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Original Article

Sub-acute toxic effects of methanol root extract of *Carpolobia alba G. Don* on testes of adult Wistar rats

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Abstract

BACKGROUND AND AIM: Over 80% of the populations in some Asian and African countries depend on traditional medicine for primary health care. For centuries men and women have attempted to enhance their sexual performances by using substances derived from plant-based natural sources known as aphrodisiacs. *Carpolobia alba G. Don* is a popular natural recreational aphrodisiac plants commonly used in Nigeria. In this study, we assessed the effects of sub-acute administration of methanol root extract of *Carpolobia alba* on the testes of adult Wistar rats.

MATERIALS AND METHODS: Twenty adult male rats (weighing 200g-250g) were randomized into four groups consisting of five animals each. Each group was treated daily with distilled water, 100, 200 and 400mg/kg body weight respectively of methanol root extract *Carpolobia alba G. Don* for 30 days. The animals were sacrificed via cervical dislocation. Histological examination (Hematoxylin and Eosin stain), hormonal profile [Testosterone (T), Leutinizing hormone (LH), follicle stimulating hormone (FSH)], Oxidative stress biomarkers (MDA, SOD, CAT) and sperm analysis were done.

RESULTS: Daily administration of the extract resulted in marked degeneration and shrunken seminiferous tubules, degeneration and vacuolization of germinal epithelium, reduction in spermatogenic cells population, absence of late stage germ cells and significant reduction (<0.05) in T, LH, FSH, sperm parameters(sperm count, motility and morphology) and induction of oxidative stress in a dose-dependent manner

CONCLUSION: This result indicate anti-fertility potential of sub-acute consumption of methanol root extract of *Carpolobia alba G.Don* in male Wistar rats.

Keywords:
Carpolobia alba; sub-acute; aphrodisiacs; anti-fertility; histoarchitecture

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INTRODUCTION

The desire to increase sexual arousal, sexual pleasure and sexual potency has existed since the beginning of life. Sexual response in male and female reflects a dynamic balance between excitatory and inhibitory signals of the autonomic nervous system within the external genitalia and throughout the central nervous system (Goldstein, 2022). Men and women have for centuries sought different ways of gaining sexual fulfillment including the use of aphrodisiacs; this quest has been shown to increase rather than decline over the years (Abdullahi and Tukur, 2013). Over 80% of the populations in some Asian and African countries depend on traditional medicine for primary health care. The World Health Organization (WHO) estimates that in many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine including Aphrodisiac substances (WHO, 2005).

Aphrodisiacs are a family of substances that stimulate sexual activity, pleasure, or desire. They also have a significant potential to treat mild to severe erectile dysfunction. *(Agrahari et al., 2021).* Though main indication of Aphrodisiac substances is erectile dysfunction (Christopher and Cindy, 2011), these drugs are excessively and recreationally use for sexual enhancement. Those with normal erectile functioning do sometimes use aphrodisiacs (Ishikura *et al.,* 2000). aphrodisiacs are used recreationally for motives and reasons like : add to the fun, maintain an erection, counteract the effects of alcohol and drugs, increase erectile rigidity, have sex for hours, impress sexual partner, enhance self-esteem, curiosity, increase sex drive, decrease refractory phase, improve sensation (Kim *et al.,* 2001). The rate of consumption of natural aphrodisiacs especially among young men is alarming and disturbing. Furthermore, there is limited scientific evidence supporting the efficacy and safety of natural aphrodisiacs which is worrisome as it could

