eISSN: 09748369, www.biolmedonline.com

The effects of sildenafil citrate on the superior colliculus of adult Wistar rats (*Rattus norvegicus*) - a histological study

***AO Eweka[1], AB Eweka[2]**

[1] Department of Anatomy, School of Basic Medical Sciences, University of Benin, Edo State, Nigeria.
[2] School of Nursing, University of Benin Teaching Hospital, Benin City, Nigeria.

*Corresponding Author: andreweweka@yahoo.com

Abstract

The histological effect of oral administration of sildenafil citrate (Viagra), commonly used as an aphrodisiac and for the treatment of erectile dysfunction on one of the visual relay centres namely the superior colliculus (SC) of adult Wistar rat was carefully studied. The rats of both sexes (n=24), average weight of 202g were randomly assigned into three treatment (n=18) and control (n=6) groups. The rats in the treatment groups 'A', 'B' and 'C' received respectively, 0.25mg/kg, 0.70mg/kg and 1.43mg/kg body weight of sildenafil citrate base dissolved in distilled water daily for 30 days, through orogastric feeding tube, while that of the control group D, received equal volume of distilled water daily during the period of the experiment. The rats were fed with growers' mash obtained from Edo Feeds and Flour Mill Ltd, Ewu, Edo State, Nigeria and were given water liberally. The rats were sacrificed on day thirty-one of the experiment. The Superior colliculus was carefully dissected out and quickly fixed in 10% formal saline for histological studies. The histological findings after H&E method indicated that the treated section of the Superior colliculus showed some varying degree of cell clustering, cellular hypertrophy, and intercellular vacuolations appearing in the stroma of the superior colliculus. Varying dosage and long administration of sildenafil citrate may have some deleterious effects on the neurons of the intracranial visual relay centre and this may probably have some adverse effects on visual sensibilities by its deleterious effects on the cells of the superior colliculus of adult Wistar rats. It is therefore recommended that further studies aimed at corroborating these observations be carried out.

Keywords: Sildenafil citrate; superior colliculus; cell clustering; cellular hypertrophy; vacuolations; Wistar rats.

Introduction

Erectile dysfunction (ED or "male impotence") is a sexual dysfunction characterized by the inability to develop or maintain an erection of the penis sufficient for satisfactory sexual performance (NIH Consensus Conference: Impotence, 1993). An erection occurs as a hydraulic effect due to blood entering and being retained in the corpus cavernosum within the penis. The process is most often initiated as a result of sexual arousal, when signals are transmitted from the brain to nerves in the pelvis. Erectile dysfunction is indicated when an erection is consistently difficult or impossible to produce, despite arousal (Eardley and Sethia, 2003). There are various and often multiple underlying causes, some of which are treatable medical conditions. The most important organic causes are cardiovascular disease and diabetes, neurological problems (for example, trauma from prostatectomy surgery), hormonal insufficiencies (hypogonadism) and drug side effects. It is important to realize that erectile dysfunction can signal underlying risk for cardiovascular disease (Hackett et al., 2007).

Sexual dysfunction is a serious medical and social problem that occurs in 10%-52% in men and 25%-63% in women (Tharakan and Manyam, 2005). Numerous central and peripheral neural circuits control sexual activity. Impairment of one or more of these functional circuits may have a significant impact on personal, social and biological relationships. Although several aspects of sexual motivation and performance are known, a complete picture of the various factors that control human sexual activity is still unknown. The available drugs and treatments have limited efficacy, unpleasant side effects and contraindications in certain disease conditions (Tharakan and Manyam, 2005).

There is often a contributing and complicating and sometimes a primary psychological or relational problem.

Biology and Medicine

Psychological impotence is where erection or penetration fails due to thoughts or feelings (psychological reasons) rather than physical impossibility; this can often be helped. Notably in psychological impotence, there is a strong response to placebo treatment. Erectile dysfunction, tied closely as it is to cultural notions of potency, success and masculinity, can have severe psychological consequences. There is a strong culture of silence and inability to discuss the matter. In reality, it has been estimated that around 1 in 10 men will experience recurring impotence problems at some point in their lives (Vinik and Richardson, 1998).

