug metabolism are released into bloodstream when cells are damaged or their functions are disrupted.

Pituitary cells of control animals were well stained, normal and numerous on histological examination, whereas cells of experimental animals were sparse with loss of cytoplasmic contents. The effect was more in groups treated for 6 weeks recording greater loss of cytoplasmic contents and free spaces (Figure 1a-e). The testes on histopathological examination showed seminiferous tubules of control possessing epithelia with well-defined Sertoli cells and germ cells at various stages of spermatogenesis. Sertoli cells showed distinct granular cytoplasm and irregular nuclei. Lumen of seminiferous tubules contained mature sperm and numerous Leydig cells in the interstitium (Figure 2a). Groups 2-5 animals showed dose and time duration dependent alteration on the testes evidenced by distortion of tubular cells, prominent spaces and severe structural disorganization (Figure 2b-e).

Epididymis of experimental groups showed thinness of epididymal epithelial lining compared to control with their lumen showing cell debris, large number of immature cells and degenerated cells (Figure 3a-e). The lumen of the prostate of experimental animals showed pinkish fluid and inflammatory cells (Figure 4a-e). The presence of debris in the lumen of the epididymis may be a reflection of degenerated testicular assault observed in the treated rats. This lesion may probably have been passed to the epididymis. Thus, it is safe to

deduce that extract of SpM has defective effect on the germ cells. The observed effect of the extract on the accessory sex gland may also be as a result of its destructive tendency on testicular tissue that led to a decrease testosterone production [48]; since decrease in testosterone production has been observed to have negating effect on accessory sex glands [49]. Therefore, it is safe to state that the low testosterone reported in our earlier work [48] may be responsible for the effect of the extract on the accessory sex glands since male accessory sex glands are known to depend on male sex hormone for development and secretory activity [50].

Table 1: Weight of vital organs of control and experimental SpM extract treated rats.

Parameters (g)	**Groups**				
	1	2	3	4	5
Brain	1.85±0.25	1.82±0.05	1.77±0.14	1.73±0.23	1.72±0.28
Heart	0.82±0.05	0.76±0.02	0.72±0.03	0.70±0.06	0.68±0.24
Lungs	1.56±0.30	1.54±0.35	1.48±0.20	1.49±0.30	1.46±0.28
Thyroid	0.060±0.002	0.060±0.002	0.050±0.002	0.048±0.006	0.046±0.006
Kidney	1.80±0.28	1.78±0.22	1.72±0.29	1.70±0.20	1.70±0.24
Liver	8.26±0.80	8.00±0.78	7.95±0.80	7.64±0.75	7.62±0.82
Adrenal	0.180±0.002	0.178±0.002	0.178±0.002	0.176±0.002	0.173±0.002
Spleen	0.62±0.20	0.60±0.27	0.60±0.18	0.58±0.22	0.55±0.21

Values are Mean ± SEM, n=5. Extract had no significant effect on the weights of vital organs.

Table 2: Body and reproductive organ weights in control and treated rats.

Parameters (g)	**Groups**				
	1	2	3	4	5
Body weight	210±2.42	208.7±3.42	214.0±2.07	204.5±3.57	210.0±1.82
Testis	3.20±0.75	2.75±0.43	1.43±0.36*	2.44±0.23*	1.26±0.15*
Epididymis	2.75±1.36	2.55±1.80	1.89±0.82*	2.24±1.08*	1.54±0.62*
Seminal vesicle	3.75±1.10	2.92±0.34	1.83±0.33*	1.72±0.64*	1.46±0.73*
Prostate	1.92±0.79	1.63±0.64	1.42±0.64*	1.26±0.48*	1.08±0.84*

Values are Mean ± SEM, n=5. *P<0.05 compared to control. Extract showed a significant effect on the weights of reproductive organs compared to body weights of animals where no significant effect was recorded.

Table 3: Haematological and biochemical parameters of control and SpM extract treated rats.

