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Effects of *Moringa oleifera* Lam. aqueous leaf extracts on follicle stimulating hormone and serum cholesterol in Wistar rats

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The study evaluated the effect of *Moringa oleifera* aqueous leaf extracts on follicle stimulating hormone and serum cholesterol in Wistar rats. Thirty six (36) mature Wistar rats (20 male and 16 female rats) were used. The male rats were grouped into four groups with five animals each, while the female animals were grouped into four made up of four animals per group, on the basis of their body weights. Graded doses (1, 5 and 10 g) aqueous extract were prepared from the *Mo* leaves as the test samples. In the first phase: The test extract was administered orally after acclimatization to individual groups: A-male and E-female rats, 1%; groups B-male and F-female rats, 5%; and groups C-male and F-female rats, 10%. These test groups also had rat chow and water *ad libitum.* The second phase of the experiment involved mating the male and female animals that had the same dose of *M. oleifera* extract. The results show that the mean body weights of the male rats increased significantly after treatment ($p<0.05$). The study also reveals that the administration of *M. oleifera* extract at different doses for the male and female rats differed significantly ($p<0.05$) with that of the control in raising the level of follicle stimulating hormone (FSH). A-1% increased by 38.52% while B-5% decreased (-21.20%); E-1% decreased (-12.96%) and F-5% (-25.64%). After mating, the % increase in FSH concentration was observed to be significantly ($p<0.05$) difference. Administration of Mo extract at different levels for the male and female rats differed significantly ($p<0.05$) in this study as compared with the control in lowering the total serum cholesterol of the rats. A-1% increased total cholesterol by 1.10%, while B-5% revealed a decrease of (-10.99%).

Key words: *Moringa oleifera*, follicle stimulating hormone, serum cholesterol, Wistar rats.

INTRODUCTION

The therapeutic use of *Moringa oleifera* parts in the Indian subcontinent dates back to antiquity. This plant is known as, a remedy to malnutrition and a vast range of ailments. *M. oleifera* is variably labelled as “Saviour of the Poor” (Mbikay, 2012). In many parts of Africa, it is widely consumed for self-medication by patients suffering from

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diabetes, anaemia, hypertension, or HIV/AIDS (Monera and Maponga, 2010; Otitoju et al., 2014; Beckman et al., 2000; Rotimi et al., 2011).

Besides culinary and other domestic uses, several biological properties ascribed to various parts of this plant have been reviewed in the past (Fahey, 2005). The leaves of *M. oleifera* have been reported to be a valuable source of both macro- and micronutrients, rich source of β-carotene, protein, vitamin C, calcium, and potassium and act as a good source of natural antioxidants; and thus enhance the shelf-life of fat-containing foods (Siddhuraju and Becker, 2003; Dillard and Bruce, 2000).

Follicle-stimulating hormone (FSH) is a glycoprotein gonadotropin secreted by the anterior pituitary in response to gonadotropin-releasing hormone (GnRH), which is released by the hypothalamus (Bowen, 2004). FSH is composed of alpha and beta subunits. The specific beta subunit confers the unique biological activity. FSH and luteinizing hormone LH bind to receptors in the testis and ovary and regulate gonadal function by promoting sex steroid production and gametogenesis (Grover et al., 2005). In women, follicle stimulating hormone stimulates the growth of ovarian follicles in the ovary before the release of an egg at ovulation, and promotes oestradiol production. In men, follicle stimulating hormone acts on the Sertoli cells of the testes to promote sperm production (spermatogenesis). Follicle stimulating hormone is one of the hormones essential to pubertal development and function of the gonads (ovaries and testes) both in women and men.

Cholesterol is a ubiquitous component of all animal tissues, where much of it is located in the membranes, although it is not evenly distributed (Maxfield and van Meer, 2010; Athenstaedt and Daum, 2006). In plants, it tends to be a minor component only of a complex mixture of structurally related phytosterols, although there are exceptions, but it is nevertheless important as a precursor of some plant hormones (John et al., 2007).

