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Original Research Article

Effect of *Momordica charantia* Leaf Extract on Female Fertility and Foetal Well-Being in Sprague-Dawley Rats

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ABSTRACT

Infertility is a public health issue and women are often blamed for the childlessness of a couple. Solution is sort, sometimes from traditional medicines usually lacking scientific proof of efficacy. This research was aimed at investigating the effect of *Momordica charantia* leaf extract on female fertility and the well-being of the conceived fetuses. *Momordica charantia* has been utilized traditionally to treat various ailments with reported biological activities. There is however a dearth of knowledge on the effects of its leaf on female fertility and foetal well-being. Ethanol extract of *M. charantia* leaf (McL) was administered orally to cyclic Sprague-Dawley (S-D) rats at 100 mg/kg in pre-mating treatment for 14-17 days. Fertility index increased by 20%, number of implantation sites and live fetuses also increased. Foetal weight and crown-rump length in McL group were significantly ($p < 0.05$) higher than control with no physical malformations observed. This study suggests that pre-mating administration of *Momordica charantia* leaf extract to Sprague-Dawley rats confers pro-fertility and pro-implantation activities without foetal death or intrauterine growth restrictions at the dose administered.

***Keywords*:** *Momordica charantia*, Pre-mating treatment, Female infertility, Foetal measurement.

Introduction

Infertility impacts negatively on the lives of infertile couples, particularly on women who are perceived to suffer from infertility, regardless of whether they are infertile or not. They often blame themselves or are blamed by society for the childlessness of a couple[1] and experience violence, social stigma, emotional stress, depression, anxiety and low self-esteem.[2] Foetal death at any stage of gestation is also traumatic for the family.[3] Infertility as a disease of the reproductive system has been recognized as a public health issue by WHO considering the global decline in rate of fertility.[4] Globally, infertility impacts 15% of couples trying to conceive[5] affecting about 72.4 million people.[6] Africa suffers from inordinately high rates of infertility especially in parts of West, Central, and Southern Africa.[7] The rate of infertility in African countries ranges from 15-30%.[8] The prevalence of infertility in women has been estimated to be one in every seven couples in the western world and one in every four couples in developing countries.[9] Infertility due to female factors and unexplained infertility account for 50-80% of the cases in Nigeria.[10] Infertility remains a woman's social burden[4] and affected women seek remedies to improve their fertility. It is estimated that over 80% of the world population depend on traditional health care practices for the treatment of diseases[7] with up to 90% of the population in developing countries relying on the use of medicinal plants to help meet their Primary Health Care needs.[11] Lack of basic healthcare, social cultural and economic factors likely encourages the use of traditional medicine among the rural populations in the treatment and management of diseases.[12]

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Traditional, folk or herbal medicine is the most ancient method of curing diseases. It remains the most easily accessible, affordable and available.[13] A plant is considered medicinal when the whole plant or at least one of its parts has one or more medical properties for the prevention, relief or cure of disease conditions. Medicinal plants used to improve reproductive functions generally have more than one property.[14] Most African medicinal plants and herbal products however lack any scientific proof of validation of their efficacy and safety.[15]
Momordica charantia L. (Curcubitaceae) known as bitter melon (English), "*ejinrin-wewe*" (Yoruba) is a tropical and subtropical vine widely grown in India, south Asia, China, Africa and the Caribbean. It is one of the plants credited with both therapeutic and nutritional values.[16] *Momordica charantia* is used to expel intestinal gas, for tumors, wound treatment, rheumatism, malaria and vaginal discharge while the seeds are used to induce abortion.[17] The bitter fruit is antidiabetic.[17] The leaf is used to treat stomach pain, diabetes, fevers, cold, cough, headache, malaria, skin complaints, menstrual disorders, aches and pains, hypertension, sore, wound, infections, pile, jaundice and ringworm.[18] Several studies of the biolological activities of *M. charantia* have been documented [19-33]. These include its hypoglycemic,[19-22] anticancer,[23-26] antiviral,[27-29] antifertility in male[30,31] and antifertility activity of the fruit pulp and seeds in females.[32, 33] There is however a dearth of knowledge on the effects of its leaf on female fertility and foetal well-being.

