

The Histopathological Effect of Sildenafil Citrate on Superior Colliculus of Adult Male Rat.

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Abstract

Objective: Preclinical and very limited clinical studies suggest that sildenafil may have therapeutic potential in selected neurological disorders. However, many neurological side effects of sildenafil have been reported. This work aimed to clarify the histopathological effect of sildenafil citrate on the superior colliculus (SC) of adult male albino rat.

Material and methods: 24 adult male albino rats were used and divided into 4 groups. The first 3 groups were received respectively sildenafil citrate orally at doses 0.25, 0.70 and 1.43mg/kg body weight daily for 30 days while the 4th group was used as control. At end of the treatment, the superior colliculi were undergone light and electron microscopic investigation.

Results: In the control group, superficial part of the SC has neural cells and myelinated nerve fibers. With least dose of sildenafil, the superficial part of SC revealed disturbance in neural tissue with dilated capillaries and vacuoles. Some neurons showed deeply stained nuclei shrunken cytoplasm. Some cells showed enlarged mitochondria and dilated endoplasmic reticulum. With medium dose of sildenafil, SC showed more disturbances; stripped myelin sheaths or widely separated myelin lamellae, dilated blood vessels with large vacuoles around them and many neurons with apoptotic criteria. However, maximum dose of sildenafil induced massive destruction of edematous neural tissue; invasion of the surface with massive blood vessels, marked decrease in thickness of myelin sheaths and the neural cells revealed degenerative and apoptotic changes. The mean number and size of cells revealed significantly progressive decrease in all treated rats with increasing doses of the drug.

Conclusion: Long-term, daily use of sildenafil can lead to pathological effect in the superior colliculus which may be implicated in visual disturbance and this effect is dose dependent, so neurological effect of sildenafil necessitates further investigations.

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INTRODUCTION

Sexual dysfunction is a major social and medical problem that may occur in both men and women. Moreover, the available drugs have limited efficacy, many side effects and contraindications in certain diseases [1].

Sildenafil citrate is widely used as an effective and safe oral treatment for erectile dysfunction of various causes [2]. It is a highly potent and selective inhibitor of phosphodiesterase type 5 enzyme that leads to breakdown of cyclic guanosine monophosphate (cGMP). Accumulation of cGMP inhibits the breakdown of nitric oxide that results in relaxation of smooth muscle within the corpora cavernosa [3].

Phosphodiesterase type 5 enzyme is present in penile tissue, skeletal muscle, visceral and vascular smooth muscle and platelets [4]. It is also present in brain tissue, mainly in cerebellum, hippocampus and superior

cervical ganglion [5]. It has been also reported to be present and active in the basilar artery [6]. Sildenafil may have an effective therapeutic role for some neurological disorders [7], via inhibition of phosphodiesterase type 5 enzyme [8].

In models of embolic rat, sildenafil increased brain levels of cGMP, angiogenesis and neurogenesis and increased the cerebral blood flow level in the ischemic region, which can contribute to enhancement of functional recovery [9, 10]. In humans, studying the effect of sildenafil citrate on cerebral blood flow and oxygenation are insufficient [11, 12].

Also, data from preclinical and very limited clinical studies suggest that sildenafil may have therapeutic potential in selected neurological disorders. However, many neurological side effects of sildenafil have been reported as cerebral hemorrhage, seizure, migraine, amnesia, macular degeneration, retinal artery occlusion, and ocular muscle palsies [13].

Superior colliculus (SC) is the intracranial visual relay center that has a major role in visual localization, accommodation and pupillary reflex [14]. SC is highly laminated structure. It consists of superficial gray and optic layer, intermediate gray and white layer and deep gray and white layer. These layers can be functionally, divided into two parts; a superficial subdivision and deep subdivision. Superficial subdivision is involved in detection of purely visual stimuli and lesion of it leads to defects in some forms of visual discriminations [15]. The stratum griseum superficiale and stratum opticum of the SC contain a dense distribution of neurons and terminals that contain gamma amino butyric acid, which contributes to the SC receptive field properties [16].

The use of sildenafil citrate by many individuals is often indiscriminately, for sexual arousal. There is a growing apprehension that sildenafil citrate abuse may have harmful effect on SC. The effects of sildenafil citrate on the SC have not been fully studied, however sildenafil citrate has been found to be implicated as a possible cause of blindness due to nonarteritic anterior ischemic optic neuropathy [17].

The current study was carried out to clarify the histopathological effect of sildenafil citrate on the superior colliculus of adult male albino rat.

