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Evaluation of Safety, Proximate and Efficacy of Graded Dose of Moringa oleifera Aqueous Seed Extract as Supplement that Improve Live-Body Weight and Scrotal Circumference in Yankasa Ram

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Abstract

Moringa oleifera seeds are used as dietary supplement in developing countries. Phytochemical, proximate constituent and acute toxicity test were determined. Aims of the study were to investigate the effects of Moringa oleifera aqueous seed extract on live body weight and scrotal circumference of Yankasa rams. 25 apparently healthy Yankasa rams aged 1-2 years weighing (18-20 kg) were purchased and kept at the experimental pen of the Department of Veterinary Parasitology and Entomology, ABU, Zaria. Rams were acclimatised and screened for endo and ecto-parasites prior to commencement of the experiment. Rams were randomly divided into five groups (A, B, C, D and E; n = 5) treatment groups and control group, respectively. The rams were fed basal diet of Digitaria smuttssi and supplemented with bean husks and maize offal. Water and salt lick were provided ad libitum. Groups A, B, C and D were given oral dose of Moringa oleifera aqueous seed extract at a dose rate of 1000, 2000, 3000 and 4000 mg/kg, respectively, while group E (control) received water (10 mL/kg), daily, for five months between 7:00 AM and 9:00 AM. Results of live-body weight showed significant (P < 0.05) monthly (April-June) increase in rams treated with different doses of M. oleifera aqueous seed extract compared to their respective initial weight in (March), with the highest and lowest in group D and A (26.671±0.3) and (24.89±0.5) respectively, compared to the control group E (24.63±0.4). There was significant increase in scrotal circumference of Yankasa rams administered with different dose of Moringa oleifera aqueous seed extract compared to the control group. Inclusion, Moringa oleifera aqueous seed extract could be used to increase live-body weight and scrotal circumference of Yankasa rams.

Keywords: Safety, Proximate, Moringa oleifera Aqueous Seed Extract, Supplement, Live-Body Weight, Scrotal Circumference, Yankasa Ram

Introduction

Moringa oleifera (Lam) is a medicinal tree that commonly grows in many tropical and subtropical countries (Gopalakrishnan *et al*., 2016). It is grown commercially in Asia, Africa, South and Central America and Mexico Panwar and Mathur (2020). It is known as the drumstick tree based on the appearance of its immature seed pods, the horseradish tree based on the taste of ground root preparations, and the ben oil tree from seed-derived oils (Razis *et al*., 2014).

In some areas, immature seed pods are eaten, while the leaves are widely used as a basic food because of their high nutrition content (Mbikay, 2012; Razis *et al*., 2014). No human clinical trials have been conducted looking at the efficacy of *M. oleifera* for treatment of malnutrition individual. Seeds, leaves, oil, sap, bark, roots, and flowers are widely used in traditional medicine. Moringa leaves have been characterized to contain a desirable nutritional balance, containing vitamins, minerals, amino acids, and fatty acids (Moyo *et al*., 2011; Razis *et al*., 2014). Additionally, the leaves are reported to contain various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics, and carotenoids (Alhakmani *et al*., 2013). According to several commentaries (Anwar *et al*., 2007; Mbikay, 2012; Razis *et al*., 2014), various preparations of *M. oleifera* are used for their anti-inflammatory, antihypertensive, diuretic, antimicrobial, antioxidant, anti-diabetic, anti-hyperlipidemic, anti-neoplastic, antipyretic, antiulcer, cardioprotectant, and hepatoprotectant activities. The therapeutic potential of *M. oleifera* leaves and seeds in treating hyperglycemia and dyslipidemia was reviewed by Mbikay (2012). Razis *et al*. (2014) summarized the potential health benefits of *M. oleifera*, focusing on their nutritional content, antioxidants and antimicrobial properties.

Adverse effects were not reported in any of the human studies that have been conducted to best of our knowledge (Razis *et al*., 2014). Furthermore, various preparations have been used around the globe as foods and medicine without any side effects in humans (Mayo *et al*., 2011). Several animal studies evaluated for the potential toxicity of *M. oleifera* preparations (Mayo *et al*., 2011). The safety of aqueous leaves extract given orally to the rats at doses of 400, 800, 1600, and 2000 mg/kg body weight was reported by Adedapo *et al*. (2009). The treatment was acute single dose and also given daily for 21 days. Various parameters assessed including blood cell counts and serum enzyme levels and it was concluded that consumption of *M. oleifera* leaves at doses of up to 2000 mg/kg were safe (Adedapo *et al*., 2009). A dose-dependent decrease in body weights of the rats occurred over the 21 days of the study. Therefore, some preparation of *M. oleifera* like the seeds can be used to improve physiological activities in livestock.