Within cells, cholesterol is the precursor molecule in several biochemical pathways (Smith, 1991). Cholesterol is an important precursor molecule for synthesis of vitamin D and the steroid hormones, including the adrenal gland hormones cortisol and aldosterone, as well as the sex hormones: progesterone, oestrogen and testosterone and their derivatives (Smith, 1991).

M. oleifera is reportedly used to alleviate menstrual pains; other reports implicated the same herb in causing abortion in rats hence its abonificient property. It has also been reported to be use in curing infertility; the actual effect of *M. oleifera* on the reproductive system and its hormones has not been fully investigated. Therefore, the objective of this study was to examine the effects of leaf extracts of *M. oleifera* on total cholesterol and follicle stimulating hormone.

MATERIALS AND METHODS

Mature Wistar rats (20 males and 16 females) were used for this study. Choice of different numbers of animals was to reduce competition among the males and for effective reproductive experiment. The animals were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria.

The *M. oleifera* leaves were gotten from the demonstration farm of Crop Science Department, University of Nigeria, Nsukka, pulverized and aqueous extract produced from them. The samples of *M. oleifera* leaves were shade-dried and subsequently ground to powder using household blender. Aqueous extract was prepared by adding 1 g of *M. oleifera* leaf to 99 ml of H_20; 5 g of *Mo* leaf to 95 ml of H_20; 10 g of *Mo* leaf to 90 ml of H_2O and each solution labelled.

Chemical and bio-chemicals

All the chemicals that were used in the research were of analytical grade. Total cholesterol assay, Randox Monza CH 200 kit was used; manufactured by Randox Laboratories Limited United Kingdom. For follicle stimulating hormone assay, Accu-Bind ELISA Microwells kit was used; manufactured by Monobind Incorporated United States of America. Chloroform, formalin was also used.

Animal treatment

Twenty (20) mature males and 16 female Wistar rats aged 10 weeks were weighed and divided into groups according to their weights; 3 test and control groups containing 5 rats each for males while the females had 4 rats each. The rats were kept in separate cages and labelled according to groups. They were acclimatized for a period of one week in the metabolic cages in the Department of Home Science, Nutrition and Dietetics, University of Nigeria Nsukka. They were fed with Vita feed Finisher (rat chow) and water *ad libitum*. The study lasted for a total duration of 51 days comprising of two phases.

Phase 1

After acclimatization, the rats were fed with rat chow and water *ad libitum*, and treated with test leaf extract A-1%, B-5% and C-10% for 14 days (these concentrations were below the LD_{50} of *M. oleifera;*15.9 g/kg body weight). The extract was administered using oral gavage except for the control group who had only rat chow and water *ad libitum*. At the end of the first phase, the rats were anesthetized and blood samples collected and analysed to determine the level of follicle stimulating hormone and total cholesterol concentration.

Phase 2

The animals were regrouped for mating, males introduced to females who were administered the same concentration of *M. oleifera* and monitored for 30 days. At the end of the duration, the rats were anaesticized with chloroform and blood samples collected and analysed to determine the follicle stimulating hormone and total cholesterol concentrations.

Sample collection

Overnight before the days of sample collection, the animals were fasted of 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 sample bottles containing no EDTA. The samples were allowed to clot and centrifuged under cold condition at 4000 rpm in a table top centrifuge. The serum layers were collected for the hormone assay.

Table 1. Mean feed intake in grams of male and female Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extracts.