An understanding of the physiological mechanism of erection has led to the development of new oral therapies for erectile dysfunction that target different sites in the sexual arousal process. Apomorphine activates the arousal center of the brain. Phentolamine increases penile blood flow. Sildenafil enhances the action of nitric oxide, an endothelial-derived vasodilator and smooth muscle relaxant. These developments constitute a significant advance in a much-neglected area of male medicine (Vinik and Richardson, 1998).

Sildenafil citrate is widely used as an effective and safe oral treatment for erectile dysfunction of various etiologies (Goldstein et al, 1998). It is a potent and selective inhibitor of phosphodiesterase type 5 enzymes that acts to break down cyclic guanosine monophosphate (cGMP) (Boolell et al., 1996). Accumulation of cGMP inhibits the degradation of nitric oxide that is responsible for smooth muscle relaxation within the corpora cavernosa. Nitric oxide is released by intracavernous nonadrenergic noncholinergic nerve terminals not only following a central or local erectogenic stimulus but also during rapid eye movement (REM) sleep (Burnett, 1997). Psychogenic erectile dysfunction (ED) patients are excellent candidates for sildenafil citrate therapy due to the intact neurovascular pathway. Nevertheless, the drug has been reported to be effective only in about 78% of patients with psychogenic ED (McMahon et al., 2000). It is likely that performance anxiety and sympathetic overtone are the cause of this unresponsiveness to sildenafil citrate during awakening, though data supporting this assumption are lacking (Rosen, 2001). The drug has been found to be effective and well tolerated in men with mild to moderate erectile dysfunction of no clinically identifiable organic cause (Eardley, 2001).

With the presence of PDE5 in choroidal and retinal vessels sildenafil citrate increase choroidal blood flow and cause vasodilation of the retinal vasculature. The most common symptoms are a blue tinge to vision and an increased sensitivity to light (Kerr and Danesh-Meyer, 2009). Adverse effects include headache, visual and retinal disturbances, dizziness and pupil-sparing third nerve palsy (Monastero et al., 2001). There have been reports of non-arteritic anterior ischaemic optic neuropathy and serous macular detachment in users of PDE5 inhibitors; although a causal relationship has not been conclusively shown. Despite the role of cGMP in the production and drainage of aqueous humor, these medications do not appear to alter intraocular pressure and are safe in patients with glaucoma. All PDE5 inhibitors weakly inhibit PDE6 located in rod and cone photoreceptors resulting in mild and transient visual symptoms that correlate with plasma concentrations. Psychophysical tests reveal no effect on visual acuity, visual fields or contrast sensitivity; however, some studies show a mild and reversible impairment of blue-green colour discrimination. PDE5 inhibitors transiently alter retinal function on electroretinogram testing but do not appear to be retinotoxic. Despite the role of cyclic nucleotides in tear production, there is no detrimental effect on tear film quality. Based on the available evidence, PDE5 inhibitors have a good ocular safety profile (Kerr and Danesh-Meyer, 2009).

It has been reported that Sildenafil citrate significantly improves nocturnal penile erections in sildenafil non-responding patients with psychogenic erectile dysfunction (Abdel-Naser et al, 2004). Several pharmacological and physiological properties of sildenafil have been described (Cheitlin et al., 1999; Aviv et al., 2004; Galie et al., 2005; Hoeper et al., 2006).

In Nigeria, most individuals often use sildenafil citrate indiscriminately for sexual arousal. There is a growing apprehension that it could be harmful or injurious to the body. Though sildenafil is currently being used to treat erectile dysfunction in patients with multiple sclerosis, Parkinson disease, multisystem atrophy, and spinal cord injury by improving their

neurologically related erectile dysfunction, conversely, it has been implicated in a number of neurological problems, such as intracerebral hemorrhage, migraine, seizure, transient global amnesia, nonarteritic anterior ischemic optic neuropathy, macular degeneration, branch retinal artery occlusion, and ocular muscle palsies. Thus, preclinical and very limited clinical data suggest that sildenafil may have therapeutic potential in selected neurological disorders. However, numerous reports are available regarding neurological adverse events ascribed to the drug. Although sildenafil shows some promise as a therapeutic agent in selected neurological disorders, well-designed clinical trials are needed before the agent can be recommended for use in any neurological disorder (Farooq et al., 2008).

