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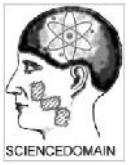

Piper guineense Effects on Testicular Histoarchitecture

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Authors' contributions

This work was carried out in collaboration between all authors. Author OCG designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author J. O. Owolabi managed the literature searches, analyses of the study and performed the histological analysis and author J. O. Ochei managed the experimental process. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Piper guineense is used as spice and herb, particularly to enhance libido. *P. guineense* has effects on penile erection and copulatory behaviour which are controlled by androgens. This study was carried out to evaluate the effects of consumption of *P. guineense* leaves on testicular histoarchitecture. The effects of *P. guineense* ingestion, in form of pelleted formulation, on the histoarchitecture of the testis of adult male Wistar rats were studied. Thirty adult male Wistar rats were divided into six groups labelled A-F. Group A animals served as the control and they were fed *ad libitum* throughout the experiment. Dried and powdered *P. guineense* leaf was added to the feed of the rats in Groups B, C, D, E and F in graded proportions. Based on the well monitored and properly evaluated rate of feed consumption, the proportion of *P. guineense* in the feed for Groups B, C, D, E and F gave approximate daily dosage of 100, 200, 300, 400 and 500 mg/kg body weight respectively. The regimen design was to observe the possible influence of dosage variations.

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Animals in Groups B, C, D, E, and F were given the pelleted food formula for 28 days. Animals were sacrificed after treatments and the testes were excised and processed using the Eosin and Haematoxylin staining histological technique. Photomicrographs for histological analyses were taken with the aid of the digital Accuscope Photomicrographic Set. Results on the testicular histoarchitecture show that *Piper guineense* consumption affected testicular structural integrity and gametogenesis. Higher doses caused disruption in seminiferous tubules epithelium and general ultra structure. Though *P. guineense* had been reported to increase libido and frequency of male sexual activities, results from this investigation showed that its consumption might not enhance or improve male fertility.

Keywords: Testis; Piper guineense; fertility; libido; male.

1. INTRODUCTION

Piper guineense is used as spice and herb [1]. Medicinal plants are commonly used for the treatment of disorders linked with male infertility-as aphrodisiacs [2,3,4,5]. Some of the factors responsible for infertility are associated with hormonal secretion, erectile impotence, disorders of ejaculation, and toxic effects on the testes and accessory sex organs. *P. guineense* is a West African species of *Piper*; the spice derived from its dried fruit is known as West African pepper, Ashanti pepper, Benin pepper, false cubeb, Guinea cubeb, uziza pepper or Guinea pepper, and called locally kale, kukauabe, masoro, sasema and sorowisa. Ashanti peppers contain 5-8% of the chemical piperine and large amounts of beta-caryophyllene, significant proportions (10%) of myristicin, elemicin, safrole and dillapiol [6].

The effects of *P. guineense* on male reproductive parameters have been observed either by extraction of its active compound, its aqueous extraction or combining it with other herbs or plants. It is often used to improve libido. However, some reports stated that *P. guineense* induced adverse and detrimental effects on the testicular function and structure [7,8,9].

Humans and mammals generally have paired testis. Histologically, each testis is divided by a connective tissue tunica albugenea into lobules. The lobules have one to four twisted seminiferous tubules in the mesh of the interstitial cells and connective tissues. The Seminiferous tubule is the functional unit of the testis and its epithelium has the primordial germinal cells that mature through the process termed spermatogenesis [10,11,12]. Damage to the seminiferous tubules would negatively affect gametogenesis cum fertility [13].

This article contains our findings on the effects of *P. guineense* leaf on the structural integrity or histoarchitecture of the testis, towards validating its usefulness and influence on male fertility, in addition to the popularly known positive influence on libido and male sexual behaviour.

2. MATERIALS AND METHODS

The fresh leaves of *P. guineense* used for this experiment was procured from Ilisan market, Ogun state, Nigeria. They were identified and authenticated in the Department of Agriculture, Babcock University, Nigeria, headed by Dr Oyekale Kehinde where voucher specimen of the dry leaves are being kept in the herbarium.

2.1 Dry Leaves of *Piper guineense* Preparation

The fresh leaves of *P. guineense* were dried and crushed into fine powder using a dry blender. The powder was weighed and stored in an air-tight container. The *P. guineense* powder was used to prepare pelleted food formula based on the research regimen.

2.2 Experimental Animals

Thirty (n = 30) adult male Wistar rats with average weight of 170 g were used. They were fed with pelletized rat feed, given water *ad libitum*, and allowed to acclimatize for two weeks before commencement of experiment. They were randomly weighed and divided into six (6) groups: Group A served as the control while the other groups [Groups B, C, D, E and F] served as the treated group. The control group animals were given water and pelletized growers feed alone. The *P. guineense* powder was used to prepare the pelleted food formula based on the predetermined average food consumption of the animals as well as the pre-designed research regimen. The animal Groups B, C, D, E, and F were given pelleted food formula for 28 days. The regimen made the Groups B, C, D, E and F animals consume 100, 200, 300, 400 and

500mg/kg body weight of *P. guineense* leaf powder respectively, daily. All doses used were considered non-toxic from prior pilot study. Ethical approval was obtained from Babcock University Health Research Ethics Committee, Nigeria. All experimentations were carried out following the guidelines for the care and use of laboratory animals obtained from the institutional animal ethics committee.

2.3 Sample Collection and Histo-pathological Analysis

Twenty four hours after last day of treatment, the animal were anesthetized with chloroform vapour and dissected. The testes were carefully dissected out and properly grossed. The tissues were fixed in 10% formal saline. The fixed tissues were dehydrated in graded concentrations of ethanol. The tissues were further cleared in xylene, infiltrated in molten paraffin wax in the oven at 58°C, embedded in wax and blocked out. Serial sections of 5 µm thick were obtained from the solid block of tissue, mounted on clean albuminized slides and stained using the Haematoxylin and Eosin staining techniques [14]. They were passed through ascending grades of alcohol, cleared in xylene and mounted in DPX (a mixture of distyrene, a plasticizer and xylene) mountant [15]. Photomicrographs were taken using the Accuscope Digital Photomicrographic set and the samples were examined and analysed.

3. RESULTS AND DISCUSSION

3.1 General Histoarchitectural Organisation

It is important to note that the functional integrity of the testis, especially with respect to spermatogenesis, (production of the gamete cells- spermatozoa) is associated with the seminiferous tubules. Their structural integrity, especially with respect to their epithelium, provides information on the tissue's functional integrity. In addition to this, the endocrine integrity is strongly associated with the Leydig cells which are found in the interstitial stroma [16]. Since these cells produce testosterone; they are vital to hormonal activities associated with libido. To this end, the testicular structure analysis would provide information primary on two vital parameters of fertility: libido and gametogenesis [16]. While the former would influence copulation and its rates, the latter determines the spermatozoa content of the product of ejaculation [semen] and this is why sperm count is often considered in determining male fertility [16].

General testicular histology or histoarchitecture is being considered across the animals groups including the Control Group A which is being used suitably as the control. Animals across the groups showed signs of deviation from the control in a progressive manner from the Group B to the last Group F. While Groups B and C closely resemble Group A in terms of the general histoarchitecture, Group F presents the most extensive structural deviation from the control Group A. This implies that at such high dosage of consumption of the leaf, the histological organisation of the testicular tissue otherwise referred to as the general histoarchitecture is being distorted. More specifically, pattern of distortion include increased heterogeneity in the seminiferous tubule diameter and circular tubular cross-sections. Seminiferous tubules distortions are particularly observed in Groups D, E and F, with the Group F being affected the most. Also, interstitial spaces appear more abundant or prominent which are not filled by the stroma or cells. It is important to note that all the seminiferous tubules are not all affected at the same time.

These observations point to the fact that the administered herb might not affect the testicular tissue organisation deleteriously when consumed moderately such as in the cases of Groups B and C that show quite much similarity to the control Group A. However, at relatively high dosages of consumption, there could be deleterious effects on the testis histoarchitecture and this could increase as the dosage of consumption is increased [7,8]. These effects could alter testicular structural integrity, by compromising the epithelia in certain seminiferous tubules, thus limiting the spermatogenesis within them.

3.2 Seminiferous Tubules Integrity

Seminiferous tubules are readily observable across all the Groups. They however have certain features that provide information on the effects of the substance being administered on the testis. The distortion of the seminiferous tubules in the animal groups given higher dosages of the herb is accompanied by a number of structural changes within the seminiferous tubules. The stratification-heights of the seminiferous tubule epithelia vary in the higher dosage groups especially Groups D, E and F (Figs. 4, 5 and 6) with Groups F being the worst affected. Some have relatively larger

central lumen than others. It is also important to note that the seminiferous tubules are not affected to the same extent; some are distorted while others are relatively preserved. The seminiferous endothelial cells are intact and provide support across the groups; as such, there is no evidence of complete tubular damage. Cells in different zones of the seminiferous tubules epithelium are also observable.

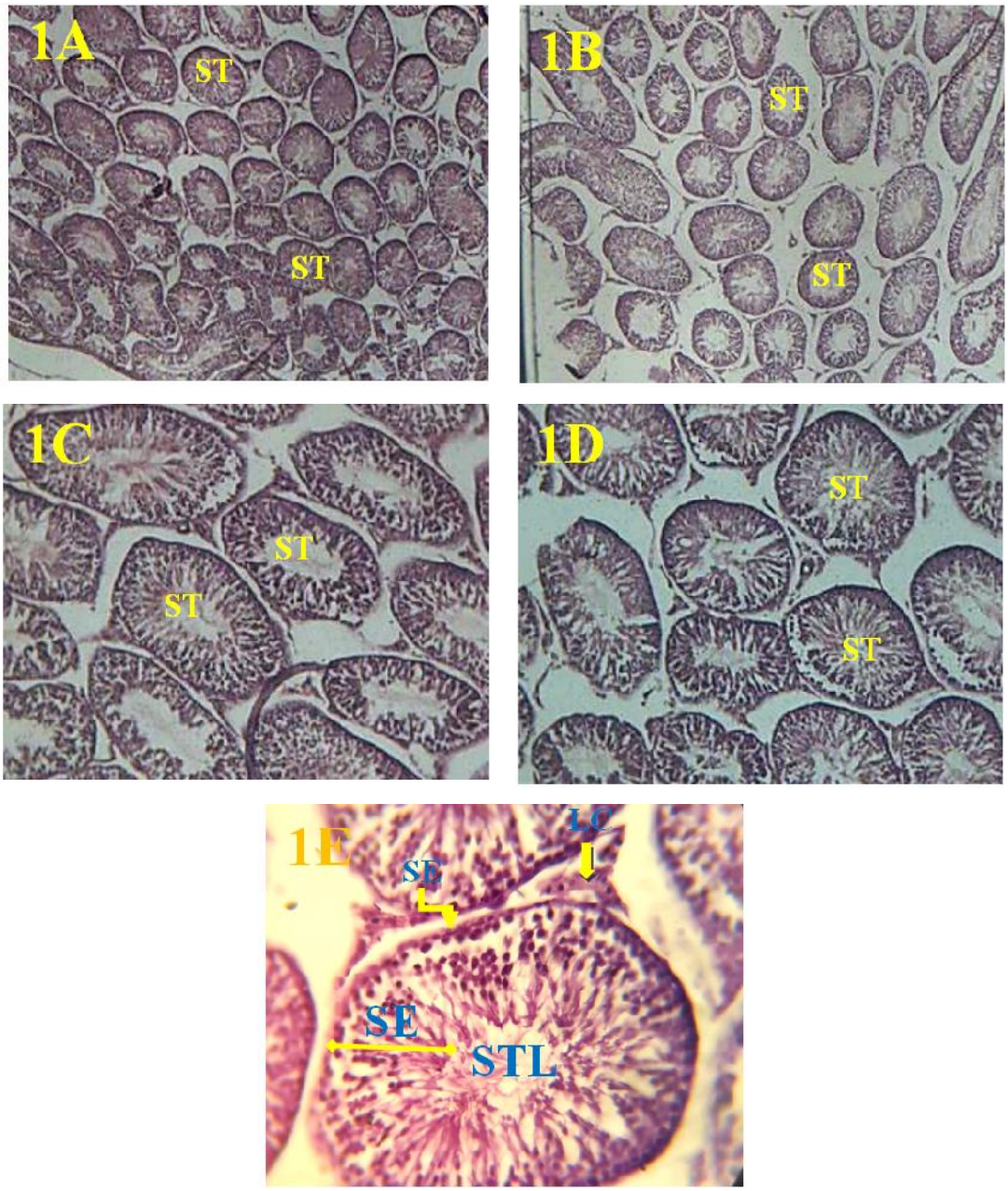

Fig. 1. Photomicrographs of the testes of the control Group A animals fed *ad libitum* at X64 [1A and 1B], X160 [1C and 1D] and X400 [1E] using the Haematoxyline and Eosin [H & E] staining technique. Tissue has normal testicular histoarchitecture; seminiferous tubules are normal in transverse section and the epithelium is well defined. Interstitial Leydig cells are also observable. [ST= Seminiferous Tubules; SE = Seminiferous Epithelium; LC = Leydig Cells; SEC = Seminiferous Endothelial Cells; STL = Seminiferous Tubule Lumen]

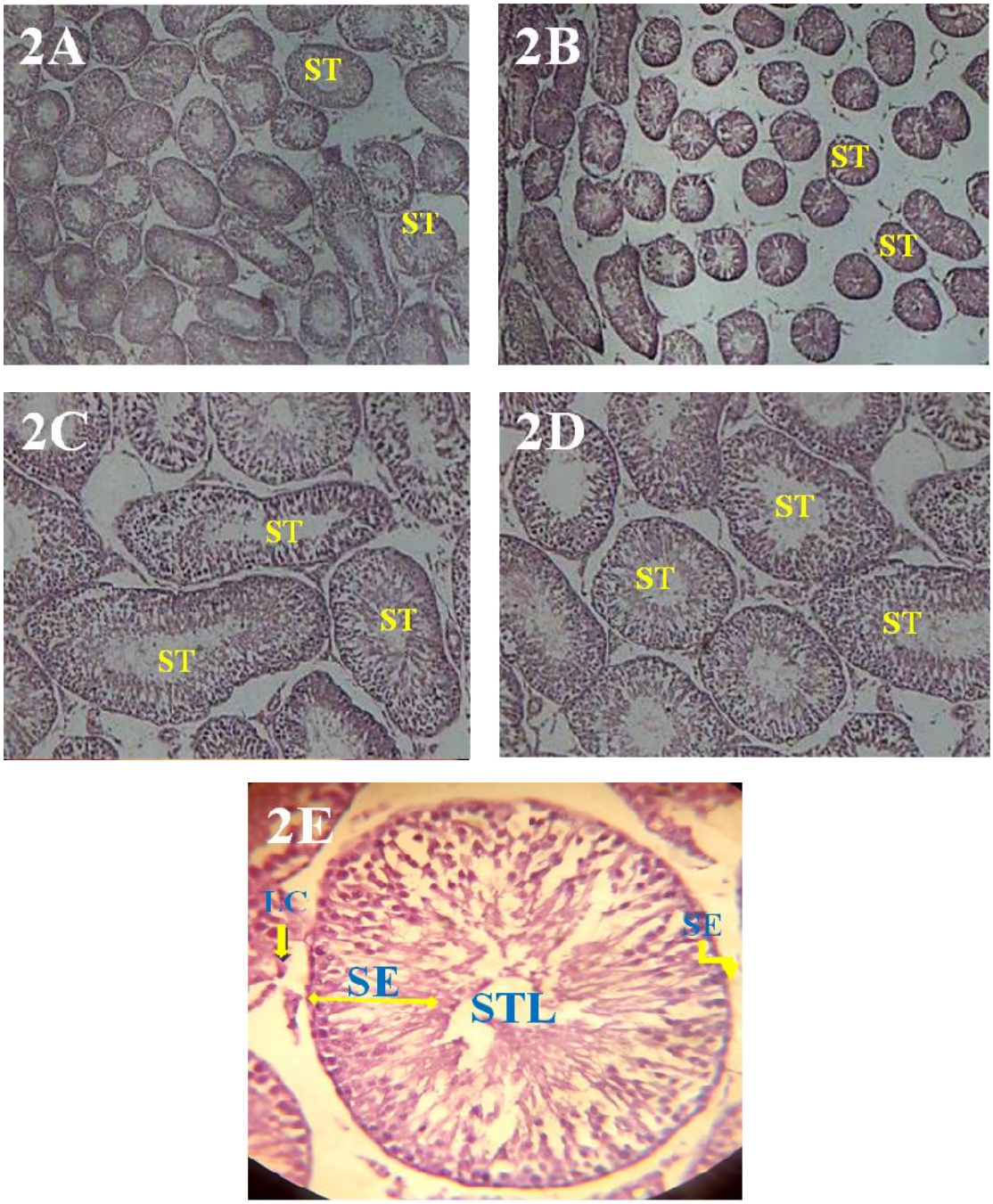

Fig. 2. Photomicrographs of the testes of the Group B animals given 100 mg/kg body weight of *P. guineense* at X64 [2A and 2B], X160 [3C and 3D] and X400 [2E]; using the Haematoxyline and Eosin [H & E] staining technique. Tissue has relatively has normal testicular histoarchitecture; seminiferous tubules are close to Control in transverse section; epithelium is defined and interstitial Leydig cells are observable. [ST= Seminiferous Tubules; SE = Seminiferous Epithelium; LC = Leydig Cells; SEC = Seminiferous Endothelial Cells; STL = Seminiferous Tubule Lumen]

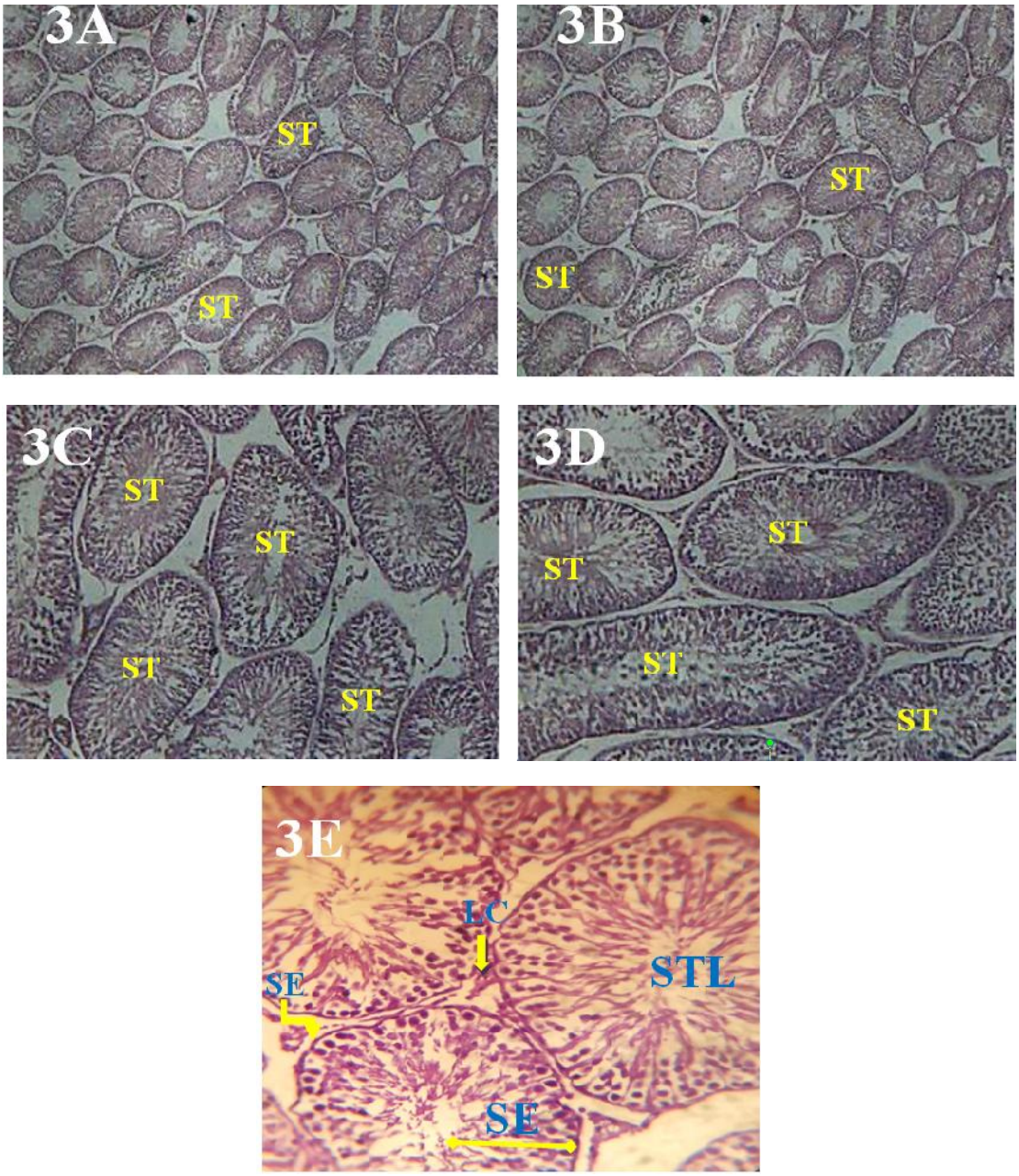

Fig. 3. Photomicrographs of the testes of the Group C animals given 200 mg/kg body weight of *P. guineense* at X64 [3A and 3B], X160 [3C and 3D] and X400 [3E]; using the Haematoxyline and Eosin [H & E] staining technique. There are no signs of seminiferous tubule distortions [ST= Seminiferous Tubules; SE = Seminiferous Epithelium; LC = Leydig Cells; SEC = Seminiferous Endothelial Cells; STL = Seminiferous Tubule Lumen]

Seminiferous tubule disruption, reduction in seminiferous epithelium thickness and accompanying widened lumen however could have negative consequences on the quantity of spermatozoa being produced. There is, therefore, the possibility that at the extreme dosage[s] of *P. guineense* ingestion, less spermatozoa could result from the process of spermatogenesis. Furthermore, extensive distortions across the tubules which make certain tubules relatively smaller in cross section could reinforce evidence of lowered sperm output on

the average per tubule. Consequently, testis exposed to higher dosage of the herb could have reduced sperm output. These findings share similarities with previous works reporting histopathological disruptions or distortions of the seminiferous tubules and certain male fertility parameters [17,18]. In contrast however, Sutyarso and Kanedi, [19] reported that fruit extract of black pepper had positive effects on male fertility, particularly sperm count and seminiferous tubule integrity.

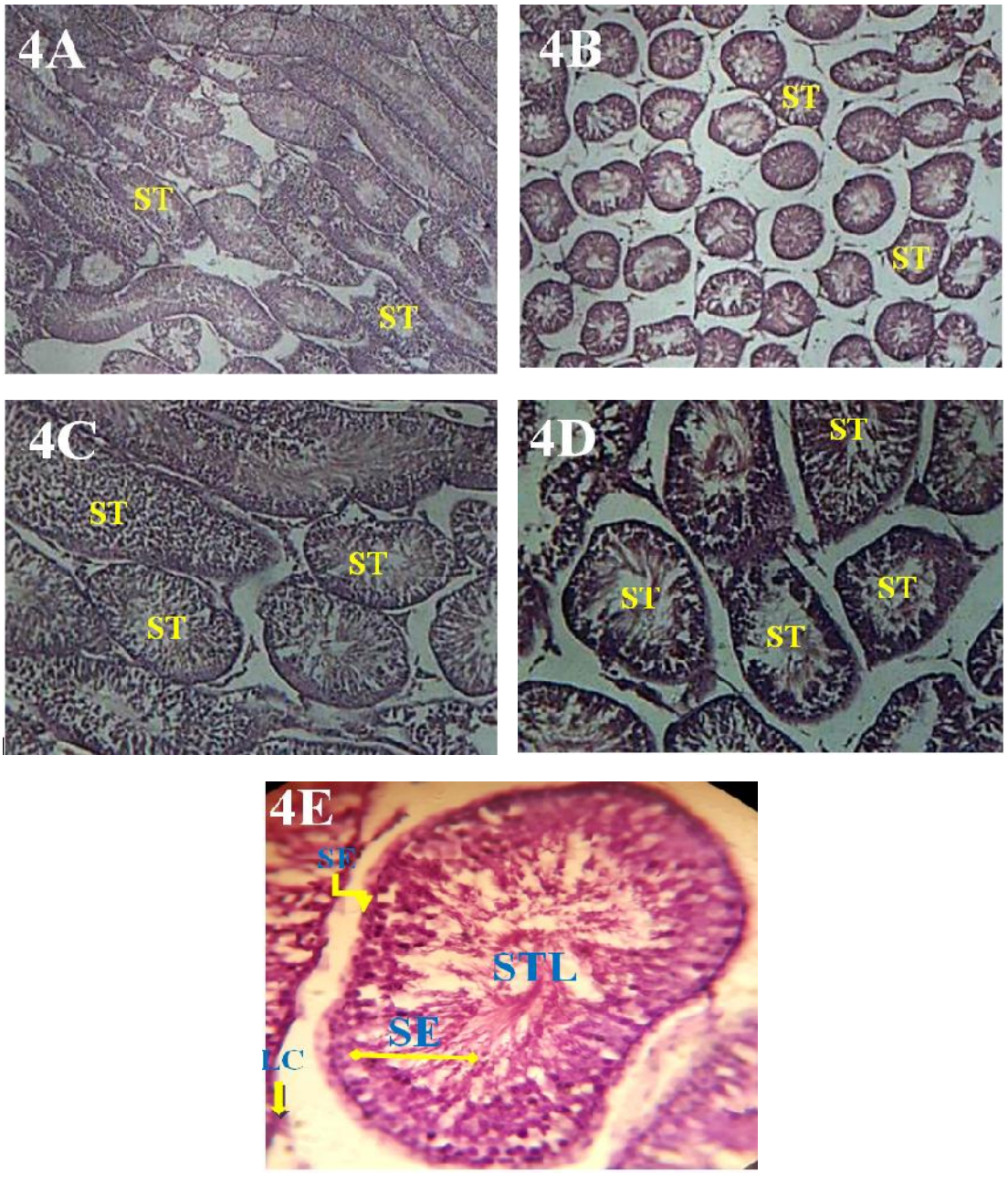

Fig. 4. Photomicrographs of the testes of the Group D animals given 300 mg/kg body weight of *P. guineense* at X64 [4A and 4B], X160 [4C and 4D] and X400 [4E] using the Haematoxyline and Eosin [H & E] staining technique. Seminiferous tubules are mildly morphologically distorted in transverse cross section. [ST= Seminiferous Tubules; SE = Seminiferous Epithelium; LC = Leydig Cells; SEC = Seminiferous Endothelial Cells; STL = Seminiferous Tubule Lumen]

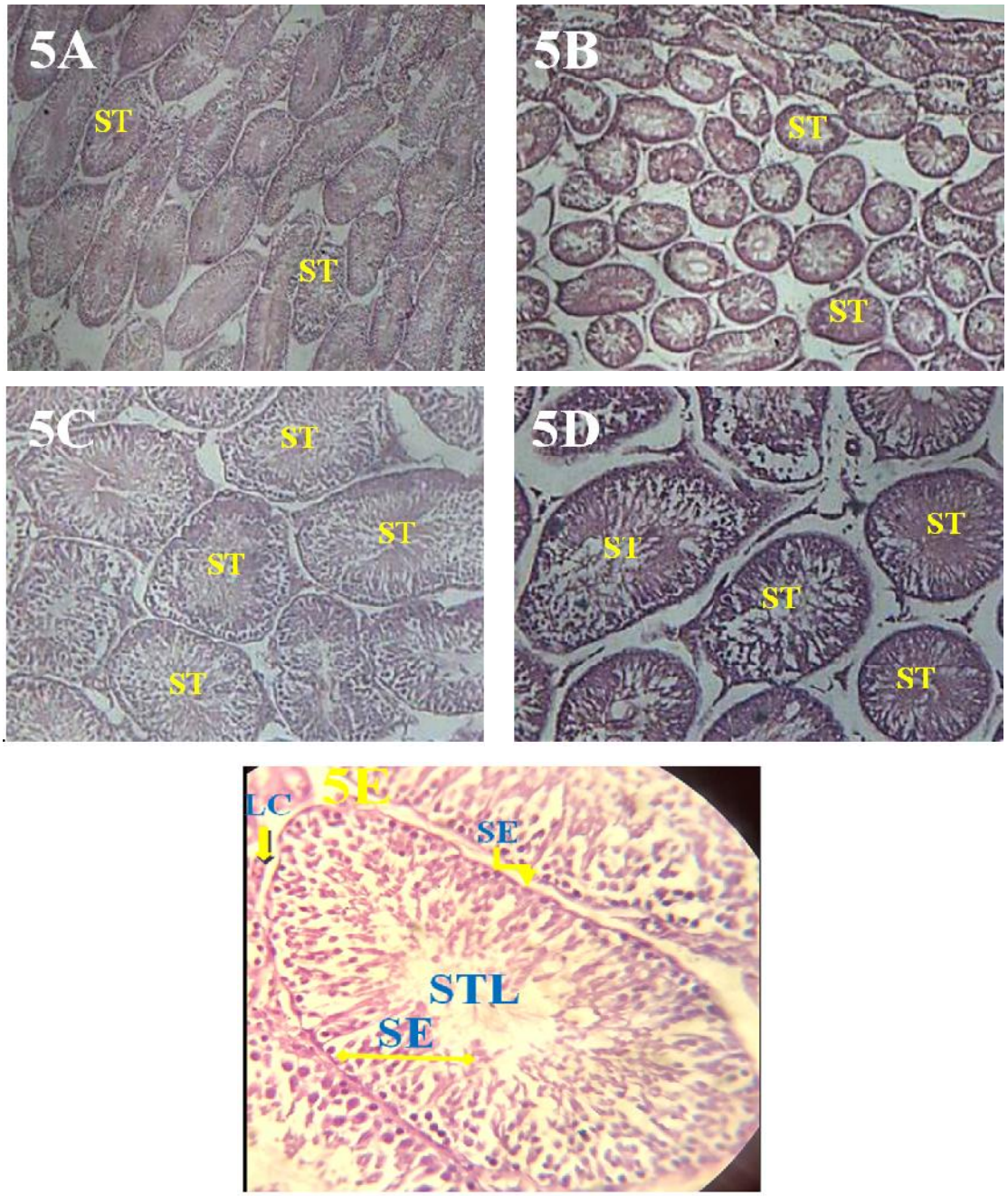

Fig. 5. Photomicrographs of the testes of the Group E animals given 400 mg/kg body weight of *P. guineense* at X64 [5A and 5B], X160 [5C and 5D] and X400 [5E]; using the Haematoxyline and Eosin [H & E] staining technique. There are signs of distortions of certain seminiferous tubules [ST= Seminiferous Tubules; SE = Seminiferous Epithelium; LC = Leydig Cells; SEC = Seminiferous Endothelial Cells; STL = Seminiferous Tubule Lumen]

3.3 Interstitial Leydig Cells

Leydig cells are found in the stroma of the interstitial spaces. Though they do not participate structurally in spermatogenesis; they are very important for producing hormones, especially testosterone- a vital reproductive hormone. It does not appear that the Leydig cells are affected more than their consequential displacement caused by the tubular distortions in

some groups as previously described. Even in Group F where the tubules are most distorted and the tissue most disrupted, interstitial Leydig cells and stroma are still observable. Therefore, there is evidence that the Leydig cells are preserved across the Groups A- F. Furthermore, this observation suggests that these cells would still perform their function of producing the hormone testosterone which is needed for vital male reproductive functions. There is the possibility that libido might not be reduced because of the herb ingestion. The

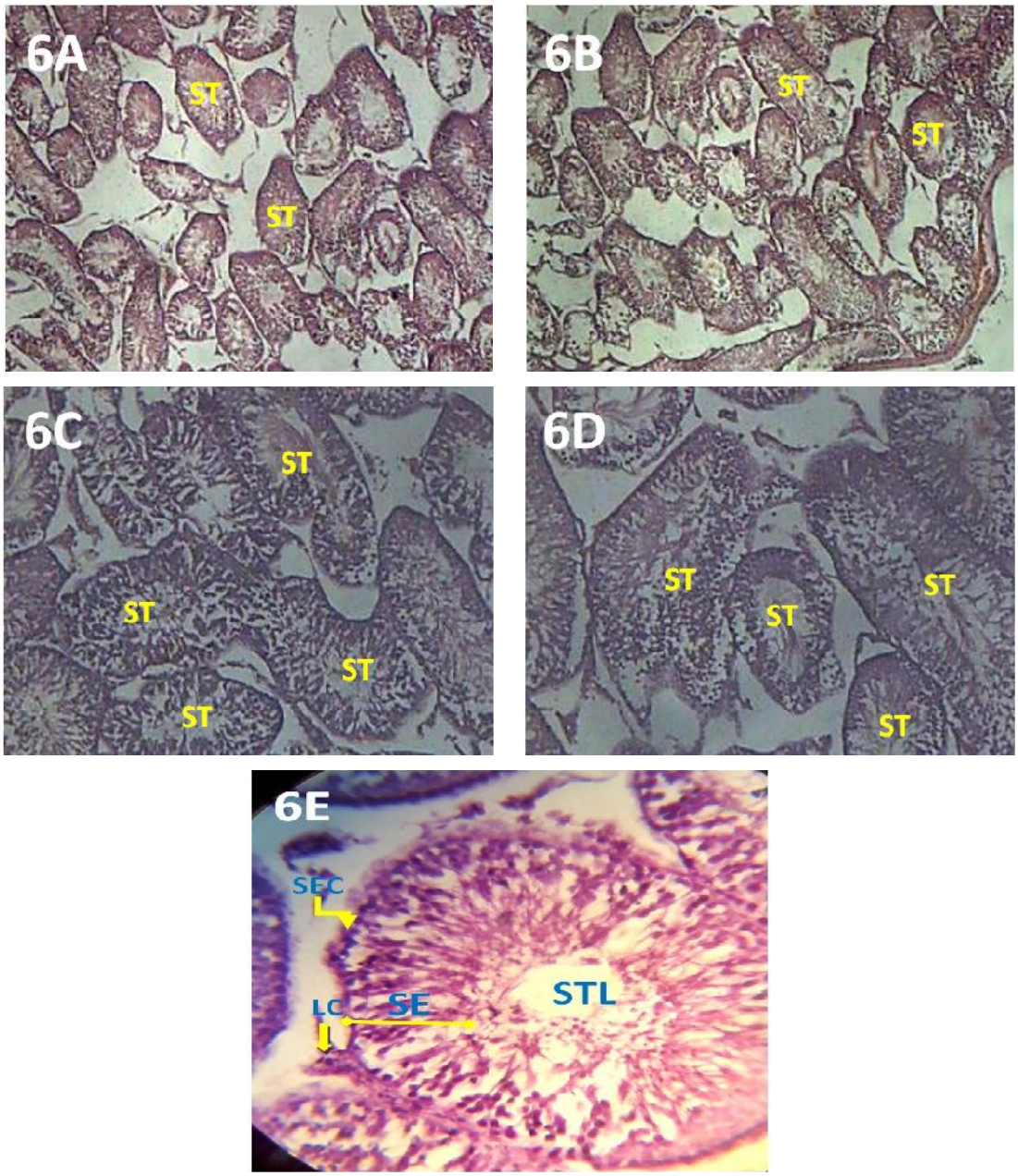

Fig. 6. Photomicrographs of the testes of the Group F animals given 500 mg/kg body weight of *P. guineense* at X64 [6A and 6B], X160 [6C and 6D] and X400 [6E]; using the Haematoxyline and Eosin [H & E] staining technique. Lobular organisation of seminiferous tubule is grossly distorted. Certain individual seminiferous tubules are also disrupted. [ST= Seminiferous Tubules; SE = Seminiferous Epithelium; LC = Leydig Cells; SEC = Seminiferous Endothelial Cells; STL = Seminiferous Tubule Lumen]

relative prominence of these cells in the high-dose Groups, especially E and F could point to enhanced hormonal activities of the cells and production of testosterone. The implication is that the animals might have increased sexual activities- increased rate of copulation or sexual mounting due to high hormonal activity [20,21, 22,23]. However, since there could be reduced spermatogenesis, increase in sexual activities might not translate to increased fertility relative to the control or untreated Group A.

Disruptions of the testicular seminiferous tubules in different forms, including luminal expansion, seminiferous epithelium thinning and cell disorganisation or disruptions are all associated with compromises in fertility through gametogenesis [13]. Generally, it is logical to infer that the extract of *P. guineense* would primarily affect sexual drive or libido as well as erectile dysfunction correction [20,21,22,23]. More so, it had been associated with rising in the testosterone level. Hence its function is more associated with the copulatory structures such as the corpus cavernosum relaxation [24,25,26] and penile erection. The observed negative effects on testicular histoarchitecture, seminiferous epithelium integrity, and consequently spermatogenesis are in line with some previous investigations [7,8,9]. Interestingly, most users of this herb are typically concerned about its effects on libido. This explains why in a previous study, *P. guineense* was found to modify experimental male rats' sexual behaviour especially by significantly increasing penile erection index, and frequencies of intromission and ejaculation [27]. Another study however, had reported that *P. guineense* seeds extract caused infertility [28]. It is also worthy of note that the substance doses as used is considered not generally toxic to the body [29] and there was no evidence of toxicity in the experimental animals.

4. CONCLUSION AND RECOMMENDATIONS

Results from the observations of the testes structures suggest that the ingestion of *P. guineense* at the high doses caused seminiferous tubules disruption and distortion of the epithelia. The consequences of this would be negative on testicular structural integrity and gametogenesis. Leydig cells prominence may influence hormonal activities and increase libido and frequency of male sexual activities. This might not necessarily translate to increased or improved fertility. Therefore, though the herb might be found potent for increasing libido and would be used for this purpose, moderate dosages should be employed. Further work should include sperm count and differential demonstration of seminiferous epithelial cells to observe progress and states of spermatogenesis. Testosterone assays in blood sample could also provide information on Leydig cells activities.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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