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Reproductive record on Ethanolic Extract of Moringa Oleifera Seed on the Testes of Adult Wistar Rats

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Abstract:

Background: All ages, racial and ethnic groups, including males from all cultural backgrounds, are vulnerable to sexual dysfunction. Although there are few publications on the importance of Moringa oleifera seeds for reproductive organs, it has been stated that they improve aphrodisiac activity. In this study, we investigated the effects of seed extract of Moringa oleifera on the reproductive organ of male Wistar rats. Methods: Sixteen male rats (120-150g, n=4) were grouped as follows: Control, and seed extract of Moringa oleifera (100, 200 and 400mg/ kg). Ethanolic extract of Moringa oleifera seed (EEMS) was given daily for 14 days and thereafter sacrificed by cervical dislocation. Sperm variables were examined microscopically while serum was analyzed for sex hormones, and testicular tissue histopathological. Data were analyzed using Students t-test and ANOVA. Results: Ethanolic extract of Moringa oleifera seed caused a significant increase in body weight, and a decrease in sperm motility, quality, and sex hormones. These declines were dose dependent. Normal histoarchitecture was observed and spermatogenesis was enhanced. Conclusion: Ethanolic seed extract of Moringa oleifera exhibit male reproductive toxicity, as observed from its deleterious effect on andrology and sperm variables.

Keywords: *Ethanolic extract, Moringa oleifera seed, reproductive toxicity, male Wistar rats.*

Introduction

Reproduction is a vital aspect of existence and is essential to the survival of the human race (Zeng et al., 2019). Advanced reproductive technology is essential for successful livestock production (Hayes, Lewin, & Goddard, 2013), and in animals, food or nutrient is a vital role in controlling reproductive performance. Some natural plants are referred to be nutraceuticals because they contain functional compounds and may be beneficial for animal reproductive (Allan & Bilkei, 2005; Guroy, et al. 2012).

Natural antioxidants can help reduce free radicals produced as a result of negative impacts (Abdelazem, 2019). Antioxidants found in plants are employed to protect against sickness and sustain health (Azzini et al., 2017). According to several investigations, Moringa oleifera (Lin.) has antioxidant properties and these plants are thought to contain qualities that are anti-inflammatory, wound-healing, anticancer, monoamine regulating, and antidiabetic (Sadek et al., 2017; Shaat et al., 2017).

In addition to having a significant nutritional value, Moringa oleifera tree is the most widely distributed species of the Moringaceae family in the biosphere (Ekong et al., 2017). It also possesses an extraordinary range of medicinal qualities. Because so many various portions of the plant have beneficial purposes, it is frequently referred to as the miracle tree. Additionally, it was mentioned as a remedy for metal intoxication. The roots, leaves, gum, and flowers are only a few of the numerous plant parts that have broad medical use for treating cardiovascular illnesses (Jaja-Chimedza et al., 2018; Khatum &Varma, 2018; Charles C. N 2021).

The seeds of the M. oleifera plant are spherical, dirty white in color, and measure around 1 cm in diameter. In the northern region of Nigeria, they can be eaten and are commonly consumed. The main bioactive components of seed infusion include nitrite, mustard oil glycosides, and thiocarbamate glycosides, which are hypothesized to be responsible for urinary excretion, decreasing cholesterol, and having antiulcer characteristics (Cuellar-Nunez et al., 2018).

Male rats' sexual behavior was evaluated by Zadeet al., (2013) for M. oleifera effects, and they found a considerable improvement in performance. Studies on the leaves extract of M. oleifera and other parts exhibit a potent antioxidant function in vivo (Fakurazi et al., 2012; Jinghua et al., 2018). This study was directed to evaluate the effect of ethanolic extract of M. oleifera seed on the histoarchitecture, physical and behavioral changes, and activity on serum hormones in the testes of male Wistar rats.

