Effects of Vitamin C on Testicular Function of Male Wistar Rats Treated with Xylopia Aethiopica

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Abstract

Xylopia Aethiopica fruits locally called "Uda" by the south eastern part of Nigeria is a highly valued plant in Igbo land, as the fruit is used as spices and aqueous decoction, especially after child birth, probably for its antiseptic properties and to arrest bleeding. Previous studies have shown that prolonged use of Xylopia Aethiopica fruits could induce reproductive impairment. In this study, effects of Vitamin C on testicular function of Wistar rats treated with Xylopia Aethiopica were investigated to see if Vitamin C can have ameliorative effects.

Five groups were used; negative control (group 1), dimethylsulfoxide (DMSO) (0.5 ml/kg) positive control (group 2), Xylopia Aethiopica fruits crude extract (300 mg/kg/day) (group 3), Vitamin C (100 mg/kg/day) (group 4), and Xylopia Aethiopica fruits crude extract (300 mg/kg/day) + Vitamin C (100 mg/kg/day) cotreatment (group 5). All treatments were administered orally for 60 days. Sperm parameters, FSH, LH, estrogen and testosterone were evaluated.

The result showed that Xylopia Aethiopica fruit extract altered spermatogenesis, hormonal profiling, hormonal fertility index, sperm capacitation, acrosomal-reaction and histoarchitecture of testes. However, Vitamin C was shown to avert Xylopia Aethiopica-induced inhibition of sperm capacitation and acrosomal-reaction. This study showed that *Xyolopia aethiopica* may cause reproductive impairment and that Vitamin C was able to ameliorate Xylopia Aethiopica-induced inhibition of spermatogenesis, hormonal profile, epididymal sperm capacitation and acrosomal reaction in rats, which may be due to increased testicular antioxidant activities. In conclusion, this result shows that the consumption of extract of Xylopia Aethiopica in male may cause reproductive impairment and administration of Vitamin C could ameliorate Xylopia Aethiopica-induced testicular impairment in rats.

Introduction

Infertility as a disease of the reproductive system is defined as the failure to achieve a clinical pregnancy after twelve months or more of regular unprotected sexual intercourse (WHO, 2010). Male factor infertility can contribute between 30-50% to this condition and may result from several factors such as physiological, systemic pathologies, genetic abnormalities, environmental pollution and oxidative stress (Oelmedo et al., 2005; Maneesh et al., 2006). The WHO reported (2010) that male infertility is commonly due to deficiencies in the semen volume, sperm concentration and sperm quality. These deficiencies often cause abnormalities such as azoospermia (absence of spermatozoa), oligozoospermia (decreased number of spermatozoa), asthenozoospermia (decreased sperm motility), teratozoospermia (abnormal sperm morphology) and or a combination of the three conditions (oligo-astheno-teratozoospermia) (Dohle et al., 2005).

Xylopia Aethiopica is a slim, tall, evergreen, aromatic tree of the Annonaceae family whose height could reach over 20 m with a stem girth up to about 60-70 cm in diameter (Burkhill et al., 1985). It is native to tropical African rainforests and moist fringe forests in the Savanna Zones of Africa (APD, 2007). Xylopia Aethiopica is used locally in Ghana as cough remedy, a carminative, a postpartum tonic, and to treat uterine fibroid and amenorrhea (Asekun and Adeniyi, 2004). It is also use as spice in the preparation of most local food in Ghana and Nigeria, notably in Igbo community where it's mostly used as a traditional spice in making pepper soup. However, previous studies have demonstrated that Xylopia aethioica fruit crude extract can impair sperm integrity, hormonal indices, reduce reproductive organ weights, and eventually resulting in testicular failure, while others shows that Xylopia aethioica fruit crude extract improved reproductive functions. Thus, this study aims at assessment of the effects of Vitamin C on the testicular function of male rats treated with Xylopia Aethiopica fruit crude extract.

Material and Method

A total of twenty (25) male adult Wistar rats weighing between 150 g and 200 g were used for this experiment. The animals were obtained and housed in the College of Medicine animal house, Enugu State University of Science and Technology for the study. The animals were housed in standard laboratory condition with 12:12 hour light and dark cycle at 25° C ±2° C and allowed free access to standard commercial rat pellets with standard composition and water ad libitum. Each group were kept in separate cage which were cleaned daily and washed weekly (during the period of the research) with proper identification. The animals were allowed to acclimatize for 2 weeks before the commencement of administration.

