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Testicular pathology in albino rats administered graded doses of ethanol leaf extract of *Cymbopogon citratus*

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Abstract

Cymbopogon citratus (*C. citratus*) has antioxidant, anti-inflammatory, and chemoprotective properties. This study investigated if increased doses of *C. citratus* ethanol extract can cause any serious adverse effect (gross, histological, hormonal, and/or inflammatory responses) on the testes of male albino rats. The sixty albino rats used in this study were randomly assigned into four groups of 15 each: 1/CENT each allowed free access to potable water, 2/CET2, 3/CET5, and 4/CET10 rats were each administered 250, 500 and 1000mg/kg b.w dose of *C. citratus*, respectively. At two-weeks intervals, the rats were examined for signs and lesions, serum samples were collected on days 0, 14 and 28 post-treatment and assayed for testosterone, luteinizing and follicle-stimulating hormones. On days 14 and 28 post treatment, gross and microscopic lesions were not observed in the testis of groups 2/CET2 and 3/CET5. Grossly, the testis of group 4/CET10 appeared reduced in size, with translucency of the visceral and parietal coats of the tunica vaginalis, while the histopathologic changes were degeneration of spermatogenic cells and fibrinous exudates expanding the intertubular spaces. Groups 2/CET2 and 3/CET5 testosterone, luteinizing and follicle stimulating hormones levels were significantly ($p < 0.05$) higher than that of 4/CET10. These findings suggest that degeneration of spermatogenic cells and fibrinous exudates expanding the intertubular spaces, and decreased reproductive hormone levels are diagnostic features of *C. citratus* toxicity in male albino rats.

Keywords: Albino rats, *Cymbopogon citratus,* Experimental, Pathology, Testes

Introduction

Cymbopogon citratus (*C. citratus*), is a perennial grass that is widespread throughout the world. It is native to Asia, Africa, and the Americas, but is widely cultivated in temperate and tropical regions of the world, being widely used for their pleasant taste and therapeutic properties (Chukwuocha *et al*., 2016; Coelho *et al*., 2016; Atawodi *et al*., 2017; Lawal *et al*., 2017; Rahhal *et al*., 2024). Popularly known as citronella grass or lemongrass, but due to its distribution, it has several names (Oladeji *et al*., 2019). *C. citratus* is one of the best known species of grass of the genus *Cymbopogon, and* belongs to the family *Poaceae* (Gramineae). *Cymbopogon* originated from the Greek word "kymbe - pogon" meaning boat-beard (due to its flower spike configuration) and *citratus* (Latin) means lemon-scented leaves (Shah *et al*., 2011; Lawal *et al*., 2017; Oladeji *et al*., 2019).

Cymbopogon citratus is a valuable aromatic, economical, medicinal and nutritional grass found in Nigeria (Atawodi *et al*., 2017; Oladeji *et al*., 2019). Owing to chemical composition of the essential oils and flav**o**noids of *C. citratus,* it is used in pharmaceutical and chemical industries being incorporated in the manufacture of perfumes, fragrances, soaps, detergents, aftershaves, cosmetics and as culinary flavour in food, beverage and confectionaries, and insecticides (bio-pesticide) (Tajidin *et al*., 2012; Oladeji *et al*., 2019).

Traditionally, the leaves of *C. citratus* have been used as tea or decoction. Among various ethno-medical remedies, lemon grass has gained significant attention for its wide range of pharmacological or biological activities, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, antinociceptive, and anticancer effects and mitigate psychosocial stress-induced neurologic diseases (memory promoting) in susceptible individuals and spent grass in agriculture (Viana *et al*., 2000; Francisco *et al*., 2014; Lawal *et al*., 2017; Oladeji *et al*., 2019; Umukoro *et al*., 2020; Mukarram *et al*., 2021).

