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Morphological and Histological Alterations in the Testes of Wistar Rats Administered Extracts of *Monodora Myristica* Seeds

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ABSTRACT

The use of plant extracts as a fertility enhancer in man is on the increase because of the shifting of attention from synthetic drugs to natural plant products. This work is focused on the effects of *Monodora myristica* seeds on testes of Wistar rats. Twenty Wistar rats were divided into 4 groups. The Control group received distilled water. Three groups represented low, medium, and high doses were given 1500 mg/kg, 3000 mg/kg, and 4500 mg/kg body weight of extracts of *M. myristica* respectively via oral administration for 28 days. The testes were excised, measured, and processed for morphological and histological analysis. Morphological results showed a statistically non-significant difference ($P>0.05$) between the mean testicular weight and dimensions in low and medium doses compared to the control. This was also supported by the normal histological features seen in sections of the testes of rats in these groups. The high dose group showed a statistically significant decrease in the testicular weight, - with shrinking and less dense spermatogenic cells in the seminiferous tubules. This study revealed that *M. myristica* has no detectable damaging effect(s) on the testes at low and medium doses but could improve the cytoarchitecture and enhance fertility. However, its effect can be said to be dose-related and usage should be controlled.

INTRODUCTION

According to the World Health Organization, approximately 80% of the World population currently uses herbal medicines in healing different ailments (WHO, 2001). During the last few decades, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world (Mbuni *et al.,* 2020, Aziz *et al*., 2018, Lev 2006, Gazzaneo *et al*., 2005, Al-Qura'n 2005, Hanazaki *et al.*, 2000, Rossato *et al.*, 1999). There are considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment of various diseases (Azaizeh *et al.,* 2003). Medicinal plants contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowara *et al.,* 2013). The use of plants for medicinal purposes usually in the form of traditional medicine is recognized by the World Health Organization (WHO) as a building block for primary health care (WHO, 2001, Akerele, 1988).

The use of plant extracts as a fertility enhancer in man is now on the increase because of the shifting of attention from synthetic drugs to natural plant products (Dada *et al.*, 2009). *Monodora myristica* (African nutmeg) is a berry belonging to the Annonaceae family, it is one of the most important trees of the evergreen forest of West Africa where it is widely used as a condiment for different delicacies (Nkwocha *et al*., 2019). The presence of alkaloids, saponins, tannins, and flavonoids in the plant makes it possible for the seeds to be used in traditional medicine as well as a spice in local foods (Agiriga and Siwela, 2017, Adewole *et al.,* 2013, Burubai *et al.*, 2009). The aromatic seeds are antiemetic, astringent, anti-inflammatory, antipyretic, aperient, stimulant, stomachic, tonic and they are added to medicines to impart stimulating properties (Bouba *et al.*, 2016; Agomuo, *et al.,* 2014, Adewole, *et al.,* 2013, Chao, *et al.,* 2005; Chao *et al.,* 1997). The seed oil is also useful as a carminative and for scenting perfumes and soaps (Dada *et al.,* 2013, Ekeanyanwu *et al.,* 2010, Ojiako *et al.,* 2010, Burubai *et al.,* 2008). Additionally, the root is munched to mitigate toothaches and arthritis and is also utilized in the management of anaemia, haemorrhoids as well as a sexual weakness (Erukainure *et al.,* 2012). The seed has been reported to contain oil: 22.79g/100g dry matter; protein: 20.79g/100g dry matter and carbohydrate: 44.29g/100g dry matter (Ajayi *et al.,* 2004). The essential oil from the leaves contains *β*-caryophyllene, *α*-humulene and *α*-pinene, while that from the seeds contains *α*-phellandrene, *α*-pinene, myrcene, limonene, and pinene. (Nguefack *et al.,* 2004). It has been reported that oils containing unsaturated fatty acids can be used to lower plasma cholesterol (El-Adawy and Khaled, 2001, Mbofung *et al.,*1994.) The oil of *M. myristica,* because of its high level of unsaturated fatty level is likely able to reduce coronary heart disease if consumed (Njoku, *et al.,* 1996). Akinwunmi *et al.,* (2014) reported that the flavonoid fraction of *M. myristica* seed extracts was observed to be non-toxic in mice with LD_{50} greater than 5000 mg/kg bwt for 28 days. Diminishing sexual performance among the male population though associated with the aging process has always been a matter of great concern to man. Studies have shown that, despite the high prevalence and psychological consequences of the disorder, relatively few men had sought for orthodox treatment prior to the introduction of sildenafil (McKinley *et al.,* 1999). A study conducted to identify potential risk factors for male infertility indicated that there were associations between male infertility and previous exposure to sexually transmitted diseases, unorthodox medication (traditional medication), and moderate to heavy alcohol consumption (Okonofua *et al.,* 2005). *Monodora myristica* is believed to possess aphrodisiac abilities, therefore, concerns on the fact that many of these herbal remedies remain untested and their use which is either poorly monitored or not monitored at all may have adverse effects on male fertility. A recent study reported 34.5% of infertility is found among the male partners of childless marriages (Ekwere, 2007). With the increasing rate of male infertility, it is pertinent to know if there are changes in the morphology and cytoarchitecture of the testes using the seeds of *M. myristica* and its effect on fertility using a rat model.