MATERIALS AND METHODS

Experimental animals

24 adult male albino rats (4-6 months old, 200-300gm weight) were used. The use of experimental animals was prospectively approved by the Committee at Mansoura University, Faculty of medicine. The rats were housed in animal care centre of Mansoura faculty of Medicine. The rats were provided with fresh food and water daily and inspected for any possible signs of inflammation or infection.

Experimental design

Animals were divided into four groups (6 rats were included in each group). First three groups were used as treated groups while the fourth group was used as control.

Sildenafil citrate administration

The rats of treated groups (1, 2 and 3) received respectively, 0.25mg/kg, 0.70mg/kg and 1.43mg/kg body weight of sildenafil citrate dissolved in distilled water daily for 30 days, through orogastric feeding tube. The giving doses were similar to the recommended human oral doses (from 25 to 100 mg.) [18]. Control group (4) received equal volume of distilled water daily during the period of the experiment through orogastric feeding tube. Sildenafil citrate was supplied from Pfizer Inc. (Pfizer, Egypt), stored at 2-4

°C and protected from sunlight.

Histological analysis

At the end of treatment, rats of each group were anaesthetized with diethyl ether, sacrificed and their brains were carefully dissected [19] and the two superior colliculi were taken. One of them was immersed in Bouin's fixative for 24 hours, processed for paraffin sections and stained with H & E stain [20] for histological examination of superficial part of SC.

Tissue preparation for ultrastructural study

The superficial part of the other SC was cut into small pieces 1mm³. The specimens were immediately fixed in cold 3.5% glutaraldehyde in 0.1 M phosphate buffer (PH 7.2), post fixed with 1% osmium tetroxide (OsO₄), processed and embedded in epon. Semithin sections (1µm thick) stained with toluidine blue were examined by light microscope. Ultrathin sections (50-70 nm thick) were cut, mounted on copper grids, stained with uranyl acetate and lead citrate [21] and examined by JEOL-100SX transmission electron microscope provided with a digital camera.

Quantitative Analysis

The number and size of neural cells in superficial part of superior colliculus were measured in a fixed field in serial sections by using Leica QWin 500 image analyzing and processing software (England). The neural cells were counted in five fixed non-overlapping microscopic fields through a 100x objective lens. Only neurons with clearly visible nucleoli were counted to differentiate from glial cells which show absence of nucleoli. The size of the neural cells was measured by 400x objective lens through obtaining two diagonal diameters of the cell in five fixed non-overlapping microscopic field.

Statistical Analysis

Statistical analysis was done using computer software SPSS program (statistical package for social science) version 10. All data were expressed as the mean ± SD. The significance level considered was P ≤ 0.05.

RESULTS

I. Light microscopic examination of superficial part of SC

Control rat

In the control rat (group 4), H&E and toluidine blue stained sections showed many small and medium sized neurons with round vesicular nuclei. The neuroglial cells appeared with small nuclei in between well-arranged nerve fibers (Figures 1A and 2A).

Sildenafil treated rats

In rat received the least dose of sildenafil citrate (group 1), H&E and toluidine blue stained sections showed disturbance in neural tissue with dilated capillaries and vacuoles. Many neurons appeared normal while others showed deeply stained nuclei, shrunken cytoplasm and surrounded by vacuoles (Figures 1B and 2B).

In rat received the medium dose of sildenafil citrate (group 2), H&E and toluidine blue stained sections showed more disturbance of neural tissue with deformed myelin sheath. More dilated and thick walled blood vessels appeared with large vacuoles around them. Many neurons showed deeply stained nuclei and

shrunken cytoplasm (Figures 1C and 2C).

In rat received the maximum dose of sildenafil citrate (group 3), H&E and toluidine stained sections showed massive destruction of neural tissue in form of loose stroma with massive vacuoles. The blood vessels appeared invading the surface, also in between the tissue with thick wall. Many neurons showed deeply stained small pyknotic nuclei and shrunken cytoplasm. Some cells showed irregular nuclear membrane with condensed chromatin. The finding revealed vacuolar cytoplasmic degeneration and apoptotic cells together with stromal edema (Figures 1D and 2D-F)