Malnutrition has been linked to a number of age-related abnormalities observed from younger to adult age (Bailey *et al*., 2015). The mechanisms involving malnourishment begins in utero and could continue throughout the lifespan of individual (Bailey *et al*., 2015). Because of this, research is necessary to remains focus on how to eliminate growing deficits linked to poor nutrition especially in developing countries where malnutrition is widespread (Ogbe and Affiku, 2020). Plant like *Moringa oleifera* is usually use as supplement that can enhance physiological performance by providing adequate amounts of vitamins, minerals, protein and energy in the diet (Bailey, 2020). Supplementing animal diets with vitamins and minerals from sustainable plant sources is critical in areas where intensive agriculture and animal production are limited due to socioeconomic challenges and climate changes (Ogbe and Affiku, 2020).

In animals, deficiencies in vitamin A, B, C and E, and certain micro and macro minerals have been shown to have a profound influence on embryonic development and various systems in the animals. In fact, studies have shown that lack of certain minerals, vitamins, and nutrients in the diet leads to significant muscular-skeletal pathologies as reported by (Lall and Lewis-McCrea, 2007) and (Manhal *et al*., 2017**)** in animal models zebra fish and rats, respectively. Likewise, diets deficient in vitamin E and antioxidants composite can affect the physiology of reproductive organ and ability to fertilized (Hisham *et al*., 2012).

A methanol extract of seeds of *M. oleifera* were screened for phytochemical components and used for acute and sub acute toxicity studies in rats (Ajibade *et al*., 2013). The phytochemical screening revealed the presence of saponins, tannins, terpenes, alkaloids, flavonoids, carbohydrates, and cardiac glycosides but the absence of anthraquinones. Although signs of acute toxicity were observed at an extract dose of 4000 mg/kg, mortality was recorded at 5000 mg/kg. No adverse effects were observed at concentrations lower than 3000 mg/kg. The authors concluded that methanol extracts of seeds of *M. oleifera* are safe for nutritional use (Ogbe and Affiku, 2020). The aim of this study was to evaluate the safety and efficacy of graded dose of *Moringa oleifera* aqueous seed extract as supplement to Yankasa rams.

Materials and Method

Source of Plant, Collection and Identification

The whole plant with fruits was collected at the harvesting period (November- March) from Anguwan Yusi, Sabongari Local Government Area of Kaduna state. The plant was confirmed and assigned with Voucher number 0571 by a taxonomist at the Herbarium, Department of Biological Sciences, Faculty of Science, ABU, Zaria, Nigeria.

Aqueous Extraction of *Moringa oleifera* Seed

The seeds were obtained from the fruits that dried under shade for 14 days to ease the shedding of the seeds. The dried seeds were made into powder (40 g) was weighed using a weighing balance and transferred into one-liter beaker. Three hundred milliliter (300 ml) of distilled cold water was added to the powder and allowed to stand for 48 hours with vigorous shaking at 6-hour interval. Thereafter, it was heated on a water bath at (60 ^oC) for 3 hours. Hot water was added continuously to the residue and subsequently filtered. The procedure was repeated three times at 10 – 15 minute interval and then the filtrate was evaporated to dryness on water bath at (60 ^oC). The liquid extract was concentrated to dryness *in vacuo* at 40 ^oC using a rotary evaporator. The dried extract was stored at 4 ^oC until required for use.

Concentration of Seed Extract

Using sterile dilution technique, 4 g of *M. oleifera* seeds aqueous extracts were dissolved separately in 10 ml of water to give concentration of 400 mg/ml (highest stock culture), followed by serial dilution with distilled water to give several concentrations. The tubes containing the various concentrations were labeled appropriately and used immediately.

Preparation of *Moringa oleifera* seed

Seeds of *M. oleifera* were sorted out manually, soaked in water overnight and rinsed, till the water was foam free. Then Moringa seeds were dried and ground in a domestic electric blender to obtain *M. oleifera* seed meal.

