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Effects of a Conventional Aphrodisiac: A Twenty Eight Day Repeated Administration of Sildenafil Citrate Graded Doses on Experimental Rats

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Abstract
Sildenafil citrate (Viagra), a phosphodiesterase-5 inhibitor (PDE5I), has long been used as a first-line oral treatment for erectile dysfunction. While it has beneficial effects on the erectile organ, it also has some negative effects on other cells and/or tissues related to the reproductive system when exposed for an extended period of time. The purpose of this study was to study the long term effect of Viagra on sperm parameters in male Wistar albino rats. Twenty (20) albino male Wistar rats were selected into four groups (n=5) randomly. The control group was administered 1 mL/kg of distilled water while 50 mg/kg, 100 mg/kg, and 200 mg/kg body weight of Sildenafil citrate were administered once in a day for 28 days to three treatment groups of rats. Oral administration was done using oropharyngeal cannula once daily for 28 days. At the end of the trial period animals were sacrificed on the 29th day, and epididymal sperm and testes were collected subject to various analytical assays. Findings showed significant reduction in sperm count and sperm normality, with a significant incremental in sperm malformations/abnormality in 28 days Viagra exposed animal when compared to the control. The testicular assay depicted histopathological alterations in testes of male rats treated with Sildenafil citrate at all dose levels. The current study clearly demonstrated that long-term use of Viagra caused changes in sperm quality and quantity, resulting in a decrease in fertility rate. It also suggested that Viagra had an effect on spermatogenesis and epididymal function. Understanding the molecular downstream events involved in long-term PDE5 inhibitor exposure can be useful for supervising infertility related issues and suggesting corrective measures.

Keywords: Sildenafil citrate, Sperm, Testicular indices, Fertility, Rats.

INTRODUCTION
The use of therapeutic agents in the treatment of diseases or any disorderly condition in human physiological phenomena dates back to time immemorial. Impotence, also known as erectile dysfunction (ED), is the inability to obtain and maintain a sexually suitable erection (Yakubu *et al*., 2007). It is a common condition that can have a negative impact on one's quality

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of life [National Institutes of Health (NIH), 1993], affecting both the elderly and the young (Feldman *et al*., 1994); and for there to be a normal sexual intercourse and sexual fulfillment in males, the male sexual organs (the copulatory organ, the penis) and factors relating to erection must function normally (Yakubu *et al*., 2007). *Sildenafil citrate* (Viagra) is the first oral drug approved for the treatment of erectile dysfunction that works pharmacologically by selective inhibition of phosphodiesterase-5 (PDE5) which is the isozyme that metabolizes cyclic guanosine monophosphate (c-GMP) in the corpus cavernosum (Boolell *et al*., 1996).

The release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation is required for penile erection (Yakubu *et al*., 2007). It is an atypical regulatory molecule having the dual role as a secondary messenger/neurotransmitter, and has been implicated in diverse physiological functions (Forstemann *et al*., 1990); as stimulus for relaxation of penile vasculature and trabecular smooth muscle, essential for penile erection (Holmquist *et al*., 1991). Relaxation of the trabecular smooth muscle of the corpus cavernosa leads to a decrease vascular resistance and increased blood flow to the penis (Holmquist *et al*., 1991). Sildenafil increased the effect of NO by inhibiting PDE5, which is responsible for cGMP corpus cavernosum degradation (Moncada and Higgs, 1993). When Viagra is taken before sexual activity, it produces consistent efficacy, good tolerability, and rapid absorption, resulting in a rapid onset of action as it has a plasma half-life that results in an appropriate duration of action while avoiding accumulation with repeated once-daily use (Boolell *et al*., 1996).

