Al-Azhar International Medical Journal

Volume 3 | Issue 12 Article 8

12-1-2022

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El-Sayed Jahin
Anatomy and Embryology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt,
drsayedhamedjahin@gmail.com

Mustafa Al-Jizawi
Anatomy and Embryology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt,
moustafa_gizawy@yahoo.com

Fayez Abd-Elfattah
Anatomy and Embryology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt,
fayezmohammed2018@gmail.com

How to Cite This Article

Jahin, El-Sayed; Al-Jizawi, Mustafa; and Abd-Elfattah, Fayez (2022) "EFFECTS OF SILDENAFIL ON THE TESTES OF THE NONDIABETIC AND DIABETIC ADULT ALBINO RATS (Light and Electron Microscopic Study)," *Al-Azhar International Medical Journal*: Vol. 3: Iss. 12, Article 8.
DOI: https://doi.org/10.21608/aimj.2022.145833.2008

OPEN AIMJ ORIGINAL ARTICLE

Anatomy and Embryology

Effects of Sildenafil on The Testes of The Nondiabetic and Diabetic Adult Albino Rats (Light And Electron Microscopic Study)

El-Sayed E Jahin, [1] MSc, Mustafa E. Al-Jizawi , [1] PhD, Fayez M. Abd-Elfattah, MD.

** Corresponding Author:*
El-Sayed E Jahin
Drsayedhamedjahin@gmail.com

Received *for publication June 20, 2022; Accepted December 29, 2022; Published online December 29, 2022.*

doi: 10.21608/aimj.2022.145833.2008.

*Citation**:** El-Sayed EJ., Mustafa EA, Fayez MA. . **Effects of Sildenafil on The Testes of** The Nondiabetic and Diabetic Adult Albino Rats (Light And Electron Microscopic Study) AIMJ. 2022; Vol.3-Issue12 .37-42.*

[1]Anatomy and Embryology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

ABSTRACT

Background: Sildenafil citrate is effective in men with erectile dysfunction. Sildenafil induces degenerative changes in seminiferous tubules and interstitial histological alterations. Diabetes mellitus may cause an end-organ damage in many systems as the genitourinary system. The testis is the primary sex organ and is formed mainly of stroma and parenchyma. It is a mixed gland that performs both endocrine and exocrine activities.
Aim of the study: This work aimed to reveal the effect of sildenafil citrate in high and toxic doses on the histological structure of the healthy and diabetic rat testes**.**
Materials and Methods: The research was carried out at the animal house at Faculty of Pharmacy, Al-Azhar University between July, and September 2021. Fifty adult rats were used. They were divided into the following groups: Group I: 10 rats received distilled water daily; group IIA: 10 rats received intraperitoneal injection of sildenafil 9mg/kg/d; group IIB: 10 rats received sildenafil 13.5mg/kg/d, group IIIA: received a single dose of an intraperitoneal injection of 150 mg/kg body weight of alloxan to induce diabetes then sildenafil citrate 9 mg/kg/d, group IIIB: received a single dose of an intraperitoneal injection of 150mg/kg of alloxan to induce diabetes then intraperitoneal injection of sildenafil citrate 13.5mg/kg/d. Animals from all groups were examined 2 months after the start of the experiment.
Results: Sildenafil had a toxic effect on the testes of adult albino rats that was more severe with the toxic dose. Also, the changes were mild in non-diabetic and moderate to severe in diabetic rats.
Conclusion: Sildenafil had toxic effects on the testes in high doses and also in diabetics.
Keywords: *Sildenafil; diabetes; testis; histology; rats...*

INTRODUCTION

Sildenafil citrate (SC) was introduced for treatment of angina pectoris, but it was observed that it induces marked penile erection. In males with mild to severe erectile dysfunction (ED) without an organic cause, the medication has been proven to be beneficial [1].

SC inhibits the breakdown of cyclic guanosine monophosphate (cGMP) in the corpus cavernosum of the penis through cGMP-specific phosphodiesterase type 5 (PDE5). This causes erection by promoting smooth muscle relaxation, vasodilation, and increased blood flow into the spongy tissue of the penis[2].

SC induces degenerative changes in the seminiferous tubules as well as interstitial histological alterations, which finally might lead to an arrest of the spermatogenesis[3].

In human, the testis is the primary sex organ of males and is formed mainly of stroma and parenchyma. It is a tubular gland that performs both endocrine and exocrine activities[4].

Diabetes mellitus (DM) is a critical disease. End-organ damage will occur in many systems, including the genitourinary system, if DM not well controlled[5].

