TESTICULAR TOXICITY CUM ACTIONS OF MORINGA *OLEIFERA SEEDS* EXTRACT FOLLOWING NUTMEG ADMINISTRATION

ABSTRACT

The study was carried out to assess the effect of *Moringa oleifera* extracts on nutmeg induced toxicity on the testes of rats. The animals were weighed before the experiment and subsequently every weekend of the experiment. The animals were acclimatized for two (2) weeks before commencement of the experiment. After acclimatization, the animals were divided into ten (10) groups designated as A, B, C, D, E1, E2, F1, F2, G1, and G2; each group consisted of three (3) animals. Group A animals (control group) were administered distilled water, Group B animals were administered oral doses of 0.70ml of nutmeg, Group C animals were administered oral doses of 1.40ml of nutmeg, Group D animals were tested with 10g of nutmeg through inhalation with the use of a desiccators, Group E1 animals were administered oral doses of 0.70ml of nutmeg for four weeks, followed with 1.5ml of ethanolic *Moringa* extract on the fourth week, Group E2 animals were administered oral doses of 0.70ml of nutmeg for four weeks, followed with 1.3ml of n-hexane *Moringa* extract on the fourth week, Group F1 animals were administered oral doses of 1.40ml of nutmeg for four weeks, followed by 3.0ml of ethanolic *Moringa* extract on the fourth week, Group F2 animals were administered oral doses of 1.50ml of nutmeg for four weeks, followed by 3.0ml of n-hexane *Moringa* extract on the fourth week, Group G1 animals were tested with 10g of nutmeg through inhalation with the use of a desiccator for four weeks, followed by oral doses of 2.2ml of ethanolic *Moringa* extract on the fourth week and Group G2 animals were tested with 10g of nutmeg through inhalation with the use of a desiccator for four weeks, followed by oral doses of 3.0ml of n-hexane *Moringa* extract on the fourth week. The rats were weighed before and after the experiment. On the 28th day, the rats were anaesthetized via chloroform inhalation, sacrificed and the testes harvested and fixed immediately in 10% buffered formalin, processed and stained with Harris Haematoxylin and Eosin (H&E) staining method. Phytochemical analysis of the extracts showed the presence of several bioactive compounds. *Moringa oleifera* extracts showed ameliorative and protective functions over the testes administered excess nutmeg which could induce a level of toxicity.

Keywords: Testes**,** *Moringa oleifera,* 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. This work hopes to unravel the impact of *Moringa oleifera and* Nutmeg extracts on the testes of Wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty (30) adult male Albino Wistar rats weighing 129g-211g were used for the study. The animals were kept and fed at the animal house of College of Health Sciences, University of Uyo, Uyo. They were fed with pelletized grower mash and clean drinking water was provided. They were acclimatized for two (2) weeks before commencement of experiment. The rats were kept under standard room temperature of 27℃ to 30℃. They were housed in ten (10) 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 was used as 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.

2.2 Preparation of *Moringa* Extract

Moringa was purchased from the local market (Itam market) in Uyo, Akwa-Ibom state, Nigeria. The seeds 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, Uyo, Akwa-Ibom state, Nigeria. The *Moringa* seeds were removed and well grounded using a manual grinder. Soxhlet extraction technique was used. 80% of ethanol was added to 500g of ground *Moringa* seeds, 80% of n-hexane was added to 500g of ground *Moringa* seeds. 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 from a local market (Itam market) in Uyo, Akwa-Ibom state, Nigeria and was 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, Uyo, Akwa-Ibom state, Nigeria. 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 animals were acclimatized for two (2) weeks before commencement of the experiment. After acclimatization, the animals were divided into ten (10) groups designated as A, B, C, D, E1, E2, F1, F2, G1, and G2; each group consisted of three (3) animals each. Group A animals (control group) were administered distilled water, Group B animals were administered oral doses of 0.70ml of Nutmeg, Group C animals were administered oral doses of 1.40ml of nutmeg, Group D animals were tested with 10g of nutmeg through inhalation with the use of a desiccator, Group E1 animals were administered oral doses of 0.70ml of nutmeg for four weeks, followed by 1.5ml of ethanolic extract of *Moringa* on the fourth week, Group E2 animals were administered oral doses of 0.70ml of nutmeg for four weeks, followed by 1.3ml of n-hexane *Moringa* extract on the fourth week, Group F1 animals were administered oral doses of 1.30ml of nutmeg for four weeks, followed by 3.0ml of ethanolic *Moringa* extract on the fourth week, Group F2 animals were administered oral doses of 1.50ml of nutmeg for four weeks, followed by 3.0ml of n-hexane *Moringa* extract on the fourth week, Group G1 animals were tested with 10g of nutmeg through inhalation with the use of a desiccator for four weeks, followed by oral doses of 2.2ml of ethanolic *Moringa* extract on the fourth week and Group G2 animals were tested with 10g of nutmeg through inhalation with the use of a desiccator for four weeks, followed by oral doses of 3.0ml of n-hexane *Moringa* extract on the fourth week.

2.5 Termination of Experiment

The animals were anaesthetized with chloroform and sacrificed. The Testes were removed and fixed in 10% buffered formalin for 48 hours. The tissues were promptly processed for paraffin wax embedding and stained with Haematoxylin and Eosin.

3. RESULTS

3.1 Phytochemical Screening of Nutmeg Seeds

Presence of bioactive compounds in Nutmeg is shown in the table I

Table I: Result of phytochemical Analysis of Active Ingredients in Nutmeg

Test	Observation	Inference
Alkaloid	red orange precipitate observed	+ +
Saponin	persistent frothing observed After heating for 10 minutes	+ +
Tannins	bluish black coloration observed	+ +
Flavonoid	orange coloration observed	++ +
Cardiac glycosides		
1. Salkawski	a brownish-red ring coloration observed	+
2. Liberman	a green ring coloration observed at the interphase	+
3. Keller Killani	brown ring observed at the interphase	+

KEY:
-- = absent
+ = slightly present
+ + = moderately present
+ + + = heavily (strongly) present

3.2 Phytochemical Screening of *Moringa oleifera* Seeds

Presence of bioactive compounds in ***Moringa oleifera*** is shown in the table 11

Table II: Result of phytochemical Analysis of Active Ingredients in *Moringa oleifera*

TEST	OBSERVATION	INFERENCE
Alkaloids		
1. Dragendof	Red precipitate observed	+ +
2. Mayer	Red precipitate observed	+ +
Tannin	Bluish-black coloration observed	+ + +
Saponin	Persistent frothing observed	+ +
Flavonoid	Orange precipitate observed	+ +
Cardiac glycoside		
1. Salkawski	Reddish-brown ring	+ + +
2 KellLillirin	Brown ring observed at the interphase	+ +
3 Lierberman	Violet ring observed at the interphase	+ +

KEY:

-- = absent
+ = slightly present
+ + = moderately present
+ + + = heavily presence