Materials and Methods

Collection and extraction of Momordica charantia leaf
Momordica charantia leaves were collected between July and October 2017 from Irolu (Ikene LGA, Ogun state; 6.52000°N, 3.43000°E). Identification and authentication was done at the herbarium of the Forestry Research Institute of Nigerian (FRIN) (FHI) where voucher specimens with herbarium number FHI 112346 was deposited.
The plant material was washed under running water, pat dried and further dried under shade. The dried material was ground to powder using a blender, weighed and extracted. Extraction was by maceration in 40% ethanol at room temperature for 72 hours with regular agitation, 300 g : 3 L. The resultant extract was filtered, concentrated at 40°C on a

water bath, oven dried at 40°C. *M. charantia* leaves yielded 20.19% of dark green crude extract (McL). The dried extract was stored in refrigerator prior to use.

Animal models

Adult sexually naïve female Sprague Dawley (S-D) rats with initial weight of 150-200 g and vigorous males of proven fertility were used in this study. The animals were kept in well-ventilated cages in the animal house of the Department of Anatomy, College of Medicine University of Lagos (CMUL) under standard environmental conditions (approximately 12hour natural light/ dark cycles; temperature: 25-29°C) and allowed 2 weeks of acclimatization. They were fed with pelleted feed and water *ad libitum*. The estrous cycles were monitored by daily examination of vaginal smears taken between 7:00am and 9:00am. A total of 10 rats with established 3-4 consecutive estrus cycles were used for this study. Pre-treatment of the animals with the extract before mating (pre-mating treatment) was used.[34] All the animals were obtained from the animal house of Nigerian Institute for Medical Research (NIMR). The Lagos University Teaching Hospital (LUTH) Health Research Ethics Committee approved the experimental procedures (Approval no. ADM/DCST/HREC/APP/189).

Experimental design

The experimental animals were randomly divided into 2 groups of 5 animals each. Group 1 (control) received distilled water (5 ml/kg) while group 2 received treatment with *Momordica charantia* leaves extract (100 mg/kg) dissolved in distilled water. Administration was done using oral feeding cannula for 14[35, 36] -17 days. Treated cyclic adult female Spraque – Dawley rats were cohabited on proestrous day with untreated male of proven fertility, 2 females: 1 male. The animals were examined daily for visible vaginal sperm plugs and vaginal smear examined for detection of spermatozoa. Sperm positive females were isolated. Day of successful mating was taken to be Day 1 of pregnancy. They were euthanized under chloroform anesthesia and caesarian session performed on Day 19 of gestation. Fertility Index was calculated using:

$$\text{Fertility Index (FI)} = \frac{No.of\ Pregnant \times 100\%}{No.of\ Mated} \quad (34, 37)$$

The uterine horns were examined for implantation and/or resorption sites, live foetuses and foetal measurements were taken.

Statistical analysis

Data were expressed as mean ± standard error of mean and analyzed with Graph Pad Prism 8 using one-way Analysis of Variance (ANOVA). Statistical level of significance was considered at $P < 0.05$.

Results and Discussion

Pre-mating treatment by oral administration of 100 mg/kg with *Momordica charantia* leaf extract increased fertility index by 20% in Sprague-Dawley (S-D) rats. All the animals treated with 100 mg/kg bw. of *Momordica charantia* aqueous/ethanol leaf extract (McL) became pregnant while the control group which receive 5 ml/kg bw. of distilled water (control) recorded 80% fertility index (Table 1).

Momordica charantia leaf extract treated group also recorded higher number of implantation sites and foetuses (Figure 1). Implantation is regarded as an indication of reproductive capacity.[38] Extracts from other parts of *Momordica charantia* administered at different stages are however reported to have potential antifertility activities. The extract from *M. charantia* seed induced irregular estrus cycle, suppress the release of ova and cause significant post-coital anti-implantation effect in early stage of pregnancy.[34] The leaves of *M. charantia* are rich in carbohydrates, proteins, vitamins, mineral elements and other nutrients[39] offering nutritional and nutraceutical compounds to promote good health. These may explain the pro-fertility activity of the leaves as opposed to the anti-fertility activity of *M. charantia* seeds.

