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Moringa Plant Parts Consumption Had Effects on Reproductive Functions in Male And Female Rat Models

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Abstract: *Moringa oleifera has become a very popular plant due to its widely acclaimed nutritional and phytomedicinal values. The leaves, seed, pods and flowers are consumed and used as ingredients and spices, while concoctions are also made from them. The leaves and flowers may also be consumed as vegetables. There are however, concerns about the effects of the various parts of the plant on fertility and reproduction. This aspect of the effects of Moringa oleifera phytochemistry has not been elaborately explored. There are findings that point to the possibility of antifertility effects of some of its parts. This research investigated the effects of the leaf, seed, flower, stem bark and root on reproductive functions of experimental animals. These are the parts of the plants that are typically used and consumed. In order to model human consumption of the plant parts; food formulas were prepared with the plant parts. Animals were divided into eleven groups labeled Groups 1-11. Groups 1 and 2 were given the high and low doses of the seed in the feed formula respectively; Groups 3 and 4 were given the high and low doses of the leaf in the feed formula respectively; Groups 5 and 6 were given the high and low doses of the flower in the feed formula respectively; Groups 7 and 8 were given the high and low doses of the root in the feed formula respectively; Groups 9 and 10 were given the high and low doses of the stem bark in the feed formula respectively while Group 11 served as the standard control and the animal were fed the normal chow. Experiment lasted 30 days and animals were thereafter sacrificed by cervical dislocation. Blood samples were collected, spermatozoa were collected from male animals and the testis in the male and uterus in the female were dissected and excised. Specific tests and analysis included packed cell volume [PCV (%)]; Sperm cell count (x10^{6}cells/ml), Sperm cell motility (%), Normal sperm cells (%), Abnormal sperm cells (%) to observe male reproductive functions; LH (mIU/ml), FSH (mIU/ml), Testosterone (ng/ml) to observe reproductive hormone functions and observation of the implantation sites to observe abortion probabilities. Results show that plant parts interfered with fertility functions to varying extents. It should be noted that the ingestion of certain moringa plant parts interfered with fertility parameters.*

Keyword: *Fertility, Reproduction, Moringa, Leaf, Flower, Bark, Seed, Root*

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I. Introduction

Moringa, especially the specie *oleifera*, has been described as one of the most nutritious and beneficial plants to human health and wellbeing ever known (Ram, 1994; Mughal *et al.*, 1999; Mishra *et al*, 2011). The exact level of benefits as well as the possible side effects and adverse effects with consequent precautions and contra-indications to the consumption of moringa are however yet to be adequately and scientifically validated by research. Adequate scientific researches that address global vital questions with regards to the safety of moringa consumption in different physiological and pathological conditions are therefore indispensable to exploring optimally the benefits of the plant. Medicinal plants are extensively used to relieve sexual dysfunction or as fertility enhancing agent, through their nutritional content known to improve sexual performance and fertility (Sumalatha *et al.*, 2010; Yakubu *et al.*, 2007), however, the safety level of consumption of each plant part, especially during pregnancy has not been ascertained. The study therefore set out to evaluate the effect moringa plant parts on blood cell levels, fertility hormones, implantation of ova in female (pregnancy assessment) and semen quality in male experimental rats.

II. Materials And Methods

Experimental animals were adult Wistar rats; 55 females and 55 males, weighing between 160g and 200g. The male and female rats were randomly grouped into 11 groups respectively, for each moringa plant part extract namely seed, leave, flower, root, stem and control respectively at 2 dosages- High dose[HD] and Low dose [LD], with doses administered through rat chow on the basis of the groupings (Table 1). HD constituted 500mg of extract per Kg body weight for all moringa plant parts except the seed, with the HD concentration at 200mg per Kg body weight, while LD constituted 200mg of extract per Kg body weight for other moringa plant parts except seed, with a HD concentration of 100mg/Kg body weight, because of its known toxicity. Male and female rats in all groups were fed with the constituted diet for 30 days.