3.3 Histological Observation

A transverse plane through a single seminiferous tubule of the testes of rat given distilled water (**Group A** -Control) under the light microscope using a magnification of X400 revealed intact

and normal histological architecture of a typical normal testes. Structures which were visible include; Sertoli cells, Seminiferous tubule, spermatogonia and Pale (early) spermatogonia, fibromusculra interstitial cells**,** connective tissues and primary spermatocytes. A transverse plane through a single seminiferous tubule of the testes of rat given 0.60 ml (low dose) Nutmeg for four weeks (**Group B),** revealed unaffected Sertoli cells, unaffected Seminiferous tubule, Dark (late) spermatogonia and pale (early) spermatogonia, fibromuscular interstitial cells, unaffected connective tissues**,** unaffected primary spermatocytes with a noticeable atrophy of connective tissue showing the testes structures were slightly affected. A transverse plane through a single seminiferous tubule of the testes of rat given 1.20 ml (high dose) Nutmeg for four weeks (**Group C),** revealed atrophy of connective tissue and necrosis of primary spermatocytes thus indicating the testes structures were adversely affected. A transverse plane through a single seminiferous tubule of the testes rat given 10g of Nutmeg via inhalation method for four weeks (**Group D**), revealed atrophy and degeneration of the connective tissue and basement membrane, hypertrophy of spermatogonia thus indicating an adverse effect on testes structures. A transverse plane through a single seminiferous tubule of the testes of rat 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, (**Group E1**) revealed intact and unaffected Sertoli cells, connective tissues and basement membrane, thus indicating no adverse effect**.** A transverse plane through a single seminiferous tubule of the testes of rat 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),** revealed slight atrophy and degeneration of the connective tissue, degeneration of the basement membrane and hypertrophy of spermatogonia, thus indicating structures were slightly affected.

A transverse plane through a single seminiferous tubule of the testes of rat given 1.20 ml (high dose) of Nutmeg for three weeks followed by 30.0 ml (high dose) ethanolic extract of *Moringa oleifera* seed for one week (**Group F1**), revealed slight atrophy of the basement membrane, necrosis of Sertoli cells and inflammation of connective tissue, thus indicating an adverse effect of the testes structures. A transverse plane through a single seminiferous tubule of the testes 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**), revealed slight atrophy of the basement membrane, atrophy of sertoli cells and degneration of connective tissues thus indicating the testes structures were adversely affected. A transverse plane through a single seminiferous tubule of the testes of 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**) revealed atrophy of the basement membrane, degeneration of Sertoli cells and degeneration of primary spermatocytes thus indicating testes structures were adversely affected.

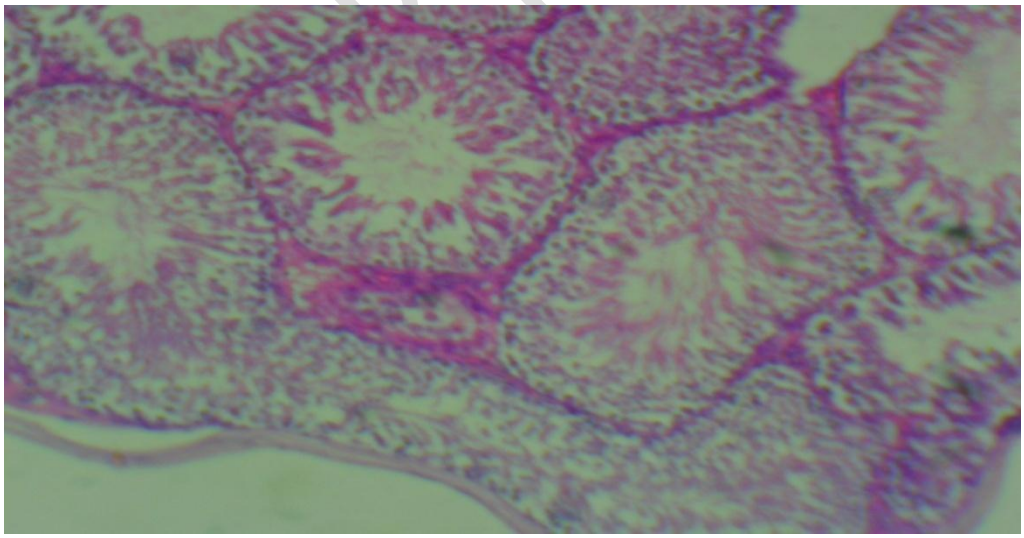

Fig I. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rat given distilled water. **Group A** (Control). (H&E method, **X100**).

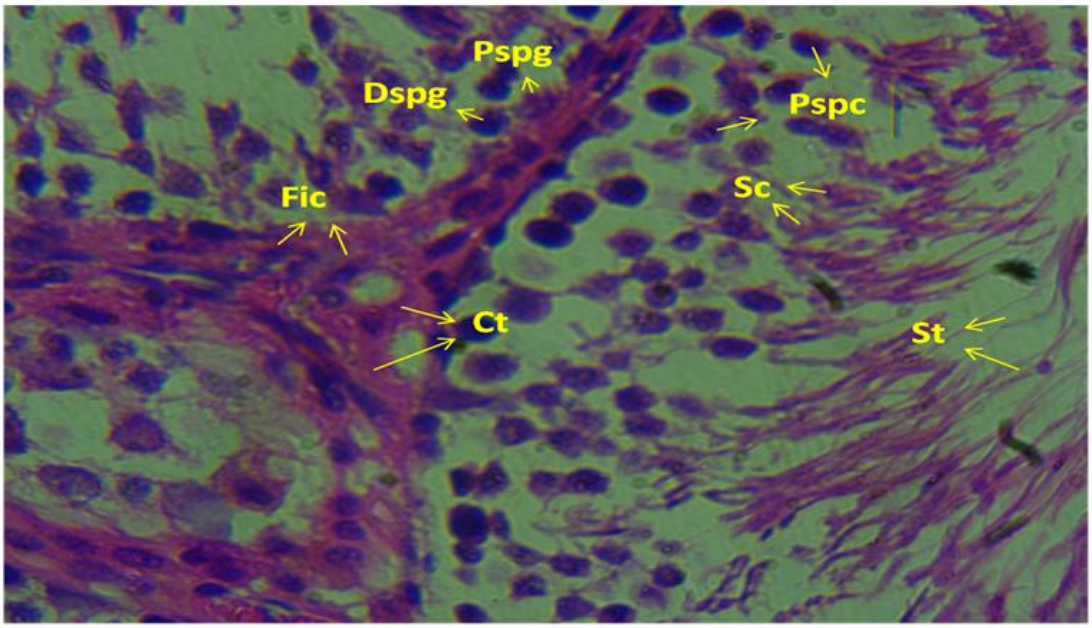

Fig. II. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rat given given distilled water. **Group A** (Control). (H&E method, **X400**). Section revealed features typical of normal testis. Sertoli cells (**Sc)**, Seminiferous tubule **St.** Dark (late)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