The superior colliculus and lateral geniculate body constitute the intracranial visual relay centres. The superior colliculus has a critical role in visual localization, orientation tracking movements, accommodation and pupiliary reflex (Reczkowski and Diamond, 1978). An analysis of effective connectivity demonstrated that the search-dependent variance in the activity of the superior colliculus was significantly influenced by the activity in a network of cortical regions including the right frontal eye fields and bilateral parietal and occipital cortices (Altman and Bayer, 1981). Cerebral nuclei such as the medial and lateral geniculate bodies, inferior and superior colliculi have higher glucose utilization than other structures (Siesjo, 1985). There is also a correlation between functional activity and metabolic rate such as in the visual and auditory system (Siesjo, 1985).

The effects of sildenafil citrate on the intracranial visual relay centre may not have been documented, but there have been reports that it may be implicated in varied symptoms of dizziness, vomiting, headaches, diarrhea, tinnitus, increase hearing loss, macular rash, neutropenia, migraine, seizure, transient global amnesia, nonarteritic anterior ischemic optic neuropathy, macular degeneration, branch retinal artery occlusion, and ocular muscle palsies. It is probable that the adverse effects of sildenafil citrate on vision such as dizziness may be due to direct effect of sildenafil citrate on this visual relay centre. This present study was to elucidate the histological effects of sildenafil citrate on the superior colliculus of adult Wistar rats.

Materials and Methods

***Animals*:** Twenty-four (24) adult Wistar rats of both sexes with average weight of 202g were randomly assigned into four groups A, B, C and D of (n=6) in each group. Groups A, B, and C of (n=18) serves as treatments groups while group D (n=-6) was the control. The rats were obtained and maintained in the Animal Holdings of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. They were fed with grower's marsh obtained from Edo feed and flourmill limited, Ewu, Edo state, and were given water liberally. The rats were allowed to gain maximum acclimatization before the actual commencement of the experiment. The Artesunate tablets were obtained from the University of Benin Teaching Hospital Pharmacy, Benin City, Edo state, Nigeria.

***Sildenafil Citrate Administration*:** The rats in the treatment groups (A, B, & C) received respectively, 0.25mg/kg, 0.70mg/kg and 1.43mg/kg body weight of sildenafil citrate base dissolved in distilled water daily for 30 days, through orogastric feeding tube, while that of the control group D, received equal volume of distilled water daily during the period of the experiment. The rats were sacrificed by cervical dislocation on day thirty-one of the experiment. The skulls were opened using bone forceps to expose the brain of the rat, and the superior colliculus was quickly dissected out and fixed in 10% formal saline for routine histological techniques.

Histological Study: The tissue was dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 microns thick were obtained using a rotatory microtome. Some of the deparaffinized sections were stained routinely with hematoxylin and eosin (H&E) method (Drury, 1967). The digital photomicrographs of the desired sections were made in the Department of Anatomy research laboratory, University of Benin, Nigeria for further observations.

Biology and Medicine

Results

Photomicrographs of the sections of the superior colliculus (SC) from the control group (D) showed normal histological features, with the neurons appearing distinct and the glial cells normal without vacuolation in the stroma (Figure 1).

The sections of the superior colliculus from the treatment (A, B, & C) groups showed some varying degree of cell clustering, cellular hypertrophy, and intercellular vacuolations appearing in the stroma (Figure 2, 3 & 4).

