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Full Length Research Paper

Suppressive effects of *Momordica charantia* on pituitary-testicular axis and sperm production in male Sprague-Dawley rats

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Momordica charantia **plant is quite common in Africa and Asia, known in folkloric medicine to affect adversely the reproductive system by mechanism yet to be fully understood. The aim is to determine the effects of** ***M. charantia*** **with and without testosterone administration; on hormonal levels and sperm production (number and motility) in rats. Thirty male Sprague-Dawley rats were used, divided randomly into six groups (MC, TP-MC, MC-TP, MC+TP, TP and DW for group I, II, III, IV, V and VI, respectively). Groups I to V were fed daily for 16 weeks, with either 50 mg/100 g body weight (b.w) /day** ***M. charantia*** **seed extract (MC) or intramuscularly injected with 0.05 mg/kg b.w testosterone propionate (TP) thrice/week. Group VI (negative control) were administered daily with distilled water (DW). Serum gonadotrophins, prolactin and testosterone concentrations and sperm production were measured.** ***M. charantia*** **extract resulted in significant reduction in serum gonadotrophins and testosterone concentrations, with an increase in prolactin level. Sperm production was decreased significantly compared to control. It produced responses that were rather modulatory when concurrently administered with TP.** ***M. charantia*** **seed extract suppresses the pituitary-testicular axis and sperm production in male rats. Thus, it could be developed to a contraceptive drug for men.**

Key words: *Momordica charantia*, testosterone, prolactin, gonadotrophins.

INTRODUCTION

Herbal preparations have been used since time immemorial to reduce female fertility. Though male anti-fertility preparations are less common, modern research has demonstrated antispermatogenic activities of extracts from at least seven plants (Naseem et al., 1998). Some plant extracts have been reported to negatively affect spermatogenesis thereby having antifertility potentials. They act by interfering with sperm production, sperm maturation and storage or with their transport in the female genital tract (Cunningham and Huckin, 1979). *Momordica charantia* (MC) has been identified in previous studies as a multipurpose medicinal plant. It has been used in folkloric medicine for various ailments such as diabetes mellitus, bacterial and viral infections (Technical data for bitter melon, 2002). Its fruit is also known for its antioxidant potential (Thenmozhi and Subramanian, 2010). A dearth of literature has reported its antifertility effect on the different parts of the plant in laboratory animals. The root has been documented to have a uterine stimulant effect (Technical data for bitter melon, 2002). There is however a paucity of literature on the effect of the seed extracts on the male reproductive system of rats.

A brief review of the physiology of male reproductive system implicates the hypothalamic stimulation of the pituitary gland to secrete the gonadotrophins: luteinizing hormone (LH) and follicular stimulating hormone (FSH) that promote spermatogenesis (Sofikitis et al., 2008; Cheng et al., 2010). The LH has an indirect effect on spermatogenesis via Leydig cell stimulation, which has surface receptors for LH (Sofikitis et al., 2008;

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Steinberger, 1975). It responds to stimulation by producing and releasing testosterone (T), which diffuses into the tubules or binds to a carrier such as albumin that delivers it across the lymphatic space into the tubules (Steinberger, 1975). LH stimulation of T production is required for development of the rat seminiferous epithetlium during the first 30 days and for maintenance of adult spermatogenesis. In rats, it has been shown that intratesticular T concentration is normally thirty-fold higher than serum concentration (Turner et al., 1984). The concentration that is minimally required to maintain spermatogenesis is also substantially 10 folds higher than the serum level (Jarow et al., 2001). However, it has also been shown that the T level in serum and plasma correlate with sperm concentration and sperm motility (Carropo et al., 2003; Osinubi et al., 2003). A reduced T level has been adduced for the significant reduction in the epididymal and testicular sperm number and therefore daily sperm production in oligospermic males (Elbeticha and Da"as, 2003). FSH stimulates maturation of sperms in seminiferous tubules and spermatogenesis. FSH enhances the production of androgen-binding protein by the Sertoli cells of the testes by binding to FSH receptors on their basolateral membranes and is critical for the initiation of spermatogenesis (Walter, 2003). A pronounced suppression of primate spermatogenesis seemingly requires inhibition of FSH rather than testicular androgen levels. Exogenous T administered daily in very high doses functions as a male contraceptive by suppressing the pituitary gonadotrophins. Low levels of these hormones decrease endogenous T secretion from the testis and deprive developing sperm of the signal required for normal maturation. Interference with sperm maturation causes a decline in sperm production and can lead to reversible infertility in man (Amory and Bremner, 1998).