Alloxan is an organic compound has the molecular formulae, C4H2N2O4. Alloxan is a diabetogenic drug that is frequently used in diabetes research to examine the antidiabetic potential of both pure

chemicals and plant extracts. Alloxan-induced diabetes is a kind of insulin-dependent diabetes that develops after animals are exposed to alloxan[6].

This work aimed to reveal the effect of sildenafil citrate in high and toxic doses on the histological structure of the normal and diabetic rat testes.

MATERIALS AND METHODS

Fifty adult male albino rats aging 12-14 weeks and weighing 150-200 grams were used. The study was done at Animal House, Faculty of Pharmacy, Al-Azhar University, Cairo, between July and September 2021.

Experimental Design:

The rats were randomly divided into three groups and had free access to water and food:

Group I (control group): 10 rats received distilled water daily for 2 months.

Group II (Nondiabetic group): 20 rats was divided into 2 equal subgroups: II-A and II-B.

Group II-A: received intraperitoneal injection (IPI) of SC 9 mg/kg/d for 2 months[7].

Group II-B: received IPI of SC 13.5 mg/kg/d for 2 months[8].

Group III (diabetic group): 20 rats were divided into 2 equal subgroups: III-A and III-B. Both subgroups had free access to water and food and were treated with a single dose of IPI of 150 mg/kg of alloxan to induce diabetes. Seventy-two hours later, blood glucose level was determined to confirm induction of diabetes (normal blood glucose level of the rats is 50-95 mg /dl fasting and 70-130 post prandial). Rats with blood glucose level >250 mg/dl were considered diabetic and included in the experiment Then,

Group III-A: received IPI of SC 9 mg/kg/d for 2 month[7].

Group III-B: received IPI of SC 13.5 mg/kg/d for 2 months[8].

Chemicals:

The chemicals used in this work were:

- SC 100mg tablets were purchased from Pfizer Egypt Pharmaceutical Company and the tablets were crushed and diluted in 50 ml of sodium chloride, so each 1 ml of sodium chloride were contained 2mg of sildenafil citrate.

- Alloxan was present in the form of alloxan hydrate in aqueous solution 1.2 g in 12 ml of normal saline. It was purchased and manufactured by Sigma Aldrich Company, UK.

Methods:

1) Testes specimens collection:

Rats in all groups were sacrificed after 2 months. The collected testes of each group were divided into two subgroups for processing.

The first subgroup was processed for paraffin sectioning and stained by Hematoxylin and eosin stain (H&E) and Masson's trichrome stain. The other half of testes were processed for transmission electron microscopic

2) Light and electron microscopic processing:

The collected testes were divided transversely, and 2 mm thick section of each specimen was immersed in 2.5% glutaraldehyde solution. Then, they fixed for 2 hours in 1% osmium tetraoxide at 4°C. Then after washing the tissue three times in distilled water, the specimens were prepared for dehydration, infiltration, ultramicrotomy. The tissues then were stained by uranyl acetate and lead citrate for transmission electron microscopic processing (TEM).

Then, the rest of each dissected testis was preserved in 10% buffered formalin and then processed for paraffin sections then stained by H&E and also Masson's trichrome stain for light microscopic examination [9,10].

RESULTS

1) External appearance and mortality:

Examination of the external appearance of the testes revealed that there was no difference between the testes excised from all groups. Regarding mortality, no deaths were recorded among the animals of all groups.

2) Effect of SC on testes histology:

A- Light microscopic finding:

Control (Gp1): testis showed normal tunica albuginea with normal sub-capsular blood vessels, normal-sized tubules with normal tunica propria, normal germinal lining and complete spermatogenic layers, and normal interstitium with Leydig cells (Figure. 1-A).

Gp II (non-diabetic + SC): testis showed thick tunica albuginea with mildly congested sub-capsular blood vessels, scattered normal-sized and distorted tubules with quietly normal tunica propria, normal germinal lining and complete spermatogenic layers, and normal interstitium with Leydig cells. These changes were more evident in high-dose (Figure. 1-C) than in low-dose sildenafil (Figure. 1-B).

Gp III (diabetic + SC): testis showed markedly thickened tunica albuginea with congested sub-capsular blood vessels, scattered small-sized distorted tubules with irregular tunica propria, thin germinal lining and diminished spermatogenic layers, and interstitium with Leydig cells. These changes were more evident in high-dose (Figure. 1-E) than in low-dose sildenafil (Figure. 1-D).

