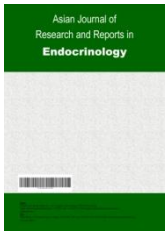

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Testicular Toxicity Cum Actions of Moringa *oleifera Seeds* Extract Following Nutmeg Administration

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study was carried out to assess the effect of *Moringa oleifera* seed extracts on nutmeg induced toxicity on the testes of adult male Albino Wistar rats. After two weeks of acclimatization, the rats were divided into ten equal groups designated as A, B, C, D, E1, E2, F1, F2, G1, and G2; each group consisted of three rats. Group A (control group) were administered distilled water, Group B were orally administered 0.70 ml of nutmeg, Group C were orally administered 1.40 ml of nutmeg, Group D were administered 10g of nutmeg via inhalation. Group E1 were orally administered 0.70 ml of nutmeg for four weeks, followed with 1.5 ml of ethanolic extract of *Moringa* seed on the fourth week, Group E2 were orally administered 0.70 ml of nutmeg for four weeks, followed with 1.3 ml of n-hexane extract of *Moringa* seed on the fourth week, Group F1 were orally administered 1.40 ml of nutmeg for four weeks, followed by 3.0 ml of ethanolic extract of *Moringa* seed on the fourth week, Group F2 were orally administered 1.50 ml of nutmeg for four weeks, followed by 3.0 ml of n-hexane extract of *Moringa* seed on the fourth week, Group G1 were administered 10g of nutmeg via inhalation for four weeks, followed by oral administration of 2.2 ml of ethanolic extract of *Moringa* seed on the fourth week and Group G2 animals were administered 10g of nutmeg via inhalation for four weeks, followed by oral administration of 3.0 ml of n-hexane extract of *Moringa* seed on the fourth week. On the 28th day, the rats were anaesthetized via chloroform inhalation, sacrificed and the testes were harvested and fixed immediately in 10% buffered formalin, processed and stained with Harris Haematoxylin and Eosin (H&E) staining method.

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Data were expressed as Mean ± standard error of the Mean (M±SEM) and subjected to one way analysis of variance (ANOVA). Significant difference between mean was accessed by student-New-Man-Keuls post hoc test. 95% level of significance ($p < 0.05$) was used for statistical analysis. At the end of the experiment *Moringa oleifera seed* extracts showed ameliorative and protective functions from the toxicity of excess nutmeg on the testes.

Keywords: Testes; extract of Moringa oleifera seed; nutmeg; albino wistar rats.

1. INTRODUCTION

Nutmeg belongs to the family Myristicacae [1]. Nutmeg is a tropical tree, commonly present in India, Indonesia and Malaysia [2]. It smells pungent and has a warm and slightly sweet taste and its flavor can vary from a sweetly spicy to a heavier taste [3]. People all over the world have used nutmeg in cooking, and it has also played a role in traditional remedies. In Asia, it has served as a traditional medicine for treating stomach cramps, diarrhea, and rheumatism. Researchers have also reported that nutmeg can have antioxidant and antimicrobial properties, as well as effects on the central nervous system. *Moringa oleifera* is a plant that is often called the drumstick tree, the miracle tree or the Ben oil tree. *Moringa* has been used for centuries due to its medicinal properties and health benefits. Although *Moringa* lowers blood pressure and slows heart rate due to presence of alkaloids in the plant, many of its health benefits are due to its rich proteins, minerals, amino acids, antioxidants and flavonoids, calcium, potassium, Zinc, Magnessium, iron and copper [4]. *Moringa* powder can be used to protect tissue (heart, kidney, liver and lungs), and to reduce pain. The objectives of this Study includes: to determine the effect of nutmeg in the histoarchitecture of the testes of adult male albino Wistar rats. To determine the effect nutmeg and *Moringa oleifera* extract on the body weight of the Wistar rats. To evaluate the effects of ethanolic extracts of *Moringa oleifera* seed extract on the testes histology of adult male albino Wistar rats following nutmeg admistration. To evaluate the effects of N-Hexane extract of *Moringa oleifera* on testes histology of adult male albino Wistar rats following nutmeg adminstration.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty adult male Albino Wistar rats weighing 129 g to 211 g were used for the study. They were fed with pelletized grower mash and clean drinking water was provided. They were acclimatized for two weeks before commence-ment of experiment. The rats were kept under standard room temperature of 27℃ to 30℃. They were housed in ten wooden cages designated as A, B, C, D, E1, E2, F1, F2, G1 and G2, measuring about 18 by 12 inches with wire gauge and saw dust beddings. The cages were cleaned daily and all the animals were handled in compliance with the applicable guidelines for the care and use of laboratory animals. Ethical clearance was obtained from the relevant ethical committee of the University.