The purpose of this present study underscores the effect of oral administration of MC seed extract on the hormonal levels (pituitary-testicular axis) and caudal epididymal fluid parameters in male Sprague-Dawley rats.

MATERIALS AND METHODS

Collection and identification of *M. charantia*

The ripe fruits of *M. charantia* of the family *Cucurbitaceae*, harvested in 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, Nigeria, where the voucher specimen was deposited (ascension number FHI 108422).

Preparation of seed extract

The seeds were extracted from the fruit; dried in an oven (temperature between 30 and 40°C) for a week. The dried seeds were weighed and Soxhlet extraction done using alcohol and water as solvents at the Pharmacognosy Department of College of Medicine, University of Lagos, Nigeria. The percentage yield obtained for the extract was 23.0 w/w.

Determination of LD_{50}

A preliminary LD_{50} of MC seed extract determined was 460 mg/100 g body weight (b.w) of rat. This was done using the fixed-dose procedure described by Walum (1998). The MC seed extract was given at one of three fixed doses at a time to five males Sprague-Dawley rats (S-D). At the dose of 460 mg/100 g b.w, there were clear signs of toxicity with mortality of 50% of the rats.

Pilot study

A prior pilot study was conducted involving a population of 15 male S-D rats weighing 110 ± 10 g divided randomly into three groups (I to III) of 5 rats /group. They were fed daily graded oral doses of MC seed extract. Groups I to III received: 25, 50 and 60 mg/100 g body weight of MC extract respectively for 16 weeks. Briefly, the results obtained revealed a dose dependent antifertility effect in the rats. Rats in group III (60 mg/100 g body weight), had the most effect, although there were no significant difference when compared to group II (50 mg/100 g body weight).

Dosage

The dosages and route of administration for *M. charantia* seed extract and T were selected in order to simulate those in human. To address the confounding effect of age and hormonal variations in the rats, the same duration corresponding to two spermatogenic cycles of 16 weeks in rats (Jegou et al., 2002) was adopted throughout the study. Guided by the dose from the pilot studies, in addition to the LD_{50} of seed extract, 50 mg/100g body weight of MC extract was chosen. A metal canula was used to administer the extract orally by gastric gavages and done between 13.00 and 16.00 h daily. Testosterone was administered as testosterone proprionate (TP), at a dose of 0.05 mg/kg b.w, three times a week: Mondays, Wednesdays and Fridays (Oremosu and Ashiru, 1996; Osinubi et al., 2006). From an ampoule of TP (25 mg/ml) suspended in arachis oil, appropriate aliquots calculated by simple proportion based on the animal"s weight were administered intramuscularly (Osinubi et al., 2006).

Experimental animals

A total of 30 male S-D rats between six to eight weeks weighing 162 ± 52 g, were used. The rats were procured from the Animal House of the College of Medicine, University of Lagos, Nigeria and authenticated by a taxonomist at the Zoology Department of the same university. They were kept in plastic cages in the animal room of the Department of Anatomy and allowed to acclimatize for two weeks under standard laboratory conditions of temperature 18 to 26°C, with a photoperiodicity of approximately twelve hours light alternating with twelve hours of darkness. They were fed with commercially available rat chow (Livestock Feeds Plc, Ikeja, Lagos Nigeria) and had unrestricted access to water.

Experimental protocol

Rats were randomly divided into six groups (I to VI), five rats/group. Group I (MC only) rats were fed daily with *M. charantia* seed extract at the dose of 50 mg/100 g b.w/day in 2 to 5 ml of distilled

water/body weight for 16 weeks. Group II (TP-MC) rats were intramuscularly injected with testosterone propionate at a dose of 0.05 mg/kg b.w in 2 to 5 ml of oil/body weight, thrice a week for eight weeks and followed with MC for another eight weeks. Group III (MC-TP) rats were administered with MC for eight weeks and followed with TP for another eight weeks. Group IV (MC+TP) rats were simultaneously administered with MC and TP for 16 weeks. Group V (TP only) rats were administered with only TP for 16 weeks. Group VI (negative control group) rats were fed daily with 2 to 5 ml of distilled water for 16 weeks.