Fruits of the Xylopia Aethiopica was bought from New Market, Enugu State, Nigeria and was used for the study. Xylopia Aethiopica was extracted according to the method described by E. Woode et al. (2011). Vitamin C was obtained from Rhenocks Pharmacy, Enugu, Enugu State, Nigerian and Vitamin C was administered at 100 mg/kg body weight per day following the administration dose used by Akorede et al., 2020. Dimethylsulfoxide (Mistral industrial chemicals, UK) was used as a vehicle to reconstitute the crude extract of Xylopia Aethiopica.

Experimental Design

After acclimization, the experimental animals were randomly divided into 5 groups (n = 5). Rats in group 1 received Distilled water (10 ml/kg) and served as normal control, group 2 received Dimethylsulfoxide (DMSO) (0.5 ml of 5%) and served as vehicle control, groups 3 had fruit extract of Xylopia Aethiopica (300 mg/kg, p.o./day), group 4 was given Vitamin C (100 mg/kg, p.o./day), while group 5 receive the combination of fruit extract of Xylopia Aethiopica (300 mg/kg, p.o./day), + Vitamin C (100 mg/kg, p.o./day). The vehicle, extract and Vitamin C were given orally for 60 days. All treatments were administered 30 minutes between each treatment.

Samples Collection

At the end of the experimental period, the animals were euthanized by cervical dislocation. Blood was collected by ventricular punctures for hormonal assay. The testes and epididymis were carefully dissected out and weighed on electronic weighing balance. The epididymis was used for semen analysis, some of the testes were used to process tissue histology and photomicrographs produced on them.

Result

The result of the effects of Vitamin C on testicular function following administration of crude extract of Xylopia Aethiopica fruit in male Wistar rat treated for 60 days is as follows.

1. Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on changes in Body Weight, Epididymal and Testicular Weights in Male Rats

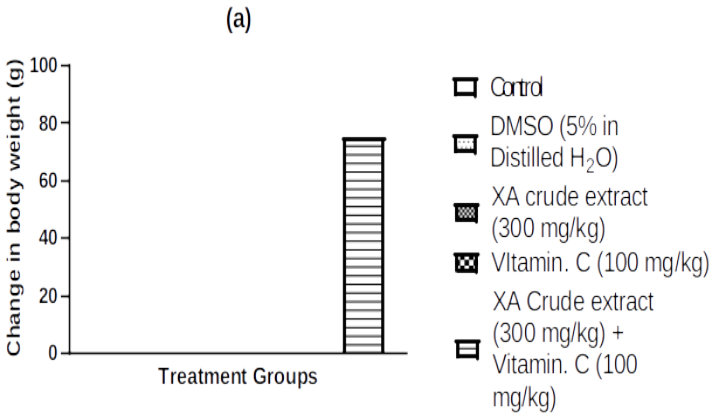


Figure 1a: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Changes in Body Weight in Male Rats

NS = Not Significant when Compared to the Control

Bars represent Mean ± S.E.M. (n = 5) (One-way ANOVA followed by Benferroni post hoc test.

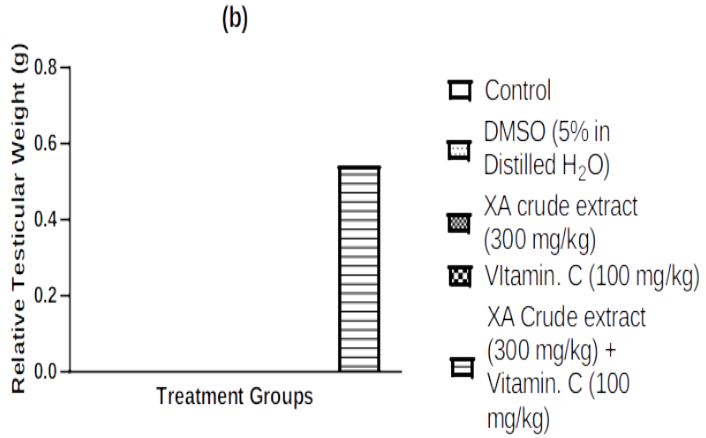


Figure 1b: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Changes in Testicular Weight in Male Rats

NS = Not Significant when Compared to the Control

Bars represent Mean ± S.E.M. (n = 5) (One-way ANOVA followed by Benferroni post hoc test)

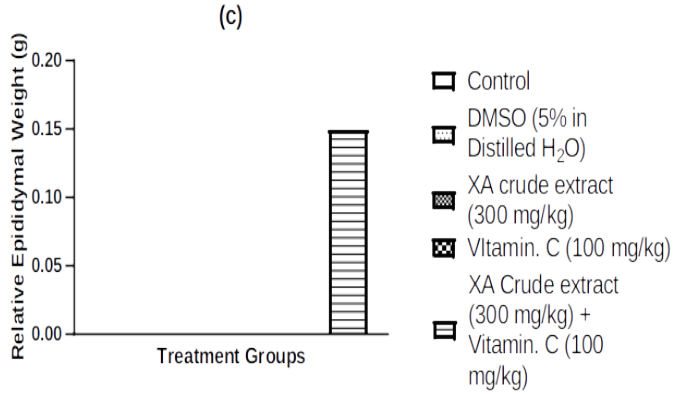


Figure 1c: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Changes in Epididymal Weight in Male Rats

NS = Not Significant when Compared to the Control

Bars represent Mean ± S.E.M. (n = 5) (One-way ANOVA followed by Benferroni post hoc test).

2. Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Sperm Indices in Male Rats

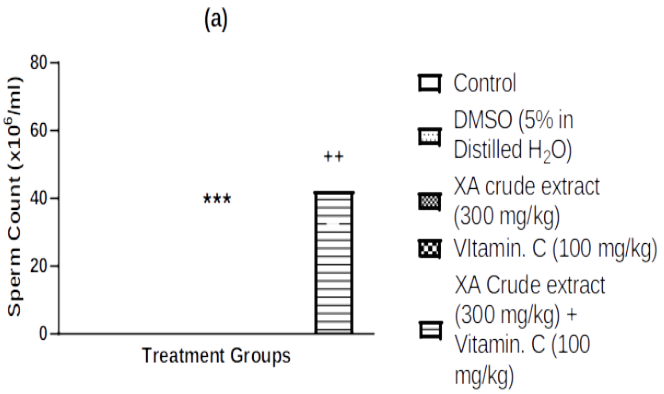


Figure 2a: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Changes in Sperm Count in Male Rats

There was a signicant decrease in sperm count compare to the control and Vitamin C combined treated rats.

Data are expressed as mean ± S.E.M. (n = 5) (One-way ANOVA followed by Benferroni post hoc test). $^{***}p < 0.0001$ when compared with controls; $^{++}p < 0.001$, when compared with Xylopia Aethiopica crude extract (treatment protocol of Vitamin C and Xylopia Aethiopica crude extract administration for 60 days).

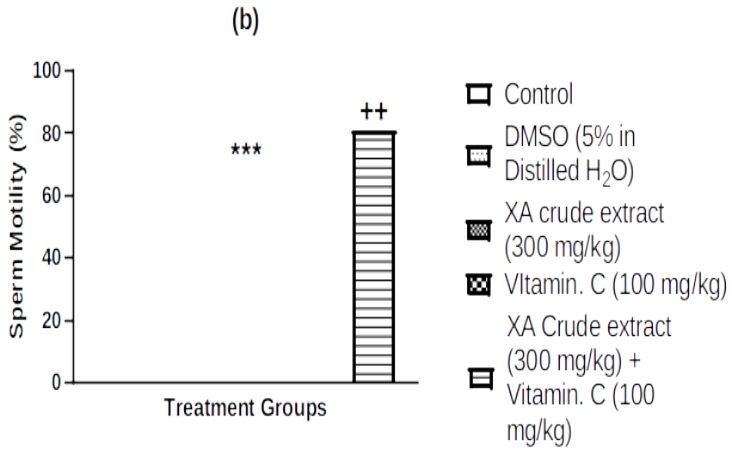


Figure 2b: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Changes in Sperm Motility in Male Eats

There was a significant decrease in sperm motility compare to the control and Vitamin C combined treated rats.

Data are expressed as mean ± S.E.M. (n = 5) (One-way ANOVA followed by Benferroni *post hoc* test). $^{***}p < 0.0001$ when compared with controls; $^{++}p < 0.001$, when compared with Xylopia

Aethiopica crude extract (treatment protocol of Vitamin C and Xylopia Aethiopica crude extract administration for 60 days).

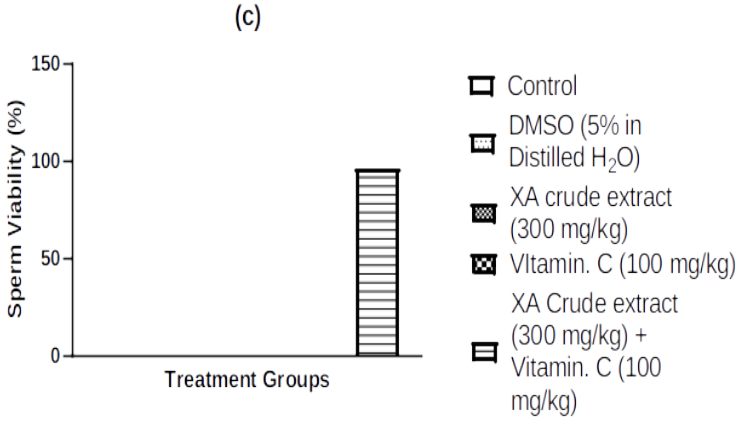


Figure 2c: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Changes in Sperm Viability in Male Rats
NS = Not Significant Compared to the Control

Data are expressed as mean ± S.E.M. (n = 5) (One-way ANOVA followed by Benferroni *post hoc* test). $^{***}p < 0.0001$ when compared with controls; $^{++}p < 0.001$, when compared with Xylopia Aethiopica crude extract (treatment protocol of Vitamin C and Xylopia Aethiopica crude extract administration for 60 days).

