CORE
Metadata, citation and similar papers at core.ac.uk
Provided by International Institute for Science, Technology and Education (IISTE): E-Journals

Advances in Life Science and Technology
ISSN 2224-7181 (Paper) ISSN 2225-062X (Online)
Vol 14, 2013

Chronic Consumption of Abelmoschus Esculentus and Piper Guineense Induce Testicular-Toxicity in Wistar Rats, Histopathological Finding.

Idorenyin Umoh, Samson Oyebadejo, Enobong Bassey and Uduakobong Uko
Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Akwa-Ibom, Nigeria.
P.M.B. 1017, Akwa-Ibom, Nigeria.
* Correspondence Author E-mail: samsonoyebadejo@yahoo.com

Abstract

Histopathology of the Testes is one of the parameters used in assessing its micro-structural integrity. In this study, the effect of the oral chronic consumption of 500mg/kg of Abelmoschus esculentus and 20mg/kg of piper guineense on the Testes of wistar rats was assessed. Twenty adult wistar male rats weighing (123-207g), divided into four groups 1, II, III &1V, group 1 as control and groups II, III &IV as experimental groups. The rats in the control group were administered with distilled water, while rats in group II and III were administered with 500mg/kg of Abelmoschus esculentus and 20mg/kg of piper guineense respectively. Group IV received a combination of the two extracts. After 28days of administration of extracts, animals were sacrificed Testes was extracted and processed to paraffin section, cut at 5micron, stained, and observed histopathologically under light microscope. Result showed numerous atrophied and damaged seminiferous tubules, degenerated myoid cells, spermatogenic lining cells, spermatogonia, spermatocytes, spermatids, spermatozoa and lumen filled with semen, degenerated interstitial cells of leydig and interstitial fibrosis against the background of connective tissues with marked area of necrosis in group II and III and IV as compared to the control group 1. Statistical value in the weight of the body and testes showed significant value ($p<0.05$) compared to control.
In conclusion, *Abelmoschus esculentus* and *Piper guineense* has severe toxicity effect on the testes of albino wistar rats.
Keywords: *Abelmoschus esculentus*, *piper guineense*, Histopathology, Testes and wistar rat.

1.0 Introduction

The consumption of leafy vegetables is part of Africa's cultural heritage. The nutrient content of different types of vegetables varies considerably and they are the major sources of vitamins, essential amino acid, minerals and antioxidants (Fasuyi, 2006). Vegetables are included in meals mainly for their nutritional value. Abelmoschus esculentus is a popular health food due to its high fiber, calcium, potassium, vitamin C and foliate content. It also contains cytopropenoid fatty acids. It is often eaten for weight loss since it is fat and cholesterol free (Duvauchelle, 2011). It also possesses ethanomedical potentials and it us used as an antioxidant and in the treatment of urinary tract infection. It is also used for prevention and treatment of gastric acidity and duodenal ulcer.
Piper guineense contributes to the iodine content in the diet of the rural and urban dwellers. It has a high content of iodine among all vegetables (Ujoroundu *et al.*, 2011). Piper guineense is a spice used in seasoning food. It's high protein content and the presence of alkaloid makes it a supplement for daily protein requirement of the body and as an antimalarial remedy (Ekanem *et al.*, 2000). The seeds are consumed by women after childbirth to enhance uterine contraction for the extrusion of placenta and other remains from the womb (Udoh *et al*,, 1999). It is also used for the control of weight and as an adjuvant in the treatment of rheumatic pains.
A study on the effect of fruit extracts of *Abelmoschus esculentus* has shown that it causes infertility in male wistar rats (Malini, 2009). Another research carried out by (Atawodi, 2003), it was observed that ethanolic extracts of *Abelmoschus esculentus* produced a reversible reaction in male fertility in rats and a significant reduction in gross sperm motility. Study carried out by (Gbile, 2006) reported that *Abelmoschus esculentus* reduces the mean weight of the testis and it is supported by histological studies which showed testicular atrophy.
The fruit of *Abelmoschus esculentus* is used for the treatment of sexually transmitted disease like gonorrhoea and other urinary problems such as painful urination. (Lengsfeld, 2004) studied the effects of extracts of young fruit of *Abelmoschus esculentus* on the adhesiveness of helicobacter pylori to gastric mucosa, he found out that pre-treatment of the bacteria with *Abelmoschus esculentus* inhibited bacteria adhesion most completely.
In a study carried out by (Ogbuewu, 2009), it was observed that animals treated with ethanolic extracts of *Piper guineense* shown an increase in mean body weight of the animals. Extracts of *Piper guineense* has also been reported by (Reifer, 1995) to damage germ cells and seminiferous tubules when administered orally to male wistar rats.