In *Homo sapiens*, reproduction is initiated by the mating of male with a female in sexual intercourse which facilitates the coming together of sperm and egg for the purpose of fertilization (Yakubu *et al*., 2007). Fertilization *in vivo* necessitates a sufficient number of spermatozoa with normal morphology and motility (Adamopoulos *et al*., 1996). Because spermatogenesis is a complex process of cellular development, any stage of developmental malformation can lead to a reduction in fertility (Adamopoulos *et al*., 1996). It based on these background information that this study was conducted to study the effects of chronic Sildenafil citrate treatment on some sperm characteristics (count and morphology) in relation to testicular damage in experimental rats.

MATERIALS AND METHODS

Animal Used

Twenty (20) male Wistar albino rats (*Rattus norvegicus*) were used for this study. Male rats of body weight (b.w.) 225～250 g were procured from the animal holding unit of the Department of Anatomy, Faculty of Basic Medical Science, University of Benin, Edo State, Nigeria and used for the research study. They were housed individually in separate standard cages and maintained under standard laboratory conditions (temperature 24～28 ˚C, relative humidity 60～70%, and 12-hour light-dark cycle) with free access to solid pellet diet and water *ad libitum* throughout the study. The study was approved by the Institutional Ethical Committee. And maintenance of experimental rats, purpose of control and supervision of experiments were undertaken according to animal's guidelines for laboratory animal facility as recommended by the Institutional Ethical Committee. Experimentations on animal were carried out after subjecting it to a quarantine period of seven (7) days.

Sildenafil Citrate Administration

Sildenafil citrate (Viagra), used for the study was a product of Pfizer Incorporated, New York, NY10017, USA. After the quarantine periods the animals were divided into four groups (n=5), group I (control) – given 1 mL/kg distilled water (vehicle), and three treatment groups. The

procured Sildenafil citrate (Viagra), was reconstituted separately in distilled water to give the required graded doses of 50, 100, 200 mg/kg b. w., and administered to the experimental rats in groups II, III, and IV respectively. The administration was done once daily for 28 days. At the end 28 days, specifically on the 29th day, the experimental rats were sacrificed and the caudal epididymis sperm parameter assay and testicular histopathology were carried out.

Sperm Analyses

Sperm Count

Sperm count was done according to the procedure described previously by Suresh *et al*., (2010). Briefly, spermatozoa were collected from caudal portion of epididymis, by mincing caudal epididymis with anatomical scissors in 5 mL of pre warmed (35 ˚C) physiological saline, placed in a rocker for 10 minutes. Supernatant fluid was diluted 1:100 with solution containing 5 g Sodium bicarbonate, 1 mL formalin (35%) and 25 mg Eosin/ 100 mL H_2O. Total sperm was determined with haemocytometer (Model S113, Olympus, Japan). Approximately 10 µL of diluted sperm suspension was transferred to each counting chamber and was allowed to stand for 5 minutes and counted under a light microscope (Model B22, Olympus, Japan) at 400 × magnification.

Sperm Morphology

The technique described by Dostal *et al*. (1996) was employed. The caudal epididymis was cut and weighed. A cell suspension was prepared by macerating the caudal epididymis in 1.0 mL of 0.85% saline. The cell suspension was kept for 24 hours at 40 ˚C. The suspension was then filtered through a double gauze layer and an aliquot of the sample was used for analysis. An aliquot of the caudal epididymis sperm suspension was smeared and stained with hematoxylin and eosin. For morphology analysis one hundred sperm were evaluated, and then examined under a light microscope (Model S45, Olympus, Japan) at magnification of x40.

Testicular Histopathology

The preparation of tissues for histopathological study was done using the standard technique outlined by Drury *et al*. (1967). And testes of experimental rats were dissected out, cut into small slices, and fixed in 10% formaldehyde buffer for 24 hours. The tissues were washed free of 10% formaldehyde and stored in 70% alcohol until being embedded. The tissues were dehydrated in alcohol series and embedded in paraffin. Tissue sections of 5 µm thickness were prepared and placed on glass slides. The sections were stained with hematoxylin and eosin and mounted in mounting medium. The slides were examined under the light microscope. For each testis several cross sections composing of 20-50 tubule sections were examined for signs of interstitial edema, somniferous tubule degeneration, and congestion.

