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Impact of Sildenafil on Smooth Muscle and Blood Vessels by Using Light and Electron Microscopy

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study aimed to examine the histological changes in the smooth muscles of corpus cavernosum and veins of rats as an eventual outcome of the ceaseless use of Sildenafil citrate. The comparison of the histological aftereffects of the veins among the control and experimental groups revealed extended thickness of tunica intima and media, as exhibited by a picture analyzer. The findings indicated that the steady usage of Sildenafil reduced smooth muscles and enabled the growth of collagen fibers in corpus cavernosum of penis and mass of veins.

Keywords: Sildenafil citrate; continuous use; corpus cavernosum; blood vessels.

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1. INTRODUCTION

Sildenafil citrate (commonly known as Sildenafil) is a phosphodiesterase type 5 (PDE-5) inhibitor that was accidently developed when PDE-5 inhibitors were being investigated for the treatment of coronary disease [1]. PDE-5 inhibitors are considered as novel remedial gadgets that can overhaul endothelial limit in individuals and upgrade mainly neighborhood circulatory system [2]. However, studies have raised doubts regarding the use of Sildenafil in cardiovascular events and did not find the drug to be promising against anginal treatment. The drug was found to be effective in the treatment of erectile brokenness or erectile dysfunction [3]. The Food and Drug Administration has approved a reformulation of Sildenafil for the treatment of aspiratory vein hypertension. In general, PDE inhibitors are effective in the treatment of Raynaud's disease, respiratory issues with ventilation/perfusion frustrate, congestive heart failure, hypertension, and strokes [4].

Moreover, the different effects of PDE-5 on skeletal muscles and trachea bronchial muscles are being considered for further studies, highlighting the potential of Sildenafil [5]. Presently, Sildenafil is the most inspected PDE inhibitor; two more PDE-5 inhibitors, Tadalafil and Vardenafil, are currently under investigation [6]. These two inhibitors have certain advantages over Sildenafil, including more selectivity for PDE-5 differentiated and individual isoenzymes, non-attendance of an effect of food on digestion, speedier onset, and more time allotment of actions [5]. In the treatment of erectile dysfunction, Sildenafil relaxes the corpus cavernosum in the penis and extends some related veins. However, Sildenafil may be associated with fibrosis in the penis [7]. The purpose of the present work was to examine the effects of Sildenafil on the histological structure of the smooth muscles in the penis and a few veins in albino rats using light microscopy. This research work will contribute to the further investigation of Sildenafil in the treatment of other restorative issues.

2. MATERIALS AND METHODS

2.1 Materials

This study was conducted on 60 male albino rats aged between 4 and 10 months and weighed between 150 and 300 grams. The animals were maintained as per the rules for the utilization and consideration of lab animals under the steady state of temperature, ventilation, mugginess, light with ordinary sustenance and water. The medication utilized as a part of this work was Sildenafil citrate, which was fed as tablets (50 mg/ml-Pfizer) that were disintegrated in distilled water. The medication was given to all animal subjects orally by means of gastric gavage. The rats were categorized into four groups of 15 rats each: The control group got the same volume of vehicle (distilled water without Sildenafil) for 3 months. In group I (therapeutic dose), each animal received a daily oral dosage of 1 mg Sildenafil for 3 months. In group II (intermediate dose), each rat got a daily oral dosage of 2 mg Sildenafil for 3 months. In group III (high-dosage), the animals received a daily oral dosage of 3 mg Sildenafil for 3 months. After 3 months, the rats were executed under anesthesia, and samples were taken from penis, aorta, femoral course and femoral vein of the animals. The samples were then subjected to light and electron minute examination.

2.2 Methods

2.2.1 Light microscopic

The acquired samples were promptly altered in 10% formal saline and then washed in rising concentrations of liquor (70%, 90%, and 100%) and cleared overnight in xylene. Implanting was done, and paraffin squares were acquired. Then 5–6 µm thick serial transverse segments were cut and mounted and recolored using hematoxylin and eosin (H and E) stain [8], Masson's trichrome stain [9], and Weigert stain [10].