2.2 Preparation of *Moringa* Extract

Moringa oleifera pods were authenticated and identified by the Botanist in charge of the herbarium unit of the Department of Botany and Ecological Studies of the University of Uyo. The *Moringa* seeds were removed and well grounded using a manual grinder. Soxhlet extraction technique was used. 80% of ethanol and 80% of n-hexane was added to 500 g of ground *Moringa* seeds respectively. Both extracts were gotten by filtration, stored in a glass beaker, covered with a foil and placed in a refrigerator to preserve the efficacy of the *Moringa* extracts.

2.3 Preparation of Nutmeg Extract

Nutmeg was purchased, authenticated and identified by the Botanist in charge of the herbarium unit of the Department of Botany and Ecological Studies of the University of Uyo. The nutmeg seeds were well grounded using a manual grinder. The ground nutmeg was portioned into two; one for the inhalation procedure and the other was extracted using the Soxhlet extraction technique. 80% of ethanol was added to 500g of ground nutmeg seeds. The extract was gotten by filtration, stored in a glass beaker, covered with a foil and placed in a refrigerator to preserve the efficacy of the nutmeg extract.

2.4 Experimental Protocol

The rats were acclimatized for two weeks before commencement of the experiment. They were divided into ten groups designated as A, B, C, D, E1,E2,F1,F2,G1, and G2; each group consisted of three rats. Group A (control group) were administered distilled water, Group B were orally

administered 0.70 ml of nutmeg, Group C were orally administered 1.40 ml of nutmeg, Group D were administered 10 g of nutmeg via inhalation. Group E1 were orally administered 0.70 ml of nutmeg for four weeks, followed with 1.5 ml of ethanolic extract of *Moringa* seed on the fourth week, Group E2 were orally administered 0.70 ml of nutmeg for four weeks, followed with 1.3 ml of n-hexane extract of *Moringa* seed on the fourth week, Group F1 were orally administered 1.40 ml of nutmeg for four weeks, followed by 3.0 ml of ethanolic extract of *Moringa* seed on the fourth week, Group F2 were orally administered 1.50 ml of nutmeg for four weeks, followed by 3.0 ml of n-hexane extract of *Moringa* seed on the fourth week, Group G1 were administered 10g of nutmeg via inhalation for four weeks, followed by oral administration of 2.2 ml of ethanolic extract of *Moringa* seed on the fourth week and Group G2 animals were administered 10g of nutmeg via inhalation for four weeks, followed by oral administration of 3.0 ml of n-hexane extract of *Moringa* seed on the fourth week.

2.5 Termination of Experiment

On the 28th day, the rats were anaesthetized via chloroform inhalation, sacrificed and the testes were harvested and fixed immediately in 10% buffered formalin, processed and stained with Harris Haematoxylin and Eosin (H&E) staining method.

2.6 Statistical Analysis

Data were expressed as Mean ± standard error of the Mean (M±SEM) and subjected to one way analysis of variance (ANOVA). Significant difference between mean was accessed by student-New-Man-Keuls post hoc test. 95% level of significance ($p < 0.05$) was used for statistical analysis.

3. RESULTS

3.1 Comparison of Changes in Body Weight of Adult Male Albino Wistar Rats

The result of the changes in body weight following the administration of Nutmeg only, Nutmeg and Ethanolic extract of *Moringa oleifera* seed, and Nutmeg and N – Hexane extract of *Moringa oleifera* seed, for twenty eight days is shown in Table 1.

Table 1. Comparison of changes in body weight of adult male albino wistar rats in grams (gm)

Groups	Week 1	Week 2	Week 3	Week4
A	205.50±6.50	220.50±8.50	313.00±8.00	313.00±7.00
B	188.33±1.20	194.33±1.86	277.33±1.86	280.00±3.51
C	177.33±2.03	182.33±2.96	267.67±3.18	269.67±2.03
D	166.33±3.93	172.00±2.00	253.67±2.03	262.33±3.67
E1	196.67±2.33	210.67±1.67	297.00±0.58	307.00±0.58
E2	164.00±3.06	177.33±4.06	264.67±4.18	265.33±4.81
F1	159.00±4.93	177.00±7.51	257.33±10.17	264.67±11.41
F2	159.00±4.93	213.00±0.00	283.00±2.31	291.67 ±3.48
G1	136.33±3.84	144.67±2.19	215.33±1.33	215.00±2.00
G2	191.00±10.02	199.67±4.68	279.67±17.67	278.33±19.55

Values are expressed as mean ± standard error of mean (S±SEM). At 95% level of significance (P < 0.05)

Key:

A = rats given distilled water Group A (Control).