Materials and Methods

Plant Collection and Extraction

Pods of Moringa oleifera seeds were collected from the plant located in an open field in Uturu, Abia State, Nigeria. The plant sample was identified and authenticated at the herbarium of the department Plant and biology sciences, Abia State University, Uturu. The pods were broken to expose the winged and coated seeds. These coated seeds were then shade dried for several weeks and thereafter individually and manually broken to separate the dried naked seeds from the seed coats. The chaff was discarded while the naked dried seeds were subjected to gravitational force so as to blow off any adhering chaff. The clean white seed were pulverized into a homogeneous powder using an electric blender and stored. Ethanolic extract of Moringa oleifera seeds (EEMS) was prepared by dissolving 200g of the grinded pulverized seed in 2 liters of 99% ethanol and then stir every 8 hours for 3 days. Thereafter, the mixture was filtered, and filtrate was then concentrated to dryness over a hot water bath at 40°c. The extract was stored in a glass container and the refrigerator prior to use.

Experimental Design and Animals Management

A total of 16 male rats (120-150g) were procured and housed in the Animal House of Department

of Anatomy, Abia State University, Uturu. Rats were randomly divided into 4 experimental groups (n=4) as follows: Control, and EEMS (100,200, and 400mg/kg). EEMS was administered daily by oral gavage for 14 weeks. They were kept in well aerated cages and were maintained under controlled atmospheric pressure, humidity and acclimatized to the environment for two weeks before experimental use and were allowed free access to clean water and standard livestock pellets (Guinea Feed Nigeria Limited). All procedures in this study conformed to the guiding principles for research involving animals as recommended by the National Research Council.

Phytochemical Analysis of Moringa Oleifera Seed Extract

Moringa Seed Extract were analyzed for Alkaloids, Steroids and terpenoids, tannins, Anthraquinones, flavones aglycones, saponins and coumarins using the standard method by Nathaniel et al. (2020) as shown in Table 1.

Sacrifice and Collection of Specimens

Blood Collection and Hormonal Assay

On the last day of the experiment, about 1ml of blood was collected from each of the 16 rats. The blood sample was immediately placed in a centrifuge (Centurion Scientific Ltd., UK) and centrifuged for 5 minutes at 12,000 revolutions per minute. The serum was quickly decanted into a test tube and stored immediately in a chest freezer (Haier Electrical Appliances Inc., Philippines). The concentration of the serum testosterone, FSH and LH. were determined shortly after using ELISA Kit.

Histological Study

The rats were sacrificed by cervical dislocation and sex organs were excised and washed in phosphate buffered saline (PBS). The testis was fixed in Bouins fluid and thereafter 10% formalin and processed for paraffin wax embedding. They were carefully sectioned using a rotary microtome, stained and the histological architecture examined. The stained tissues were micrographed and interpreted by a pathologist at the University of Port Harcourt teaching hospital.

Statistical Analysis

Data are expressed as mean ± standard error of the mean. Significance difference was analyzed using one-way analysis of variance (ANOVA)followed by Tukey's post-hoc test. Differences were considered statistically significant at $P<0.05$.

Results

Result of Phytochemical Analysis

The phytochemical analysis of the ethanolic seed extracts of Moringa oleifera revealed high presence of flavones aglycones, slight presences of steroids and terpenoids, anthraquinone sand trace amount of tannin, and saponinbut alkaloids and coumarins were not detected. (Table 1).

Table1. Qualitative Analysis of Ethanolic Extract of M. Oleifera

Phytochemical	Ethanolic extract
Tannins	+
Steroids and terpenoids	++
Saponins	+
Anthraquinones	+ +
Alkaloids	-
Flavones aglycones	+++
Coumarins	-

Keys: - = Not detected ++ = Moderate concentration + = Low concentration +++ = High concentration

Effect of Moringa Oleifera Seed Extract on Body Weight of Male Wistar Rats

This showed that there was significant increase in the body weight of the wistar rats (Table 2).

Table 2. Effect of Moringa Oleifera Seed Extracts on the Body Weight of the Male Rats

Periods	Group A (mean ± std)	Group B (mean ± std)	Group C (mean ± std)	Group D (mean ± std)
Before	93± 4.79	101.25± 2.50	120.00±8.18	110.00±8.16
After	182.50± 6.46	153.00 ±10.13	155.00±5.79	170.00±2.73

Effects of Ethanolic Seed Extract of Moringa Oleifera on Sperm Variables

The result of the sperm quality test on the male wistar rats are shown in (Table 3a). There was increase number of normal sperm at 200mg/kg, and rise in number of abnormal sperm in 100 and 400mg/kg intake of Moringa oleifera. The number of active motile sperm increased as well in group C and D, slit sluggish motility was observed in groups C and D unlike group B, non-motile sperm also was majorly seen in group B (Table 3b).