2.2.2 Masson's trichrome stain

The segments were taken to water, and the nuclei were recolored with Weigert’s iron hematoxylin. They were washed well in water, and the nuclear stain was separated with 0.5% HCL in 70% alcohol. They were washed well with faucet water, flushed in distilled water and then recolored with the red cytoplasmic stain for 5–10 minutes. Then the samples were washed in distilled water. They were then separated with 1% phosphomolybdic corrosive until the collagen was stained and the muscles, red platelets, and fibrin were red and flushed in distilled water. The samples were counterstained in aniline blue for 2–5 minutes and washed admirably in 1% acidic corrosive for 1 minute and then smeared, dried out in outright liquor, cleared in xylene, and mounted in Canada resin.

2.2.3 Weigert stain

The samples were conveyed to 95% liquor and then stained with weight's versatile tissue stain in a secured recoloring jug for one hour in the broiler at 50℃ and then for one hour at room temperature. Then they were separated in 1% corrosive liquor and washed with faucet water and then washed with clean water. The samples were then dried out in absolute alcohol and cleared in xylol and mounted in Canada amber.

The amount of collagen fibers in the corpus cavernosum in the penis as well as in the blood vessels was measured. In addition, the thickness of each of the internal tunica media and vascular tunica media were also examined.

2.3 The Samples were Subjected to the below Procedures

2.3.1 Fixation

The penis, aorta, vein and coronary samples were first placed in newly arranged 3% phosphate cushioned glutaraldehyde pH 7.3 for 3–4 minutes and kept at room temperature. The samples were then washed twice with phosphate buffer for 10 minutes each and left overnight in the phosphate support at 4℃. The settled samples were then post-altered in 1% osmium tetroxide in phosphate cushion for 1–2 hours. The examples ought to turn tarnish dark after post-obsession in osmium to guarantee legitimate entrance of the fixative. The samples were then flushed three times with PBS and arranged for the next dehydration step.

2.3.2 Dehydration

Parchedness was examined at room temperature in reviewed ethanol (30%, 50%, 70%, 90% and 100%) two times in every evaluation of 15 minutes each.

2.3.3 Clearing

The clearing was done in propylene oxide for 15 min.

2.3.4 Infiltration and inserting

Equal volumes of epon and acetone were left on the samples for 60 minutes to encourage the penetration of the resin. The hardness of the last square could be balanced by the proportion of the two blends:

2.3.4.1 Arrangement (An)

Upon 3 ml and dodecyl succinic Anhydride [DDSA] 50 ml. Method (B): Upon 100 ml and Methyl Nordic Anhydride [MNA] 89 ml. The standard proportion utilized was 7 ml of the method (B) and 3 ml of arrangement (A) which were combined with the last expansion of six major drops of the quickening agent Tridimethyl amino methyl phenol [DMP-30, Ciba Labs]. The last altered examples were discarded in pillar container (BDH EM grade, England), a little drop of weapon was set at its base.

2.3.5 Polymerization

This was done at 60℃ for 24 hours.

2.3.6 Ultramicrotomy

In the first place, semi-thin segments (1 µm thick) were cut, gathered on clean slides and left to dry on a hot plate. They were then recolored with Toluidine blue for one minute on the hot plate (60–100℃) (Dawes, 1980). The segment was immediately flushed with distilled water and dried on the hot plate. After drying, the areas were analyzed by the drenching oil force of the light magnifying instrument to guarantee the vicinity of the required samples in the cut segments. Second, ultrathin areas (50–60 nm thick) with gold silver shading were cut. Segments were singled out in a copper matrix and left to dry on a channel paper in a petri dish.

2.3.7 Staining

Matrices were recolored by a two-fold recoloring procedure of uranyl acetic acid derivation, trailed by lead citrate [11,12-16]. The frameworks were then washed with purified water and dried on channel paper. The networks were examined utilizing JOEL JEM-1400 Electron magnifying instrument [Faculty of Agriculture, Cairo University].