Acute Toxicity Study of Aqueous Seed Extract of *Moringa oleifera*

The acute toxicity effects of the aqueous extract of the seeds of *M. oleifera* were determined by the method described by Lorke (1983). The experiment was done in two phases. In phase I trial, nine rats were randomly divided into three groups of three rats each. Groups 1, 2 and 3 were given *M. oleifera* seeds extracts at dose rates of 10, 100 and 1000 mg/kg, respectively, orally. In the second phase (II) of the trials, three rats were placed at random into three groups of one rat each. The groups were individually treated with three different doses of *M. oleifera* seeds extract based on the outcome of the initial trial. The median lethal dose (LD_{50}) of *M. oleifera* seeds extract as an indication of its acute toxic effect was determined by taking the geometric mean of the highest dose that did not produce death and the lowest dose that produced death.

Mineral Analysis

The samples (*Moringa oleifera* seed) were weighed (5 g) and subjected to drying in well cleaned porcelain, crucible at 550 ^oC in a muffle furnace. The resultant ash was dissolve in 5.0 ml of HNO_3/HCL/H_2O (1:2:3) and heated gently on a heating muffle until brown fumes disappeared, then, distilled water (5.0 ml) was added to the sample and heated until colourless solution was obtained. The mineral solution was filtered into a 100 ml volumetric flask through filter paper, and the volume was made to the mark with distilled water. The solution was analyzed in triplicate for its elemental composition using Parking Elmer 403 model of Atomic Absorption Spectrophotometer (New York, USA).

Proximate Analysis of *Moringa oleifera* Seed

The proximate composition of the *Moringa oleifera* seeds was determined as described by Association of Official Analytical Chemists, AOAC (2005). The sample was analyzed for moisture content (MC), crude protein (CP), crude fat (CF), crude fibre (CF), mineral matter (total ash) and Carbohydrate (CHO).

Estimation of Moisture Content

Exactly (2 g) of the powdered sample was weighed in a beaker of known weight. The sample was then placed in hot air oven at 105 ^{o}C for 3 h. The sample was cooled and weighted again to determine water loss in powdered sample.

Estimation of Fat Content

Apparatus used for estimation of fat was Soxhlet extractor. To determine the percentage of fat, the dried sample of plant was extracted with petroleum ether. It was then distilled off completely and dried. The oil weight and percentage of oil were calculated

Estimation of Crude Fiber

The acid and subsequent alkali treatment, oxidative hydrolytic degradation of native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight is the crude fibre content.

Estimation of Ash Percentage

For estimation of ash, the sample was incinerated at higher temperature. Briefly, 2 g of sample in a crucible was incinerated in to the Muffle furnace at 600 ^{o}C for 5 hours. The crucible was then cooled, the sample was reweighed and the percentage of ash calculated.

Estimation of Crude Protein

The Kjeldahl method was used for nitrogen estimation. Sample was digested with concentrated sulfuric acid in the presence of digestion mixture. The ammonia was distilled by the addition of excess sodium hydroxide. Released ammonia was collected in boric acid and titrated with standard hydrochloric acid using methylene blue as an indicator. Total protein was obtained by multiplying nitrogen percentage by 6.25.

Determination of Carbohydrate (CHO)

Nitrogen free extract (NFE) was determined by calculation using this formula. NFE = 100 – (% CP +% CF + % EE + % Ash + % moisture). Estimation of Ca (Calcium) and P (phosphorous) value in *M. oleifera* seed meal was determined by Thimmaiah (2004) method. Determination of mineral energy (ME) values. The ME value of *M. oleifera* seed meal was calculated by the equation of Pauzenga (1985) according the method described by Obidah *et al*. (2020). NFE = 100 – (% CP +% CF + % EE + % Ash + % moisture), NFE = COH.

Phytochemical Screening

The presence of various constituents in the seed extract of *M. oleifera* was determined by preliminary phytochemical screening as described by Thimmaiah (2004). The tests for phlobatanins, cardiac glycosides, carbohydrates, flavonoids, anthraquinones, saponins, cyanogenetic glycosides, alkaloids, saponins, steroids and tannins were carried out according to the method described by Trease and Evans (1983).

Detection of Alkaloids

The amount of alkaloids was tested using the method reported by Oluduro, (2012). Exactly (10 mL) of the extract were evaporated to dryness and 2 ml of 2 % HCl acid were added to the dry residue. One-two drops of Wagner's reagent were added to the solution. The presence of alkaloids was confirmed when reddish brown precipitate occurred.

Detection of Flavonoids

The test was carried out according to the method described by Pamar, (2012). Few drops of NaOH were added to 2 ml of the extract and intense yellow colour appeared. Few drops of dilute HCl were added and the solution turned colourless as indicator of presence of flavonoids.

