IJMS
Vol 36, No 4, December 2011

Original Article

Histomorphological Alterations in the Prostate Gland and Epithelium of Seminiferous Tubule of Sprague-Dawley Rats Treated with Methanolic Extract of *Momordica charantia* Seeds

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Abstract

Background: There is yet a dearth of literature on the antifertility effect of *Momordica charantia* on the male reproductive system. The aim of this study was to determine the effect of graded oral doses of methanolic seed extract of *Momordica charantia* on the histology of prostate gland and seminiferous tubules of rats.

Methods: Forty male Sprague-Dawley rats, weighing 176±7 g were assigned randomly into four main groups A to D of 10 rats per group. Groups A to C received daily oral doses of15, 25 or 50 mg/100 g body weight of the seed extract for 56 days. Group D (control) received physiological saline. In each group, five rats were sacrificed on day 57, the remaining half on day 113 (56 days after withdrawal of the extract). The testes and prostate were processed for histological examination.

Results: There was a dose-related alteration in the cytoarchitecture of seminiferous tubules with marked reduction in spermatogenic series. The prostate gland showed dilatation as well as increased intraluminal secretions with increasing dose. Moreover, there was a significant recovery of prostate tissue as the sections were similar to their control counterpart.

Conclusion: the findings of the present study indicate that methanolic extract of *Momordica charantia* seeds caused reversible histological alterations in the prostate and testes of Sprague-Dawley rats.

Iran J Med Sci 2011; 36(4): 266-272.

Keywords ● *Momordica charantia* ● Sprague-Dawley rats ● prostate ● testes ● seminniferous tubules

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Received: 8 January 2011
Revised: 11 May 2011
Accepted: 19 June 2011

Introduction

There is currently a shift in the consumption of synthetic formulations to medications prepared from natural product. The insufficiency of current therapies to combat some ailments, combined with the lack of trust in conventional remedies and an inability of the economy to afford the cost of synthetic medicines accounts for the growing public interest in the natural products.[1,2] There has also been a great fascination for herbal medicines and dietary food supplements in the developed countries, since they are believed to possess minimal side effects.[3]

In the same vein, there has been a rise in the incidence of male infertility today as supported by growing evidence from clinical and epidemiological studies.[4,5] Earlier investigations have, however, implicated certain locally-consumed ethnopharmacological preparations/extracts as a positive source of environmental toxicants that may contribute to decline in male fertility.[6] Some other etiological aspects are still under investigation, and the knowledge of exogenous factors affecting the male reproductive system still limited.

Momordica charantia (MC) is a plant that has gained popularity in recent years. It is widely consumed in about 8-10 countries and at least in two continents as an ethnopharmacological preparation.[7] It is indicated in the management of diabetes mellitus, and several other ailments in these countries.[7-10] Its' blood sugar lowering property is perhaps the best-researched property of the plant.[7] It is known to improve glucose tolerance to a degree similar to the conventional oral hypoglycemic agent, tolbutamide.[11]

Although the application of MC to illnesses is universal, there are gaps in our knowledge in regards to the understanding of how it affects the testes, as indicated by paucity of literature. The general objective of the present study was, therefore, to evaluate the effect of the crude methanol extract of the dried seed of MC on the male reproductive system of Sprague-Dawley rats.

Materials and Methods

The ripe fruits of MC, harvested in month of June, were purchased from the local market in Lagos Nigeria. It was authenticated by Professor J. Olowokudejo, a taxonomist in the Botany Department of the University of Lagos, where the voucher specimen was deposited (Voucher number FHI 108422).

Preparation of Seed Extract

The seeds were dried in an oven (temperature of between 30–40°C) for a week. The dried seeds were weighed, and Soxhlet extraction done using absolute methanol. Water was used as solvents for the preparation of the various concentrations. Experiment was carried out at the Pharmacognosy Department of Faculty of Pharmacy, University of Lagos. The percentage yield was 23.0% w/w. The doses (15, 25 and 50 mg/100 g body weight) were administered orally.

Sources and Maintenance of Rats

Forty male Sprague-Dawley rats (6–8 weeks old) weighing 176±70 g were used for this study. They were randomly divided into four main groups of A, B, C and D of 5 rats each. Animals in each main group were further divided into two sub-groups including A_1, A_2, B_1, B_2, C_1, C_2, D_1 and D_2. The rats were procured from the Animal House of the College of Medicine University of Lagos, and authenticated at the Zoology Department of the same University. They were kept in well-ventilated metal cages in the animal room of the Department of Anatomy College of Medicine University of Lagos under normal standard conditions of a temperature between 35–37°C and a 12:12 photoperiodicity. The animals were weighed at procurement and at weekly interval. They had access to rat chow and water *ad libitum*. The animals were left to acclimatize for two weeks.