B = rats given 0.60 ml (low dose) Nutmeg

C = rats given 1.20 ml (high dose) Nutmeg

D = rats given 10g of Nutmeg via inhalation method for four weeks.

E1 = rats given 0.70 ml (low dose) Nutmeg for three weeks and 1.5 ml (low dose) ethanolic extract of Moringa oleifera seed for one week.

E2 = rats given 0.60 ml (low dose) of Nutmeg for three weeks followed by 1.3 ml (low dose) N – Hexane extract of Moringa oleifera seed for one week.

F1 = rats given 1.20 ml (high Dose) of Nutmeg for three weeks followed by 30.0 ml (high dose) ethanolic extract Moringa oleifera seed for one week

F2 = rats given 1.30 ml (high Dose) of Nutmeg for three weeks followed by 30.0 ml (high dose) of N – Hexane extract of Moringa oleifera seed for one week

G1 = rats given 10 g of Nutmeg via inhalation method for three weeks followed by 2.20 ml of ethanolic extract Moringa oleifera seed for one week

G2 = rats given 10g of Nutmeg via inhalation method followed by 3.0 ml of

N – Hexane extract Moringa oleifera seed.

3.2 Histological Observation

Hematoxylin and Eosin (H&E) Method for General Demonstration of testis

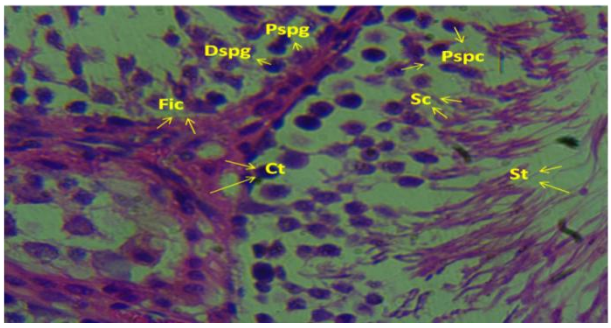

Fig. 1. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rat given given distilled water. Group A (Control). (H&E method, X400). Section revealed features typical of normal testis. Sertoli cells Sc. Seminiferous tubule St. Dark (late) spermagonia Dspg and Pale (early) spermatonia Pspg. Fibromuscular interstitial cells Fic. Connective tissues Ct. Primary spermatocytes Pspc.

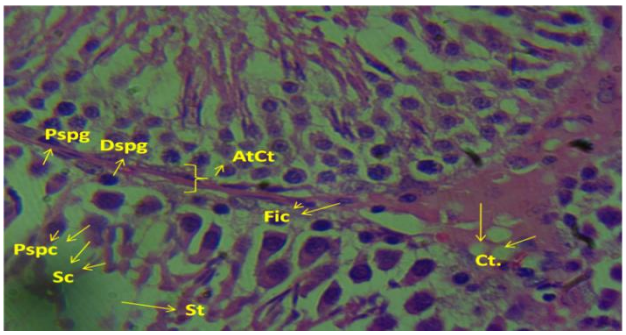

Fig. 2. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rat given 0.60 ml (low dose) Nutmeg for four weeks (28 days). Group B. (H&E method, X400). Section revealed unaffected Sertoli cells Sc.Seminiferous tubule St. Dark (late) spermagonia Dspg andPale (early) spermatonia Pspg. Fibromuscular interstitial cells Fic. Connective tissues Ct. Primary sprmatoyts Pspc.,with Atrophy of connective tissue AtCt. Inference: Slightly affected