Anaesthesia and autopsy schedule for the study

At the end of the experiments, the rats were sacrificed a day after the last dose of administration of the extract, TP or distilled water. All sacrifices were done under mild anaesthesia with intraperitoneal ketamine hydrochloride at a dose titrated against consciousness starting with 0.01 ml. Laparotomy was done, and the testes delivered per abdomen. Fat and connective tissue around the testes cleared off. The testes were blotted dry and weighed; the caudal epididymis neatly excised and the epididymal fluid obtained by the „„swim up"" technique immediately analysed. Blood samples were taken from the left ventricle, centrifuged at 1000 g, 25°C for 10 min in an angle head centrifuge. Blood sera were separated and immediately assayed for LH, FSH, PRL and T levels.

Animal ethics

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 (American Physiological Society, 2002) and were approved by the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

Testosterone assay

Serum T was assayed from blood obtained from left ventricular puncture. The samples were assayed in batches from a standardized curve using the enzyme linked immunosorbent assay (ELISA) method (Tietz, 1995). The microwell kits used were from Syntro Bioresearch Inc., California USA. Using 10 µl of the standard, the samples and control were dispensed into coated wells. 100 µl T conjugate reagent was added followed by 50 µl of anti-T reagent. The contents of the microwell were thoroughly mixed and then incubated for 90 min at room temperature. The mixture was washed in distilled water and further incubated for 20 min. The reaction was stopped with 100 µl of 1N hydrochloric acid. Absorbance was measured with an automatic spectrophotometer at 450 nm. A standard curve was obtained by plotting the concentration of the standard versus the absorbance and T concentration was determined from the standard curve.

Luteinising hormone assay

The BioCheck LH ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (Clinical Guide to Laboratory Tests, 1995). The assay system utilizes sheep polyclonal anti-LH for solid phase (microtiter wells) immobilization, and a mouse monoclonal anti-LH in the antibodyenzyme (horseradish peroxidase) conjugate solution. The test sample was allowed to react simultaneously with the antibodies, resulting in the LH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 45 min incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. A solution of Tetramethylbenzidine was added and incubated for 20 min, resulting in the development of a blue colour. The colour development was stopped with the addition of HCl, and the resulting yellow colour measured spectrophotometrically at 450 nm. The concentration of LH is directly proportional to the colour intensity of the test sample.

Follicle stimulating hormone assay

This assay was carried out using double antibody radio immunoassay. A rat recombinant FSH {I^{125}} from Amersham, UK was used. The sensitivity of the assay was 0.9 ng/ml (Clinical Guide to Laboratory Tests, 1995).

Prolactin hormone assay

This assay was carried out using the GenWay, Inc. Prolactin ELISA kit was used for the quantitative measurement of prolactin. The principle of the test was based on a solid phase sandwich ELISA method. Upon the addition of the substrate (sera), the intensity of colour was proportional to the concentration of prolactin in the samples. A standard curve was prepared relating colour intensity to the concentration of the prolactin (Amenomori et al., 1970; Clinical Guide to Laboratory Tests, 1995).

Sperm number and motility analysis

Several small cuts were made in the cauda epididymis which was then placed in a sterile universal specimen bottle, containing 1 ml of normal saline to allow motile sperm to swim up from the epididymis. 5 µL of epididymal fluid was delivered onto a glass slide covered with a 22 × 22 mm cover slip (World Health Organization, 1999) and examined under the light microscope at a magnification of ×400. The microscopic field was scanned systematically and each spermatozoon encountered was assessed. Motility was determined by counting the number of immotile spermatozoa and subtracting from the total count × 100%. The motility was simply classified as either motile or non-motile. The procedure was repeated and the average of the two readings taken.

The sperm number was determined using the Neubauer improved haemocytometer. A dilution ratio of 1: 20 from each well-mixed sample was prepared by diluting 50 µl of epididymal spermatozoa suspended in physiological saline with 950 µl diluent. The diluent was prepared by adding 50 g of sodium carbonate and 10 ml of 35% (v/v) formalin to distilled water and making up the final solution to a volume of 1000 ml (World Health Organization, 1999; Barratt, 1995). Both chambers of the haemocytometer were scored and the average count calculated, provided that the difference between the two counts did not exceed 1/20 of their sum (that is, less than 10% difference). When the two counts were not within 10%, they were discarded, the sample dilution re-mixed and another haemocytometer prepared and counted. To minimize error, count was conducted three times on each epididymis. The average of all the six counts (3 from each side) from a single rat was taken and this constituted one observation for the sperm number.