The testis is round in nature; it is surrounded by a thick collagenous fiber known as tunica albuginea. In the posterior part of the testis, the connective tissue of the albuginea expands into a thick mass that project into the substance of the testis. The projection is known as the mediastinum testis.
From the mediastinum testis, there is a septa arising and entering into 250 compartments called testicular lobules and each of the lobules contained coiled structure known as semniferous tubule (Singh, 2004). The tunica vaginalis is the outermost covering of the testis. The testis develops from the posterior abdominal wall and as it descends, it drags along the peritoneal sac which forms a sac known as tunica vaginalis. The tunica vaginalis has an outer parietal layer and inner visceral layer (Singh, 2004). The inner part of the seminiferous tubule has so many cells known as the myoid cells. The spermatogenic lineage cells are that precursors of spermatozoa. The spermatogenic cells of the testis support and nourish the sperm and also play a role in phagocytosis (Singh, 2004). . In this study, the histopathological changes of the testicular tissue (testicular-toxicity) associated with chronic oral exposure of Abelmoschus esculentus and piper guineense were assessed in albino wistar rats.

2.0 Materials and methods

2.1 Drugs and Chemicals

Sodium chloride, formaldehyde, sodium trioxocarbonade V, sodium bicarbornate, xylene, 70% alcohol, 90% alcohol, absolute alcohol, haematoxylin, eosin, egg albumin, distilled water, paraffin wax were all procured from BDH Chemicals, England. All other chemicals were of analytical grade.

2.2 Animals

20 wistar rats (123-207g) were obtained from the University of Uyo animal house. They were maintained on standard pellets (guinea feed) and water *ad libitum.* Permission and approval for animal studies were obtained from the college of health sciences animal ethnics committee, University of Uyo.

2.3 Sourcing of Plant material

Freshly fruit of Abelmoschus esculentus and piper guineense were obtained in July, 2012 from Itam market, Uyo, Akwa Ibom State, Nigeria. The plant was identified and authenticated by the Botanist in the Department of Botany, University of Uyo, Uyo, Nigeria.

2.4 Preparation of Extract.

Abelmoschus esculentus was chopped and air dried. *Piper guineese* (seeds) was also air dried and after being dried they weighed 600g for *Abelmoschus esculentus* and 800g for *Piper guineense*. They were then macerated in 97% ethanol (SIGMA CO., UK) in a flat bottom flask and were kept for 72hrs at room temperature. At the end of 72hrs it was filtered. The filtrates were concentrated in water bath at 45 degree Celsius. The concentrated extract was preserved in refrigerator till commencement of research.
The weight of the extracts was 40.25g for *Abelmoschus esculentus* and 24g for *Piper guineense.*

2.5 Acute Toxicity testing.

The acute toxicity of *Abelmoschus esculentus* and *Piper guineense* on Wistar Albino rats were determined in two (2) stages for the two extracts.
For *Abelmeschus esculentus*, in stage one animals received 1000, 2000, 3000, 4000 and 5000mg/kg body weight while in stage two, animals received 2300, 2400, 2500, 2600, 2700mg/kg body weight.
And in acute toxicity of *Piper guineense the same two stages was observed,* in stage one; animals received 10, 50, 100, 200, 300 mg/kg body weight. Stage 2; received 85, 90, 95, 100, 105 mg/kg body weight.
All experimental animals were observed for physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, body limb and death within 24hours. The extract was administered intraperitoneally (i.p). The LD50 was found to be 2500mg/kg for *Albemoschus esculentus* and 100mg/kg for *Piper guineense* .
According to the modified lorke's method.500mg/kg and 20mg/kg per body weight were calculated respectively as middle doses for the *Albemoschus esculentus* and *Piper guineense*. Doses were considered as stock solution, they were calculated further using 20 mls of distilled water for *Albemoschus esculentus* and 10 mls of distilled water for *Piper guineense* to obtain working solution.

