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Original Research Article

Significance of graded doses of aqueous seed extract of *Moringa oleifera* (L) on live body weight, gonadal, extragonadal dimensions and sperm reserves of Yankasa rams

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ABSTRACT

The aim of the study was to investigate the effects of *Moringa oleifera* aqueous seed extract on live body weight, gonadal and extragonadal dimensions and sperm reserves of Yankasa rams. Twenty five apparently healthy Yankasa rams aged 1-2 years and weighing 19.0 ± 2.1 Kg were used for the study. The rams were randomly selected into five groups: A, B, C, D and E with five rams in each group as treatment and control groups respectively. Groups A - D were given oral dose of *Moringa oleifera* aqueous seed extract at a dose rate of 1000, 2000, 3000, 4000 (mg/kg), respectively while group E was given 10 ml/kg water orally, daily for five months. Live body weight, gonadal and extragonadal reserves were determined according to standard techniques. The results showed a significant increase in live body weight in the months of April to June among rams treated with different doses of *Moringa oleifera* aqueous seed extract compared with the control group. The control group showed no significant differences in the body weight, gonadal and extragonadal dimensions and sperm reserves. In conclusion, the treatment of Yankasa rams with *Moringa oleifera* aqueous seed extract increased live body weight, but had no significant effects on gonadal and extragonadal dimensions and sperm reserves, it is recommended that *M. oleifera* aqueous seed extract can be used at doses of 2000mg/kg to 3000mg/kg in Yankasa rams for optimum gain in live body weight.

Keywords: Gonadal and extragonadal sperm reserves, live-body weight, testicular dimension

INTRODUCTION

Moringa is a plant that grows throughout the tropics with a single genus having 14 known species. *Moringa oleifera* (*M. oleifera*) is the most widely known and utilised plant by humans and livestock (Manju *et al.*, 2018). *Moringa oleifera* is native to sub-Himalayan and is commonly found in Africa. It is also well recognized in Pakistan, Bangladesh and Afghanistan in folkloric medicine (Mughal *et al.*, 1999). *Moringa oleifera* is a small or medium-sized tree up to 10 m tall, with thick, soft, corky, deeply fissured bark, growing mainly in

semiarid, tropical and subtropical areas (Jahn, 1996). Moringa is a multipurpose tree of significant economic

importance, as it has vital nutritional, industrial, and medicinal properties (Manju *et al.*, 2018). *Moringa oleifera* thrives well in both tropical and subtropical climates under hot, humid and wet conditions with rainfall in excess of 3000 mm per annum. Moringa grows in a variety of soil conditions ranging from sandy, loamy and clays. The tree can be planted for forage production under intensive farming systems and can yield up to 3.0 tones seed/ha. Pod bearing usually start 6-8 months after planting and the plant can survive up to 40 years (Lalas & Tsaknis, 2002). Moringa

plant is drought resistant and will grow even during the dry season. Owing to its drought tolerance, the tree is most suitable in desert as a valuable source of food for livestock. Farmers consider Moringa as one of the most useful trees, as almost every part of the tree can be used as food, medicine or livestock feed (Lalas & Tsaknis, 2002).

Moringa oleifera (Horse-radish tree or Drumstick or Magic tree) is a medicinally important plant, belonging to the family Moringaceae. The leaves and the pods are known to have a high content of protein, minerals and vitamins (Council of Scientific and Industrial Research, 1962). Moringa oleifera leaves (Asma et al., 2005), seeds (Lalas & Tsaknis, 2002) and roots (Faizi et al, 1998; Ashok & Pari, 2003) are excellent sources of antioxidants. The leaves of M. oleifera are used as purgative; applied to sores as poultice, rubbed on the temples for headaches. They are also useful in treatment of piles, fevers, sore throat, bronchitis, eye and ear infections, scurvy and catarrh (Ghasi et al., 2000). The leaf juice is believed to lower glucose levels, and reduces glandular swelling (Makonnen et al., 1997). The stem bark is used as an abortifacient and antioxidant (Nath & Sethi, 1992; Ghasi et al., 2000).