MATERIALS AND METHODS

Preparation of Extract:

The seeds of *Monodora myristica* were harvested, identified, and authenticated in the Botany Department, University of Calabar,

Calabar, and with Voucher number: BOT/UC/HERB/067. The seeds were de-shelled, air-dried at room temperature and pulverized to obtain a coarse powder which was then used for ethanolic extract preparation. The extraction was carried out in the Endocrine laboratory of the Biochemistry Department, University of Calabar. The Ethical approval number (FAREC/PA/018A50118) was given to carry out the research and all the guidelines of the Ethical Committee were fully followed during the research.

Animal Care and Experimental Design:

Twenty male albino Wistar rats weighing between 170g and 210g bred in the animal house of the Department of Anatomy, University of Calabar was used for this study. The animals were grouped into 4 groups of 5 rats per cage and maintained under standard laboratory conditions. The rats were fed with normal rat chow, and water was provided *ad libitum* throughout the duration of the experiment. The Control group received 0.5 ml of distilled water orally for the same period with the experimental groups. Low, medium, and high dose groups were administered orally with 1500 mg/kg, 3000 mg/kg, and 4500 mg/kg body weights of ethanolic extract of *M. myristica* respectively via oral administration daily for twenty-eight days. The animals were sacrificed a day after the last dose of administration. The testes were excised, measured, and fixed for morphological and histological analysis (using Haematoxylin and Eosin staining technique).

RESULTS

Testicular Weights and Dimensions:

The result showed a non-significant difference (P<0.05) in the average testicular weights of rats in the Low (1.10 ± 0.01) and Medium doses (1.05 ± 0.05) compared to the Control group (1.05 ± 0.05). Whereas, a significant decrease (P<0.05) was observed in the high dose (0.80 ± 0.01) when compared to the different groups (Table 1). Morphologically, (Table 2) there were no statistically significant (P<0.05) differences in the length and width of the testes of rats in both control and treated groups (Figs. 1 & 2). Whereas, the height of the testes in the low and medium dose treated groups (0.80 ± 0.01) was significantly increased (P<0.05), and non-significant increase was observed in the high dose treated group (0.70±0.01) compared to the control group (0.65 ± 0.05) (Fig. 3).

Table 1: Mean Testicular weights of the different groups

Groups	Weight of Testes(g)
Control	1.05±0.05
Low dose (1500mg/kg)	1.10±0.01
Medium dose (3000mg/kg)	1.05±0.05
High dose (4500mg/kg)	0.80±0.01*

Values are expressed as mean ± SEM, n = 5.
*significantly different from control at P<0.05;

Table 2: Testicular dimensions of experimental groups

Groups	Length cm	Width [AP] cm	Height [TR] cm
Control	1.75±0.05	0.75±0.15	0.65±0.05
Low dose (1500mg/kg)	1.70±0.10	0.80±0.00	0.80±0.01*
Medium dose (3000mg/kg)	1.65±0.05	0.80±0.00	0.80±0.01*
High dose (4500mg/kg)	1.65±0.05	0.75±0.05	0.70±0.01[a,b]

Values are expressed as mean ± SEM, n = 5.
*significantly different from Control at $P<0.05$;
a = significantly different from Low dose at $P<0.05$;
b = significantly different from Medium dose at $P<0.05$.
AP=anteroposterior
TR=transverse

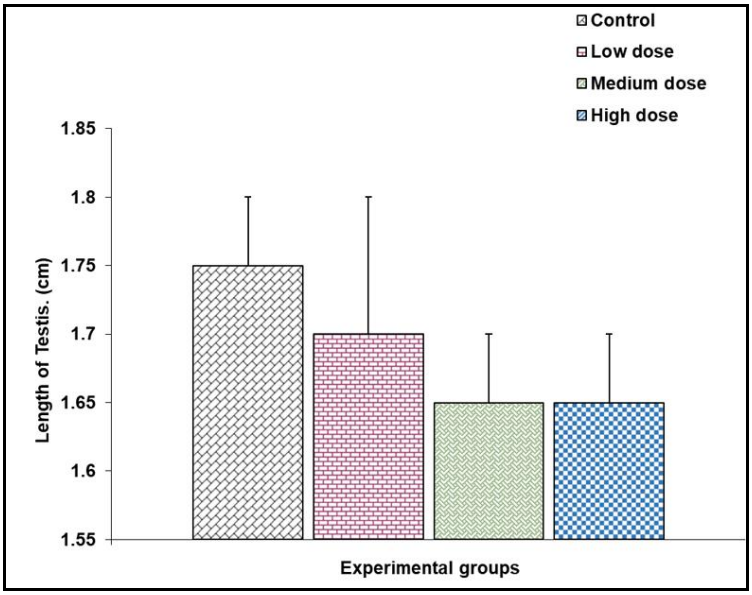

Fig.1: Comparison of length of testis in the different experimental groups
Values are expressed as mean ± SEM, n = 5.
No significant different between groups at $P<0.05$