2.6 Experimental Design

Matured 20 albino wistar male rats weighing between 123g-207g were obtained from the faculty of Basic Medical Sciences Experimental Research Animal House of the University of Uyo, Uyo Nigeria. They were fed with standard laboratory diet and water ad libitum. Illumination was 12h light /dark cycle and room temperature was 25±2^{o}C. The animals were divided into four groups, one control (I) and three experimental groups (II, III

and IV), which consisted of 10 normal abino wistar rats per per group. The control group was given distilled water while the experimental group II, III and IV were exposed daily to 500mg/kg body weight of Abelmoschus esculentus alone, 20 mg/kg of body weight piper guinenese alone and 500 mg/kg of Abelmoschus esculentus combined with 20 mg/kg of piper gineense respectively by oral administration for 28 days. In this study, all the animals' experimentations were carried out following the guidelines for the care and use of laboratory animals obtained from the institutional animal ethics committee.

2.7 Sample collection and Histopathological analysis.

Twenty four hours after last exposure, the animal were anesthetized with chloroform vapour and dissected. The harvested testes were carefully dissected out, trimmed of all fat and connective tissue blotted dry to remove any blood. The tissues were fixed in 10% formal saline, and then transferred to a graded series of ethanol. On day 1, they were placed in 70% alcohol for 7 hours, then transferred to 90% alcohol and left in the latter overnight. On day 2, the tissues were passed through three changes of absolute alcohol for an hour each then cleared in xylene. Once cleared, the tissues were infiltrated in molten paraffin wax in the oven at 58°C. Three changes of molten paraffin wax at one-hour intervals were made, after which the tissues were embedded in wax and blocked out. Prior to embedding, it was ensured that the mounted sections to be cut by the rotary microtome were orientated perpendicularly to the long axis of the kidney, liver and pancreas. The sections were designated "vertical sections". Serial sections of 5 μm thick were obtained from a solid block of tissue, fixed on clean albuminized slides to prevent sections coming off the slides and later stained with Haematoxylin and Eosin staining techniques, after which they were passed through ascending grade of alcohol, cleared in xylene and mount in DPX mountant, allowed to dry at room temperature and observed Histopathologically under digital light microscope.

2.8 Gross morphometrical analysis

The initial and final weight of the rats and the weight of the Testes in each group were taken using the weighing balance. The values of all the morphometric analysis were compared statistically using SPSS 17 Software.

2.9 Photomicrography

Records of the Histological and histochemical results were obtained by photomicrography using digital photomicrographic microscope at the Gross Anatomy Research Laboratory, Department of Human Anatomy, College of Health sciences, University of Uyo,Uyo,Akwa- Ibom, Nigeria as illustrated in Plate 1 to 5.

3.0 Results

3.1 Statistical Analysis result.

All result were analyzed using one way ANOVA and using SPSS version 17.P-values of less than 0.05 were considered statistically significant.

3.2 Histopathological findings

Plate 1 Control group of Testes showed normal numerous seminiferous tubules containing myoid lining cells, spermatogenic lining cells, spermatogonia, spermatocytes, spermatids, spermatozoa and lumen filled with semen, in between the seminiferuos tubules are the interstitial cells of leydig, and interstitium against background of connective tissues.

Plate 2 Group II: Testes treated with *Abelmochus esculentus-500mg/kg for 28 days* show numerous atrophied and damaged seminiferous tubules distortion, degenerated myoid cells, spermatogenic lining cells, spermatogonia, spermatocytes, spermatids, spermatozoa and lumen filled with semen, degenerated interstitial cells of leydig and interstitial fibrosis against the background of connective tissues with marked area of necrosis as compared to the control group.

Plate 3 Group III: Testes treated with *Piper guineense*-20mg/kg for 28 days showed numerous atrophied and damaged seminiferous tubules distortion, degenerated myoid cells, spermatogenic lining cells degeneration, degenerated interstitial cells of leydyg and interstitial fibrosis against the background of connective tissues with marked area of necrosis as compared to the control group.