Detection of Saponins

Precipitation and foam test method was used as described by Devmurari, (2010). Two drops of (1 %) lead acetate solution were added to 1 ml of the extracts. Intense white precipitate appeared due to presence of saponins. Foam test also confirmed presence of saponins in the extract, in which 20 ml of distilled water was added to one ml of the extract in graduated cylinder. The solution was shaken for 5 to15 minutes and formation of stable foam indicated the presence of saponins.

Detection of Steroids

The test was performed based on the method described by Solihah *et al*. (2012). Two ml concentrated sulphuric acid were added to two ml of the extract. Formation of red precipitate indicated presence of steroids.

Detection of Tannins

The test method was described by Ugochuchwu *et al*. (2013). Exactly 1 ml of 3 % of ferric chloride was added to one ml of the extract. Brownish green colour development indicated presence of tannins.

Quantitative Analysis of the Bioactive Constituents

Different methods were used to determine the amount of the required constituents in the seeds based on gravimetric tests. Three replicates were used for each test to ensure the accuracy.

Estimation of Alkaloids

Alkaloids were quantified by the method described by Agoreyo *et al*. (2012). Five-gram sample was weighed into a beaker and 200 ml of 10% acetic acid in ethanol were added. The mixture was covered and allowed to stand for four hours. After that the mixture was filtered and the filtrate was concentrated on water bath (Thermostat, model AI-748) at 100 ^{o}C, to one-quarter of the original volume. Concentrated ammonium hydroxide was added to the extract in the form of drops until precipitation was completed. After settlement, the extract was filtered and the precipitate washed with dilute ammonium hydroxide and then dried and weighed using sensitive weight balances (Eassy®).

Estimation of Flavonoids

The method was described by Bharathidasan *et al*. (2013). A total of (100 ml) of 80% aqueous methanol was used to extract 10 g of the sample. The mixture was filtered by using (Qualigen-Germany) Whatman filter paper No. 42 (125 mm). The filtrate was evaporated until dried, and then weighed until constant weight was achieved.

Estimation of Saponins

The method was described by Gupta (2013), 20 g of plant sample was put into a conical flask and 100 ml of 20 % ethanol was added. The solution was heated over water bath at 100 ^{0}C for 4 hours with continuous stirring at 55 ^{o}C. The solution was then filtered and the residue re- extracted with another 200 mL of 20 % ethanol. The combined extracts were reduced to 40 ml over a water bath. The concentrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added to the extract and was vigorously shaken. The aqueous layer was recovered while the ether layer was discarded and the purification process was repeated, 60 mL of N-butanol was added, the combined extract was washed twice with 10 ml of 5% NaCl. The remaining solution was heated in a water bath till after evaporation. The sample was dried in the oven at 105 ^{o}C to a constant weight.

Estimation of Steroids

The method described by Araujo *et al*. (2013), was used with some modification to get the gravimetric weight. The extract was cooled to room temperature at 25 ^{o}C, filtered on cotton, and the residue (cotton and plant materials) re-extracted twice, using 30 ml of chloroform for 15 min. The two filtrates were collected and dried under reduced pressure at 40 ^{o}C, rotary evaporator. The residue was then placed in an oven at 80 ^{0}C until it reached constant weight.

Estimation of Tannins

This method was described by Saxena *et al*. (2020) and Elzein *et al*. (2018) and 100 ml of distilled water were added to 2 g of the sample. The solution was kept in water bath at 90 ^{o}C for one hour. The mixture was filtered using (Whatman's paper No. 1) and the residue was re-extracted. The two filtrates were collected together and allowed to cool down. Distilled water was added to the filtrates up to 500 ml. One hundred ml of the solution transferred to a beaker, and then 10 ml of 40 % formaldehyde and 5 ml of concentrated sulphuric acid were added respectively. The whole mixture was refluxed for 30 minutes and was left to cool down. The mixture was filtered and the precipitate dried and weighed.