Male group	Feed intake (g)	Female group	Feed intake (g)
A-1%	70.33^{b}±14.03	E-1%	61.07^{b}±9.47
B-5%	62.80^{b}±17.09	F-5%	52.33^{b}±14.27
C-10%	51.00^{b}±17.20	G-10%	54.20^{b}±9.64
D-control	151.60^{b}±20.21	H-control	110.33^{b}±34.09

Mean scores with the same superscripts are statistically different at $p < 0.05$. Values are mean±SD of five variants for male and four variants for female. A, Male group, 1% of *M. oleifera* aqueous leaf extract was administered; B, male group, 5% of *M. oleifera* aqueous leaf extract was administered; C, male group, 10% of *M. oleifera* aqueous leaf extract was administered; D, male control group, no *M. oleifera* aqueous leaf extract was administered; E, female group, 1% of *M. oleifera* aqueous leaf extract was administered; F, female group, 5% of *M. oleifera* aqueous leaf extract was administered ;G, female group, 10% of *M. oleifera* aqueous leaf extract was administered; H, female control group, no *M. oleifera* aqueous leaf extract was administered.

Table 2. Body weight of Wistar rats fed graded doses of *M. Oleifera* aqueous leaf extract and % Mean Increase at end of the third Week.

Male group	Mean	Increase (%)	Female group	Mean	Increase (%)
A-1%	269.16±22.26	4.40	E-1%	157.73±39.54	8.47
B-5%	223.72±71.94	46.40	F-5%	142.02±7.23	14.84
C-10%	233.38±40.84	30.38	G-10%	147.01±2.59	15.33
D-control	221.34±29.85	1.07	H-control	156.59±16.13	1.38

Mean scores with the same superscripts are statistically different at $p < 0.05$. Values are mean±SD of five variants for male and four variants for female. A, Male group, 1% of *M. oleifera* aqueous leaf extract was administered; B, male group, 5% of *M. oleifera* aqueous leaf extract was administered; C, male group, 10% of *M. oleifera* aqueous leaf extract was administered; D, male control group, no *M. oleifera* aqueous leaf extract was administered; E, female group, 1% of *M. oleifera* aqueous leaf extract was administered; F, female group, 5% of *M. oleifera* aqueous leaf extract was administered; G, Female group, 10% of *M. oleifera* aqueous leaf extract was administered; H, female control group, no *M. oleifera* aqueous leaf extract was administered.

Hormone assay

The concentrations of follicle stimulating hormone and total cholesterol were determined in the serum. The concentration of follicle stimulating hormone was carried out using Accu-bind Enzyme Linked Immunosorbent Assay kits from Monobind Incorporated, Lake Forest, USA. Total cholesterol concentration was spectrophotometrically determined using Randox cholesterol kits.

Assay principle

The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidise.

Data analysis

The mean, standard deviation and analysis of variance (ANOVA) test were used as the statistical tools for analysing the results between the treated groups and control groups.

RESULTS

Table 1 shows the mean feed intake by the different male and female groups of Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extract. Group A treated male rats with 1% *M. Oleifera* leaf extract had the highest mean feed intake but not compared to the control hence was significantly ($p<0.05$) different.

For female Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extract; the result showed that group E administered 1% *M. oleifera* aqueous leaf extract consumed 61.07 g of the feed, a value highest among the treated groups but not compared to the control. There was significant difference ($p<0.05$) between the means of the various female groups.

Table 2 shows the mean body weight (g) of Wistar rats that were fed graded doses of *M. oleifera* aqueous leaf extract. Male rats in group B that were administered 5% of *M. oleifera* aqueous leaf extract showed more weight gain of 46.40% than C fed 10% *Mo* (30.38%) at the end of the third week. Females in group F (5%) were observed to have weight gain of 14.84% than G 10% with 15.33%.

Table 3 shows the mean concentration in mIU/ml of follicle stimulating hormone (FSH) of male and female Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extracts. Male rats in group A that were administered 1% of *M. oleifera* aqueous leaf extract

Table 3. Concentration of follicle stimulating hormone of male and female Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extracts.