Table 3a. Effects of Ethanolic Seed Extract of Moringa Oleifera on Sperm Variables

Parameters	Group A (Normal control)	Group B (100mg/kg extract)	Group C (200mg/kg extract)	Group D (400mg/kg extract)
Normal Sperm cell	85.00 ± 5.00	80.00 ± 0.00	88.33 ± 3.33	75.00 ± 2.88
Abnormal Sperm cell	15.00 ± 5.00	20.00 ± 0.00	11.66 ± 3.33	25.00 ± 5.00
P-value	0.00	0.00	0.00	0.00

Note: Values expressed as mean ±SD, n=4

Table 3b. Effects of Ethanolic Extract of Moringa Oleifera Seed on the Sperm Variables

Parameters	Group A (Normal control)	Group B (100mg/kg extract)	Group C (200mg/kg extract)	Group D (400mg/kg extract)
Active motility (%)	84.00±2.88	81.00±11.43	89.00±2.88	91.00±5.77
P-value	0.001	0.009	0.021	0.023
Sluggish motility (%)	6.66±1.66	11.50±1.45	7.05±1.05	8.00±6.66
P-value	0.669	0.887	0.100	0.608
Non-motile (%)	9.33±66	7.50±2.88	3.05±1.45	1.00±0.00
P-value	0.00	0.00	0.00	0.00

Note: Values expressed as mean ±SD, n=4

Effect of Seed Extracts of Moringa Oleifera on Sexual Hormones (FSH, LH and testosterone)

The levels of FSH, LH and testosterone in the serum of the animals fed with the seed extract were elevated at the end of the observatory period but only the rise in groups C and D for testosterone, B and C groups for FSH and LH was statistically significant when compared with their respective control values (Table 4).

Table 4. Effects of Ethanolic Seed Extract of Moringa Oleifera on Sexual Hormone Levels

Parameters	Group A (Normal control)	Group B (100mg/kg extract)	Group C (200mg/kg extract)	Group D (400mg/kg extract)
TESTOSTERONE (ng/ml)	4.45±0.13	3.83±0.21	4.92±0.35[a]	5.23±0.18[a]
LH miu/ml	2.37±0.11	2.48±0.37[a]	2.77±0.27[a]	0.75±0.07
FSH miu/ml	0.71±0.05	0.74±0.05[a]	0.71±0.06[a]	2.46±0.09

Note: Values expressed as mean ±SD, n=4a $p < 0.05$ vs normal control

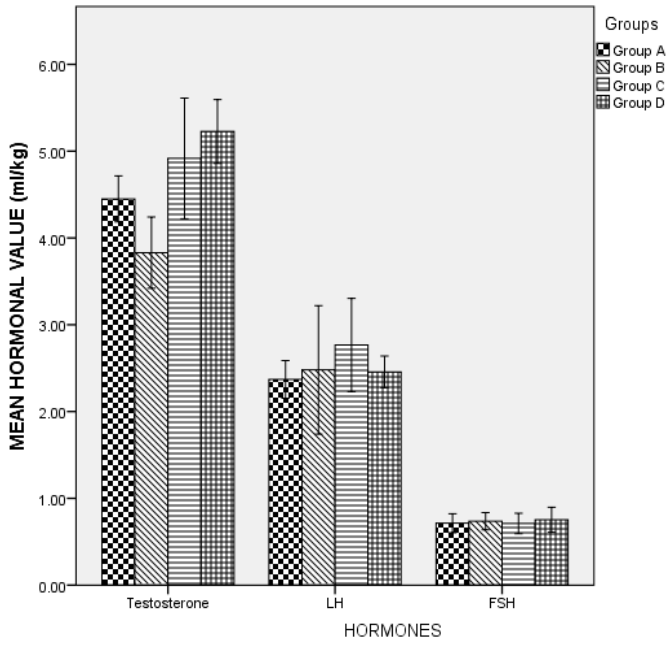


Figure 1. Effects of Ethanolic Seed Extract of Moringa Oleifera on Sexual Hormone Levels of Male Rats