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lead to abuse of aphrodisiacs (Manortey *et al*, 2018). One of such common aphrodisiacs traditionally used in Nigeria is *Carpolobia alba*. *Carpolobia alba* is a shrub or small tree which is 3 to 6 metres (9.8 to 19.7 ft) tall. Its branches are querulous or shortly pubescent. Its leaves are membranous or slightly leathery. This type of plant belongs to the milkwort family (Polygalaceae) and is native to Western Tropical and Central Africa's rainforests, forest edges, and savanna parks at elevations lower than 400 meters (1,300 feet). (Lucky et al., 2015). *Carpolobia alba* is a popular natural recreational aphrodisiac plants commonly used by pygmies of the forest region (Ipassa of Garbon, South-Cameroon; and the Yoruba, Efik, Ibo,and Ijaw peoples of Nigeria,) and other part of sub-Sahara Africa (Nwidu *et al*., 2015). It is commonly called Cattle stick in English, *Egbo Oshunshun* in Yoruba, *Angalagala* in Igbo, *Ikpafum* in Ibibio (Nwidu *et al.,* 2015). *Carpolobia alba* root has long been used to treat male sterility, increase virility, and increase male fertility. It has been reported to have aphrodisiac and androgenic effects. (Manfo *et al.,* 2014). Ethnomedicinal reports reveal that the leaf has anti-inflammatory and anti-arthritic, and effective in treating wounds, leprosy, venereal diseases, fever that comes with diarrhea, and diabetes mellitus. (Nwidu *et al.,* 2015). The Phytochemical screenings of *Carpolobia alba* confirmed the presence of various phytochemicals such as saponins, tanins, flavonoids, Anthraquinones, Terpenes and Terpenoids, Polyphenols, Alkaloids, cardiac glycosides and simple sugars (Nwidu and Nwafor, 2009; Sun *et al.,* 2011; Ettebong *et al.,* 2011; Chan *et al.,* 2013; Tagousop *et al.,* 2018).

The effects of sub-acute administration of *Carpolobia alba* on male reproductive functions has not been reported. This study aims to fill this gap by assessing the effects of sub-acute administration of methanolic extract of *Carpolobia alba* on testicular histoarchitrcture, hormonal profile and sperm parameters of male Wistar rats.

MATERIALS AND METHODS

Plant Harvest and Extraction

The *Carpolobia alba* root was obtained from Ayede Ogbese in Akure North Local Government, Ondo state, Nigeria and it was authenticated in the herbarium of Department of Plant Biology and Biotechnology, University of Medical Sciences Ondo state, Nigeria with voucher number UNIMED P.B.T.H No 034. After air drying, the *Carpolobia alba* root (2.20kg) was pulverized into powder after which Carpolobia alba root was extracted using Soxhlet with pure methanol as the solvent. After filtering the methanol containing the extract, the solvent was vacuum-distilled in a rotary evaporator at 4^{o}C. To verify that all remaining solvent was gone, the remaining extract was lastly dried for two hours at 30^{o}C in a vacuum oven. 90.22g (1.69% yields) of the powdery mass were obtained which was stored at room temperature for the study. Distilled water was used to prepare a fresh solution of the extract.

Phytochemical screening

Qualitative phytochemical screening of the extract was done following the method of Odebiyi & Sofowora (1978) and Trease & Evans (1989).

Animal experimentation and design

Twenty male Wistar rats (weighing 200-250g) were procured from the Animal House of University of Medical Sciences, Ondo State, Nigeria. The rats were acclimatized for two weeks before the commencement of the experiment. The experimental animals were housed in standard laboratory conditions. The animals were exposed to light and dark cycle of 12 hours and fed rat show and water *ad libitium*. Twenty adult Wistar rats were randomly divided into four groups of five rats each. Group 1 (control) received only distilled water; Group 2,3 and 4 received 100mg/kg, 200mg/kg and 400mg/kg body weight respectively of methanol root extract of *Carpolobia alba* for 30 days.

Sample Collection

Twenty-four hours after the last administration, the animals were anesthetized with 50 mg/kg body weight of sodium thiopentone. Blood samples were collected by cardiac puncture, transferred into plain bottles under aseptic conditions, allowed to clot and retract then centrifuged for 5 minutes at 4000 revolutions per minute to obtain serum, used for hormone profile. The testes and epididymis of the animals were harvested and used for sperm analysis and histological studies.

Determination of serum male reproductive hormones

The concentrations of testosterone, FSH and LH in the serum of the animals were determined via the enzyme-Linked immunosorbent Assay (ELISA) method. The procedure used in the determination of the concentration followed the method described by the manufacturer of the Kit (Calbiotech Inc, USA) and met the WHO research program standard for reproductive studies.