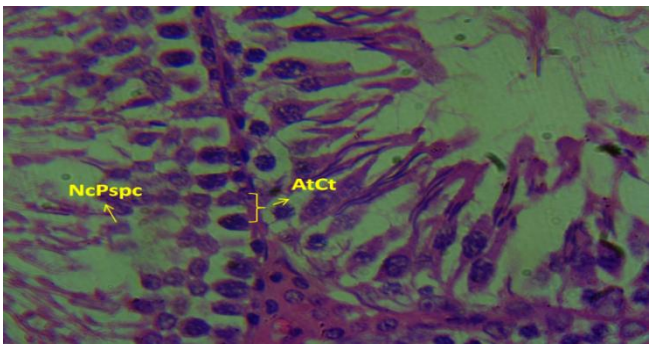

Fig. 3. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rat given 1.20 ml (high dose) Nutmeg for four weeks (28 days). Group C. (H&E method, X400). Section revealed Atrophy of connective tissue AtCt., and necrosis of primary spermatocytes NcPsc Inference: Affected

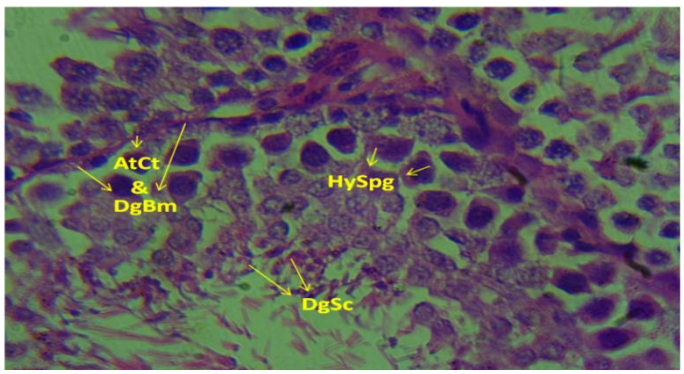

Fig. 4. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rat given 10 gm of Nutmeg via inhalation method for four weeks (28 days). Group D. (H&E method, X400). Section revealed: Atrophy and degeneration of the connective tissue and basement membrane AtCt. & DgBm. Hypertrophy of spermatogonia HySpg. Inference: Affected

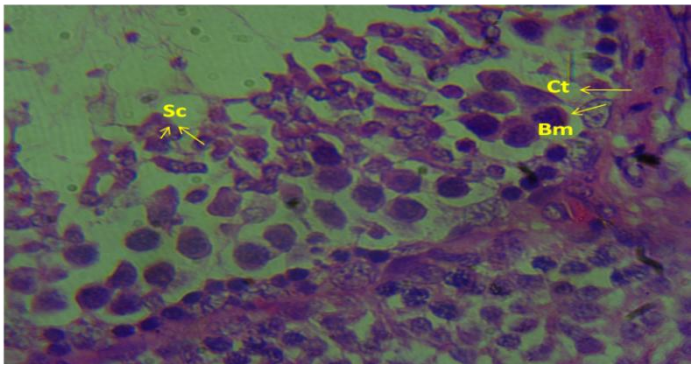

Fig. 5. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rats given 0.70ml (low dose) Nutmeg for three weeks and 1.5 ml (low dose) ethanolic extract of *Moringa oleifera* seed for one week.. Group E1. (H&E method, X400). Section revealed intact and unaffected: Sertoli cells Sc. Connective tissues Ct. Basement membrane Bm

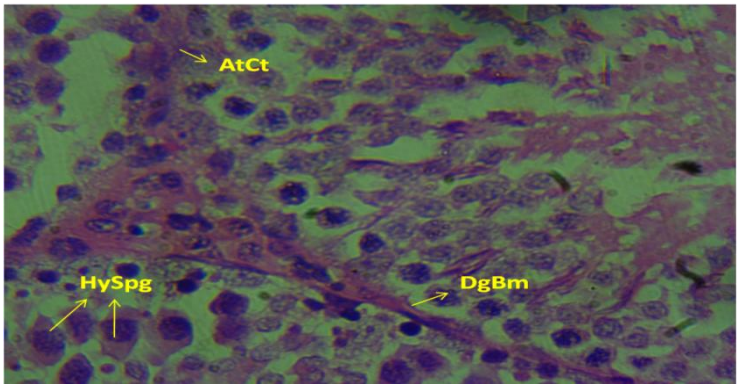

Figure VI. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rats given 0.60 ml (low dose) of Nutmeg for three weeks followed by 1.3 ml (low dose) N – Hexane extract of *Moringa oleifera* seed for one week. Group E2. (H&E method, X400). Section revealed: Slight Atrophy and degeneration of the connective tissue and basement membrane AtCt. & DgBm. Hypertrophy of spertogonia HySpg. Inference: Affected