B- Electron microscopic Finding:

Control (Gp1) : testis showed seminiferous tubules with spermatogonia resting on normal tunica propria with normal myoid cells, nucleus with dispersed chromatin and rounded mitochondria, primary spermatocytes with nucleus showing dispersed chromatin, well-formed endoplasmic reticulum small cytoplasmic vacuoles, Sertoli cells with indented nucleus showing prominent nucleoli and numerous lysosomes (Figure. 2-A).

Gp II (non-diabetic + SC): testis showed seminiferous tubules with spermatogonia resting on thick irregular tunica propria with smaller myoid cells, nucleus with dispersed chromatin in the periphery and swollen and distorted mitochondria with irregular cristae, primary spermatocytes with small nucleus, large cytoplasmic vacuoles, swollen endoplasmic reticulum and distorted mitochondria with irregular cristae, and rounded and elongated spermatids, and portions of distorted spermatozoa with no acrosomal cap, directed towards Sertoli cell with nucleus showing prominent nucleolus. These changes were more evident in high-dose (Fig. 2-C) than in low-dose sildenafil (Figure. 2-B).

Gp III (diabetic + SC): testis showed seminiferous tubules with scattered primary spermatocytes showing small nucleus showing clumped chromatin in the periphery, small, rounded mitochondria and large cytoplasmic vacuoles, many rounded and elongated spermatids with less-formed acrosomal cap, and portions of well-formed spermatozoa with well-formed acrosomal cap directed towards Sertoli cells with numerous rounded mitochondria and numerous lysosomes. These changes were more evident in high-dose (Figure. 2-E) than in low-dose sildenafil (Figure. 2-D).

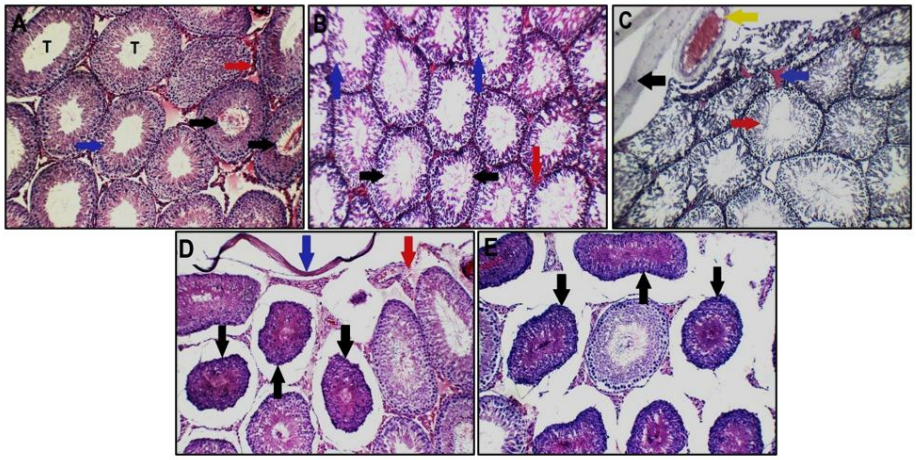

Fig 1: A photomicrograph of a section of the testis of **A)** control group showing normal sized tubules (T) with normal germinal lining (blue arrow), full layers of germinal lining (black arrows) and normal interstitium (red arrow) occupying spaces between the tubules. **B)** Non-diabetic + SC 9mg group showing quietly normal-sized tubules and normal interstitium (red arrow). Some of tubules appear normal with normal germinal lining and complete spermatogenic layers (black arrows), while others have distorted and separated germinal lining (blue arrows). **C)** Non-diabetic + SC 13.5mg group showing thick tunica albuginea (black arrow) with mildly congested sub-capsular blood vessels (yellow arrow), quietly normal-sized tubules with distorted & spaced germinal lining (red arrow), and normal interstitium (blue arrow). **D)** Diabetic + SC 9mg group showing disturbed tunica albuginea (blue arrow), sub-capsular accumulation of fluid (red arrow) and widely separated distorted tubules (black arrows). **E)** Diabetic + SC 13.5mg group showing widely spaced distorted seminiferous tubules with obliterated lumen (black arrows) (H & E, X 200).

