

Full Length Research Paper

Effects of *Moringa oleifera* Lam. (Moringaceae) on the reproduction of male mice (*Mus musculus*)

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The effects of *Moringa oleifera* Lam. on the reproduction in male mice were studied. Twenty four male ICR mice divided equally into four groups were administered with the hexane extract of the leaves of *M. oleifera* by gavage at doses of 0.5, 5 and 50 mg/30 g BW daily for 21 days. The vehicle corn oil was used as control. Data on body weights, weights of reproductive organs, diameter of seminiferous tubule, stage of maturity, and levels of serum luteinizing hormone and follicle stimulating hormone were obtained and analyzed using one-way ANOVA and Tukey's test. Significant findings were: increased weights of testis (at medium and high doses); epididymis (at all doses); seminal vesicle (at the high dose) and also increased seminiferous tubule diameter (at all doses); increased thickness of epididymal wall (at medium and high doses); higher score for lumen formation (at the high dose) and epididymal maturity (at all doses). No significant effects on the level of the 2 hormones were obtained.

Key words: *Moringa oleifera*, reproductive organs, epididymal maturity, LH and FSH levels, antioxidant system, spermatogenesis.

INTRODUCTION

Moringa oleifera Lam. is a rapidly growing tree that is native to India (Tsaknis et al., 1999), endemic in Asia (Duke, 1982) and the most widely cultivated species of a mono generic family, the Moringaceae (Fahey, 2000). This plant is extraordinary because all of its parts are edible and possess nutritional and medicinal values.

In the ancient times, the *Moringa* tree has gained popularity as a nutrition power plant that can feed the needy and save lives. Its leaves are an excellent source of vitamin A (four times the amount in carrots), vitamin C (seven times the amount in oranges), vitamin B, calcium (four times the amount in milk), protein (twice the amount in milk), and potassium (three times the amount in bananas). In addition, *Moringa* contains specific plant pigments with demonstrated potent antioxidant properties such as the carotenoids - lutein, alpha-carotene and beta-carotene, xanthins, and chlorophyll; other phytochemicals with known powerful antioxidant ability - kaempferol, quercetin, rutin and caffeoylquinic acids; powerful antioxidant vitamins - C, E, and A and essential

micronutrients with antioxidant activity - selenium and zinc (Fuglie, 1999).

M. oleifera L. is used in a number of tropical countries for medicinal purposes (Foidl et al., 1999). Extracts of various *Moringa* tissues have been used as anti-cancer (Guevarra et al., 1999), anti-trypanosomal (Mekonnen et al., 1999), antimicrobial (Caceres et al., 1991) and anti-inflammatory and hepatoprotective (Kurma and Mishra 1998) agents. In addition, leaf extracts have been shown to regulate thyroid status (Tahiliani et al., 2000) and cholesterol levels in rats (Ghasi et al., 2000).

Recently, novel derivatives of thiocarbamates and nitriles which stimulate insulin release in animals have been found in the plant explaining its anti diabetic mechanism of action (Fahey, 2000). Indeed, science continues to validate the ancient traditional therapeutic uses of *Moringa* (Figure 1).

To date, there is an ongoing scientific investigation on some of the reported therapeutic and medicinal properties of the plant. Just recently, the role of the plant in improving male reproduction has been the focus of interest.

This accounts to the fact that male infertility is one of the problems faced by 10% of couples in the society and within a population; the incidence rate for male infertility is

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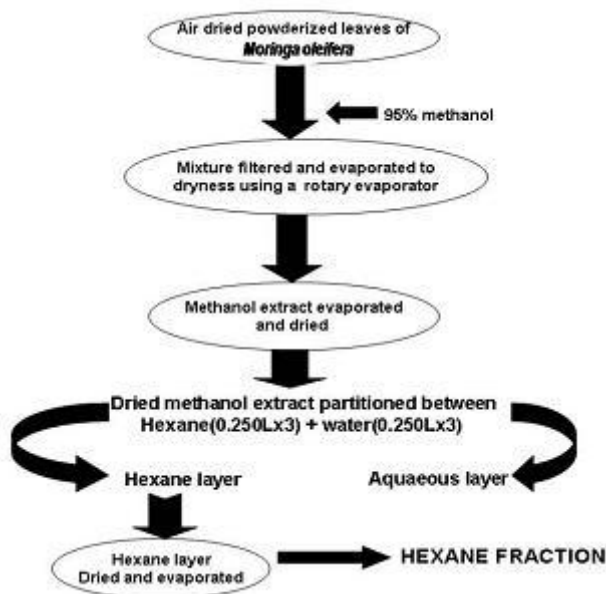