Plate 4 Group IV: Testes treated with a Combination of *Abesmochus esculentus and Pipper guineense*-for 28days showed numerous atrophied and damaged seminiferous tubules, degenerated myoid cells, spermatogenic

lining cell lining clumping, tubular necrosis, atrophy and degenerated interstitial cells of leydig and interstitium against background of connective tissues with marked area of necrosis as compared to the control group
Finally, the result of the microscopic examination showed histopathological damage to the Testicular tissues of rats exposed to Abelmoschus esculentus and piper guineense, compared to the tissues from the control rats (plate 1), the testes of rats in the test groups were observed to have developed seminiferous tubules distortion, degenerated myoid cells, spermatogenic lining cells, spermatogonia, spermatocytes, spermatids, spermatozoa and lumen filled with semen, degenerated interstitial cells of leydig and interstitial fibrosis rats of each group (plate 2-4). This indicated chronic consumption of to Abelmoschus esculentus and piper guineense may induce cellular alterations of normal Testes. Cytoarchitecture, distorting the functional integrity of the testicular tissues. The observations made from the tissue microscopic analysis, indicated testicular-toxicity induced by chronic consumption of Abelmoschus esculentus and piper guineense in healthy rats.

4.0 Discussion

This study investigated the effect of Abelmoschus esculentus and piper guineense on the Testicular structure in rat model. In assessing the testicular-toxicity effects of these extracts, the histopathology of the testicular tissues were examined. This study showed a significant increase in weight of animals and a significant decrease in organ weight in each group. The decrease in the weight of the testes of the experimental groups may have occurred as a result of tubular necrosis and loss of cytoplasmic constituent of the seminiferous tubule. Significant distortions in the architectural integrity of the spermatogenic lining cell structural status and the interstitium were observed. Specifically, the cytostructure of the tubules, degeneration with damaged myoid cell, migration of the polymorms, interstitial odema and fibrosis. The results of this study therefore provide a clear indication that Abelmoschus esculentus and piper guineense contain some chemical substances with testiculo-toxic potential. The specific chemical constituents and mechanisms responsible for the testicular-toxicity effect reported in this study are not clear. It may be assumed that the reactive metabolites of Abelmoschus esculentus and piper guineense constituents could have interacted with the testicular injury in testicular structure and this could impair the reproductive function of the testes. The combination of the two extracts showed severe testicular-toxicity effects on the testes, indicating that in combination their effect potentiates each other. The interaction of these metabolites with the testicular tissues may be responsible for cellular injury and subsequent damage to the tissues. However functionality of the testes in terms of function and structure may be compromised due to damage of the testicular tissues.

5.0 Conclussion

Results obtained in this study show that exposure to Abelmoschus esculentus and piper guineense induced adverse and detrimental effects on the testicular function and structure at microscopic level in rat model. These observations indicated that exposure to Abelmoschus esculentus at doses as high as 500mg/kg body weight and piper guineense at doses of 20mg/kg body weight and above is a risk factor for testicular function impairment and the associated disorders revealing high level of cellular distortion in all experimental groups. Further study is recommended with isolated components of these extracts at various concentration of extract of Abelmoschus esulentus and piper guineense to confirm the underlying mechanism and active constituents responsible for the observed activity documented by the results of this study.

6.0 Fundings

This research received no specific grant from any funding agency in the public, commercial, or not – for profit sectors.

ACKNOWLEDGEMENT

We wish to acknowledge Miss Osibajo Kehinde Adefowope, computer scientist for her help in the computer typesetting of the research work.

References

Atawodi, S.E., Bulus,T., Ibrahim,S., Ameh, D.A. (2003). In vitro trypanocidal effect of methanol extract of some Nigeria savannah plants. *African Journal of Biotechnology* 2(9): 317-321.

Duvauchelle,J.,(2011);"OkraNutritionInformation".LiveStrong.com. http://www.livestrong.com/article/371351-okra-nutrition-information/.Retrieved 24 June 2012.

Ekanem, A., Obiekezie, A. (2000). Antiparasitic effects of leaf extract of piper guineense on the jurveules of hetorobranchus longitilis (cuvier and valencieness). Afr. J. Fisher Aguacult, 8:74.

Fasuyi, A. (2006). Nutritional potentials of some tropical vegetable leaf meals: chemical characterization and functional properties. Afri. J. Biotechnol., 5: 49-53.

Gbile, B. A and Adesina, D.C. (2006) Therapeutic effects of medicinal plants. *Journal of Herbal Medicine*. 20:81-88.

Lengsfeld, C., Tigemeyer, F. Faller, G., Hensel, A. (2004): Glycosylated Compound from Okra inhibit adhesion of Helicobacfer pylori, The most common bacterial infection in Africa, a random, serological study. *American journal of Gastroenterology* 87:28.

Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983, 54, 275.

Mbongue, F., Kamtchouing, P., Essame, O., Yewah, P., Dimo, T. Lontsi, D., (2005). Effect of the aqueous extract of dry fruits of piper guineense on the reproductive function of adult male rats, Indian. J. Pharmacol. 7: 30-32.
Udoh, F. Theodore, V., Braide, V. (1999). Effects of extract of seed and leaf of piper guineense on skeletal muscle activity in rat and frog, phytother. Res., p. 110.

Singh SK, Chakravartys, (2000): Histologic changes in the mouse of testis after administration of ethanolic extracts of Okro.J.Androl,2115

Ujowundu, C., Kalu. F., Nwosunjoku, E., Nwaoguikpe R., Okechukwu, R. Igwe , K. (2011). Iodine and inorganic mineral content of some vegetables, spices and grains consumed in southeastern Nigeria. African Journal of Biochemistry Research vol. 5(2), pp. 57-64.

Table 1: Showing the effect of *extracts* on initial and final body weights

Groups	**Drug administered**	**Initial body weight(g)**	**Final body weight (g)**
1	Control (no treatment)	137.90±6.16	169.50±6.53*
2	*Abelmochus esculentus-500mg/kg-28 days*	167.80±7.81	200.10±11.40**
3	*Piper guineense*-20mg/kg -28 days	181.10±8.56	198.10±12.22**
4	Combined *A.esculentus* + *P.guineense*-28days	151.78±7.86	205.44±12.73***

Result shown as=Mean ± SEM, **=p<0.05, **=p<0.01 ***=P<0.001* compare to control.

Table 2: Showing the effect of *extracts* on percentage terminal testes weights.

Groups	**Extracts administered**	**Testes Weight(g)**	**% Testes Weight (g)**
1	Control(no treatment)	2.02±0.05	1.5
2	*Abelmochus esculentus-500mg/kg-28 days*	5.09±0.34	3.0
3	*Piper guineense*-20mg/kg -28 days	4.63 ± 0.28^{b}	2.5
4	Combined *A.esculentus* + *P.guineense*-28days	4.88 ± 0.28^{a}	3.2

Mean ± SEM, ***=*P<0.05* compare to control, n=10 no of animal in a group

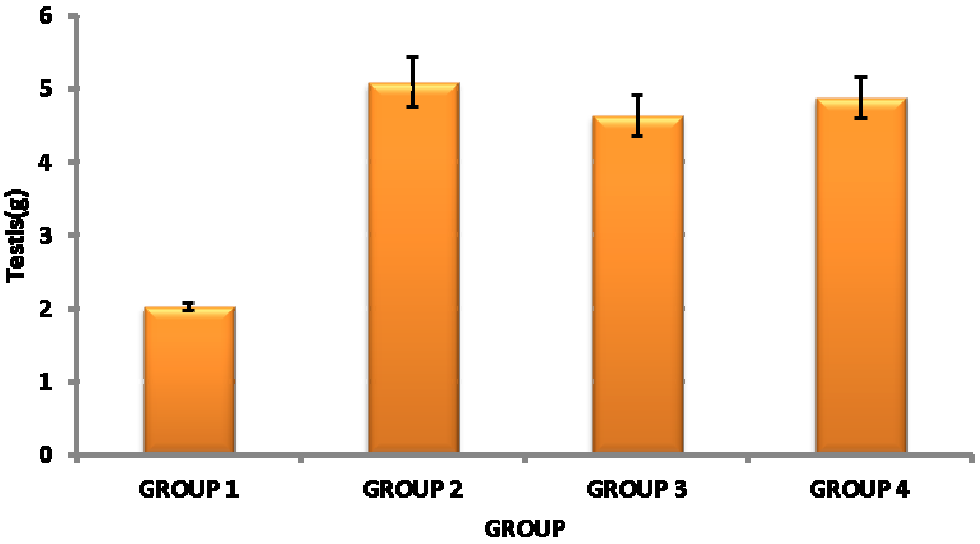

Fig 1: showing mean Testes weight.