Parameters	Groups				
	1	2	3	4	5
RBC (10^4/µL)	7.44±0,38	7.93±0.22	8.82±0.12*	8.72±0.10*	8.85±0.23*
WBC (10^3/µL)	19.58±3.50	18.78±1.40	16.64±2.10	19.32±1.96	18.42±2.46
HB (g/dL)	11.68±0.59	12.20±0.27	15.42±0.21*	13.28±0.26*	18.38±1.05*
PCV (%)	45.78±2.64	49.74±1.78	47.16±0.62	54.30±1.45*	47.58±0.94
ALP (µL)	27.05±1.77	17.70±0.36*	0.66±0.03*	12.96±1.06*	0.19±0.07*
AST (µL)	373.42±47.45	294.88±17.07*	447.01±8.05*	218.41±50.03*	442.67±14.75*
ALT (µL)	170.76±9.66	154.15±6.31	171.56±6.95	151.78±4.17*	171.62±2.14
TP(mg/L)	0.51±0.13	0.40±0.06	0.64±0.08	0.27±0.03	0.59±0.13
ALB (mg/L)	5.31±0.74	3.90±1.58	1.79±0.34*	2.73±0.93*	1.58±0.48*
CRT (µmol/l)	2.06±0.28	2.92±0.59	2.77±0.79*	2.74±0.64	3.95±0.73*
UREA (mg/L)	32.08±0.93	29.72±1.42	16.03±0.71*	34.86±1.87	14.50±3.83*

Values are Mean ± SEM, n=5. P<0.05

RBC: Red blood cell WBC: White blood cell HB: Hemoglobin PCV: Packed cell volume
ALP: Alkaline phosphatase AST: Aspartate aminotransferase ALT: Alanine aminotransferase
TP: Total protein ALB: Albumin CRT: Creatinine

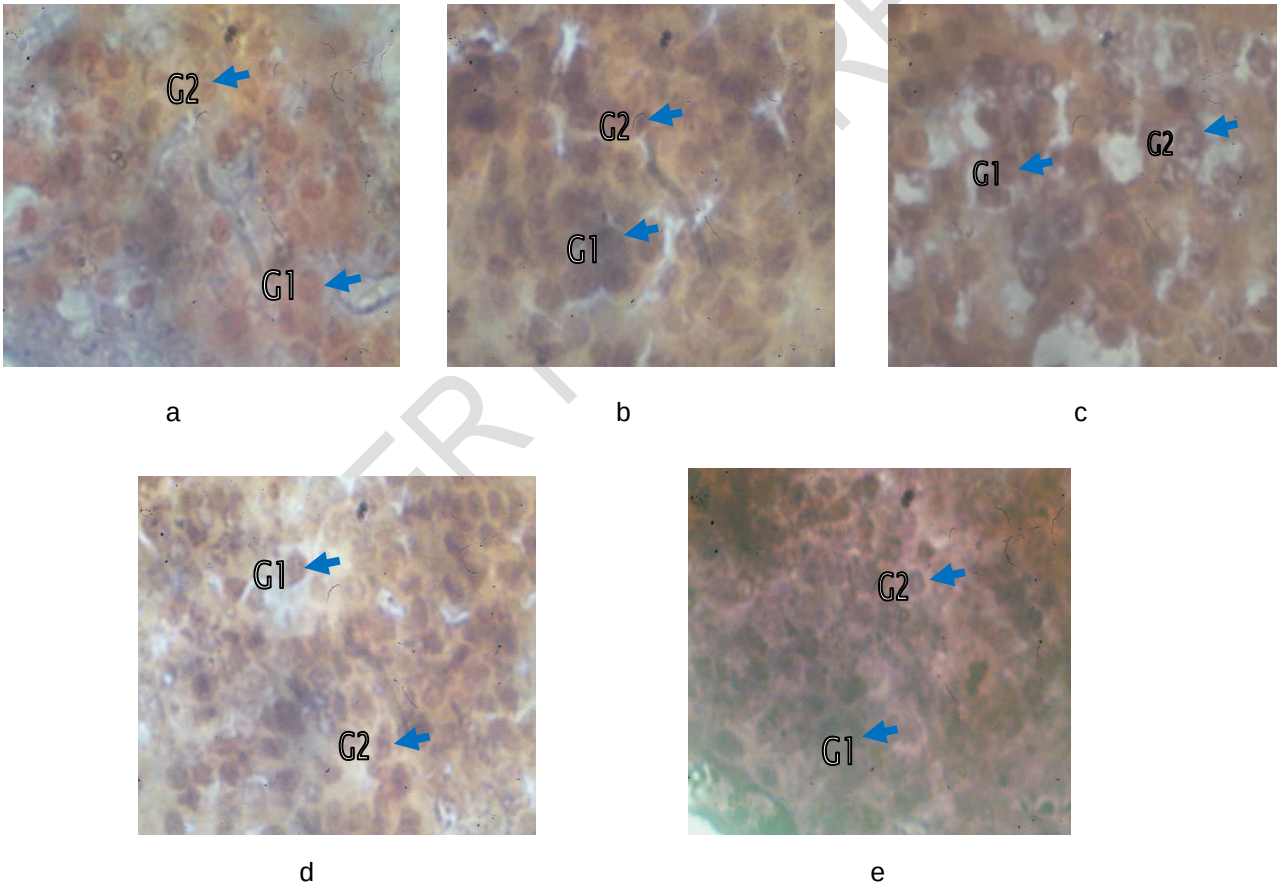
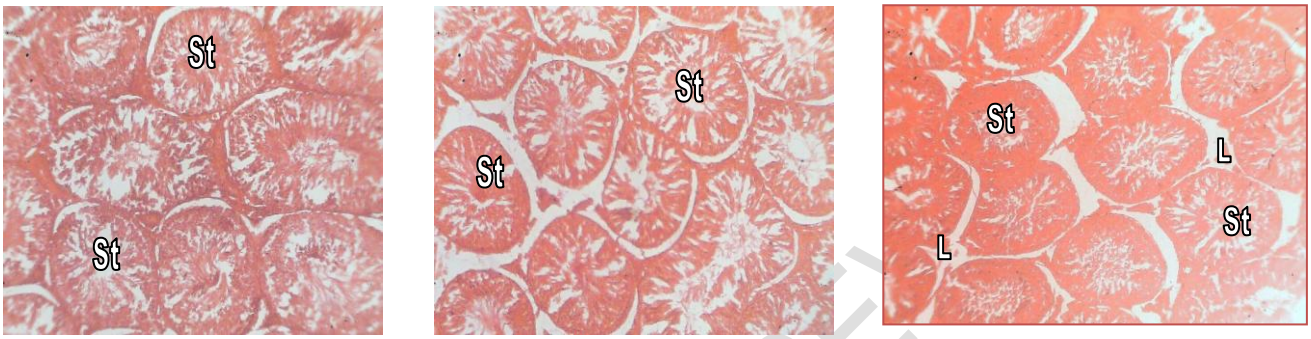