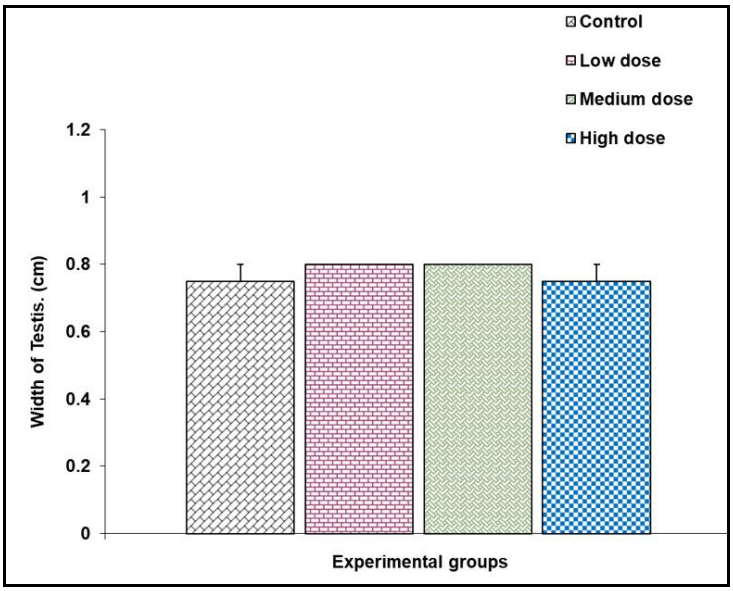

Fig.2: Comparison of width of testis in the different experimental groups
Values are expressed as mean ± SEM, n = 5.
No significant different between groups at $P<0.05$

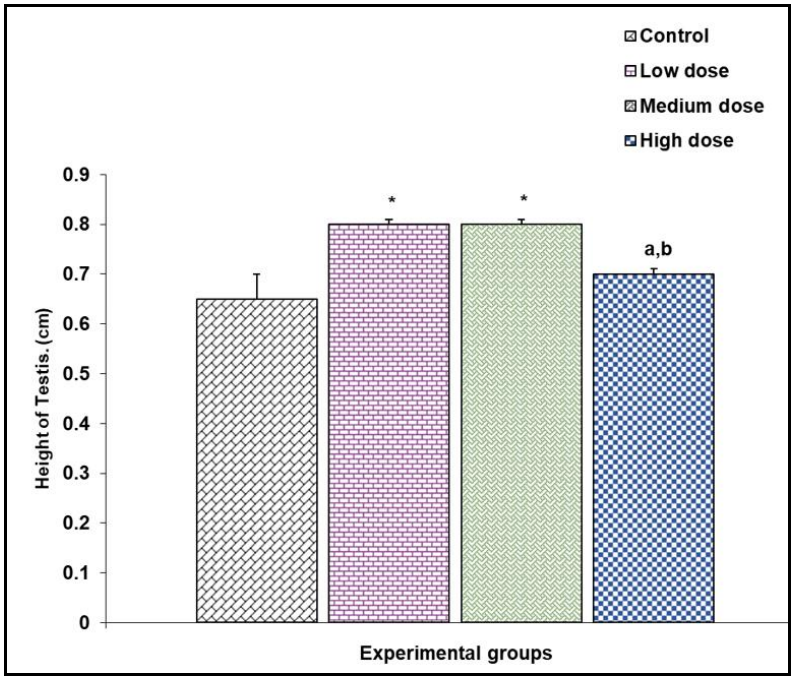

Fig.3: Comparison of height of testis in the different experimental groups
Values are expressed as mean ± SEM, n = 5
*significantly different from Control at $P<0.05$;
a = significantly different from Low dose at $P<0.05$;
b = significantly different from Medium dose at $P<0.05$

Histological Observations:

In the control group, the testes showed normal cytoarchitecture with seminiferous tubules, spermatogonia at various stages of maturation (Fig. 4a), Leydig and Sertoli cells, and intact basement membrane (Fig. 4b). The Low dose group showed closely packed seminiferous tubules with spermatogonia at various stages of maturation. A rich interstitial connective tissue was also observed (Fig. 5a). The cells within the seminiferous tubules are moderately populated and are thick with deeply stained nuclei and abundant Sertoli cells. The early series spermatogonia cells located close to the basement membrane are densely populated and the late series are scanty consisting mainly of spermatid with sparsely populated spermatozoa (Fig. 5b). The medium dose group showed seminiferous tubules are densely packed with spermatogonia at various stages of maturation (Fig. 6a). The interstitial connective tissue containing Leydig cells was richer than those of the low dose. The cells within the seminiferous tubules are also thick with deeply stained nuclei and Sertoli cells (Fig. 6b). The high dose group showed loosely scattered and less dense early spermatogonia cells close to the basement membrane while the spermatids and spermatozoa population are reduced compared to the other groups (Fig.7a). The late series consisting of the spermatid and spermatozoa are also scanty and not as densely packed as those of the control and other treated groups (Fig. 7b).