2.4 Quantitative Morphometric

All quantitative morphometric estimations were performed utilizing picture analyzer (Lecia Imaging System Ltd., Cambridge, England). The pictures were taken live on to the screen from segments under a light magnifying lens (Olympus Bx-40, Olympus Optical Co.Ltd., and Japan) with a fastened camcorder (Panasonic shading CCTV camera, Matsushita Communication Industrial Co. Ltd., Japan). The video pictures were digitized utilizing "Lecia Twin 500C," which is a Lecia's Windows-based picture investigation toolbox fitted to an IBM-good PC with a shading screen.

2.4.1 Electron microscopic examination of rat penis

2.4.1.1 Control group

Electron microscopic examination of penis sections of control rats revealed normal ultrastructural features of cavernous tissue. The nucleus of smooth muscles was centrally placed, rounded or oval in shape. The nuclear chromatin was evenly distributed, sometimes slightly condensed along the nuclear membrane. The nucleoli were easily distinguishable. The smooth muscles were rich in cytoplasm organelles, mainly rod-shaped or rounded mitochondria. They were bounded by two unit membranes separated by a narrow space. The inner membrane was invaginated to form lamellar cristae. They were having a medium electron dense homogenous matrix with electron dense granules and were surrounded by abundant rough endoplasmic reticulum. Abundant rough endoplasmic reticulum, forming branching network in the form of cisternae were observed in smooth muscles cells. The great part of cisternae is parallel to each other and continuous with the outer nuclear membrane. The outer surface of rER was studded with ribosomes. The sarcoplasmic reticulum appeared as irregular branching tubular structures and not studded with ribosomes. They were noticed in glycogen-rich areas mostly distant from the nucleus. They had a vesicular form composed of a mesh of delicate tubules. Free ribosomes and polyribosomes were found evenly distributed in the cytoplasm of smooth muscle. The cytoplasm of smooth muscle contained glycogen particles which usually form minor to major aggregation in rosette-like assays in close association with the smooth endoplasmic reticulum. The sarcolemma of smooth muscles was intact surrounding by few elastic fibers and collagen fibers from outside and cytoplasmic caveolate on inner side (Fig. 10). Vascular spaces lined by endothelial cells with flat nucleus and thin basal lamina underneath endothelial cells.

2.4.1.2 Experimental groups

Group I and Group II showed minimal changes by electron microscopic examination but considering Group III. Examination of the corpus cavernosal smooth muscle of rats group III was showing large euchromatic nucleus, large amount of cytoplasmic vesicles and large amount of glycogen particles with increased intra cytoplasmic myofilaments. Collagen fibers were detected in between smooth muscles. Sarcoplasm was rich in mitochondria, ribosomes & microtubules (Fig. 11).

2.4.2 Morphometric results and statistical analysis of collagen fibers of rat penis

Quantitative morphometric measurements of the Masson's Trichrome stained sections revealed that mean area percentage of collagen content in corpus cavernosum in the different experimental groups (I, II, III), (9.93, 11.649, 20.912) showed significant increase (P.0001) which was manifested in Group III and Group II but Group I revealed no significant increase (P=.069) comparing to Control group (8.938) (Fig. 12).

2.5 Statistical Analysis

Every parameter was investigated utilizing the mathematical mean, standard deviation (SD), and the "t" test taken after the Hock post-test. The objective examination was done on an IBM PC utilizing the measurable programming "insights for Windows SPSS" form 9. The results considered significant when the p-value was ≤ 0.05.