Statistical analyses

Data were entered and analyzed using Epi6 info and SPSS 10.0 software packages. The outcome variables were expressed as mean ± standard deviation. In view of small sample size, we performed Kruskall-Wallis tests to test whether the groups compared came from the same distribution. If the P-values were

less than 0.05, post-hoc analysis using Mann-Whitney were done to assess whether each two groups come from the same distribution. The level of significance was set at 0.05 (2-sided) or otherwise mentioned.

RESULTS

Serum testosterone level

In comparison to group VI (DW negative control), serum T levels in groups III (MC-TP), IV (MC+TP) and V (TP only) were not significantly different. However, serum T levels in groups I (MC only) and II (TP-MC) tended to be lower ($p < 0.05$) than that of the group VI *viz* 0.10 ± 0.05, 0.15 ± 0.07 and 0.31 ± 0.11 ng/ml for groups I, II and VI, respectively (Table 1).

The mean serum T concentration within the groups showed significant difference ($p < 0.05$), rats treated with the extract alone and those pre-treated prophylactically with TP, before the extract administration showed a decline (Table 2).

Serum gonadotrophins (LH and FSH) concentration

The mean serum LH level declined significantly ($p < 0.05$) from 1.75 ± 1.33 (negative control) to 0.31 ± 0.25 mIU/ml, in group I (MC only). a significant decrease compared to control was also recorded in rats in group II (TP-MC) 1.75 ± 1.33 *vs* 0.13 ± 0.07 mIU/ml. The mean serum LH concentrations of rats in groups III, IV and V were1.88 ± 0.71, 1.86 ± 2.03 and 1.70 ± 0.63 mIU/ml respectively; these values were not significantly different from negative control ($p > 0.05$; Table 1). On the other hand, comparison of the mean serum LH concentrations within the groups showed a significant difference ($p < 0.05$; Table 2). The mean serum FSH levels of experimental animals follow similar pattern to those of serum LH, groups I (1.18 ±1.59) and II (1.71 ± 0.66) showed a significant decrease ($p < 0.05$) compared to 3.52 ± 1.41 mIU/ml of control (Table 1). For groups III, IV and V, the mean FSH were not significantly ($p > 0.05$) different from negative control: 3.21 ± 0.55, 2.96 ± 1.48 and 2.26 ± 2.50 respectively *vs.* 3.52 ±1.41 mIU/ml (Table 1). There were no significant differences of the serum FSH within the groups ($p > 0.05$; Table 2).

Prolactin concentration

Compared to the gonadotrophins, the mean serum prolactin (PRL) levels showed a reversed pattern in animals treated with extract alone and those pre-treated with TP before administration of extract. These groups recorded a significant increase ($p < 0.05$) to 1.50 ± 0.39 and 1.24 ± 0.41 mIU/ml compared to 0.54 ± 0.38 mIU/ml of negative control. The mean serum PRL of animals in Group III (0.52 ± 0.14) and V (0.68 ± 0.47mIU/ml) were not significantly different ($p > 0.05$) from animals in the negative control groups. In rats administered concurrently the extract and TP, the mean serum PRL were elevated from 0.54 ± 0.38 (control) to 0.78 ± 0.82 mIU/ml though increase was not statistically significant ($p > 0.05$; Table 1). The variation in mean PRL concentration within the groups did not show any significant difference ($p > 0.05$; Table 2).

Sperm number and motility

There was a significant ($p < 0.01$) reduction in sperm production (number and motility) in rats treated with the extract alone and those pre-treated with TP before administration of extract compared to control. This reduction was more pronounced in the former than in the latter (Table 1). The mean sperm number and motility for animals in Group III (MC-TP) and IV (MC+TP), show a significant ($p < 0.05$) reduction compared to negative control (Table 1) although these values were within the normal range for rats. Lastly, rats in group V (TP only) had values similar to control (Table 1). The reduction in sperm production within the groups were however statistically significant ($p < 0.01$; Table 2).

DISCUSSION

The pituitary-testicular axis is a central regulatory conduit for testicular function that culminates in the production of spermatozoa (Cheng et al., 2010). The integrity of which is crucial for a healthy procreative life. In this study, the experimental rats had revealed varying degrees of perturbation in this axis. This is evidenced by the evaluated serum hormonal levels compared to their control counterparts (Group VI: DW negative and V: TP), although the exact mechanism is yet to be fully understood.