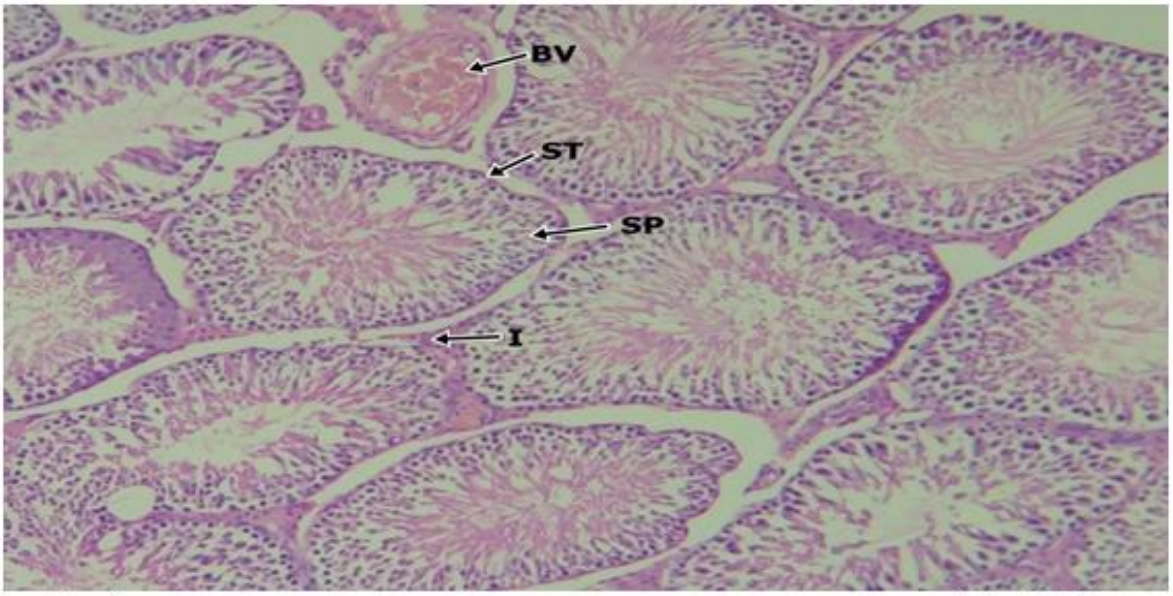

Fig. 4a H&E X100

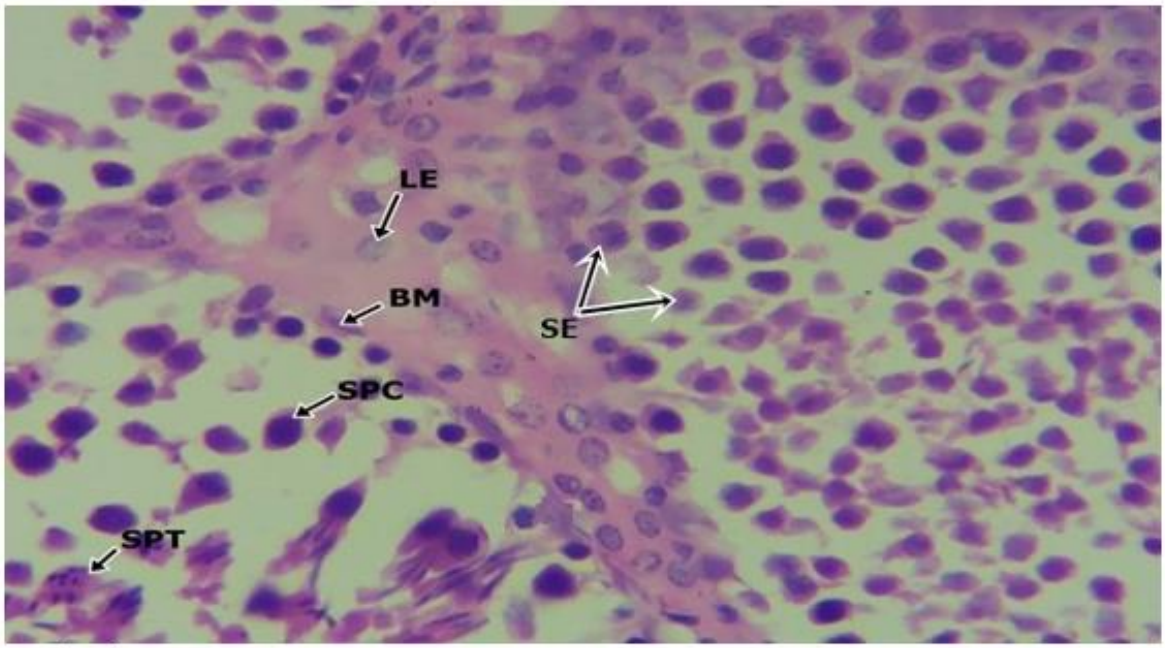

Fig. 4b H&E X400

Fig. 4a&b: Photomicrograph showing the histological section of the testis of the adult male Wistar rat control group showing closely packed seminiferous tubules with abundant spermatogenic and Sertoli cells. **BV**- Blood Vessel, **ST**- Seminiferous Tubule, **I**- Interstitium, **BM**- Basement Membrane, **LE**- Leydig Cell**, SP**- Spermatogonia, **SPC**- Spermatocyte, **SE**- Sertoli Cell, **SPT**- Spermatid