Experimental Protocol and Necropsy Schedule

The animals in groups A (A_1, A_2), B (B_1, B_2) and C (C_1, C_2) were treated for 56 days with 15, 25 and 50 mg/100 g body weight/day of MC seed extract, respectively. The group D (D_1, D_2) was used as control and given equal volumes of physiologic saline for 56 days. A metal canula was used for the oral administration by gastric gavages, which was done between 13.00–16.00 hours daily.

The rats in sub-groups A_1, B_1, C_1 and D_1 were sacrificed on the 57th day, while those in sub-groups A_2, B_2, C_2 and D_2 were allowed to recover for 56 days, and sacrificed on day 113.The animals were sacrificed by cerebral dislocation, following which a ventral was laparotomy done. The testes and ventral prostate were procured and processed for histology.

Tissue Processing for Histological Studies

The harvested organs were carefully dissected out, and trimmed of fat and connective tissue. The tissues were processed by the method described below with slight modification.[12] The steps involved in tissue processing included fixation, dehydration, clearing, infiltration, embedding, blocking, sectioning, and staining. The tissues were fixed in 10% formaline, and then transferred to a graded series of ethanol (50%, 70%, 90%, absolute alcohol), and cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and made into blocks of wax. Microtome whose sectioning size knob was adjusted to five µm thick was used to section the block. The sections were fixed on clean slides and later stained with hematoxylin and eosin.

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals,[13] and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

Results

Microscopic sections of prostate showed intergroup variations including, varying degrees of dilatations of the prostatic gland as well as of their intraluminal secretions (figures 1-4). There appear, however, to be an increased dilatation resulting in crowding of the glands in those given doses of 25 and 50 mg/100 g body weight of the extract. A lesser degree of crowding and dilatation than that of the control was seen in those given 15 mg/100 g of the extract.

Microscopic sections of testes showed that the seminiferous tubules of the control had regular cytoarchitecture with all cells of the spermatogenic series represented (figures 5-8). The tubular lumen showed numerous spermatozoa. The cellular interstitium revealed normal interstitial cells. The testes of rats treated with 50 mg/100 g of the extract revealed a marked reduction in spermatids and spermatozoa in about 20% to 30% of tubules. Less than 10% of tubules were similarly affected in the group given 25 mg/100 g of the extract compared to rats in the control group or those receiving 15 mg/100 g.

There was no difference between microscopic sections of testes or prostate of all groups 56 days after the discontinuation of treatment with the extract (figure 1-8).

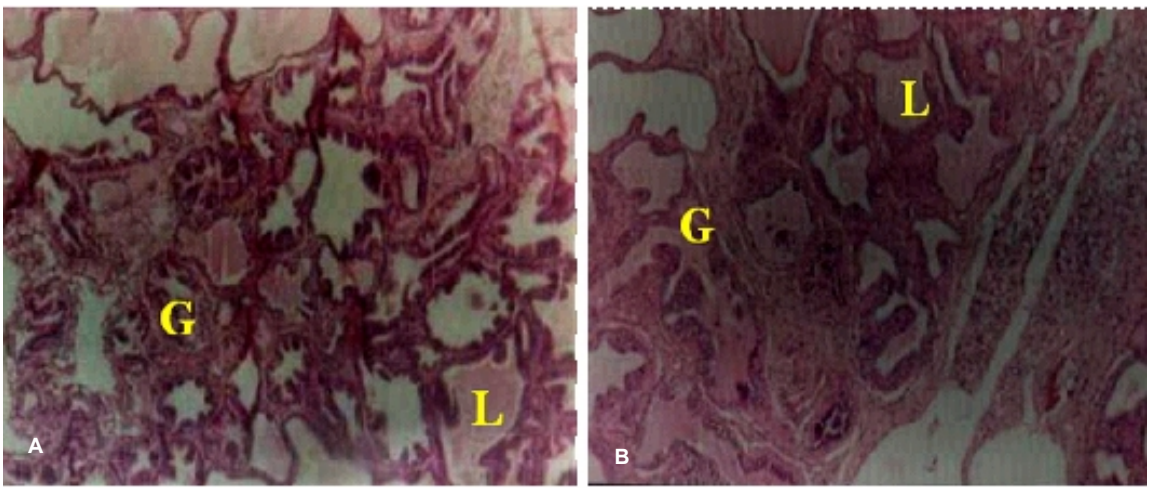

Figure 1: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the prostate of control rats (receiving normal saline) sacrificed at the end of 8 weeks (a) and 16 weeks (b). L=lumen of gland; G=prostate gland