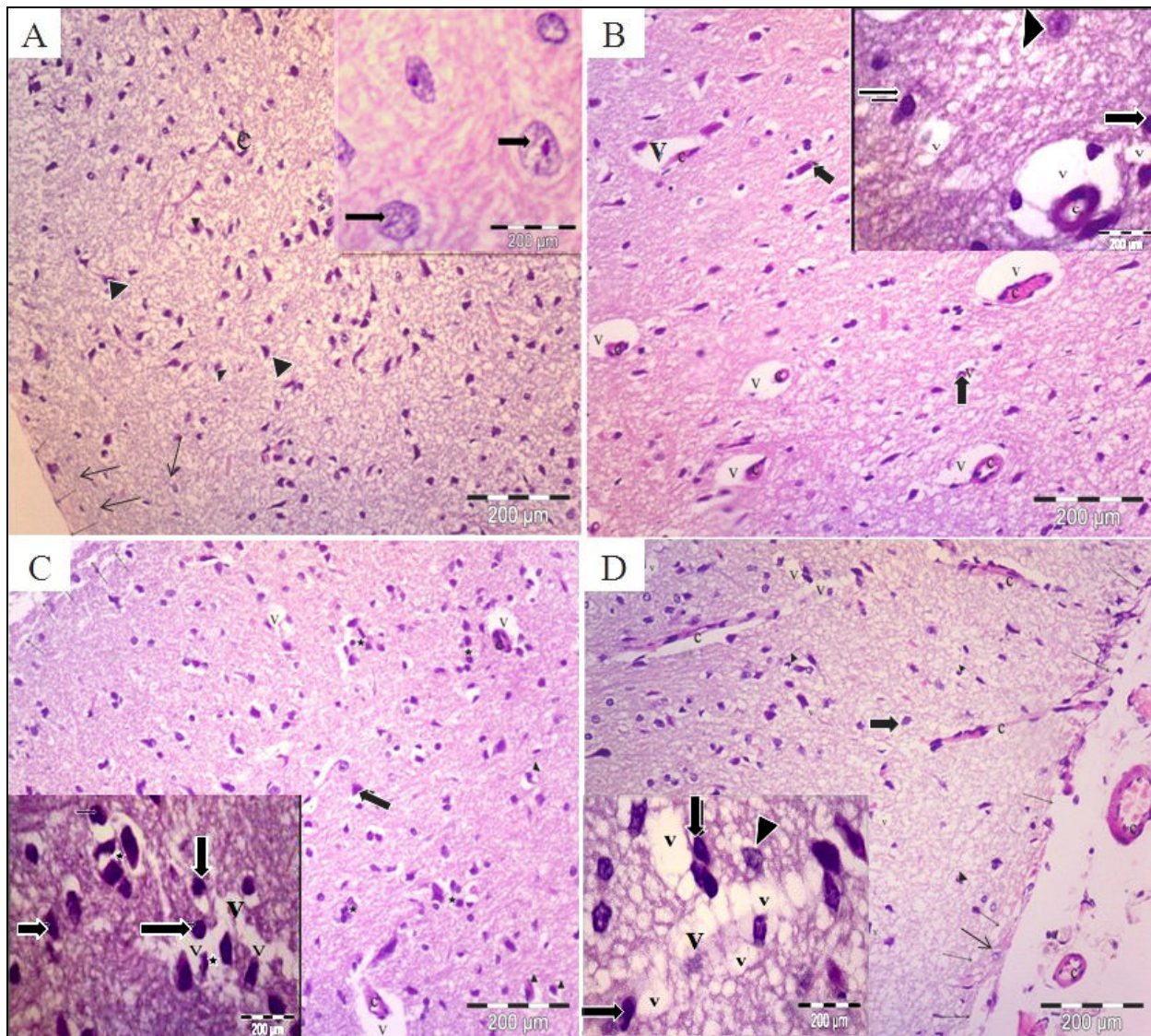


Figure 1: Photomicrographs of H&E stained sections of superficial region of superior colliculus show: **(A):** in control rat, nerve fibers (thin arrows), neural cells (arrow heads) with few capillaries (c) in between, neurons with vesicular nuclei (thick arrows) in the inset. **(B):** in group (1), vacuoles (v) appear around dilated capillaries (c) and shrunken neural cells with deeply stained nuclei (arrows), neuron with vesicular nuclei (arrow head) **(C):** in group (2), more disturbances in neural tissue with spaces in between (thin arrows). Large vacuoles (v) appear around blood vessels (c) and many cells (thick arrows) with deeply stained small nuclei. **(D):** in group (3), destroyed neural tissue (thin arrows), Congested blood vessels (C) appear on the surface, invading the surface and also, in between the neural tissue. Many cells reveal deeply stained nuclei (thick arrows) surrounded by vacuoles (v).