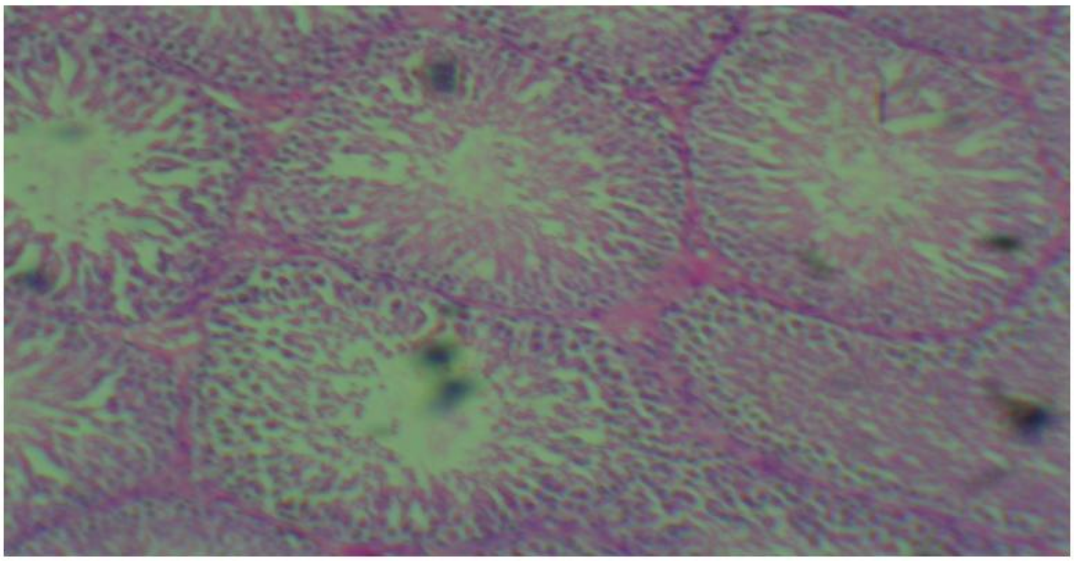

Fig. III. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rat given 0.60 ml (low dose) Nutmeg for four weeks (28 days**). Group B**. (H&E method, **X100**). Section revealed features typical of normal testes.

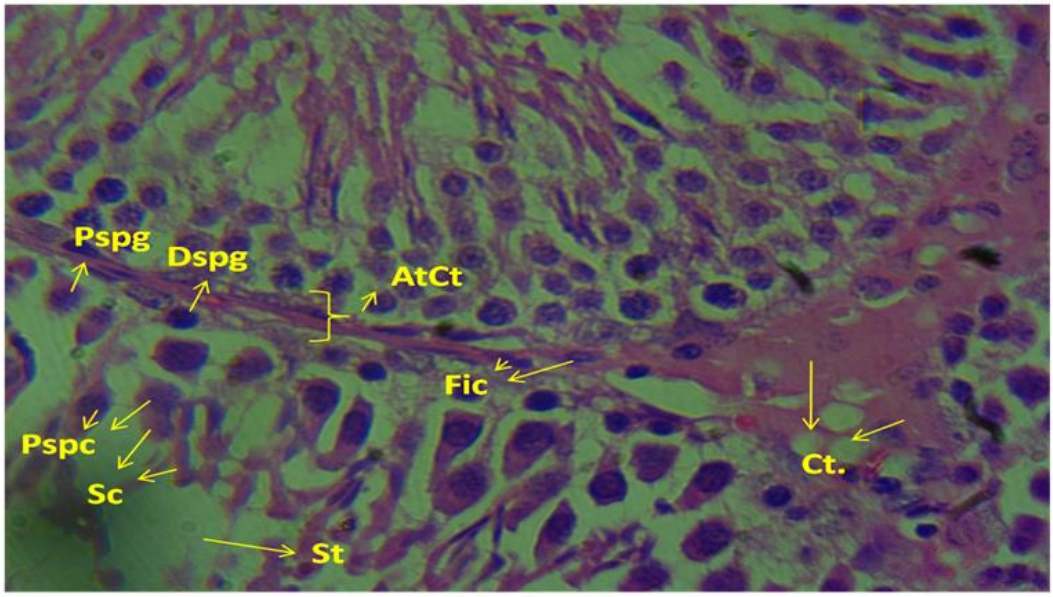

Fig. IV. A transverse plane through a single seminiferous tubule of the testAs of adult male albino Wistar rat given 0.60 ml (low dose) Nutmeg for four weeks (28 days). **Group B.** (H&E method**, X400).** Section revealed unaffected Sertoli cells (**Sc)**, Seminiferous tubule (**St),** Dark

(late spermatogonia **(Dspg)** and Pale (early) spermatogonia **(Pspg)**, Fibromuscular interstitial cells **(Fic),** Connective tissues **(Ct),** Primary spermatocytes **(Pspc)** with Atrophy of connective tissue **(AtCt). Infernce:** Slightly affected.

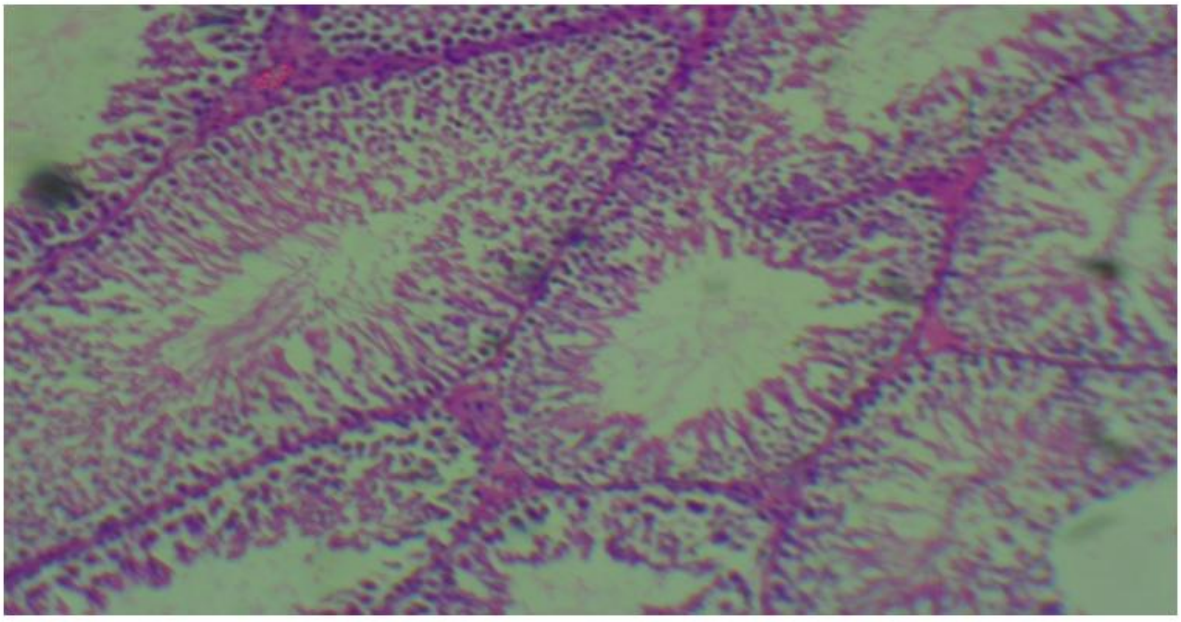

Fig. V. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rat given 1.20 ml (high dose) Nutmeg for four weeks (28 days) **Group C**. (H&E method, **X100**).