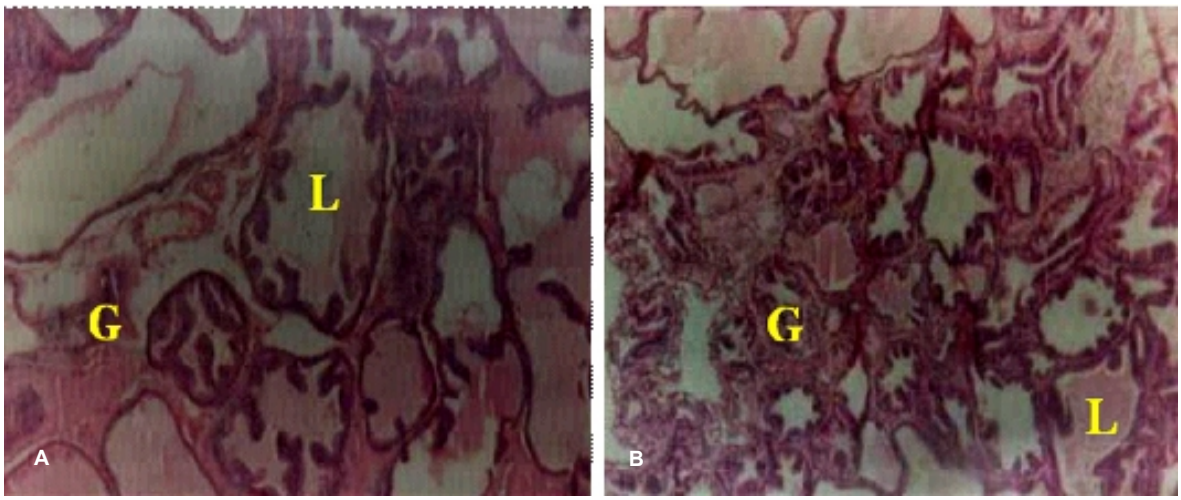

Figure 2: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the prostate of rats treated with 15 mg/100 g of Momordica charantia seed extract for eight weeks (a) and the extract for eight weeks followed by physiological saline for eight weeks after withdrawal of extract (b). L=lumen of gland; G=prostate gland

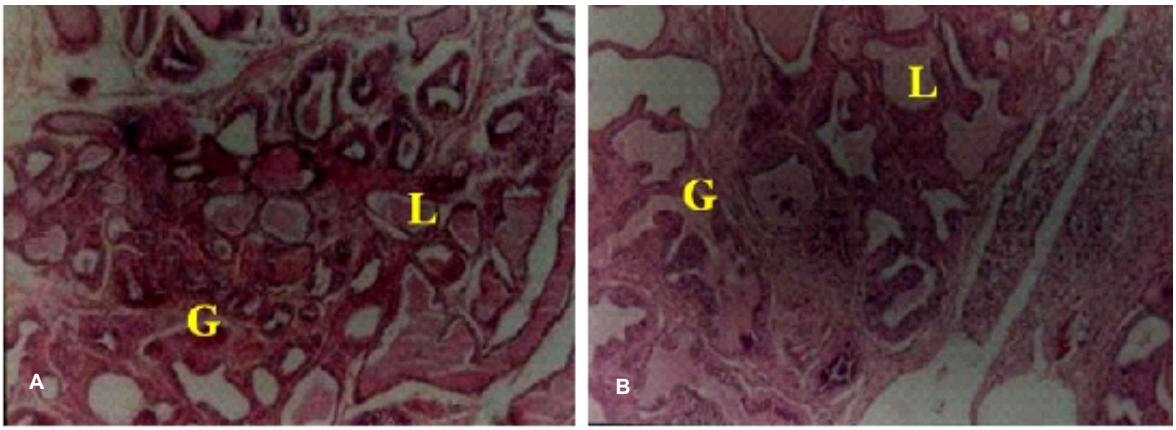

Figure 3: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the prostate of rats treated with 25 mg/100 g body weight of Momordica charantia seed extract for 8 weeks (a) and the extract for eight weeks followed by physiological saline for eight weeks after withdrawal of the extract (b). Stains: Haematoxylin & Eosin. Mag. x100; L=lumen of gland; G=prostate gland