Sperm Analysis

Sperm count: The epididymides were harvested, quickly cleared of fatty tissue. Sperm suspensions were prepared by placing the right caudal epididymis in a pre-warmed (37°C) cell-culture dish with 1mL of Phosphate Buffered Saline. The left caudal epididymis was then minced with a surgical blade, kept at 37°C for 10 minutes to allow spermatozoa to swim out into the solution. The solution was aspirated using a Pasteur pipette and placed on a glass slide and observed at ×100 magnification under an Olympus light microscope equipped with a Makler counting chamber (Sefi-Medical Instruments, Haifa, Israel). The sperm count, motility and morphology were evaluated.

Sperm motility: All progressive motile, non-progressive motile, and immotile spermatozoa were counted in 10mL of sperm suspension. The percentage of motile spermatozoa was visually estimated using a microscope set to 400x magnification.

Spermatozoa that did not move were considered as nonmotile. After that, the percentage of motile spermatozoa was estimated.

Sperm morphology: A drop of sperm suspension was added into an equal volume 1% eosin-y 5% nigrosin, which was then mixed together and smeared on pre-warmed clean glass slides and air-dried. Using an Olympus light microscope, two hundred sperm cells were examined at × 400 magnifications per animal to determine the morphological abnormalities. Morphology of the sperm cells was categorized based on the presence of one or more abnormal features (Teratospermia) such as tail defects (short, irregular, coiled or multiple tail); neck and middle piece defects (distened, irregular, bent middle piece , abnormally thin middle piece); and head defects (round head, small or large size, double or detached head). The morphology was in percentage.

Harvest of organ and Tissue processing

Using a midline abdominal incision, abdominal cavity was opened to access the two testes which were excised and fixed in a 4% buffered paraformaldehyde, dehydrated in ascending grades of ethanol, cleared in xylene, infiltrated and embedded in paraffin wax. The tissue blocks were mounted on a wooden block and trimmed to size at 20µ thick. They were sectioned on a rotatory microtome at 7µ thick. The sections were stained with Haematoxylin and Eosin. Photomicrographs were taken using the 5 mega pixel Amscope digital scope, mounted on an Olympus microscope.

Statistical Analysis

The analysis was done with GraphPad Prism software version 10.1. Both descriptive and inferential statistics (one-way ANOVA) were done on all obtained data. Turkey's multiple comparison was used to test for statistical significant difference between control and experimental groups. The data were represented as Standard Error of Mean and the level of differences were considered significant at $p<0.05$.

RESULT

Phytochemical Constituent

Result obtained confirmed the presence of chemical constituents such as steroids, flavonoids, alkaloids, tannins, saponins, phenol, cardiac glycosides and trapenoids.

Hormone profile

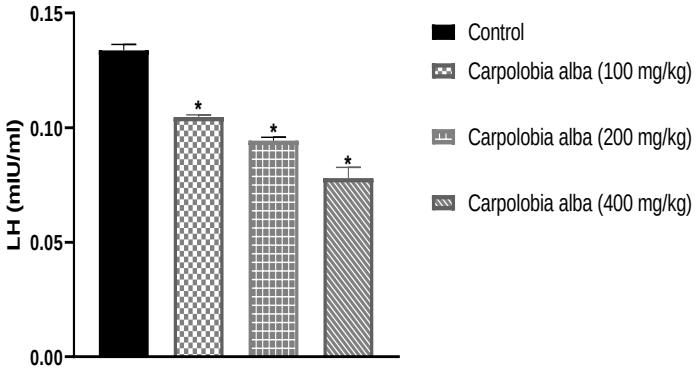


Figure 5: Effect of methanol root extract of *Carpolobia alba* on serum LH level. Bars represent Mean ± SEM (n=5) (one-way ANOVA followed by Tukey *post hoc test*). *p< 0.05 relative to control

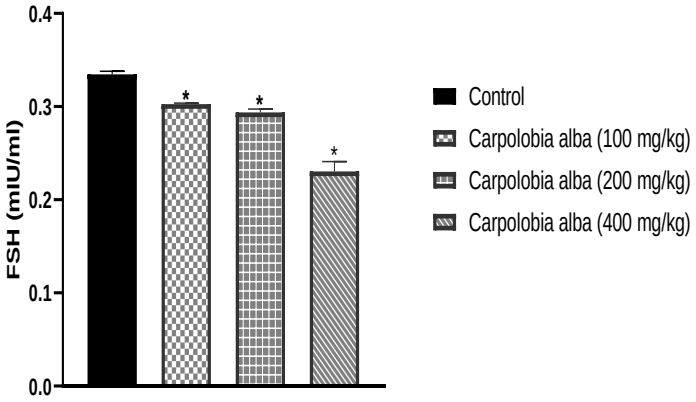


Figure 6: Effect of methanol root extract of *Carpolobia alba* on serum FSH level. Bars represent Mean ± SEM (n=5) (one-way ANOVA followed by Tukey *post hoc test*). *p< 0.05 relative to control.