II. Transmission Electron Microscopic Examination of superficial region of SC

Control rat

Ultrastructural study revealed regularly arranged myelinated nerve fibers with well-formed myelin sheath. Nerve cells appeared with normal cytoplasmic organelles and euchromatic nuclei with regular envelop (Figures 3A and 4A).

Sildenafil citrate treated rats

In rat received the least dose of sildenafil citrate (group 1), SC exhibited some disturbance in the nerve fibers. Myelin sheaths revealed irregular folding, herniation and separated from nerve fibers by small vacuoles. Nerve cell showed euchromatic nucleus. The cytoplasm revealed swelling mitochondria and slightly dilated endoplasmic reticulum (Figures 3B and 4B).

In rat received the medium dose of sildenafil citrate (group 2), SC showed marked disturbance of nerve fibers. Myelin sheaths revealed irregular folding,

compression, stripping from nerve fibers or widely separated myelin lamellae. Neural cells showed apoptotic cytoplasmic vacuoles and ballooned mitochondria. The nucleus exhibited irregular envelop with blebs and condensed chromatin. Some dendrites showed defect in their wall and revealed light–electron dense vacuoles (Figures 3C and 4C).

In rat received the maximum dose of sildenafil citrate (group 3), SC revealed marked decrease in thickness of myelin sheaths of the most nerve fibers. Ballooned mitochondria appeared in nerve fibers. Also neural cells revealed signs of degeneration or apoptosis in form of vacuolated or depleted cytoplasm, ballooned mitochondria and many electron dense masses (may be apoptotic bodies). Moreover dilated Golgi apparatus, vesicles with materials of different electron density and many lysosomes were shown. The nuclei exhibited irregular envelop (in form of indentation or blebs) and condensed or clumped chromatin (Figures 3D and 4D-F).