Statistical Analysis

Data were analysed using SPSS (version 20) statistical package and Microsoft Excel (2013) software. The differences between test groups, and control were analysed by one-way analysis of variance (ANOVA), and significant means were separated using Duncan's multiple range tests. $p < 0.05$ was regarded as significant.

RESULTS

Effect of Sildenafil Citrate (Viagra) on Sperm Count of Male Rats

The twenty eight day repeated administration of Sildenafil citrate (50 mg/kg, 100 mg/kg and 200 mg/kg) on sperm count of experimental male rats caused a significant reduction in number of sperms comparing with control group (Table 1). The results also indicated that the

treatment of male rats with 100, and 200 mg/kg of Viagra had more depletion and deleterious effect than those rats treated with 50 mg/kg of Viagra.

Table 1: Effect of twenty eight day repeated administration of Sildenafil citrate on sperm count of experimental rats.

Treatments	Sperm count (mm^3)
Control group	95.2±1.22
Group treated with 50 mg/kg Sildenafil citrate	62.5* ±1.52
Group treated with 100 mg/kg Sildenafil citrate	23.5** ± 1.45
Group treated with 200 mg/kg Sildenafil citrate	21.3**±1.32

** There is a significant difference compare with control group at P<0.05.*
*** There is significant difference compare with group treated with 50 mg/kg Sildenafil citrate at P<0.05.*

Effect of Sildenafil Citrate (Viagra) on Sperm Morphology of Male Rats

Sperm normality percentage levels in rat decreased, while abnormality increased as the dose increased when compared with the control group of rats. The observed percentage levels of normal sperm form were (35.3±0.32), (17.5±0.29), and (8.40±0.21) when compared with the control value of (90.56±0.28), while abnormal sperm form percentage levels in male rats administered Sildenafil citrate at the various graded doses were (62.3±0.46), (71.40±0.35), and (91.42±0.31), respectively when compared with the control value of (9.44±0.21). From these findings, it can be seen that the chronic treatment of male rats with sildenafil citrate revealed a significant increase in abnormal sperms compared with control group. The results indicated that these cellular changes were more effective in the rats treated with 100, and 200 mg/kg compared with rats treated with 50 mg/kg of Viagra.

Effect of Sildenafil Citrate (Viagra) on Testes (Testicular Histopathology)

The results of the present study indicated that the administration of 50 mg/kg, 100 mg/kg, and 200 mg/kg of Viagra for male rats caused histopathological changes in testis of male rats. All sections of testes collected from rats administrated 50 mg/kg, 100 mg/kg, and 200 mg Sildenafil citrate had necrosis of both somniferous tubules and the interstitial tissue, congested blood vessels, hypertrophy of the interstitial leydig cells and degeneration of the spermatogonial cells (Plates 2, 3, and 4). However, the control rats did not record these histological abnormalities, but possessed normalcy of testicular cyto-architecture (Plate 1).

Plate 1: Cross section of the seminiferous tubules in the testis of the control rat: noticeable features of normalcy were regularly arranged tubules with no necrosis. (H&E staining 40X).

