

Reduction in Some Reproductive Indices of *Momordica Charantia* Treated Male Wistar Rats

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Abstract

Original Research Article

The effect of *Momordica charantia* leaf extract was evaluated by determining blood follicle stimulating hormone (FSH), Interstitial cell stimulating hormone (ICSH) and testosterone (TET) levels as well as sperm quality and organ weight in male wistar rats, after 30 days of oral administration. The animals were randomly assigned into three (3) groups of six (6) rats each. Group one (1) served as control and received distilled water. Group two (2) and group three (3) received 200mg/kg and 400mg/kg of the hydromethanol (20%:80%) extract respectively. Results obtained showed that the extract caused no significant ($P < 0.05$) alteration in the levels of FSH and ICSH. But there was a significant decrease in plasma TET level. There were no significant effects on testicular and epididymal weights. There was significant impairment of sperm quality evidenced by a reduction in percentages of viable sperms, sperms with normal morphology and actively motile sperm as well as sperm count. The decrease in plasma testosterone and abnormal sperm parameters may suggest the ability of the plant extract to cause a direct toxic effect on the seminiferous tubule which may damage testosterone secreting cells thereby affecting testosterone synthesis and the production of healthy sperm. This study proved that, the leaf extract of *Momordica charantia* caused anti fertility effects in male wistar rats.

Keywords: *Momordica charantia*, hydromethanol, antifertility, wistar rats.

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INTRODUCTION

Plants are known to play different roles that enhance the wellbeing of humans. Apart from their use as sources of food, fuel, industrial and agricultural raw materials, some are also used for medicinal purposes. As a result, much value is placed on plants with medicinal properties. *Momordica charantia* (*M. charantia*) Linn has gained considerable popularity by virtue of its medicinal value. *M. charantia* contain active ingredients responsible for its characteristic therapeutic and nutritional effects. Some of the compounds identified in the plant include, charantin, momorcharins, momordicin, momordenol, momordin, momordicilin, momordicins, erythrodiol, charine, cryptoxanthin, cucurbitins, galacturonic acids etc. [1, 2]. Other phytochemical compounds found in *M. charantia* are saponins, alkaloids, glycosides, triterpenes and steroids [3]. These biologically active compounds have been reportedly responsible for various pharmacological effects of *M. charantia*. *M. charantia* have demonstrated hypoglycemic activity in

normal animals [4]; and antihyperglycemic activity in drug induced diabetic animals [5, 6]. The hypoglycemic actions of *M. charantia* are due to a mixture of charantins, (a steroidal saponin), insulin-like peptides and alkaloids [7][8], momordin Ic, oleanolic acid 3-O-glucuronide and oleanolic acid 3-O-monodesmoside [9]. Extracts of *M. charantia* have also demonstrated antibacterial properties [10]; hypotensive [11]; anti-inflammatory [12] and anti-ulcer effects [13]. On human reproduction, several reports exist on its effect on the female reproductive system. The benzene extract of *M. charantia* showed anti-ovulatory [14] and anti-estrogenic [14, 15] properties. In earlier experimental studies, some proteins derived from *Momordica* were found to induce abortion in early and midterm pregnancy [16, 17].

In effect, *M. charantia* produced antifertility and abortifacient properties in female animal models. However, fertility regulation using medicinal plants in traditional medicine is not a new practice but dates back to ancient times [18]. *M. charantia* is widely used

traditionally to reduce fertility in both males and females [19]; however, there is paucity of scientific studies on its effect on male reproduction, especially, elaborating any possible influence on male reproductive hormone regulation. The objective of this study is to investigate the effects of *M. charantia* leaf extract on some reproductive parameters of male wistar rats.

MATERIALS AND METHODS

Preparation of Plant extract

The leaves of *M. charantia* were obtained from Choba community in Obio Akpor Local Government Area of Rivers State, Nigeria and authenticated in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. Firstly, the leaves were washed to remove dirt, then later dried and blended to fine powder. The crude extraction was done with hydromethanol (20:80) at 60 – 70°C with the aid of the soxhlet apparatus.

Following filtration of the solution which was done after 24 hours, the filtrate was concentrated with the rotary evaporator to a semi solid form under reduced pressure of 60°C. The net yield was weighed and the extract preserved in a refrigerator at 4°C.

To enable animal oral administrations the extract was reconstituted to obtain 200mg/ml of solution.

Animal models

Adult male rats were obtained from the animal house of the Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The handling of the animals conformed to the guiding principles in the care and the use of experimental animals by the American Physiological society [20].