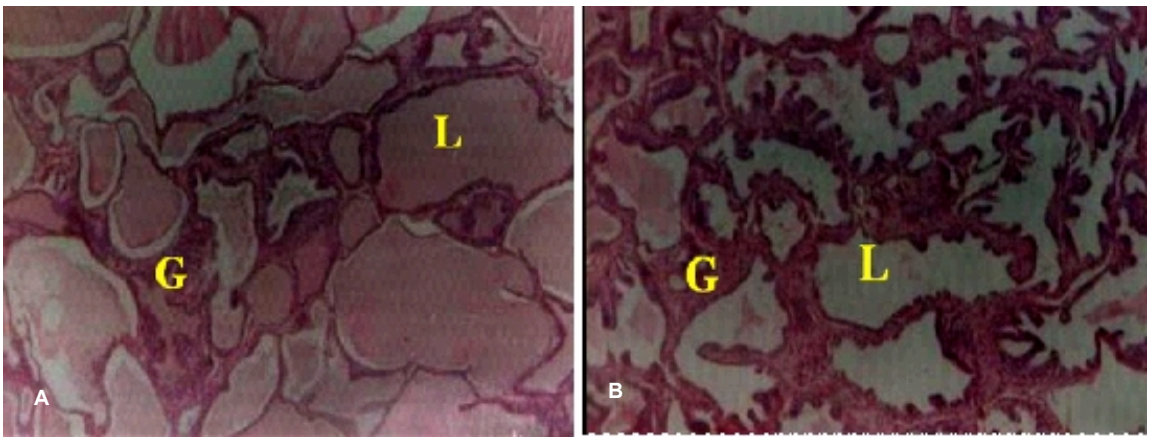

Figure 4: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the prostate of rats treated with 50 mg/100 g of Momordica charantia seed extract for 8 weeks (a) and the extract for eight weeks followed by physiological saline for eight weeks after withdrawal of the extract (b). L=lumen of gland; G=prostate gland

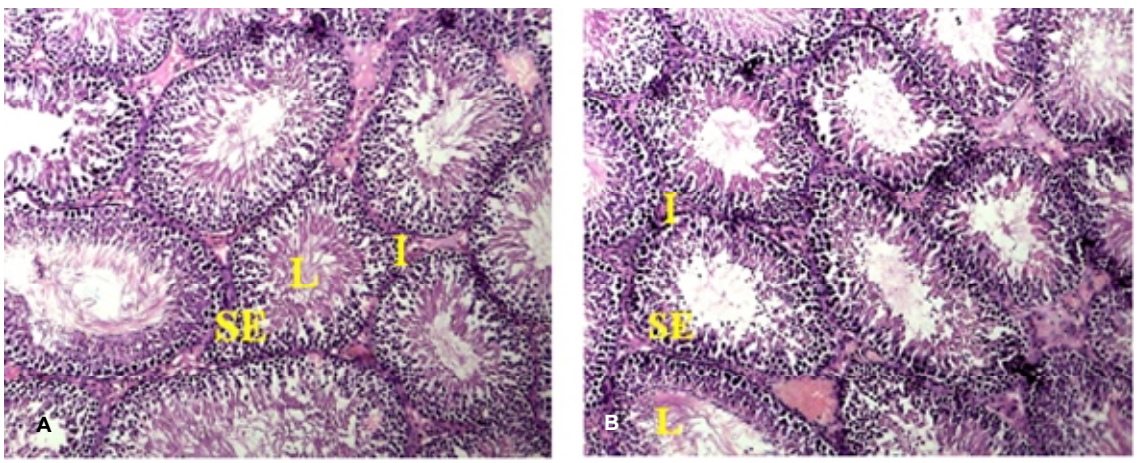

Figure 5: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the seminiferous tubules of control rats (receiving normal saline) sacrificed at the end of eight weeks (a) and 16 weeks (b). L=lumen of seminiferous tubule; SE=seminiferous epithelium; I=testicular interstitium seminiferous epithelium; I=testicular interstitium