The qualitative and quantitative phytochemical analysis of *C. citratus* revealed that it has important bioactive chemical compounds such as ketones, alcohols, phenols, terpenes, flavonoids, saponins, steroids, tannins, alkaloids, geranial, terpernoids, polyphenols, esters, aldehyde and fatty acids, which could be linked to the therapeutic potency of the plant. The most essential compounds in *C. citratus* according to literatures are essential oil and flavonoids, which contributed to the pronounced therapeutic and pharmacological activities of the plant (Shah *et al*., 2011; Oladeji *et al*., 2019). Proximate analysis revealed that *C. citratus* contains low moisture content (5.7%) (responsible for the marked antimicrobial activities and storage capacity), crude fiber (9.28%) (aids digestion of food and makes food well absorbed by the body), crude fat, crude ash, crude protein and 55% carbohydrate (energy supplier or booster) (Asaolu *et al*., 2009; Oladeji *et al*., 2019). However, despite its popularity and potential health benefits, the impact of lemon grass on reproductive health remains largely unexplored.

Testes perform two highly organized and intricate functions, called spermatogenesis and steroidogenesis, which are vital for the perpetuation of life. Any disruption in testicular function or morphology can lead to impaired fertility and reproductive disorders in males. Previous studies have investigated the effects of various herbal compounds on testicular health, providing valuable insights into their potential benefits or adverse effects (D'Cruz *et al*., 2010). Rodent testes have been established as a useful model for studies on male reproductive system (Nagano *et al.,* 2002; Xie *et al*., 2014). Under normal conditions, it is comprised of a mixture of Sertoli and germ cells that work together to accomplish reproductive functions. Therefore, considering that *C. citratus* has a wide range of beneficial pharmacological or biological activities, understanding the effects of *C. citratus* on the gross and histomorphology of rat testes is of utmost importance to determine its potential impact on male reproductive health.

Materials and Methods

Location of study

The study was carried out at Umudike, Abia State, which is located about 10 kilometers Southeast of Umuahia, the State capital, within the Lowland Rain Forest Ecological Zone of Nigeria, between latitude 5°32'N, and longitude 7°29'E; the average rainfall of this zone is 3,500 mm per annum, and the average temperature ranges from 22^0C to 32^0C (FRoN-UNFCCC, 2019).

Experimental animals

Sixty adult male albino rats (*Rattus norvegicus)* (10-12 weeks old), weighing between 150 g and 180 g, procured from the Laboratory Animal Unit of the Animal Production Unit, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike were used for the study. The albino rats were housed in stainless steel cages in a fly-proof animal house at room temperature between 24 and 28°C under a 12 h dark/light cycle, and allowed 2 weeks to acclimatize before the commencement of the study. They were fed commercial pelleted grower's feed (Vital feed, Grand Cereals Ltd, Jos, Nigeria) and provided with portable water *ad libitum*. The albino rats were humanely handled and well cared for all through the study period. Guidelines for the use of animals for laboratory experiments were strictly adhered to (Zimmermann, 1983; Ward & Elsea, 1997). The protocol for the laboratory animal experiment was approved by the College of Veterinary Medicine Institutional Animal Care and Use Committee, Michael Okpara University of Agriculture, Umudike (MOUAU/CVM/REC/202323).

Collection and identification of plant material and preparation of plant extract

Fresh leaves of matured *C. citratus* collected from Umudike in Ikwuano Local Government Area, Abia

State, Nigeria, were used for the study. The leaves were identified and authenticated by a plant taxonomist and Forest Manager in the Department of Forestry, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. A voucher number MOUAU/ZEB/HERB//19/005 was assigned to the sample which was deposited at the Michael Okpara University of Agriculture, Umudike herbarium.

The leaves were spread under shade to dry for 14 days, and then ground into powder using a grinding machine. Five hundred grams (500 g) of the powdered leaves were extracted with 80% ethanol using the cold maceration extraction technique, with intermittent shaking at 2-h interval for 48 h and afterwards filtered twice, first with a sterile handkerchief and then with Whatman (Number 1) filter paper. The filtrate was concentrated to dryness in hot air at low temperature (40^{o}C) to obtain a light green crude extract which weighed 26.82g and represented 5.36% extract yield. The extract obtained was preserved in a refrigerator (4^{o}C) until needed (Adeneye & Agbaje, 2007; Anaga *et al*., 2010), and was referred to as *C. citratus* ethanolic extract (CCE).

