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Infecund evaluation of cycling female Sprague–Dawley rats: An aftermath treatment with *Momordica charantia* seed extract

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KEYWORDS

Momordica charantia;
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Abstract *Introduction:* Bitter melon (*Momordica charantia*) grows in tropical areas including parts of the Amazon, Africa, Asia and the Caribbean. It has an array of biologically active plant chemicals including triterpenes, proteins and steroids.

Aim: The aim is to evaluate the effect of methanolic seed extract of *M. charantia* (MC) on ova count, implantation and the fetus of Sprague–Dawley rats.

Methodology: Thirty adult cyclic female Sprague–Dawley (S–D) rats divided into three groups (A, B and C) of 10 rats/group were used for the study. The female rats in Groups B and C were made pregnant by cohabiting with male S–D rats. In all the groups, MC extract was administered in the morning (9.00 a.m.) at a dose of 25 mg/100 g b.w./oral. In Group A, rats (in proestrous phase) were treated with a single dose and sacrificed the following day (estrous phase). Rats in Group B were fed once daily from day 1 to 10 of gestation and sacrificed on the 12th day. Rats in Group C were fed once daily from day 6 to 19 of gestation and sacrificed on the 20th day of gestation. The

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following were assessed: ova count, anti-implantation, early abortifacient properties and possible teratogenicity.

Result: The extract completely suppressed the release of ova and exhibited highly significant anti-implantation activity. Significant ($p < 0.05$) changes were seen in the mean body weight, mean crown rump length and mean tail length of the fetuses.

Conclusion: In conclusion, MC (25 mg/100 g b.w.) exhibited anti-ovulatory and anti-implantation (early abortifacient) properties. These are certainly desirable anti-fertility actions. It also resulted in prenatal growth deficiencies.

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1. Introduction

Several cultures around the world have practiced extreme methods of contraception or birth control including castration and female circumcision (1). Use of goat bladder as condoms was seen among the Romans (2,3). In the Nigerian context, traditional methods were usually in the form of mixtures of plants, herbs, charms, copper or zinc rings or pendants, sponge or cloth soaked in vinegar, potassium salts and even suprapubic incisions rubbed with locally made medication (4). For example, in Nigeria, the Efik tribe as reported by UNPFA survey 1994 (5) practiced the use of spiritual 'Padlock', drinking of herbal extracts and abstinence. Similarly the Fulanis tribes wore amulets called 'layaru' and 'guruwol' around their waists (5). While the Igbos wore amulets (magical padlocks) around the waist and also ingested herbal extracts of brush nuts (5). The contraceptive property of the castor oil plant (*Ricinus communis* Linn.) used from ancient times for contraception among the Rukuba tribe in the Plateau State of Nigeria has also been confirmed (6,7).

In contemporary Nigeria, government's posture on contraception has changed to a rather positive attitude, hence the inclusion of family planning as an integral component of maternal and child health programmes. There is presently awareness by the government that the use of contraceptive as a means of regulating family size is an essential element of socio-economic development and is a key element in the population strategies of most developing countries because it addresses important health problems, rather than its strict impact on fertility.

National and international evidences indicate that contraception has a positive influence on maternal and child health by reducing high risk pregnancies, such as: Pregnancies before the age of 18 and after the age of 35 years and also pregnancies after the 4th birth and less than 2 years apart (4).

From time immemorial, plants and herbal preparations have provided a major source of food, useful drugs, additive colorants, binders, lubricants and flavoring agents. In fact about 30% of the total plant species on earth have a medicinal value (8). Modern scientific research has confirmed anti-fertility effect of numerous plants (9). *Momordica charantia* (MC) has been shown in folkloric medicine to possess antifertility property although there is paucity of scientific documentation.

Many plant preparations used today to control fertility have the advantage of availability, accessibility, affordability, reversibility and are associated with fewer side effects

compared to synthetic contraceptives currently in use which though effective, are saddled with side effects (10).

The search for an alternative contraceptive agent therefore continues. This study attempts to underscore the antifertility potentials of MC on the female rat of reproductive age.

2. Materials and methods

2.1. Plant materials (collection and identification)

Fresh fruits of MC were procured in Mushin a local market in the Lagos State of Nigeria. Identified and authenticated by a taxonomist Professor J. Olowokudejo in the Botany Department of the University of Lagos, where the voucher specimen was deposited (ascension number FHI 108422).

