

## CONTRACEPTIVE EFFECT OF METHANOLIC EXTRACT OF *MOMORDICA CHARANTIA* SEED IN MALE SPRAGUE-DAWLEY RATS

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### ABSTRACT

The development of a new male oral contraceptive of herbal origin has moved at a slow agonizing pace. Despite years of research, there is yet no widely acceptable modern contraceptive drug that currently exists for men, whose most effective choices are limited to condoms or vasectomy. The aim of this study is therefore to investigate the oral contraceptive effects of methanolic seed extract of *Momordica charantia* (MC) in male Sprague-Dawley (S-D) rats. A total of 160 adult male S-D rats weighing between 110–214 g, were used for the study. The animals were divided randomly into 3 main groups A, B and C. Rats in groups A and B were further subdivided into A<sub>1</sub> to A<sub>6</sub> and B<sub>1</sub> to B<sub>4</sub> subgroups. Group C rats were the controls. Each subgroup comprised 10 male S-D rats. The rats in subgroups A<sub>1</sub> to A<sub>6</sub> were administered MC at a single daily dose of 50 mg/100 g body weight (bw) for 6 different durations 6, 8, 16, 24, 32 and 40 weeks, respectively. While rats in subgroups B<sub>1</sub> to B<sub>4</sub> were all pre-treated with MC at a single daily dose of 50 mg/100 g bw for 8 weeks (one complete spermatogenic cycle) and then allowed to convalesce for 4 intervals (16, 24, 32 and 40 weeks) fed distilled water during this period. The animals in group C (control) were administered distilled water alone throughout the experiment. The rats were sacrificed under mild anaesthesia using low dose intraperitoneal ketamine at the end of the durations. The parameters evaluated include mating test/fertility assessment, testicular morphometry, sperm count and motility. None of the male rats fed the MC seed extract (from the 8<sup>th</sup> week) were able to fertilize the females exposed to them despite successful mating. The testicular weight and volume were markedly reduced ( $p < 0.05$ ) in the extract treated groups compared to control. The rats in groups in which the extracts were withdrawn after an initial treatment showed substantial recovery. The data from the present study suggest that MC seed extract produced a male contraceptive effect in S-D rats.

**Keywords:** *Momordica charantia*, sperm count, sperm motility, mating test fertility assessment, morphometry

### INTRODUCTION

Male contraceptives have become a popular topic in journalistic and medical communities as men are looking for more control over their reproduction<sup>1</sup>. It is recently the subject of in depth research with emphasis on the development of a 'pill' for men which will ensure that virility is not compromised<sup>2</sup>. The search for a safe, effective, cheap and reversible hormonal contraceptive for men has been a slow one and for more than forty years since women got the "pill", birth-control methods available to men have not changed much<sup>3</sup>. Although there are currently more than a dozen forms of male contraceptives under development, only few are devoid of side effects. Some herbal extracts have been found to have an inhibitory action on spermatogenesis. Incidentally some of these extracts suppress libido while others cause irreversible loss of fertility<sup>4</sup>. Men's contraceptive options are not only imperfect, but there are very few of them. Other than withdrawal, which leads to a 27% pregnancy rate, men have only two contraceptive methods to choose from: vasectomy, which is inappropriate for men who still want children and the condom which, though important for disease prevention, is unpopular among committed couples and can sometimes fail<sup>1</sup>. Neither of them is ideal. The world population explosion has caused political leaders to look upon national and regional birth control projects as vital. Support for regulation of individual fertility has been evident in all cultures, and at all times, even in those societies in which social and religious rules have favored unrestrained reproduction<sup>5</sup>.

The earliest record of birth control use is an ancient Egyptian set of instruction for creating a contraceptive pessary<sup>6</sup>. Different methods of birth control have varying characteristics. Condoms for example are the only methods that provide significant protection from sexually transmitted disease<sup>7, 8</sup>. As the secularization of western society and scientific enquiry gained momentum, the knowledge of reproduction has increased and applied to control human population growth. In poorer countries, control over fertility is essential from a socio-economic standpoint because of poverty and high maternal mortality rate. Each approach to fertility control has its advantages and disadvantages since no one method is perfect for everyone, for every clinical setting, and in every culture. This implies that the border the option for couples the better the chances of choosing a safe method.