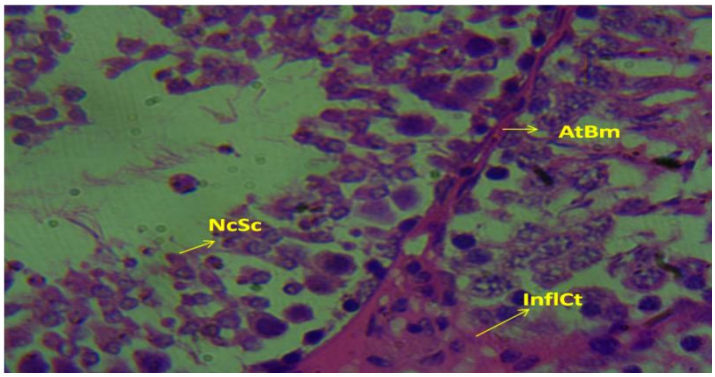

Figure VII. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rats given 1.20 ml (high Dose) of Nutmeg for three weeks followed by 30.0 ml (high dose) ethanolic extract *Moringa oleifera* seed for one week. Group F1. (H&E method, X400). Section revealed: Slight Atrophy of the basement membrane AtBm. Necrosis of Sertoli cells NcSc. Inflammation of Connective tissue InflCt. Inference: Affected.

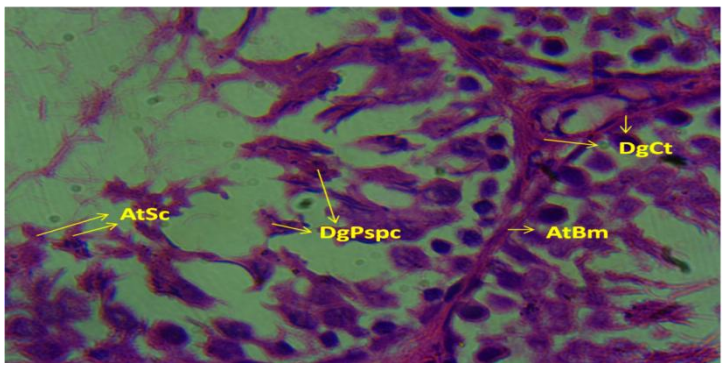

Figure VIII. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rats given 1.30 ml (high Dose) of Nutmeg for three weeks followed by 30.0 ml (high dose) of N – Hexane extract of *Moringa oleifera* seed for one week. Group F2. (H&E method, X400). Section revealed: Slight Atrophy of the basement membrane AtBm. Atrophy of Sertoli cells AtSc. Degeneration of Connective tissue DgCt. Inference: Affected

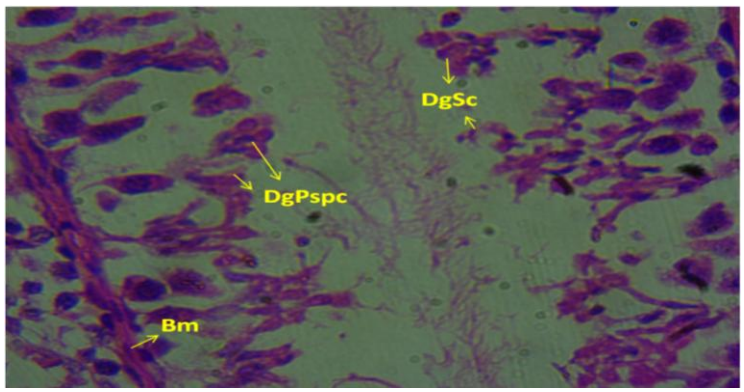

Fig. 9. A transverse plane through a single seminiferous tubule of the testis of adult male albino Wistar rats given 10g of Nutmeg via inhalation method for three weeks followed by 2.20 ml of ethanolic extract *Moringa oleifera* seed for one week. Group G. (H&E method, X400). Section revealed: unaffected basement membrane Bm. Dgeneration of Sertoli cells DgSc. Degeneration of primary spermatocytes DgPspc. Inference: Affected

