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Effects of *Moringa Oleifera* Lam Aqueous Leaf Extracts on Progesterone Hormone in Wistar Rats

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Abstract

This study evaluated the effect of Moringa oleifera aqueous leaf extract on progesterone in adult wistar rats. Thirty-six adult wistar rats consisting of twenty males and sixteen females were used. In the first phase of the study, the rats were divided into eight groups (groups A-H), based on sex and body weight. Groups A-D consisted of males and groups E-H consisted of females. Male groups of A, B and C and female groups E, F and G were respectively administered 1%, 5% and 10% Moringa oleifera aqueous leaf extracts for 14 days following acclimatization for 7 days. Groups D (males) and H (females) served as control. All the rats were fed rat chow and water ad libitum throughout the study. The second phase of the study was for further 30 days and involved mating the male and female rats that had the same dose of Moringa oleifera aqueous leaf extract. The data obtained were analyzed using means, standard error, standard deviation and analysis of variance (ANOVA). The result showed that progesterone concentration in groups A (28.30 ng/ml) and D (23.47 ng/ml) differed significantly ($p<0.05$) from B (8.80 ng/ml) and C (2.50 ng/ml). In the female groups, G (9.40 ng/ml) differed significantly ($p<0.05$) from E (34.60 ng/ml), F (28.50 ng/ml) and H (23.83 ng/ml). In the second phase of the study, group E_2 had the highest number of litters. The study concludes that Moringa oleifera can influence progesterone levels in a dose dependent manner and can be used as supplement especially for individuals experiencing low progesterone levels. The researcher therefore recommends that individuals should be cautious in the intake of Moringa oleifera because excess intake can lead to inability to conceive.

Keywords: **Moringa oleifera, edible plant, hormone, progesterone, wistar rats**

Introduction

Plants provide the richest source of natural compounds, which are used directly or indirectly for a wide range of applications for the wellbeing of humans and animals alike (Gupta *et al.*, 2017; Khanuja, 2012). *Moringa oleifera* Lam, a member of the *moringaceae* family of perennial angiosperm plants, is a fast-growing multipurpose miracle tree extensively grown in tropics and subtropics of Asia and Africa. It is widely distributed in India, Egypt, Philippines, Sri Lanka, Thailand, Malaysia, Burma, Pakistan, Singapore, West Indies, Cuba, Jamaica and Nigeria (Ponnuswami and Rani, 2019). It is an edible plant with a variety of nutritional and medicinal benefits attributed to its roots, bark, leaves, flowers, fruits and seeds (Falowo *et al.*, 2018; Mbikay, 2012). *M. oleifera* leaves have been reported to contain appreciable amount of fatty acids, minerals, and amino acids. (Teixeira *et al.*, 2014; Valdez-Solana *et al.*, 2015). A single gram of *M. oleifera* is important because its leaf powder contains twenty-five times the iron of spinach, ten times the vitamins of carrots, nine times the protein of yoghurt, half of the vitamins of oranges, fifteen times the potassium of banana fruits, and seventeen times the calcium of milk (Ajuogu *et al.*, 2018; Singh *et al.*, 2019). The leaves of *M.*

oleifera tree also exhibits antioxidant activity and contain high amounts of phenolic compound like quercetin and kaempferol (Singh *et al.,* 2019). Fresh leaves of *M. oleifera* contain 6.6–6.8 mg/100 g of â-carotene, greater than apricots, pumpkin and carrots (Kidmose *et al.,* 2006). Bark of *M. oleifera* is boiled in water and soaked in alcohol to acquired drinks and infusions that can be used to cure various ailments such as joint pain, diabetes, hemorrhoids hypertension, poor vision, anemia, tooth ache, and uterine disorder (Yabesh *et al.,* 2014).

M. oleifera has been found to contain relatively low amount of antinutrients such as phytates, saponins, tannins and oxalates (Shih *et al.,* 2011). These antinutrients, though not necessarily toxic or harmful, may interfere with digestion and absorption of other nutrients such as zinc, iron, calcium and magnesium when consumed in high quantities (Falowo *et al.,* 2018). Stevens *et al.,* 2015) however reported that phytate and saponin content in *M. oleifera* seed (2.23% and 3.89% respectively) and leaf (2.5% and 5.0% respectively) was lower than those found in other legumes, thus indicating its safety for consumption.

The medical applications of *M. oleifera* in food systems has been reported (Leone *et al.,* 2015; Dhakar *et al*., 2011). In many parts of Africa, it is generally consumed (either raw, dried or as an extract in an aqueous form) by patients suffering from malaria, typhoid fever, arthritis, swellings, cuts, diseases of the skin, genito-urinary ailments, hypertension, diabetes, HIV/AIDS and cancer (Al-Asmari *et al.,* 2015; Falowo *et al.,* 2018; Gandji *et al.,* 2018; Leone *et al.,* 2015; Nwamarah, *et al.,* 2015; Otitoju *et al*, 2014; Singh *et al.,* 2019; Popoola & Obembe, 2013).