Source of Experimental Animals

Twenty-five, apparently, healthy Yankasa rams with an average body weight of 18 - 20 kg were purchased from Kafur central market, Kafur local government area of Katsina State, Nigeria. 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. The animals were kept under intensive management and fed on *Digiteria smuttssi*, groundnut leaves and beans husks. Salt lick and water were provided *ad libitum*. All experimental protocols were approved by Ahmadu Bello University Committee on Animal Use and Care with clearance number **ABUCAUC/2017/029.**

Experimental Design

The Yankasa rams were divided randomly into five groups (A, B, C, D and E) as presented in Fig I. Group E served as untreated control was given 10 ml of water, while Groups A, B, C and D were given total doses of 1000, 2000, 3000 and 4000 mg of *M. oleifera* aqueous seed extract, respectively. The extract was administered orally in the morning between 7:00-9:00 am daily for 5 months. Body weights were taken once every week throughout the experimental period. Semen were collected once every week using electro ejaculator as described by Iliyasu *et al*. (2014a). Semen characteristics were evaluated as described by Iliyasu *et al*. (2014a). Blood samples were collected once every week to evaluate haematological parameters and sera harvested to assay for gonadotropins and testosterone profiles. At the end of the experiment, 2 rams from each group were humanely sacrificed and postmortem examination was conducted. Testes were examined grossly and samples taken for histopathological examination.

Measurement of Live Body Weight and Scrotal Circumference

The scrotal circumference was measured with a flexible tape at the broadest part of the scrotum according to the method described by Iliyasu *et al*. (2014b). This was done by applying gentle pressure with hand above the head of the epididymides thereby forcing the testes into the scrotum. The rams were restrained in a standing position during measurements. Live weights of the rams were also determined weekly by weighing balance (Generic®) throughout the experimental period according to the method described by Yusuf *et al*. (2014).

Blood Collection for Haematological Parameters

For haematological studies, 2 ml of blood were collected aseptically via jugular vein using 21 gauge hypodermic needles and 5 ml syringe (Darinject®) once every week for 5 months and decanted to EDTA bottles.

Serum for Gonadotropins and Testosterone Profiles

To obtain serum samples for gonadotropins and testosterone assay, 3 ml of blood were collected aseptically via jugular vein three times at 30-minute interval between 7:00-800 am once every week for 5 months. The blood was decanted into plain test tubes and allowed to stand for 15-30 minutes before centrifuged at 3000 × g for ten minutes. Thereafter, the harvested serum was decanted into a micro tube and stored at -20 ^{0}C until analysed.

Serum for Biochemical Analysis

To obtain sera for biochemical analysis, 3 ml of blood were collected aseptically via jugular vein once every week. The blood was decanted into plain test tubes and allowed to stand for 15-30 minutes before centrifuged at 3000 × g for 10 min. Thereafter, serum was decanted into a micro tube and stored at -20 ^{0}C until analysed.

Statistical Analysis

Data generated were presented in means (± SEM) and values of $P < 0.05$ were considered significant. Data from individual groups were subjected to one-way analysis of variance (ANOVA) and Dunnett control as Posthoc test to determine their differences. All the data were analyzed using special package of statistical analysis GraphPad prism version 5, (2000) Windows 4.03 (GraphPad prism software, San Diego, CA, USA).

Results and Discussion

Extraction and Phytochemical Screening of *Moringa oleifera* Seeds

A total of 6.67 kg of the dried *Moringa oleifera* seeds (MOS) powder yielded 918.8 g of the crude extract. The extract was a golden brown, oily liquid. Preliminary phytochemical screening for seeds extract of *M. oleifera* revealed the presence of alkaloids, flavonoids, saponins, steroids and tannin (Table 1). Phytochemical bioactive quantification revealed the presence of components and its quantity as summarised in (Table 2).

Table 1: Extraction and phytochemical analysis of aqueous seed extract of *Moringa oleifera*

Phytochemical /Components	Presence or Absence of Secondary Metabolites
Flavonoids	++
Glycosides	++
Steroids	++
Tannins	+
Saponins	+
Reducing sugar	++
Phenolics	_
Tarpenoids	_
Carbohydrates	++
Alkaloids	++
Eugenols	+

(+) = slightly present; (++) = largely present; (-) = absent

Table 2: Quantitative analysis of secondary metabolites in aqueous seed extracts of *Moringa oleifera*

Phytoconstituents	Quantity (%)
Alkaloid	13.3 ± 0.04
Glycosides	11.3 ± 0.4
Flavonoids	15.4 ± 0.03
Reducing sugar	16.3 ± 0.2
Steroids	16.5 ± 0.3
Carbohydrates	18.6 ± 0.1
Eugenols	1.2 ± 0.03
Saponins	2.8 ± 0.06
Tannins	4.6 ± 0.03

Proximate Analysis of *Moringa oleifera* Seeds

Proximate analysis of *M. oleifera* seed results revealed an appreciable amount of crude protein (44.5 ± 2.5), crude fat (43.2 ± 1.6), crude fibre (17.3 ± 1.9), carbohydrate (8.3 ± 1.3) others are moisture and ash as presented in Table 3.