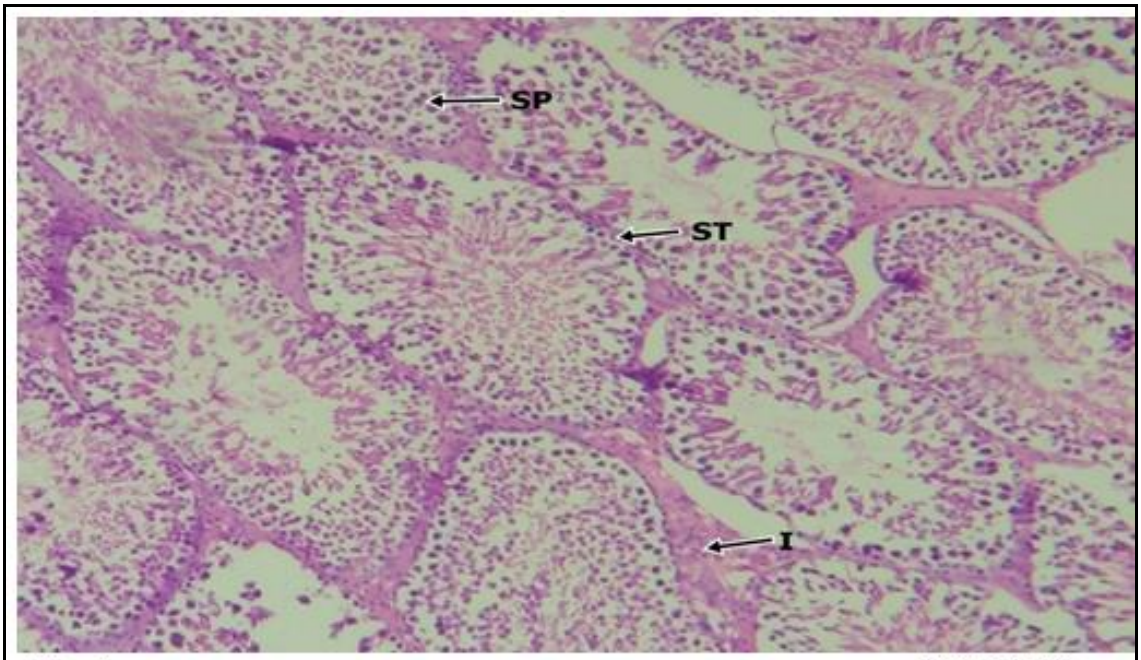

Fig. 5a H&E X100

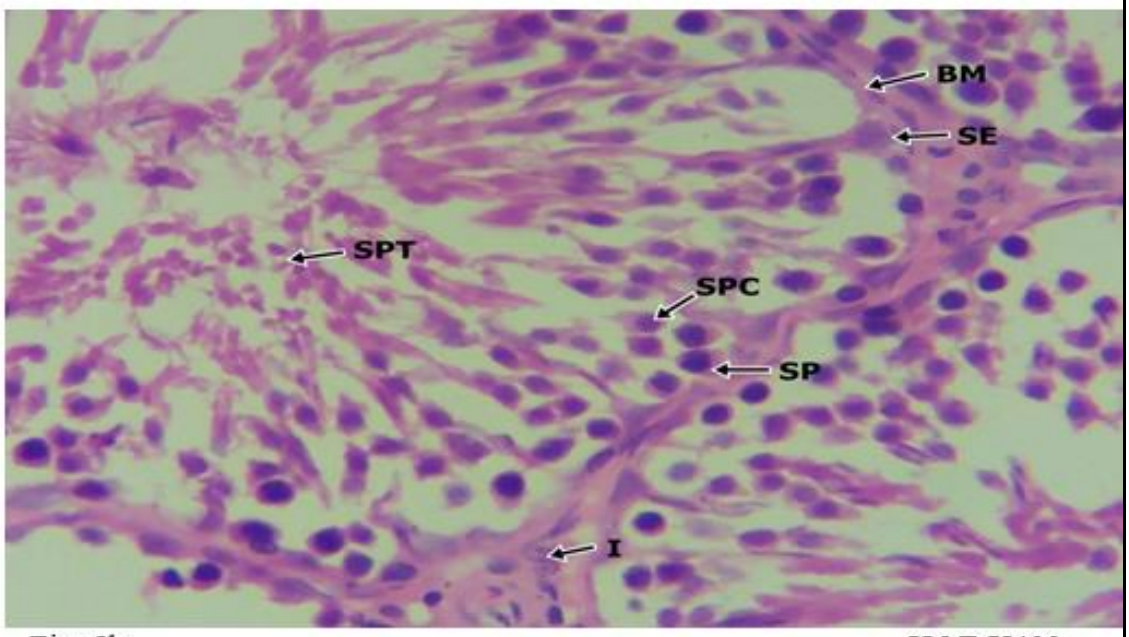

Fig. 5b H&E X400

Fig. 5a&b: Photomicrographs of testis of Low Dose Group rats showing a rich interstitial connective tissue containing Leydig cells. **BM**- Basement Membrane**, ST**- Seminiferous Tubule, **SE**- Sertoli Cell**, I**- Interstitium containing Leydig cells**, SP**- Spermatogonia**, SPC**- Spermatocyte, **SPT**- Spermatid