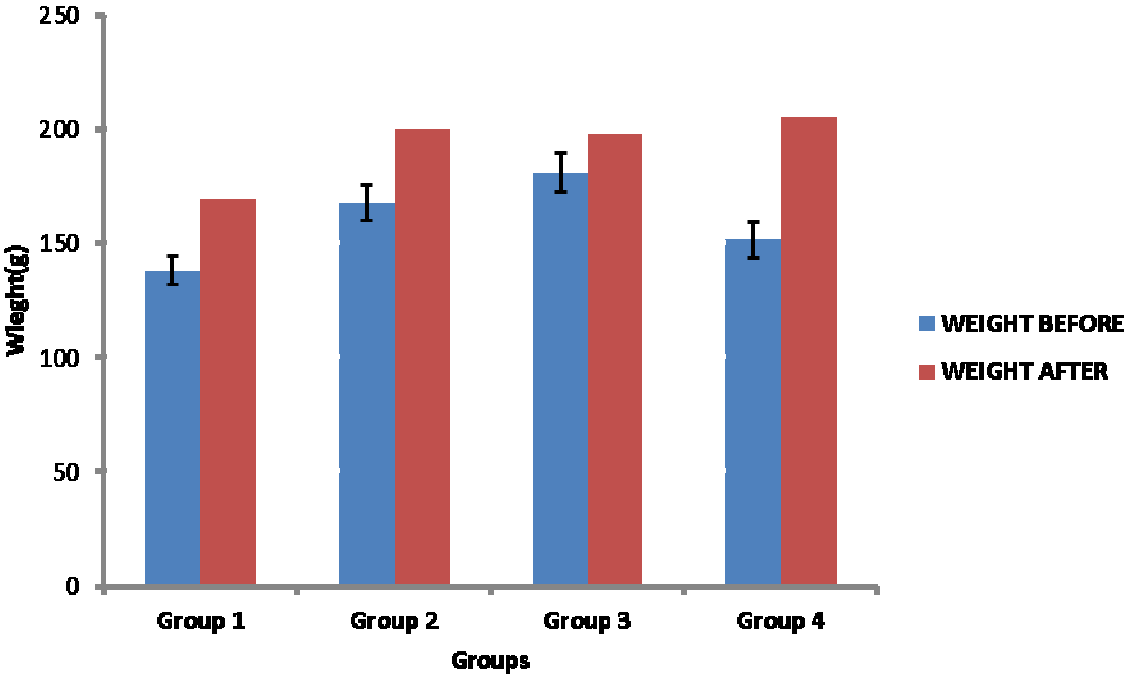

Fig 2: showing comparison between initial and final body weight.

Histopathological findings.

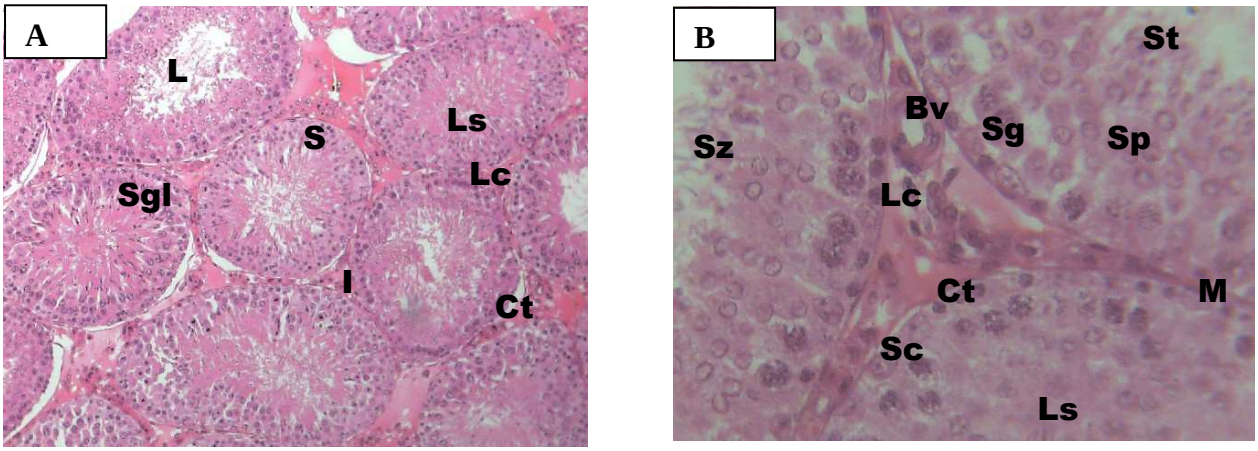

PLATE 1 Control Testes at magnification A(x100) & B(x400) stained with H & E technique.