Herbal therapy is applicable for alleviation of male infertility (Anthony *et al.*, 2006). A large number of plants have been tested for their possible fertility regulatory properties (Bhatia et. al., 2010). Some medicinal plants are extensively used as aphrodisiac to relieve sexual dysfunction or as fertility enhancing agents (Fatoba *et al.*, 2013). They provide a boost of nutritional value thereby improving sexual performance and libido (Yakubu *et al.*, 2007; Sumalatha *et al.*, 2010).

Several conditions/factors can reduce sperm quality and interfere with the process of spermatogenesis (Iliyasu, *et al.*, 2014a). Such factors include infectious and non-infectious diseases, drug treatments, chemotherapy, toxins, air pollution, and insufficient vitamin intake with consequential harmful effects on spermatogenesis (Mosher & Pratt, 1991; Iliyasu, *et al.*, 2014a). Stress is known to compromise reproductive performance (Ayobami *et al.*, 2013).

There is paucity of scientific report on the use of Moringa aqueous seed extract in Yankasa rams as weight or fertilityenhancing substance. Hence the need to determine the effects of aqueous seed extract of *Moringa oleifera* on live body weight, gonadal and extragonadal dimensions and sperm reserves in Yankasa rams.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Whole plants with fruits were collected at the end of the rainy season (November- March, 2018) from Anguwan Yusi, Sabongari Local Government Area of Kaduna state. The plant was authenticated and assigned with Voucher number 0571 by a taxonomist at the Herbarium, Department of Biological Sciences, Faculty of Science, Ahmadu Bello University, Zaria. All experimental protocols were approved by Ahmadu Bello University Committee on Animal Use and Care with clearance number ABUCAUC/2017/029.

AQUEOUS EXTRACTION OF *MORINGA OLEIFERA* SEED

The seeds of *M. oleifera* were obtained from the fruits and dried under shade for 14 days to ease the shedding of the seeds. The dried seeds were made into powdered form (40 g) which was weighed using a weighing balance and transferred into one litre beaker. Three hundred milliliter (300 ml) of distilled water were added to the powder and allowed to stand for 48 hours. Thereafter, it was heated in a water bath at 60° C for 3 hours. Hot water was added continuously to the residue and subsequently filtered. The procedure was repeated three times at 10-15 min intervals, and then the filtrate was evaporated to dryness in water bath at 60° C. The liquid extract was concentrated to dryness in *vacuo* at 40° C using a rotary evaporator. The dried extract was stored at 4° C until required.

EXPERIMENTAL ANIMALS

Twenty five, apparently healthy Yankasa rams aged 1-2 years with an average body weight of 19.0 ± 1.2 kg were purchased from the market. The animals were examined to ensure that they were in good physical health. In addition, the animals were acclimatized for two weeks during which blood and faecal samples were collected for laboratory investigations of ecto and endoparasites. The animals were kept under intensive management and fed on *Digiteria smuttssi*, groundnut leaves and bean husks. Salt lick and water were provided ad libitum.

EXPERIMENTAL DESIGN

The rams were randomly selected into five groups (A, B, C, D and E) of five rams per group. Groups E served as untreated control and was administered 10 ml of clean water orally, while Groups A - D were administered via oral route different doses: 1000, 2000, 3000, 4000 mg of M. oleifera aqueous seed extract, respectively. The extract was administered in the morning between 7:00 - 9:00 am, daily for 5 months. Body weight was recorded once every week throughout the experimental period. At the end of the experiment, 2 rams were selected randomly from each group and humanely sacrificed and postmortem examination was conducted. The testes were examined grossly and evaluated for testicular weight, volume, gonadal sperm reserves and extragonadal. The right and left testes as well as the right and left epididymis from a particular group were included and evaluated individual such that each group had a total of 4 testes and 4 epididymes. The epididymides were further divided into corpus, caput and cauda and used for the extragonadal sperm reserves analyses.