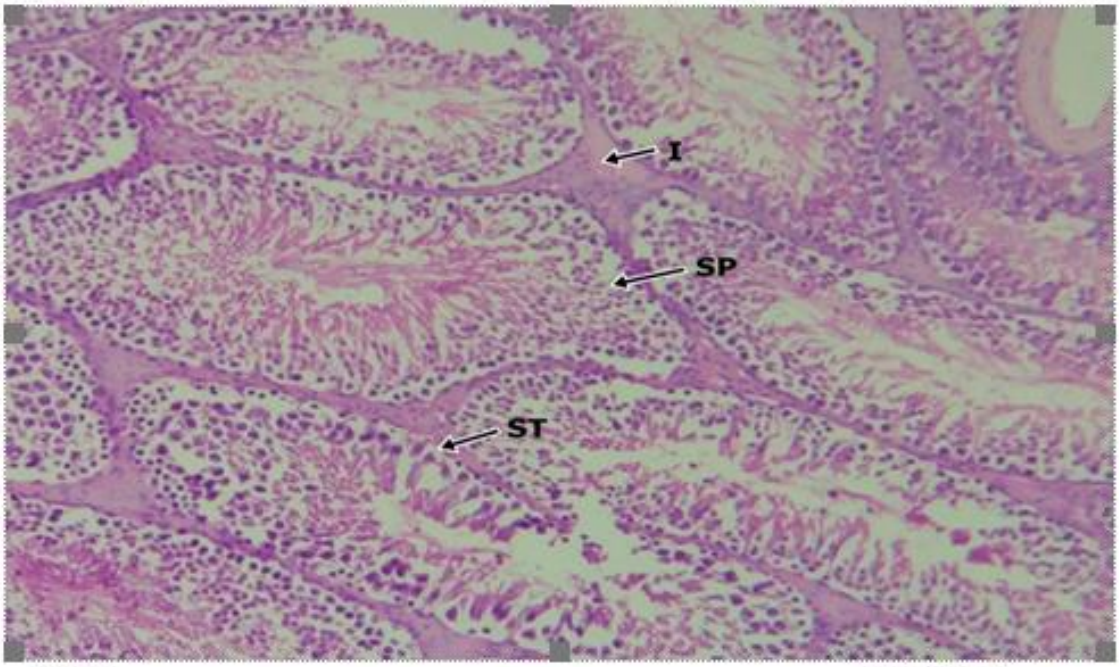

Fig. 6a H&E X100

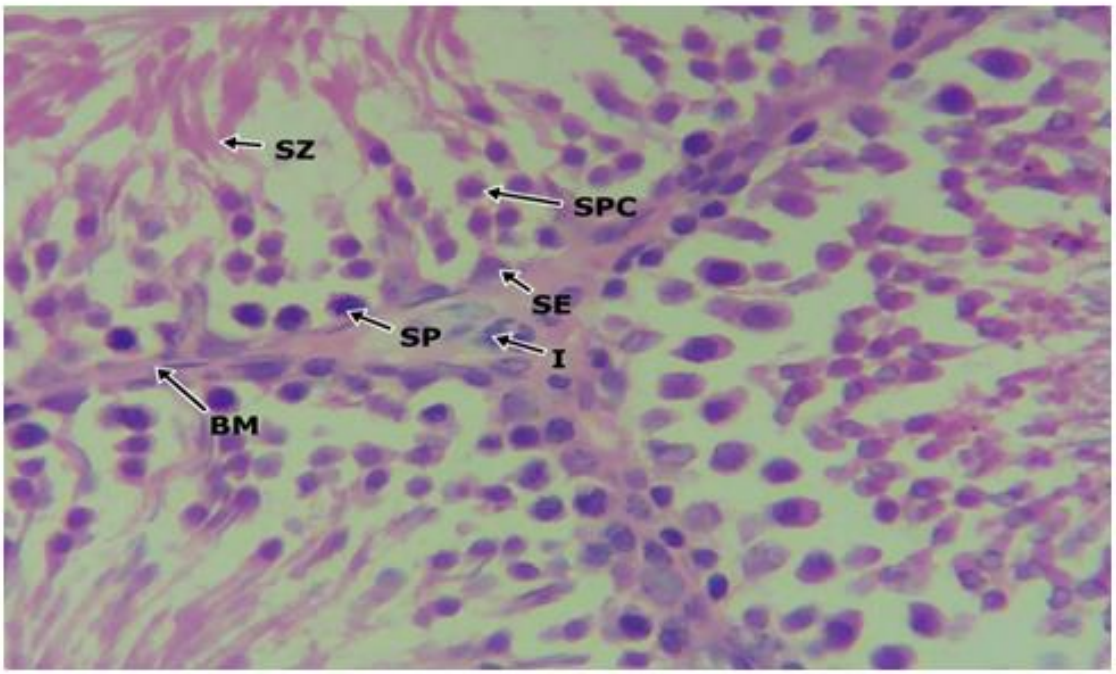

Fig. 6b H&E X400

Fig.6a&b: Photomicrographs of testis of Medium Dose Group of rats showing a rich interstitial connective tissue containing Leydig cells and densely packed seminiferous tubules. **BM-** Basement Membrane, **ST-** Seminiferous Tubule, **I-**Interstitium containing Leydig cells, **SE-** Sertoli Cell, **SP-**Spermatogonia **SPC-** Spermatocyte, **SZ-** Spermatozoa

