Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://saudijournals.com/

Histological Assessment of the Testes and Serum Testosterone of Adult Male Albino Wister Rats Following Oral Administration of Ground Nutmeg Seed

Christopher Chiedozie Mbadugha[1*], Ebenezer Reuben Oke[1], Leko Bankole Japhet[2]
[1]Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria
[2]Department of Anatomy, Faculty of Basic Medical Sciences, Madonna University, Elele, Rivers State, Nigeria

Original Research Article

***Corresponding author**
Christopher Chiedozie Mbadugha

Article History
Received: 13.10.2018
Accepted: 21.10.2018
Published: 30.10.2018

DOI:
10.36348/sjmps.2018.v04i10.021

Abstract: This research was designed to assess the effects of oral administration of varying doses of ground Nutmeg seed on the histomorphology of the testes and the hormonal profile of thirty adult male albino wister rats weighing between 140 g and 250 g. The rats were divided into six equal groups, namely A,B,C,D,E and F. Group A served as the control and were administered only water and 100 g of rat feed; while groups B,C,D,E and F, the treatment groups, were administered varying doses (1 g, 5 g, 10 g, 15 g and 20 g/kg body weight, respectively) of the ground Nutmeg seed, mixed with 100 g of rat feed, respectively, for 26 days. The weight measurement was done every seven days. The testes were harvested immediately after sacrifice, fixed in 10 % buffered formalin, and was later made to undergo Hematoxylin and Eosin (H&E) staining method. Blood samples were collected via cardiac puncture for serum testosterone analysis. Data were expressed as Mean ± standard error of the Mean and subjected to one way analysis of variance. Significance different between mean was assessed by student-New-Man-Keuls post hoc test. 95 % level of significance (P = 0.05) was used for statistical analysis and Microsoft Excel 2010 package was used for graph and error bars. The histopathological results revealed no structural defect on the testes of rats administered 1 g and 5 g of ground nutmeg. There was dose dependent alteration of the histomorphology of the testes of rats given 10 g and above. There was also persistent elevation in serum testosterone level in treatment groups B to F, compared to the control Group A. Hence, consumption of high dose of nutmeg is toxic to the testes.

Keywords: Albino Wister Rats, Ground Nutmeg seed, *Myristica fragrans*, Serum Testosterone, Testes.

INTRODUCTION

Nutmeg is the seed of the tree scientifically classified as *Myristica fragrans.* Nutmeg is widely used in cuisines around the world. It has numerous health benefits, which include its ability to relieve pain, soothe indigestion, improve cognitive function https://dx.doi.org/10.1016/j.foodchem.2005.10.058 [1]; detoxify the body, boost skin quality, alleviate oral conditions, and reduce insomnia. Nutmeg strengthens the immune system, prevents leukemia, and improves blood circulation as well. https://www.ncbi.nlm.nih.gov/pubmed/1040 [2].

The extracts of the nutmeg is implicated in the stimulation of mounting behaviour in male mice, and also in significant increase in their mating performance [3]. Evidences abound about its hypnotic, analgesic and hypotensive activities [4]. Pharmacologically and therapeutically, nutmeg seed oil is useful in the treatment of diarrhea and is employed in the management and control of blood pressure [4]. According to the United States of America Food and Drug Administration, nutmeg oil is of low acute toxicity based on results from studies on rodents; and as such accorded it its recognition.

The literature also reports adverse effects associated with the consumption of Nutmeg. Nutmeg ingestion is implicated in acute psychosis and anticholinergic-like episodes with a wide variety of symptoms, characterized by abdominal pain, central nervous system excitation, cutaneous flushing, decreased salivation, fever, nausea, tachycardia, and vomiting, which occurred within 0.5 to 8 hours following ingestion [5-8]. Hepatocarcinomas in mice is associated with safrole, which is a minor component of nutmeg oil [9-11].

The testes, are a pair of ovoid glandular organs that are central to the function of the male reproductive system. Each testis is ovoid in shape, and is covered with the slick, visceral portion of the tunica vaginalis [12]. Weighing 10 g to 15 g each, the testes are suspended outside the body in a fleshy sac called the

scrotum. The scrotum attaches to the body between the base of the penis and anus [13]. In males the testes continually produce sperm that accumulate in the epididymis and vas deferens until they and seminal fluid from accessory glands are ejaculated through the penis. The testes are also responsible for the production of male sex hormone testosterone.

