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HISTOMORPHOMETRIC EFFECTS OF SILDENAFIL CITRATE ON THE TESTIS OF NORMOGLYCAEMIC AND HYPERGLYCAEMIC ADULT WISTAR RATS

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ABSTRACT: *Histo-morphometric effects of sildenafil citrate was investigated on the testes of adult Wistar rats. Twenty five male Wistar rats were weighed before and after the experiment and categorized into four treatment groups and one control group of five rats per group (n=5). They were treated with the following regimen for eight weeks duration. The control group (A) received feed mash and water ad libitum. Treatment groups B and C were the normoglycaemic groups and received low dose (1mg/kg body weight) and high dose (2mg/kg body weight) of sildenafil citrate, respectively. Groups D and E were the hyperglycaemic groups, treated with low dose (1 mg/kg body weight) and high dose (2 mg/kg body weight) of sildenafil citrate, respectively. Blood samples were collected for hormonal assay and the testes were excised and processed for morpholological changes.The results obtained showed significant (P<0.05) loss in both body and testicular weight in the hyperglycaemic groups, but insignificant (P>0.05) body weight gain in the normoglycaemic groups and insignificant (P>0.05) epididymal weight difference loss in all the treatment groups, compared to control. The hormone assay showed significant (P<0.05) difference in the levels of FSH, LH, testosterone and oestrogen in the hyperglycaemic groups compared to control. Testicular and epididymal tissues revealed mild distortions in hyperglycaemic treatments but with dose dependent improvement while the changes in the normoglycaemic treatments were essentially non-remarkable. The results suggest relative safety of sildenafil citrate in normoglycaemic and hyperglycaemic states, with beneficial testicular effects in the latter condition.*
KEYWORDS: Sildenafil Citrate, Normoglycaemia, Hyperglycaemia, Testis, Wistar rats

INTRODUCTION

A very well-known endocrine and metabolic disorder that affects males and females across diverse age range in whom sexual dysfunction can occur is diabetes mellitus (Ballester *et al.*, 2004; Akef *et al*., 2012). In the male, infertility and impotence are two threatening complications associated with diabetes mellitus (Zhao *et al.*, 2011; Ataman and Osinubi, 2014; Oyelade *et al*., 2016). Thus, diabetes might be co-morbidity in erectile dysfunction (Brown *et al.*, 2005; El-Sakka *et al.*, 2008). There is increasing evidence that diabetes is closely associated with male reproductive dysfunctions with observed pathological changes in Leydig cells, interstitial connective tissues and seminiferous tubules of the testis (Nascimento Silva et al., 2014; Ataman and Osinubi, 2017). Compared with non-diabetic people, male diabetic patients show an increasing incidence of erectile dysfunction and infertility with erectile dysfunction occurring about ten years earlier in the diabetics than in the non- diabetic counterparts (Kiskac *et al.*, 2015). Experimental diabetic animals tend to suffer from testicular dysfunction such as reduced sperm count, low serum testoterone levels and decreased fertility (Mallick *et al.*, 2010; Ataman and Osinubi, 2017).

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Erectile dysfunction has been identified as a possible complication in about 50 % of diabetic men (Spollett, 1999). Poor glycaemic control has been related to the pathogenesis of diabetes mellitus which results in complications as vasculopathy, neuropathy and myopathy (Kiskac *et al.,* 2015; Anwar *et al.*, 2017), which interferes with the normal stimulation of noradrenergic and noncholinergic nerves in the pelvic parasympathetic plexuses that cause release of nitric oxide across the neuromuscular junction of the penile arteries and cavernosa smooth muscles (Spolletti, 1999; McCullough, 2000). With diabetes mellitus as a known risk factor in erectile dysfunction, decreased endothelial levels of nitric oxide synthase has been implicated in its pathogenesis and mediated phosphorylation of this enzyme reportedly, promotes penile erection (Hurt *et al.*, 2002). Current treatment modality for it with tremendous acceptance is seen in the role of drug formulations as sildenafil citrate, a phosphodiesterase- 5 (PDE-5) inhibitor which helps in potentiating the levels of nitric oxide by binding to the PDE-5 enzyme, preventing PDE-5 breakdown of cyclic guanosine monophosphate (cGMP) through competitive inhibition. Nitric oxide causes increase in cGMP which results in the relaxation of penile smooth muscles and increased cavernosa blood flow to promote penile erection (McCullough, 2002; Montague *et al.*, 2005).

It has been reported that sildenafil citrate served a cytoprotective role via reduction in oxidative stress in a study with compromised vascular supply to the testis, but not without some morphological distortions to some of the testicular tissues following graded dosage (Yildiz *et al.*, 2011). Sildenafil citrate has also been reported to have positive effects on spermatogenesis, sperm production, and semen quality (Beheshtian *et al.*, 2008).

It is known that poor glycaemic control in men of reproductive age leads to attendant infertility challenge and vulnerability to erectile dysfunction (Brown *et al.*, 2005; Agbaje *et al.*, 2007), that might require long term treatment with sildenafil citrate (Vardi and Nini, 2007; Schouten *et al.*, 2010). An important issue for consideration is does sildenafil ameliorate infertility challenge posed by diabetes in its effects on testicular functions or does it worsen it? In this study, the morphometric and morphological effects of sildenafil citrate on normal and induced hyperglycaemia in Wistar rats is evaluated to provide a clue.