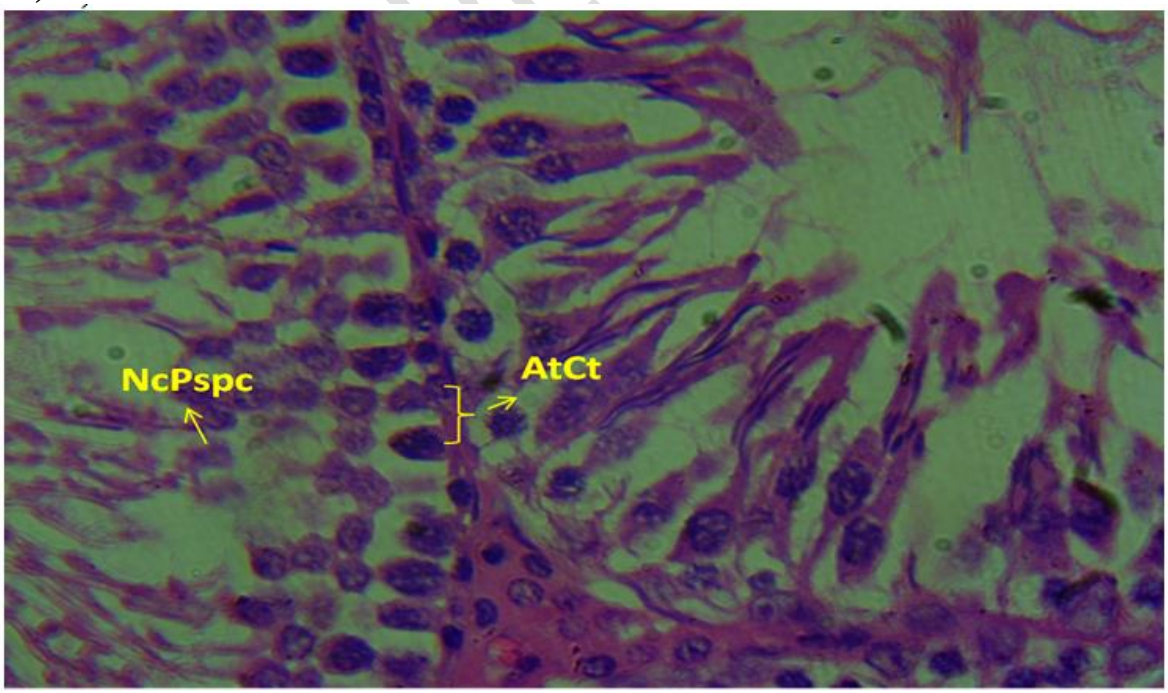

Figure VI. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rat given 1.20 ml (high dose) Nutmeg for four weeks (28 days). **Group C**. (H&E

method, **X400**). Section revealed Atrophy of connective tissue (**AtCt)** and necrosis of primary spermatocytes **(NcPsc). Infernce: A**ffected.

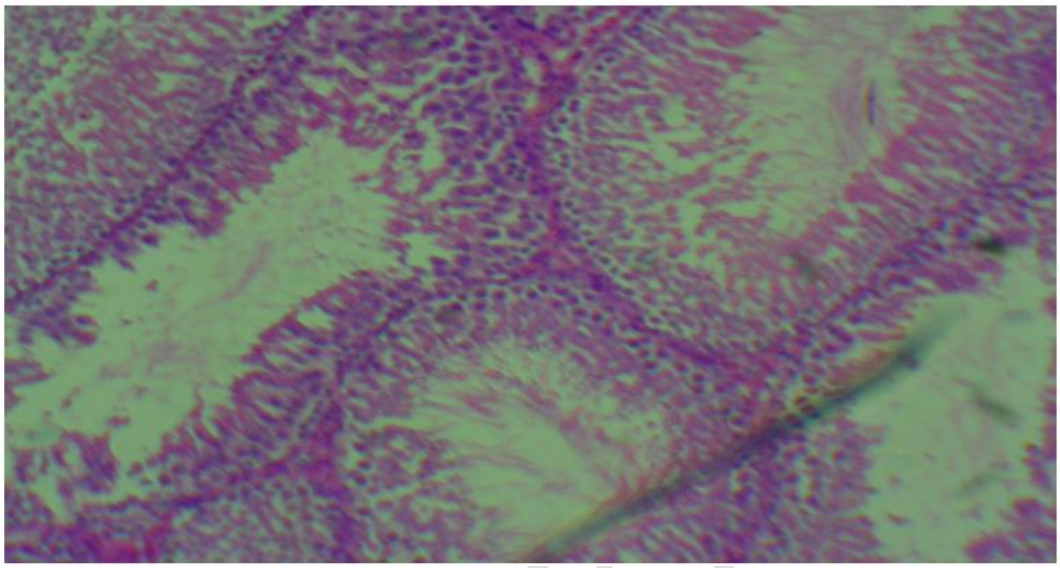

Fig. VII. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 10g of Nutmeg via inhalation method for four weeks. **Group D**. (H&E method, **X100**).

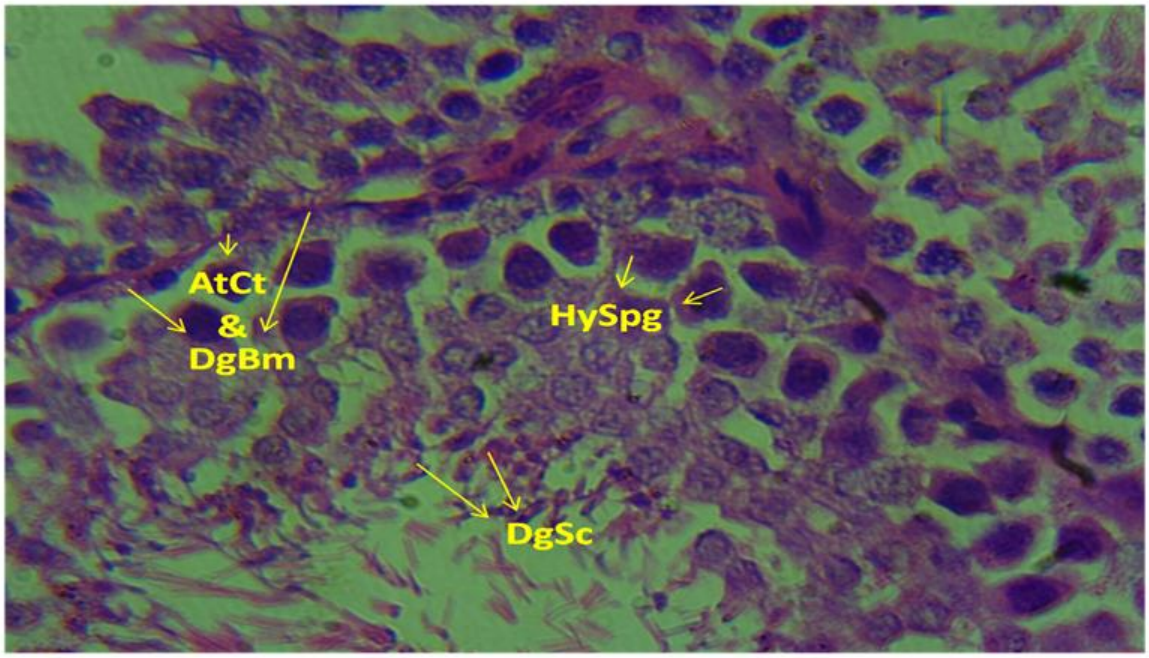

Fig. VIII. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rat given 10g of Nutmeg via inhalation method for four weeks (28 days). **Group D**. (H&E method, **X400**). Section revealed: Atrophy and degeneration of the connective tissue and basement membrane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