Cymbopogon citratus ethanol extract treatment

After acclimatization, the albino rats were randomly divided into 4 groups of 15 rats each. The groupings and their treatments were:

Group 1 consisted of CCE-non-treated rats (1/CENT), allowed free access to potable water.

Group 2 consisted of CCE-treated daily with low dose of CCE, 250 mg/kg b.w (2/CET2).

Group 3 consisted of CCE-treated daily with middle dose of CCE, 500 mg/kg b.w (3/CET5).

Group 4 consisted of CCE-treated daily with high dose of CCE, 1000mg/kg b.w (4/CET10).

Treatment was done orally for 28 days. The choice of these doses was based on rational decision and to maintain a non-lethal dose based on findings from acute toxicity evaluation (Orieke *et al*., 2018). All the groups were observed for effects of CCE treatment daily for 28 days.

Pathology

A total of five (5) albino rats per experimental group treatment were randomly selected in each group, humanely sacrificed for the collection of testes at 0, 14, and 28 days treatment. They were necropsied and examined for gross lesions. Samples of the testes were fixed in 10% formal saline for 48 h and processed for histopathology as described by Suvarna *et al*. (2018). Sections (5µm) were stained with haematoxylin and eosin (H&E) and examined under the light microscope.

Hormonal assay

The five randomly selected albino rats per treatment in each group were used for blood sample collection. On 0, 14 and 28 days post-treatment, respectively, blood samples were collected into clean test tubes in each group and allowed to stand at room temperature for 30 minutes to clot. It was then centrifuged at 3000 rpm for 10 min at 4°C, after which the serum was aspirated with a syringe into clean labeled test tubes, and used immediately for the hormonal analysis. The concentrations of testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in each serum sample were determined by the chemiluminescence intra-immunoassay techniques using kits (Autobio Diagnostics Co., Ltd. Zhengzhou). The protocols and procedures used for the assay were as described by the manufacturer.

Data analysis

Data obtained from the study were analysed using a one way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., 2011). Variant means were separated post-hoc using the least significant difference method (Okafor, 1992). Probabilities less or equal to 0.05 were accepted as statistically significant. Significance was accepted at $p < 0.05$. All the data were presented as the mean ± standard deviation (SD).

Results

No clinical signs were observed in any albino rat in all the groups. Grossly, the testes of sacrificed albino rats in 4/CET10 groups showed mild atrophy, soft and translucency of the visceral and parietal coats of the tunica vaginalis, associated with small amounts of serous-like fluid in the cavity of the tunica vaginalis, compared to groups 1/CENT (the control), 2/CET2 and 3/CET5 (Plates I, II) on 14 and 28 days CCE post treatment.

On day 14 post treatment, groups 1/CENT (the control), 2/CET2 and 3/CET5 showed the normal histologic architecture of the tissues, revealing the normal progression of spermatogenesis which showed the formation of mature spermatids with few attached to Sertoli cells, and few mature spermatids mixed with proteinaceous matrix substance were

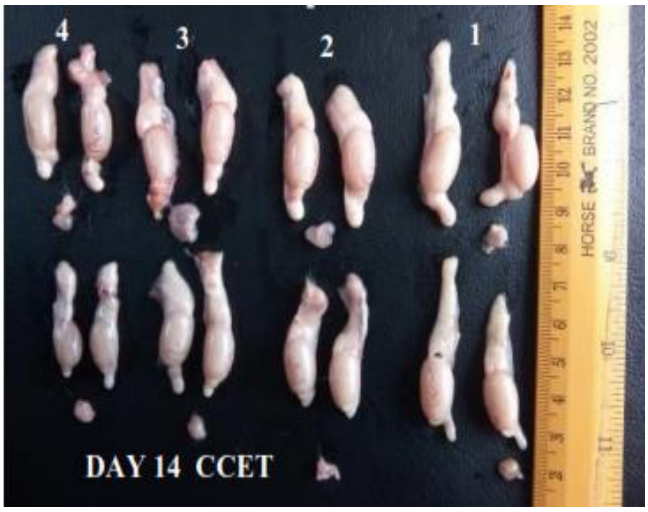

Plate I: Testes of group 4/CET10 albino rats showing mild atrophy, soft and translucent visceral and parietal coats with serous-like fluid in the cavity of the tunica vaginalis, compared with the groups 1/CENT (the control), 2/CET2 and 3/CET5 normal pale pink testes on day 14 post-treatment

