International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Testicular Weight and Histology following Administration of Ethanolic Leaf Extract of *Lophira lanceolata* to Wistar Rats

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Abstract: ***This research work is focused on the effects of Lophira lanceolata leaves on testicular weight and histology of wistar rats. Twenty adult male wistar rats were divided into four groups of five rats each. Group A served as control group and was administered normal saline; Group B, C and D was treated with 100mg/kg, 200mg/kg and 300mg/kg body weight of Lophira lanceolata leaf extract respectively for seven days. The rats were sacrificed a day after seven days and the testes were excised weighed and preserved in Neutral buffered formalin for histological processing using haematoxylin and eosin staining method. The results showed the control group had normal histological features such as seminiferous tubules (ST) of various sizes with an intact basement membrane and containing spermatogonia cells at various stages of maturation. The cells have thick layers with numerous spermatozoa within their lumen (L). The spermatogonia cells include the spermatogonia A and B, primary spermatocytes (S1), secondary spermatocytes (S2) and spermatids (S3). The cells have prominent oval to round deeply stained basophilic nuclei and thin rim of cytoplasm. Leydig cells lie within the interstitium (I). Experimental group B and C also showed normal histological features but treated group D showed increased intercellular space between somniferous tubules indicating a loss of Leydig cells and hence decrease in spermatogenesis. Morphological result obtained showed decrease in the weight of the testes in the group B and C and weight gain in group D. The extract produced a significant and dose dependent increase (P<0.05) in the weight of the testes.***

Keywords: Testicular weight, testicular histology, *Lophira lanceolata*

1. Introduction

Many medicinal plant have been used in modern medicine where they occupy a very significant place as raw materials for important drugs. *Lophira Lanceolata* is a multipurpose herb commonly found in the west and central Africa, including Northern states in Nigeria (Abdulahi *et al*., 2003). Different part of this plant like the roots, leaves, stem, barks, and fruits have many medicinal uses.

Ethno botanical uses of plant include treating toothache in Cameroon, liver infection in Togo, female sterility, and cough in Nigeria (Ghogomu *et al*., 1967). A decoction of the fresh leaves is used for the treatment of cough, abdominal pain, diarrhea, cardiovascular disease, and hypertension. It is also said to be used for the treatment of menstrual pain (Blessing, 2009). The plant stem bark is commonly used by herbalist for the treatment of fertility related problem in male (Etuk and Mohammed, 2009). Studies on the methanolic extract of L. lanceolata leaves showed a significant effect in decreasing the transient hypertension caused by adrenaline (Koukou et al., 2013). Studies have also shown that the methanolic extract of lophira lanceolata leaves has an anti-diarrhea and antispasmodic activity (Nneka et al., 2015). Studies have also shown that the aqueous stem bark extract of l. lanceaolata possesses some activity constituents that have anti-hetotoxic potentials (Patrick et al., 2014). A research was conducted on the nephro-protective effect of aqueous extract of L. Canceolata leaves in albino coistar rat poisoned with gentamcin. Histological analysis showed that the aqueous extract of Cophira Canceolata leaves reduced the nephrotoxic effects of gentamicin (Doughon et al., 2015). Lophira canceolata extract is widely used to enhance performance among male population in Sokoto state. A study was conducted to evaluate the safety of oral administration of the plant extract in dawley rats. Result showed that the single dose of the extract did not cause any death or adverse effect. In the repeated dose study, the extract produced an increase in body weight gain of the rat but a high dose of this extract may damage the testes, thereby leading to infertility (Etuk Mohammed, 2009).

2. Material and Methods

Twenty (20) male albino Wistar rats were grouped and housed in cages with 5 animals per cage and maintained under standard laboratory condition at a temperature of 25± 3℃ with dark and light (12/12 hrs) and well ventilated. They were allowed to acclimatize to the laboratory condition for two weeks before proceeding with administration in the animal house of Human Anatomy Department University of Calabar. They were fed with standard animal pillet obtained from vital feed (Nig) and free access to water throughout the period. The animals in the Group A (control) received 0.5ml of 10% normal saline while Group B, C and D the low, medium and high dose respectively, received oral administration of 100, 200, 300mg/kg body weights of ethanolic leaf extract of *lophira lanceolata* respectively. The total duration of treatment with lophira lanceolata leaves was seven days. The animals were weighed after the administration of the extract. The animal sacrifice was done a day after the last dose of administration was carried out. The rats were anesthetized with a chloroform vapor. The testes were accessed removed and fixed in Neutral buffered formalin for histological analysis.

3. Results and Observation

Morphological Observation

Table 1: Showing mean body weights (initial and final) in the different experimental group.