MATERIALS AND METHOD

Drug Preparation and Administration

The drug, sildenafil citrate is manufactured by HAB Pharmaceuticals Limited was obtained from Thelver Pharmacy, Benin City. Drug preparation was done by diluting a tablet of 100mg in 50mls of distilled water to obtain 2mg/ml, and administration was 1mg/kg body weight and 2mg/kg body weight.

Experimental animals

Twenty-five Wistar rats bred at the Animal house of the Department of Anatomy, University of Benin, Benin City were used for this study under ethical approval. The animals were kept in standard environmental conditions of humidity of 65±5%, room temperature of between 25 to 26^0C and 12:12 hours of day and night photoperiodicity. Experimental procedures involving the animals and their care were conducted in conformity with International and Institutional guidelines

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for the care of laboratory animals in Biomedical Research, as promulgated by Canadian Council of Animal care (Canadian Council of Animal Care, 1985). Further, the animal experimental models used were in conformity to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the care and use of Animals (American Physiological Society, 2002).

They were categorized into four treatment groups and one control group of five rats per group. The duration of the experiment was two months. The control group (A) was fed on feed mash only and water throughout the period. The four treatment groups (B, C, D and E) received feed mash, water and sildenafil citrate but groups D and E were induced with diabetes using 50mg/kg body weight of streptozotosin manufactured by Sigma Aldrich Company, USA. Confirmation of diabetes at 250mg% was done with accu check glucometer and strips before the administration of sildenafil Citrate. The treatment regimen is shown in Table 1. The body weights of the animals were measured at the beginning and end of the experiment and recorded.

After eight weeks of administration, the animals were sacrificed by cervical dislocation. Blood samples were collected for hormone assay using ELISA kits in line with the guiding principles of the tests in DRG Diagnostics User's Manual. The abdomen was excised to retrieve the testes and the epididymis which were processed through prior 24 hours fixation in bouin`s fluid. The fixed tissues were passed through ascending grades of alcohol and the dehydrated tissues were cleared in xylene before infiltration with molten paraffin wax and thereafter allowed to cool and solidify. The embedded tissues were trimmed and mounted on a wooden chauck and sectioned at 5 microns using a rotary microtome. Staining with hematoxylin and eosin was done (Drury and Wallington, 1980) and the stained slides were mounted with Canada balsam and coverslipped for microscopy which was carried out at x400 magnification.

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Data Analysis

Data were presented as Mean ± SEM. Means separation and significant differences between the means (Duncan, 1957) were determined at ($P<0.05$) using ANOVA.

Table 1: Showing Experimental Groups and the Treatment Regimen

GROUPS	TREATMENT REGIMEN
A	Received Feed mash and water ad libitum
B	Feed mash and low dose of sildenafil citrate (1mg/kg body weight) in normoglycaemic rats.
C	Feed mash and high dose of sildenafil citrate (2mg/kg body weight) in normoglycaemic rats.
D	Hyperglycaemic rats: received water ad-libitum, feed mash and low dose of sildenafil citrate (1mg/kg body weight)
E	Hyperglycaemic rats: received water ad-libitum, feed mash and high dose of sildenafil citrate (2mg/kg body weight)

RESULTS

From the results of the effects of treatments on the mean weight values of experimental rats as shown in Table 1, the initial mean body weight was 246.00±9.14 g, while the final mean body weight value was 257.00±7.68 g for group A Control) animals. In group B, the initial mean body weight value changed from 189.00±6.40 g to the final mean body weight value of 209±6.78 g. In Group C, the initial mean body weight value was 208.00±9.82 g while the final mean weight value was 219.00±7.31g. In Group D, the initial mean body weight value of 229.00±5.34 g dropped to final mean weight value of 214.00±5.34 g. In Group E, the initial mean body weight value of 227.00±6.44 g became 223.00±0.02 g at the end of the experiment. The findings revealed weight gain in groups B and C, but not significantly ($P>0.05$) different from control. Conversely, there was significant ($P<0.05$) weight loss in the hyperglycaemic groups D and E compared to control. The results on mean testicular weight of the rats post-sacrifice was significant ($P<0.05$) in groups D (1.22±0.02 g) and E (0.88±0.32 g) compared to control (1.41±0.18 g) but there was however no significant difference ($P>0.05$) between the mean testicular weight of the other treatments and the control. Also the results of the mean epididymal weight revealed no significant difference ($P>0.05$) between that of the treatment groups and the control (Table 2).