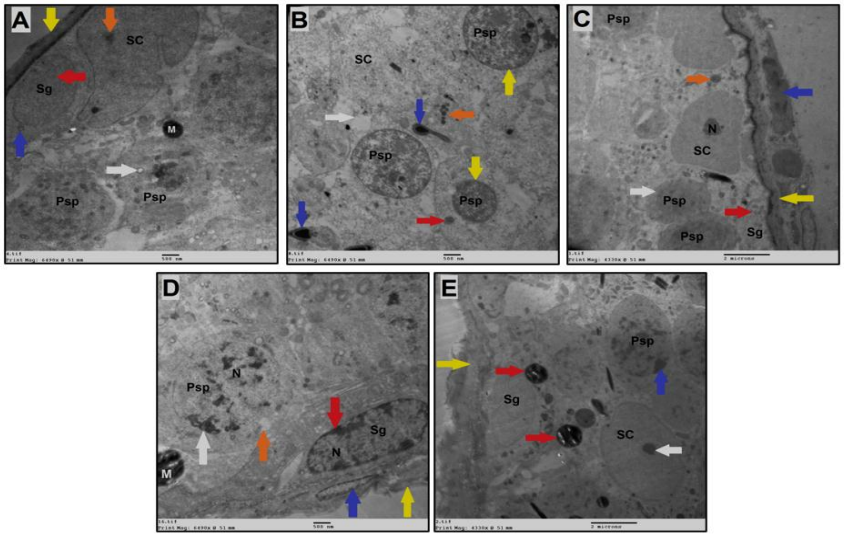

Fig 2: An electron photomicrograph of sections of the testis of **A)** control group showing seminiferous tubules with spermatogonia (Sg) resting on normal tunica propria (yellow arrow) with nucleus showing dispersed chromatin (red arrow) and rounded mitochondria (blue arrow), primary spermatocytes (Psp) with swollen mitochondria (M) and small cytoplasmic vacuoles (white arrow), Sertoli cells (SC) with indented nucleus showing prominent nuclei (orange arrow). **B)** Non-diabetic + SC 9mg group showing seminiferous tubules with primary spermatocytes (Psp) with nucleus (N) showing dispersed chromatin (yellow arrow), rounded mitochondria (red arrow) and large cytoplasmic vacuoles (white arrow), and few distorted spermatozoa with less formed acrosomal cap (blue arrow) directed towards Sertoli cell (SC) with numerous lysosomes (orange arrow). **C)** Non-diabetic + SC 13.5mg group showing seminiferous tubules with spermatogonia (Sp) resting on thick tunica propria (yellow arrow) with myoid cell (blue arrow) and nucleus with dispersed chromatin (red arrow), primary spermatocytes (Psp) with nucleus (N) showing dispersed chromatin (white arrow), and Sertoli cell (SC) and small rounded mitochondria (orange arrow). **D)** Diabetic + SC 9mg group showing seminiferous tubules with spermatogonia (Sp) resting on thick irregular tunica propria (yellow arrow) with smaller myoid cell (blue arrow), nucleus (N) with clumped chromatin in the periphery (red arrow), primary spermatocytes (Psp) with small nucleus (N) and clumped chromatin (white arrow), large cytoplasmic vacuoles (orange arrow), and distorted mitochondria with irregular cristae (M). **E)** Diabetic + SC 13.5mg group showing seminiferous tubules with spermatogonia (Sg) resting on markedly thickened irregular tunica propria (yellow arrow) and markedly swollen mitochondria with irregular cristae (red arrow), primary spermatocytes (Psp) with small nucleus (N) and clumped chromatin in the periphery (blue arrow), and Sertoli cell with nucleus showing prominent nucleolus (white arrow). (TEM, X 6000).

DISCUSSION

In the present study, the histological examination of testicular sections from the control group showed that the seminiferous tubules had regular outlines and lined by layers of germinal epithelium at different stages of spermatogenesis. Germinal cells and Sertoli cells made up the lining epithelium. The germinal epithelium was made up of spermatogonia cells on a basement membrane, big rounded primary spermatocytes, and spermatids, all of which were distinguished by their darkly stained rounded nuclei and location toward the lumen. Spermatozoa seemed to be elongated and pointed at the end. The seminiferous tubules' lumens were filled with the flagella of mature sperms. Leydig cells were found in the interstitial areas between tubules. Control group showed normal tunica albuginea with normal sub-capsular blood vessels, normal-sized tubules with tunica propria, normal germinal lining and complete spermatogenesis, and normal interstitium with normal Leydig cells. These results were in agreement with Elkerdasy and Mohamed[7] who showed that testes of control group had normal architecture.

In the present study, the histological examination of testicular sections from the non-diabetic group treated with low-dose sildenafil (9 mg) showed thick tunica albuginea with mildly congested sub-capsular blood vessels, scattered small-sized and distorted tubules with quite normal tunica propria, germinal lining and complete spermatogenic layers, and near normal interstitium with Leydig cells. These results were in disagreement with Salwa et al.[11] who showed decreased spermatogenic layers, thinning of germinal lining and interstitial edema.