Male group	FSH conc. (mIU/ml)	Increase (%)	Female group	FSH conc. (mIU/ml)	Increase (%)
A-1%	3.92^{a}±2.28	38.52	E-1%	2.38^{b}±0.30	-12.96
B-5%	2.23^{b}±0.12	-21.20	F-5%	2.03^{c}±0.05	-25.64
C-10%	3.50^{a}±0.00	23.67	G-10%	2.55^{b}±0.52	-6.59
D-Control	2.83^{b}±0.15		H-control	2.73^{a}±0.25	

Mean scores with the same superscripts are statistically different at $p < 0.05$. Values are mean±SD of five variants for male and four variants for female. A, Male group, 1% of *M. oleifera* aqueous leaf extract was administered; B, male group, 5% of *M. oleifera* aqueous leaf extract was administered; C, male group, 10% of *M. oleifera* aqueous leaf extract was administered; D, male control group, no *M. oleifera* aqueous leaf extract was administered; E, female group, 1% of *M. oleifera* aqueous leaf extract was administered; F, female group, 5% of *M. oleifera* aqueous leaf extract was administered; G, female group, 10% of *M. oleifera* aqueous leaf extract was administered; H, female control group, no *M. oleifera* aqueous leaf extract was administered.

Table 4. Concentration (Mmol/l) of total cholesterol (TC) of male and female Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extract.

Male group	TC conc. (Mmol/L)	Increase (%)	Female group	TC conc. (Mmol/L)	Increase (%)
A-1%	2.76^{a}±0.43	1.10	E-1%	2.65^{c}±0.19	-4.33
B-5%	2.43^{b}±0.50	-10.99	F-5%	2.35^{b}±0.24	-15.16
C-10%	2.55^{b}±0.07	-6.59	G-10%	2.10^{c}±0.08	-24.19
D-control	2.73^{a}±0.40		H-control	2.77^{a}±0.45	

Mean scores with the same superscripts are statistically different at $p < 0.05$. Values are mean±SD of five variants for male and four variants for female. A, Male group, 1% of *M. oleifera* aqueous leaf extract was administered; B, male group, 5% of *M. oleifera* aqueous leaf extract was administered; C, male group, 10% of *M. oleifera* aqueous leaf extract was administered; D, male control group, no *M. oleifera* aqueous leaf extract was administered; E, female group, 1% of *M. oleifera* aqueous leaf extract was administered; F, female group, 5% of *M. oleifera* aqueous leaf extract was administered; G, female group, 10% of *M. oleifera* aqueous leaf extract was administered; H, female control group, no *M. oleifera* aqueous leaf extract was administered.

had the highest concentration of FSH (3.92 mIU/ml) with a percentage increment of 38.52%, followed by the rats in group C that were administered 10% of *M. oleifera* (3.50 mIU/ml) (23.67%) increase. The rats in group B which were administered 5% *M. oleifera* leaf extract had the lowest FSH concentration (2.23 mIU/ml) with a decrease of 21.20%. There was significant statistical difference ($p<0.05$) between the mean FSH concentration of the groups compared with control group.

Table 4 shows the mean concentration of total cholesterol of Wistar rats that were administered graded doses of *M. oleifera* aqueous leaf extract. Group A which was administered 1% of *M. oleifera* aqueous leaf extract, had the highest mean concentration, of total cholesterol (2.76 Mmol/L), with a percentage increase of 1.10% while group B which was given 5% of *M. oleifera* aqueous leaf extract had 2.43 Mmol/L with a decrease mean percentage of -10.99%. There was statistically significant difference ($p<0.05$) in the mean concentration of total cholesterol between the group.