Table 3: Proximate analysis of *Moringa oleifera* seeds

Proximate Analysis	Percentage (%)
Moisture	6.05 ± 1.2
Ash	3.48 ± 3.7
Crude protein	44.5 ± 2.5
Crude fibre	17.3 ± 1.9
Crude fat	43.2 ± 1.6
Carbohydrate	3.38 ± 1.3

Mineral Compositions of *Moringa oleifera* Seeds

The mineral constituents of *Moringa oleifera* seed revealed significant amounts of minerals such as calcium Ca (1.50 mg), magnesium Mg (5.23 mg), iron Fe (0.96 mg), copper Cu (0.38 mg) manganese Mn (0.65 mg) and some other compounds as presented in Table 4.

Table 4: Mineral composition of *Moringa oleifera* seeds

Minerals	Concentration mg/100 g
Ca	1.5
Mg	5.23
Fe	0.96
Cu	0.38
Mn	0.65
Pb	0.04
Cr	0.03
Cd	0.03

First Phase Evaluation of LD_{50} of *Moringa oleifera* Aqueous Seeds Extract

No death was recorded during or post oral administration of *Moringa oleifera* aqueous seed extract in any group of the Wistar rats as presented in Table 5.

Table 5: First phase of acute toxicity evaluation of *Moringa oleifera* aqueous seed extract

Extract	Doses (mg/kg) per os	Mortality per group
Moringa oleifera aqueous seed extract	10	0/3
	100	0/3
	1000	0/3

Second Phase Evaluation of LD_{50} of *Moringa oleifera* Aqueous Seeds Extract

No death was recorded during or post oral administration of *Moringa oleifera* aqueous seed extract in any of the group of the Wistar rats as presented in Table 6.

Table 6: Second phase of acute toxicity evaluation of *Moringa oleifera* aqueous seed extract

Extract	Doses (mg/kg) per os	Mortality per group
Moringa oleifera aqueous seed extract	1600	0/1
	2900	0/1
	5000	0/1

Live-weight of Yankasa Rams Treated with *M. oleifera* Aqueous Seed Extract

There was marginal increase in live-weight of rams treated with *M. oleifera* aqueous seed extract in group A from May, 21.4 ± 0.4 kg up to June 25.6 ± 0.5 kg post oral administration of *M. oleifera* seed extract compared to the control group 21.5 ± 0.4 kg. Significant increase ($P < 0.05$) in live-weight was observed among rams in groups A, C and D compared to group E in May as presented Table 7. The live-weight of the rams among the treated groups with *M. oleifera* aqueous seed extract progressively decreased to 21.5 ± 0.2 kg in July as shown in Table 7.

Table 7: Effects of graded dose of *M. oleifera* aqueous seed extract administered orally on live body weight of Yankasa ram post experiment

Parameters/ BW/kg	MOASE (Dose) mg/kg	March	April	May	June	July
A	1000	21.43±0.4	23.42±0.3^a	24.89±0.5	22.53±0.2^a	21.93±0.2^a
B	2000	21.60±0.4^a	23.91±0.3^a	25.61±0.3^a	21.96±0.2^a	21.50±0.2^a
C	3000	21.93±0.5^a	23.99±0.3^a	25.612±0.4^a	23.93±0.6^a	22.8±0.4
D	4000	21.77±0.4^a	23.91±0.4^a	26.671±0.3^a	22.95±0.2^a	23.1±0.3
E	10 ml	20.97±0.4	22.56±0.2	24.63±0.4	24.76±0.3	23.3±0.2

^{ab} Means in the same columns for each parameter with different superscripts between the groups are statistically ($^a p<0.05$) different. Key: MOASE= M. oleifera aqueous seed extract; BW= body weight

Table 8: Effects of graded dose of *M. oleifera* aqueous seed extract administered orally on scrotal circumference of Yankasa ram post experiment

A	1000	10.1 ± 0.4	13.0 ± 0.1^a	15.1 ± 0.5	16.5 ± 0.2^a	17.9 ± 0.2^a
B	2000	12.1 ± 0.4^a	15.1 ± 0.3^a	18.2 ± 0.3^a	20.2 ± 0.2^a	20.1 ± 0.2^a
C	3000	14.9 ± 0.5^a	16.9 ± 0.3^a	20.6 ± 0.4^a	21.0 ± 0.6^a	21.8 ± 0.4
D	4000	16.7 ± 0.4^a	18.1 ± 0.4^a	20.6 ± 0.3^a	21.5 ± 0.2^a	22.1 ± 0.3
E	10 ml	11.7 ± 0.4	12.5 ± 0.2	14.3 ± 0.4	16.7 ± 0.3	17.3 ± 0.2