Effect of Seed Extracts of Moringa Oleifera on Histo-Architecture of Male Wistar Rats

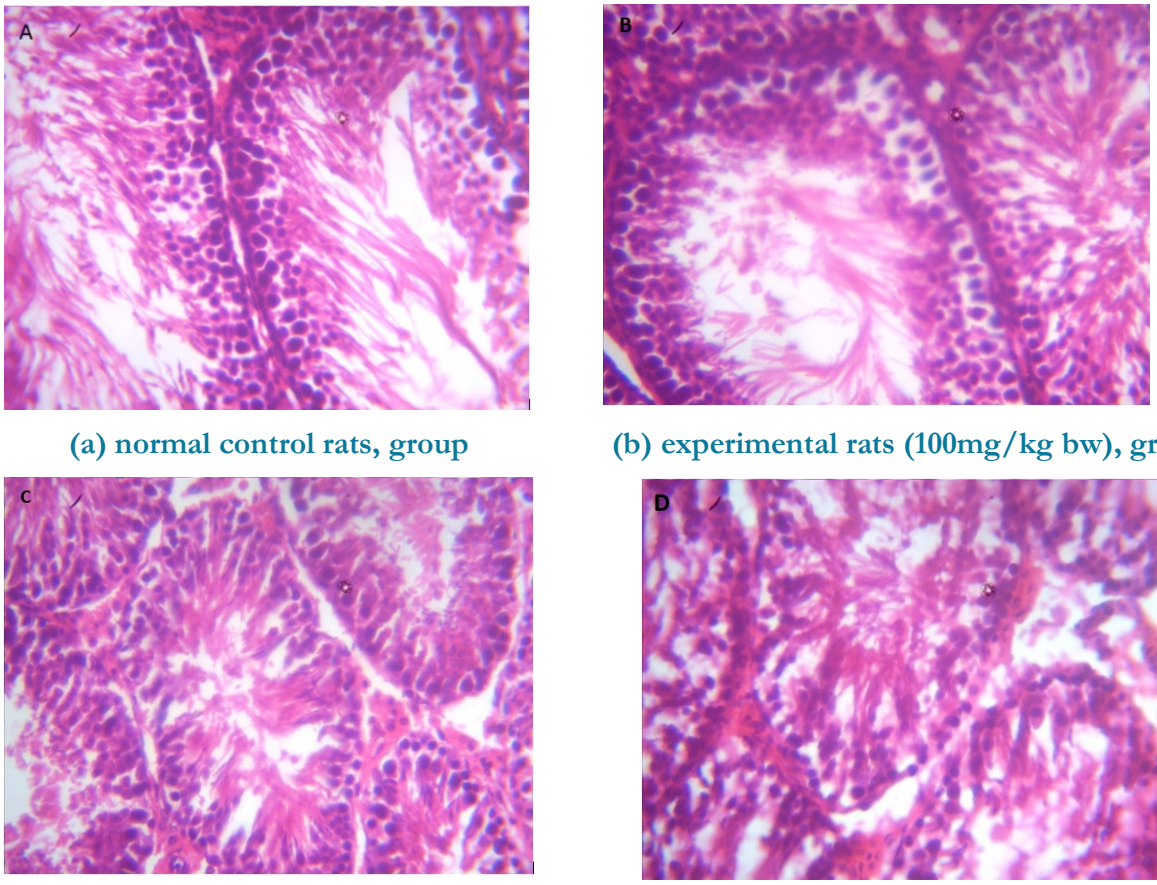
The testicular sections of rats in all the groups show normal testicular features, consisting of seminiferous tubules (ST), lined by spermatogenic cells including the spermatogonia cells, spermatocytes, spermatids as well as the spermatozoa that fill the lumen of the tubules. ST are held together by connective tissue interstitium consisting of interstitial cells and leydig cells. In the experimental groups (b), (c) and (d), administered with 100mg/kg, 200mg/kg and 400mg/kg doses of the seed extracts respectively, there were increased tubular size, more compact ST enhanced spermatogenesis when compared to the normal control group (a). These increases were most pronounced in the group (c) H&E x400.