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Table 3 showed the hormonal profile of the experimental groups. From the results, the values of FSH (0.08± 0.32 IU/ml), LH (0.91±0.01 IU/ml) and testosterone (1.02± 0.12 ng/ml) in the hyperglycaemic group were significantly lesser (P<0.05) in group D compared to control where the values were 0.13±0.44 IU/ml, 1.23± 0.1 IU/ml and 2.72± 1.31 ng/ml, respectively. The oestrogen value was unaffected by sildenafil treatment in groups B (13.13±1.24 ng/ml) and C (14.25±1.32 ng/ml), but insignificantly (P>0.05) higher in the hyperglycaemic groups D (17.36±2.04 ng/ml) and E (15.34±1.50 ng/ml) compared to control (14.22±0.03 ng/ml). The prolactin values for groups B (0.82±0.42 ng/ml), C(1.01±0.02 ng/ml), D (0.83±0.11 ng/ml) and E (0.77±0.23 ng/ml) were not significantly different (P>0.05) from control (0.94±0.55 ng/ml). Also, there was no significant difference (P>0.05) in the values of progesterone between the treatment groups B (2.35±0.12 ng/ml), C(2.02±0.02 ng/ml), D (1.89±0.45 ng/ml) and E (1.99±0.26 ng/ml) and the control (2.84±0.33 ng/ml).

Table 2: Mean values of the body weight, weight of the testes and epididymis following the administration of sildenafil citrate

	Group A (mean± S.E.M)	**Group B (mean ± S.E.M)**	**Group C (mean ± S.E.M)**	**Group D (mean± S.E.M)**	**Group E (mean± S.E.M)**
Initial body Weight	246.00±9.14	189.00±6.40	208.00±9.82	229.00±5.34*	227.00±6.44*
Final body Weight	257.00±7.68	209.00±6.78	219.00±7.13	214.00±5.34*	223.00±9.02*
Weight of Testes	1.41±0.18	1.37±0.09	1.41±0.19	1.22±0.02*	0.88±0.32*
Weight of epididymis	0.14±0.02	0.12±0.00	0.13±0.01	0.12±0.00	0.11±0.01

Values are Mean ± SEM. Means with asterick remark are significantly different from control: (P<0.05). Horizontal comparisons only

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Table 3: Mean hormonal profile and standard error of mean of control and treatment animals

Groups	Oestrogen (ng/ml)	FSH (IU/ml)	LH (IU/ml)	Prolactin (ng/ml)	Progesterone (ng/ml)	Testosterone (ng/ml)
A:	14.22±0.03	0.13±0.44	1.23±0.10	0.94±0.55	2.84±0.33	2.72±1.31
B:	13.13±1.24	0.11±0.02	1.07±0.47	0.82±0.42	2.35±0.12	2.14±0.32
C:	14.25±1.32	0.13±0.22	1.22±0.22	1.01±0.02	2.02±0.02	2.36±0.02
D:	17.36±2.04	0.08±0.32*	0.91±0.01*	0.83±0.11	1.89±0.45	1.02±0.12*
E:	15.34±1.50	0.10±0.11	1.09±0.13	0.77±0.23	1.99±0.26	2.82±1.20

* Values are Mean ± SEM. Means with asterick remark are significantly different from control: ($p<0.05$). Vertical comparisons only

Group A (Control): Received feed mash and water only

Group B: Normoglycaemic with low dose sildenafil

Group C: Normoglycaemic with high dose sildenafil

Group D: Hyperglycaemic with low dose sildenafil

Group E: Hyperglycaemic with high dose sildenafil

FSH: Follicle stimulating hormone

LH: Luteinizing Hormone, Ng/ml: nanogram/milliliters

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Histological findings

The histological outlook of the processed testicular and epididymal specimens from the various experimental groups are shown in Figs. 1-10. The control slides of group A (Figs. 1 & 2) revealed testis with seminiferous tubules in normal sequential maturation, separated by interstitial space and enclosed tunica albuginea while the epididymis showed ducts packed with mature spermatozoa. The normoglycaemic rat testis of group B treated with low dose sildenafil citrate showed apparently normal seminiferous tubules, interstitial vascular hypertrophy, mild congestion and moderate tissue separation while its epididymal tissue had mildly reduced luminal spermatozoa population (Figs. 3 & 4). Group C normoglycaemic rat testis treated with high dose sildenafil citrate similarly had apparently normal seminiferous tubules and moderate tissue separation while its epididymal lumen is enriched with spermatozoa storage but with mild infiltrates of chronic inflammatory cells in the interstitial space (Figs. 5 & 6). Group D hyperglycaemic rat testis treated with low dose sildenafil citrate showed sequential maturation of sperm cells and its epididymis had mildly reduced luminal spermatozoa population (Figs. 7 & 8). Group E hyperglycaemic rat testis treated with high dose of sildenafil citrate revealed moderate improvement in sequential maturation of spermatozoa and the epididymis showed fairly improved luminal storage of spermatozoa (Figs. 9 & 10).