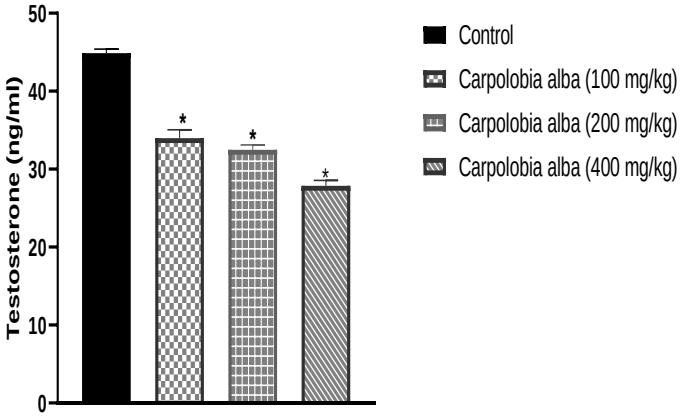


Figure 7: Effect of methanol root extract of *Carpolobia alba* on serum testosterone level. Bars represent Mean ± SEM (n=5) (one-way ANOVA followed by Tukey *post hoc test*). *p< 0.05 relative to control.

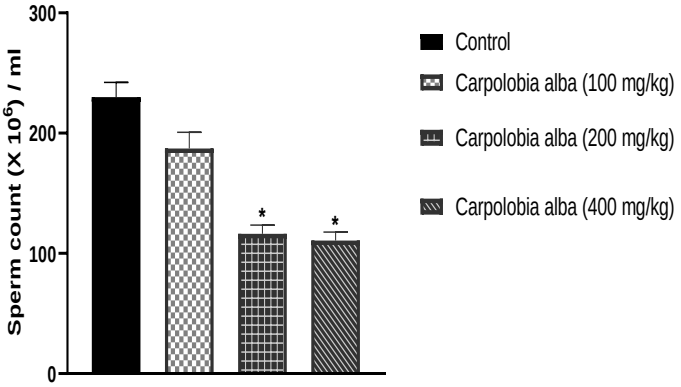


Figure 8: Effect of methanol root extract of *Carpolobia alba* on sperm count. Bars represent Mean ± SEM (n=5) (one-way ANOVA followed by Tukey *post hoc test*). *p< 0.05 relative to control.