Figure 1. Preparation of methanol extraction and hexane partition fraction of dried leaves of *Moringa oleifera* Lam.

approximately 1 out of 136 individuals or 0.74% (based on NWHIC).

In the country, a work by the Bureau of Plant Industry (BPI) showed that a steady diet of *Moringa* fruit boosts the sperm count of men thus, improves their chances of fertilizing an egg (Cabacungan, 2008). Thereafter, on September 14, 2007, Senator Loren Legarda campaigned for the popularization of moringa. Interestingly, in India, the moringa fruit is said to increase sexual libido in male. Serrano and Pocsidio (2008, unpub.) reported an increase in the sperm count in male mice when 1% concentration of *Moringa* ethanolic leaf extract (MOLE) was administered subcutaneously for two weeks. However, the effects of the extract on the histoarchitecture and hormonal profile of the reproductive organs have not been examined.

The aim of this study was, therefore, to investigate the effect of the hexane fraction obtained from *M. oleifera* leaves on the male reproduction in relation to changes in the weight of testes, epididymides, maturity of seminiferous tubules and sperm density using *Mus musculus* as test animal. Since spermatogenesis is a hormone-dependent phenomenon the measurement of FSH and LH levels was included.

MATERIALS AND METHODS

Animals

Twenty four male ICR mice (8 week old, 28 - 32 g) were obtained from the Animal Laboratory of Bureau of Food and Drug Administration (BFAD). After a quarantine period of 14 days, the

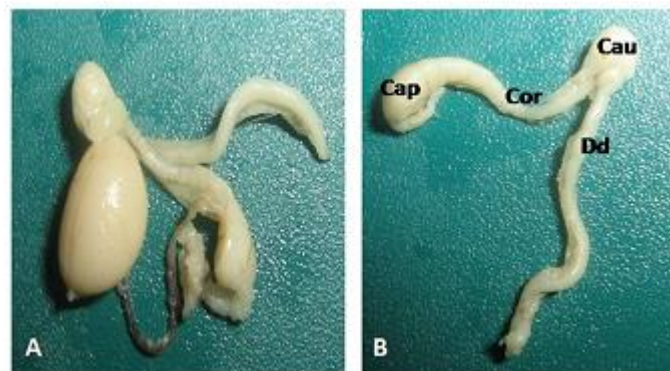


Figure 2. Gross photographs of partially dissected (A) testis and epididymis; (B) epididymis showing parts: Cap (Caput); Cor (Corpus); and Cau (Cauda). The ductus deferens (Dd) has been left attached to the cauda epididymis.

mice were randomly divided into 4 groups (n=6) and gavaged with 0, 0.5, 5 and 50 mg/30 g BW/day of *M. oleifera* L. leaves hexane fraction for 21 days. The experimental animals were housed three per cage (polyethylene) with free access to food (Purina Bio 300) and tap water. The use of animals and the experimental protocol were in compliance to the guidelines set by the Animal Care and Use Committee of the University of the Philippines.

Plant material and preparation of extract

M. oleifera L. leaves were harvested from a plantation in Cavite City and processed at the Institute of Biology University of the Philippines-Diliman. The leaves were dried, powdered, dissolved in 95% methanol in 1:2 ratio (w/v) and allowed to stand for 48 h. The mixture was filtered and evaporated to dryness using a rotary evaporator. The methanolic extracts were dried and extracted three times with hexane and water (Figure 1) to obtain the hexane partition. Air drying was done to obtain the solid residue which was later on weighted to obtain 1, 10 and 100 mg/ml *M. oleifera* hexane fraction dissolved in corn oil.