Numerous studies on Nutmeg were carried out on its oil extract. There are lack of studies focusing on the ground Nutmeg seed and its effects on the histology of the testes and serum testosterone level of adult male Albino Wister rats. Hence, the present work is designed to investigate the effects of ground Nutmeg seed on the histology of the testes of adult male albino Wister rat, to evaluate the effects on the body weight, and to determine the effects on the serum testosterone. Routine laboratory method involving H and E staining technique will be used, in addition to light microscopy and immunoenzymatic method with an ELISA reader.

Nutmeg Seed
Source: https://images.google.com

EXPERIMMENTAL SECTION/ MATERIALS AND METHODS

Nutmeg seed was obtained from Itam Market, Itu Local Government Area of Akwa Ibom State, Nigeria. It was identified by a plant taxonomist in the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria and a voucher specimen deposited at the herbarium of the same department. Herbarium No: Oke, UUH 3726 (Malaysia).

The Nutmeg seeds were ground into powder at the Department of Pharmacognosy and Natural Medicines, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria. Out of the total gram of ground nutmeg seed obtained, a given gram was subjected to phytochemical analysis to determine its phytochemical properties. The method of Odebiyi and Sofowora [15] was employed in the analysis.

Thirty adult male albino wister rats weighing between 180 g to 200 g were used for the study. The rats were purchased from the animal house of the Faculty of Basic Medical Sciences, University of Uyo, Nigeria, and were fed standard growers mash feed, produced by grand cereals limited, Nigeria The rats were divided into six equal groups, namely A,B,C,D,E and F. Group A served as the control and were administered only distilled water and 100 g of rat feed; while groups B,C,D,E and F, the treatment groups, were administered varying doses (1 g, 5 g, 10 g, 15 g and 20 g/kg body weight, respectively) of the ground nutmeg seed mixed with 100 g of rat feed, respectively, for 26 days.

The animals were weighed on the 26th day of the experiment and anaesthetized using light ether. The testes were harvested and fixed immediately in 10% buffered formalin for routine histological techniques.

The tissues were stained using Hematoxylin and Eosin; Drury and Wallington, method [16]. Blood samples were collected by cardiac puncture in EDTA bottle and plain bottle for hematological and serum Testosterone assessment. Serum concentration of testosterone was measured following an immunoenzymatic method with an ELISA reader (Merck, Japan), according to the standard protocol given by the National Institute of Health and Family Welfare [17].

This research received a go-ahead-order to be carried out, from the Department of Anatomy Research and Ethics Committee. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

STATISTICAL ANALYSIS

Data were analyzed using descriptive statistical tool, Primer, version 3.0, and were expressed as mean ± standard error of the mean (M±SEM) and subjected to one way analysis of variance. Significant difference between means was assessed by Student - Newman-Keuls post hoc test. 95% level of significance (P = 0.05) was used for the statistical analysis. Microsoft excel 2010 package was used for graphs and error bars.

RESULT

There was increase in body weight of the control group (group A), which is directly proportional to the number weeks involved in the research. However, in the test groups, B to F, the weight did not increase proportionally with the number of weeks rats were fed; instead, there was fluctuating increase and decrease in their body weight, as shown in table-2. Dose dependent marked elevation in serum testosterone level in groups B to F were observed compared to the control group. This was clearly demonstrated in table-3. The outcome of histopathological analysis point to the fact that nutmeg consumption at higher doses above 10 g/kg bw per day is toxic to the testes. These are depicted succinctly in figures 3 to 14.

Table-1: Result of Phytochemical Analysis of Active Ingredients in Nutmeg Seed

Test	Observation	Inference
Alkaloids	Red orange colour precipitate observed	++
Flavonoids	Orange colour precipitate observed	+++
Tannins	Bluish – black colouration observed	++
Saponnins	Persistent frothing observed	++
Cardiac Glycosides		
(1) Lieberman	A green ring observed	+
Salkowski test	A brownish-red ring observed	+
(2) Keller killiani test	Brown ring observed	+

Key: + = slightly present; + + = moderately present; + + + = heavily (strongly) present

Table-2: Comparison of Changes in Body Weight of Albino Rats

Group	Week 1	Week 2	Week 3	Week 4
A	167.50±17.69	185.25±22.73	193.75±23.93*	205.50±26.38*
B	155.00±8.79	164.00±7.84	169.20±9.00	159.60±7.46*
C	192.40±13.92*	201.60±15.44*	191.00±16.97	198.60±18.85
D	148.20±7.13*	154.80±9.77*	153.40±10.77*	160.60±12.70
E	174.25±22.50	185.00±25.09	185.50±24.39	179.67±26.09
F	182.80±11.49	188.40±12.14	178.40±13.57	175.60±13.95
	P = 0.293	P = 0.411	P = 0.591	P = 0.432