Fig 1: Photomicrographs of anterior pituitary of control and experimental animals treated with 250mgkg^{-1} and 500mgkg^{-1} ethanolic extract for 4 and 6 weeks (Br. AB/OFG X 400).

a. Anterior pituitary of control showing normal gonadotrophs FSH (G_1) and LH (G_2) respectively.

b. Anterior pituitary of 250mg/kg ethanol extract treated for 4 weeks showing hypertrophied gonadotrophs FSH (G_1) and LH (G_2).
c. Anterior pituitary of 250mg/kg ethanol extract treated for 6 weeks showing regressed gonadotrophs FSH (G_1) and LH (G_2).
d. Anterior pituitary of 500mg/kg ethanol extract treated for 4 weeks showing hypertrophied gonadotrophs FSH (G_1) and LH (G_2).
e. Anterior pituitary of 500mg/kg ethanol extract treated for 6 weeks showing regressed gonadotrophs FSH (G_1) and LH (G_2) with loss of cytoplasmic contents.



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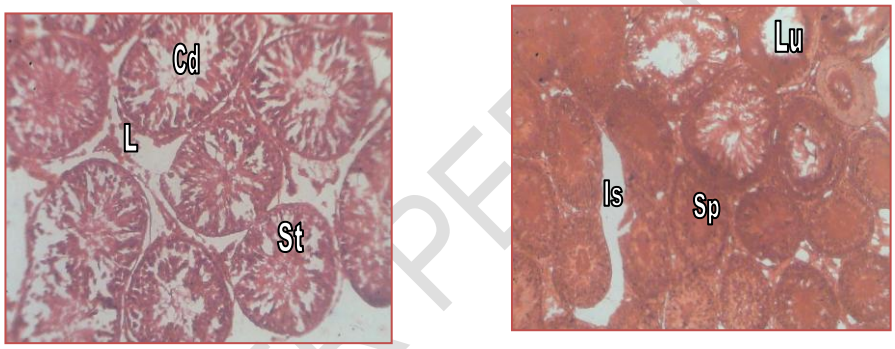

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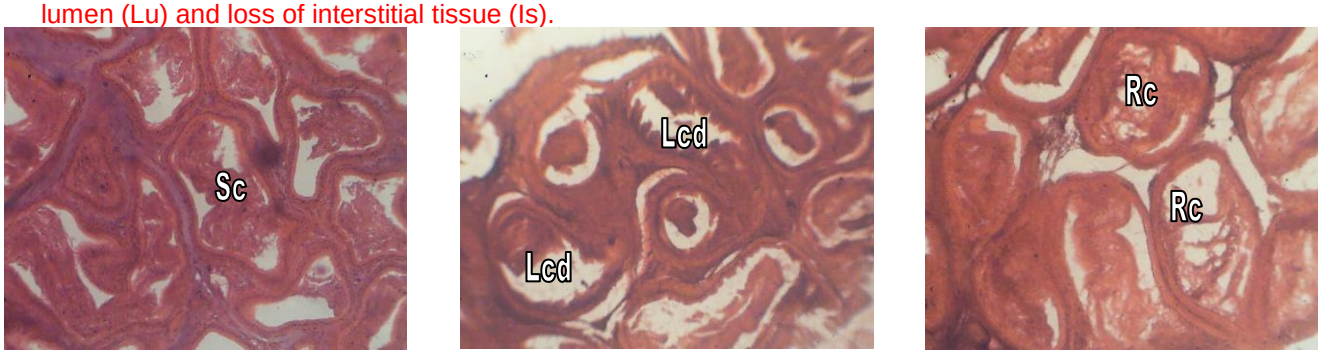
Fig 2: Photomicrographs of testis of control and experimental animals treated with 250mgkg^{-1} and 500mgkg^{-1} ethanolic extract for 4 and 6 weeks (H & E X 400).

a. Testis of control animal showing well arranged seminiferous tubules (St) and normal process of spermatogenesis.
b. Testis of 250mg/kg ethanol extract treated for 4 weeks showing loosely arranged seminiferous tubules (St).
c. Testis of 250mg/kg ethanol extract treated for 6 weeks showing shrunken seminiferous tubules (St) and loss of Leydig cells (L).
d. Testis of 500mg/kg ethanol extract treated for 4 weeks showing distorted seminiferous tubules (St), loss of Leydig cells (L) and cell debris (Cd).
e. Testis of 500mg/kg ethanol extract treated for 6 weeks showing arrest of spermatogenesis (Sp), empty lumen (Lu) and loss of interstitial tissue (Is).



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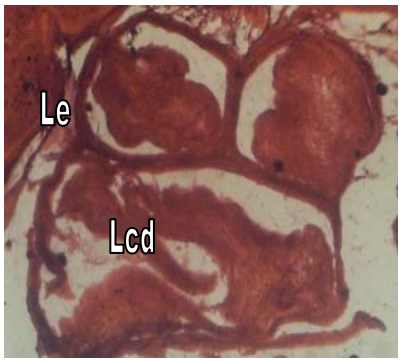