Dissection of organs and blood collection for hormone analysis

The mice were anesthetized with ketamine and cardiac puncture was performed to obtain blood samples (samples were pooled, n = 6) for FSH and LH analysis. The anesthetized animals were then killed by cervical dislocation. Testes, epididymides, seminal vesicles (Figures 2 and 3) were collected, cleared from the surrounding fat, and weighed; organ weights were assessed relative to animal body weight.

Histological examination of fixed testis

Three sections from each of 3 - 5 fixed testes from each treatment group were examined. The sections were scored for the presence of lumina and elongating spermatids in a blinded fashion. Tissue sections with no evidence of lumen formation in any tubules received a score of 0, sections where less than 50% of tubules containing a lumen received a score of 1, sections where less than 50 - 95% of tubules had lumina received a score of 2, and sections where all tubules contained a lumen received a score of 3. A similar

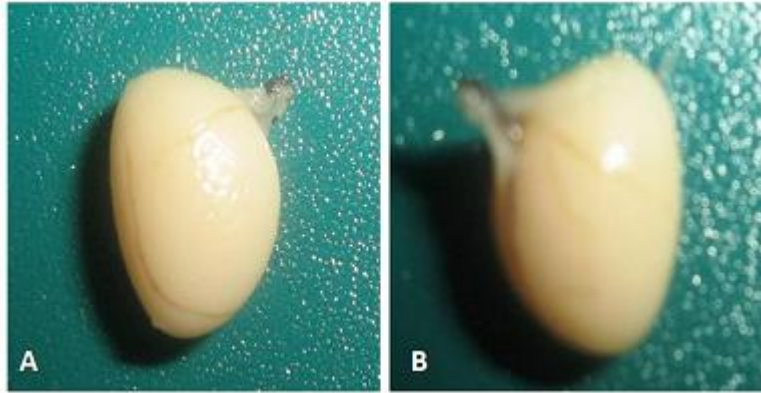


Figure 3. Gross photograph of a partially dissected mouse testis showing caudal (A) and cranial (B) aspects.

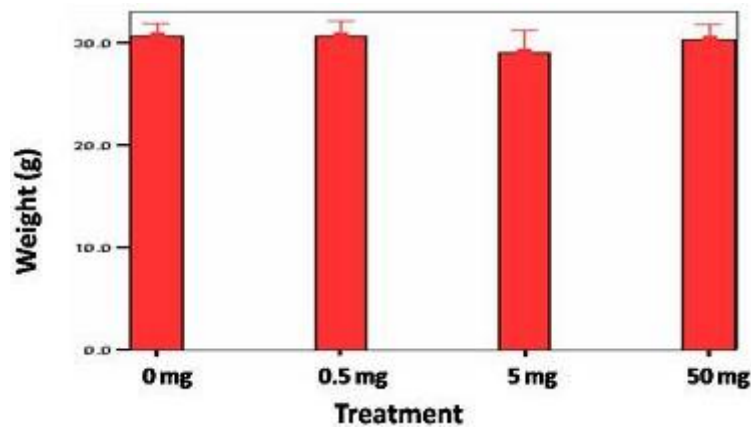


Figure 4a. Body weights of mice administered with varying doses of *M. oleifera* L. leaves hexane fraction. Data represents mean \pm SD; n = 6; no significant difference between treatments.

scoring system was used to estimate maturity which is defined as the condition where spermatogenesis was actively proceeding in the testes and spermatozoa were abundantly present in all regions of the epididymides.

Those which met the above definition of maturity were given a rating of 3; those which had many spermatozoa in the distal cauda epididymidal tubules were rated 2; those which had few spermatozoa in the caudal tubules were rated 1; and those which completely lacked caudal spermatozoa were rated 0. The mean score for each testis was then calculated, and used to calculate the group means score. The diameters of ten centrally located seminiferous tubules were recorded and the thickness of the epithelial lining of 10 randomly chosen epididymides was measured using ocular micrometer at 100 magnifications.

Statistical analysis

Results were expressed as the mean \pm standard deviation. Comparisons of means were analyzed by one-way analysis of variance (ANOVA) to determine inter group differences. If the results of the ANOVA were significant ($p \leq 0.05$), Tukey's honestly significant

difference (HSD) was applied to the data to compare the treated groups with control group.