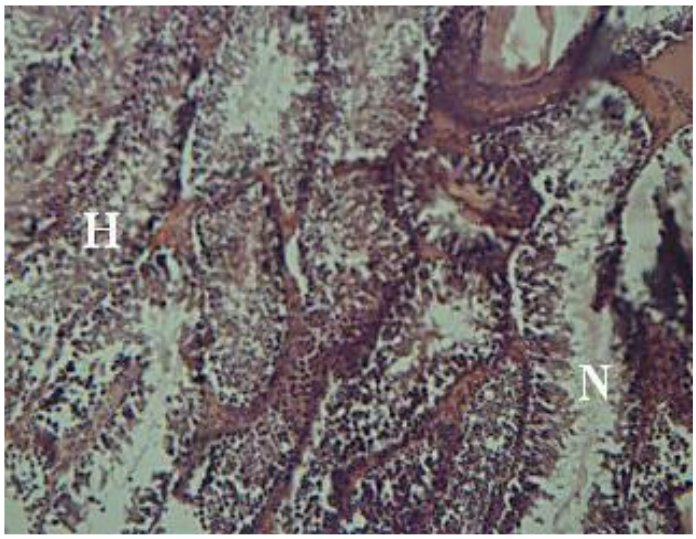

Plate **2**: Cross section of the seminiferous tubules in the testis of rat administered 5ppt of 50 mg/kg Sildenafil citrate (Viagra): Noticeable histo-degenerative features were hypertrophy cells (H), and necrosis of seminiferous tubules (N). (H&E staining 40X).

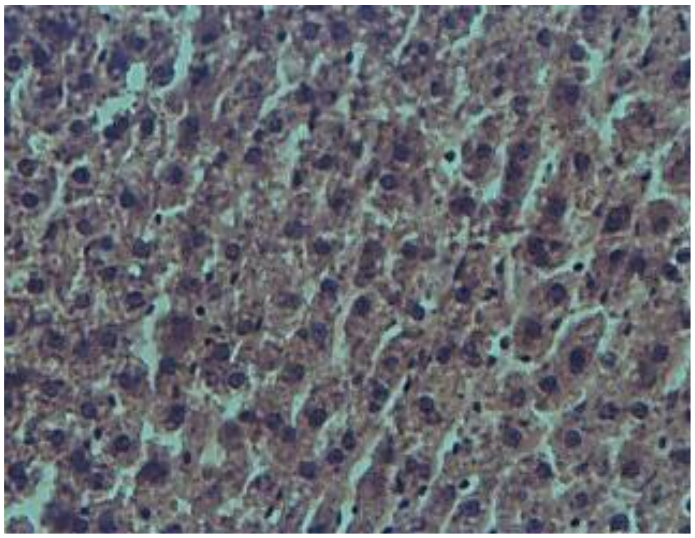

Plate **3**: Cross section of the seminiferous tubules in the testis of rat administered 5ppt of 100 mg/kg Sildenafil citrate (Viagra), depicts distortion of seminiferous tubules with dark spotted patches of inflammatory emanation. (H&E staining 40X).

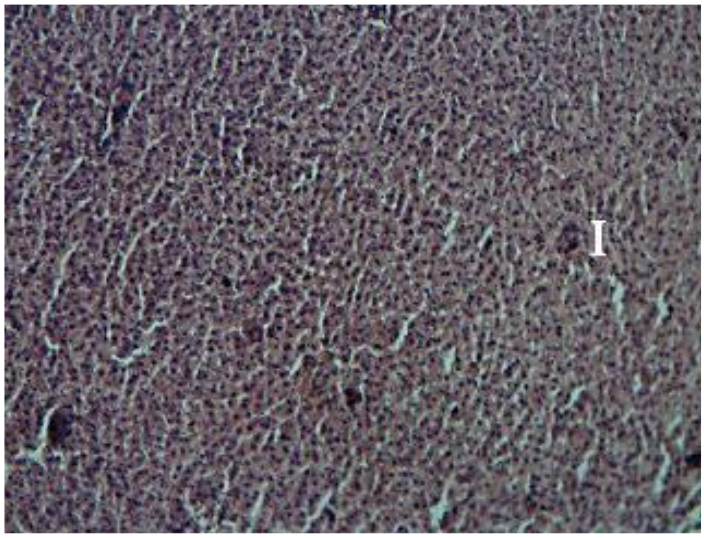

Plate 4: Cross section of the seminiferous tubules in the testis of rat administered 5ppt of 200 mg/kg Sildenafil citrate (Viagra), depicts complete destruction of seminiferous tubules: Noticeable histo-degenerative features were inflammatory cells, denoted with I. (H&E stain. 40X).