The use of herbs and other means of traditional contraceptive are not new; it cuts across the world both in century past and present. Some herbs have been reported to possess antifertility properties such as date palm, wheat and *Carica papaya*. Apart from the hypoglycemic effect of *Momordica charantia* (MC) which is perhaps the most researched pharmacologic effect of this plant, there is a paucity of literature on its effect on the testes. However this present study has shown this plant extract to have an enormous potential for an effective male oral contraceptive in the male Sprague-Dawley (S-D) rats.

### MATERIALS AND METHODS

#### Collection and identification

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

#### Preparation of *M. charantia* 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 alcohol and water as solvents at the Pharmacognosy department of College of Medicine, University of Lagos. The percentage yield was 23.0% w/w, from which a dose of 50 mg/100 g body weight in distilled water was calculated and administered orally.

#### Experimental animals

A total of 160 adult male S-D rats were used. The rats were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos and were authenticated by a taxonomist<sup>9</sup> in the Department of Zoology of the University of Lagos. 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 27-30°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

The animals were divided randomly into 3 main groups A, B and C. Animals in group A referred to as suppression phase group were fed with MC at a single daily dose of 50 mg/100 g. There were 6 different treatment durations (6, 8, 16, 24, 32 and 40 respectively) in weeks which indicate a particular subgroup designated A<sub>1</sub> to A<sub>6</sub>. Rats in group B were divided into 4 subgroups B<sub>1</sub> to B<sub>4</sub> all pre-treated orally with MC extract at a single daily dose of 50 mg/100 g for 8 weeks and then allowed to recover before sacrifice. The periods of recovery were for 4 various durations (16, 24, 32 and 40 weeks respectively) which depicted a particular subgroup designated B<sub>1</sub>-B<sub>4</sub>. Thus B<sub>1</sub> (fed MC daily for 8 weeks and permitted to recuperate for another 8 weeks), B<sub>2</sub> (fed MC daily for 8 weeks and allowed to recuperate for another 16 weeks), B<sub>3</sub> (fed MC daily for 8 weeks and allowed to recuperate for another 24 weeks) and B<sub>4</sub> (fed MC daily for 8 weeks and allowed to recuperate for another 32 weeks). During the periods of recuperation, animals were fed distilled water. These subgroups are referred to as the reversibility group. In all, each experimental subgroup comprised 10 rats. The rats in group C (totaling 60 rats) served as controls and used to compare the events in the other groups. They were administered daily 4-5 ml distilled water at the different time intervals ranging 6-40 weeks. A metal canula were used to administer distill water and extract orogastrically. At the end of these different periods, 5 rats in each subgroup (control and experimental) were sacrificed while the other half kept alive for mating test. The *caudal epididymal* fluids from the sacrificed rats were processed for sperm count and motility. Testicular morphometric analysis (weight and volume) were also assessed.

### Anaesthesia and necropsy schedule for the study

At the end of the experimental durations, the male rats were sacrificed a day after the last dose of extract or distilled water was given, while pregnant females were sacrificed on day 20 of gestation. All sacrifices done under anaesthesia with intra-peritoneal 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 were cleared off. The testes were blotted dry and weighed; the *caudal epididymis* neatly excised and *epididymal* fluid harvested from the testes was immediately analyzed. The pregnant females had ventral laparotomy and their uteri were examined for fetal number.

### Evaluation of fertility capability of treated male rats

The best test of a contraceptive is to study the agent in couples who use it as a sole means of contraception. It was decided in this study to extend investigation of the extract to include "coupled rat" models to assess the contraceptive capability of the extract on the male rats. Therefore at the end of the different stipulated treatment periods, 5 male S-D rats in each group were left alive for mating. One male rat was mated with two sexually mature normal female S-D rats of tested fertility; they were housed in a single cage. Female rats with normal 4-day estrus cycles were used and proestrus females were exposed to the experimental males. The males were then removed on the morning of estrus. The coupled rats were kept for 10 days during which two estrus cycles should have elapsed<sup>10</sup>. The presence of sperm plug at the estrus indicated successful mating and this day was taken as day zero of gestation.

### Sperm count and motility analysis

The *caudal epididymides* from both sides were dissected out and several small cuts of about 1 mm were made and the tissue was suspended in 1 ml Ham F-10 solution to allow the spermatozoa to swim up. The solution was diluted 1:20 with Ham F-10 solution. Aliquots of the solution were loaded into an improved Neubauer counting chamber using a pipette. *Caudal epididymal* fluid analysis was done using the World Health Organisation (WHO) 1999 criteria<sup>11</sup>. Motility was determined by counting the number of immotile spermatozoa and subtracting from the total count x 100 %.

### Testicular morphometry

The testicular volume was estimated by water displacement method using the Archimedes principle<sup>12</sup>, while the testicular weight was measured with an electronic balance. Briefly, the two testes of each rat were measured and the average values obtained for both regarded as one observation.

### Statistical analysis

Results were expressed as mean ± standard deviation. Analysis was carried out using analysis of variance (ANOVA) with Scheffé's post hoc test. The level of significance was considered at  $p < 0.05$ , except where otherwise stated.

## RESULT

### Sperm count and motility patterns in Sprague-Dawley rats

There was a marked duration dependent statistically significantly ( $p < 0.05$ ) decrease in sperm count and motility compared to the control (Figures 1 and 2). The sperm count of the rats treated with distilled water and extracts for 6, 8, 16, 24, 32 and 40 weeks were  $139.80 \pm 41.70$ ,  $17.9 \pm 21.33$ ,  $1.36 \pm 1.56$ ,  $0.42 \pm 0.43$ ,  $0.50 \pm 0.71$ ,  $0.58 \pm 0.88$  and  $0.38 \pm 0.52 \times 10^6/\text{ml}$  respectively. The sperm motility of the rats treated with distilled water and extracts for 6, 8, 16, 24, 32 and 40 weeks were  $93.6 \pm 7.89$ ,  $29.20 \pm 13.25$ ,  $9.60 \pm 7.09$ ,  $7.40 \pm 7.89$ ,  $3.60 \pm 4.93$ ,  $5.60 \pm 7.67$  and  $3.60 \pm 4.93\%$  respectively. The sperm counts (and motility) of rats treated with extract for 8 weeks and then allowed a recovery period of 16, 24, 32 and 40 weeks were  $139.80 \pm 41.70$  ( $93.6 \pm 7.89$ ),  $98.75 \pm 8.54$  ( $81.00 \pm 12.78$ ),  $132.50 \pm 20.62$  ( $93.25 \pm 12.20$ ),  $125.75 \pm 21.42$  ( $92.25 \pm 11.03$ ) and  $112.50 \pm 26.30 \times 10^6/\text{ml}$  ( $88.50 \pm 12.26\%$ ) respectively.

### Percentage population of Sprague-Dawley rats achieving targeted sperm count parameters

The following percentage experimental targets in the suppression phase were achieved for animals which completed the 40 weeks span (i.e. 6-40 weeks); 40%, became azoospermia, the earliest by week 8 and the latest after 24 weeks of treatment. Some 20% of animals suppressed to  $< 1$  million/ml and another 20% to  $< 3$  million/ml, after 32 weeks, while 16.7% suppressed to  $> 3 < 50$  million/ml. A few of the S-D rats fed with MC extract were relatively unresponsive to treatment, with nadir possible sperm density remaining at  $> 50$  million/ml at week 6 and this constituted a population of 3.3% (Figure 3).

### Contraceptive efficacy of extract in male Sprague-Dawley rats

The results showed none of the male rats treated with the extract for  $\geq 8$  weeks were able to fertilize the females exposed to them compared to male rats in the control (Table 1). In the Reversibility group however, it is interesting to note that by the 16<sup>th</sup> week of discontinuation  $\geq 80\%$  of extract pre-treated animals showed a significant level of recovery (Figures 1 and 2).

### Testicular Morphometry in Sprague-Dawley rats

The mean testicular weights (in grammes) of the testes were very similar to the values obtained for the testicular volume (in milliliters), giving a mean testicular density of one. The mean testicular volume of the control rats was  $1.14 \pm 0.22$  ml. The testicular volume and weight of rats treated with MC in the suppression phase showed a steady decline beginning from the 6<sup>th</sup> week ( $p < 0.05$ ). This decline continued throughout the suppression phase at a mean difference of approximately 0.04, 8 weekly from the 8<sup>th</sup> week till termination of the experiment at 40<sup>th</sup> week. After cessation of MC extract treatment for 8 weeks, the testicular volume and weight recovered to baseline control value (Table 2).

## DISCUSSION

The process of spermatogenesis begins after 20 days in rats and studies have also shown that the time taken for a complete spermatogenic cycle is 51.6–56 days and normal sperm counts for fertility to occur vary from 50–350 million sperm *per* ml in rats<sup>13</sup> while the time taken for spermatogonia to evolve into spermatozoa is about 64 days in man<sup>14</sup>.

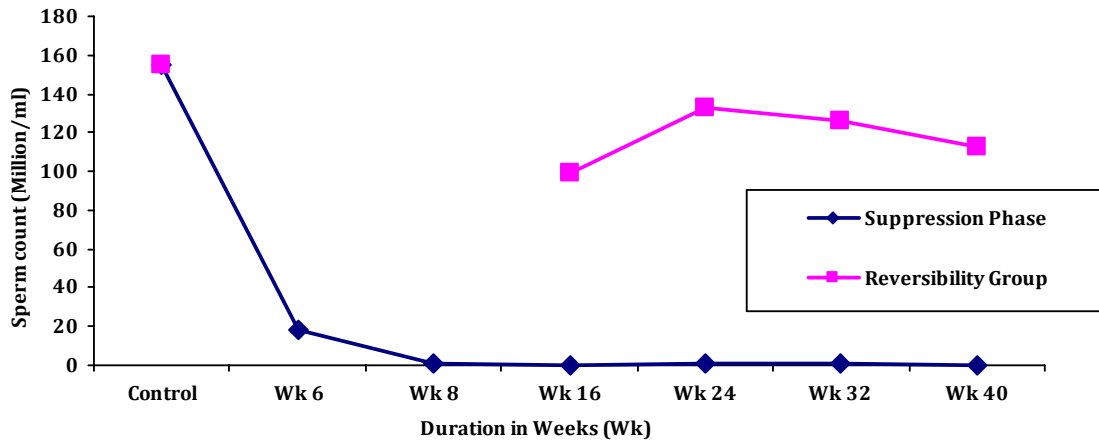


Figure 1: Sperm count in Sprague-Dawley rats fed distilled water and 50 mg/100 g *Momordica charantia* extract in control, suppression phase and reversibility groups

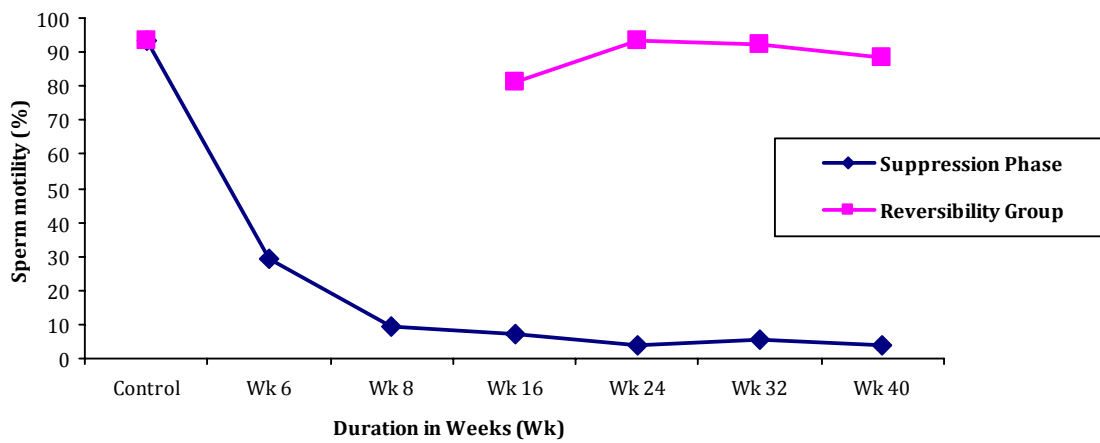


Figure 1: Sperm motility in Sprague-Dawley rats fed distilled water and 50 mg/100 g *Momordica charantia* extract in control, suppression phase and reversibility groups

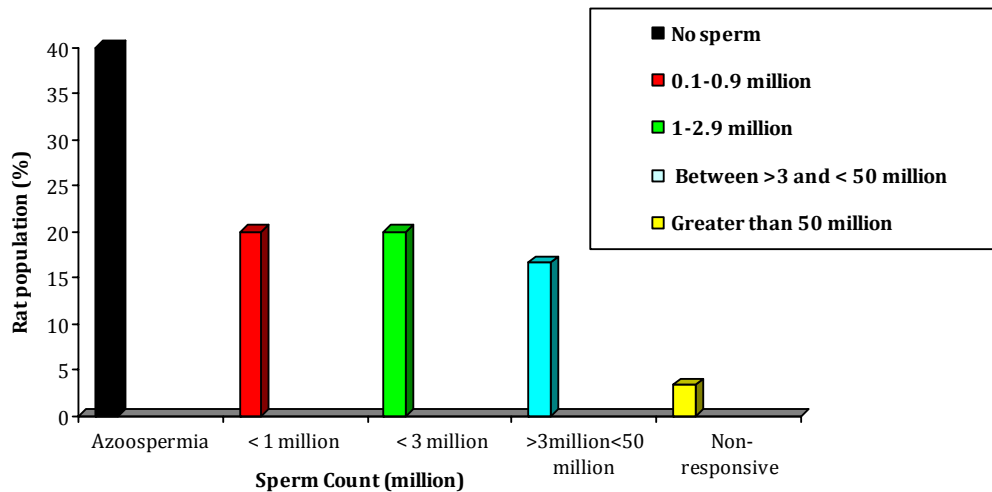


Figure 3: Percentage population of Sprague-Dawley rats achieving sperm count parameters in suppression phase group alone

Table 1: Fertility data in the "coupled rat model" in experimental and control groups

Groups (n=55)	Group detail	of pregnant rats	Mean # Foet
Suppression Phase Groups	Wk 6	2	3.00 ± 0.00 <sup>a</sup>
	Wk 8	0	0.00 ± 0.00 <sup>b</sup>
	Wk 16	0	0.00 ± 0.00 <sup>b</sup>
	Wk 24	0	0.00 ± 0.00 <sup>b</sup>
	Wk 32	0	0.00 ± 0.00 <sup>b</sup>
	Wk 40	0	0.00 ± 0.00 <sup>b</sup>
Reversibility Groups	Wk 16	4	6.00 ± 0.82
	Wk 24	5	5.60 ± 1.14
	Wk 32	5	5.40 ± 0.55
	Wk 40	5	6.60 ± 1.14
Control Group	Distilled water	4	5.75 ± 0.50

Values given represent mean ± standard deviation; <sup>a</sup> Significant difference at  $p < 0.05$ , <sup>b</sup> Significant difference  $< 0.001$ . Wk: week. Suppression phase groups: 50 mg/100 g of *Momordica charantia* extract given for duration of 6 to 40 weeks. Reversibility group: distilled water given for duration of 16 to 40 weeks after an initial 8 weeks treatment with 50 mg/100 g of *Momordica charantia* extract. #: Number. n: population of rats.

Table 2: Mean weight and volume of testes of experimental and control Sprague-Dawley rats

Groups (n =55)	Group details	Weight (g)	Volume (ml)
Suppression Phase Group	Wk 6	0.66 ± 0.11 <sup>a</sup>	0.65 ± 0.12 <sup>a</sup>
	Wk 8	0.32 ± 0.27 <sup>b</sup>	0.33 ± 0.29 <sup>b</sup>
	Wk 16	0.18 ± 0.08 <sup>b</sup>	0.18 ± 0.09 <sup>b</sup>
	Wk 24	0.32 ± 0.19 <sup>b</sup>	0.32 ± 0.19 <sup>b</sup>
	Wk 32	0.30 ± 0.16 <sup>b</sup>	0.30 ± 0.16 <sup>b</sup>
	Wk 40	0.30 ± 0.14 <sup>b</sup>	0.30 ± 0.14 <sup>b</sup>
Reversibility Group	16 Wk	1.40 ± 0.42	1.42 ± 0.42
	24 Wk	1.12 ± 0.24	1.14 ± 0.24
	32 Wk	1.12 ± 0.24	1.11 ± 0.25
	40 Wk	1.12 ± 0.24	1.14 ± 0.24
Control	Distilled Water	1.14 ± 0.22	1.14 ± 0.23

Values given represent mean ± standard deviation; <sup>a</sup> Significant difference at  $p < 0.05$ ; <sup>b</sup> Significant difference at  $p < 0.001$ ; Wk: week; Suppression phase groups: 50 mg/100 g of *Momordica charantia* extract given for duration of 6 to 40 weeks; Reversibility group: distilled water given for duration of 16 to 40 weeks after an initial 8 weeks of treatment with 50 mg/100 g of *Momordica charantia* extract. n: population of rats.