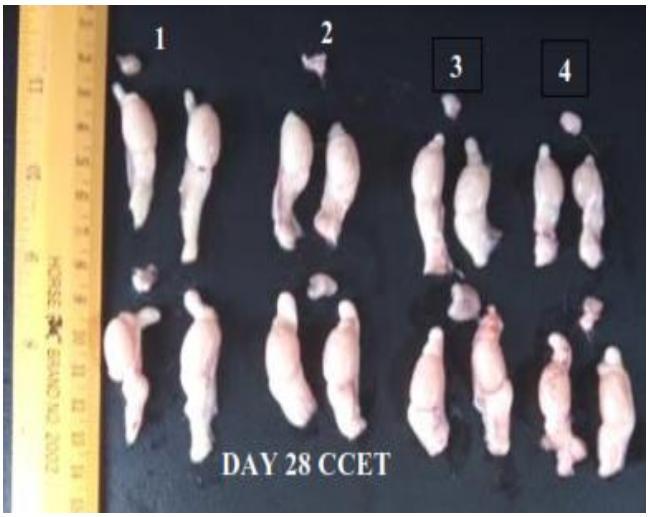

Plate II: Testes of albino rats showing a reduction in size of testes of group 4/CET10, compared with groups 1/CENT (the control), 2/CET2 and 3/CET5 on day 28 post treatment

present in the lumen of seminiferous tubules of the albino rats. (Plate IIIA, B & C). The main microscopic lesions in group 4/CET10 consisted of degenerative lesions such as mildly disorganized numerous spermatogenic columns, depleted amount in the generation of sperm cells, multifocally, only a single layer of spermatogonia remained in the basal layer of the seminiferous tubules. Fibrinous exudates and mild vacuolation of the interstitium or fibrovascular stroma were observed in the testes of albino rats (Plate IIID).

On day 28 post treatment, groups 1/CENT (the control), 2/CET2 and 3/CET5 testes, showed normal seminiferous tubules lined with a stratified epithelium composed of supporting Sertoli and spermatogenic cells and normal interstitial histological arrangement of cellular components (Plates IVA, B and C). Group 4/CET10 showed mild depleted amount in the generation of spermatogenic cells in seminiferous tubules and fragmentation of spermatids in seminiferous tubule. The interstitium or fibrovascular stroma mildly expanded with fibroblasts (Plate IVD).

At day 0 of the study, the testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels did not show any significant difference ($p > 0.05$) between the treated groups and their controls (Tables 1-3).

At 14 and 28 days post treatment, testosterone levels in groups 2/CET2 and 3/CET5 were observed to significantly increase ($p < 0.05$) compared with these of groups 1/CENT (the control) and 4/CET10, while the levels of this hormone in group 4/CET10 significantly decreased ($p < 0.05$) compared with those of groups 1/CENT (the control), 2/CET2 and 3/CET5 (Table 1).

At 14 and 28 days post treatment, LH and FSH levels in group 4/CET10, were significantly decreased ($p < 0.05$) compared to the level of these hormones recorded in groups 1/CENT (the control), 2/CET2 and 3/CET5 (Tables 2 and 3).

Discussion

The results of the present study showed that grossly, *C. citratus* ethanolic extract at dose levels of 250mg /kg b.w and 500 mg/kg b.w has a reproductive function-enhancing potential by causing significant increase testosterone and normal morphology/architecture of testes. This is likely to translate to protection of reproductive organs and positive effects on the reproductive performance. This could be due to the phytochemicals which are essential bioactive compounds commonly found in *C. citratus* linked to the therapeutic, pharmacological and biological activities of the plant (Akande *et al*., 2011; Shah *et al*., 2011; Oladeji *et al*., 2019). This is consistent with the prior use of the aqueous extracts of *C. citratus* as a chemoprotective agent against hydrogen peroxide-induced oxidative stress in the reproductive system of male rats (Rahim *et al*., 2013). However, the ethanolic extract of the *C. citratus* at

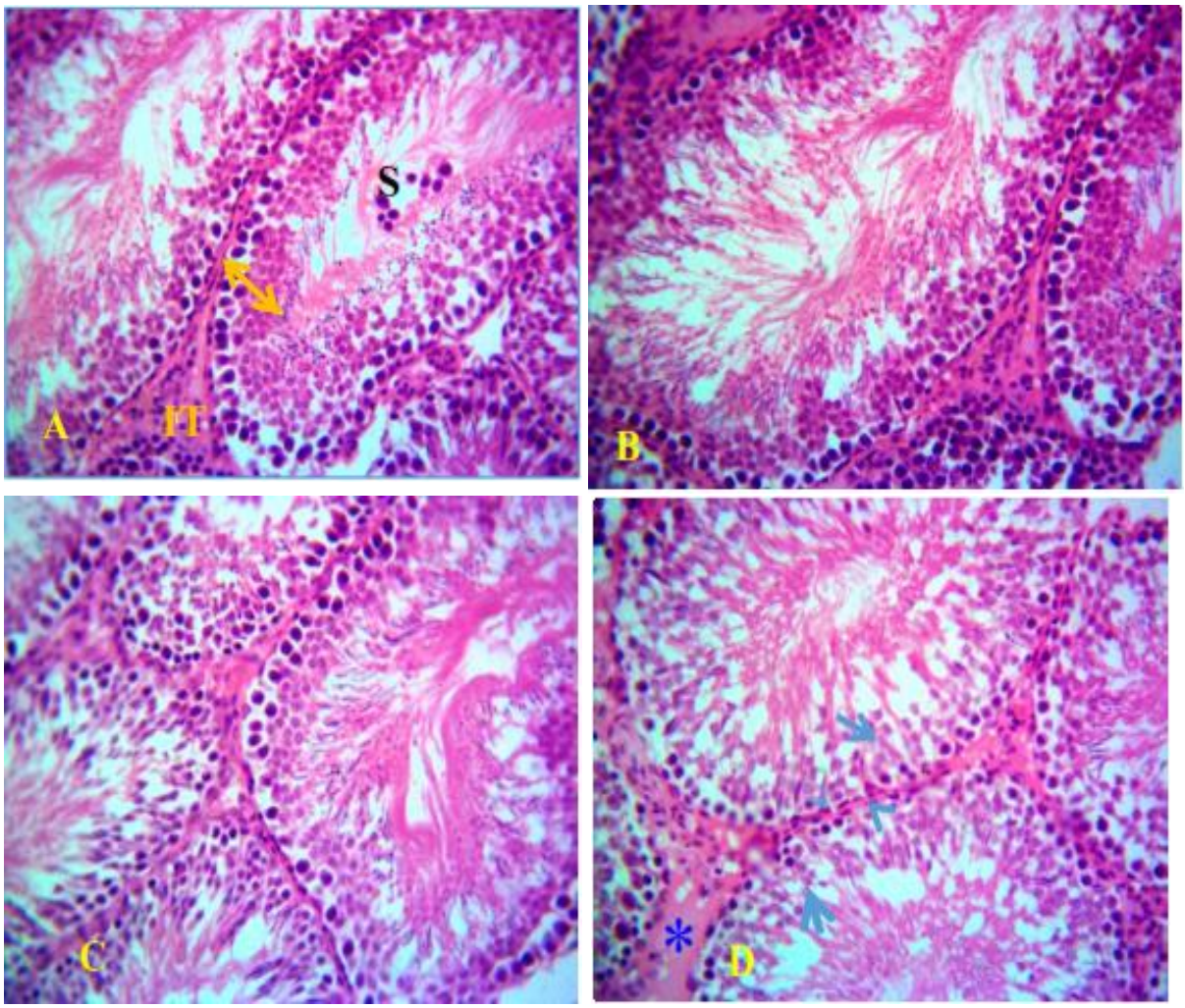

Plate III: (A) Normal testis of albino rats showing interstitium or fibrovascular stroma (IT), progression of spermatogenesis (double arrow covering various stages)and seminiferous tubule lumen (S) of group1/CENT, the control, on 14 days post treatment. H&E, X200. (B, C). Normal histologic architecture of testes in groups 2/CET2 and 3/CET5 on 14 days post treatment. H&E, X200. (D) Mild degeneration of spermatogenic cells in seminiferous tubules (arrows), multifocal shrunken spermatogonia at the basal layers of the seminiferous tubules (arrow heads) and moderate fibrinous exudates expanding the IT (*) in group 4/CET10 on 14 days post treatment. H&E, X200

1000mg/kg caused atrophy and colour change of testes in group 4/CET10, indicating its toxic effect on the testes when consumed at a higher dose. Toxicosis has been reported as one of the potential causes of testicular atrophy in animals (Foster, 2016).

The observed normal testicular histoarchitectural integrity of the rats in groups 2/CET2 and 3/CET5 showed that *C. citratus* has the capacity to enhance reproductive function by maintaining and improving the sperm quality in male albino rat. This agrees with the prior use of such extracts as a chemoprotective agent against hepatotoxicity (Rahim *et al*., 2014), and suggests that *C. citratus* can be used as potent antioxidant agent and also can enhance reproductive function in male albino rats due to its potent or higher flavonoid and phenolic components (Cheel *et al*., 2005; Rahim *et al*., 2013).

The observed degenerative lesions in the testes of rats in group 4/CET10 on 14 and 28 days post-treatment are evidence of altered structural and functional integrity of testicular tissues due to the toxicity of the *C. citratus* ethanolic extract at dose level of 1000mg/kg b.w to the male reproductive system, and might have directly interfered in the process of spermatogenesis. Interference of a toxic substance with spermatogenesis has been reported

to induce degenerative changes in the seminiferous tubule and interstitial cells in the testis of rats (Thakur *et al*., 2014). It is, therefore, possible for the extract at 1000mg/kg b.w to have directly interfered with spermatogenesis in the testes of the rats in group 4/CET10 and this could probably be responsible for the observed abnormal sperm morphology produced by the mildly atrophic testes. Testicular degeneration is manifested clinically and grossly as atrophy, mineralization, and fibrosis (Foster, 2017). Microscopically, it begins as a reduction in spermatogenesis, shrinking of tubular diameter,