DETERMINATION OF GONADAL AND EPIDIDYMAL SPERM RESERVES

Gonadal and epididymal sperm reserves were determined as described by Kwari & Waziri (2001). The testicular parenchyma was sliced and homogenized with a high-speed blender for two minutes with 50 ml of 0.9 % NaCl solution containing antibiotics (sodium penicillin G, 100 IU/ml and streptomycin sulphate 1 mg/ml) to prevent bacterial growth. For determining the epididymal sperm reserves, the caput, corpus and cauda epididymides were isolated, and minced with a pair of scissors separately in 20 ml of 0.9 % NaCl solution. All tissues were homogenised for 2-6 hours after minced. Testicular homogenates and epididymal samples were refrigerated overnight. After 24 hours, the samples were filtered through gauzed and the filtrate volumes were measured. The gonads was homogenised with 50 mls of normal saline and filtered. The filtrate was diluted at a ratio of 1:2; one millilitre of filtrate was diluted separately with 2 ml of normal saline solution prior to examination as described by Rekwot et al. (1987).

Sperm concentration of the testicular and epididymal sample was determined using a haemocytometer and light microscope, as described by (Kwari & Waziri 2001; Iliyasu *et al.*, 2014b).

MEASUREMENT OF GONADAL AND EPIDIDYMIS ORGAN DIMENSIONS

Two rams were selected randomly from each treated groups and the control group and humanely sacrificed. The testicular parenchyma was weighed using digital weighing balance (Essae[®]) while the dimensions were measured using a flexible tape. Thereafter, the volumes of the right and left testes and the epididymides were determined using the Archimedes principle of water displacement, as previously described (Hughes, 2006).

STATISTICAL ANALYSES

Data were analyzed using GraphPad Prism version 5 (GraphPad, 2000). Data were subjected to one-way ANOVA and Dunnett Posthoc test. Values were presented as mean \pm SEM and the results were considered significant at p < 0.05.

RESULTS

A dose-dependent increase was observed in the live-weights of rams treated with *M. oleifera* aqueous seed extract among group A, B, C, D in April, post oral administration of *M. oleifera* seed extract, compared to control group. A significant increase was also observed in the month of May in groups B, C and D, compared with control group as presented in Table 1. A progressive decrease in live-weight in the months of June and July were also observed in rams in groups treated with *M. oleifera* aqueous seed extract as shown in Table 1.

There was significant (P < 0.05) increase in scrotal circumference among groups A, B, C and D treated with *M*. *oleifera* aqueous seed extract in a dose-dependent manner, compared with group E (control). There was a significant decrease (P < 0.05) in the gonadal and extragonadal dimensions in groups A and B treated with 1000 and 2000 mg/kg of *M. oleifera* aqueous seed extract respectively, when compared to the control (group E). However, there were significant increase (P < 0.05) observed in groups C and D treated with 3000 and 4000 mg/kg of *M. oleifera* aqueous seed extract, respectively (Table II).

There was significant (P < 0.05) increase in the gonadal, epididymal, caput, corpus and caudal sperm reserves among the treated groups A, B, C and D compared with the control group (group E) as presented in Table III.

The left and right epididymides were separated into the caput, corpus and cauda portions based on gross anatomy and component each was measured and weighed using flexible tape and digital weighing balance, respectively. Thereafter they were placed in normal saline for onward estimation of epididymal sperm reserves.

 Table I: The effects of graded doses of *M. oleifera* aqueous seed extract administered orally on live body weight of Yankasa ram

Parameters/ BW/kg	MOASE (Dose) mg/kg	March	April	May	June	July
А	1000	21.43±0.4 ^b	$23.42\pm0.3^{\circ}$	24.89±0.5 ^c	22.53±0.2 ^b	21.93±0.2 ^b
В	2000	$21.60{\pm}0.4^{b}$	23.91±0.3 ^c	$25.61 \pm 0.5^{\circ}$	21.96±0.2 ^b	21.50±0.2 ^b
С	3000	21.93±0.4 ^b	23.99±0.3 ^c	$25.612 \pm 0.5^{\circ}$	23.93 ± 0.2^{b}	22.8±0.2 ^b
D	4000	21.77±0.4 ^b	23.91±0.3 ^c	26.671±0.5 ^b	22.95±0.2 ^b	23.1±0.2 ^a
E	Control (10 ml H ₂ 0)	20.97±0.4 ^a	22.56±0.3ª	24.63±0.5 ^a	24.76±0.2ª	23.3±0.2 ^a

^{a,b,c} Means in the same columns for each parameter with different superscripts between the groups are statistically (p < 0.05) different. MOASE (*M. oleifera* aqueous seed extract)

There was irregular significant (P < 0.05) pattern of decrease and increase in the gonadal (testicular), extragonadal

(epididymis) weight and volume among the treated groups compared to the control group, as presented in Table IV.