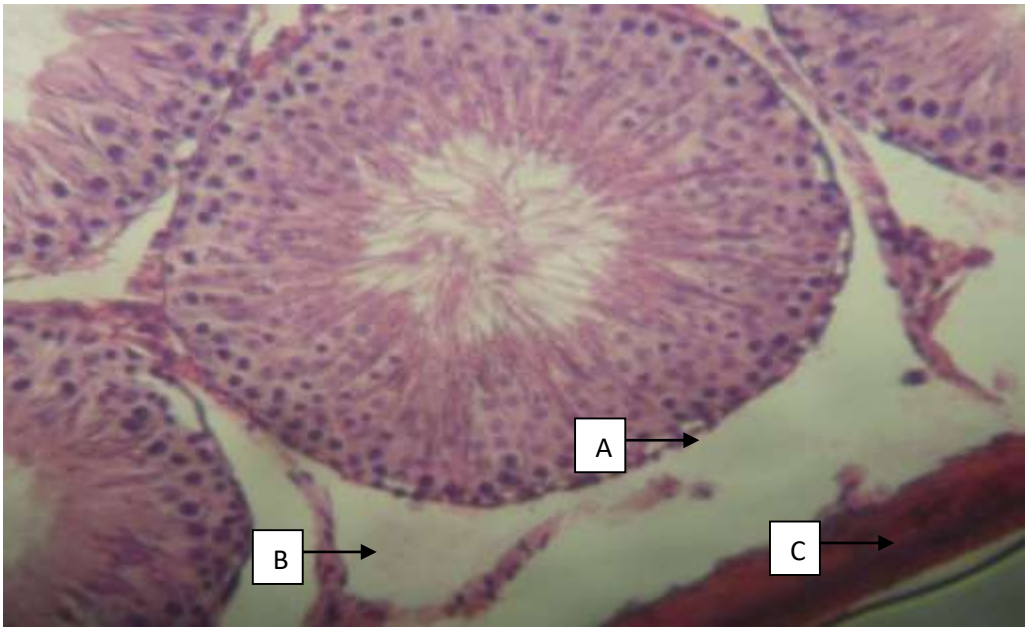

Fig 1: Section of control testis: Seminiferous tubules with normal sequential maturation A, separated by interstitial space B and enclosed tunica albuginea C (H&E x 400)

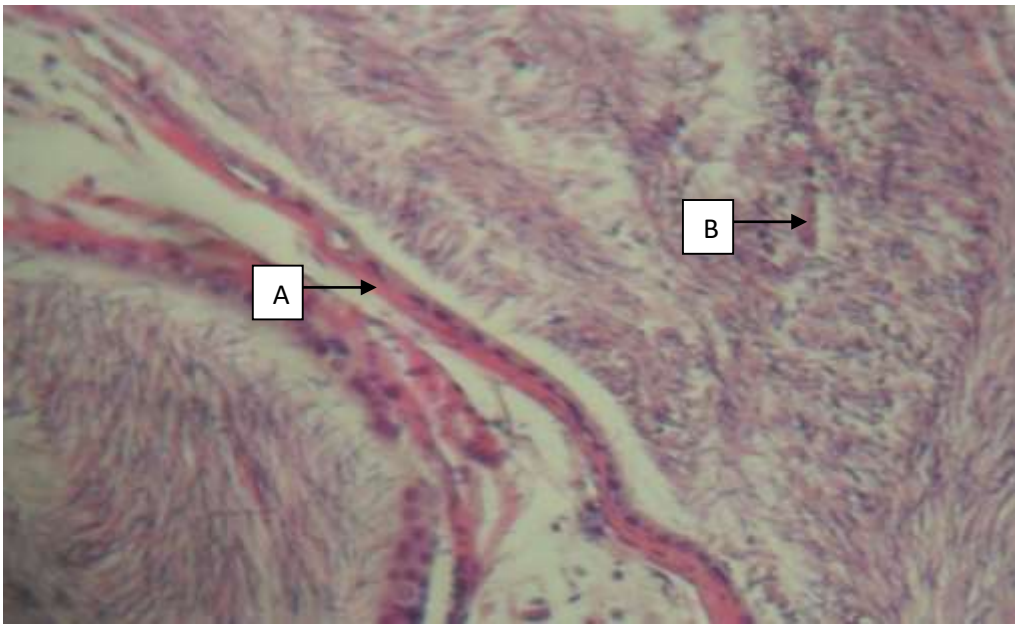

Fig 2: Control rat epididymis showing epithelial lining with columnal cells A, lumen packed with mature spermatozoa B (H&E x 400)

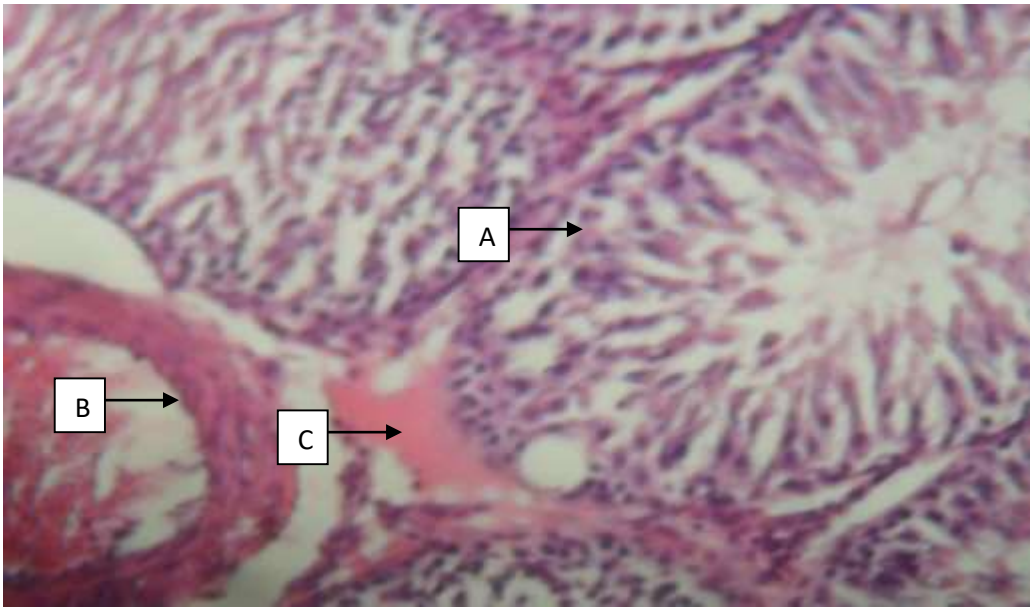

Fig 3: Normoglycaemic rat testis treated with low dose sildenafil citrate showing apparently normal seminiferous tubule A, interstitial vascular hypertrophy and mild congestion B and moderate tissue separation C (H&E x 400).