3. RESULTS AND DISCUSSION

The results of this research have shown that the use of Sildenafil may lead to an increase in collagen fibers and a decrease in smooth muscles in the corpus cavernosum and the vascular wall. It was observed that there was a growing positive correlation between the dosage value and the changes in both the collagen and smooth muscle fibers; these changes were more pronounced in the third experimental group. It was also observed that the drug enhanced the thickness of the tunica media and vascular tunica. No change was observed in the elastic fibers. However, the present study demonstrated that the high doses of the drug and longer periods of its administration without interruption led to the occurrence of fibrosis in the corpus cavernosum of the penis, as well as in blood vessels. Therefore, it is advised that the drug should be used only in cases of necessity and under medical supervision.

3.1 Histological Results: I-Light Microscopic Examination of Rat Penis

3.1.1 A-Control group

The examination of the penile areas of control rats demonstrated the presence of a hard

structure called ‘os penis’ or ‘penis bone’. The external piece of os penis was shaped of conservative bone while its inside was framed of light bone. At the glans penis, the os changed into a cartilaginous structure. The urethra was available under os penis and was encompassed by corpus spongiosum. Its mucosa had transitional epithelium.

The corpus cavernosum was found as a distinct layer wrapping the corpus spongiosums and was enclosed by the epidermis of the penis (Fig. 1).

A corpus cavernosum penis is a mass of erectile tissues (caves) that was lined by endothelial cells and was encompassed by smooth muscles, and flexible and collagen filaments (Fig. 2).

Masson's Trichrome recolored areas of control penis uncovered the huge tissues surrounding blood sinusoids and was rich in smooth muscles with few collagen strands (Fig. 3).

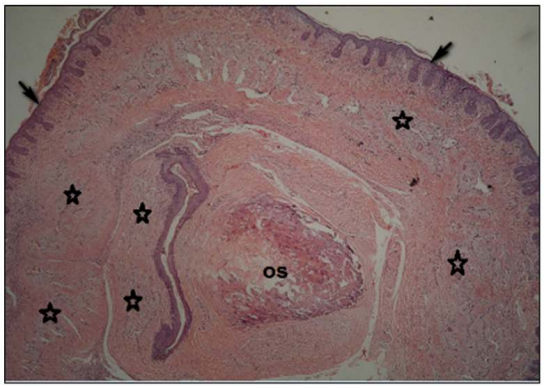

Fig. 1. A photomicrograph of a section of the penis (covered by skin (arrow) in a control rat. The cavernous tissue (stars) surrounds both corpus spongiosum including urethra and the os penis. (H & E X 40)

3.2 Test Groups

3.2.1 B-Group I

The examination of the areas of the penis demonstrated that the corpus cavernosum uncovered stamped widened congested blood sinusoids isolated by smooth muscles, few collagen strands and fibroblast as showed in Masson's Trichrome (Figs. 4 and 5).

3.2.2 C-Group II

In this group, the penile examination revealed less widened congested blood sinusoids that were isolated from less smooth muscle and collagen packs (Figs. 6 and 7).

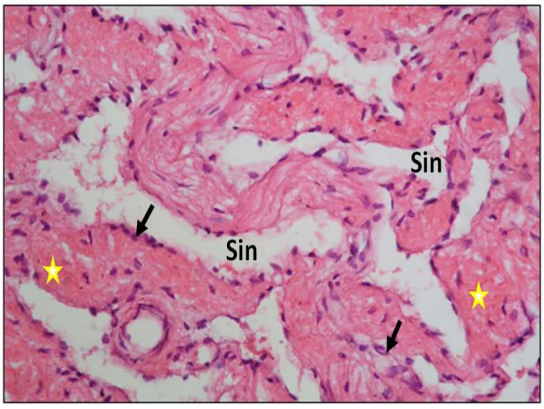

Fig. 2. A photomicrograph of a section of the penis in control rat showing corpus cavernosum with dilated blood sinusoids (Sin) separated by smooth muscles (arrows) with a minimal amount of collagen fibers (stars). (H & E X 400)