*Note: **L**-Lumen, **S**-spermatogonia, **LS-luminal semen**, **I**-intrerstitial, **CT**- connective tissue, **SZ**-spermatocyte, **LC**-leydig cells, **Sgl**- spermatogenic layer, **BV**-blood vessel, **ST**-spermatids, **M**-myoid cells, **SP**-spermatocyte, **Sg**-spermatogonia.*

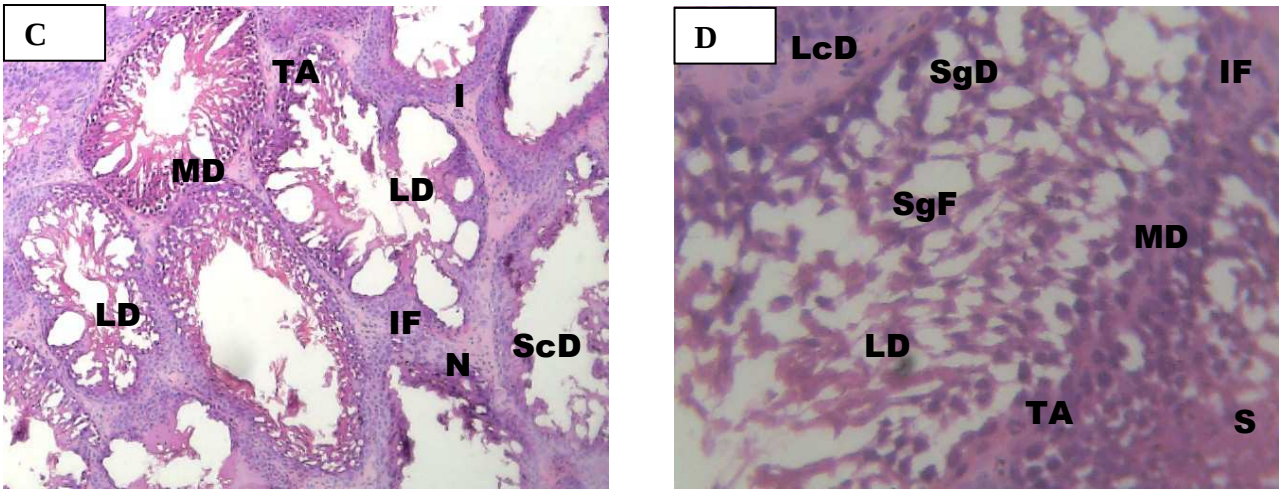

***PLATE 2 Testes treated with extract of ABELMOSCHUS ESCULENTUS (500mg/kg) at magnification* C(x100) & D(x400) stained with H & E technique.**

*Note: **MD**-myoid degeneration, **LD**-luminar degeneration, **I**-interstitial, **TA**-tubular atrophy, **N**-necrosis, **TN**-tubular necrosis, **S**-semniferous tubules, **LCD**-leydig cell degeneration, **SgD**-spermatogenic degeneration*