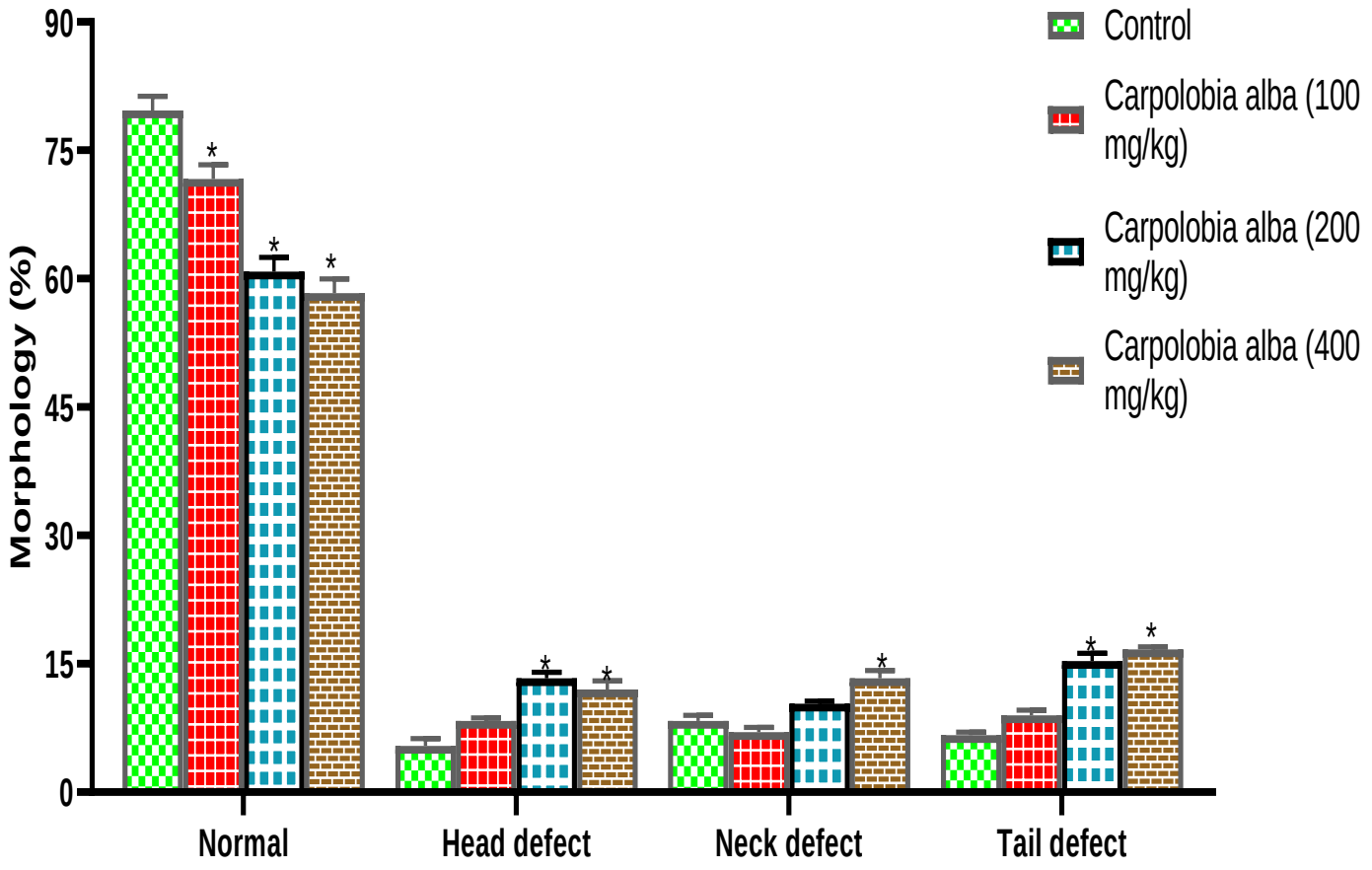


Figure 9: Effect of methanol root extract of *Carpolobia alba* on sperm morphology. Bars represent Mean ± SEM (n=5) (one-way ANOVA followed by Tukey *post hoc test*). *p< 0.05 relative to control.