Parameters	A MOSE (1000mg/kg)/ body weight	B MOSE (2000mg/kg)/ body weight	C MOSE (3000mg/kg)/ body weight	D MOSE (4000mg/kg)/ body weight	E Control group (10 ml H ₂ 0)
SC (cm)	19.3 ± 3.2^{a}	$20.1\pm1.5^{\rm c}$	$21.5\pm2.8^{\text{b}}$	$22.7\pm2.6^{\text{b}}$	18.3 ± 2.4^{a}
Testis (g)	98.7 ± 3.2^{b}	105.0 ± 1.5^{a}	113 ± 2.8^{b}	$116 \pm 2.6^{\circ}$	106.7 ± 2.4^{a}
Epididymis (g)	27.6 ± 3.2^{b}	$30.0\pm1.5^{\rm c}$	$32.7\pm2.8^{\text{d}}$	34.1 ± 2.6^{a}	33.7 ± 2.4^{a}
Caput (g)	6.3 ± 3.2^{b}	$6.1\pm1.5^{\text{b}}$	$8.2\pm2.8^{\rm c}$	$10.6\pm2.6^{\text{b}}$	$9.8\pm2.4^{\mathrm{a}}$
Corpus (g)	$7.1 \pm 3.2^{\circ}$	$8.1\pm1.5^{\rm c}$	$9.1\pm2.8^{\rm c}$	$12.9\pm2.6^{\text{b}}$	11.3 ± 2.4^{a}
Cauda (g)	25.6 ± 3.2^{e}	$28.8 \pm 1.5^{\text{d}}$	$35.4\pm2.8^{\rm c}$	10.6 ± 2.6^{b}	$22.6\pm2.4^{\rm a}$

Table II: Effects of graded doses of *Moringa oleifera* aqueous seed extract administered orally on body weight, scrotal circumference, gonadal and extragonadal dimensions of Yankasa rams

^{a,b,c,d} Means in the same row for each parameter with different superscripts between the groups are statistically (p<0.05) different. MOASE (*M. oleifera* aqueous seed extract); SC = Scrotal circumference

Parameters	A MOSE (1000mg/kg)/ body weight	B MOSE (2000mg/kg)/ body weight	C MOSE (3000mg/k g)/ body weight	D MOSE (4000mg/kg)/ body weight	E Control group (10 ml H ₂ 0)
Gonadal sperm reserves (×10 ⁶ /g)	19.3 ± 3.2^{a}	20.1 ± 1.5^{c}	21.5 ± 2.8^{b}	22.7 ± 2.6^{b}	212.3 ± 2.0^a
Epididymis sperm reserve (×10 ⁶ /g)	98.7 ± 3.2^{b}	$105.0\pm1.5^{\rm a}$	$113\pm2.8^{\text{b}}$	$116 \pm 2.6^{\circ}$	98.2 ± 2.0^{a}
Caput sperm reserve (× 10^6 /g)	27.6 ± 3.2^{b}	$30.0\pm1.5^{\rm c}$	32.7 ± 2.8^{d}	34.1 ± 2.6^a	70.2 ± 2.0^a
Corpus sperm reserve (×10 ⁶ /g)	$6.3\pm3.2^{\text{b}}$	$6.1\pm1.5^{\rm b}$	$8.2 \pm 2.8^{\circ}$	10.6 ± 2.6^{b}	29.2 ± 2.0^{a}
Cauda sperm reserve (× 10^6 /g)	$7.1\pm3.2^{\text{c}}$	$8.1 \pm 1.5^{\circ}$	$9.1 \pm 2.8^{\circ}$	12.9 ± 2.6^{b}	74.2 ± 2.0^a
Gonadal sperm reserves (×10 ⁶ /g)	25.6 ± 3.2^{e}	28.8 ± 1.5^{d}	35.4 ± 2.8^{c}	10.6 ± 2.6^{b}	

Table III: Effects of graded doses of *Moringa oleifera* aqueous seed extract administered orally on body weight, scrotal circumference, gonadal and extragonadal dimensions of Yankasa rams