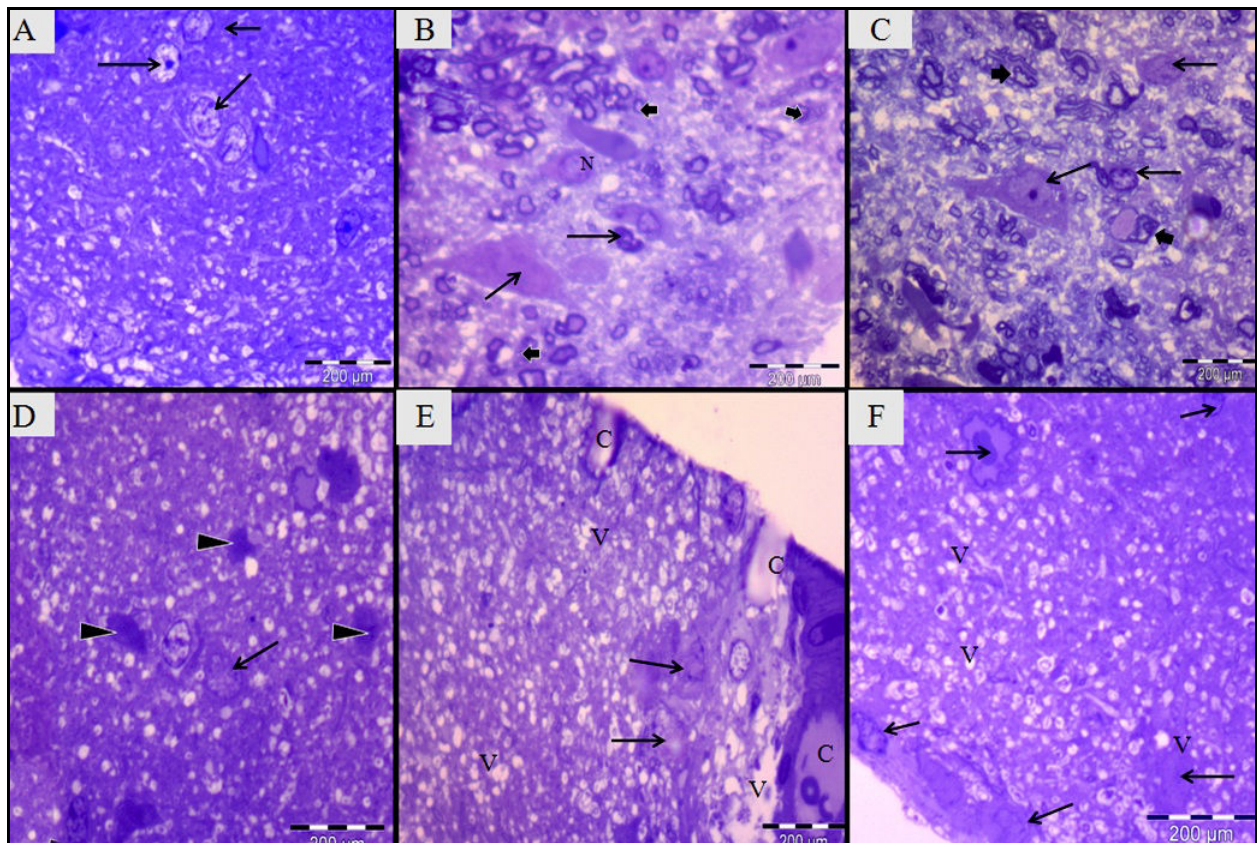


Figure 2: Photomicrographs of toluidine blue stained semithin sections of superficial region of superior colliculus show: **(A):** in control rat, neurons with vesicular nuclei (arrows). **(B):** in group (1), cells with well-formed nuclei (N), other cells revealing shrunken cytoplasm and irregular nuclear membrane (arrows) and nerve fibers reveal some irregularities (short arrows). **(C):** in group (2), cells with shrunken cytoplasm and irregular nuclei (arrows) and the myelin sheath are deformed (short arrows). **(D-F):** in group (3), massive destruction of neural tissue in form of loose stroma with massive vacuoles (v), many cells with deeply stained pyknotic nuclei (arrow heads) or with irregular nuclear membrane (arrows) and blood vessels(C) appear invading the surface, also in between the tissue with thick wall.