DISCUSSION

This study reveals that during administration of the aqueous *M. oleifera* leaf extract, as shown in Table 1, aqueous *M. oleifera* leaf extract had significant ($p<0.05$) effect on the feed intake of the male Wistar rats. This shows that aqueous *M. oleifera* leaf extract altered the appetite and subsequently the weight of the rats. This confirms the reports by Chandra (2012) and Oyewo (2013) who stated that one of the reasons behind hunger is decreased amounts of vitamins, minerals and other needed nutritional elements. Because *M. oleifera* is so nutritionally dense, it provides many of these without a large amount of calories. This can keep the hunger urges from striking when they are unwanted. Based on the fact that the leaves themselves provide 42% of one's recommended daily protein, you will feel full and get the vitamins and minerals you need.

In addition to catering for basic nutritional needs, *M. oleifera* contains a unique blend of antioxidants and complex proteins that work together to provide a host of health benefits, including mental clarity, improved feelings of emotional wellbeing, increased energy and stamina. Many people who have weight problems found out that the enhanced emotional feelings go a long way in keeping away cravings for certain sugary foods (Chandra, 2012).

The use of *M. oleifera* as a potential herb for the treatment of infertility among its consumers is on the high

increase with antecedents of complaints relating to various observations such as irregular menstruation, loss of menstruation, heavy menstrual flow, and decrease sexual urge especially in men. The decrease in the concentrations of FSH gives a lot of information with respect to its biological functions. Since FSH and luteinising hormone LH bind to receptors in the testis and ovary and regulate gonadal function by promoting sex steroid production and gametogenesis (Grover et al., 2005), any decrease may in turn decrease its function relating to gametogenesis. Biologically in men, follicle stimulating hormone acts on the Sertoli cells of the testes to promote sperm production (spermatogenesis). Follicle stimulating hormone is also one of the hormones essential to pubertal development and function of the gonads (ovaries and testes) both in women and men (Grover et al., 2005).

In women, follicle stimulating hormone stimulates the growth of ovarian follicles in the ovary before the release of an egg at ovulation, and promotes oestradiol production (Grover et al., 2005). The rise in follicle stimulating hormone stimulates the growth of the follicle in the ovary. With this growth, the cells of the follicles produce increasing amount of oestradiol and inhibin (en.wikipedia.org/Wiki/Hypothalamic-pituitary-gonada-axis, cited 2014). The findings of the study also reveals that the administration of *M. oleifera* extract at different percentages for the male and female Wistar rats differed significantly ($P<0.05$) with that of the control in raising the follicle stimulating hormone (FSH) even after they were regrouped for mating. However, rats administered with the high and medium doses of *M. oleifera* leaf extract are reproductively superior to those that were given low doses and no dose. This is in contrast with a similar work done by Cajuday and Pocsido (2010) where they found out that the effect of *M. oleifera* had clearly manifestations enhancing male reproduction in all the treated groups compared with the control. Sudwan et al. (2007) found that the ethanolic extract of another plant, *Boesenbergia rotunda,* does not affect sexual behavior or serum androgen levels, but enhances seminiferous tubule, testis and seminal vesicle in the treated male rats. Similarly, Watcho et al. (2001) found that the rats treated with *Mondia whitei* for eight days induced an increase in the testicular weight, testicular testosterone level and sperm density without affecting the accessory gland weights whereas, Salman et al. (2013) and Gonzales et al., (2001) observed that rats treated with honey had significantly higher sperm counts compared to those in control group but there were no significant differences for sperm morphology, seminiferous tubule diameter, weights of reproductive organs and in the levels of reproductive hormones.

The loss in weight with increase in *M. oleifera* leaf extract implicates the product as agent of anti-obesity as reported by other studies which is a consequence hypocholesterolemia (Ghasi et al. 2000).

Conclusion

In conclusion, prolong oral and possible high dosages administration of aqueous leaf extract of *M. oleifera* may adversely affect the production process of follicle stimulating hormone. The observed cholesterol-reducing action of the crude leaf extract of *M. oleifera* indicates that this leafy vegetable possesses some potential medicinal value and could validate and explain its ethno-medicinal use on the obese and heart disease patients, but this observed positive effect may affect reproductive capacity if consumed over a long period of time.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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