Experimental design

This study was designed to investigate the effects of the leaves of *Momordica charantia*. Male wistar rats were divided into three (3) groups of six (6) rats each. Group one (1) which served as control received distilled water. Group two (2) and group three (3) were treated with 200mg/kg bw and 400mg/kg bw of the hydromethanol leaf extract of *Momordica charantia* respectively. The extracts were administered as single oral doses per day using animal feeding

hypothermic syringes for duration of 30 days. The rats were sacrificed under chloroform anaesthesia 24hours after last administered dose.

Collection of blood

Blood was collected through cardiac puncture into dry sample tubes and left standing for about 15 minutes to clot. The samples were later centrifuged at 3000 rev/min for 10-15 minutes using a table centrifuge machine. The sera were separated using a pasteur pipette into sterile sample tubes and stored at -4°C until used.

Semen collection/ Sperm quality analysis

Semen was collected by making a small incision at the inguinal region to access the caudal epididymis where an incision of about 1mm was made. Semen was gently squeezed through the vas deferens. The method of cytometry using the improved Neubauer cytometer was used to obtain the epididymal sperm count which was expressed as million/ml [21, 22]. The procedures employed in the determination of all sperm parameters have been documented [23].

Hormone assay

The assay for Interstitial cell stimulating hormone (ICSH), Follicle stimulating hormone (FSH) and Testosterone (TET), was done in accordance with established principles [24]; using appropriate hormone kit. The assay for testosterone depended on competitive binding of the hormone on immobilized antibody. The procedure involved was based on a solid phase enzyme linked immunosorbent assay (ELISA).

STATISTICAL ANALYSIS

Statistical analysis of data was carried out on Statistical Package for Social Sciences (SPSS) version 20.0 using analysis of variance (ANOVA). Results were expressed as Mean \pm standard error of mean and level of significance was considered at $p < 0.05$.

RESULT

Result presentation

The result of the study is presented in tables 1 to 3 and figure I.

Table-1: Effect of *Momordica charantia* leaf extract on some hormones.

Groups	Hormones		
	ICSH (IU/L)	FSH (IU/L)	TET (ng/ml)
Group 1 (Control)	0.95 \pm 0.17	0.40 \pm 0.03	2.09 \pm 0.35
Group 2 (200mg/kg)	0.59 \pm 0.13	0.35 \pm 0.04	0.88 \pm 0.15*
Group 3 (400mg/kg)	0.81 \pm 0.07	0.48 \pm 0.06	1.45 \pm 0.30

Values expressed as Mean \pm SEM. n=6. Significant at [$* (P < 0.05)$] when compared with control group.

Table-2: Effect of *Momordica charantia* leaf extract on some sperm parameters

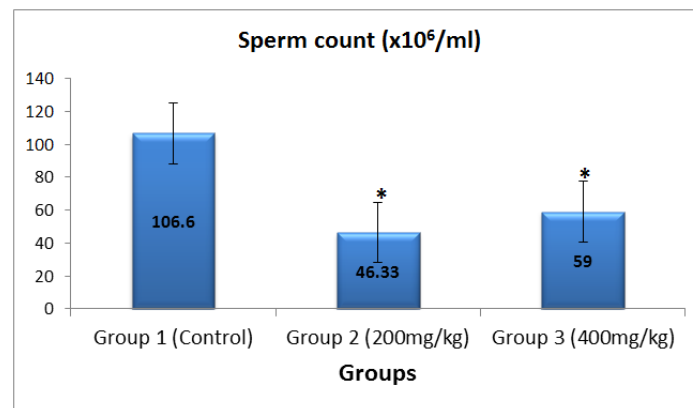
Groups	SPERM PARAMETERS		
	VIABLE SPERM CELLS (%)	NORMAL MORPHOLOGY (%)	ACTIVELY MOTILE (%)
Group 1 (Control)	78.83±2.77	73.33±2.47.	70.00±2.88
Group 2 (200mg/kg)	60.83±3.96*	60.00±3.41*	55.00±5.16*
Group 3 (400mg/kg)	66.66±4.94*.	63.33±4.01	58.33±5.72

Values expressed as Mean ± SEM. n=6. Significant at [* (P<0.05)] when compared with control group.

Table-3: Effect of *Momordica charantia* leaf extract on tissue/organ weights

Groups	tissue/organ weights (g)	
	TESTIS	EPIDYDIMIS
Group 1 (Control)	1.45±0.02	0.20±0.02
Group 2 (200mg/kg)	1.14±0.08	0.12±0.02
Group 3 (400mg/kg)	1.26±0.19	0.19±0.04

Values expressed as Mean ± SEM. n=6.

**Fig-1: Effect of *Momordica charantia* leaf extract on Sperm count**

RESULT ANALYSIS

Hydromethanolic extracts of the leaves of *Momordica charantia* was administered for a period of 30 days as low dose of 200mg/kg bw (group 2) and higher dose of 400 mg/kg bw (group 3).