2.2. Processing of MC seed extract

The fruits were dried to get seeds which were weighed. The percentage yield of 230 g of MC in 1000 ml of methanol was prepared. The processes leading to the constitution of this formulation was done in the Pharmacognosy Department of College of Medicine, University of Lagos (CMUL). The dose of the extract administered orally was 25 mg/100 g body weight (11).

2.3. Animals

Thirty healthy adult female albino rats of S-D strain were used for this study. These rats weighing 125 ± 15 g and 8–10 weeks old were obtained from the Animal Breeding Laboratory Centre of CMUL. They were housed under standard animal house conditions (temperature: 28–31 °C; light: approximately 12 h natural light alternating with 12 h darkness per day; humidity: 50–55%) in well-ventilated plastic cages in the rat room, Anatomy Department of CMUL. Animals were allowed free access to pelleted food (Pfizer Nigeria Limited) and water *ad libitum*. Sun rays (natural light) provided light in the room which reflected through the glass windows. Illumination periodicity plays a dominant role in the incidence and duration of the stages of the ovarian cycle (12). These rats were also allowed to acclimatize to the laboratory environmental condition for 2 weeks.

The rats were weighed at procurement and weekly subsequently. Each animal has a 4-day estrous cycle, which was confirmed through vaginal smears taken and examined daily between 9.00 and 10.00 a.m. every morning for 16 days (4 cycles).

2.4. Experimental protocol

The 30 adult cyclic (4-day cycles) female S–D rats, weighing between 110 and 140 g were randomly divided into three groups (A, B and C) of 10 rats/group. A metal cannula was used to administer the extract (25 mg/100 g b.w.) at any given time by gastric gavages.

2.5. Effect of MC on ova count

Rats in Group A were divided further into two subgroups, designated treatment and control groups, each having five rats. On the morning (9.00 a.m.) of proestrous, rats in the treatment group received a single oral dose of MC (25 mg/100 g b.w.) while rats in the control group received the equal volume of distilled water. The rats were sacrificed the following day (estrus) by cervical dislocation. The upper third of both oviducts was dissected out and examined under a light microscope for the number of ova shed.

2.6. Effect of MC on implantation/early abortifacient activity

In Group B, 10 rats were used and were also divided into two, treatment and control groups. These female rats in the proestrous phase were allowed to mate with male rats of proven fertility from Animal Breeding Laboratory Centre of CMUL in the ratio of 2:1. The presence of vaginal sperm plug in the vaginal smear on the morning after was considered as a positive proof of mating and that day was designated as day 1 of pregnancy. These pregnant rats were weighed and MC (25 mg/100 g b.w.) administered orally at 9.00 a.m. daily, from day 1 to 10 of gestation. They were sacrificed on the 12th day of gestation by cervical dislocation (as in Group A). The two uteri horns were examined for implantation sites. The numbers of corpora lutea and resorption sites were recorded.

2.7. Effect of MC on fetal parameters

Again, the 10 rats in Group C were randomly divided into treatment and control groups of five rats each. They were made pregnant (as in Group B) and the possible teratogenic properties of MC evaluated. These pregnant rats were weighed at the beginning of the experiment, and then administered MC (25 mg/100 g b.w.) from the 6th to 19th day of gestation every morning at 9.00 a.m. These rats were weighed weekly during this period, after which they were all sacrificed on the 20th day of gestation by cervical dislocation under light chloroform anesthesia. The uterine horns were examined for dead and live fetuses. The live fetuses were counted and weighed. They were also examined with a magnifying lens for gross external malformations (limbs, mouth, face and spine). The crown rump and tail length as well as their individual weights were measured.

2.8. Statistical analysis

Data were expressed as mean \pm SD. Students' *t*-test and the one-way analysis of variance (ANOVA) test were used to analyze statistical data. The levels of significance were taken at $p < 0.05$. 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 (13) and were approved by

the Departmental Committee on the Use and Care of Animals in conformity with international acceptable standards.

3. Results

3.1. Number of ova shed

The administration of a single oral dose of MC (25 mg/100 g b.w.) in the morning (9.00–10.00 a.m.) of proestrus completely suppressed the release of ova. There were no ova seen in the excised fimbrial end of the oviducts of the treated rats on the morning of estrus compared to that of control. Table 1 shows the mean number of ova shed by the control and treated groups.

3.2. Implantation/the early abortifacient property of MC

The extract exhibited highly significant anti-implantation activity. There were observed *per* vagina bleeding (slight) in all the rats when the extract was administered to pregnant rats on the post-coital days 1–10. There were no resorption and post implantation sites in all the treated animals sacrificed on the 12th day of gestation.