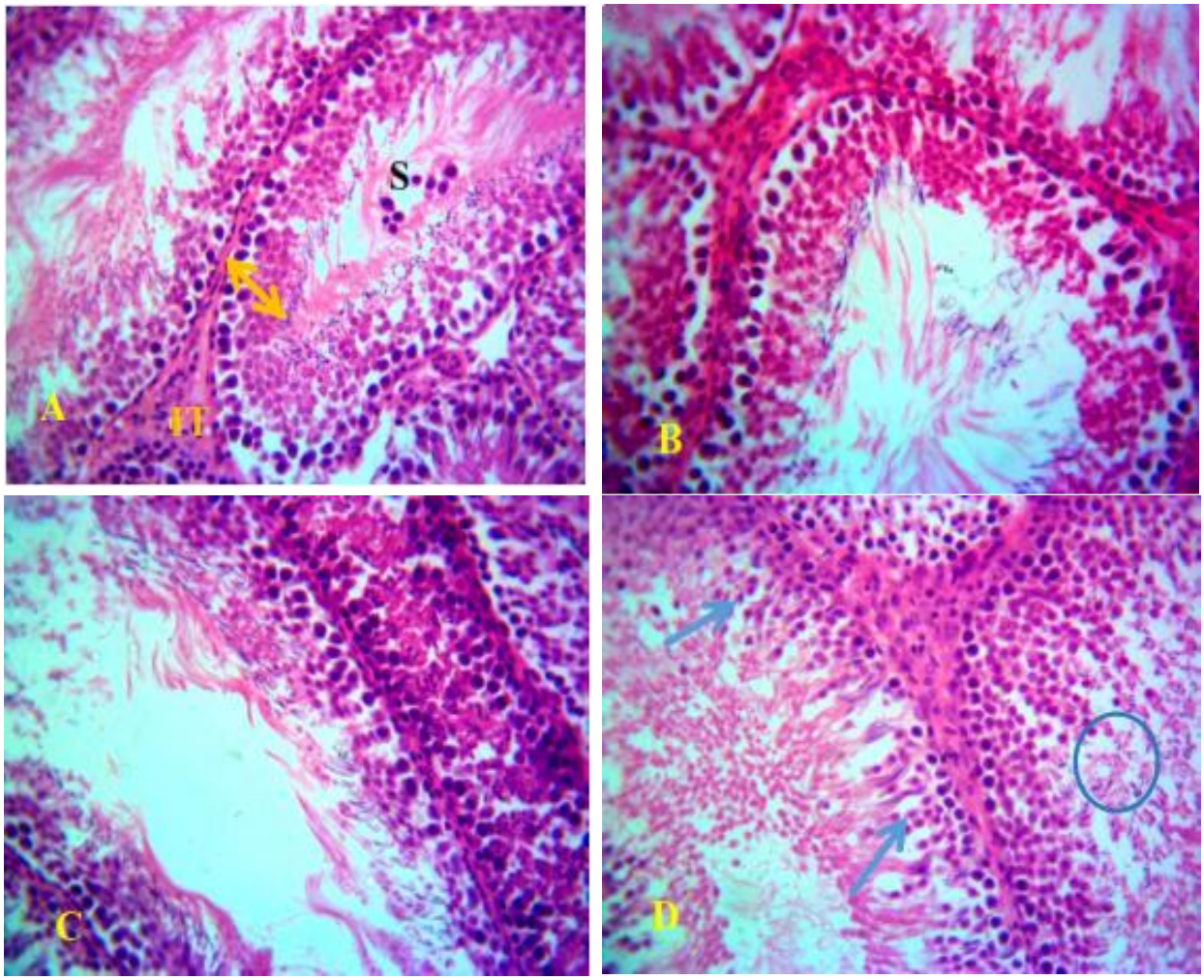

Plate IV: (A) Normal testis of albino rats showing interstitium or fibrovascular stroma (IT) , progression of spermatogenesis (double arrow covering various stages)and seminiferous tubule lumen (S) of group1/CENT, the control, on 28 days post treatment. H&E, X200. (B, C). Normal histologic architecture of testis in groups 2/CET2 and 3/CET5 on 28 days post treatment. H&E, X200. (D) Mild depleted amount in the generation of spermatogenic cells in seminiferous tubules (blue arrows) and fragmentation of spermatids (blue circle) in seminiferous tubule on the right, in group 4/CET10 on 28 days post treatment. H&E, X200.

Table 1: Comparison of serum testosterone values at various doses of *Cymbopogon citratus* in the treated groups compared to the control, Mean ± SD

Experimental groups	Day 0 (ng/ml)	Day 14 (ng/ml)	Day 28 (ng/ml)
Control, 1/CENT	14.25 ± 0.21^{a}	14.14 ± 0.32^{b}	14.94 ± 0.29^{a}
250mg/kg, 2/CET2	14.37 ± 0.14^{a}	17.31 ± 0.28^{c}	17.75 ± 0.73^{c}
500mg/kg 3/CET5	14.33 ± 0.29^{a}	17.13 ± 0.48^{c}	16.59 ± 0.40^{c}
1000mg/kg, 4/CET10	14.38 ± 0.29^{a}	12.40 ± 0.31^{a}	13.19 ± 0.22^{b}

$^{a, b, c}$ Different superscripts in a column indicate significant differences between the groups, $P < 0.05$

Table 2: Comparison of serum luteinizing hormone values at various doses of *Cymbopogon citratus* in the treated groups compared to the control, Mean ± SD