Values are expressed as mean ± standard error of mean (M±SEM). 95% level of significance (P = 0.05)

Key: A = rats given distilled water (Control).
B = rats given 1g of nutmeg/ kg bw/day mixed with 100g of feed.
C = rats given 5 g of nutmeg/ kg bw/day mixed with 100g of feed.
D = rats given 10 g of nutmeg/ kg bw/day mixed with 100g of feed.
E = rats given 15 g of nutmeg/ kg bw/day mixed with 100g of feed.
F = rat given 20 g of nutmeg/ kg bw/day mixed with 100g of feed.

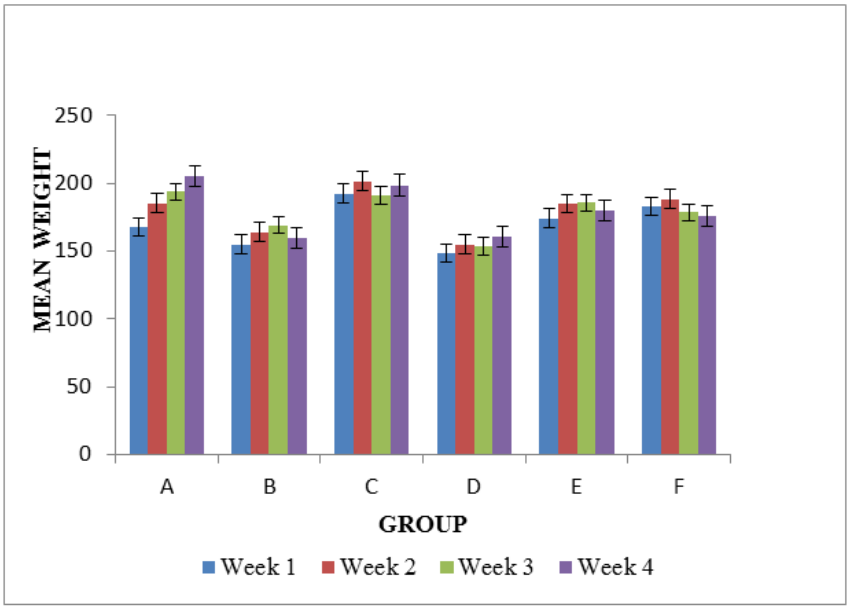

Fig-1: Comparison of changes in body weight of albino rats in Mean and Standard Error of Mean (M±SEM)

Table-3: Comparison of the Effect of Ground Nutmeg seed on the Level of Serum Testosterone

Group	Treatment	Serum testosterone (ng/mL)
A	control (distilled water) for 26 days	0.10
B	1 g of nutmeg/kg bw/day for 26 days.	0.50
C	5 g of nutmeg/kg bw/day for 26 days.	0.62
D	10 g of nutmeg/kg bw/day for 26 days.	0.71
E	15 g of nutmeg/kg bw/day for 26 days.	0.80
F	20 g of nutmeg/kg bw/day for 26 days.	1.03

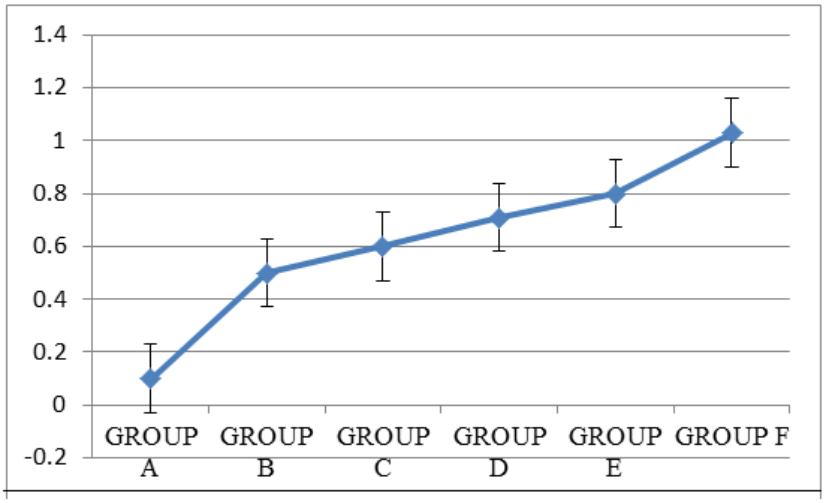

Fig-2: Comparison of serum testosterone level following oral administration of Ground Nutmeg seed