Moringa oleifera has also been reported to be used as an abortifacient, in the treatment of menstrual pain, as an aphrodisiac and also in fertility enhancement as it promotes the production of follicle stimulating hormone, testosterone and progesterone (Ajuogu *et al.,* 2018; Nwamarah *et al.,* 2015; Zade *et al.,* 2013) Progesterone regulates a variety of functions in the body; playing a role in maintaining pregnancy, preparing the body for conception and regulating the monthly menstrual cycle (Fish, 2019; Goldstein, 2019). It is primarily produced in the ovaries (by the granulosa-lutein cells of the corpus luteum), the adrenal glands (near the kidney) and by the placenta during pregnancy. It is stored in the adipose tissue (Al-Asmakh, 2007; Fish, 2019; Goodson *et al.,* 2007).

Progesterone decreases as women approach menopause or due to a variety of health disorders (Caufriez, *et al.,* 2017). The effects of low levels of progesterone may range from mild symptoms such as fluid retention, vaginal dryness or migraine headaches to some serious effects including an increased risk of high blood pressure, certain forms of cancer as well as miscarriages (Gotter, 2018).

The objective of this study was to examine the effects of aqueous leaf extracts of *Moringa oleifera* on progesterone hormone in wistar rats.

Materials and Methods

Material Procurement

Mature male and female Wistar rats used for this study were procured from the faculty of veterinary medicine, University of Nigeria, Nsukka. The test sample, *M. oleifera* was obtained from the experimental farm of Crop Science Department, University of Nigeria, Nsukka. The samples were shade-dried and ground into powder using a household blender. All the chemicals used in this study were analytically graded. Accu-Bind ELISA Microwells kit was used for progesterone hormone assay.

Sample Preparation

Aqueous extract of 1% (1 g of *M. oleifera* leaf to 99 ml of water), 5% (5 g of *M. oleifera* leaf to 95 ml of water) and 10% (10 g of *M. oleifera* leaf to 90 ml of water) concentration were labeled as samples A, B and C respectively for males and E, F and G respectively for females. The control sample contained no extract (sample D and H).

Animal Treatment: Thirty-six mature wistar rats (20 males and 16 females) aged 10 weeks were used for the study. They were weighed and sorted into four test groups according to their weights—groups A-D consisted of males and groups E-H consisted of females. Groups D and H served as the control groups for males and females respectively. Each group contained five (5) males and four (4) females. They were acclimatized for a period of one week. The study comprised of two phases and lasted for of 51 days.

Phase 1: After acclimatization, the animals were fed with rat chow and water *ad libitum* and treated with the test leaf extract for 14 days. The concentrations of test sample administered were below the lethal dose (LD_{50}) of *M. oleifera* (15.9g/kg body weight). No test sample was administered to the control group. The extract was administered using a syringe attached to a cannula through the oral route. At the end of the first phase, the rats were anaesthetized, and blood samples analyzed to determine the level of progesterone hormone concentration.

Phase 2: The animals were regrouped for mating and were labelled E_2–H_2. Males introduced to females who were administered the same concentration of the test sample. This implied that group E_2 was a combination of groups A and E; F_2 was a combination of groups B and F; G_2 was a combination of groups C and G whereas H_2 was a combination of the control groups D and H. This phase lasted for 30 days. During this period, the animals were fed rat chow and water *ad libitum*. At the end of this phase, the number of offspring produced from each group was observed and noted.

Sample collection: Overnight, prior to sample collection, the animals were fasted from solid food. Blood (5 ml) was collected from the ocular median cantus vein of the rats with the aid of capillary tubes and transferred into two different test tubes, one containing EDTA (0.1ml) and the other containing no EDTA. Blood containing EDTA was centrifuged at 2000xg for 5 minutes and plasma samples were collected thereafter while blood without EDTA was allowed to clot and subsequently was centrifuged to obtain the serum component.

Hormone assay: The concentration of progesterone hormone was determined in the serum. This was done using Accu-Bind Enzyme Linked Immunosorbent Assay kits (Monobind Incorporated, Lake Forest, USA). All samples were frozen and stored in the Freezer at 20^0C until the time of analysis.

Data analysis: Data was subjected to analysis of variance (ANOVA). Means were separated using Duncan's Multiple Range test and significance accepted at 5% level of probability. Results were presented as means ± standard deviation.