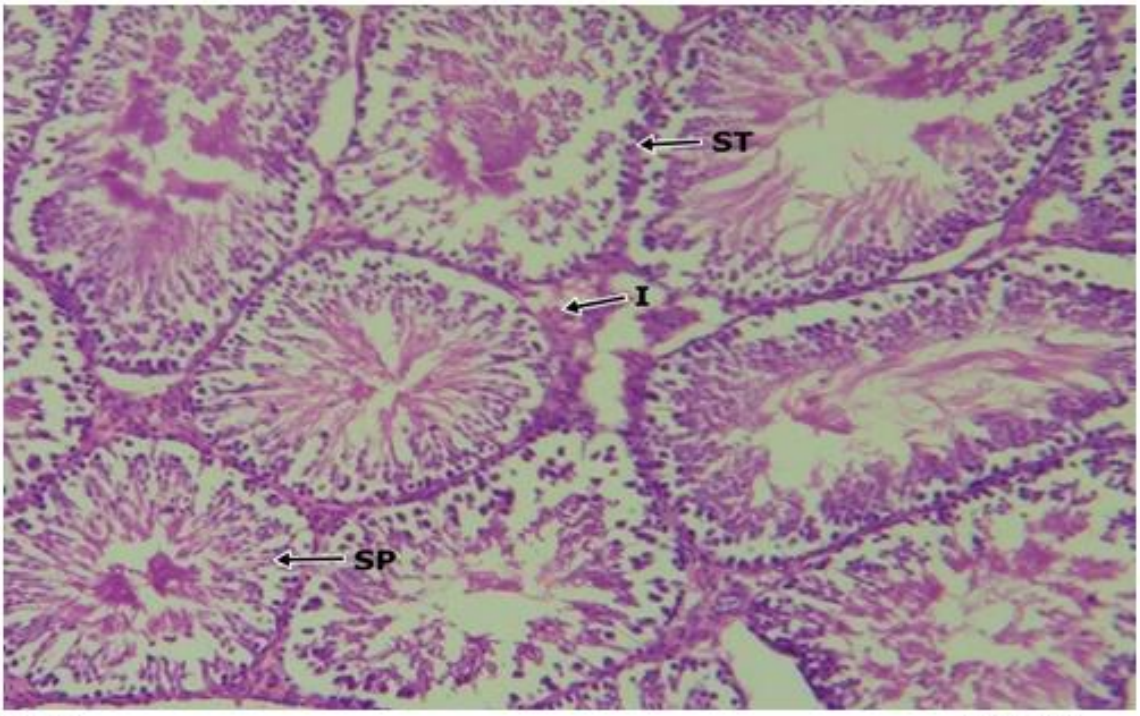

Fig 7a H&E 100

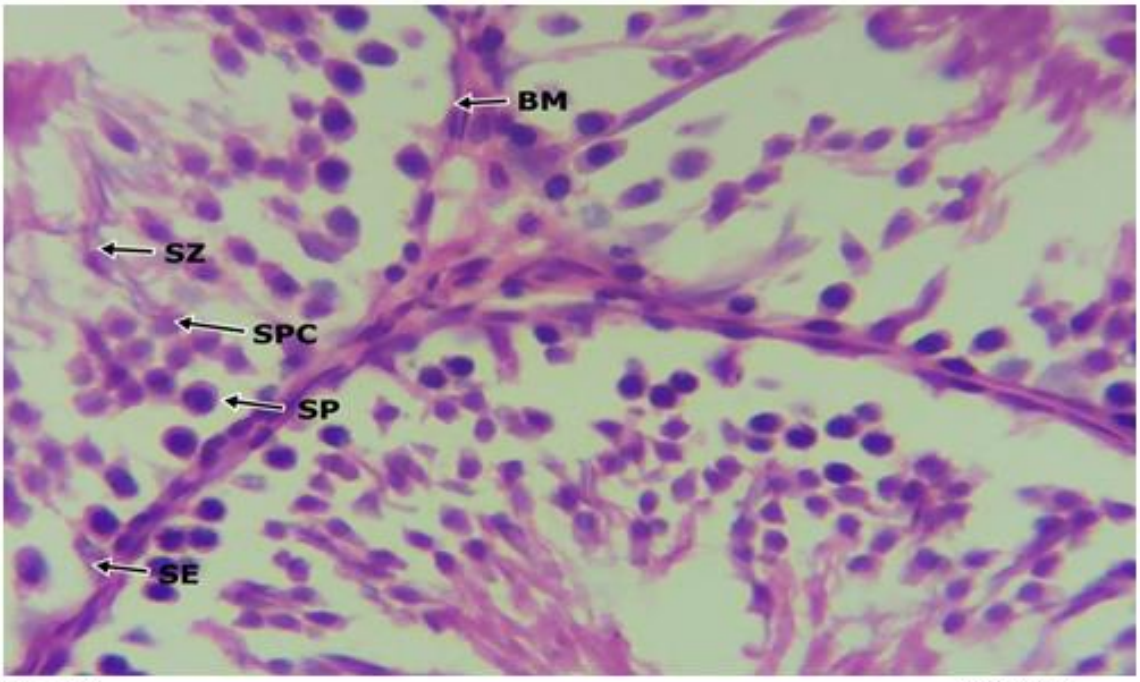

Fig. 7b H&E 400

Fig.7a&b: Photomicrographs of testis treated of High Dose Group rats showing loosely scattered and less dense early spermatogonia cells close to the basement membrane while the spermatids and spermatozoa population are reduced compared to the other groups. **BM-** Basement Membrane, **I-**Interstitium containing Leydig cells, **ST-** Seminiferous Tubule, **SE-** Sertoli Cell, **SP-**Spermatogonia, **SPC-** Spermatocyte, **SZ-** Spermatozoa

DISCUSSION

The morphological result obtained showed there was no significant difference (P>0.05) between the mean testicular weight and dimensions of rats in the Low and Medium dose groups that received 1500 mg/kg and 3000 mg/kg body weight of ethanolic extract of *M. myristica* respectively compared to the control. This was also supported by the normal histological features seen in the histological sections of the testes of the rats in the low and medium dose groups. This is in-line with the findings by Akinwunmi *et al.,* (2014) which reported no adverse effect on the liver, brain, kidney, and lungs of rabbits following administration of extracts of *M. myristica*. However, a slight increase in the mean testicular weight in the low dose group and denser interstitial connective tissue were observed in the low and medium dose groups compared to the high dose group which is suggestive of a positive effect of this extract on the testes. In a recent study carried out by Okonko *et al.,* (2019) on sperm profile and testicular weight assessment of albino rats, the result for sperm profile analysis revealed that *M. myristica* extract increased sperm motility, viability and count significantly (P<0.05) and dose-dependently. Erukainure *et al.