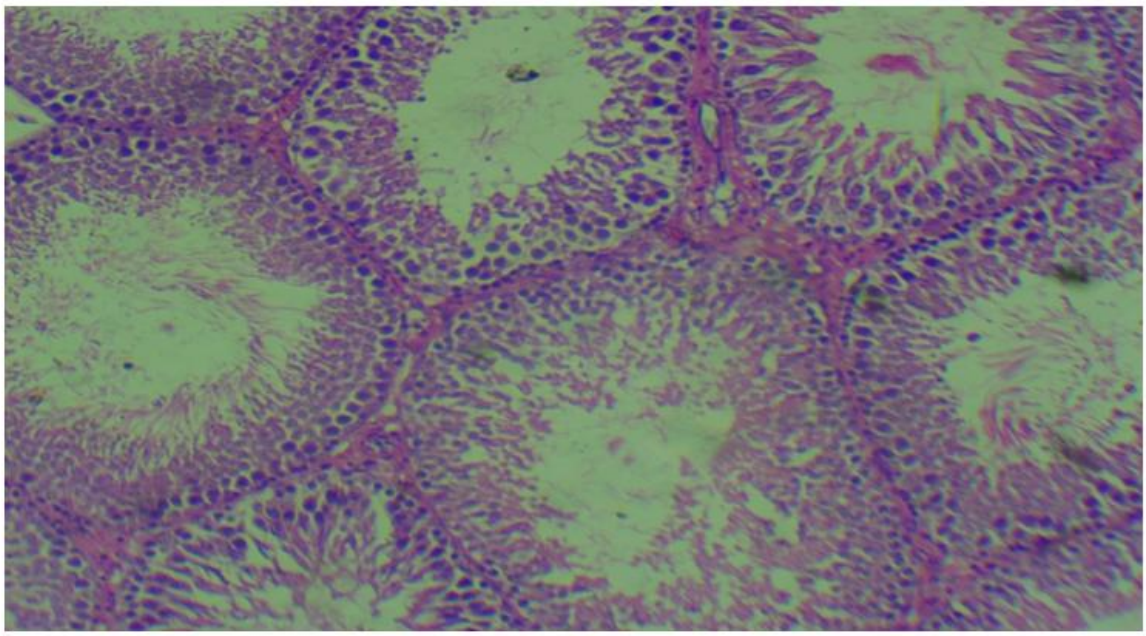

Fig. IX. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 0.70 ml (low dose) Nutmeg for three weeks and 1.5 ml (low dose) ethanolic extract of *Moringa oleifera* seed for one week. **Group E1**. (H&E method, **X100**).

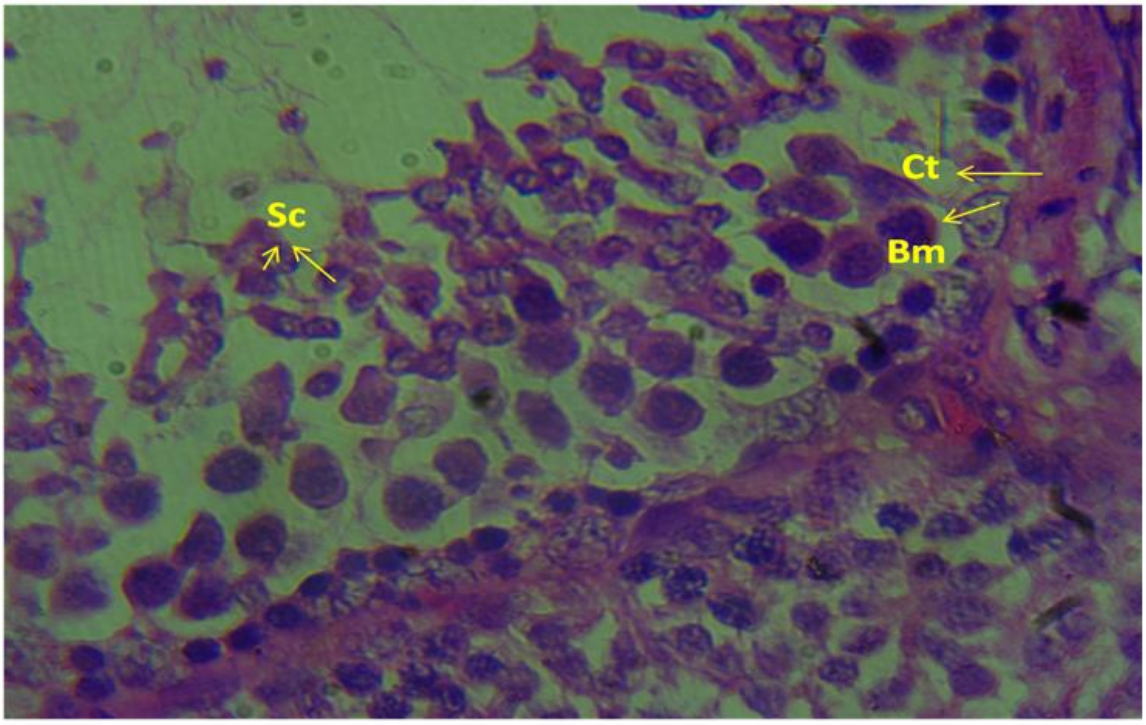

Fig. X. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 0.70 ml (low dose) Nutmeg for three weeks and 1.5 ml (low dose) ethanolic extract of *Moringa oleifera* seed for one week.. **Group E1** (H&E method, **X400**). Section revealed intact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