Table 1: Animal grouping and Moringa plant part dosing

MORINGA PLANT PART	GROUP FEED FORMULA
SEED	Group 1 [HDS] High Dose of Seed Group 2 [LDS] Low Dose of Seed
LEAF	Group 3 [HDL] High Dose of Leaf Group 4 [LDL] Low Dose of Leaf
FLOWER	Group 5 [HDF] High Dose of Flower Group 6 [LDF] Low Dose of Flower
ROOT	Group 7 [HDR] High Dose of Root Group 8 [LDR] Low Dose of Root
STEM	Group 9 [HDM] High Dose of Stem bark Group 10 [LDM] Low Dose of Stem bark
CONTROL	Group 11 Normal Chow

LD= Low Dose; HD= High Dose (S-seed, L-leaf, F-flower, R- root, M- stem)

The rat feed was constituted using the formula

$$\text{Diet dose (mg/Kg)} = \frac{\text{Daily dose (mg/Kg) X body weight (g)}}{\text{Daily food intake (g)}}$$

Blood cell volume

At the end of the experiments, blood samples were collected by cardiac puncture, and a portion placed in a capillary tube and centrifuged at 10,000rpm for 10 minutes in a micro-hematocrit centrifuge. The packed cell volume (PCV) values were obtained with a micro-hematocrit reader and recorded.

Hormonal assays

Blood samples obtained from rats in all the groups were centrifuged at 3000 rpm for 15 minutes and serum separated and kept frozen till hormonal assay was carried out. Serum levels of progesterone, testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured by enzyme-linked immunoassay (ELISA) technique (Elabscience, P.R.C).

Mating and induction of pregnancy

The stages of the oestrous cycle of the rats were determined by microscopic analysis of the predominant cell type in vaginal smears obtained daily for a two - week period (Marcondes *et al.,* 2002). Only female rats showing two consecutive oestrous cycles of the same length were used. Pregnancy was induced by mating a pro-oestrus female rat with a mature and proven adult male rat overnight. This was to ensure that copulation occurred at oestrus, the period when the female rat is receptive to the male rat. Successful mating was confirmed by the presence of sperm cells in the vaginal smear carried out the following morning and the day was regarded as day 1 of pregnancy (Ratnasooriya *et al.*, 2003).

Implantation studies

Implantation studies were carried out on day 8 of pregnancy in rats from all the groups. The tail veins of the rats were dilated using Xylene after which 0.5ml of Evans Blue dye (1mg/ml) was injected into the vein. This dye when injected intravenously combines with albumin and therefore stains all structures in the extra cellular space. The dye was allowed to equilibrate in the rat for 15 minutes after which cervical dislocations were carried out on the animals and were placed in the supine position on the dissecting board. This was followed by laparatomy, the lower abdominal region was cut open and the implantation sites of the blastocyst stained by Evans Blue dye were counted in the rat uterus.

Sperm cell count and motility

50 mg of caudal epididymis was minced in 2.5 ml of physiological saline. One drop of an evenly mixed sample was applied to a Neubauer's counting chamber under a cover glass. Sperm cell motility was expressed in percentage relative to the total sperm cells per unit area. Epididymal sperm cell counts was made by routine procedure and expressed as million/ml of suspension, as previously described by Prasad *et al.,* 1972.

Sperm cell morphology

Sperm smears were prepared by placing a drop of minced sample of caudal epididymis and one drop of eosin stain on a clean slide, and spread using a slide spreader. The smears were allowed to air dry and then examined using high power (100X) microscope oil immersion objective. 100 sperm cells from different fields were examined and the number of normal and abnormal sperm cell forms were estimated in percentage.

III. Results

Table 2: PCV, number of implantation sites and levels of reproductive hormones in female rats

GROUP	PCV (%)	Implantation sites (n)	Progesterone (ng/ml)	Estradiol (pg/ml)
1	27.0±3.0*	Nil	8.1±3.2	13.7±5.0
2	29.5±2.5	Nil	13.1±3.5	18.0±4.6
3	48.5±4.0*	9.0±2.0*	30.5±5.1*	44.1±5.7*
4	39.5±3.5	6.0±1.0	23.1±4.5*	35.5±4.1*
5	36.5±5.0	Nil	19.8±3.2	28.9±3.6*
6	42.5±4.5*	Nil	19.3±3.0*	24.1±4.5*
7	24.5±6.0*	Nil	6.0±2.8*	12.4±3.8
8	26.0±3.5*	Nil	7.6±2.4	14.0±5.4
9	35.5±4.0	Nil	6.3±3.9*	12.4±4.8
10	33.0±2.5	Nil	9.1±3.8	14.1±3.5
11	36.0±3.0	6.0±1.5	10.9±8.5	16.0±5.2

Results are presented in mean ± SEM *P < 0.05 vs. Control

Group 1: HD seed, Group 2: LD seed, Group 3: HD leave, Group 4: LD leave, Group 5: HD flower, Group 6: LD flower, Group7: HD root, Group 8: LD root, Group 9: HD stem, Group 10: LD stem, Group 11: Control. (HD: 500mg per kg body weight of Moringa plant part except seed: 200mg per kg body weight; LD: 200mg per kg body weight of Moringa plant part except seed: 100mg per kg body weight).