DISCUSSION

Sperm structural and functional integrity is critical for fertilization and progeny quality. Male infertility is heavily influenced by sperm quality and quantity. In the current study, a significant reduction in sperm count was observed in experimental rat exposed to Sildenafil citrate for 28 days, indicating that Sildenafil has a toxic effect on spermatogenesis and/or spermiogenesis at the testicular level, leading to increased production of defective sperm. Sperm reproductive capacity (count and viability) is an important factor in determining the success of in vitro fertilization and insemination procedures. Previous human studies have shown that using Sildenafil citrate for a short period of time can improve spermatic functions and activate the acrosomal reaction (Burger *et al*., 2000; Mostafa, 2007). Results generated in this study show a significant reduction in sperm functional status test in long term Sildenafil citrate treated rats, indicating that long term exposure might cause reduced acrosomal functional status, as well as sperm cell malformation and malfunctioning (Glenn *et al*., 2007). Consistent with previous research, the current findings showed that long-term Sildenafil

citrate exposure significantly altered sperm fertilizing capacity (Glenn *et al*., 2009). The relationship between reduced acrosomal functional status and decline in fertility indicated the role of Sildenafil citrate's long-term influence on rat sperm.

The decreased concentration of sperm count and increased amount of abnormal sperm in Sildenafil citrate treated rats observed in this study could be attributed to the administered graded doses, route of administration, and long term administration effect of Sildenafil on Sertoli cell function, resulting in impaired spermatogenesis or spermiation, which according to Suresh and Prakash (2010), can result in sperm cell malfunctioning or malformation. Increased morphological defects and cytoplasmic remnants in sperm following long-term Sildenafil citrate exposure in rats suggest that it also affected caudal epididymis and epididymal functions of the experimental rats studied. The significant decrease in sperm count associated with increased sperm malformation can impair motility and reduce fertilizing capacity, as extra defects for the sperm may cause it to travel further to reach the ampullary part of the fallopian tubes for fertilization (Cooper, 2011). Long-term administration of Sildenafil citrate was found to have an infertility effect in this study.

The observed changes in sperm parameters suggested that long-term Sildenafil citrate consumption can cause severe alterations in the testis (causing testicular cyto-architectural degeneration, which can impair motility and reduce spermatozoa fertilizing capacity), caudal epididymis, and epididymis and induce sperm damage, resulting in infertility. Similar findings had been reported by Glenn *et al*. (2009). In recent decades, both old and young men have used PDE5 inhibitors to improve penile erection and sexual life. Although Sildenafil citrate may be beneficial in the short term, long-term administration may increase the risk of infertility, as evidenced by the reproductive toxicity in caudal epididymis sperm observed in the current study. Following the preceding of this study, there are significant amount of findings creating awareness on the histological adverse effect of Sildenafil citrate in rats following oral administration, which could be used justifying human circumstances. Histopathology in visceral organs involves membrane structural and functional integrity degeneration/distortion. Sildenafil citrate was implicated as a toxin in this study, as it had a negative effect on the studied visceral organ, the testes of rats. This finding is consistent with the findings of Ezekwesili *et al*. (2011), who discovered significant histological changes in the visceral organs of rats treated intraperitoneally for 28 days. These findings, however, could be explained in part by dose relativity, route of administration, or duration of administration (Odigie and Odigie, 2014).

CONCLUSION

Long-term administration of Sildenafil citrate in an animal model, justifying human dose experimental circumstance, was found to have an infertility effect on the experimental rats employed in this study. Sildenafil citrate thus can be implicated to have effect on spermatogenesis as well as the caudal epididymis and vas deferens functions. Understanding the molecular downstream events involved in long-term PDE5 inhibitor exposure through basic and clinical research can be useful in supervising this infertility issue so as to improve on corrective measures through a short term administration of Sildenafil citrate at low doses.

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Conflicting Interest
No conflict of interest is associated with this manuscript.

Authors Declaration
The authors hereby declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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