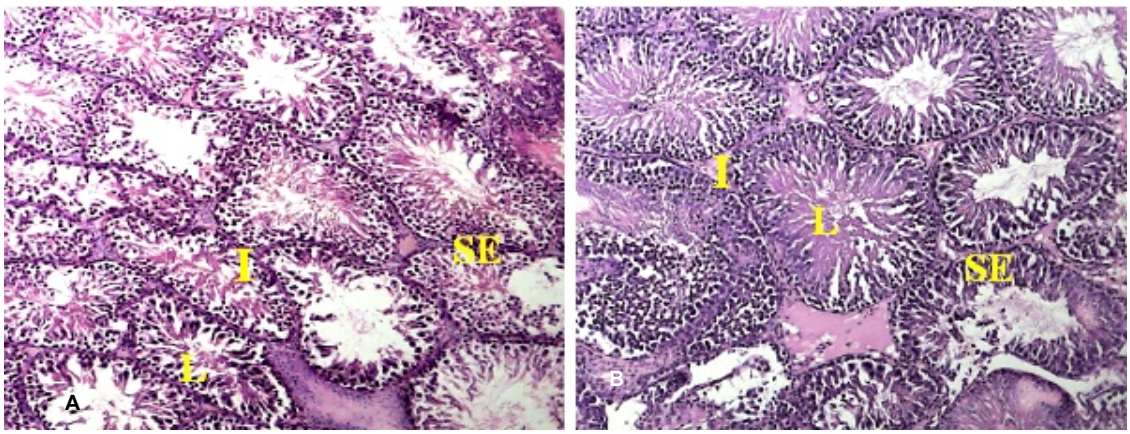

Figure 6: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the seminiferous tubules rats treated with 15 mg/100 g of Momordica charantia seed extract for eight weeks (a) and the extract for eight weeks followed by physiological saline for eight weeks after withdrawal of the extract (b). L=lumen of seminiferous tubule; SE=seminiferous epithelium; I=testicular interstitium

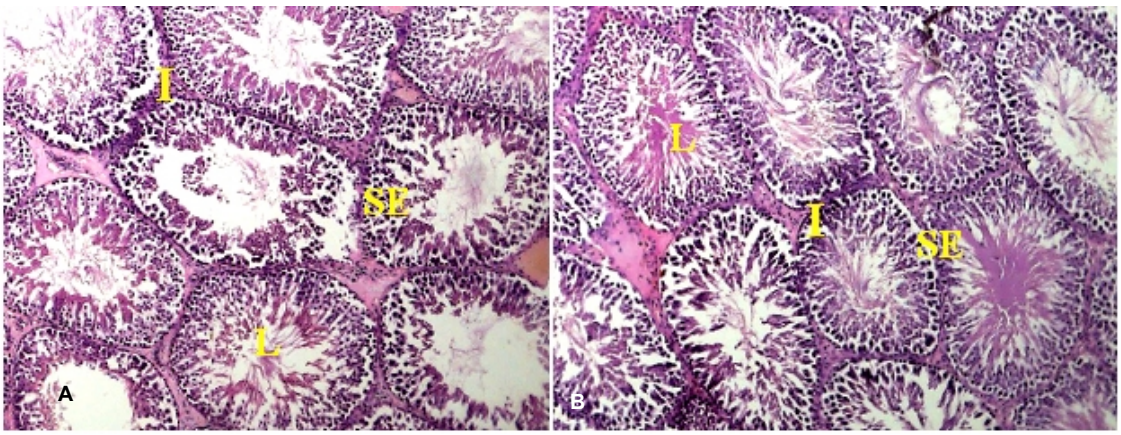

Figure 7: Microscopic sections (Haematoxylin & Eosin staining, Mag. x100) of the seminiferous tubules rats treated with 25 mg/100 g of Momordica charantia seed extract for eight weeks (a) and the extract for eight weeks followed by physiological saline for eight weeks after withdrawal of the extract (b). L=lumen of seminiferous tubule; SE=seminiferous epithelium; I=testicular interstitium