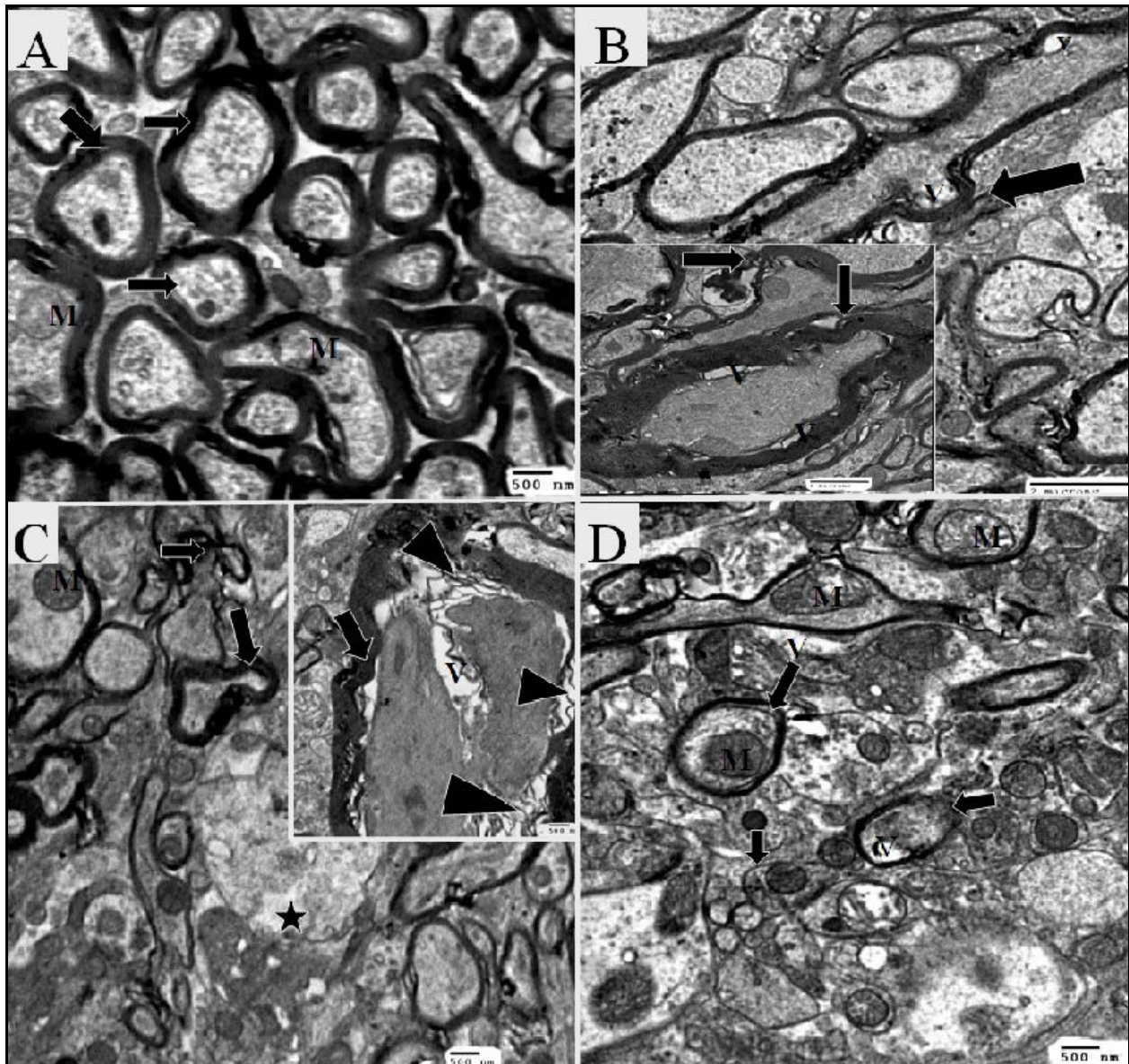


Figure 3. Electron micrographs of ultrathin sections of superficial region of superior colliculus show: **(A):** in control rat, nerve fibers with well-formed myelin sheath (arrows) and normal mitochondria (M). **(B):** in group (1), myelin sheaths revealing irregular folding, herniation (arrows) or separated from nerve fibers by small vacuoles (V). **(C):** in group (2), myelin sheaths revealing irregular folding, compression (arrows) stripping from nerve fibers or widely separated myelin lamellae (arrow heads in inset). Dendrite shows membrane lost (asterisk) and light–electron dense vacuoles (V). **(D):** in group (3), decrease in thickness of myelin sheaths of most of nerve fibers (arrows) with spaces spreading between the tissues (v). Ballooned and damaged mitochondria (M) appear.