RESULTS

Weights of male mice and reproductive organs

The administration of *M. oleifera* L. hexane fraction at any dose did not alter the body weight of the animals (Figure 4a). The male mice receiving the plant extract at medium ($p = 0.011$) and high ($p = 0.001$) doses had significantly higher relative testes weight than the control (Figure 4b). The weight of the epididymides in all the moringa-treated groups of mice was significantly increased in contrast to the control group (Figure 4c). *M. oleifera* L. extract at the dose of 50 mg/30g BW also significantly induced the relative weights of the seminal vesicles of the male mice (Figure 4d). These weight gains may signify a selective

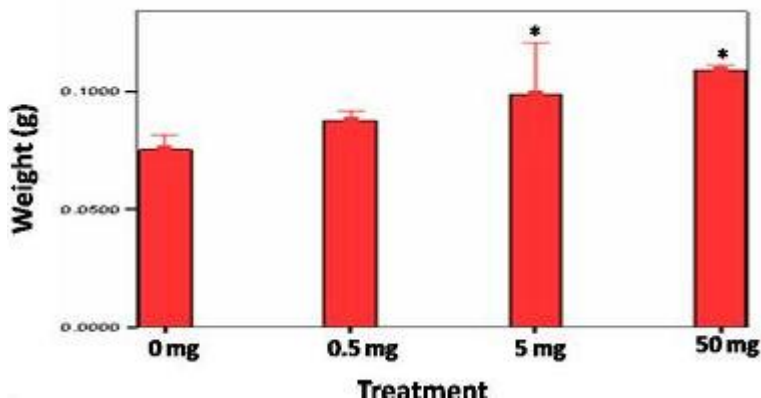


Figure 4b. Testes of mice administered with varying doses of *M. oleifera* L. leaves hexane fraction. Data represents mean \pm SD; n = 6; *p \leq 0.05 (compared to control).

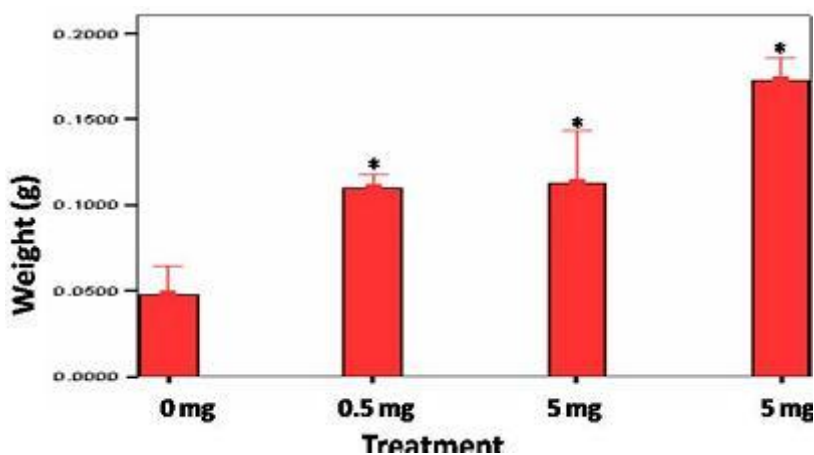


Figure 4c. Epididymides of mice administered with varying doses of *M. oleifera* L. leaves hexane fraction. Data represents mean \pm SD; n = 6; * p \leq 0.05 (compared to control).

effect of *M. oleifera* as reported in *B. rotunda* (Sudwa et al., 2007) and *Lepidium meyenii* (Gonzales et al., 2001) and reflect activation of spermatogenesis as a result of the presence of elongated spermatids in the seminiferous tubules.

Testicular and epididymal histology

For all groups of male mice receiving *M. oleifera* L. extract, the diameters of seminiferous tubule were significantly larger and relative maturity was significantly higher than that of the control group (Table 1). Spermatogenesis was enhanced in all the treated groups as manifested in terms of the significant increases in the relative maturity of the epididymides observed in the different groups given with low (P \leq 0.05), medium (P \leq 0.01) and high (P \leq 0.001) doses. This is noticed by the presence of

abundant spermatids in their seminiferous tubules (Figure 5) and the thickened epididymal epithelial lining (Figure 6) compared to the control group. Lumen formation which is also an indication of the degree of spermatogenesis was highly significant (P \leq 0.001) in mice given 5 and 50 mg/30 g BW of the plant extract.