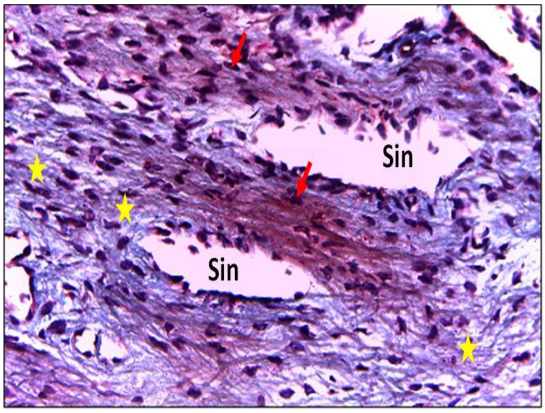

Fig. 3. A photomicrograph of a section of the penis in control rat showing corpus cavernosum with dilated blood sinusoids (Sin) separated by cavernous tissue containing collagen fibers (stars) and rich smooth muscles (arrows). (Masson’s Trichrome X 400)

3.2.3 D-Group III

The examination of the penile areas demonstrated that the corpus cavernosum uncovered a few wrecked sinusoids while others indicated a couple of red blood corpuscles. The blood sinusoids were isolated by many collagen groups and few smooth muscles (Figs. 8 and 9).

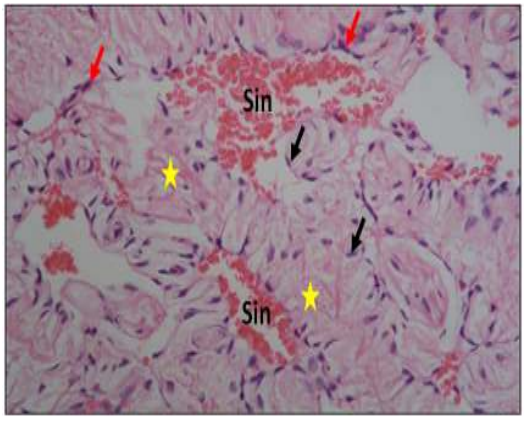

Fig. 4. A photomicrograph of a section of the penis in rat group I showing corpus cavernosum with marked dilated congested blood sinusoids (Sin) separated by smooth muscles (red arrows), a minimal amount of collagen fibers (stars) and fibroblasts (black arrows). (H & E X 400)

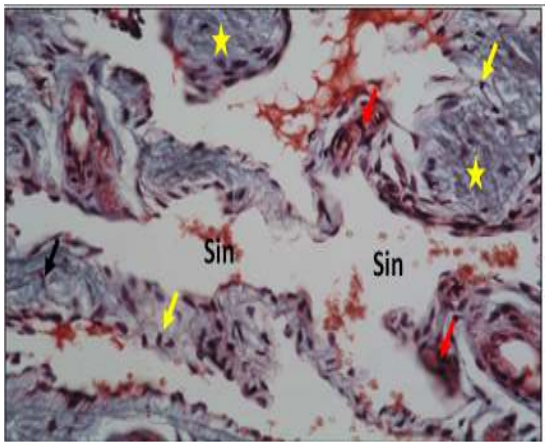

Fig. 5. A photomicrograph of a section of the penis in rat group I showing corpus cavernosum with marked dilated congested blood sinusoids (Sin) separated by collagen fibers (stars), smooth muscles (Red arrows) and fibroblasts (yellow arrows). (Masson's Trichrome X 400)