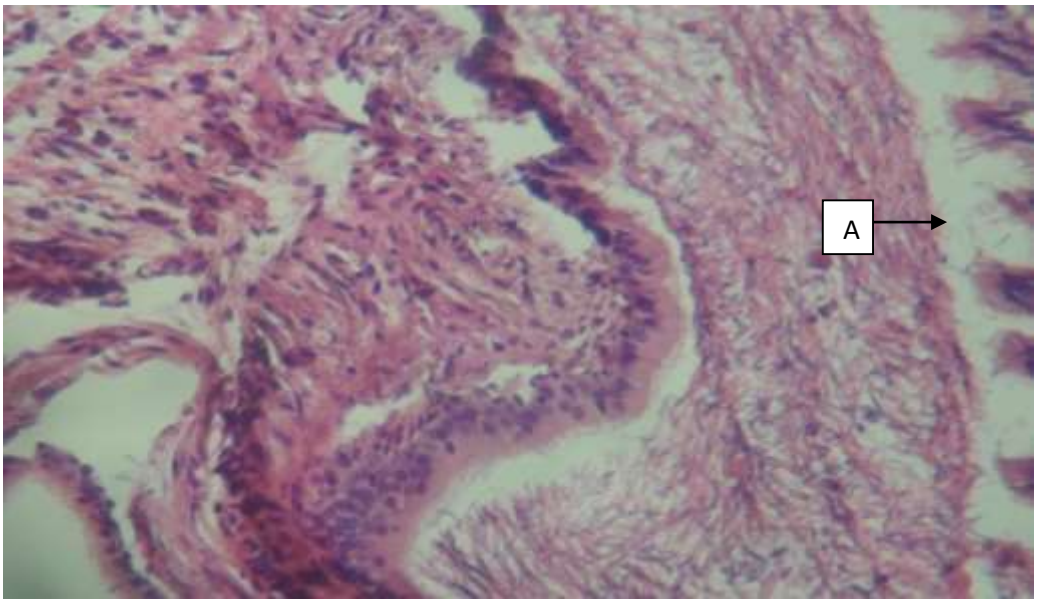

Fig 4: Normoglycaemic rat epididymis treated with low dose sildenafil citrate showing mildly reduced luminal spermatozoa population A (H&E x 400).

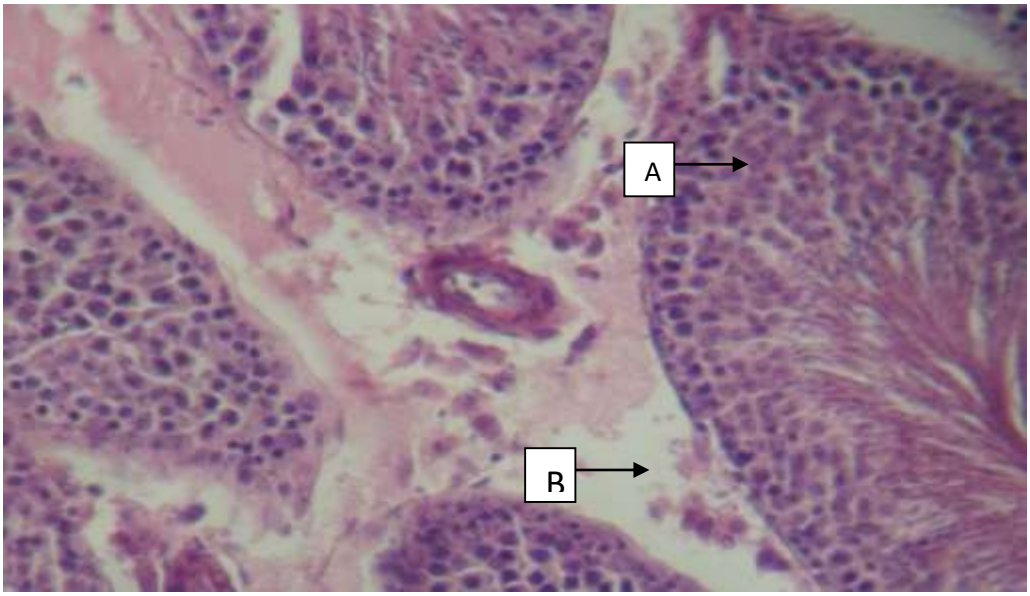

Fig 5: Normoglycaemic rat testis treated with high dose sildenafil citrate showing apparently normal seminiferous tubules A and moderate tissue separation B (H&E x 400).