Hormone levels

M. oleifera Lam. treatment did not appear to affect serum LH and FSH levels on male mice since the values obtained did not differ among treatments (<0.100 mIU/ml). A word of caution is warranted, however, as the researcher collected only a single blood sample, and this may have been insufficient to reveal true changes because of marked fluctuations of the reproductive hormones concentration.

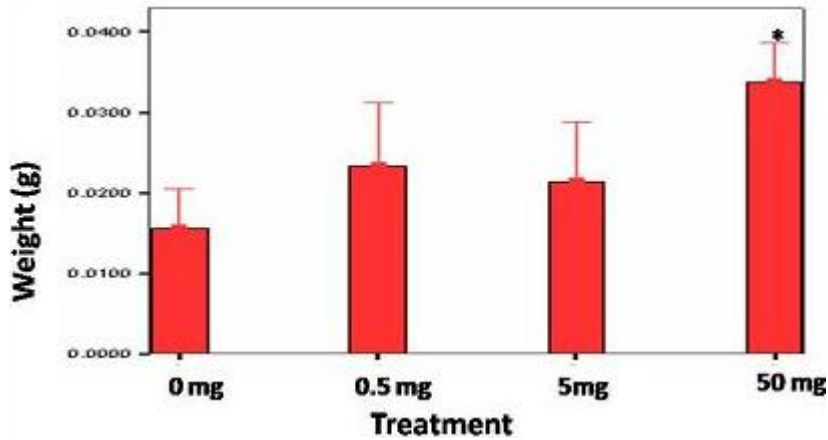


Figure 4d. Seminal vesicles of mice administered with varying doses of *M. oleifera* L. leaves hexane fraction. Data represents means \pm SD; $n = 6$; * $p \leq 0.05$ (compared to control).

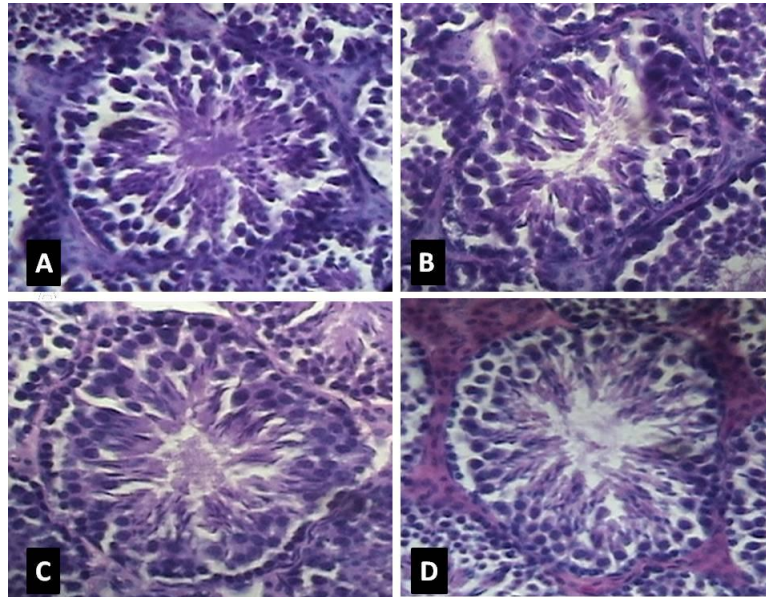


Figure 5. Representative H and E samples of testicular histology from pubertal mice given with *M. oleifera* HF at A) 0 mg; B) 0.5 mg; C) 5 mg and D) 50 mg / 30 g BW; 400X.

DISCUSSION

In the present study, the body weight of the moringa-treated animals remained unchanged, which showed that the doses selected did not exert any harmful effect and the metabolic processes of the treated animals were normal. The effect of *M. oleifera* Lam. in enhancing male reproduction is clearly manifested in all the treated groups compared with the control. However, mice administered with the high and medium doses of the plant extracts are reproductively superior to those that were

given only the low dose. Observations of testicular and epididymal weights, relative maturity ratings, lumen formation, and seminiferous tubule diameters constituted supporting evidence.