3.3. Fetal parameters (teratogenic effects)

It was observed that there was a statistically significant ($p < 0.05$) decrease in the mean body weight, mean crown rump length and mean tail length of fetuses compared to that of control (Table 2). There was also a significant difference ($p < 0.05$) in the number of fetuses (Table 2). No gross abnormalities of the limb, tail or head region were observed compared to control. No death was recorded as a result of the test extract.

4. Discussion

There are three main routes to preventing or ending pregnancy they are: the prevention of fertilization of the ovum by sperm cells (contraception), the prevention of implantation of the blastocyst (contragestion) and the chemical/surgical induction of abortion of the developing embryo (14). In common usage,

Table 1 Effect on ovulation and number of ova shed.

Groups ($n = 30$)	Dose of extract	Number of ova
Experimental	MC at 25 mg/100 g b.w.	0 (none)
Control	2–5 ml of distilled water	3–4

Significantly different from value of control $p < 0.05$; MC: *Momordica charantia* seed extract; b.w.: body weight of Sprague–Dawley rat.

Table 2 Effect on fetal parameters.

Groups ($n = 30$)	No. of fetuses	CRL (cm)	TL (cm)	FW (g)
Experimental	3.0 \pm 0.05	2.5 \pm 0.10	1.0 \pm 0.01	1.8 \pm 0.02
Control	7.0 \pm 0.50	3.5 \pm 0.10	1.50 \pm 0.05	4.0 \pm 0.02

Significantly different from the value of control $p < 0.05$; CRL: crown rump length; TL: tail length; FW: fetal weight.

the term contraception is often used for both contraception and contragestion (14).

Many plants have been investigated for their antifertility potentials (7,15–17) with most exhibiting estrogenic or progestogenic activities (18) as the main contraceptive mode of action. However a recent study (19) demonstrated significant post-coital anti-implantation activity in the ethanolic root extract of *Momordica cymbalaria* fenzl which was not be due to these hormonal activities. This study is similar to our findings in which the orally administered MC seed extract was demonstrated to have a significant post-coital anti-implantation and early abortifacient effect.

The process of ovulation is comparable to an inflammatory process (20). Anti-inflammatory drugs have been employed in blocking ovulation (21). In this study, MC extract completely blocked ovulation as there were no ova seen in the oviduct of the treated rats on the morning of estrous. MC is known to have anti-inflammatory properties (22) which could actually be responsible for its ability to inhibit ovulation completely. Inhibition of ovulation is certainly a desirable contraceptive action. Other common plant agents in the sub-Saharan region that have been documented to possess similar anti-inflammatory property that also block ovulation are: *Garcinia kola* seed extract and cottonseed oil (16,23).

Studies have shown that ovulation follows a complex course initiated by the surge of the luteinizing hormone (LH). This is typified by the resumption of meiosis and the restructuring of the follicular wall, resulting in the follicular rupture and the release of a mature fertilizable ovum (24,25). Since the MC extract completely interfered with ovulation when administered on the morning (9.00 a.m.) of proestrus, it follows that the extract may have disrupted the proestrous surge of the LH that is responsible for the processes/events of ovulation.

MC has 'enjoyed popularity' in the treatment of diabetes being known to improve glucose tolerance to a degree similar to the oral hypoglycemic agent, tolbutamide (26). However its management in gestational diabetes still remains a controversial issue since even a minor degree of hypoglycemia can adversely affect the reproductive outcome (27). Significant morphological changes were observed in the developing fetus in the female S–D rats treated with MC seed extract on days 6–19 of gestation. Induced prenatal growth deficiencies observed in the litters include low mean birth weights, low mean crown rump length and low mean tail length of fetuses compared to their control counterpart. This indicates intrauterine growth retardation (IUGR). The IUGR in this study was not due to the reduction of gestational length or preterm delivery, and was probably not due to intrinsic fetal factors such as chromosomal abnormalities or other malformations. However, it may be attributed to impaired glucose supply to the fetuses.

In conclusion, this research work has demonstrated that MC at a dose of 25 mg/100 g b.w. has both contraceptive and contragestic properties in female S–D rats. An ideal female oral contraceptive agent that is easily available and affordable could be developed for humans if the antifertility potentials of the extract are harnessed. The implication would be a healthier mother and child as a result of reduced high risk pregnancies.

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