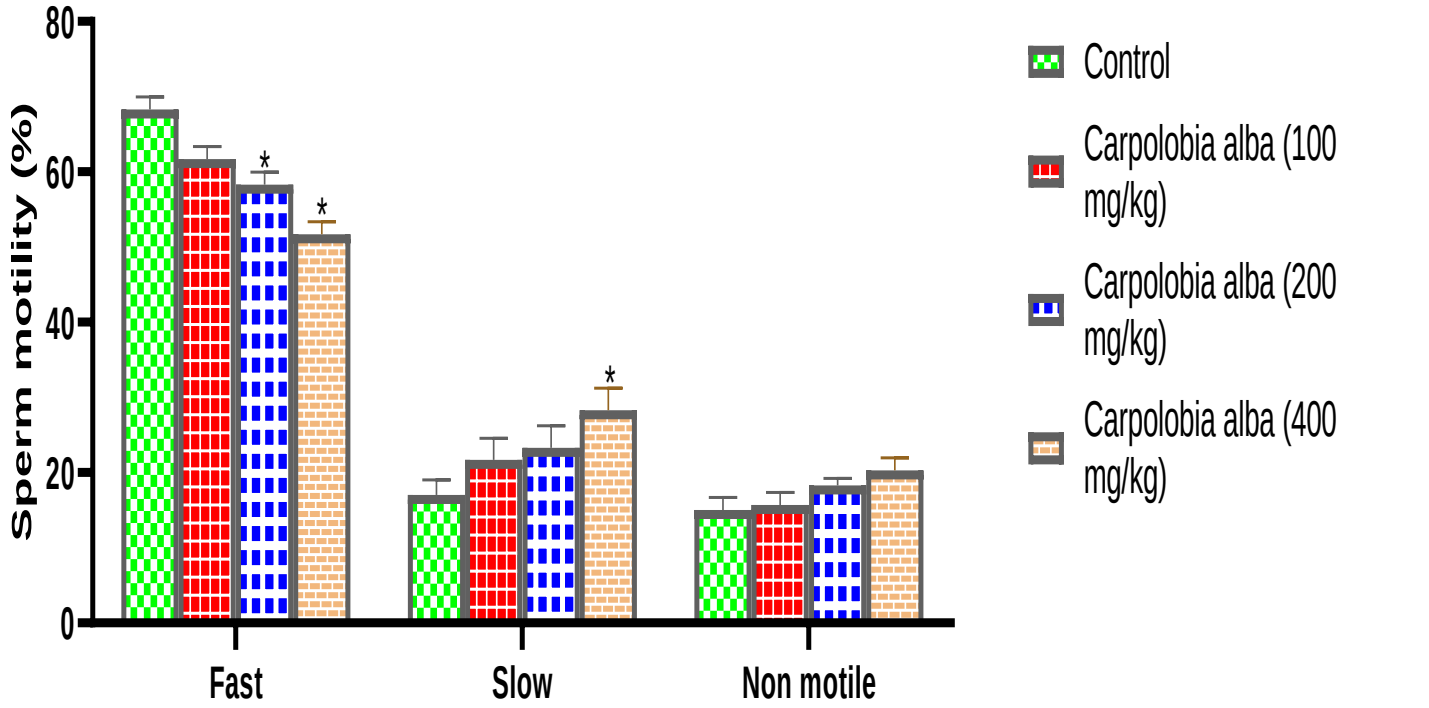
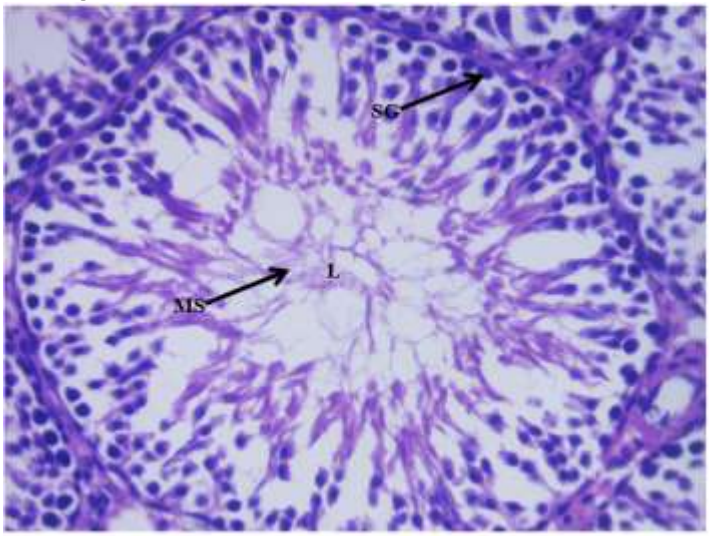