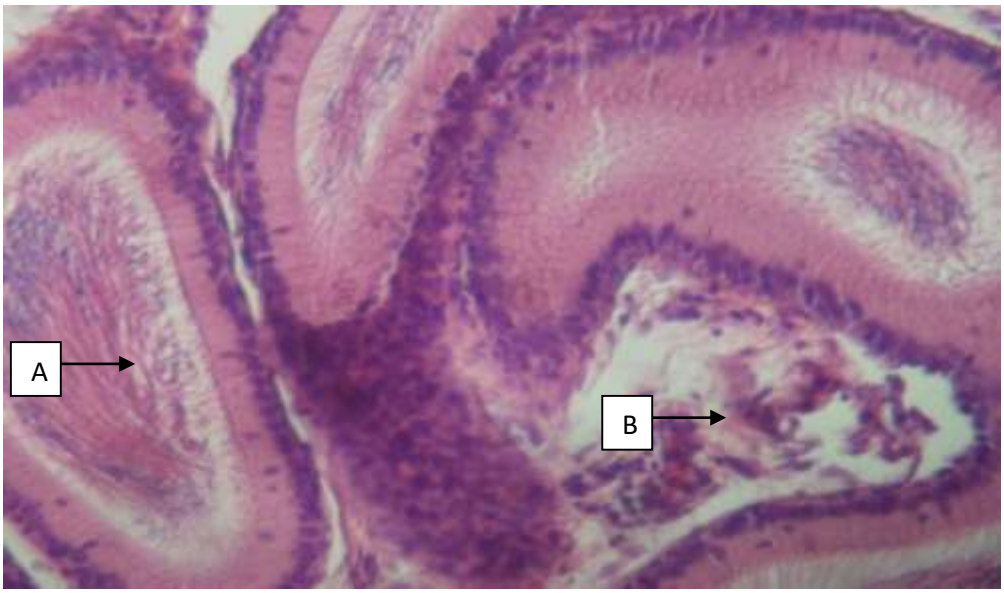

Fig 6: Normoglycaemic rat epididymis treated with high dose sildenafil citrate showing lumen with enriched spermatozoa storage A and mild infiltrates of chronic inflammatory cells in the interstitial space B (H&E x 400).

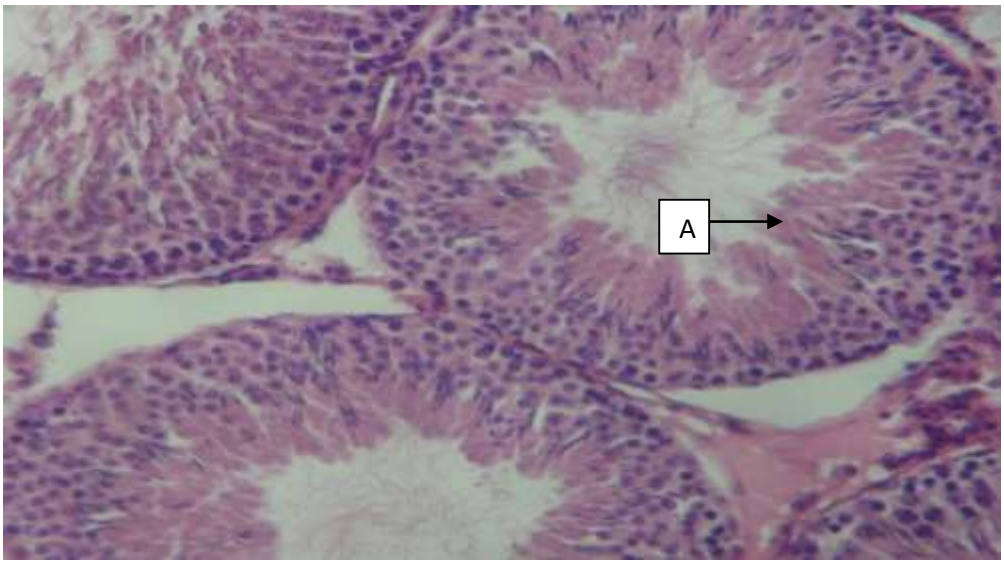

Fig 7: Hyperglycaemic rat testis treated with low dose sildenafil citrate showing normal sequential maturation of sperm cells A (H&E x 400)