There were no deaths, physical malformations or anomalies observed in any of the feotuses from both *Momordica charantia* leaves extract treated and control groups. Stillbirths generally account for half of all perinatal mortality. It is estimated that 4 million occur annually worldwide and greater than 30 per 1000 births among the least developed countries, especially in Sub-Saharan Africa and Southeast Asia.[3] There was a statistically significant increase ($p < 0.05$) in mean foetal weight and crown-rump length of the foetuses borne by the treatment group in relation to the control group at 19 days of gestation. However, there was no significant difference in mean tail length of the fetuses (Figure 2). This is an indication of no intrauterine growth restrictions (IUGR). It further indicates the non-toxic profile of the extract on foetal development when orally administered at 100 mg/kg pre-conception. Intrauterine growth restriction (IUGR) is the single largest contributing factor to perinatal mortality in non-anomalous foetuses. Suboptimal intrauterine growth affects up to 10% of pregnancies and confers an increased risk of perinatal morbidity and mortality. The perinatal outcome of IUGR fetuses is largely dependent on the severity of growth restriction.[40]

Table 1: Effect of *Momordica charantia* leaf extract (McL) on fertility of pre-mating treated female Sprague-Dawley rats.

Effect/ Treatment	100 mg/kg McL	5 ml/Kg Distilled water
Mated females	5	5
Sperm positive females	5	5
Pregnant females	5	4
Fertility index (%)	100	80

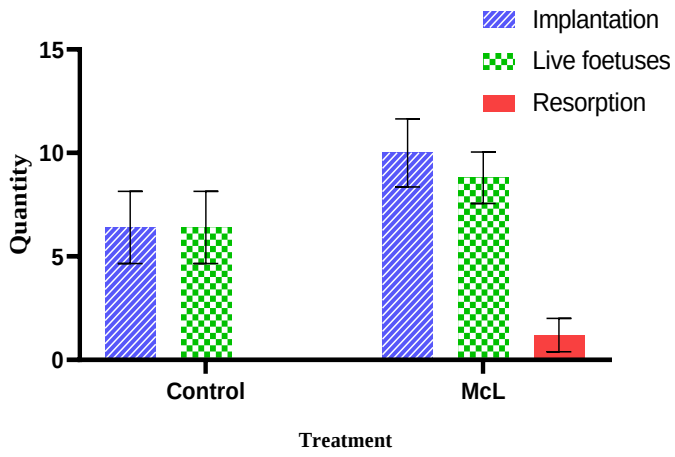

Figure 1: Effect of *Momordica charantia* leaf extract (McL) on number of implantations, foetal count and resorption in pre-mating treated female Sprague-Dawley rats.

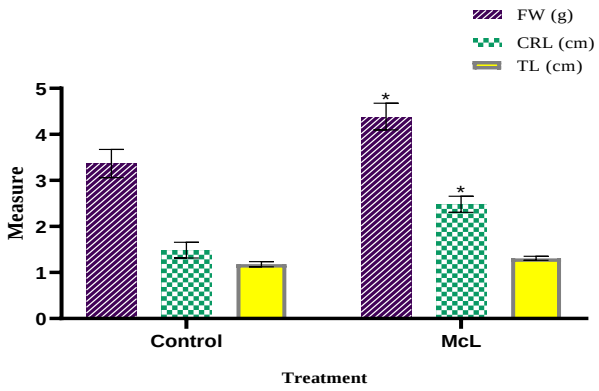

Figure 2: Effect of *Momordica charantia* leaf extract (McL) on foetal weight (FW), crown-rump length (CRL) and tail length (TL) in pregnant pre-mating treated Sprague-Dawley rats. Values are expressed as mean ± SEM. Bars with different subscripts are significantly different ($p < 0.05$).

Conclusion

Pre-mating administration of aqueous/ethanol extract of *Momordica charantia* leaf to Sprague-Dawley rats seems to confer pro-fertility and pro-implantation effects without foetal death or intrauterine growth restrictions at the dose administered. These were achieved by increasing fertility rate, implantation, foetal count, weight and size.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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