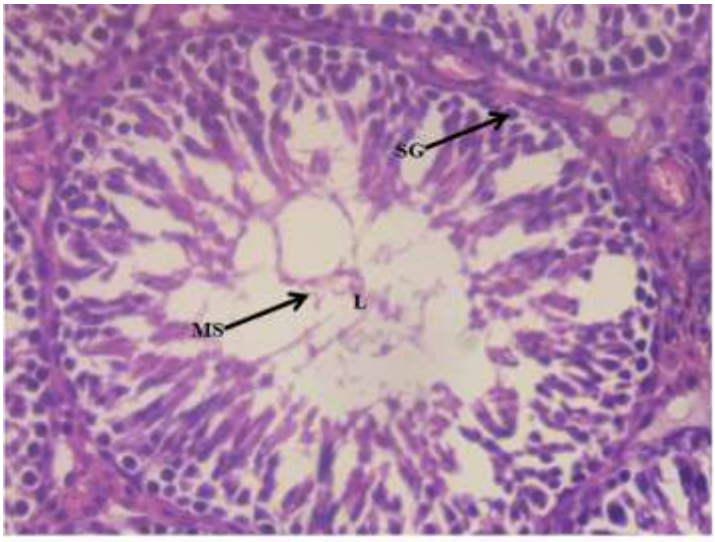
Figure 10: Effect of methanol root extract of *Carpolobia alba* on sperm motility. Bars represent Mean ± SEM (n=5) (one-way ANOVA followed by Tukey *post hoc test*). *p< 0.05 relative to control.

Histological Assessment



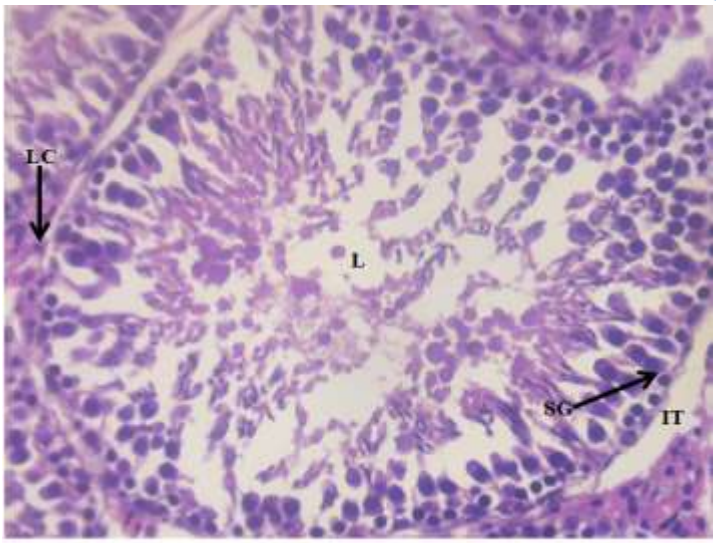
X 400

Figure 1:Representative micrograph (Hematoxylin and Eosin stain) of testes of Wistar rat in control showing normal testicular microarchitecture with normal spermatogenic series from spermatogonia (SG) to mature sperm cell (MS) in the lumen (L) indicating normal germinal epithelium and spermatogenic activity.



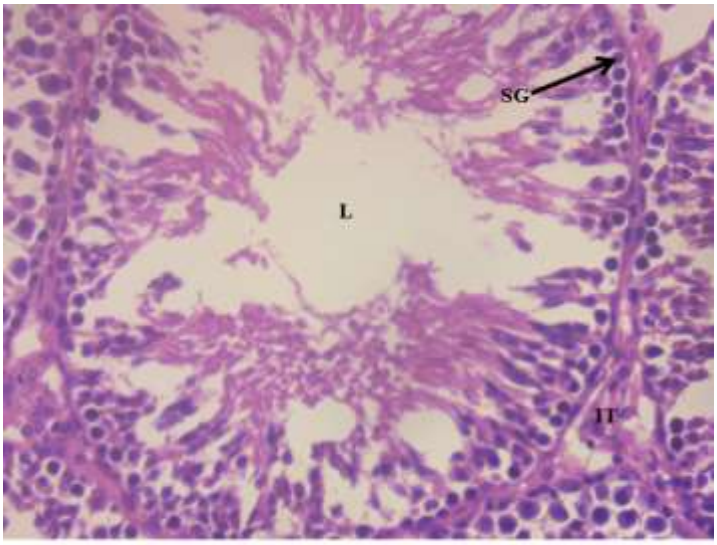
X400

Figure 2: Representative micrograph of Hematoxylin and eosin stained testicular sections of group that receive 100mg/kg body weight of *Carpolobia alba* has similar microarchitecture with the control group showing normal regular crossly sectioned seminiferous tubules having a lumen (L) which appears to have slightly fewer cell population of spermatogenic cell series from spermatogonia (SG) and mature sperm cell (MS) when compared with control.