Sudwa et al. (2007) found that the ethanolic extract of *B. rotunda*, does not affect sexual behavior or serum androgen levels, but enhances seminiferous tubule, testis and seminal vesicle in the treated male rats. Gonzales et al. (2001) also observed significantly higher testicular weights in the rats treated with *Lepidium meyenii*. Similarly, Watcho et al. (2001) found that the rats treated

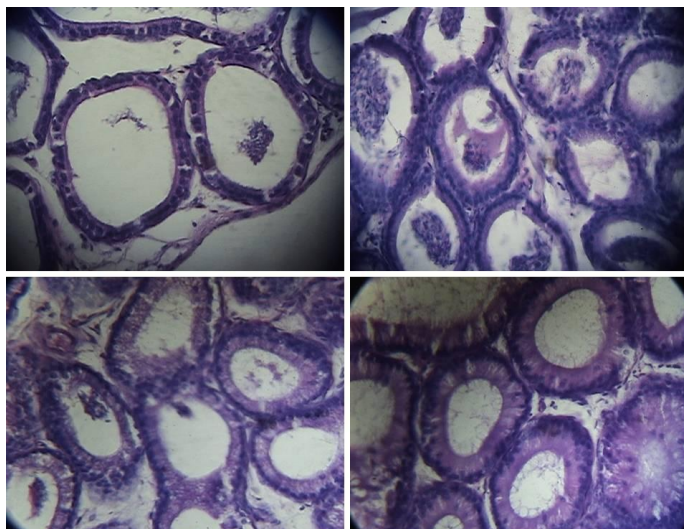


Figure 6. Representative H and E samples of epididymal histology from pubertal mice given with *M. oleifera* HF at A) 0 mg; B) 0.5 mg; C) 5 mg and D) 50 mg / 30 g BW; 400X.

Table 1. Reproductive histology of mice administered with varying doses of *M. oleifera* L. leaves hexane fraction (mean \pm SD).

	Control	0.5 mg/30 gBW	5 mg/30 gBW	50 mg/30 gBW
Seminiferous tubule diameter	8.55 \pm 0.725	10.05 \pm 0.926**	11.65 \pm 1.055*	11.20 \pm 1.229*
Thickness of the epithelial lining of epididymis	1.00 \pm 0.236	1.20 \pm 0.258	1.40 \pm 0.459**	2.450 \pm 0.438*
Lumen formation	0.80 \pm 0.422	1.00 \pm 0.471	2.10 \pm 0.568*	2.40 \pm 0.516*
Relative maturity	0.70 \pm 0.483	1.20 \pm 0.422***	1.90 \pm 0.738*	2.50 \pm 0.527*

*P \leq 0.001; **P \leq 0.01; ***P \leq 0.05 (compared to control).

with *Mondia whitei* for 8 days induced an increase in the testicular weight, testicular testosterone level and sperm density without affecting the accessory gland weights. Whereas, Manaheem et al. (2007) 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.

It is apparent that *M. oleifera* can enhance sexual activity in mice and may have an opportunity to exert its effect depending on certain conditions, as have been reported in the experiments with other plant species in various doses and times (Watcho et al., 2001; Watcho et al., 2004) and in sexual condition of male animals (Gauthaman et al., 2002). It is also likely that the antioxidants present in the leaves of the plant, acting in concert with the antioxidant system present in the epididymis further preserved and enhanced the process of spermatogenesis. Numerous studies now point to the elevation of a variety of detoxication and antioxidant enzymes and biomarkers as a result of treatment with *Moringa* or with phytochemicals isolated from *Moringa*

(Kumar and Pari 2003; Faizi et al., 1994). D'cruz and Mathur (2005) proved that the sperm cytoplasm contained very low concentrations of scavenging enzymes therefore an increase in the antioxidant enzyme system levels by *Moringa* treatment can favor the reproductive process.

Conclusion and Recommendation

The hexane fraction obtained from the leaves of *M. oleifera* does not affect serum FSH and LH levels but enhances seminiferous tubule, epididymis, testis and seminal vesicle. It is recommended to determine the effects *M. oleifera* extract on sexual behavior in male mice.

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