Discussion

This study identified flavones, aglycones, steroids, and terpenoids, including anthraquinones, as the main components in Moringa oleifera seed. According to earlier research, Moringa oleifera's phytochemical components may improve fertility (Carrera-Chávez et al., 2020). The improved testicular histo-architecture seen in this study may have resulted from these. Aphrodisiac activity has been attributed to tannin, saponin, alkaloids, and coumarins, which are also included in this extract, among other phytochemicals that have

been linked to improving sexual performance in male rats (Zade et al., 2013). However, some of these components, like flavonoids and saponins, have been linked to toxicity of the male reproductive system (Obembe and Raji, 2018).

The Moringa oleifera seed extracts in this study significantly increased body weights. This is in accordance with the study by Ogunlade et al. from 2022. However, in this present study, ethanolic seed extract from Moringa oleifera increased the body weight. According to our study's findings (Table 2), the presence of flavonoids found in the ethanolic extract of Moringa oleifera seeds could be an explanation.



(a) normal control rats, group **(b) experimental rats (100mg/kg bw), group**

(c) experimental rats (200mg/kg bw), and group **(d) experimental rats (400mg/kg bw)**

Figure 2. Photomicrograph of Coronal Section through the Testis of Group

Contrary to earlier claims, this study's findings revealed that, when compared to the control, EEMS at various doses significantly reduced sperm motility and quality (Table 3a and b). This is in line with the reports from Obembe and Raji (2018). Although an improved effect was seen on sperm motility at doses of 200 mg/kg and 400 mg/kg, as well as normal sperm cells at 200 mg/kg, moringa seeds demonstrate male reproductive toxicity in these sperm variables. Sperm motility and sperm count are reproductive variables that are androgen dependent (Ovie F.O et al., 2019).

The observed decrease in these rat sperm variables is most likely caused by the identified decline in serum testosterone levels in rats that received various dosages of EEMS which is support of (Ejikeme Suzan. N 2022). The result of the hormonal assay in this work showed significantly increased levels of testosterone (200 and 400mg/kg), FSH and LH (100 and

200mg/kg) and non-significant increase in testosterone at 100mg/kg, FSH and LH at 400mg/kg. According to reports, the levels of the (gonadotropins) LH and FSH are correlated with testosterone levels, meaning that increasing levels of the gonadotropins cause a corresponding increase in testosterone (Peper et al., 2010) (NN Chukwudi-Emelike et al., 2022). Although luteinizing and follicle-stimulating hormones were affected, it is possible that the decline in testosterone at 400 mg/kg in the rats was caused by its action on the pituitary gland (Table 4).

EEMS's phytochemical components may probably serve as a mediator for its effects on testosterone. However, some of these components, such flavonoids and saponins, have been linked to toxicity of the male reproductive system. Rutin, a flavonoid that often occurs in nature, has anti-mitotic properties. When it is hydrolyzed into quercetin, it enhances inhibition of spermatozoa motility, lowers epididymal organ weight, and increases testosterone and dihydrotestosterone levels (Maoxin et al., 2014). The weight of genital organs, sperm count, sperm motility, and sperm density have all been shown to decrease as a result of saponin exposure in males (Gupta et al., 2005).

Also, histopathological examination of the testes revealed normal histo-architecture with enhanced spermatozoa that filled the lumen of the tubules (figure 1). This could be due to elevated FSH levels, which are directly related to spermatogenesis. To enhance spermatogenesis, FSH interacts with Sertoli cells. This is consistent with the research by Mutwedu et al. (2022).

Conclusion

This study concluded that the Moringa oleifera seed ethanolic extract is harmful to male reproduction as seen by increased body weights, effects on testicular androgen and sperm parameters. However, it has no alteration on testicular morphology, hence enhancing spermatogenesis. The extract, therefore, its aphrodisiac effects may vary depending on the dose.

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