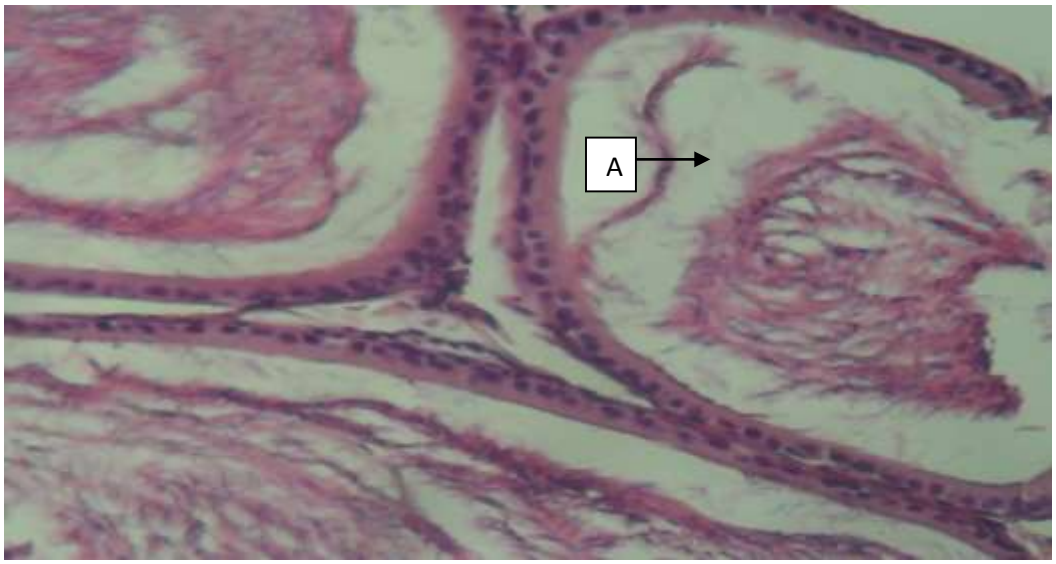

Fig 8: Hyperglycaemic rat epididymis treated with low dose sildenafil citrate showing mildly reduced luminal spermatozoa population A (H&E x 400)

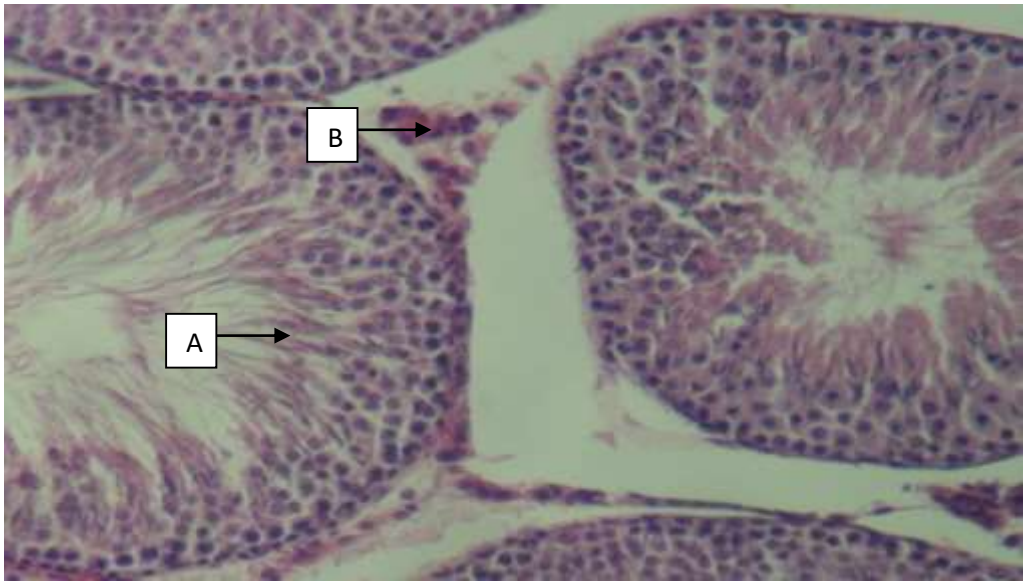

Fig 9: Hyperglycaemic rat testis treated with high dose sildenafil citrate showing moderate improvement in sequential maturation A and medullary interstitium with Ley dig cell B (H&E x 400).

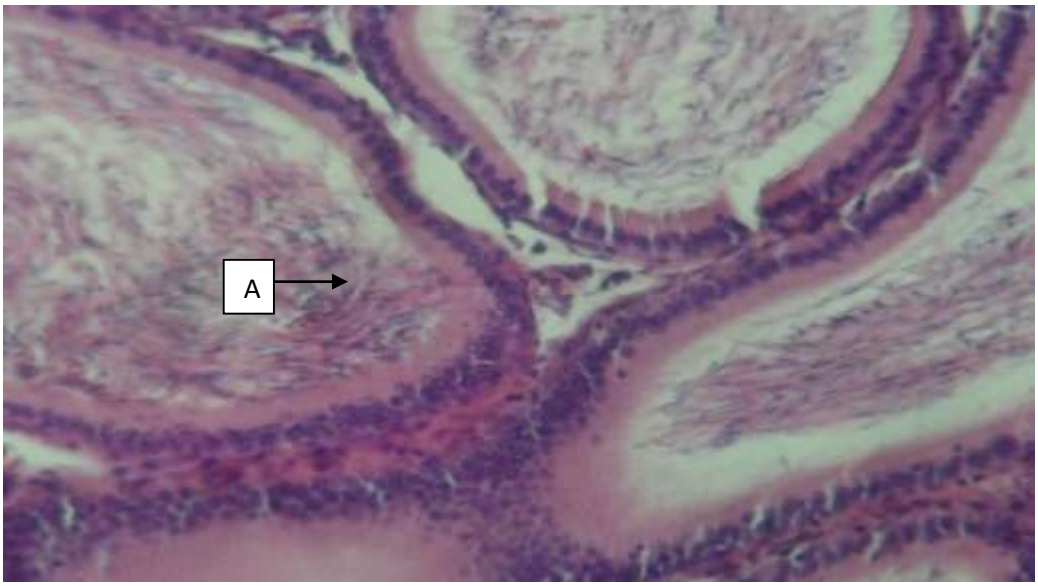

Fig 10: Hyperglycaemic rat epididymis treated with high dose sildenafil citrate showing fairly increased luminal spermatozoa population A (H&E x 400).