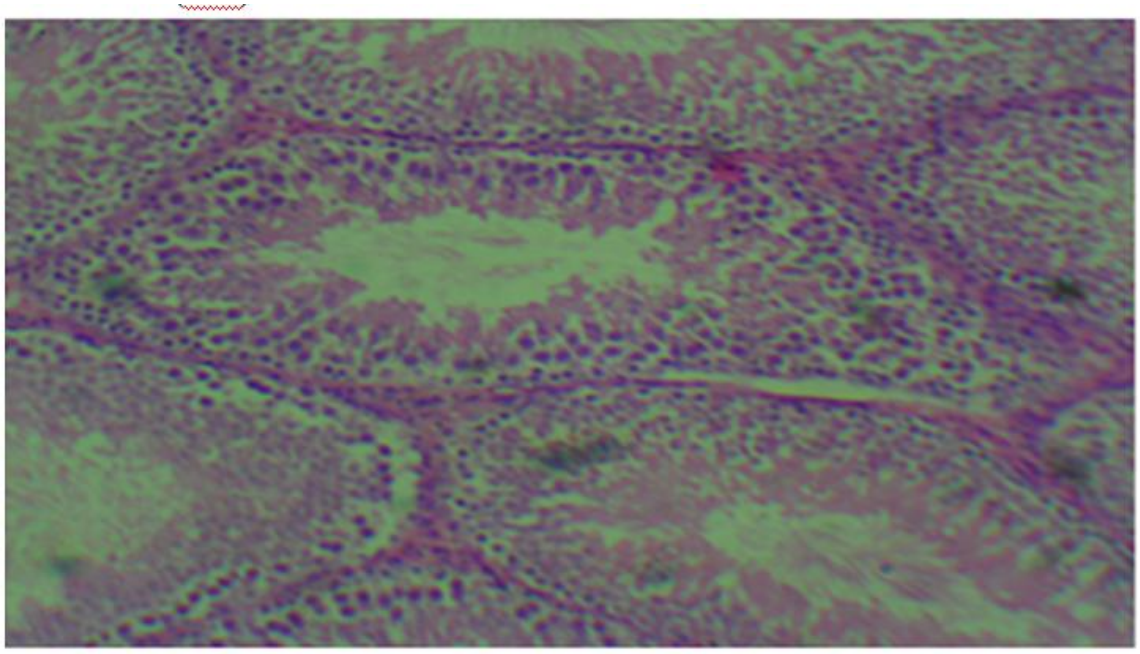

Fig. XI. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 0.70 ml (low dose) of Nutmeg for three weeks followed by 1.3 ml (low dose) N – Hexane extract of *Moringa oleifera* seed for one week. **Group E2**. (H&E method, **X100**).

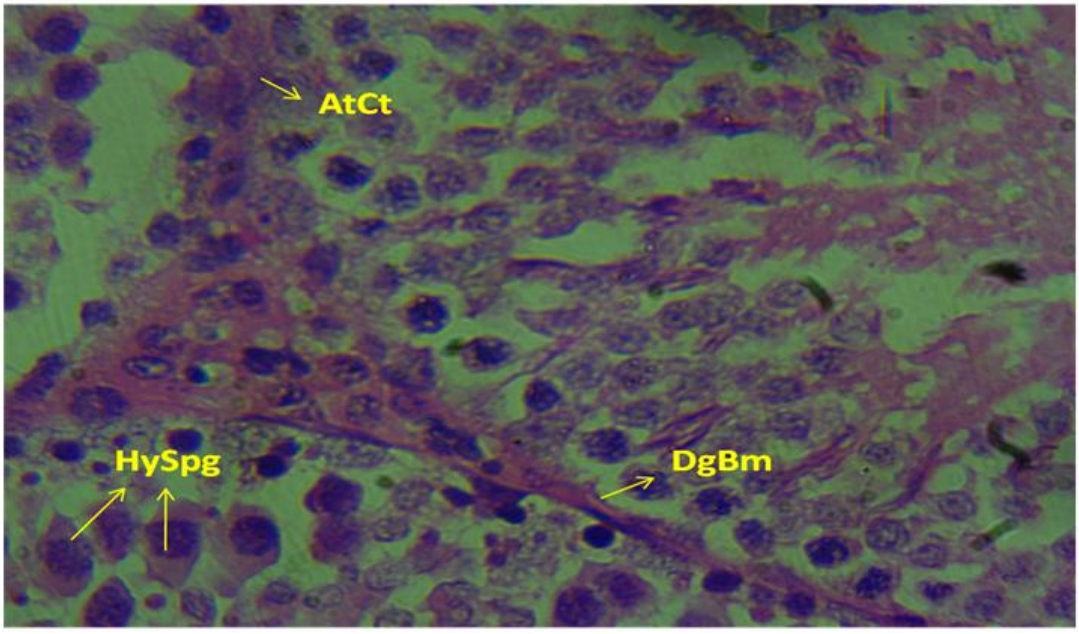

Fig. XII. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 0.70 ml (low dose) of Nutmeg for three weeks followed by 1.3 ml (low dose) N – Hexane extract of *Moringa oleifera* seed for one week. **Group E2**. (H&E method, **X400**). Section revealed: Slight Atrophy and degeneration of connective tissue and basement membrane (**AtCt. & DgBm),** Hypertrophy of spermatogonia **(HySpg).** Inference: Affected.

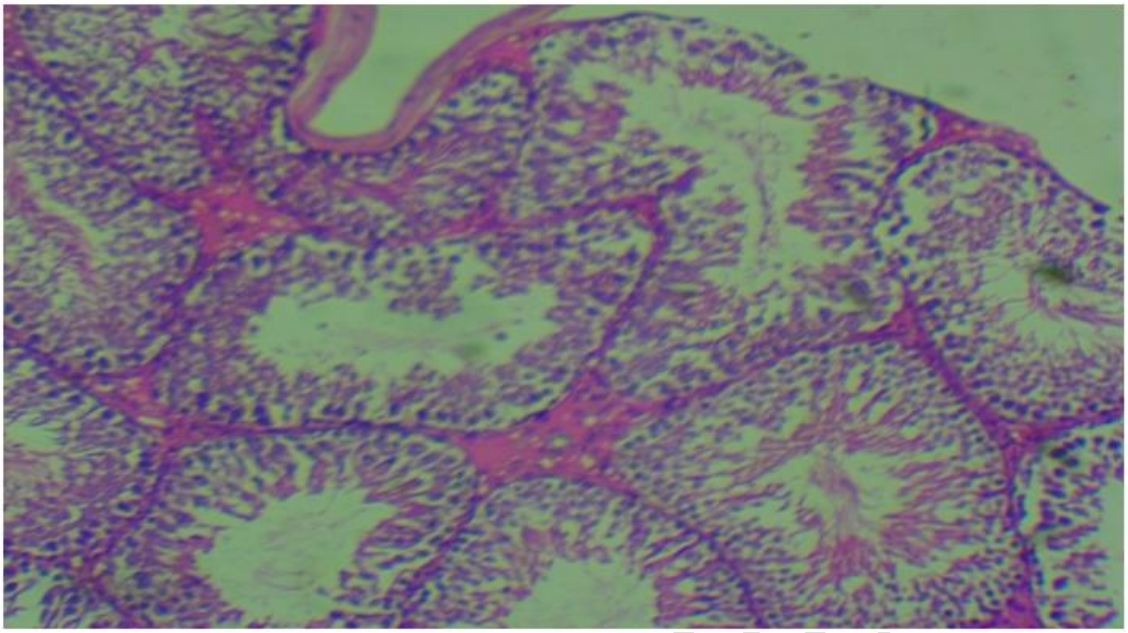

Fig. XIII. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 1.20 ml (high Dose) of Nutmeg for three weeks followed by 30.0 ml (high dose) ethanolic extract *Moringa oleifera* seed for one week. **Group F1**. (H&E method, **X100**).