Experimental groups	Day 0 (MIU/ml)	Day 14 (MIU/ml)	Day 28 (MIU/ml)
Control, 1/CENT	0.84 ± 0.02[a]	0.92 ± 0.01[b]	0.94 ± 0.02[b]
250mg/kg, 2/CET2	0.84 ± 0.04[a]	1.03 ± 0.03[b]	1.13 ± 0.02[c]
500mg/kg 3/CET5	0.88 ± 0.02[a]	0.91 ± 0.03[b]	1.26 ± 0.01[c]
1000mg/kg, 4/CET10	0.87 ± 0.02[a]	0.86 ± 0.02[a]	0.65 ± 0.02[a]

[a, b, c] Different superscripts in a column indicate significant differences between the groups, P < 0.05

Table 3: Comparison of serum follicle stimulating hormone values at various doses of *Cymbopogon citratus* in the treated groups compared to the control, Mean ± SD

Experimental groups	Day 0 (IU/ml)	Day14 (IU/ml)	Day 28 (IU/ml)
Control, 1/CENT	1.60 ±0.24[a]	1.59 ± 0.01[b]	1.67 ± 0.06[b]
250mg/kg, 2/CET2	1.64 ± 0.05[a]	1.65 ± 0.02[bc]	1.92 ± 0.11[b]
500mg/kg 3/CET5	1.64 ± 0.05[a]	1.81 ± 0.03[c]	2.05 ± 0.12[c]
1000mg/kg, 4/CET10	1.64 ± 0.03[a]	1.43 ± 0.02[a]	1.55 ± 0.03[a]

[a, b, c] Different superscripts in a column indicate significant differences between the groups, P < 0.05

reduction in the number of Sertoli cells, reduction in interstitial endocrine cell size, and a wavy and thickened hyaline basement membrane (Foster, 2016). However, the later lesions were mild in the present study, which were not evident in the lower doses in this study.

The serum concentrations of testosterone, LH and FSH in rats in groups 2/CET2 and 3/CET5 were observed to increase when compared to groups 1/CENT (the control) and 4/CET10 on days 14 and 28 post-treatment. Our finding partly agrees with Rahim *et al*. (2013) who observed improved secretion of GSH and testosterone in serum with *C. citratus* aqueous extracts in experimental rats, but the group treated with *C. citratus* alone did not show any significant difference from that of the control rat group. This suggests the *C. citratus* extract's protective and therapeutic properties, as previously reported (Avoseh *et al*., 2015; Oladeji *et al*., 2019). These hormones have been reported to play a major role in the improvement of reproductive function in male reproductive system (Oduwole *et al*., 2021). Part of the reproductive function is the enhancement of steroidogenesis as recorded in the present study.

The lowered levels of reproductive hormones are well correlated with microscopic lesions in group 4/CET10.Testosterone initiates and maintains spermatogenesis, preserves the reproductive organs, stimulates nitrogen retention, and anabolic metabolism, and promotes body growth (Rahim *et al*., 2013; Pawlina, 2016). The significant decline in testosterone level in group 4/CET10 following administration of 1000mg/kg b.w of *C.citratus* was associated with impaired spermatogenesis caused by reduced testosterone secretion. These degenerative microscopic lesions might have decreased the serum hormone levels, as the affected cells were associated with the production of testosterone. LH stringently controls steroidogenesis. In the present study, the serum levels of LH and testosterone were decreased in group 4/CET10, which might have inhibited spermatogenesis in the spermatid stage, decreased testosterone levels, and reduced several key steroidogenic enzymes (Xu *et al*., 2007). In rodents, a strong positive correlation exists between circulating FSH and testis development, with decreased FSH leading to impaired spermatogenesis (Allan *et al*., 2004).

In conclusion, the results of the present study showed that *C. citratus* ethanolic extract at 250mg/kg b.w and 500mg/kg b.w has spermatogenesis and steroidegenesis-enhancing potentials of the testis, while 1000mg/kg b.w may be toxic to the proper functioning of the testis.These findings suggest that degeneration of spermatogenic cells and fibrinous exudates expanding the intertubular spaces, and decreased reproductive hormone levels are diagnostic features of *C. citratus* toxicity in males.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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