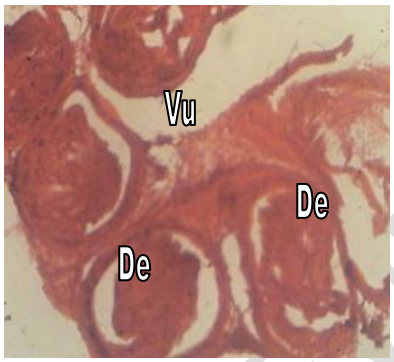

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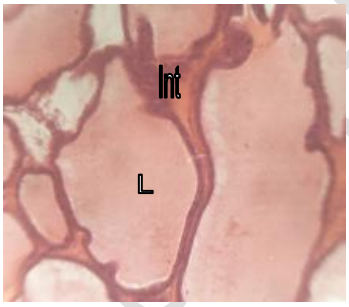
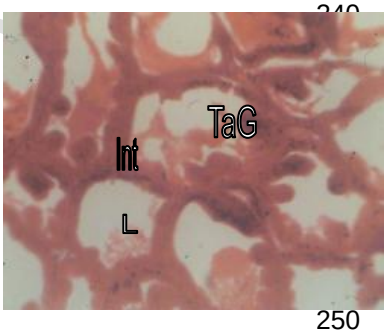
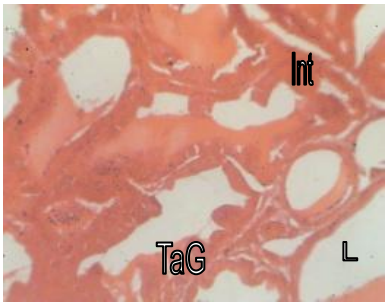
Fig 3: Photomicrographs of epididymis of control and experimental animals treated with 250mgkg^{-1} and 500mgkg^{-1} ethanolic extract for 4 and 6 weeks (H & E X 400).

a. Epididymis of control animal showing tubules filled with sperm cells (Sc).
b. Epididymis of 250mg/kg ethanol extract treated for 4 weeks showing lumen containing cell debris (Lcd).
c. Epididymis of 250mg/kg ethanol extract treated for 6 weeks showing regressive changes (Rc).
d. Epididymis of 500mg/kg ethanol extract treated for 4 weeks showing loss epithelium (Le) and cell debris (Lcd) in lumen.
e. Epididymis of 500mg/kg ethanol extract treated for 6 weeks showing distortion of epididymal tissue (De) and presence of vacuoles (Vu).







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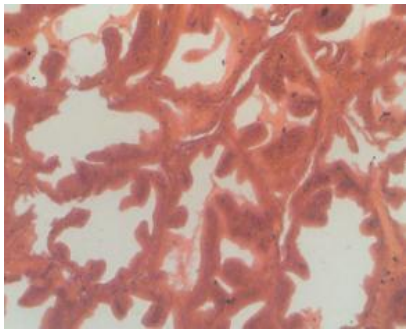

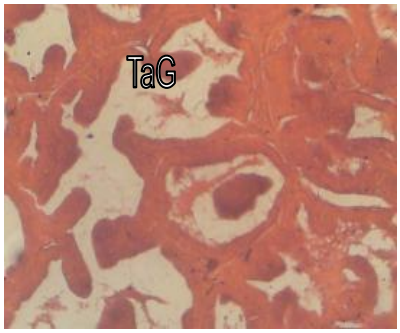

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Fig 4: Photomicrographs of prostate gland of control and experimental animals treated with 250mgkg^{-1} and 500mgkg^{-1} ethanolic extract for 4 and 6 weeks (H & E X 400).

a. Prostate of control animal showing normal architecture with well defined interstitial tissue (Int) and lumen (L) filled with prostatic secretions.
b. Prostate of 250mg/kg ethanol extract treated for 4 weeks showing lumen (L) with less secretions, thinned out interstitial tissue (Int) and tubuloalveolar glands (TaG) .
c. Prostate of 250mg/kg ethanol extract treated for 6 weeks showing regressive changes in cytoarchitecture.
d. Prostate of 500mg/kg ethanol extract treated for 4 weeks showing changes in the shape of glands (TaG), wider and empty lumen (L).
e. Prostate of 500mg/kg ethanol extract treated for 6 weeks showing distortions of gland (TaG), lumen (L) and interstitial tissue (Int).

4. CONCLUSION

This study concludes that the effect of extract of SpM is dose and duration dependent with its effect localized to the pituitary and male reproductive system which supports its use locally to stall conception in male. The mechanism through which this is mediated is not known. Further research will be based on the mechanism through which SpM mediate this action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

Approval was given by the Faculty of Basic Medical Sciences Committee on animal use and care, Universiity of Calabar to carry out this research work following laid down rules and guidelines of the institution in the use of medicinal plants and animal models.

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