4. DISCUSSION

The existence of humanity on earth was accompanied by the presence of ailments and certain therapeutic plants have been found to positively ameliorate if not completely eradicate these ailments [5]. The histological findings from this investigation showed that animals in the control group administered 1.0ml of distilled water revealed normal and unaffected architecture of testicular structures which include Sertoli cells, seminiferous tubule, spermatogonia and pale (early) spermatogonia, fibromuscular interstitial cells**,** connective tissues and primary spermatocytes. Animals administered with Nutmeg revealed unaffected testicular components**,** although a noticeable atrophy of connective tissue was seen, this shows that the testicular structures were slightly affected indicating that nutmeg may have a deleterious effect on the testes. Animals administered of 1.20 ml (high dose) Nutmeg for four weeks revealed atrophy of connective tissue and necrosis of primary spermatocytes. This is in line with a research conducted by [6] which proves that in large quantities, Nutmeg is toxic to the reproductive system. Animals administered 10g of Nutmeg via inhalation method for four weeks, revealed atrophy/degeneration of the connective tissue and basement membrane, hypertrophy of spermatogonia. Several researches have indicated the deleterious impact of certain substances on the testes, for example, Edem et al., [7] revealed that *Nauclea latifolia* significantly reduced glycogen granule level in the testes as well reducing the levels of testosterone and follicle stimulating hormones. Similarly, Chaunan et al., [6] stated that toxic effect of nutmeg causes hypertrophy of the spermatogonia confirming that Nutmeg can have some stimulating effects and increase sexual functions. Animals administered 0.70 ml (low dose) Nutmeg for three weeks and 1.5 ml (low dose) ethanolic extract of *Moringa oleifera* seed for one week, revealed sections with intact and unaffected testicular structures thereby inferring that ethanolic extract of *Moringa oleifera* has an ameliorative function in curbing the effect of nutmeg on the seminiferous tubules at low doses. Sections of animals administered 0.7 ml (low dose) of Nutmeg for three weeks followed by 1.3 ml (low dose) N – Hexane extract of *Moringa oleifera* seed for one week**,** revealed slight atrophy and degeneration of the connective tissue, degeneration of the basement membrane and hypertrophy of spermatogonia thus indicating structures were slightly affected. Further histological observations revealed slight atrophy of the basement membrane, necrosis of sertoli cells and inflammation of connective tissue structures, this finding has shown that in high doses, N-hexane extracts of *Moringa oleifera* has ameliorative functions on nutmeg induced toxicity of the testes. In group E1 animals administered 10g of nutmeg via inhalation method for three weeks followed by 2.20 ml of ethanolic extract *Moringa oleifera* seed for one week, revealed atrophy of the basement membrane and degeneration of sertoli cells. Several studies have demonstrated the beneficial effects in humans. *Moringa oleifera* has been recognized as containing a great number of bioactive compounds. The most used parts of the plant are the leaves, which are rich in vitamins, carotenoids, polyphenols, phenolic acids, flavonoids, alkaloids, glucosinolates, isothiocyanates, tannins and saponins [8]. The high number of bioactive compounds might explain the pharmacological *Moringa oleifera* leaves. Many studies, in vitro and in vivo, have confirmed these pharmacological properties [8]. The leaves of *Moringa oleifera* are mostly used for medicinal purposes as well as for human nutrition, since they are rich in antioxidants and other nutrients, which are commonly deficient in people living in undeveloped countries. *Moringa oleifera* leaves have been used for the treatment of various diseases from malaria and typhoid fever to hypertension and diabetes. Fresh leaves from *Moringa oleifera* are a good source of vitamin A [9]. It is well established that vitamin A has important functions in vision, reproduction, embryonic growth and development, immune competence and cell differentiation. *Moringa oleifera* leaves are a good source of carotenoids with pro-vitamin A potential [9]. Findings from phytochemical screening revealed the presence of antioxidants such as flavonoid, tannin and saponin. These antioxidants have been found to possess various therapeutic tendencies. Anti-oxidants are biotic compounds that can inhibit or delay the oxidation of molecules [10]. Plant extracts have been found to show powerful anti-oxidant capacities both in vitro and in vivo, and these extracts can be considered a good source of natural antioxidants and antimicrobials for ameliorating and treating various ailments [11]. Different studies have isolated tannins and saponins from some medicinal plants, testing the antibacterial activity against various pathogens such as *Klebsiella pneumonia* [12,13].

5. CONCLUSION

This study has shown that nutmeg indeed has toxic effects on the histology of the testes.

Ethanolic extract of *Moringa oleifera* has protective effect on the testes if nutmeg is consumed in low doses. Both ethanolic extract and N-hexane extract of *Moringa oleifera* cannot protect the testes if nutmeg is consumed in large doses.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical clearance was obtained from the relevant ethical committee of the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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