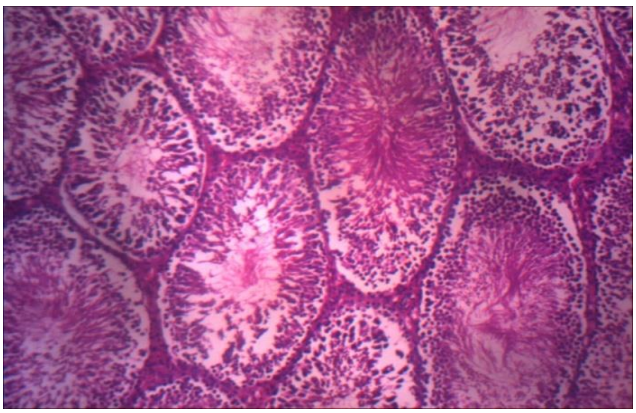

Fig-3: Group A (Control). (H&E method, X100)

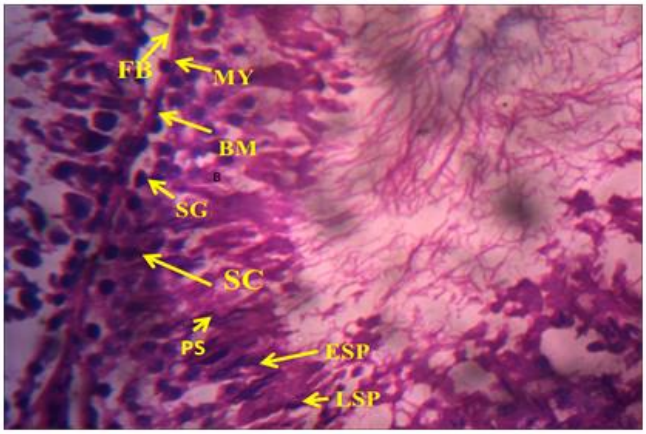

Fig-4: Group A (Control). (H&E method, X400)

Cross section of the testes of rats given distilled water. Section revealed intact, normal histological structure of the testes. fibroblast FB, Myoid cells MY, Basement membrane BM, Spermatagonia SG, Sertoli cell SC, Primary spermatocyte PS, Early spermatids ES, and Late spermatids LS.

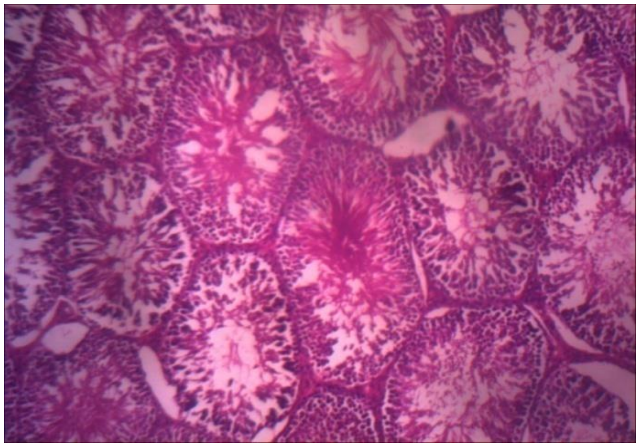

Fig-5: Group B. (H&E method, X100)

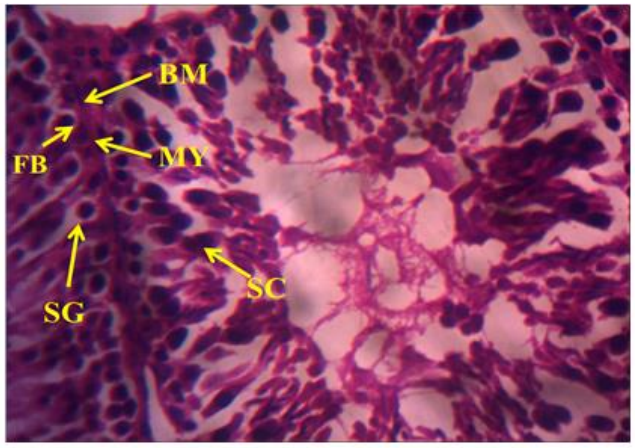

Fig-6: Group B. (H&E method, X400)

Cross section of the testes of rats given 1 g of nutmeg/ kg bw/day mixed with 100g of feed. Section revealed intact, normal histological structure of the testes. fibroblast FB, Myoid cells MY, Basement membrane BM, Spermatagonia SG, Sertoli cell SC, Primary spermatocyte PS, Early spermatids ES, and Late spermatids LS. Inference: not affected by 1 g of nutmeg.