Table 1: Effect of Vitamin C and Xylopia Aethiopica Crude Extract on the Possible Changes in Sperm Acrosome Integrity after Incubation in Sperm Capacitation Medium using a Commassie Brilliant Blue in Male Rats

Group	Acrosome Intact Uncapacitated Sperm (%)	Acrosome Intact Capacitated Sperm (%)	Acrosome Reacted Capacitated Sperm (%)
Control	2.80 ± 0.37	2.25 ± 0.95	95.86 ± 0.91
DMSO (5% in DW)	3.40 ± 1.03	2.75 ± 0.48	94.17 ± 1.08
XA crude extract (300 mg/kg)	24.60 ± 2.06*	28.75 ± 1.18*	45.65 ± 0..15*
Vitamin C (100 mg/kg)	1.75 ± 0.47 +	2.75 ± 0.95 +	95.75 ± 0.85 +
XA crude extract (300 mg/kg) + Vitamin C (100 mg/kg)	2.25 ± 0.63 +	1.25 ± 0.95 +	96.50 ± 1.19 +

Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{*}p < 0.05$ when compared with control; $^{+}p < 0.05$ when compared with Xylopia Aethiopica crude extract.

3. Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on the Possible Changes in Hormonal Indices in Male Rats

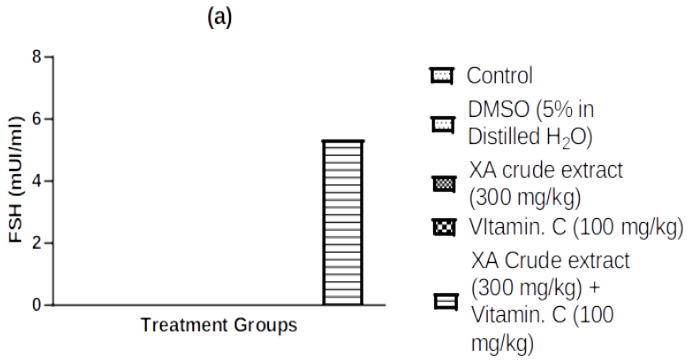


Figure 3a: Effect of Vitamin C and Xylopia Aethiopica Fruit Ethanolic Crude Extract on FSH in Male Rats
NS = No Significant Changes in FSH Compare to the Control Group Animals

Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{***}p < 0.001$ when compared with control; $^{++}p < 0.01$ when compared with Xylopia Aethiopica crude extract (300mg/kg).

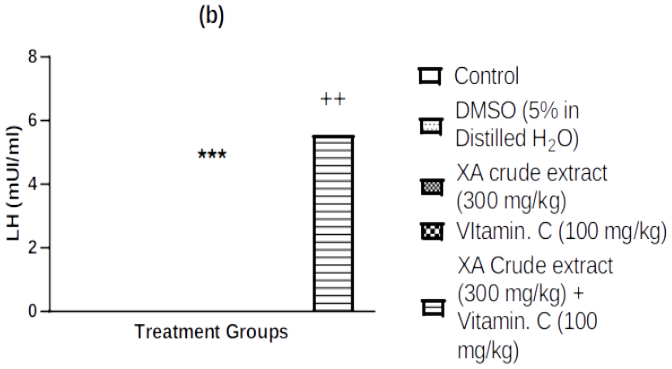


Figure 3b: Effect of Vitamin C and Xylopia Aethiopica Fruit Ethanolic Crude Extract on LH in Male Wistar Rats

LH significantly decreased compare to the control and Vitamin C combined treated rats
Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{***}p < 0.001$ when compared with control; $^{++}p < 0.01$ when compared with Xylopia Aethiopica crude extract (300mg/kg).

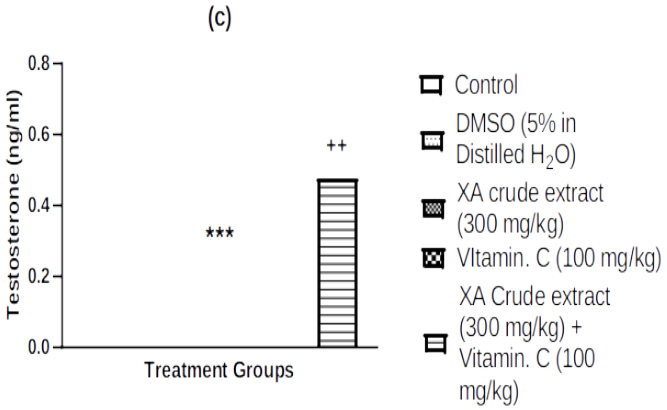


Figure 3c: Effect of Vitamin C and Xylopia Aethiopica Fruit Ethanolic Crude Extract on Testosterone in Male Wistar Rats

Testosterone significantly decreased compare to the control and Vitamin C combined treated rats Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{***}p < 0.001$ when compared with control; $^{++}p < 0.01$ when compared with Xylopia Aethiopica crude extract (300mg/kg).

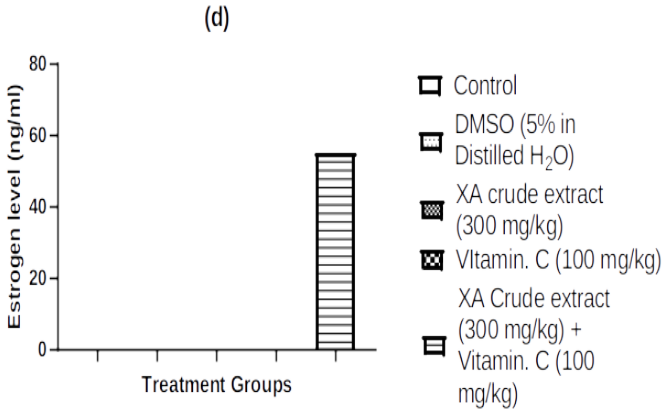


Figure 3d: Effect of Vitamin C and Xylopia Aethiopica Fruit Ethanolic Crude Extract on Estrogen in Male Wistar Rats
NS = No Significant Changes Compare to the Control and Vitamin C Combined Treated Rats

Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{***}p < 0.001$ when compared with control; $^{++}p < 0.01$ when compared with Xylopia Aethiopica crude extract (300mg/kg).

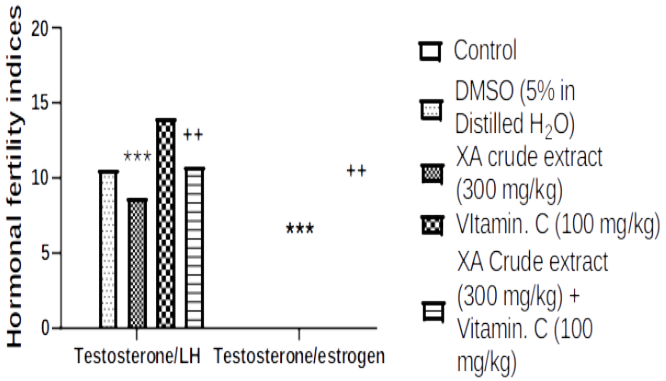


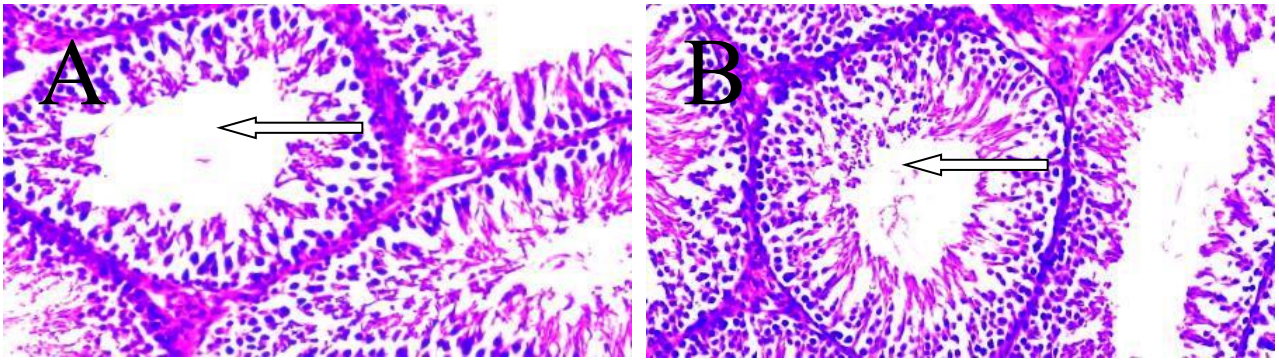
Figure 3e: Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Hormonal Fertility Indices in Male Rats

Testosterone/LH ratio and Testosterone/Estrogen ratio significantly decreased compare to the negative control and Vitamin C combined treated rats.

Data are expressed as mean ± S.E.M. (n = 6) (One-way ANOVA followed by Bonferroni *post hoc* test). $^{***}p < 0.001$ when compared with control; $^{++}p < 0.01$ when compared with Xylopia Aethiopica crude extract (300mg/kg).

4. Effect of Vitamin C and Xylopia Aethiopica Ethanolic Crude Extract on Histopathological Changes in Testes of Male Rats

The effect of Vitamin C and Xylopia Aethiopica ethanolic Crude extract on histopathological changes in testes of Male rats are shown in Plates 1. Vitamin C alone produced no changes on the testicular architecture, seminiferous tubules and spermatozoa (spermatogenesis) when compared to normal control group. In this study, rats treated with Xylopia Aethiopica fruit extract (300 mg/kg/day) had a reduced spermatogenesis with presence of few number of spermatocytes in most of the seminiferous tubules, as evidenced by degenerated seminiferous tubules and necrosis with presence of atrophy, few dead pyknotic cells, homogenous and vascular congestion when compared with normal controls. However, as shown in the Xylopia Aethiopica + Vitamin C combined treatment, Vitamin C, ameliorated Xylopia Aethiopica fruit extract -induced changes on testicular architecture, seminiferous tubules and spermatozoa (spermatogenesis) relative to control group.



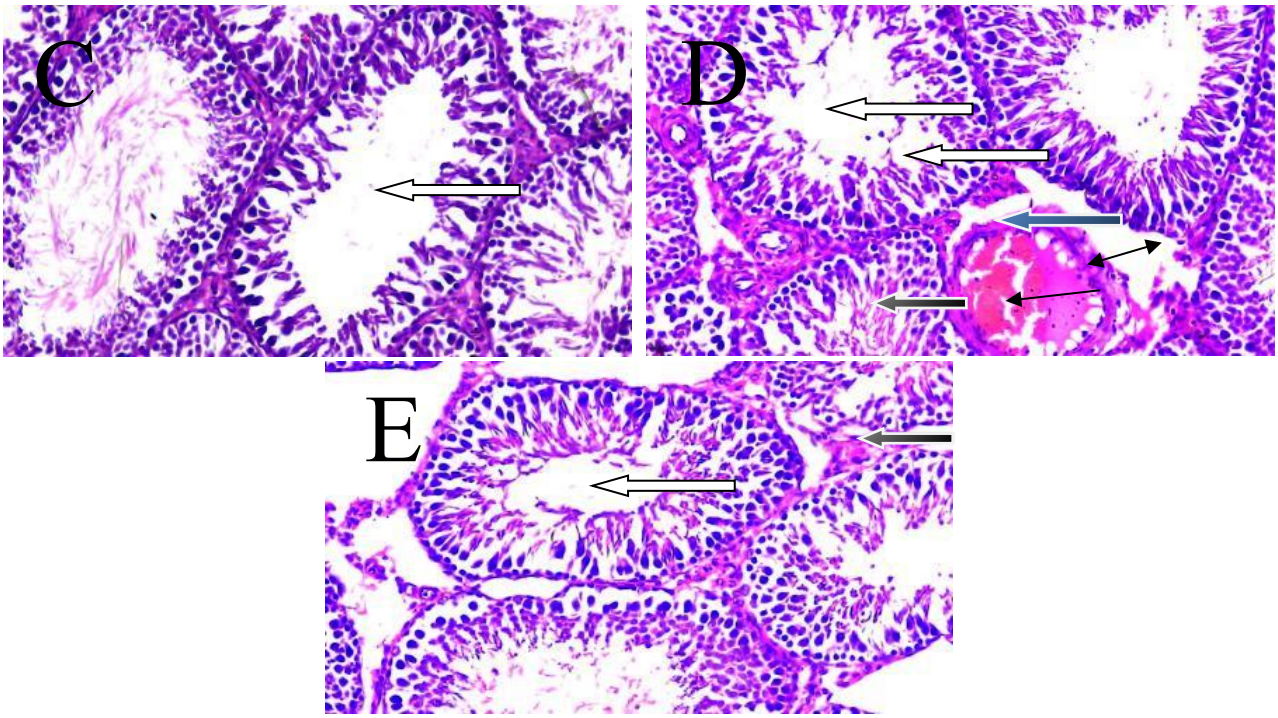


Plate 1a-e: Photomicrographs Showing the Effect of Vitamin C and Xylopia Aethiopica Fruit Extract on Histopathological Changes in Testes of Male Rats
A: Negative Control (Distilled Water)
B: Positive Control (0.5 mL of 5% DMSO v/v)
C: Vitamin C (100 mg/kg)
D: Xylopia Aethiopica Fruit Extract (300 mg/kg)
E: Xylopia Aethiopica Fruit Extract (300 mg/kg) + Vitamin C (100 mg/kg)
Haematoxylin-eosin Stain: Original Magnification x400
Calibration Bar = 0.01 mm (10 µm) for all Plate

For Plate 1, slides A, B and C revealed normal normal testicular architecture with seminiferous tubules, normal sperm maturation stage. Slide D revealed some degenerated germ cell layer and less presence of spermatozoa within their lumen and also shows presence of pinkish homogenous mass and vascular congestion. several atrophic seminiferous tubules, thickened pyknotic propria enveloping the tubular cells, degenerated and necrotic germ cells of the seminiferous tubules.. Slide E are associated with showing normal testicular architecture with a mild degenerated seminiferous tubules containing slough cell. Blue arrow – few seminiferous tubules showing maturation arrest at secondary level; Slender arrow – presence of pinkish homogenous mass and vascular congestion; Spanned–degenerated germ cell layer; White arrow – seminiferous tubule with normal sperm maturation; Black arrow – seminiferous tubule containing slough cell.

Discussion

A multitude of factors, such as physical obstruction of sperm release, reduced sperm count or motility, altered sperm morphology, infections, and hormonal imbalances, have been identified to

contribute to male infertility. Anatomic defects, endocrinopathies, immunologic problems, ejaculatory failures, and some environmental exposures are significant causes of infertility.

Plant products are known to exert their protective effects by scavenging free radicals, modulating detoxification of toxicant and antioxidant defense system. Vitamin C has been shown to exert its antioxidative property either by directly scavenging free radicals or indirectly by quenching singlet oxygen, donating hydrogen compound, chelating metal ions and inhibiting lipid peroxidation (Akorede et al., 2020).

The results of the present study revealed that oral administration of Vitamin C, Xylopia Aethiopica and their combinations for the period of 60 days did not produce any significant changes on body weight, testicular weight and epididymal weight. The result obtained for the body weight and organ weight of Xylopia Aethiopica fruit extract are not in agreement with the findings of Alhassan et al. (2013) which reported that ethanolic extract of Xylopia Aethiopica caused an increase in animal body weight as well as weights of sexual organs such as testis and epididymis. Also, this is contrary to the findings of Nwangwa et al. (2012) and Ameyaw and Owusu-Ansah (1998) whose studies revealed a significant dose dependent decrease in body weight of the animals treated with the extract. According to Raji et al., (2005), the weight of male reproductive organ usually provides a useful fertility/reproductive risk assessment in experimental studies. Testicular size is the best primary assessment for spermatogenesis since the tubules and germinal elements account for approximately 98% of the testicular weight (Sherines and Howard, 1978). Thus, decrease in testicular weight can be linked to the degeneration of tubules and loss of germinal elements which was observed in this study. The weights of testes and accessory sex organs are known to be reliable indices of testicular androgen production (Rind et al., 1963). However, in this present study, there was no alteration in the body and organ weight of the animal treated with Xylopia Aethiopica and coadministration with Vitamin C.

The significant reduction in total epididymal sperm count and sperm motility, with respective different doses of Xylopia Aethiopica fruit extract may be due to the toxic effects of Xylopia Aethiopica fruit extract on spermatozoa. Low caudal epididymal sperm density may also be due to alteration in androgen metabolism. One of the factors which may cause decrease in sperm motility may be androgen deprivation effect of the Xylopia Aethiopica fruit extract, as evident by decreased steroidogenesis. Impaired sperm motility may result in infertility due to the failure of the sperm to reach the site of fertilization as well as their ability to penetrate zona pellucida (Aly et al., 2009). These alterations were effectively prevented by Vitamin C in the coadministration.

Histopathological observations indicated degeneration of seminiferous tubules, disturbed spermatogenesis and degenerative changes of Sertoli cells. It has been suggested that Sertoli cell damage may be responsible for germ cell degeneration (Pant et al. 1995; Srivastava et al. 1990, 1992). The result obtained here is in line with the findings of Nwangwa et al. (2012) who observed that from the testicular photomicrograph rats exposed to Xylopia Aethiopica showed dose dependent degenerative changes.