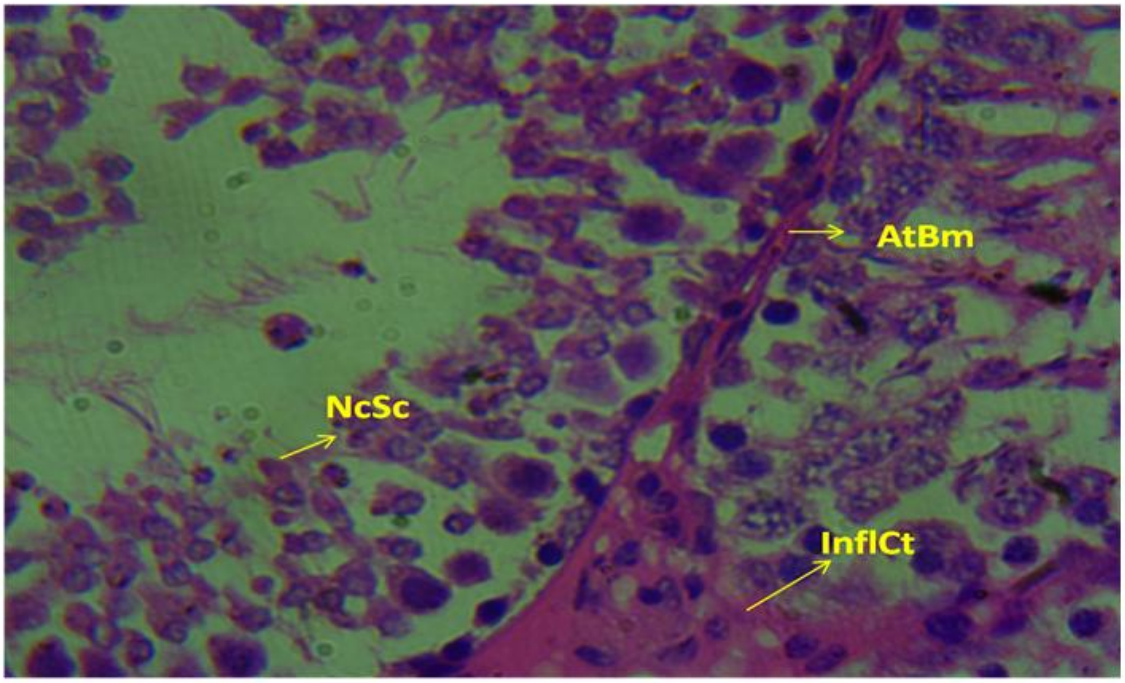

Fig. XIV. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 1.30 ml (high Dose) of Nutmeg for three weeks followed by 3.0 ml

(high dose) ethanolic extract *Moringa oleifera* seed for one week. **Group F1**. (H&E method, **X400**). Section revealed: Slight Atrophy of basement membrane (**AtBm).** Necrosis of Sertoli cells **(NcSc),** Inflammation of Connective tissue (**InflCt). Infernce:** Affected.

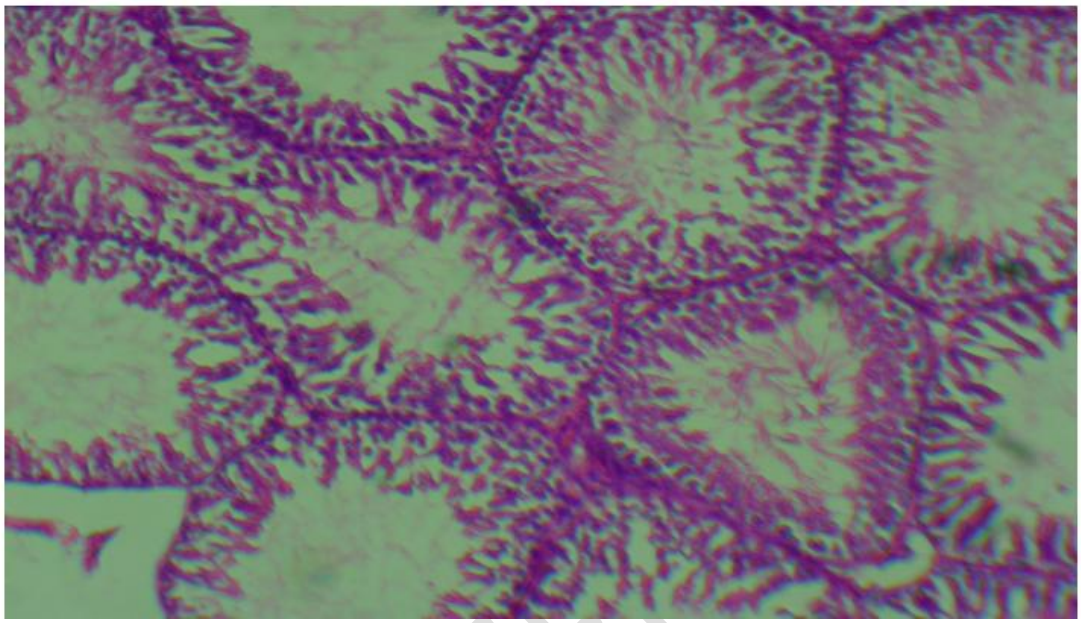

Fig. XV. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 1.30 ml (high dose) of Nutmeg for three weeks followed by 3.0ml (high dose) of N – Hexane extract of *Moringa oleifera* seed for one week. **Group F2** (H&E method, **X100**).