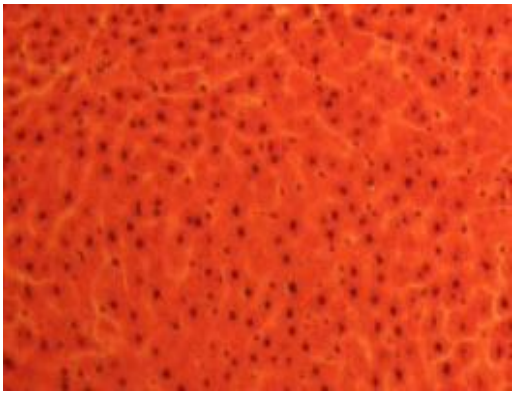

FIGURE 1 (GROUP D): Control section of the superior colliculus (Mag. x400).

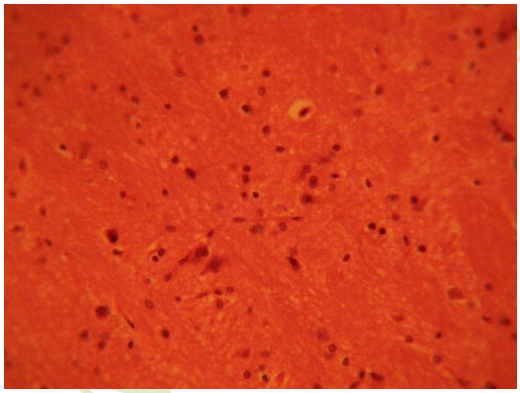

FIGURE 2: Photomicrograph of treatment section of the superior colliculus of rats that received 0.25mg/kg of--sildenafil citrate base dissolved in distilled water daily for 30 days (Mag. X400)

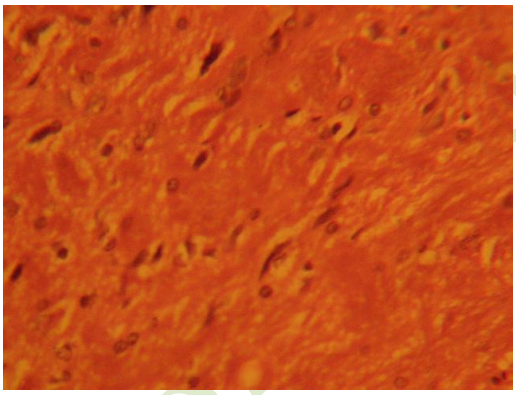

FIGURE 3: Photomicrograph of treatment section of the superior colliculus of rats that received 0.70mg/kg of sildenafil citrate base dissolved in distilled water daily for 30 days (Mag. X400).

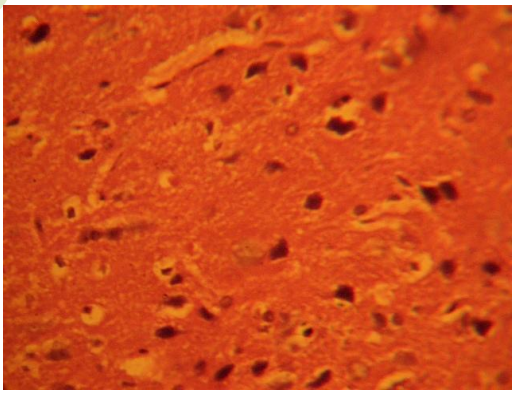

FIGURE 4: Photomicrograph of treatment section of the superior colliculus of rats that received 1.43mg/kg of sildenafil citrate base dissolved in distilled water daily for 30 days (Mag. X400).

Discussion

The results (H & E) revealed that administration of sildenafil citrate showed some varied degree of cellular degenerative changes, cellular hypertrophy, clustering of cells and intercellular vacuolations appearing in the stroma of the treatment groups compared to the control section of the superior colliculus of the adult Wistar rat. Neuronal degeneration has been reported to result in cell death, which is of two types, namely apoptotic and necrotic cell death. These two types differ morphologically and biochemically (Wyllie, 1980). Pathological or

accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects (Farber, 1981). It was reported that cell death in response to neurotoxins might trigger an apoptotic death pathway within brain cells (Waters, 1994).