DISCUSSION

Sildenafil citrate is a known drug of choice in the treatment of erectile dysfunction which is the inability to sustain a satisfactory erection (Boolell *et al.*, 1996; Vardi and Nini, 2007). One of the major causes of erectile dysfunction is diabetes mellitus (Montague *et al.*, 2005) which has also been known to be deleterious to the testis, affecting male fertility (Zhao *et al.*, 2011). Experimental diabetic animals tend to suffer from testicular dysfunction such as reduced sperm count, low serum testoterone levels and decreased fertility (Mallick *et al.*, 2010).

The result of this study revealed that sildenafil citrate does not significantly affect body, testicular and epididymal weight of treatments compared to control. The observed body, testicular and epididymal weight loss in the hyperglycaemic group is consistent with previous reports (Roy *et al.*, 2013). The normoglycaemic groups treated with low and high dose sildenafil citrate showed relatively normal testicular and epididymal cytoarchitecture with progressive spermatogenesis in the seminiferous tubules and good spermatogenic storage in the epididymis.

The treatment group with induced hyperglycaemia and low dose sildenafil citrate showed evidence of distorted seminiferous epithelium due to tubular oedema and luminal depletion of spermatozoa which is indicative of impaired spermatogenesis. Similar picture was depicted in the lumen of the epididymis of this treatment group with mildly reduced luminal store of spermatozoa. These findings are consistent with earlier report (Khalid, 2009) onsignificantly reduced sperm count, abnormal morphology and distorted testicular architecture with sildenafil. However, in contrast, the hyperglycaemic group with higher dose of sildenafil citrate in this study showed seminiferous tubules in relatively normal spermatogenesis evidenced with germ cell serie in sequential maturation from the basal surface towards the adluminal compactment and matured spermatozoa abutting the lumen. The interstitial compactment is composed of Leydig cells with no cytoarchitectural abnormality. The lumen

of the epididymis in contrast to that of the low dose sildenafil citrate hyperglycaemic group is richly enclosed with spermatozoa with no cytoarchitectural distortion.These results gave the picture of improved spermatogenesis with increasing dose of sildenafil citrate from 1 to 2mg/kg body weight to 2mg/kg. This is supportive of previous reports on the role of sildenafil in apparently compromised testis (Beheshtian *et al*., 2008; Sivasankaran *et al.,* 2008; Yildiz *et al*., 2011).

The morphological observations in the testis and epididymis are reflective of the hormonal interplay of the pituitary-hypo-gonadal as reporetd. Impaired hypothalamo-pituitary function with low basal levels of FSH and LH with associated normal or low response to stimulation has been observed as characteristic of diabetic state in animals (Kirchick *et al.,* 1982). The functions of the testes are influenced by gonadotropic hormones produced by the anterior pituitary. Follicle stimulating hormone initiates spermatogenesis and the Luteinizing hormone (LH) results in testosterone release. The presence of both testosterone and follicle-stimulating hormone (FSH) is needed to support spermatogenesis. It has also been shown in animal studies that if testes are exposed to either too high or too low levels of estrogens (such as estradiol; E2), spermatogenesis can be disrupted to such an extent that the animals become infertile (Sierens *et al.,* 2005). Sub-chronic treatment of sildenafil citrate (Viagra) on some enzymatic and non-enzymatic antioxidants in testes and brain of male rats was said to have caused significantly increased MDA levels in testes and significantly reduced brain levels but with observed significant increase in GSH content of testes and brain that suggested therapeutic dose of sildenafil citrate to have elicited modulatory roles by stabilizing/boosting antioxidant defense systems in male rat (Akintunde *et al.,* 2012).

In this study, it was remarkable that impaired testicular spermatogenesis was noted in the hyperglcaemic treatments with altered oestrogen and testosterone levels compared to control, while undistorted or insignificantly different levels of FSH, LH and testosterone levels compared to control facilitated spermatogenesis. This finding may be adducible to the possible role of oxidative stress and endothelial dysfunction in induced diabetes (Molnar *et al.,* 2005; Ataman and Osinubi, 2017), which this study considers remediable with the use of sildenafil citrate. This position is supported by previous findings (Ayala *et al.,* 2007; Oudot *et al*., 2009 Mammi *et al*., 2011; Akef *et al*., 2012) .

CONCLUSION

The objective of this study to ascertain the safety of the use of sildenafil citrate, moreso on long term basis in the management of erectile dysfunction in males of reproductive age is justifiable from these reports as no significantly deleterious effect was noticed in the testicular and epididymal functions following sildenafil administration. Also, the findings from this study did not particularly elicit testicular functional impairment with the use of sildenafil in hyperglycaemic state, but a comparative advantage of possible synergistic effect with its use that is dose dependent. This calls to mind the need to emphasize discretional use of sildenafil (Smith and Romanelli, 2005) preferably with physician's prescription and monitoring so as to avert its abuse and deleterious effects.

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