* (2012) reported *M. myristica* exhibited high antioxidant activities in vitro, signifying the protective potential of the spice against free radicals. This report is also supported by Akinwunmi & Oyedapo (2015). The phytochemicals, phenolic and flavonoids, in particular, may be responsible for these effective antioxidant properties (Moukette, *et al*., 2015, Akinwumi *et al*., 2014, Adewole, *et al*., 2013). This increase in spermatogenic cells could be due to the ability of *M. myristica* to trigger the production of hormones responsible for spermatogenesis (Okonko *et al.,* 2019). A dose-related effect of *M. myristica* extract was also observed in this study as the high dose group rats (4500 mg/kg) which showed a significant decrease in the testicular weight, seminiferous tubules appeared shrunken and conspicuous intercellular spaces within them with less dense spermatogenic cells. Etuk and Muhammad (2009), reported increases in the intercellular spaces within the seminiferous tubules to suggest the degeneration of the germinal epithelium and consequent reduction of spermatozoa in the lumen of the seminiferous tubules.

Conclusion

The present investigation has shown that *M. myristica* has no detectable damaging effect(s) on the morphology and histology of the testes at low and medium doses but rather could improve the cytoarchitecture of the testes, a pointer that it has aphrodisiac properties and could enhance fertility. However, consumption of high doses over a prolonged period of time may result in testicular damage thereby causing infertility.

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