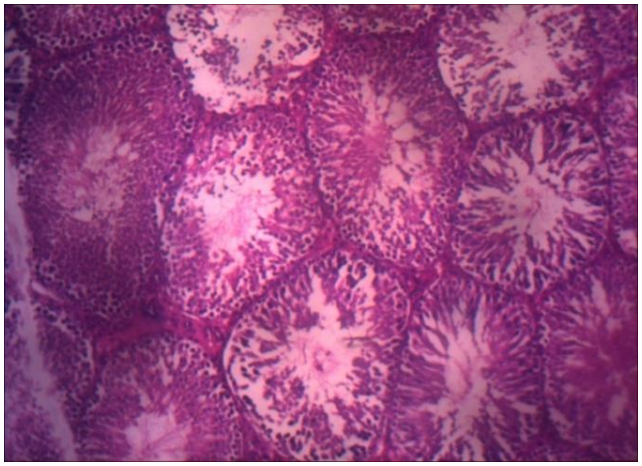

Fig-7: Group C. (H&E method, X100)

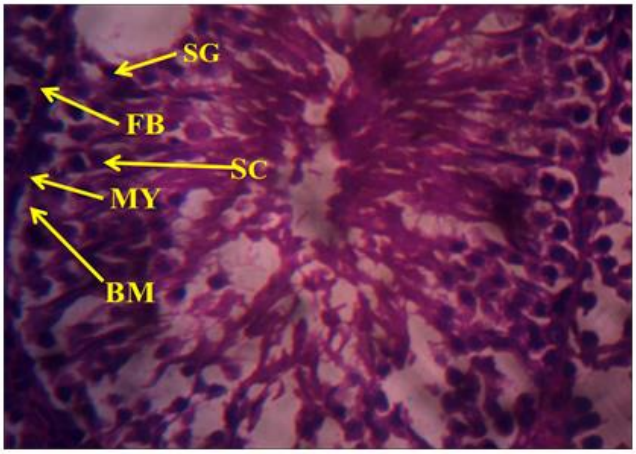

Fig-8: Group C. (H&E method, plate 6 X400)

Cross section of the testes of rats given 5 g of nutmeg/ kg bw/day mixed with 100g of feed. Section revealed intact, normal histological structure of the testes. Fibroblast FB, Myoid cells MY, Basement membrane BM, Spermatagonia SG, Sertoli cell SC, Primaryspermatocyte PS, etc Inference: not affected by 5 g of nutmeg.

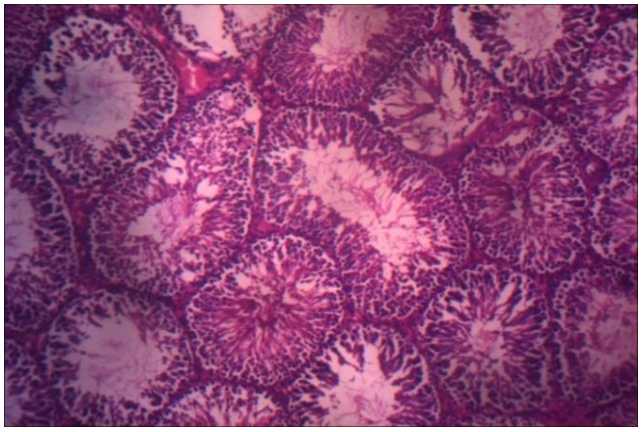

Fig-9: Group D. (H&E method, X100)

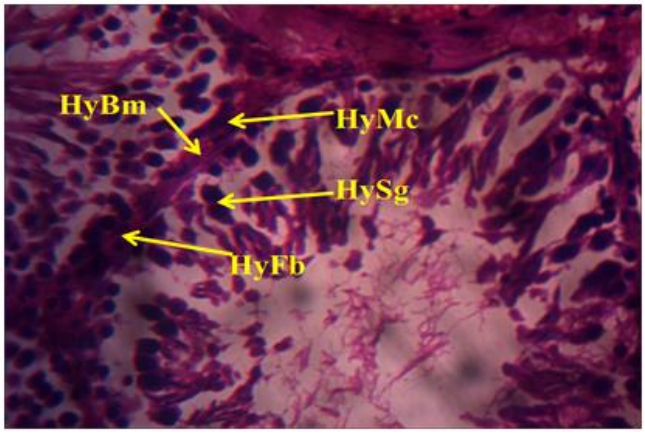

Fig-10: Group D. (H&E method, X400)

Cross section of the testes of rats given 10 g of nutmeg/ kg bw/day mixed with 100g of feed. Section revealed alteration of some histological structures of the testes, namely: hypertrophy of the fibroblast HyFb, hypertrophy of the myoid cells HyMc, Hypertrophy of spermatogenic cells HySp.and hypertrophy of the basement membrane HyBm. Inference: slightly affected.

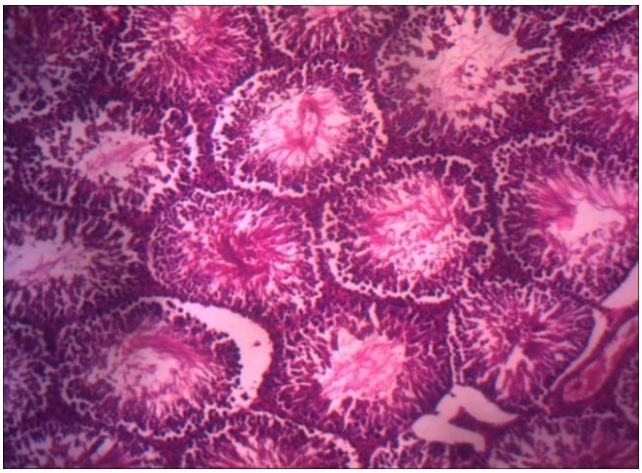

Fig-11: Group E. (H&E method, X100)

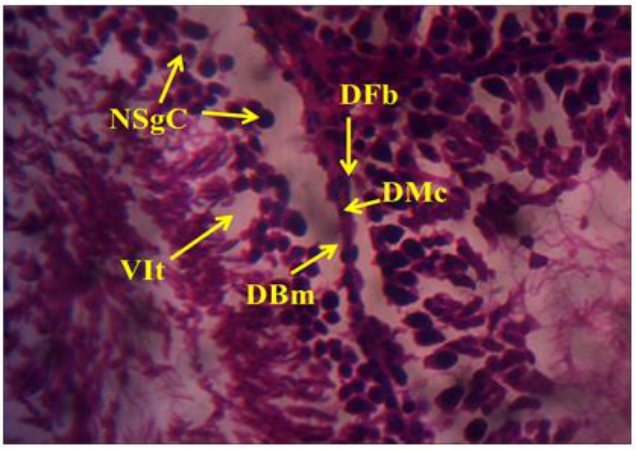

Fig-12: Group E. (H&E method, X400)

Cross section of the testes of rats given 15 g of nutmeg/ kg bw/day mixed with 100g of feed. Section revealed necrosis of the spermatogenic cells NSgc, atrophy of the interstistial tissue AIt, degeneration of the basement membrane DBm, degeneration of the fibroblast DFb, and degeneration of the myoid cells DMc. Inference: severely affected.

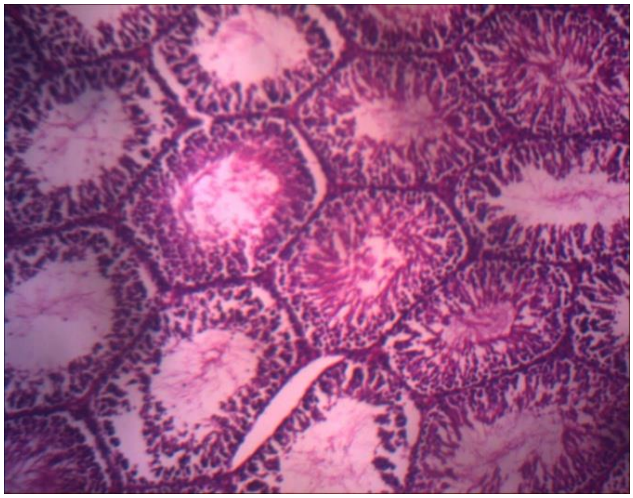

Fig-13: Group F. (H&E method, X100)

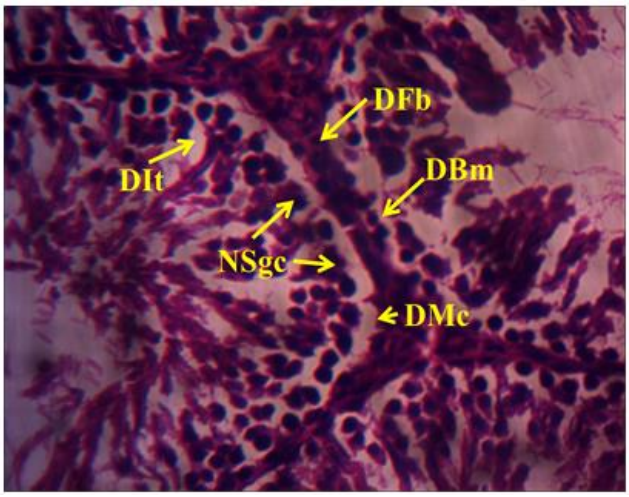

Fig-14: Group F. (H&E method, X400)

Cross section of the testes of rat given 20 g of nutmeg/ kg bw/day mixed with 100g of feed. Section revealed the necrosis of the spermatogenic cells NSgc, Degeneartion of the interstistial tissues VIt, degeneration of the basement membrane DBm, degeneration of the fibroblast DFb, and degeneration of the myoid cells DMc. Inference: severely affected.

DISCUSSION

In this study, we assessed the effects of varying doses of ground nutmeg seed in grams on the histology of the testes and serum testosterone level in adult male albino Wister rats.

Our results suggested that consumption of ground nutmeg seed in high doses, from above 10 g per kg body weight, resulted in testes structural alterations; hence, reduction in the number of spermatogenic cells, myoid cells and fibroblast in the treated groups with higher doses of nutmeg (groups D to F), compared to the control group A and test/experimental groups B and C, with 1 g and 5 g per kg body weight of ground nutmeg seed, respectively. There were several diffuse degeneration and necrosis of spermatogenic cells, the basement membrane, interstitial tissues, myoid cells and fibroblast in the testes of the treated rats in groups D to F, which might have resulted to the observed reduction of these cells and probably contributed to the dose dependent marked elevation in serum testosterone level in the tested/experimental groups. The varying levels of serum testosterone were directly proportional and significantly correlated to the quantity in grams per kg body weight of ground nutmeg seed administered. However, the quantity in grams per kg body weight of ground nutmeg seed administered were inversely proportional to the weights of the rats in grams in all the test/experimental groups (groups B to F), throughout the duration of the test, with the exception of weight result in the week two. There was observed marked increase in weights across and within groups in week two, before the gradual decrease in weight across and within groups in weeks three and four. The factor (s) and the probable mechanism responsible had yet to be understood. On a few occasions the skewed values (indicated with asterisk *) for weight obtained were significant.

The factors and mechanism by which ground nutmeg seed caused degeneration and atrophy in various cellular components of the testes were not clearly understood, hence, was not demonstrated in this research work. Actual cell count by application of stereological procedures, if done in this work, would had thrown more light on the actual numbers of cells viable.

In their experiments, Lee, *et al.*, [18] explored the cytotoxic and apoptotic effects of nutmeg and concluded that Cell viability was reduced by exposure to nutmeg in a dose and time dependent manner. This corroborated our own findings that nutmeg consumption at higher doses above 10 g/kg bw per day is toxic to the testes.

In our study, the effects of varying doses of ground nutmeg seed in grams on the histology of the testes were assessed. The study results tally with the histological findings of Olaleye *et al*., [19] which showed a progressive increase in degeneration of germinal epithelial cells of the testes across the groups with increase in doses. Hence, it suggested that large quantity of this extract when consumed can cause low sperm count, and that low sperm count in itself results from either an impairment in the germinal epithelial cells or in the mature sperm cells; notwithstanding the reported suggestion that nutmeg is capable of improving sexual activities.

Finally, Olaleye, Akinmoladun, and Akindahunsi, reported about observed degeneration of germinal centers of lymphoid cells [19]. They equally drew attention to observed gradual increase in

pathological changes of the spleen of the treated rats ranging from mild to severe lymphoid depletion in the follicles and necrosis of the lymphoid cells depending on the dosage. According to Ryabchikova *et al*., small-sized and degenerated germinal centers of lymphoid cells are indicators of their waning function [20]. Also, Olaleye and colleagues reported that there was degeneration and necrosis of myocardial focal areas in the hearts and degeneration and necrosis of the tubular epithelial cells in the kidneys of the treated animals.

CONCLUSION

Consumption of nutmeg in high doses is injurious to the testes. No doubt nutmeg seed is very important and useful in the preparation of some foods, confectionaries and medicinal drugs; our findings suggest that it should be used in moderation and sparingly. The actual mechanism by which nutmeg induced cellular degeneration observed in this experiment needs further investigation. More research works involving the application of immunohistochemistry and stereology should be carried out on these findings to better understand and appreciate the study.

REFERENCES

1. https://dx.doi.org/10.1016/j.foodchem.2005.10.058
2. https://www.ncbi.nlm.nih.gov/pubmed/1040
3. Ahmad, S., Latif, A., & Qasmi, I. A. (2003). Aphrodisiac activity of 50% ethanolic extracts of Myristica fragrans Houtt.(nutmeg) and Syzygium aromaticum (L) Merr. & Perry.(clove) in male mice: a comparative study. *BMC Complementary and Alternative Medicine*, *3*(1), 6.
4. Grover, J. K., Khandkar, S., Vats, V., Dhunnoo, Y., & Das, D. (2002). Pharmacological studies on Myristica fragrans--antidiarrheal, hypnotic, analgesic and hemodynamic (blood pressure) parameters. *Methods and findings in experimental and clinical pharmacology*, *24*(10), 675-680.
5. Forrester, M. B. (2005). Nutmeg intoxication in Texas, 1998–2004. *Human & experimental toxicology*, *24*(11), 563-566.
6. Sangalli, B. C., Sangalli, B., & Chiang, W. (2000). Toxicology of nutmeg abuse. *Journal of Toxicology: Clinical Toxicology*, *38*(6), 671-678.
7. Demetriades, A. K., Wallman, P. D., McGuiness, A., & Gavalas, M. C. (2005). Low cost, high risk: accidental nutmeg intoxication. *Emergency medicine journal*, *22*(3), 223-225.
8. Kelly, B. D., Gavin, B. E., Clarke, M., Lane, A., & Larkin, C. (2003). Nutmeg and psychosis. *Schizophrenia research*, *60*(1), 95-96.
9. Barceloux, D. G. (2009). Nutmeg (Myristica fragrans Houtt.). *Disease-a-Month*, *55*(6), 373-379.
10. De Vincenzi, M., Silano, M., Stacchini, P., & Scazzocchio, B. (2000). Constituents of aromatic plants: I. Methyleugenol. *Fitoterapia*, *71*(2), 216-221.
11. Miller, E. C., Swanson, A. B., Phillips, D. H., Fletcher, L., Liem, A., & Miller, J. A. (1983). Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. *Cancer research*, *43*(3), 1124-1134.
12. Lindsay, D. T. (1996). Functional Human Anatomy. Missouri, USA. Mosby inc., 789-793.
13. Marieb, E. N., & Mallat, J. (2003). The reproductive system. *Human Anatomy. Benjamin Cummings*, 701-742.
14. Odebiyi, O. O., & Sofowora, E. A. (1978). Phytochemical screening of Nigerian medicinal plants II. *Lloydia*, *41*(3), 234-246.
15. Irobi, O. N., & Daramola, S. O. (1993). Antifungal activities of crude extracts of Mitracarpus villosus (Rubiaceae). *Journal of ethnopharmacology*, *40*(2), 137-140.
16. Drury, R. A., & Wallington, E. A. (1980). Carltons Histological Techniques, 5th edn. New York, Oxford University Press, 206.
17. Srivastava, T. G. (2001). Enzyme linked immunosorbent assay for steroid hormones. *Orientation training course on research methodology in reproductive medicine*, 55-58.
18. Lee, B. K., Kim, J. H., Jung, J. W., Choi, J. W., Han, E. S., Lee, S. H., ... & Ryu, J. H. (2005). Myristicin-induced neurotoxicity in human neuroblastoma SK-N-SH cells. *Toxicology letters*, *157*(1), 49-56.
19. Olaleye, M. T., Akinmoladun, C. A., & Akindahunsi, A. A. (2006). Antioxidant properties of Myristica fragrans (Houtt) and its effect on selected organs of albino rats. *African Journal of Biotechnology*, *5*(15).
20. Ryobehikora, E., & Kolesnikora, L. R. (1996). Viral Biotechnol. *J. Russian Med*. 4: 430-434.