Table 3: PCV, Sperm cell counts, motility, morphology and reproductive hormone levels in male rats

GROUP	PCV (%)	Sperm cell count (x10^{6}cells/ml)	Sperm cell motility (%)	Normal sperm cells (%)	Abnormal sperm cells (%)	LH (mIU/ml)	FSH (mIU/ml)	Testosterone (ng/ml)
1	42.5±6.0	58.5±7.5*	65.0±5.0*	96.5±1.5	3.5±2.0	11.3±5.0	4.8±1.8*	10.0±3.2*
2	38.0±4.5	68.0±4.0	80.0±4.5	95.0±2.0	5.0±3.0	13.4±6.5	5.8±0.9	5.9±1.1*
3	48.0±5.5*	76.5±2.5*	85.0±5.0	94.5±1.5	5.5±1.5	20.2±4.1*	12.3±1.5*	11.3±2*
4	45.0±3.5*	72.0±5.5	80.0±7.5	97.0±2.0	3.0±2.5	15.3±3.8*	9.3±2.2	8.3±2.4*
5	40.0±3.0	65.5±2.5	85.0±5.0	98.5±3.0	1.5±0.5	17.1±4.4*	7.2±3.4	6.9±1.7*
6	44.5±5.5	68.5±4.0	70.5±5.0	95.5±3.5	4.5±2.5	13.2±3.9	6.5±2.1	5.5±1.3*
7	41.0±2.5	56.0±6.5*	60.5±2.5*	85.0±2.5*	15.0±2.0*	4.4±2.0	3.1±1.1*	1.2±0.5
8	42.5±3.0	59.5±8.0*	65.0±4.5*	80.0±2.0*	20.0±2.5*	6.2±2.5	4.2±1.4*	1.8±0.6
9	38.5±4.5	60.5±3.5	75.0±5.0	90.0±1.5	10.0±1.5*	5.8±3.1	5.1±1.2	3.4±1.4
9	40.5±4.0	64.0±5.5	70.5±2.5	95.0±1.5	5.0±2.0	4.3±2.9	4.8±1.7*	4.5±1.8*
10	44.0±3.5	68.0±2.5	75.0±5.0	95.0±2.0	5.0±2.5	6.1±2.3	6.4±2.0	2.6±0.9
11	39.5±5.0	69.5±5.0	82.5±4.5	98.5±1.5	1.5±0.5	9.9±3.0	7.3±1.9	2.0±0.4

Results are presented in mean ± SEM *P < 0.05 vs. Control

Group 1: HD seed, Group 2: LD seed, Group 3: HD leave, Group 4: LD leave, Group 5: HD flower, Group 6: LD flower, Group7: HD root, Group 8: LD root, Group 9: HD stem, Group 10: LD stem, Group 11: Control. (HD: 500mg per kg body weight of Moringa plant part except seed: 200mg per kg body weight; LD: 200mg per kg body weight of Moringa plant part except seed: 100mg per kg body weight).

IV. Discussion

Findings from the present studies show that administration of Moringa leaves at doses of 200mg and 500mg per kg body weight for 30 days, significantly increased blood cell volume in both male and female rats. A significant decrease in blood cell volume was however observed in female rats administered with Moringa seed at 200mg per kg body weight and Moringa root at both 200mg and 500mg per body weight respectively (Table 2). The findings on Moringa leaves corroborate previous report that moringa leaves boost blood cell production, hence it can be used as hematinic in both males and females. Administration of Moringa seed and root at a dose 200mg per kg body weight and 500mg per kg body weight (Moringa root) however appear to impact negatively on blood cell production in females as blood cell volume was significantly reduced in female rats administered Moringa seed and root at these doses. No significant difference was however observed in the

blood cell volume of both male and female rats administered with a lower dose of Moringa seed (100mg per kg body weight).