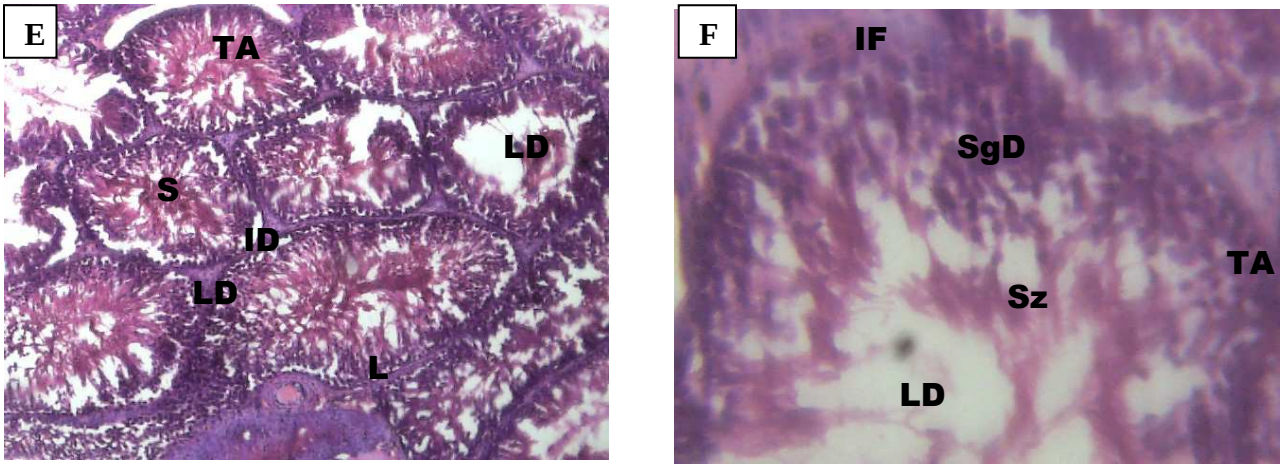

PLATE 3 Testis treated with *PIPER GUINEENSE (20mg/kg)* at magnification E(x100) & F(x400) stained with H & E technique.

*Note: **TA**-tubular atrophy, **LD**-luminar degeneration, **L**-lumen, **SgD**-spermatogenic degeneration, **S**-spermatogonia, **IF**-Interstitial fragment, **Ld**- luminal degeneration and Sz- spermatozoa.*

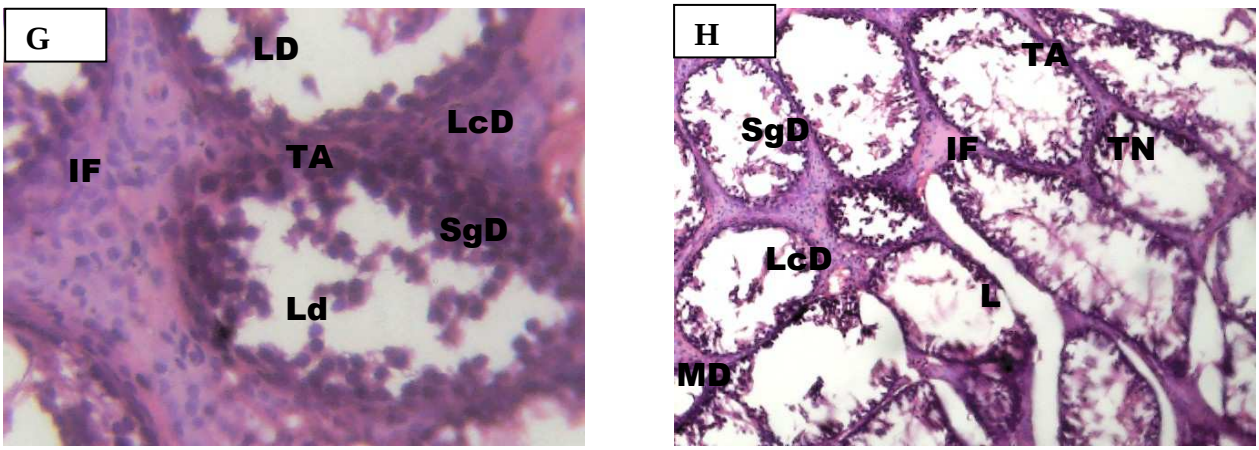

***PLATE 4 Testis treated with combined extract of ABELMOSCHUS ESCULENTUS (500mg/kg) and PIPER GUINEENSE (20g/kg) at magnification* G(x400) & H(x100) stained with H & E technique.**

*Note: **LD**-luminal degeneration, **LcD**-leydig cell degeneration, **TA**-tubular atrophy, **SgD**-spermatogenic degeneration **L**-lumen, **TN**-tubular necrosis, **IF**-Interstitial fragment and Ld- luminal degeneration.*

This academic article was published by The International Institute for Science, Technology and Education (IISTE). The IISTE is a pioneer in the Open Access Publishing service based in the U.S. and Europe. The aim of the institute is Accelerating Global Knowledge Sharing.

More information about the publisher can be found in the IISTE's homepage: http://www.iiste.org

CALL FOR JOURNAL PAPERS

The IISTE is currently hosting more than 30 peer-reviewed academic journals and collaborating with academic institutions around the world. There's no deadline for submission. **Prospective authors of IISTE journals can find the submission instruction on the following page:** http://www.iiste.org/journals/ The IISTE editorial team promises to the review and publish all the qualified submissions in a **fast** manner. All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Printed version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Recent conferences: http://www.iiste.org/conference/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar