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Priapism in Sprague–Dawley rat: Evidences for spermatotoxic, histomorphological changes in Corpora Cavernosa and the protective efficacy of ascorbic acid and testosterone

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Abstract
The histomorphological changes in the corpora cavernosa following priapism remains an interesting observation due to its pathological and reproductive implications. We investigated the attenuating effect of testosterone and vitamin C on the corpora cavernosa induced fibrosis in priapism of rats.
Twenty five Sprague – Dawley male rats were randomly allocated into 5 groups of rat each Group 1 served as the control. Group 2 had only priapism. Group 3 was induced with priapism and then treated with vitamin C. Group 4 was induced with priapism and then treated with testosterone. Group 5 was induced with priapism but were treated with both vitamin C and testosterone. Priapism was induced for one week followed by 6 hours post priapism administration of testosterone and vitamin C.
Results showed fibrotic corpora cavernosa in all groups except the control group. Sperm parameters indicated oligospermia and reduced sperm motility especially in group 2 and 3 when compared with the control group. The results indicated that testosterone and vitamin C ameliorated priapism-induced ischemia – reperfusion injury at different time intervals in rats.

Keywords: Priapism, Sprague-Dawley rats, Corpora cavernosa, Infertility, Testosterone, Vitamin C

Introduction
Priapism is a urological emergency characterised by an unrelenting and painful erection without sexual stimulation usually lasting for longer than 4h to 6h despite orgasm and basically involving only the corpora carvernosa [1-2].
It is most often drug induced and the antidepressant drug Traxodone has been frequently implicated [3]. Other psychoactive substances involved in the aetiology of priapism include marijuana, cocaine, ethanol, the atypical antipsychotics; Risperidone, Olanzapine, Clozapine and Quetiapine. Erection is determined by both neuronal and vascular factors with the latter involving essential components of arterial dilatation, relaxation of cavernosal smooth muscle and reduced venous outflow [4]. Divergence from the coordination of this mechanism alters the response of the erectile tissue and results in Erectile Dysfunction [5]. Ischemias, thrombosis, damaged blood vessels of the penis, impair erectile function or impotence has been associated with priapsm.
Ischemia-reperfusion injury is a complex phenomenon that causes destruction in both local and remote tissues [6]. Although reperfusion of ischemic tissue is necessary for reparative mechanisms, it has been shown to worsen the injury caused by ischemia via release of reactive oxygen species to the systemic circulation [7]. Oxidative injury develops when there is excessive production of reactive oxygen species and/or free radicals, which exceeds the natural antioxidant defence mechanisms in the body [7-8]. Oxidative stress produces destructive changes in tissues by causing alterations and irreversible cellular damage [9].
Ischemic reperfusion injury post treatment with various antioxidants has been evaluated in many organs [10].
Concomitant administration of androgens and antioxidants has been a subject of debate as it relates to the management of priapism induced impotence. The controversy over the years has been on the efficacy of testosterone.
This study was designed to investigate the role of testosterone and vitamin C administered 6 hours after the induction of priapism on the spermatozoa and histomorphology of the corpora cavernosa.

Materials and Methods

Animals

Thirty adult male Sprague–Dawley rats weighing between 250 and 300g were housed in solid plastic cages in animal house of Anatomy department of the College of Medicine, University of Lagos and allowed to acclimatize for 2 weeks. The animals were later grouped into five rats per cage and maintained at temperatures between 25 – 28 °C. The rats were allowed to eat standard rodent chow and water *ad libitum* throughout the experimental period.

Experimental Protocol

Induction of Priapism

It is a delicate procedure that demands intensive precautionary measures. The penis of rat is a very tender organ and could be easily injured. When traumatised, either by the use of too tight constriction band or rough handling could strangulate the penis and impair micturiction process resulting in damming of urine within the urinary bladder, ureter or hydronephrosis and uraemia. This destabilises the kidneys and becomes a source of infection and renal failure which could lead to death of the rats.

Undoubtedly, the following procedure occurred earlier in this index study and 90% of all the first set of rats died. The subsequent use of 2mm slices of size 16F catheter and minimal trauma to the penis was successful.

All operations were performed under sterile conditions. The animals were anesthetized with ketamine injection (50 mg/kg, ip). Priapism was induced with the method described by Sanli *et al*, [11]. The tip of a 60-cc syringe was applied to the base of the flaccid penis, so a vacuum erection device was created. Before the application of vacuum to the penis, a constriction band, which was cut from 16 Fr Foley catheter in 2-mm slices, loaded around the tip of the vacuum erection device. Then the tip of the syringe was placed at the base of the penis and withdrawn gently to induce erection in the rat penis. After induction of erection in sufficient grade, the constriction band was then placed at the base of the penis by slipping off the syringe. Testosterone was administered intramuscularly at 2.5mg/kg body weight three times weekly [12] and oral Vitamin C at 25mg/kg body weight daily [13] throughout the one week of priapism induction.

Animal groupings

The animals were divided into five groups containing 5 rats each.

Group 1: Served as the control and was treated with 5ml/kg body weight of normal saline

Group 2: In this group, priapism was induced for one week but no drug was administered

Group 3: Priapism was induced and 6 hours later 25 mg/kg of Vitamin C was injected intraperitoneally.

Group 4: Priapism was induced and 6hrs later 2.5mg/kg body weight of testosterone was injected intramuscularly at three times weekly.

Group 5: Priapism was induced and 2.5mg/kg body weight of testosterone and 25mg/kg body weight of vitamin C injected via same route as above.

The animals were sacrificed one week after priapism induction. The penis and the seminiferous tubules were harvested for histology and seminal fluid analysis.

Tissue preparation for histological analysis

All rats were penectomized 1 week after the induction of priapism and the penis were fixed in Bouin's solution for 24 hours and then dehydrated by passing through ascending grades of alcohol (70%, 80%, 90% and absolute alcohol). After dehydration, tissues were cleared in xylene, infiltrated, and then embedded in paraffin wax. Each penis was sectioned along horizontal axis in 5um thickness. Two sections from each rat were blocked in paraffin. Two sections of each block (total 4 sections for each testis) were stained with Haematoxylin and Eosin (H & E) according to routine light microscopic procedures.

Semen analysis

The caudal aspect of the epididymis of each rat was minced in normal saline. Spermatozoa were counted and recorded for just five random fields and the values recorded in million (10^6) [14]. Sperm motility using rapid forward progression, medium forward progression and slow forward progression was assessed**.** For consistency, all readings were carried out at 37 °C [15]. The sperm morphology was assessed as described by *Menkveld et al.* [16].

Statistics

Statistical analysis was done using SPSS statistical software (version 7). Paired T test was used to compare mean values between the control group and other groups in the study.

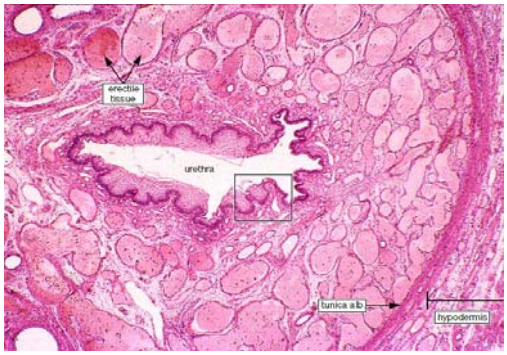
Results and Discussion

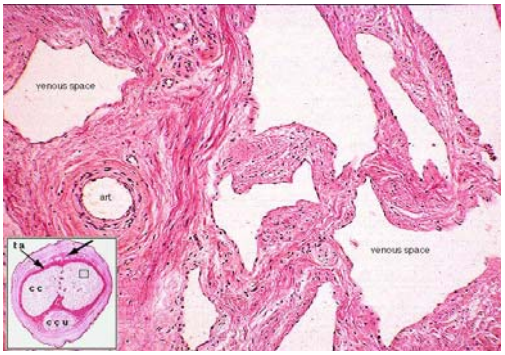
The pathophysiology of priapism can be simplistically viewed as dysfunctional hemodynamic process of the penis, whereby the genital organ excessively endures blood engorgement and the major point of this phenomenon is the anoxia of penile tissue [17].

In anoxic state, smooth muscle of corpus cavernosum has minimal basal tension and has no spontaneous contractile activity. From the therapeutic view, this study also focused on the responsiveness of corpora cavernosa to testosterone and vitamin C in priapism.

Histomorphological profiles

Histological sections of the penis of control group revealed two pale corpora cavernosa occupying most of the central portion surrounded closely by dense connective tissue, the tunica albuginea (Figure 1 and 2). Also noted is the corpus spongiosum situated ventromedially and containing the slit – like penile urethra surrounded by connective tissue trabeculae and muscle strands, as well as blood vessels and nerves. Histological assessment of group 2 rats (Figure 3 and 4) showed distortions evidenced by extensive fibrosis with thickening of elastic and collagen fibres. Some of these fibres were already transforming into fibroblast. However, testosterone and vitamin C administered 6 hours after priapism induction in P + vitamin C group, P + Testosterone group and P + vitamin C + Testosterone group showed similar fibrotic changes though at a very minimal extent (Figure 5-10). This revealed that testosterone and vitamin C has some level of protective function on the corpora cavernosa.

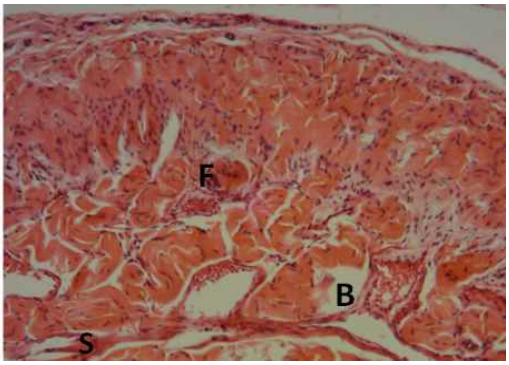


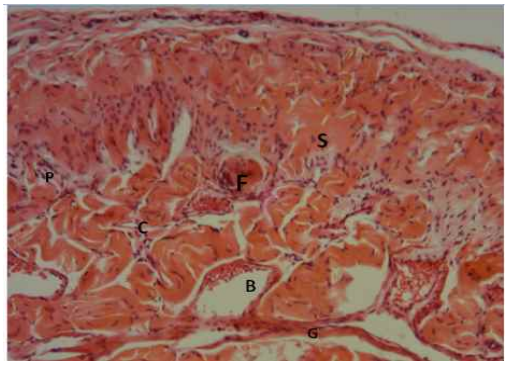


ta: Tunica albuginea, **cc:** Copora Cavernosa.

Fig 1: Photomicrograph of penis in control group of rat. Mag.: X100

Fig 2: Photomicrograph of penis in control group of rat at X400 magnification.

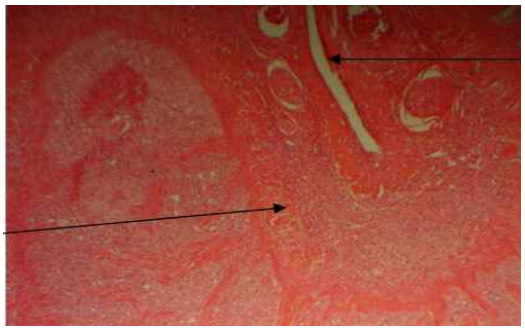


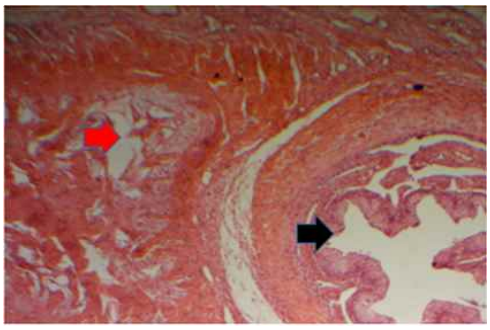


F: Copora carvernosa fibrosis, **C**: Connective tissue, **S**: Smooth muscle, **P**: Lamina propria, **B**: Dilated blood vessel space

Fig 3: Photomicrograph of penis in group 2 rat at X100 magnification.

Fig 4: Photomicrograph of penis in group 2 rat at X400 magnification.



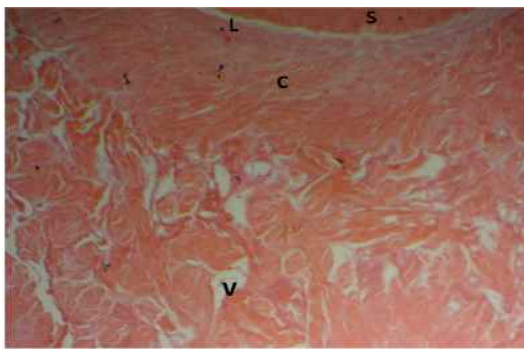


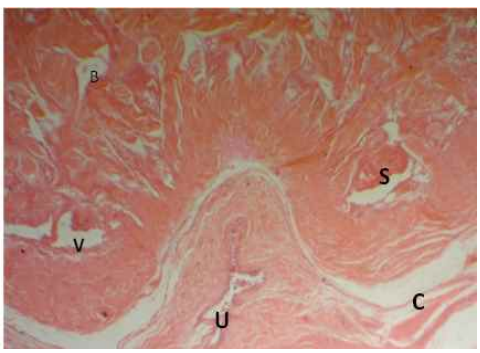
Arrows: Spongiform copora carvernosa with dilated vascular spaces,

Black arrow: Urethra**, Red arrow:** Fibrotic copora carvernosa

Figure 5: Photomicrograph of penis in group 3 rat at X100 magnification.

Figure 6: Photomicrograph of penis in group 3 rat at X400 magnification.

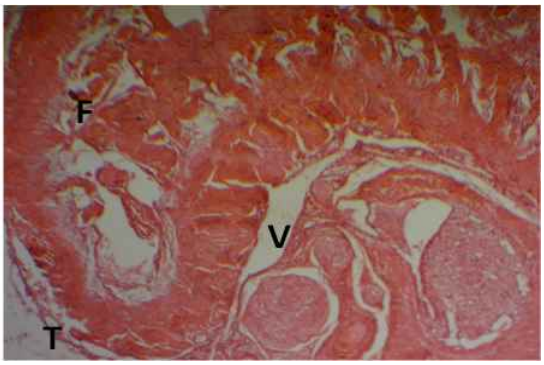


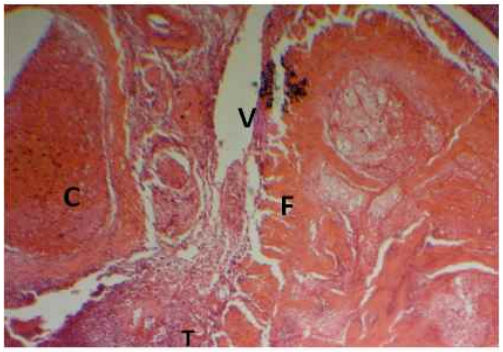


B: Blood vessels, **C**: Connective tissue, **U**: Urethra, **S**: Smooth muscle fibrosis, **V**: Venous spaces

Fig 7: Photomicrograph of penis in group 4 rat at X100 magnification.

Fig 8: Photomicrograph of penis in group 4 rat at X400 magnification**.**





F: Fibrotic copra cavernosa, **T**: Connective tissue, **V**: Dilated venous spaces, **C**: Copora spongiosum

Fig 9: Photomicrograph of penis in group 5 rat at X400 magnification.

Fig 10: Photomicrograph of penis in group 5 rat at X400 magnification.

Seminal Fluid Analysis

As shown in table 1 semen fluid analysis revealed that testosterone administration had potential for boosting up sperm count and motility. The group that received testosterone (group 4 and 5) had optimal values of sperm count and motility when compared to the control group. The mean values of sperm count for group 1, 2, 3, 4 and 5 were 154 x 10^6 cell/ml, 4.9 x 10^6 cell/ml, 4.5 x 10^6 cell/ml, 146 x 10^6 cell/ml and 111 x 10^6 cell/ml, respectively. The sperm motility also showed increase in the recorded values between group 1, 4 and 5. The mean values of fully active sperm motility for group 1, 2, 3, 4 and 5 were 80%, 5%, 20%, 50% and 60% respectively (Table 2). The mean values of normal and abnormal sperm morphology for group 1, 2, 3, 4, and 5 were 80% and 20%, 60% and 40%, 60% and 40%, 70% and 30%, 60% and 40% respectively (Table 3). This revealed that the Testosterone + priapism group had higher normal sperm morphology when compared with the control group.

The above results indicate that testosterone has a positive effect on spermatogenesis hence counter the impotent predisposition of the said groups in priapism.

Table 1: Sperm count (x10^6 cell/ml) with mean and standard deviation for various groups

Group	Sperm count (x 10^6 cell/ml)
Group 1(Control)	154 ± 2.71
Group 2 (P-alone)	1.9 ± 0.25**
Group 3(P+Vitamin C)	4.5 ± 0.60**
Group 4 (P+Testosterone)	146 ± 6.88
Group 5 (P+Vitamin C+Testosterone)	111 ± 11.54*

Data was expressed as mean ± S.D. Statistics involve the use of t-test *($p<0.05$) and ** ($p<0.005$)

Table 2: Sperm motility (%) with mean and standard deviation for various groups

GROUPS	Fully Active (%)	Slightly Active (%)	Dead (%)
Group 1 (Control)	80 ± 1.33	10 ± 0.98	10 ± 0.67
Group 2 (P-alone)	5 ± 0.56 **	45 ± 2.33	50 ± 1.89 **
Group 3 (P+Vitamin C)	20 ± 0.78 *	40 ± 1.23	40 ± 088 **
Group 4 (P+Testosterone)	50 ± 1.22	40 ± 2.11 *	10 ± 0.46
Group 5 (P+Vitamin C + Testosterone)	60 ± 2.41	30 ± 0.55	10 ± 0.43

Data is expressed as mean ± S.D. Statistics involve the use of t-test *($p<0.05$) and ** ($p<0.005$)

Table 3: Sperm morphology (%) with mean and standard deviation for various groups

GROUPS	**Normal** (%)	**Abnormal** (%)
Group 1(Control)	80 ± 3.23	20 ± 1.66
Group 2(P-alone)	60 ± 2.34 *	40 ± 0.98 **
Group 3(P+Vitamin C)	60 ± 3.27 *	40 ± 1.34 *
Group 4 (P+Testosterone)	70 ± 2.35	30 ± 3.44 *
Group 5 (P+Vitamin C + Testosterone)	60 ± 2.43 *	40 ± 2.55 *

Data is expressed as mean ± S.D. Statistics involve the use of t-test *($p<0.05$) and ** ($p<0.005$)

Conclusion

We provided evidences for marked carvernosal fibrosis of the penis, oligospermia and reactional anaemia as a result of experimentally induced priapsm. This implies that besides erectile dysfunction resulting from carvernosal fibrosis, patient may suffer low sperm counts (oligospermia) and severe haemolytic anaemia. Concomitant administration of testosterone and vitamin C significantly reversed the fibrotic effect of priapism on corpora cavernosa and the changes in all of these parameters after testosterone and vitamin C administration were in agreement with previous studies [18]. These results demonstrated the beneficial effects of testosterone and vitamin C in spermatogenesis and ischaemic reperfusion injury, as evidenced by changes in seminal fluid analysis and biochemical parameters. This preliminary study will enlighten the researchers with erectile dysfunction to construct more comprehensive studies on the effects of fibrosis, ischemia and reperfusion in cavernosal tissue. Thus, the usage of antioxidant agents in such conditions and clinical implications of antioxidant administration can be improved in

the future.

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