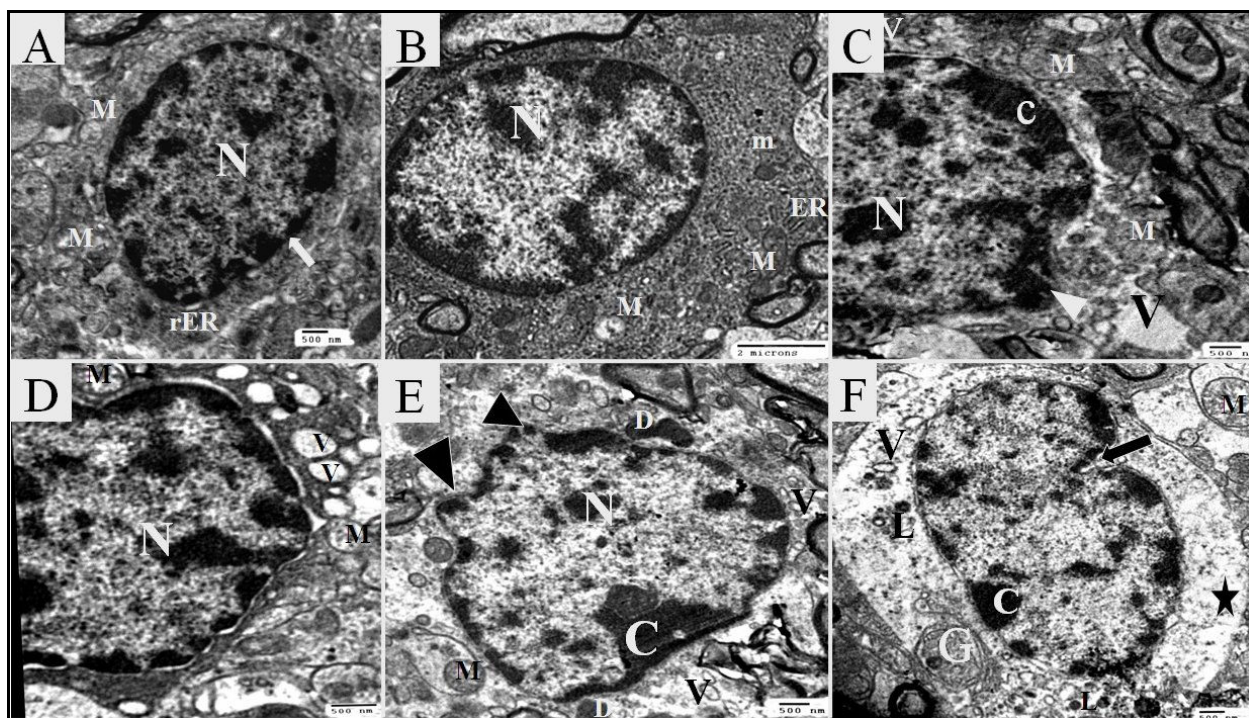


Figure 4: Electron micrographs of ultrathin sections of superficial region of superior colliculus show: **(A):** in control rat, normal neural cell with mitochondria (M), rough endoplasmic reticulum (rER) and euchromatic nucleus (N) with regular envelop (arrow). **(B):** in group (1), neural cell with euchromatic nucleus but the cytoplasm reveals slightly dilated endoplasmic reticulum (ER) and some mitochondria appear normal (m), others show dilatation or abnormal shape (M). **(C):** in group (2), neural cells with apoptotic signs; cytoplasm with different electron- dense vacuoles (v) and ballooned mitochondria(M) and the nucleus (N) exhibiting irregular envelop with bleb (arrow head) and condensed chromatin(C). **(D-F):** in group (3), apoptotic neural cells; cell with vacuolated cytoplasm(v) and ballooned mitochondria (M) in **(D)**, cell with cytoplasm showing electron dense masses (D) and vacuoles (V) and nucleus (N) with blebs (arrow heads) and clumped chromatin (c) in **(E)**, cell revealing depleted cytoplasm (asterisks), with vesicles of different electron dense materials (V) and localized area with dilated Golgi apparatus (G), lysosomes (L) and indented nucleus (arrow) with clumped chromatin(C) in **(F)**.

Statistical results

The present study recorded a highly significant decrease in the mean number and size in treated groups compared with those of control group and the decrease is significantly progressive with increasing doses of the drug (Table-1; Figures 5 and 6).

Table 1. The mean number & size (µm) of neural cells in experimental groups.

Groups	Number	P value	Size (µm)	P value
Control (4)	417±36.6		135 ± 97.9	
Group (1) (received 0.25mg/kg, body weight of sildenafil)	398.5±46.7	p<0.0001	99. 7± 46.6	p<0.0001
Group (2) (received 0.70mg/kg body weight of sildenafil)	350±23.6	p< 0.0001	79.9± 57.4	p<0.0001
Group(3) (received 1.43mg/kg body weight of sildenafil)	326±37.4	p< 0.0001	66.4±22.	p<0.0001

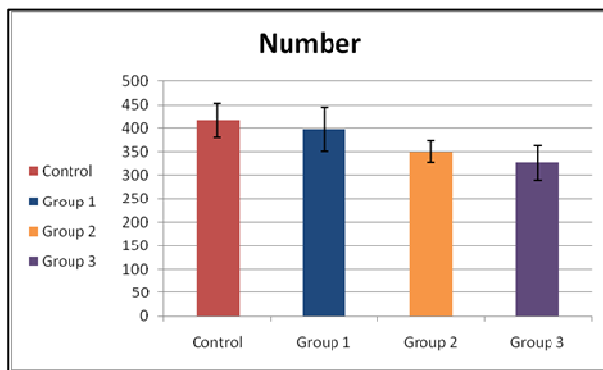


Figure 5. Histogram showing the mean number of neural cells in superficial part of superior colliculus of experimental groups.

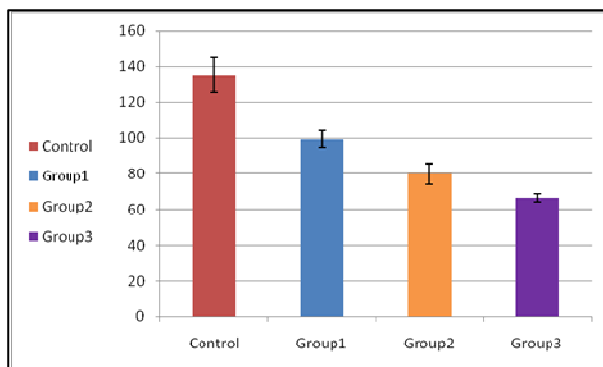


Figure 6. Histogram showing the mean size (µm) of neural cells in superficial part of superior colliculus of experimental groups.

DISCUSSION

Although sildenafil shows some promise as a therapeutic agent in some neurological disorders, well-designed clinical trials are required before its recommendation for use in any neurological disorder [13].

In this work, the histological and ultrastructural study of the effect of sildenafil citrate on the superficial part of superior colliculus (SC) in adult male rat was carried out. In control rat, SC revealed small and medium sized neural cells and myelinated nerve fibers. It is reported that, the stratum griseum superficiale and stratum opticum of the superior colliculus receive dense inputs from the retina and the visual cortex [16].