The diminished sinusoidal blood space due to the expanded collagen strands is demonstrated by recoloring areas and picture analyzer, or the expansion action of both fibroblasts and smooth muscles. The protein union movement of smooth muscles expanded as distinguished by the small electron study in this work. A previous study [17] affirmed the above clarifications as they reported that the fibroblasts in the penis were expanded and actuated, prompting amassing of collagen strands that may result in penile fibrosis. Another study [6] reported that patients utilizing Sildenafil for erectile dysfunction for a longer duration may develop irreversible fibrosis as showed by packs of collagenous strands among penile tissues. Increased vascular porousness happens amid the early periods of wound repair, hypothetically permitting the statement of the fibrin-rich lattice fundamental for cell relocation and expansion. Previous studies [18,19] expressed that Sildenafil had associated antagonistic neighborhood impacts, such as fibrosis in the corpus cavernosum of the penis and vasodilatation bringing about migraine, flushing, dyspepsia, and nasal congestion. This is in agreement with the results of our study. We observed dilatation and blockage of blood sinusoids in group I and to a lesser degree in group II. The histological results and picture analyzer of this study uncovered diminished vascular spaces and smooth muscles with expanded sinewy tissue in rats treated with Sildenafil for three months (1 month in a rodent's life is equivalent to 2.5–3 years of human life). This is in agreement with a previous study [20,21-23] that regular utilization of the drug for two years promoted loss of proficiency, which requires expanded measurements. In the present study, the comparison of the histological aftereffects of veins among the groups revealed expand 21-23ed thickness of tunica intima and media as recognized by a picture analyzer. The basal lamina of endothelial cells was thickened and collapsed as recorded by electron microscopy. Similarly, the collagen filaments both in tunica media and adventitia were found to be expanded. However, the smooth muscles were found to be diminished in the tunica media. There was no progression of flexible strands substance of veins. The increment in collagen content inside of veins and that recorded in this study may allude to the same clarification of expanded action of fibroblasts and smooth muscles [17]. In the present study, the reduction in the smooth muscles may be because of the direct impact of the drug, resulting in endothelial dysfunction. We observed an irregular endothelial reaction that prompted the lessening of the bioavailability of NO, which disabled vasodilatation and impelled smooth muscles multiplication. It has been reported that Sildenafil treatment of patients with heart disease and type II diabetes mellitus can lead to an enhanced vasomotor part of endothelium dysfunction, thus, enhancing the other rare element going with endothelial dysfunction.

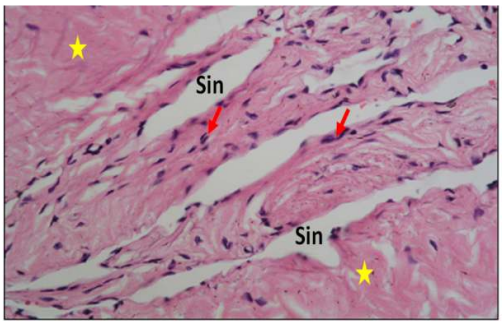

Fig. 6. A photomicrograph a section of the penis in rat group II showing corpus cavernosum with mildly dilated blood sinusoids (Sin) separated by a large amount of collagen bundles (stars) and smooth muscles(arrows). (H & E X 400)

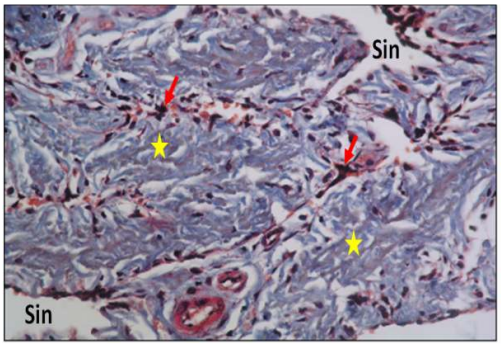

Fig. 7. A photomicrograph a section of the penis in rat group II showing corpus cavernosum with mildly congested blood sinusoids (Sin) separated by a large amount of collagen bundles (stars) and less smooth muscles (arrows). (Masson's Trichrome X 400)

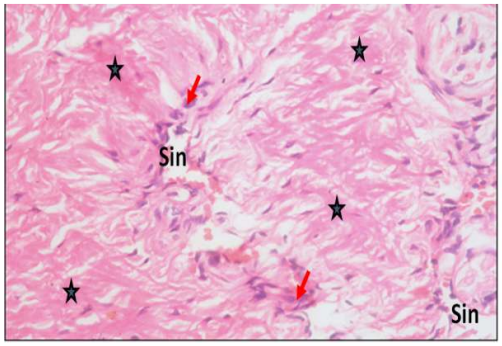

Fig. 8. A photomicrograph a section of the penis in rat group III showing corpus cavernosum with minimal congested partially obliterated blood sinusoids (Sin) separated by a large amount of collagen bundles (stars) and few smooth muscles (arrows). (H & E X 400)