^{a,b,c,d} Means in the same row for each parameter with different superscripts between the groups are statistically (p<0.05) different. MOASE (*M. oleifera* aqueous seed extract); SC = Scrotal circumference

Group Parameters	A MOSE (1000mg/kg)/ body weight	B MOSE (2000mg/kg)/ body weight	C MOSE (3000mg/kg)/ body weight	D MOSE (4000mg/kg)/ body weight	E Control group (10 ml H ₂ 0)
RTW (g)	55.1 ± 0.1^{a}	50.6 ± 0.3^{b}	$60.8\pm0.6^{\text{b}}$	60.1 ± 2.1^{b}	$56.3\pm1.5^{\rm a}$
LTW (g)	50.0 ± 0.1^{a}	48.9 ± 0.3^{a}	53.7 ± 0.6^{b}	56.6 ± 2.1^{b}	$50.8 \pm 1.5^{\rm a}$
REW (g)	17.1 ± 0.1^{a}	14.6 ± 0.3^{b}	$17.5\pm0.6^{\rm a}$	$19.5\pm2.1^{\mathrm{a}}$	$18.3\pm1.5^{\rm a}$
LEW (g)	$13.3\pm0.1^{\text{b}}$	13.1 ± 0.3^{b}	$15.3\pm0.6^{\rm a}$	15.4 ± 2.1^{a}	$15.3\pm1.5^{\rm a}$
$RTV (cm^3)$	$55.3\pm0.1^{\rm a}$	$50.9\pm0.3^{\rm b}$	61.0 ± 0.6^{b}	60.4 ± 2.1^{b}	$56.5\pm1.5^{\rm a}$
LTV (cm ³)	50.4 ± 0.1^{a}	$49.3\pm0.3^{\rm a}$	$53.9\pm0.6^{\text{b}}$	56.9 ± 2.1^{b}	$51.2\pm1.5^{\rm a}$
REV (cm ³)	15.0 ± 0.1^{b}	$17.8\pm0.3^{\mathrm{a}}$	$17.9\pm0.6^{\rm a}$	19.8 ± 2.1^{a}	$18.7\pm1.5^{\rm a}$
LEV (cm ³)	$13.5\pm0.1^{\text{b}}$	$13.4\pm0.3^{\rm b}$	$15.5\pm0.6^{\rm a}$	15.8 ± 2.1^{a}	$15.5 \pm 1.5^{\mathrm{a}}$

Table IV: Effects of *M. oleifera* aqueous seed extract administered orally on gonadal and extragonadal weight and volume in Yankasa rams

^{a,b} Means in the same rows for each parameter with different superscripts between the groups are statistically (P < 0.05) different. n=5; **Key:** Right testicular weight = RTW; Left testicular weight = LTW; Right epididymides weight = REW; Left epididymides weight = LEW; Right testicular volume = RTV; Left testicular volume = LTV; Right epididymides volume = REV; Left epididymides volume = LEV; MOASE= *M. oleifera* aqueous seed extract

DISCUSSION

In the present study, oral administration of aqueous seed extract of *M. oleifera* to Yankasa rams at graded doses induced a progressive increase in the live body weight of the treated animals. This could be as a result of the high dietary value of M. oleifera seed. This agrees with the findings reported by Andrea *et al.* (2015) and Yusuf *et al.* (2018). The high nutritional value of *M. oleifera* seed was confirmed from the proximate and phytochemical analysis of the seed extract as similarly reported by Iliyasu *et al.* (2020a). Secondary metabolites found to be present in *M. oleifera* include flavonoids, glycosides, steroids, tannins, saponins and reducing sugar. Others are carbonhydrate, phenolics and tarpenoids. Most of these substances are acknowledged to possess anti-oxidant properties

Furthermore, the increase in the live body weight of the treated Yankasa rams within the first three-month treatment with *M oleifera* is in agreement with the findings reported by Yusuf *et al.* (2018). This might be linked to the ability of the seed extract to reduce pathogenic microbial activities in the rumen, thereby improving digestibility and enhance the assimilation of essential nutrients required for muscle building. The reduction in the live body weight recorded after three months of treatment with *M. oleifera* aqueous seed extract might be attributed to the enhanced reproductive activities of *M. oleifera* aqueous seed extract that resulted in

decreased gonadal sperm reserves among the treatment groups.