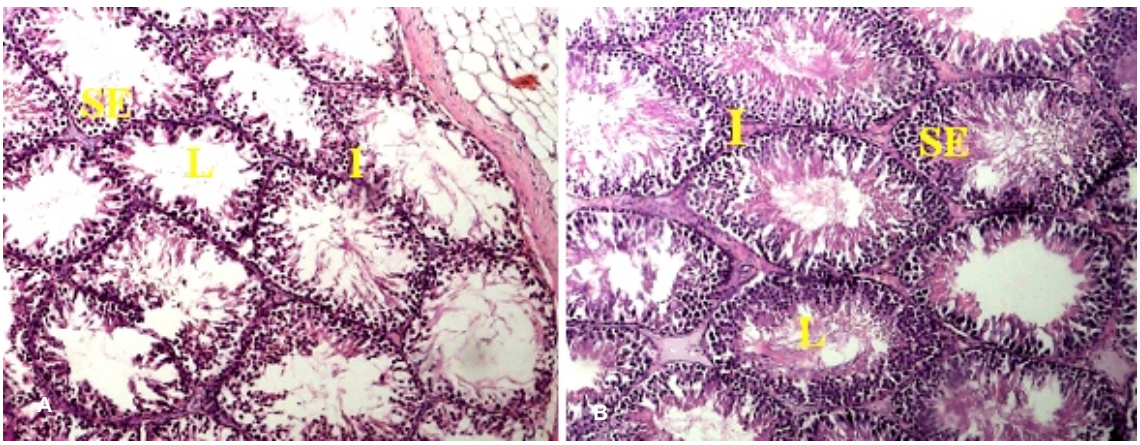

Figure 8: Cross-section of the seminiferous tubules of rat treated with 50 mg/100 g of Momordica charantia seed extract for 8 weeks (a) and treatment with physiological saline for another 8 weeks after withdrawal of extract (b). Stains: Haematoxylin & Eosin. Mag. x100; L=lumen of seminiferous tubule; SE=seminiferous epithelium; I=testicular interstitium

Discussion

The histology of the testes and prostate showed dose-dependent degenerative changes as a result of the treatment with graded doses of methanolic extract of MC seed. On the seminif-

erous tubular epithelium, the changes ranged from a decrease in the number of germ cells to a reduction in the sizes of interstitial connective tissue/Leydig and Sertoli cells. There is also associated widening of the seminiferous tubules as well as decrease in percentage spermatozoa populating the tubular lumen. The prostate showed an increased luminal secretions and dilatation resulting in the crowding of the glands. These observations suggest degenerative changes in the prostate and testes.

The germinal epithelium of the testes produces sperm cells, whereas the interstices occupied by Leydig cells are responsible for testosterone production required for germ cell maturation and function.[14] The present study showed a dose-dependent decrease in the interstitial stroma cells, which connote compromised Leydig cells and hence testosterone levels. This is supported by previous studies in which MC extract resulted in a dose-dependent decrease in the testicular testosterone and testicular volume in rats,[15] as well as suppression of the sperm production.[15,16] Similar reports have shown that a decrease in androgen level usually leads to the disruption of spermatogenic series.[17-19] Also the integrity of the tubular interstitium correlates directly with the quality of spermatogenesis.[14] Thus, the disrupted spermatogenic cell lines seen in the present study could probably be a reflection of a decreased androgen level that accompanied the depleted interstitium.

Sertoli cells are known to be involved in the biosynthesis of non-protein substances mainly nutrients.[20] The elaborate differentiation of the germ cells from spermatogonia to spermatozoa is known to occur in intimate association with Sertoli cells.[20] Strong evidences observed in this present study suggest a likely compromise in secretory activity as the depletion of Sertoli cell lines were observed in the generalized tubular hypocellularity of the tissue section

The gonadotropins are necessary for meiosis and development of spermatids.[21] The androgens are necessary to induce meiosis, formulation, and development of spermatids in response to follicle-stimulating hormone.[21] The observed reduction in the number of spermatogonia, spermatocytes and spermatids may indicate lowered availability of gonadotropins, which are essential for initiation and maintenance of spermatogenesis. It is also known that without a continuous androgen supply sperm production cannot proceed to optimal completion.[15] The resulting antispermatogenic response produced by the extract, as was observed on the histology, may have been via the suppression of the gonadotropins. This, however, remains mere speculation as the gonadotropins were not assayed in this study.

The other halve of the animals in each group were investigated for possible reversibility of the effect of MC extract. They were first administered varied doses of the extract and then later physiological saline for 56 days, which is the time taken for one complete spermatogenic cycle of rats.[22] The prostate and testicular tubular epithelia revealed an appreciable regenerative pattern with microscopic features compared to control. This gives credence to the fact that the extract could be responsible for the observed destruction in the first instance.

Conclusion

The administration methanolic extract of MC seed to male rats resulted in a dose-dependent reversible alterations in the histology of the prostate and testes. These findings are of immense importance, because substances intended as contraceptive agents are expected to possess reversible effects.

Acknowledgement

We wish to acknowledge Mr Adeleke of the Pharmacognosy Department, Faculty of Pharmacy, University of Lagos, Nigeria for the help with the preparation of the herbal decoction and support of this work.

Conflict of Interest: None declared

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