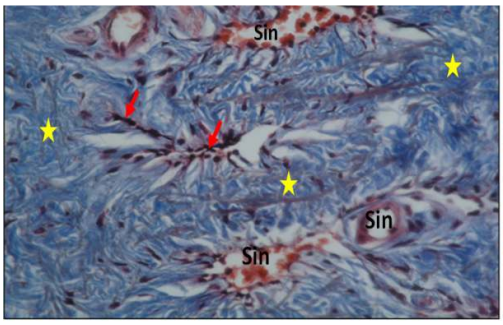

Fig. 9. A photomicrograph a section of the penis in rat group III showing corpus cavernosum with nearly obliterated blood sinusoids (Sin) separated by a large amount of collagen bundle (stars) and few scattered smooth muscles (arrow). (Masson's Trichrome X 400)

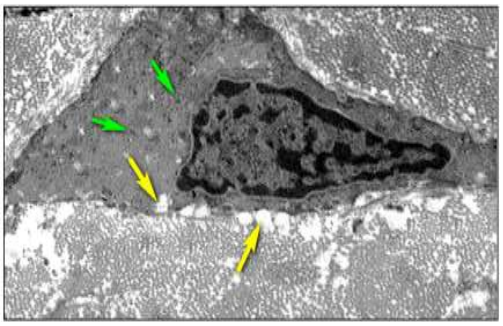

Fig. 10. Electron micrograph of corpus cavernosum of control rat showing smooth muscle cell is filled with dense body's (green arrow) & caveolate near by the sER to (yellow arrow). (Original magnification X 10000)

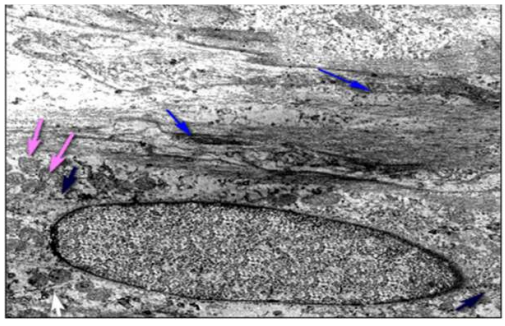

Fig. 11. Electron micrograph of corpus cavernosum of group III showing increased intra cytoplasmic myofilaments & collagen fibers in between smooth muscles (blue arrow), Sarcoplasm is rich in mitochondria (violet arrow), ribosomes (blue black arrow), & microtubules (white arrow), (Original magnification X 8000)

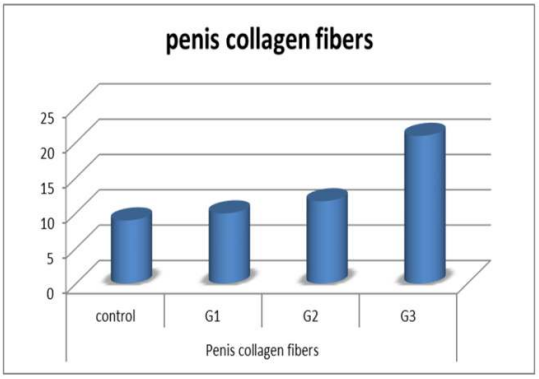

Fig. 12. Mean area percentage of collagen content in control and experimental groups

4. CONCLUSION AND RECOMMENDATION

The results of the study showed that high doses of the drug and longer periods of uninterrupted administration can lead to fibrosis in the corpus cavernosum of the penis, as well as in the blood vessels. Therefore, it is advised that the drug should be used only in cases of necessity and under medical supervision.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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