The absence of spermatozoa in the *caudal epididymis* (azoospermia) makes fertilization impossible and confers excellence to a contraceptive method<sup>21</sup>. In humans, in most trials of male hormonal contraception the majority of men have sperm counts suppressed to azoospermia, while some have a partial reduction in their sperm counts, (oligozoospermia)<sup>15</sup>. There was a markedly significant decrease in both the sperm count and motility (Figures 1 and 2) of the rats treated with MC seed extract when compared to the normospermic values obtained for the control animals. These were as a result of hypospermiogenesis and consequent hypospermatogenic effect of MC extract in the suppression phase group (Figures 1 and 2; Table 1). The semen quality is a measure of the ability of semen to accomplish fertilization thus, a measure of fertility. Since 100% of the rats treated with MC extract for ≥ 8 weeks (Table 1), did not fertilize the female rats it means that semen quality of rats in these groups were poor compared to control.

The most effective time regimen of MC seed extract fed daily at a dose of 50 mg/100 g was ≥ 16 weeks. At this period the sperm count became azoospermic, although there was no statistically significant difference in the number or percentage of pregnant S-D rats in the coupling assessment test for the four targets (azoospermia; < 1; < 3 and > 3 < 50 million/ml) in the suppression phase group (Table 1). Two (40%) of five rats mated in the rats treated with the extract for 6 weeks were able to achieve pregnancy. It was in this group, that 3.3% populations of rats were classified in the non-responsive group that had sperm counts > 50 million/ml (normospermic range). The reduced fertility (in rats treated with the extract for 6 weeks) may have been as a result of residual semen (17.9 ± 21.33 x 10<sup>6</sup>/ ml) in the *caudal epididymis*, since treatment was yet to cover one spermatogenic cycle. In all the reversibility groups the sperm density started to recover after 8 weeks of discontinuation of

treatment, and all animals achieved the recovery criteria of baseline control values by end of week 24 (that is 16 weeks after the end of treatment with the extract). The evidence of recovery of sperm functions following withdrawals of extract of MC shows that it was the extract that caused the contraceptive effect in the first place.

It is not entirely understood how the extract may have brought about the contraceptive effect. An understanding of the anatomic physiology of the male reproductive system shows that the germinal epithelium of the testes produces sperm cells whereas the interstitium have Leydig cells that are responsible for the production of testosterone required by the seminiferous tubules for cell maturation and function<sup>15</sup>. It therefore means that substances acting on the germinal epithelium and seminiferous tubular stroma will have a direct or indirect effect on semen parameters that may either enhance production or become deleterious to it. The latter being the case in this present study resulting in marked oligospermia and azoospermia. Azoospermia could also have resulted from a selective action of the extract on the developing cells. Further studies are however needed to substantiate these.

Another possible mechanism of contraceptive effect of MC might be via the inhibition of protein synthesis. The testicular morphometry of all the testicular samples harvested from rats fed MC seed extract showed a significantly reduced weight and volume compared to control counterpart indicating a wide spread damage which could be ascribed to a reduced protein contents in the testes. In another study researchers have shown that testicular volume correlate positively with testicular function<sup>16</sup> as well as the testosterone level<sup>17</sup>. It therefore means that the decreased testicular volume caused by the extract lead to the altered testicular function as seen

in both decreased sperm quality and inability to achieve pregnancy in the mated male rats treated with the extract above 8 weeks.

It is concluded from obtained data that methanolic seed extract of MC at an oral dose of 50 mg/100 g produced reversible sterility in male rats. It therefore holds a "strong promise" as a contraceptive agent, based also on the observed reversibility outcome of male fertility within a predictable time frame.

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