The process of cellular necrosis involves disruption of the membranes structural and functional integrity. Cellular necrosis is not induced by stimuli intrinsic to the cells as in programmed cell death (PCD), but by an abrupt environmental perturbation and departure from the normal physiological conditions (Martins, 1978). There is the need to further investigate the actual mechanism by which sildenafil citrate induced neuronal degeneration in the superior colliculus of adult Wistar rat in this study.

Extensive cell death in the central nervous system is present in all neurodegenerative diseases (Waters, 1994). The type of nerve cell loss and the particular part of the brain affected dictate the symptoms associated with an individual disease (Waters, 1994). In this study sildenafil citrate may have acted as toxin to the cells of the superior colliculus, affecting their cellular integrity and causing defect in membrane permeability and cell volume homeostasis.

In cellular necrosis, the rate of progression depends on the severity of the environmental insults. The principle holds true for toxicological insult to the brain and other organs (Martins, 1998). The prime candidates for inducing the massive cell destruction observed in neurodegeneration are neurotoxins (Waters, 1994). The latter when present at a critical level can be toxic to the brain cells they normally excite (Waters, 1994). It is inferred from the results that prolonged and high dose of sildenafil citrate resulted in increased toxic effects on the SC.

The vacuolations observed in the stroma of the superior colliculus in this experiment may be due to sildenafil citrate interference. The cellular hypertrophy observed in this experiment may be due to the adverse effects of sildenafil citrate on the superior colliculus. This study may underlie the possible neurological symptoms such as dizziness and tinnitus. Sildenafil citrate has been implicated as a possible cause of blindness—diagnosed as nonarteritic anterior ischemic optic neuropathy (Cunningham and Smith, 2001; Pomeranz et al., 2002; Pomeranz and Bhavsar, 2005).

Conclusion

Our study revealed that high doses and long term administration of sildenafil citrate caused some varied degree of cellular degenerative changes, cellular hypertrophy, clustering of cells and intercellular vacuolations in the superior colliculus of adult Wistar rats. These results may probably affect the functions of the superior colliculus in visual sensibility in adult Wistar rats. It is recommended that further studies be carried out to examine these findings.

References

Abdel-Naser MB, Imam A, Wollina U, 2004. Sildenafil citrate significantly improves nocturnal penile erections in sildenafil non-responding patients with psychogenic erectile dysfunction. International Journal of Impotence Research, 16: 552–556.

Altman AS, Bayer CS, 1981. Time of origin of neurons of rat superior colliculus in relation to other components of the visual and visiomotor pathways. Experimental Brain Research, 42: 424-434.

Aviv A, Shelef A, Weizman A, 2004. An open-label trial of sildenafil addition in risperidone-treated male schizophrenia patients with erectile dysfunction. Journal of Clinical Psychiatry, 65: 97–103.

Boolell M, Allen MJ, Ballard SA, Gepi-Attee S, Muirhead GJ, Naylor AM, Osterloh IH, Gingell C, 1996. Oral sildenafil: an orally active type 5 cyclic GMP specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. International Journal of Impotence Research, 8: 47–52.

Burnett AL, 1997. Nitric oxide in the penis: physiology and pathophysiology. Journal of Urology, 157: 320–324.

Cheitlin MD, Hutter AM, Brindis RG, Ganz P, Kaul S, Russell RO, 1999. Use of sildenafil (Viagra) in patients with cardiovascular disease. Technology and Practice Executive Committee. Circulation, 99: 168–177.

Cunningham AV, Smith KH, 2001. Anterior ischemic optic neuropathy associated with Viagra. Journal of Neuro-Ophthalmology, 21: 22–25.

Biology and Medicine

Drury RAB, Wallington EA, Cameron R, 1967. Carleton's Histological Techniques: 4th ed., Oxford University Press, NY, U.S.A. 279-280.

Eardley I, Sethia K, 2003. Erectile Dysfunction: Current Investigation and Management: A Textbook, 2nd edition; Mosby-Elsevier Ltd, pp1-5. ISBN 10: 0723433658.