^{ab} Means in the same columns for each parameter with different superscripts between the groups are statistically ($^a p<0.05$) different. Key: MOASE= M. oleifera aqueous seed extract, SC= scrotal circumference

The results of the proximate and phytochemical analyses of *M. oleifera* seed indicate the potential of the seeds in terms of nutritional and therapeutics values. The seed has been reported by several authors on its therapeutics uses. The low moisture content in the moringa seed of the present study, agreed with the findings reported by Manju *et al*. (2018) and Adeyeye and Adejuyo (2013), who reported same range of values. The low moisture content in this study is an indication that the activity of the microbes would be reduced and thereby increases the shelf life of the moringa seeds. The high crude fat found in this study suggests that the kernel is a good source of quality food for domestic use. The crude protein found was greater in the present study than what was reported by Verma and Nigam, (2014); Olagbemide and Alikwe (2014) and Moreki and Gabanakgosi (2014). The variations in the crude protein percentage may be attributed to variation in geographical location and conditions, soil composition, ripening stage, harvesting period and extraction methods used.

The seeds of *M. oleifera* are used by herbal practitioners of many developed and developing countries for treating sexual inadequacy (Omara *et al*., 2020). The practitioners use the seed to stimulate sexual vigor but without recourse to the scientific validity of the dosage and possible side-effects on the reproductive system (Omara *et al*., 2020). Basically, phytochemical screening of the aqueous seed extract of *M. oleifera* revealed the qualitative and quantitative constituents of the plant extract. In the present study, preliminary phytochemical screening of the seed extract of *M. oleifera* revealed the presence of secondary metabolites: alkaloids, flavonoids, saponins, steroids and tannin. This is in agreement with the findings of Patel *et al*. (2014), who reported similar constituents with insignificant variation in the quantity of the bio-active component of the seeds, following 70 % hydro alcoholic extraction of *M. oleifera* seed. The slight variation in the bio-active components of the moringa seed might be attributed to the different method of extraction. However, 70 % hydro alcoholic extraction of *M. oleifera* seed has negligible impact on the constituents of moringa seeds.

The secondary metabolites of the seed extract of *M. oleifera* revealed the presence of alkaloids, flavonoids and glycosides others are tannins, reducing sugar, carbohydrates, steroids and saponins. This was contrary to the findings of Varsha *et al*. (2013), who reported the presence of phenolics and tarpenoids in secondary metabolites of moringa seeds this might be attributed to the difference in geographical location. The secondary metabolites have been reported as potential antioxidant agents (Elzein *et al*., 2018). Natural antioxidant comprises of phenolics acid (phenols), flavonoids bioflavonoid and tannic acid (tannins) also known as polyphenols (Omara *et al*., 2020). These compounds are normally found in significant quantities in *Moringa oleifera* seeds, fruits, vegetables and spices.

The chemical constituents of the seed extract that predominates like tannins, carbohydrates and glycosides over the others consist of some important bioactive agents used in the synthesis of some useful pharmacological compounds used as fertility drugs (Harbone, 1998). It has been reported that steroids and saponins constituents found in many plants possess fertility potentiating properties, and they are useful in the treatment of impotence that occurred due to reproductive hormonal imbalance (Shukla and Khanuja, 2004). Saponins found primarily in the leaf of *Tribulus terrestris* L. have been used as an aphrodisiac agent used on rats. Flavonoids are one of well-known antioxidants that ameliorate oxidative stress-induced testicular impairments in animals (Kujo, 2004). It is also known to stimulate testicular androgenesis, essential for testicular differentiation, integrity, and steroidogenic functions (Luck, 1995; Salem *et al*., 2001). The present findings also corroborate with the report of Mukhallad *et al*. (2009), who studied the effects of *Nigella sativa* on spermatogenesis and fertility activities of male albino rats, with great success due to the potential influence of flavonoids and saponins available in the constituent of the plant, however the mechanism of action is not clearly understood but it was linked to the hormonal activities along the pituitary- gonadal axis.