Testosterone is the main male gonadal hormone produced by the interstitial cells of the Leydig in the testis. It is also the major index of androgenicity. Testosterone in testis is essential for normal spermatogenesis as well as for the maintenance of structural morphology and normal physiology of seminiferous tubules. Testosterone is required for the attachment of different generations of germ cells in seminiferous tubules and therefore, low level of intratesticular testosterone may lead to detachment of germ cells from seminiferous epithelium and may initiate germ cell apoptosis (Blanco-Rodriguez and Martinez-Garcia, 1998). In the absence of testosterone, germ cells are unable to progress and result in failure of spermatogenesis and infertility (Walker, 2010; Aly et al., 2017). The testicular steroidogenesis can be interfered by many exogenous factors (chemicals, drugs, etc) acting in many different ways due to the complex physiological mechanism that regulate Leydig cell function (Cooke, 1998). As a consequence of impaired Leydig cell activity, male infertility may result (Payne et al., 1980). Spermatogenesis is a process under hypophyseal hormonal control that involves gonadotropin synthesis of LH and FSH, and they act on Leydig and Sertoli cells, respectively (Steinberger, 1971). Also, interactions between Leydig cells-Sertoli cells are necessary for normal intratesticular testosterone production (Skinner, 1991). The Leydig cell produces testosterone that is needed in the seminiferous tubules to induce the differentiation of spermatogonia to spermatozoa. Alterations in the testosterone synthesis could be due to many different pathological or experimental situations (Dufau et al., 1979). Intratesticular testosterone levels that promote normal spermatogenesis are 100-fold higher than those found in blood plasma and they are accomplished through normal Leydig and Sertoli cell activity (Dufau et al., 1979). Similarly, Xylopia Aethiopica fruit extract treatment has been shown to increase cell death, as well as immature and decreased immotile sperm velocity with significant decrease in serum testosterone in rats (Nwangwa et al., 2012). In a nutshell, the decreased serum testosterone in the Xylopia Aethiopica fruit extract-treated rats observed may be due to Leydig cell impairment caused by ROS generation (Chen et al., 2008). Although, this was contrary to the view of Alhassan et al. (2013) when working with the crude extract. Moreover, treatment with Vitamin C prevented reduction in serum testosterone level in this study.

Hormones play a vital role in initiating and maintenance of male reproductive function (Meeker et al., 2007) and it is known that gonadal dysfunction is one of the most common side effects of toxicant or chemotherapeutic drug (Oyovwi et al. 2021) affecting both the endocrine and exocrine functions of the testis (Spermon et al., 2006). Xylopia Aethiopica fruit extract treatment produced no significant changes in estrogen and FSH levels but significantly reduced serum concentrations of testosterone and LH. The pulsatile secretion pattern of GnRH induces the cyclic release of LH and FSH. Of note, it has been reported that inhibition of GnRH results in reduced LH and FSH levels (Dickson et al., 2000). Similar results were obtained in male mice studies with decreased plasma testosterone due to inhibition of pituitary LH (Nwangwa et al., 2012) Therefore, the depletion of these hormones may pose serious reproductive disorders in male. However, these findings were observed to be contrary to the findings of Alhassan et al. (2013) who reported an increase in LH, FSH and testosterone after treatment with the axtract. The ability of Vitamin C to significantly

prevent Xylopia Aethiopica fruit extract-induced testosterone and LH reduction in the ameliorative studies may suggest their potential utility in the management of testicular dysfunction.

Hormonal fertility indices are known to be Testosterone/Luteinising hormone (T/LH) and Testosterone/Estrogen (T/E_2). T/LH is a known index of Leydig cell function (Foster et al., 1993), while high T/E_2 is a pointer to fertility (Roa et al., 2009). Notably, the assessment of T/LH provides a more meaningful information than comparisons of testosterone and LH levels separately (Foster et al., 1993). In this study, we found that these hormonal fertility indices decreased in Xylopia Aethiopica fruit extract treated rats, indicating impaired Leydig cell function, which was manifested by suppression of serum testosterone level. However, the finding that treatment with Vitamin C normalized serum testosterone level in rats treated with Xylopia Aethiopica fruit extract may also suggests beneficial role in the management of reproductive dysfunction as evidence by increase in hormonal fertility inces.

The extent of severity of infertility depends on the degree of capacitation by spermatozoa and acrosomal reaction (Pilch, and Mann, 2006). Capacitation is the penultimate process in the maturation of spermatozoa and is required to render them competent for successful fertilization of ovum. (Du Plessis et al., 2015). Xylopia Aethiopica fruit extract administration was shown to significantly decrease the number of capacitated sperm cells and about 24.60% to 28.75% of the cells had their acrosome intact, despite the decrease in capacitattion. Thus over 71.25% to 74.6740% of the spermatozoa obtained from the caudal epididymis of the treated group were functionally impaired and may be incapable of fertilizing an ovum, as only 45.65 of these were capacitated and had their acrosome react. Notwithstanding, co-treatment with Vitamin C markedly prevented the adverse effect of Xylopia Aethiopica fruit extract induced changes on sperm acrosomal status when compared to the Xylopia Aethiopica fruit extract group.

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