Groups	*Initial Body Weight (g)*	*Final Body Weight (g)*
Control	215.24 ± 12.42	223.88 ± 11.43
Low Dose	208.86 ± 18.23	197.26 ± 17.34
Medium Dose	226.12 ± 5.81	215.48 ± 10.68
High Dose	239.06 ± 10.11	254.00 ± 10.19

Values are expressed as Mean ± SEM, n=5

Body weight changes in all groups showed insignificant differences between initial and final at $p<0.05$

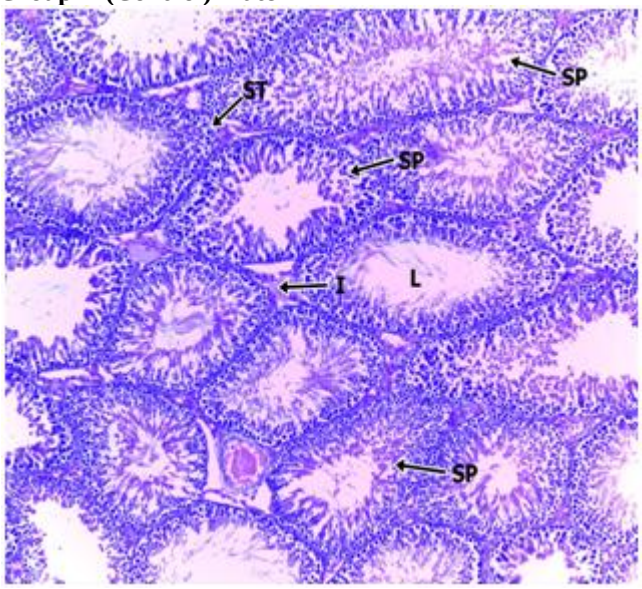
Table 2: Showing the Mean weights of the testes in different experimental groups.

Groups	*Weight of Testes (g)*
Control	2.58 ± 0.04
Low Dose	2.60 ± 0.08
Medium Dose	2.50 ± 0.04
High Dose	2.82 ± 0.03 ^{*,a,b}

Values are expressed as Mean ± SEM, n=5.
Weight changes in all groups were compared at 95% confidence level.
*Significantly different from control at $p<0.05$
a. significant different from low dose value at $p<0.05$
b. significantly different from medium value at $p<0.05$