Administration of Moringa leaves at 200mg and 500mg per kg body weight for 30 days significantly increased the number of implantation sites on day 8 of pregnancy in rats. No site of implantation was however observed in the high and low dose group of rats administered with other Moringa plant parts namely the seed, flower, root and stem. This finding suggests that the consumption of Moringa leaves increases female reproductive function and fecundity in rats, as evidenced by the increase in the number of embryo successfully implanted in the rats' uterus. Moringa leaves may enhance reproductive function in females by increasing the secretion or availability of ovarian hormones namely progesterone and estrogens as observed in this study. Progesterone is traditionally known to be essential for pregnancy maintenance as it keeps the uterus quiescent preventing premature onset of labour, hence its name 'pro-gestation', while estrogens are essential for ovulation, implantation, pregnancy maintenance and childbirth. This finding also implies that the consumption of Moringa leaves is safe, and does not cause abortion during pregnancy. Consumption of the other Moringa plant parts namely the seed, flower, root and stem is however not advised, as they may be antifertility or abortifacient, as observed in this study.

Sperm cell count was significantly increased in male rats administered with 500mg of Moringa leaves per kg body weight for 30 days in this study (Table 3). This finding suggests that the consumption of Moringa leaves increases reproductive function in males as evidenced by the increased levels of reproductive hormones namely LH, FSH and testosterone in male rats administered with Moringa leaves at 200mg and 500mg per kg body weight. Moringa leaves may enhance testosterone secretion allowing better availability of the hormone to the gonads. The testes, epididymis and other reproductive organs are structurally and physiologically dependent on LH, FSH and testosterone for sperm cell production and maturation. LH, FSH and testosterone stimulates the growth and secretary functions of the male reproductive organs, therefore, a significant increase in these hormones therefore lead to increased sperm cell count and motility as observed in this study. Similar findings were reported by previous studied (Akunna *et al.,* 2012; Dafalla *et al.,* 2015). Rats treated for 8 weeks with ascorbic acid, a potent antioxidant present in Moringa leaves, showed a significantly increased epididymal sperm cell concentration (Sonmez *et al*., 2005). Flavonoids also present in Moringa leaves are well known antioxidants that prevent oxidative stress and testicular impairments in animal (Kujo, 2004).

Sperm cell count, motility and viability are regulated by the gonadotropins, LH and FSH. FSH binds with receptors in the sertoli cells of the testes and directly stimulates spermatogenesis. Moringa oleifera leaves administered at 50 mg per kg body weight orally for 100 days have been reported to improve plasma testosterone levels by Akunna *et al.,* (2012). Saponins, also present in moringa leaves have been reported to boost testosterone levels (Gauthaman and Adaikan, 2008). LH stimulates the production of testosterone in Leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and indirectly stimulates spermatogenesis through testosterone (Singh *et al*., 1995). An increase in LH hormone concentration therefore increases testosterone secretion from leydig cells (O'Donnel *et al.*, 1994; Lilibeth and Glorina, 2010).

Administration of Moringa seed at a dose of 200mg per kg body weight, Moringa root at 200mg and 500mg body weight respectively, significantly reduced sperm cell count and motility. Alterations in sperm cell morphology were also observed in these groups as the percentages of abnormal shaped sperm cells were significantly higher in these groups. These findings suggest that the consumption of Moringa seed and root at doses higher than 100mg per kg body weight impairs reproductive function in male rats. A significant decrease was also observed in FSH levels in male rats administered with 200mg per kg body weight of Moringa seed, 200mg and 500mg per kg body weight of Moringa root and 500mg per kg body weight of Moringa stem. This finding suggests that these Moringa plant parts impair sperm cell count, motility and structure by reducing FSH levels essential for sperm cell production.

Moringa plant has significant variations in the distributions of its phytochemical substances across its various parts (Williams *et al.,* 2016). These are expected to affect the effects that were produced by the consumption of the feed formula from its various parts. Another possible factor is the variations in the concentrations of the phytochemicals in the various parts.

V. Conclusion And Recommendation

The consumption of moringa leaf did not have negative effects on male and female fertility indices; rather, it produced positive or enhancing effects on most tested parameters. However, other parts of the plant affected fertility negatively. This indicated that various parts of the plants could affect fertility differently and this should be considered when consuming the plant parts. These variations are attributable to variations in phytochemicals in the various arts as well as their proportions or concentrations.

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