X400

Figure 3: Representative micrograph of Hematoxylin and eosin stained testicular sections of the group that received 200mg/kg body weight of *Carpolobia alba* showing crossly sectioned seminiferous tubules lined with spermatogonia (SG) and fewer layers of spermatogenic series when compared to control. The lumen seems to be filled with less spermatozoa with increased amount of insterstitial tissue (IT), increased amount of small and shrunken leydig cells (LC) when compared to control.



X 400

Figure 4: Representative micrograph of Hematoxylin and eosin stained testicular sections of the group that received 400mg/kg body weight of *Carpolobia alba* showing crossly sectioned seminiferous tubules lined with spermatogonia (SG) and more fewer layers of spermatogenic cell series, with empty lumen (L) and much more increased amount of interstitial tissue when compared to control.

DISCUSSION

The root of *Carpolobia alba* (G. Don) has been reported to have aphrodisiac properties, androgenic effect and used traditionally in curing male sterility, enhancement of virility and male fertility (Manfo et al., 2014). In recent times, there have been increased desire and quest for sexual pleasure and satisfaction which has led to indiscriminate use of plant-based aphrodisiacs. Although, some authors have reported that methanol extract of CA increased mating behavioral (Kenmongne *et al.,* 2015), there are no scientific report on the effects of sub-acute administration of methanol root extract of *Carpolobia alba* on testicular histology and functions. In view of this, this study was designed to provide histoarchitectural and functional evidences on the effects of this plant extract on male reproductive functions in adult male Wistar rat.

One of the important techniques in evaluating the impact of substances on the body's internal environment is histological studies. It gives insight into the state of the cells and tissues in healthy and diseased conditions. Histological results from this study shows normal histology of the testes in the control group with healthy spermatogenic cell lineage, germinal epithelium, sertoli cells and leydig cells. Animals that received 100mg/kg body weight of *Carpolobia alba* show similar testicular microanatomy with the control. However, slight reductions in the population of late spermatogenic cells were observed. Group that received 200mg/kg body weight of *Carpolobia alba* show moderate degeneration of seminiferous tubule and interstitial edema with slight vacuolization of germinal epithelium. At 400mg/kg, severe degeneration and shrinkage of the seminiferous tubule, wider vacuolization and atrophy of the germinal epithelium were observed. The structural changes are due to abundant presence of steroid in the extract, prolonged usage of steroid are known to cause adverse effects which could potentially lead to histological damage in various organs. This result agrees with the work reported by Tousson *et al.* (2012) who documented that steroids leads to testicular shrinkage and decrease in the cellular components.

Male reproductive functions are largely controlled by reproductive hormones. These hormones which include testosterone, luteinizing and follicle-stimulating help in regulating spermatogenesis. In this study, testosterone, luteinizing and follicle-stimulating hormones reduced significantly ($p<0.05$) in the groups treated with methanol extract of *Carpolobia alba*. This is consistent with the study done by Akintunde *et al.* (2020), who reported that administration of methanol extract of *Carpolobia lutea* significantly reduced serum testosterone level. This suggests that the extract prevent the stimulation of endocrine cells in the hypothalamic-pituitary-testicular axis that is responsible for the secretion of male reproductive hormones. This could also be as a result of the abundant steroid present in the extract which has been reported to suppress natural testosterone production.

On the semen analysis, there was significant decrease in the sperm count, and motility in the 200 and 400mg/kg body weight groups when compared to the control group. Also, animals in the 200 and 400mg/kg group have more sperm cells with head, neck and tail defects. This findings is consistent with the work of Saddick (2017) and Lewis et al.,(2021) who reported that Prolonged use of steroids suppress the body natural antioxidant defense system , induce oxidative stress hereby significantly reducing sperm count and motility with morphological defects.

The anti-fertility effects observed in the extract-treated group could be as a result of the chemical constituent of the plant. Steroids and saponin contained in the extract has been found induce poor reproductive functions in rats.

Conclusion

Result obtained from this study suggests that sub-acute administration of methanol extract of *Carpolobia alba* (G. Don) disrupts testicular histoarchitecture, inhibit the production of reproductive hormones and induces poor sperm qualities. This implies that indiscriminate use of *Carpolobia alba* as an aphrodisiac can possibly lead to male infertility.

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