The sera of animals treated with the MC extract alone and those pre-treated with TP before administration of the extract showed a significant decrease in the level of assayed gonadotrophins (FSH and LH) compared to control. This indicates interference of pituitary-testicular axis. The analysis of *caudal* epididymal fluids of these rats revealed a concomitant decrease in the sperm number (oligospermia) and motility (asthernozospermia). Conversely a reversed effect was observed when T "replacement" was done following administration of the extract. This is in harmony with previous research in which suppression of secretion of pituitary gonadotrophins decrease endogenous T secretion (Gooren et al., 1984). Also, the gonadotrophins repression deterred the signal involved in the induction and completion of spermatogenesis in the normal maturation of developing sperm (Neuman et al., 2002). Similarly, studies by

Table 1. Comparison of serum hormonal levels and sperm production in control and experimental Sprague-Dawley rats.

Group	Variable	Experimental group		Control group (Group VI)		Mann-Whitney U	P
		n	Mean ± SD	n	Mean ± SD		
I ((MC only)	Testosterone	4	0.10 ± 0.05	5	0.31 ± 0.11	1.00	0.01*
	Prolactin	4	1.26 ± 0.39	4	0.54 ± 0.38	1.00	0.04*
	FSH	5	1.18 ± 1.59	5	3.52 ± 1.41	3.00	0.05*
	LH	5	0.31 ± 0.25	5	1.75 ± 1.33	2.00	0.03*
	Sperm number	5	1.40 ± 1.20	5	139.8 ± 41.7	0.00	0.01*
	Sperm motility	5	9.60 ± 7.10	5	93.80 ± 8.0	0.00	0.01*
II (TP-MC)	Testosterone	4	0.15 ± 0.07	5	0.31 ± 0.11	0.10	0.04*
	Prolactin	5	1.24 ± 0.41	4	0.54 ± 0.38	2.00	0.05*
	FSH	4	1.71 ± 0.66	5	3.52 ± 1.41	2.00	0.05*
	LH	4	0.13 ± 0.07	5	1.75 ± 1.33	0.00	0.01*
	Sperm number	4	11.5 ± 7.2	5	139.8 ± 41.7	0.00	0.01*
	Sperm motility	4	17.5 ± 11.9	5	93.8 ± 8.0	0.00	0.01*
III (MC-TP)	Testosterone	4	0.38 ± 0.17	5	0.31 ± 0.11	9.00	0.81
	Prolactin	4	0.52 ± 0.14	4	0.54 ± 0.38	8.00	1.00
	FSH	4	3.21 ± 0.55	5	3.52 ± 1.41	6.00	0.33
	LH	3	1.88 ± 0.71	5	1.75 ± 1.33	7.00	0.88
	Sperm number	3	78.3 ± 25.7	5	139.8 ± 41.7	0.50	0.04*
	Sperm motility	3	74.7 ± 4.5	5	93.8 ± 8.0	0.00	0.02*
IV (MC+TP)	Testosterone	5	0.46 ± 0.25	5	0.31 ± 0.11	11.5	0.83
	Prolactin	3	0.78 ± 0.82	4	0.54 ± 0.38	5.50	0.86
	FSH	4	2.96 ± 1.48	5	3.52 ± 1.41	8.00	0.62
	LH	5	1.86 ± 2.03	5	1.75 ± 1.33	11.00	0.75
	Sperm number	3	84.0 ± 21.2	5	139.8 ± 41.7	0.50	0.04*
	Sperm motility	3	74.3 ± 9.2	5	93.8 ± 8.0	1.00	0.05*
V (TP only)	Testosterone	4	0.37 ± 0.06	5	0.31 ± 0.11	7.50	0.54
	Prolactin	3	0.68 ± 0.47	4	0.54 ± 0.38	4.00	0.48
	FSH	5	2.26 ± 2.50	5	3.52 ± 1.41	12.00	0.92
	LH	4	1.70 ± 0.63	5	1.75 ± 1.33	9.00	0.81
	Sperm number	3	146.7 ± 25.2	5	139.8 ± 41.7	6.50	0.76
	Sperm motility	2	94.0 ± 1.4	5	93.8 ± 8.0	3.00	0.42

*Significant. FSH (follicle stimulating hormone), LH (luteinizing hormone). Groups: I =Treated with 50 mg/100 g b.w/day *Momordica charantia* seed extract (MC), II = Pre-treated with 0.05 mg/kg Testosterone propionate (TP) then post-treated with MC, III = Administered MC discontinued and post-treated with TP, IV = Treated with MC and TP concurrently, V = Treated with TP alone (positive control) and VI = Administered distilled water alone (negative control). In all the total treatment duration was for 16 weeks.