The decrease in gonadal sperm reserves could be due to decreased feed intake. This concurs with the findings reported by Iliyasu *et al.* (2020) on aphrodisiac effects of *M. oleifera* aqueous seed extract in wistar rats. The progressive decrease in the gonadal and epididymal weight observed in the treatment groups could be linked to the decrease in the gonadal and epididymal sperm reserves as observed among the rams. These findings agree with the findings of Iliyasu *et al.* (2020a) who reported a positive influence of *M. oleifera* seed extracts on reproductive indices of livestock.

In the present study, the increase in body weight observed from the first three months of post-treatment with *M. oleifera* seed extract might have been responsible for the improvement of gonadal and extra gonadal sperm reserves observed during the course of the present research. The increase in body weight and gonadal sperm reserves was a sign of normal physiology that might perchance influence the process of spermatogenesis in bucks or rams and these findings are in accord with the findings of the current study and the study conducted by Marai *et al.* (2007).

In the present study, *M. oleifera* aqueous seeds extract have supported breeding sound fitness in rams as is evidenced by increase in scrotal circumference. This agreed with the findings reported by Hoogenboezem & Swanepoel (2000), who reported 10-20 cm scrotal circumference in rams as good dimension to be used when choosing rams for breeding programme. Similar findings were reported by Beggs *et al.* (2018), who reported significant increase in scrotal circumference in goats fed 20% inclusion of Moringa seeds in their normal diet.

The increase in testicular weight observed in the present study could be connected to the nutritional properties of the M. oleifera seed extract which is also in agreement with the report of Ogbuegbu et al. (1985) and Iliyasu et al. (2014b). Also, Okwun et al. (2006) affirmed that rams with larger testes tend to produce more spermatozoa, a finding which was similar to what was observed in the present study. Similarly, Oyeyemi et al. (2002) reported a positive correlation between testicular weights and gonadal sperm reserves in West African Dwarf bucks. It was also observed in the present study that the right testes were heavier than the left testes in all the groups treated with M. oleifera aqueous seed extract, regardless of the doses of the extract administered orally. This contradicts the report of Oveyemi et al. (2002) where the left testicles were found to be heavier than the right testicles in rabbits. Obviously, the different experimental animal in the later study and the present study is a factor to be considered. Testicular weight in small ruminant is far bigger than any of laboratory animal. In agreement with our results, Raji & Njidda, (2014) reported that right testicles were heavier with high sperm reserves in the Red Sokoto bucks fed with Moringa oleifera supplemented diets. Similar finding regarding the right testes having higher sperm reserves was also observed in camel slaughtered at Maiduguri central abattoir as reported by Stephen et al. (2019). The increase in weight of the right testes may be due to a higher proportion of Sertoli cells in the seminiferous tubules of the right testes, which have been reported by Raji & Njidda, (2014) to be responsible for nourishment that support spermatogenesis under the influence of available antioxidant bioactive components present in the M. oleifera aqueous seed extract.

The corpus of the epididymis observed in the current study has the lowest sperm reserves than the control group. This agrees with the findings reported by Sekoni (1994). However, a low sperm reserve was observed among groups treated with different doses of M. *oleifera* aqueous seed extract.

The corpus epididymis in the present study had the minimum sperm reserves compared to other parts of epididymis. This might be attributable to the anatomical structure of the corpus with low tendency to store spermatozoa. This agrees with the findings reported by Taiwo *et al.* (2018). A similar finding was reported by Yunusa *et al.* (2016) who stated that the corpus epididymis had the lowest sperm reserves in goats. This showed that nutritional supplement is positively correlated with testicular weights, gonadal and extra gonadal sperm reserves output, which could be as a result of an increase in the population of Sertoli cells in the seminiferous tubules that also improved the process of spermatogenesis as reported by Sultana *et al.* (2016).

CONCLUSION

Oral administration of *M. oleifera* aqueous seed extract at different graded doses for a period of five months could serve as a dietary inclusion that may improve performance in rams as evidenced by live bodyweight gain and good outcome of testicular dimensions, gonadal and extragonadal sperm reserves in Yankasa rams.

CONFLICT OF INTEREST

Authors declared no conflict of interest.

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