Flavonoids and saponins are one of the biological indicators that improved the origin of steroidal hormones (Varsha *et al*., 2013). Since androgenic effect is attributed to the pharmacological properties of *M. oleifera* seed extract as reported by Wattanathorn *et al*. (2012). Hence, the increase in the spermatogenesis activities is a reflection of pharmacological properties of *M. oleifera* seeds. It is likely that the *M. oleifera* aqueous seed extract might have a role in stimulating testosterone secretion, which allowed availability of hormone along the pituitary gonadal axis. In addition to the intensity of orgasm and ejaculations which agreed with the conclusion recorded by Watcho *et al*. (2005), while working on hexane extract of *Mondia whitei* on the reproductive performance of male rats. Similar finding was observed on Yankasa rams treated with *M. oleifera* aqueous seed extract in the present study and the success observed can be linked to the pharmacological activities of moringa seed. However, the mechanism of action is not clear but can be ascribed to the influence of the bioactive constituents of moringa seed on hormones along pituitary-gonadal axis as reported by Watcho *et al*. (2005).

The results obtained following oral administration of aqueous seed extract of *M. oleifera* to Yankasa rams at the graded dose, demonstrate a progressive increase in live body weight of the treatment groups. This agrees with the findings reported by Andrea *et al*. (2015) and Yusuf *et al*. (2018), which was attributed to the high nutritional quality of *M. oleifera* seed as confirmed from the proximate and phytochemical analysis of the seed extract in this study. The seeds extract has the ability to reduce pathogenic microbial activities in the rumen, thereby improving digestibility and enhance assimilation of vital nutrients required for muscle building. However, the decrease in live body weight observed three-month post treatment with *M. oleifera* aqueous seed extract may be ascribed to the vigorous sexual desire exhibited among the treatment groups that resulted to frequent mounting and ejaculation which distract them from normal feed intake. Decreased in body weight was reported by Ayobami *et al*. (2013) who studied the effects of trypanosome infection on rams and this can be ascribed to fluctuation of hyperthermia that interfered with feeding attitude of the rams. The progressive decrease in the live body weight of the treatment groups can be linked to the decreased in feed intake and high interest in sexual enthusiasm exhibited by the rams (George *et al*., 2012). *Moringa oleifera* aqueous seed extract had significantly increased the quality of the semen parameters of the treated groups, this agreed with the findings of George *et al*. (2012) who reported positive influence of *M. oleifera* seeds extract on reproductive indices of livestock.

Nutritional supplement is known to improve hormonal function in the testes, thereby affecting the process of spermatogenesis (Barth *et al*., 2008). Both the interstitial cells of Leydig and the germinal epithelium are optimally active at a temperature 3-5 ^{0}C below the body temperature and in the presence of adequate micronutrient loaded with antioxidant agent Patel *et al*. (2020). The increase in body weight observed from the first three-month post treatment with *M. oleifera* seed extract in this study might have been responsible for the improvement of semen quality observed during the course of this research. Marai *et al*. (2007) reported that increase in body weight was a sign of normal physiology that has positive correlation with scrotal circumference and this might influence the process of spermatogenesis and render bucks or rams fertile. Hoogenbozem and Swanepoel (2000) reported that testicular degeneration and poor sperm quality is known to decreased scrotal circumference and render rams infertile (George *et al*., 2012). This might be due to nutritional imbalance and management-related factors. However, degenerative changes in the seminiferous tubules, testicular degeneration, low semen output, low sperm concentration, high percentage of dead spermatozoa, and sperm abnormalities have also been reported by Iliyasu *et al*. (2014b) in Yankasa rams experimentally infected with fasciolosis. *Moringa oleifera* aqueous seeds extract had support breeding sound fitness in rams, this agreed with the findings reported by Hoogenbozem and Swanepoel, (2000). Who reported 10-20 cm scrotal circumference in rams is good to be presented for breeding programme. Similar findings were reported by Yakan *et al*. (2016), who reported significant increase in scrotal circumference in goats fed 20% inclusion of moringa seeds in their normal diet.

Conclusion

The result of this study revealed that *Moringa oleifera* aqueous seed extract may be used as supplementary substance that can improve testicular physiology and body weight of Yankasa rams. Henceforth, *M. oleifera* aqueous seed extract can be used as potential supplement that can replace or compute with the conventional supplements currently in used as substance that can enhance quality performance in Yankasa rams. Therefore, dietary inclusion of *M. oleifera* seed is recommended as its improved weight gain and testicular quality in Yankasa rams.

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Conflict of Interests

There is no conflict of interest.

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