Table 2. Comparison of serum hormonal levels and sperm production in Sprague-Dawley rats within Experimental groups.

Variable	Mean ± SD					Kruskal Wallis χ^2	Df	P
	Group I	Group II	Group III	Group IV	Group V			
Testosterone	0.10±0.05	0.15±0.07	0.38±0.17	0.46±0.25	0.37±0.06	10.65	5	0.05*
Prolactin	1.50±0.39	1.24±0.41	0.52±0.14	0.78±0.82	0.68±0.47	10.24	5	0.07
FSH	1.18±1.59	1.71±0.66	3.21±0.55	2.96±1.48	2.26±2.50	8.20	5	0.15
LH	0.31±0.25	0.13±0.07	1.88±0.71	1.86±2.03	1.70±0.63	14.11	5	0.02*
Sperm number	1.4±1.2	11.5±7.2	78.3±25.7	84.0±21.2	146.7±25.2	20.18	5	0.00*
Sperm motility	9.6±7.1	17.5±11.9	74.7±4.5	74.3±9.2	94.0±1.4	18.54	5	0.00*

*Significant. FSH (follicle stimulating hormone), LH (luteinizing hormone). Group I: Treated with 50 mg/100 g b.w/day *Momordica charantia* seed extract (MC). Group II: Pre-treated with 0.05 mg/kg Testosterone propionate (TP) then post-treated with MC. Group III: Administered MC discontinued and post-treated with TP. Group IV: Treated with MC and TP concurrently. Group V: Treated with TP alone. In all the total experimental duration was for 16 weeks.

Amatruda et al. (1978) showed that normal or low levels of gonadotrophins in the presence of low level of serum T signify suppression of the hypothalamic-pituitary-testicular axis, resulting in a subclinical hypogonadotropic hypogonadism producing compromised fertility in man.

For animals in which MC extract and TP were administered concurrently (MC+TP) the mean T and gonadotrophins values were similar to control. This means “repletion” of serum T level by the administered exogenous TP against the suppressive effects of the extract. This is also supported by the normal sperm range in these rats. An appreciable restoration of gonadotrophins and sperm production in these rats compared to group I, MC only gives credence to the fact that the extract could be responsible for the observed T depletion in the first instance. That is, MC extract resulted in decreased T in extra-testicular axis of these rats. The present study is in consonance with previous reports where a decrease in androgen resulted in a decrease in the number of spermatozoa in rats (Mohri et al., 1975; Russell et al., 1991).

The complex inter-relationship of prolactin with the gonadotrophins could be another likely pathway. Studies have shown that an increase in serum prolactin level (hyperprolactinaemia) tends to inhibit the secretion of gonadotrophins releasing hormone and T production and may have a direct effect on the central nervous system. Though the inhibitory effects of prolactin on the testicular release of T have been demonstrated, their mechanisms still remain unclear (Huang et al., 2003). Recent evidence suggests that in conditions of hyperprolactinaemia, the response of testicular tissue to the release of T is influenced by the cytokines (tumour necrosis factor-alpha TNF-α) secreted by testicular interstitial macrophages (Huang et al., 2003). It was observed that prolactin rise was proportional to increased release of TNF-α by testicular interstitial macrophages. The accumulation of TNF-α in turn, decreased the release of T by Leydig cells (Huang et al., 2003). This is in consonance with findings in this present study where rats fed the seed extract of MC (and those pre-treated with TP before administration of extract) induced a significant increase in serum prolactin levels. This may have affected negatively the levels of T and ultimately led to the disruption of spermatogenesis via a rise in the level of TNF-α (although serum level was not estimated in this present study).

Conclusion

In conclusion, this study showed that *M. charantia* seed extract (50 mg/100 g b.w/day) administered orally for two spermatogenic cycles, produces a pronounced inhibition of serum T and gonadotrophins concentration with a converse elevation of prolactin. The summation of which had resulted in a suppression of sperm production. The result from the study would serve as a preliminary template for further comparative study and subsequent research work on the extract on male reproductive organs and thus expected to spring forth new frontiers to the development of a new contraceptive agent for men.

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