Eardley I, 2001. Efficacy and safety of sildenafil citrate in the treatment of men with mild to moderate erectile dysfunction The British Journal of Psychiatry, 178: 325-330.

Farber JL Chein KR, Mittnacht S, 1981. The pathogenesis of Irreversible cell injury in ischemia. American Journal of Pathology, 102: 271-281.

Farooq MU, Naravetla B, Moore PW, Majid A, Gupta R, Kassab MY, 2008. Role of sildenafil in neurological disorders. Clinical Neuropharmacology, 31(6): 353-362.

Galie N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G, 2005. Sildenafil citrate therapy for pulmonary arterial hypertension. New England Journal of Medicine, 353: 2148-57.

Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA, 1998. Oral sildenafil in the treatment of erectile dysfunction. New England Journal of Medicine, 338: 1397–1404.

Hackett G, Kell P, Ralph D, Dean J, Price D, Speakman M, Wylie K, 2007. British Society for Sexual Medicine in: *Guidelines on the Management of Erectile Dysfunction*. Journal of Sexual Medicine, 5(8): 1841–1865.

Hoeper MM, Welte T, Izbicki G, Rosengarten D, Picard E, Kuschner WG, Galiè N, Rubin LJ, Simonneau G, 2006. Sildenafil citrate therapy for pulmonary arterial hypertension. New England Journal of Medicine, 354: 1091-1093.

Kerr NM, Danesh-Meyer HV, 2009. Phosphodiesterase inhibitors and the eye. Clinical and Experimental Ophthalmology, 37(5): 514–523.

Martins LJ, Al-Abdulla NA, Kirsh JR, Sieber FE, Portera-Cailliau C, 1998. Neurodegeneration in excitotoxicity, global cerebral ischaemia and target deprivation: A perspective on the contributions of apoptosis and necrosis. Brain Research Bulletin 46(4): 281-309.

McMahon CG, Samali R, Johnson H, 2000. Efficacy, safety and patient acceptance of sildenafil citrate as treatment for erectile dysfunction. Journal of Urology, 164: 1192–1196.

Monatero R, Pipia C, Camarda LKC, Camarda R, 2001. Intracerebral haemorrhage associated with sildenafil citrate. Journal of Neurology, 248 (2): 141-142.

NIH Consensus Conference on Impotence, 1993. NIH Consensus Development Panel on Impotence. Journal of American Medical Association, 270 (1): 83–90. doi:10.1001/jama.270.1.83. PMID 8510302.

Pomeranz HD, Bhavsar AR, 2005. Nonarteric ischemic optic neuropathy developing soon after use of sildenafil (Viagra): A report of seven new cases. Journal of Neuro-Ophthalmology, 25: 9–13.

Pomeranz HD, Smith KH, Hart WM, Jr, Egan RA, 2002. Sildenafil-associated nonarteric anterior ischemic optic neuropathy. Ophthalmology, 109: 584–587.

Reczkowski D, Diamond D, 1978. Cells of origin of several efficient pathways from the superior colliculus in *Galago senegalensis*. Brain Research, 146: 351-357.

Rosen RC, 2001. Psychogenic erectile dysfunction. Classification and management. North American Journal of Clinical Urology, 28: 269–278.

Siesjo BK, 1985. Acid–base homeostasis in the brain: physiology, chemistry and neurochemical pathology. In *Molecular Mechanisms of Ischemic Brain Damage. Progress in Brain Reseach* 121–154. Amsterdam: Elsevier Science Publications.

Tharakan B, Manyam BV, 2005. Botanical therapies in sexual dysfunction. Phytotherapy Research, 19(6): 457-63.

Vinik A, Richardson D, 1998. Erectile dysfunction in diabetes: pills for penile failure. Journal of Clinical Diabetes, 16(3): 108-130.

Waters CM, 1994. Glutamate induced apoptosis of striatal cells in rodent model for Parkinsonism. Neuroscience, 6(3): 1-5.

Wyllie AH, 1980. Glucocorticoid-induced thymocyte apoptosis in associated and endogenous endonuclease activation. Nature, 284: 555-556.

Biology and Medicine