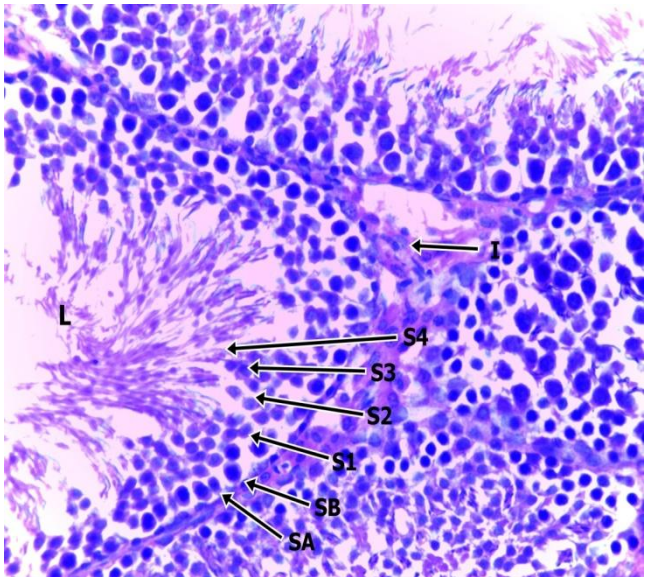
4. Histological Observation

Group A (Control) Plate I



TESTIS X100

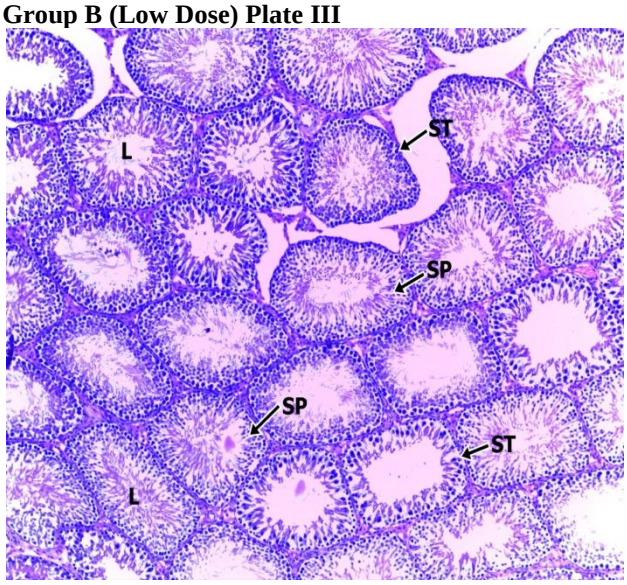
Plate II



Testis X400

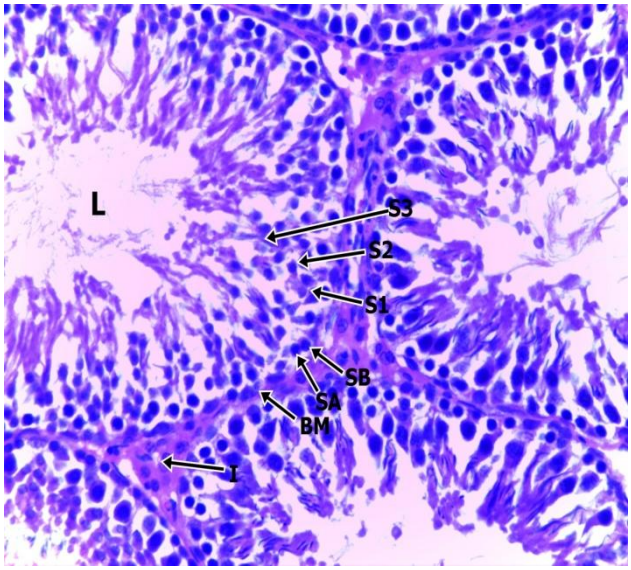
Section of the testis shows seminiferous tubules (ST) of various sizes with intact basement membrane and containing spermatogonia cells at various stages of maturation. The cells have thick layers with numerous spermatozoa within their lumen (L). The spermatogonia cells include the spermatogonia A and B, primary spermatocytes (S1), secondary spermatocytes (S2) and spermatids (S3). The cells have prominent oval to round deeply stained basophilic nuclei and thin rim of cytoplasm. Leydig cells lie within the interstitium (I)

Group B (Low Dose) Plate III



Testis X100

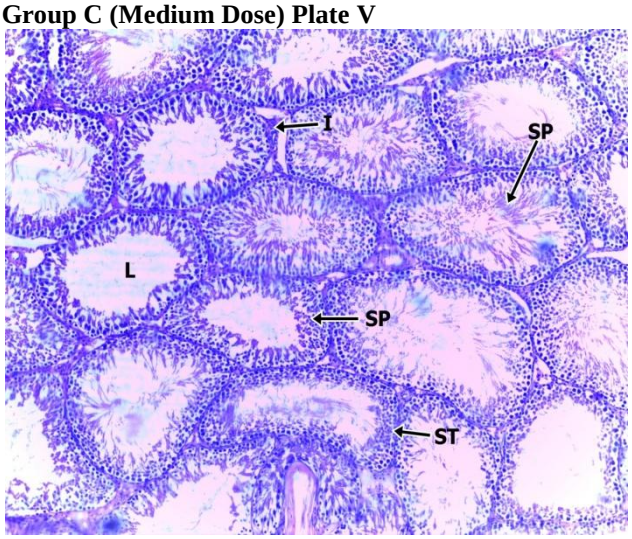
Plate IV



TESTIS X400

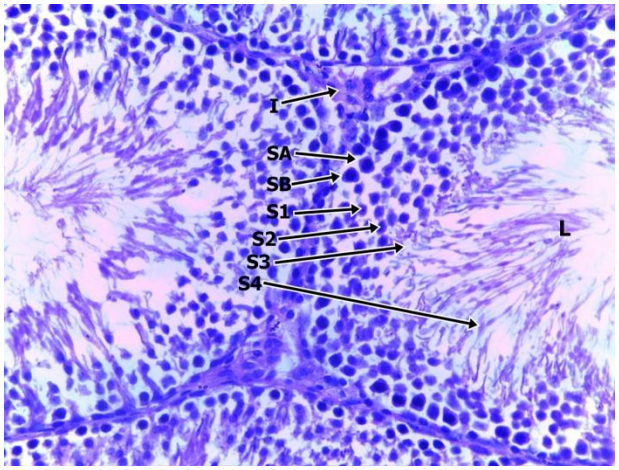
Section of the testis shows prominent seminiferous tubules with intact basement membrane (BM), containing proliferating spermatogonia cells at various stages of maturation. The cells have thick layers with deeply stained round to oval nuclei and a thin rim of cytoplasm. The supporting sertoli cells are intact. The spermatogonia includes the spermatogonia A and B closed to the germinal epithelium, the primary spermatocytes (S1), secondary spermatocyte(S2) and the spermatids(S3). Their lumen (L) contains scanty spermatozoa.