Table 1 showed that ICSH and FSH levels were not affected significantly at p<0.05 for the low dose of 200mg/kg and higher dose of 400mg/kg. The level of serum testosterone reduced significantly at p<0.05 in group 2.

In table 2, the effects of the extracts on viable sperms, normal morphology and actively motile sperms are highlighted. The difference when compared with controls was significant (p<0.05) in both test groups for the viable cells and in group 2 for normal morphology and actively motile cells. Also, there were no significant (p<0.05) differences found in the weights of the testis and epididymis (table 3) in comparison with control.

Figure 1 shows reductions in sperm count. This was found to be significant (p<0.05) at the lower (200mg/kg) and higher doses (400mg/kg) of the extract.

DISCUSSION

A thorough understanding of the hormonal regulation of spermatogenesis is critical in the proper assessment and management of problems associated with male fertility as well as issues pertaining to the development of an ideal male contraceptive [25]. The pathogenesis of infertility still remains quite unclear. To this end, efforts are being made to characterize possible mechanisms of disruption in endocrine and reproductive functions which are aimed at providing informations on safety. The focus of this study was to investigate any interference of graded doses of hydromethanolic leaf extract of *Momordica charantia* on some physiological indices of fertility, including plasma levels of FSH, ICSH and TET, sperm quality and reproductive organ characteristics in male wistar rats.

The anterior pituitary gland secretes FSH and ICSH which are glycoprotein hormones in response to hypothalamic gonadotropin releasing hormone (GnRH), and they act directly on the testes to stimulate somatic cell function in support of spermatogenesis [26]. Both hormones also regulate the testicular secretion of TET which play important role in the initiation and maintenance of spermatogenesis [27]. There are no significant changes in the serum levels/concentrations

of the pituitary gonadotropins (FSH and ICSH) following administration of extracts of *Momordica charantia*. There was a significantly ($p < 0.05$) reduced TET level observed with the lower dose of the extract. The production of matured sperm is important to male fertility. The growth and maturation of spermatozoa in addition to testosterone levels in testis are mainly controlled by FSH and ICSH. FSH activate spermatogenesis through its receptor (FSH – R) expressed in the sertoli cell, while the ICSH enhance testosterone synthesis in the leydig cells of testis [28-30]. The epididymal sperm parameters showed that the extract significantly reduced the percentages of viable sperms, sperms with normal morphology as well as actively motile sperm. Furthermore, the extract caused significant reduction in the sperm count. The decrease in testosterone output in this study may be responsible for the reduction in spermatogenesis leading to a reduction in sperm count. These changes may be due to *Momordica charantia* extract at given doses, interfering with spermatogenesis in the seminiferous tubules or altering epididymal function or activities, or failure of hypothalamus to cause the release of hypothalamic releasing hormones and anterior pituitary secretion of gonadotropin as a feedback response to declining testosterone levels; which may have resulted in impairment of spermatogenesis, and poor sperm quality [31, 32]. In other words, declining testosterone levels may be due to inhibitory effect of *Momordica charantia* on pituitary gonadotropins or direct toxic effect on the Leydig cells of the seminiferous tubules. Impaired sperm production may lead to male infertility. It is suggested that pathological changes in seminiferous tubules and epididymis caused disturbance of testicular and epididymal functions giving rise to the reduction in quality and quantity of spermatozoa, including daily sperm output and caudal epididymal spermatozoa, percentage of motile sperm, live sperm, normal sperm morphology which all has the tendency to cause infertility. There are several reports regarding infertility being related to reduction in quantity and quality of spermatozoa and testicular and epididymal damages. Infact, the sperm count is a very important test of spermatogenesis and is directly associated with fertility [21].

The findings on the effect of this extract on testosterone level and decreased sperm quality is in agreement with reported findings in a similar study [33]. In another study, on male fertility, a decline in testosterone secretion was reported as the reason for an observed impairment of spermatogenesis [34]. The testicular and epididymal weights were not affected despite the fact that the plant extracts impaired spermatogenesis. This particular finding agrees with the findings in a similar study [35].

The decrease in the density of spermatozoa has been shown to be the possible mechanism in which

natural substances used in the form of plant based contraceptive, inhibits male fertility [36].

However, the reversibility of a depressed or inhibited fertility is an essential feature of an ideal plant based male contraceptive.

Although, we did not include a recovery period in the design of our study, a complete reversal of the antifertility effects of *Momordica charantia* has been demonstrated in some other studies [35, 37] upon withdrawal of extract.

CONCLUSION

The leaf of *Momordica charantia* possesses anti fertility effects which were demonstrated at 200mg/kg body weight of extract. The decrease in plasma testosterone and abnormal sperm quality suggest the plant extract may have the ability to cause a direct toxic effect on the seminiferous tubule.

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