Results

Table 1 show the mean concentration in ng/ml of the progesterone hormones of male and female wistar rats administered doses of *M. oleifera* aqueous leaf extracts. The result shows that the rats administered 1% of the test sample had the highest mean progesterone concentration (28.30 ng/ml for males and 34.60 ng/ml for the females) while the rats administered 10% of the test sample had the lowest mean progesterone concentration (2.50 ng/ml and 9.40 ng/ml for the male and female rats respectively). Generally, there was significant difference ($p<0.05$) in the mean concentration of progesterone of animals treated with *Moringa oleifera* aqueous leaf extract when compared to the control (23.47 ng/ml and 23.83 ng/ml for the males and female rats respectively).

Table 1: Mean concentration of progesterone (ng/ml) in Wistar rats administered with graded doses of *Moringa oleifera* aqueous extract

Group (males)	Progesterone concentration	Group (females)	Progesterone concentration
A (1%)	$28.30^{b} \pm 5.26$	E (1%)	$34.60^{a} \pm 15.03$
B (5%)	$8.80^{a} \pm 10.57$	F (5%)	$28.50^{a} \pm 6.35$
C (10%)	$2.50^{a} \pm 3.54$	G (10%)	$9.40^{b} \pm 18.80$
D (Control)	$23.47^{b} \pm 1.27$	H (Control)	$23.83^{a} \pm 14.70$

N=5 for males, n=4 for females
Mean ± S.D

Means carrying similar superscripts in the same column are not significantly different $p>0.05$

Table 2 shows the fecundity of wistar rats administered graded doses of *M. oleifera* aqueous leaf extract. The result shows that the group administered 1% of *M. oleifera* had the highest number of offspring (13) while group administered 5% of *M. oleifera* aqueous extract had the lowest number of offspring (7). Group administered 10% *M. oleifera* aqueous extract had no offspring while the control group had 9 off-springs.

Table 2: Fecundity in Wistar rats administered graded doses of *M. oleifera* aqueous extract

Group	Number of Litter
E_2 (1%)	13
F_2 (5%)	7
G_2 (10%)	No litter
H_2 (Control)	9

Discussion

The study revealed that aqueous *M. oleifera* leaf extract had no significant ($p>0.05$) effect on the concentration of progesterone when administered in 1% dosage when compared to the control, but had significant ($p<0.05$) effect on the concentration of progesterone when administered in 5% and 10% dosages (Table 1). This shows that aqueous *M. oleifera* leaf extract altered the progesterone concentration in the wistar rats' systems. The study also showed that *M. oleifera* has a dose dependent effect on the progesterone production in the wistar rats' system. Lower doses appeared to have more beneficial effects, suggesting that *M. oleifera* aqueous leaf extract may be helpful in supporting pregnancy as well as improving mammary gland development and milk production, at low doses, which are the fundamental physiological functions of progesterone in female animals; and also aids in the production of testosterone in the male animals (Nichols, 2017). This result is however in contrast with that of Ajuogu *et al.* (2018) who revealed that progesterone concentration increased with increasing dosage of the test sample in the diet of the subjects.

The result from the second phase of the study (Table 2) shows that rats that were administered the highest dose of aqueous *M. oleifera* leaf extract (group G_2) did not produce any litter. This could be as a result of the toxic effect caused by the high dose of the leaf extract administered. This effect could be compared to the study carried out by Seith *et al.* (1988) and Nwamarah *et al.* (2015). Seith *et al.* (1998) reported that abortifacient effect was observed in female albino rats that were administered aqueous

oral solution of *M. oleifera*, for 5-10 days post-mated. Nwamarah *et al.* (2015) hypothesized that this could be as a result of the low progesterone concentration recorded in the male and female wistar rats. This also confirms the report of Kate (2012) and Ajuogu *et al.* (2018) that *M. oleifera* leaves and flowers should not be used during pregnancy and may only be used in low doses while trying to get pregnant. The observed trend in the current study could also be due to the influence of *M. oleifera* on progesterone production. Progesterone levels in rats treated with 10% aqueous extract was very low (2.5 ng/ml for males and 9.4 ng/ml for females) compared with progesterone level of wistar rats treated with 1% aqueous extract (28.3 ng/ml for males and 34.6 ng/ml for females).

Conclusion and Recommendations

This study revealed that *Moringa oleifera* can influence progesterone levels in a dose dependent manner and can be used as supplement especially for individuals experiencing low progesterone levels and miscarriages using suitable doses. It also shows that excessive intake of *Moringa oleifera* can lead to inability to conceive, justifying caution in the use of the product. Abuses should be avoided. However, at lower doses, *Moringa oleifera* extracts can boost fertility but at higher doses act as contraceptive, preventing pregnancy. Based on the conclusion, the following recommendations are made:

1. Lower doses of *Moringa oleifera* leaf extract (0-5%) should be examined (using shorter dosage intervals) to determine the appropriate dose with the most beneficial effects.
2. The ability of high doses of *Moringa oleifera* leaf extract to prevent pregnancy should be further studied to understand its contraceptive potential.

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