Group C (Medium Dose) Plate V



Testis X100

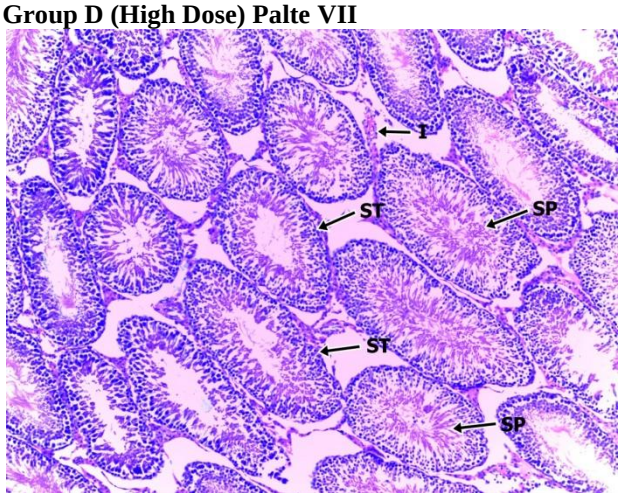
Plate VI



TESTIS X400

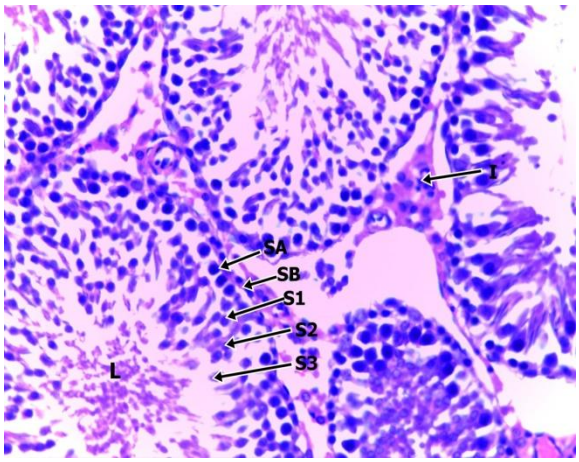
Section of the testis shows prominent seminiferous tubules with intact basement membrane (BM), containing proliferating spermatogonia cells at various stages of maturation. The spermatogonia are of 3 to 5 cell layer thick with deeply stained round to oval nuclei and a thin rim of cytoplasm. The supporting sertoli cells are intact. The intervening interstitium contains clusters of round leydigcells. Their lumen contains numerous spermatozoa. No destruction of the basement, spermatogonia cells seen.

Group D (High Dose) Palte VII



Testis X100

Plate VIII



TESTIS X400

Section of the testis shows prominent seminiferous tubules of various sizes and shape with intact basement membrane, containing proliferating spermatogonia with intact supporting sertoli cells. Their lumen contains numerous spermatozoa and the intervening interstitium contains Leydig cells and blood vessels. However there are more intercellular spaces, seminiferous tubules are smaller suggesting a reduction in spermatozoa.

5. Discussion

The effect of the ethanolic extract of *Lophira lanceolata* leaves on the histology and morphology of the testes was investigated. From this research it was observed that experimental group B, C and D which received 100 and 200 mg/kg body weight of Lophira lanceolata leaves extract respectively showed normal histological features of the testes such as seminiferous tubules (ST) containing spermatogenic cells (SP) at various stages of maturation resting on an intact basement membrane, the seminiferous tubules (ST) have thick layers with numerous spermatozoa within their lumen (L) and Leydig cells lie within the interstitium (I). However, it was observed that the seminiferous tubules in experimental group D were shrunken and intercellular spaces were prominent suggesting reduction in spermatozoa in this group. The increase in the intercellular space suggest the degeneration of the seminiferous epithelium and consequent reduction of spermatozoa in the lumen of the seminiferous tubules in this group (Etuk and Mohammed, 2009).

Morphological result obtained showed weight loss in experimental group B and C, but weight gain in experimental group D (high dose) when compared to the control. The extract also produced a significant and dose dependent increase ($p<0.05$) in the weight of the testes. Literature has shown that repeated administration of the extract at high dose causes increase in body weight of the experimental animal (Etuk and Mohammed, 2009). The histological appearance of the experimental animals in group D showed prominent intercellular space suggests a reduction in spermatozoa within the lumen. Morphologically, it was observed that there was an increase in body weight of the control group and experimental group D while experimental group B and C showed weight losses. The extract produced a significant and dose dependent increase ($p<0.05$) in the weight of the testes.

6. Conclusion

Lophira Lanceolata leaves extract may be relatively safe as shown from the result of this study. The extract is associated with increase in body weight, but repeated administration of high dose of the extract may result in testicular damage thereby causing infertility.

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