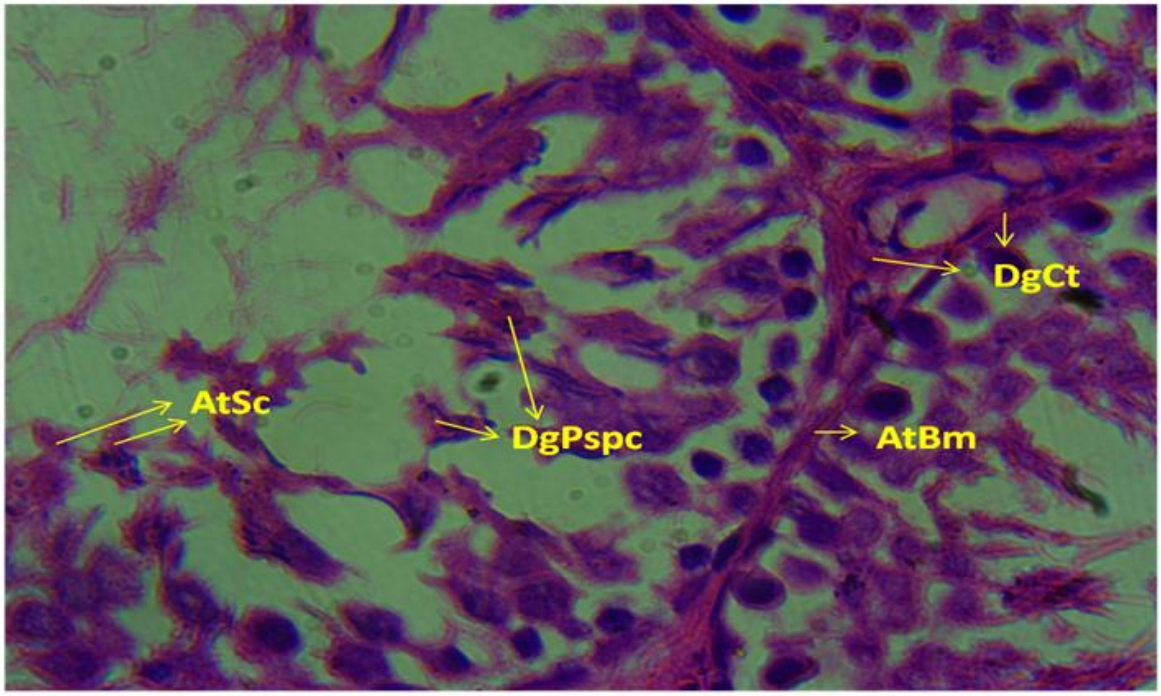

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Fig. XVI. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 1.30 ml (high dose) of Nutmeg for three weeks followed by 3.0 ml (high dose) of N – Hexane extract of *Moringa oleifera* seed for one week. **Group F2** (H&E method, **X400**). Section revealed: Slight Atrophy basement membrane (**AtBm),** Atrophy of Sertoli cells **(AtSc),** Degneration of Connective tissue **(DgCt). Infernce:** Affected.

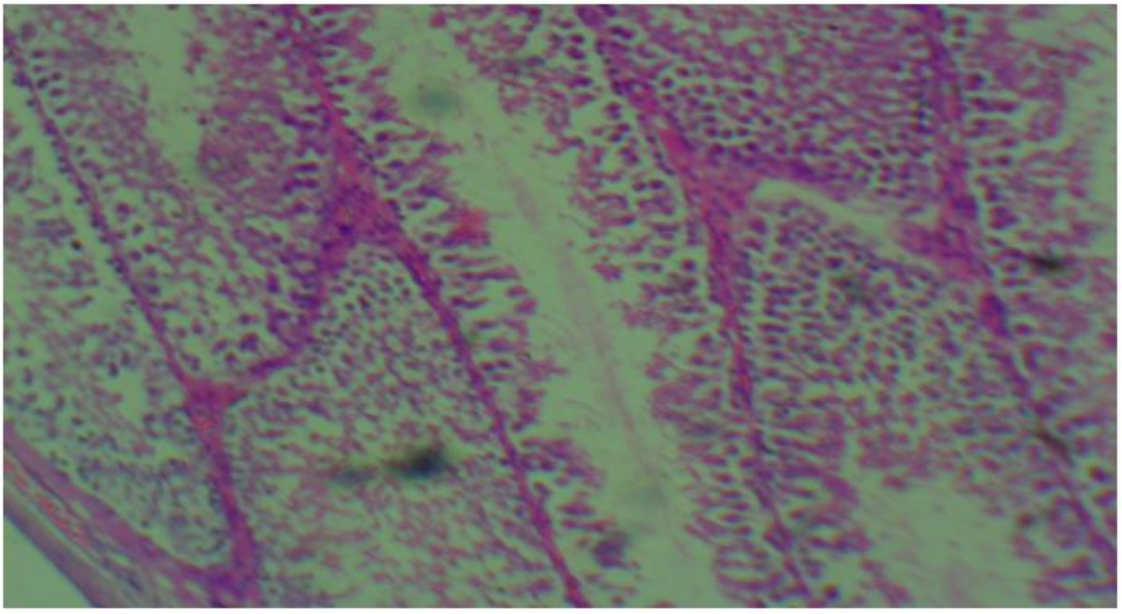

Fig. XVII. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 10 g of Nutmeg via inhalation method for three weeks followed by 2.20 ml of ethanolic extract *Moringa oleifera* seed for one week. **Group G.** (H&E method, **X100**).

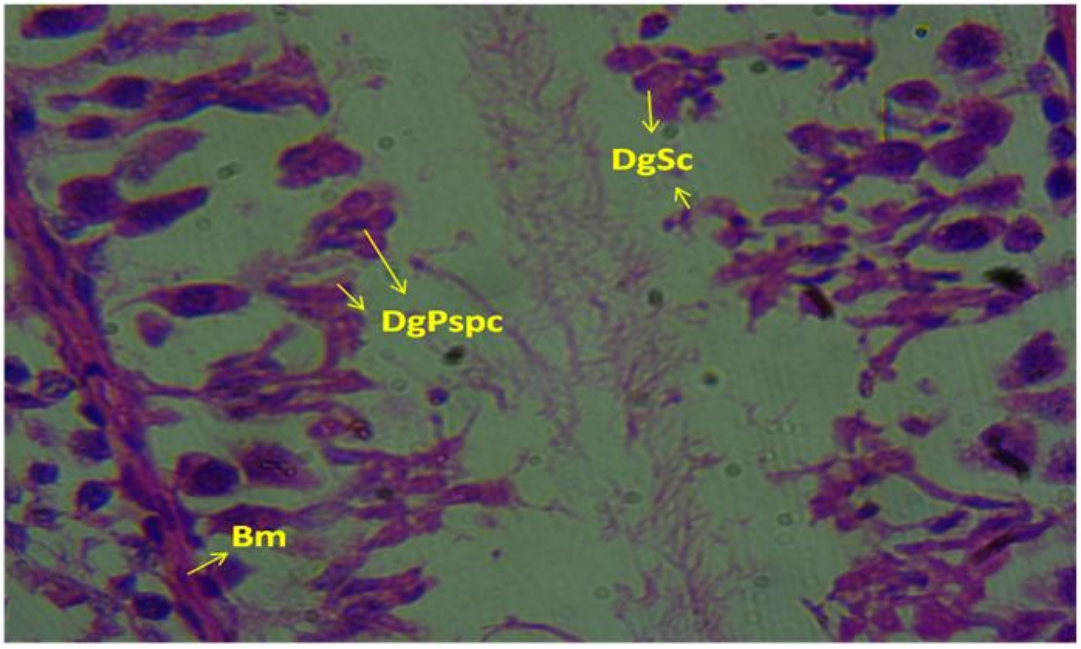

Figure XVIII. A transverse plane through a single seminiferous tubule of the testes of adult male Albino Wistar rats given 10g of Nutmeg via inhalation method for three weeks followed by 2.20 ml of ethanolic extract *Moringa oleifera* seed for one week. **Group G.** (H&E method, **X400**). Section revealed unaffected Atrophy basement membrane (**AtBm),** Degeneration 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, spermagonia and pale (early) spermatogonia, fibromuscular interstitial cells**,** connective tissues and primary

spermatocytes. Animals administered with Nutmeg revealed unaffected testiticular components**,** although a noticeable atrophy of connective tissue, 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.,* 2021[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,* 2014 [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 planet 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 properties of *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 celldifferentiation. *Moringa oleifera* leaves are a good source of carotenoids with pro-vitamin A potential [9].

5. CONCLUSION

Moringa oleifera extracts both showed ameliorative and protective potentials in the testes of Albino Wistar rats induced with Nutmeg toxicity.

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