In rat received the least dose of sildenafil citrate (group 1), SC revealed disturbance in neural tissue with dilated capillaries and vacuoles. Some cells showed deeply stained nuclei, shrunken cytoplasm. Electron microscopic study exhibited some disturbances in the myelin sheaths, while, nerve cell showed euchromatic nucleus with slightly enlarged mitochondria and dilated endoplasmic reticulum.

In consistent with the present result, in the sildenafil citrate treated rats, sildenafil induced an angiogenic

effect in striatal neurons that showed significantly marked dilatation and congestion of the blood vessels [22]. Also, in embolic stroke rat models, sildenafil administration enhanced angiogenesis and selectively increased the cerebral blood flow level in the ischemic region, and improved neurological functional recovery [10].

The finding of this study can be explained by the evidence that sildenafil citrate crosses the blood-brain barrier and that phosphodiesterase 5 enzyme is present in the brain [23] and also is proved to be present and active in the basilar artery [6]. As sildenafil citrate is a highly selective inhibitor of the cGMP-degrading intracellular phosphodiesterase 5 enzyme, it has been suggested that adverse events associated with sildenafil may be attributed to its vasodilator effect [24].

In rat received the medium dose of sildenafil citrate (group 2), the superficial part of SC showed more disturbance of neural tissue with dilated blood vessels, vacuoles and deformed myelin sheath. Many neurons showed deeply stained nuclei and shrunken cytoplasm. The ultrastructural study revealed marked pathological changes in myelin sheaths and some cells showed apoptotic criteria.

In agreement with the present results, it is reported that, administration of sildenafil citrate resulted in cellular degenerative changes, cellular hypertrophy, clustering of cells and intercellular vacuolations appearing in the stroma of the SC of treated rats with varied degree related to the dose of the drug [18].

Also, it is evident that, oral administration of sildenafil citrate (5mg/kg/day) for three weeks revealed dilated congested choroidal blood vessels together with histopathological changes in the retina [25]. Moreover, it is reported that sildenafil citrate administration resulted in dilatation of the choroidal vessels with increase in volume and diameter of the vessels resulting in congestive effects within the eye that affect the retina and, if severe, may predispose to retinal detachment, or edema, or even to glaucoma [26].

Some investigators supported that, phosphodiesterase 5 enzyme inhibition causes dose-dependent effects of visual disturbance in men receiving sildenafil and the implications of the long-term daily use of sildenafil in men are unclear [27,28]. These histopathological effects of sildenafil citrate on SC may be due to vasodilatation of increased blood vessels and subsequent edema with compressing effect on neural tissue. Similar effect was reported in eye of sheep that showed increased choroidal volume and blood flow. Such flow could result in a higher leak of plasma-like fluid from the fenestrated capillaries following sildenafil ingestion [29].

Rats received the maximum dose of sildenafil citrate (group 3), showed massive destruction of neural tissue and invasion of the surface with massively increased blood vessels. The neural cells showed different pathological changes in form of degenerative and apoptotic changes with stromal edema and marked decrease in thickness of myelin sheaths of most nerve fibers. Many reports confirm the results of the present study; sildenafil citrate treatment has been associated with central serous chorioretinopathy [30], serous macular detachment and nonarteritic anterior ischemic optic neuropathy (NAION) [17]. Moreover, some cases of blindness have been attributed to NAION due to misuse of sildenafil citrate [31].

In addition, in consistent with the present result, histopathological effects of sildenafil citrate administration on penile tissue of rats in form of vasodilatation with increased number and surface area of blood vessels and thickened basement membranes are reported [32].

The mean number and size of neural cells were significantly decreased in all treated rats compared with those of control group and the decrease is significantly progressive with increasing doses of the drug. In contrast to these results, it is reported that, sildenafil elevates the brain levels of cGMP, inducing neurogenesis and angiogenesis in normal rats and in embolic rat model [9]. This toxic effect of increased dose of sildenafil citrate may be due to increased neovascularization and edematous effect that induced destructive effect in stroma of SC. In addition, phosphodiesterase 5 enzyme inhibitors, such as sildenafil, were reported to promote angiogenesis through increasing endothelial cell cGMP [33].

It can be concluded that, long-term daily use of sildenafil citrate can lead to noticeable pathological effect in the superior colliculus which may be implicated in visual disturbance and this effect is dose dependent, so neurological effect of sildenafil necessitates further investigations.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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