In the present study, the histological examination of testicular sections from the non-diabetic group treated with high-dose sildenafil (13.5 mg) showed thickened tunica albuginea with mild sub-capsular edema, slightly distorted tubules with tunica propria, slightly diminished germinal lining and nearly complete spermatogenic layers in most of tubules, and normal interstitium with Leydig cells. These results were in agreement with Al-Fartosi[12]; Alp et al.[13]; and El- Sheikh et al.[14] who reported that most of tubules had normal architecture with some tubules had decreased germinal lining.

On the other hand, these were in contrary to Elkerdasy and Mohamed[7]; and Salwa et al.[11] who mentioned that the rat testicular sections from experiment which received 13.5mg/kg of sildenafil

for a long time showed that almost total affection of testicular tissue was apparent as compared to that of control group. The testicular tissues showed that most of the seminiferous tubules appeared depleted of most of the spermatogenic cells as there were large empty spaces in between germ cells that appeared detached and shrunken with pyknotic nuclei.

In the present study, the histological examination of testicular sections from the diabetic group treated with low-dose sildenafil (9 mg) showed markedly thickened tunica albuginea with mildly congested sub-capsular blood vessels, scattered small-sized distorted tubules with irregular tunica propria, thin germinal lining with diminished spermatogenic layers, and edematous interstitium with Leydig cells and this was in agreement with Zimmermann et al.[15]; and Uslu et al.[16]. On the other hand, these results were in disagreement with Elkerdasy and Mohamed[7] who reported that there was no histological difference between diabetic and non-diabetic groups.

In the present study, the histological examination of testicular sections from the diabetic group treated with high-dose sildenafil (13.5 mg) showed markedly thickened tunica albuginea with congested sub-capsular blood vessels, thickened tunica propria, thinned germinal lining, diminished spermatogenic layers, and edematous interstitium.

These results were obtained by Al-Fartosi[12]; and Uslu et al.[16] who studied long term administration of sildenafil citrate on testicular histopathological changes of male rats. Sildenafil citrate produced sperm abnormalities (fewer sperm and more sperm deformities), as evidenced by histological alterations in the testis (hypertrophy cells, necrosis of seminiferous tubules, testis destruction, and the presence of inflammatory cells).

Regarding electron microscopic results, the present study revealed that, the electron microscopic examination of rat testicles in the control group showed normal seminiferous tubules with spermatogonia resting on tunica propria with normal myoid cells, nucleus with dispersed chromatin and rounded mitochondria, primary spermatocytes with nucleus showing dispersed chromatin, normal mitochondria, well-formed endoplasmic reticulum, Sertoli cells with indented nucleus showing prominent nucleoli, numerous lysosomes, and many rounded and elongated spermatids and well-formed spermatozoa with acrosomal cap. These results were in agreement with Salwa et al.[11]; Sofikitis et al.[17]; and Sivasankaran et al.[18].

The present study also revealed that, the electron microscopic examination of rat testicles in the non-diabetic group treated with low-dose sildenafil (9 mg) showed seminiferous tubules with spermatogonia resting on thick irregular tunica propria with smaller myoid cells, nucleus with clumped chromatin in the periphery and swollen and distorted mitochondria with irregular cristae, primary spermatocytes with small nucleus and clumped chromatin, large cytoplasmic vacuoles, swollen endoplasmic reticulum and distorted mitochondria with irregular cristae, and rounded and elongated spermatids, and portions of distorted spermatozoa with no acrosomal cap, directed towards Sertoli cell with nucleus showing prominent nucleolus. These results were comparable to those obtained by Salwa et al.[11]; and Sofikitis et al.[17].

The present study also revealed that, the electron microscopic examination of rat testicles in the non-diabetic group treated with high-dose sildenafil (13.5 mg) showed seminiferous tubules with spermatogonia resting on markedly thickened irregular tunica propria with smaller myoid cells, markedly swollen mitochondria with irregular cristae and swollen endoplasmic reticulum, primary spermatocytes with pyknotic small nucleus and clumped chromatin in the periphery, markedly swollen mitochondria with irregular cristae and small cytoplasmic vacuoles, and distorted elongated spermatids and portions of dead and markedly distorted spermatozoa with less-formed acrosomal cap directed towards Sertoli cell with nucleus showing prominent nucleolus and markedly swollen mitochondria with irregular cristae**.**

These were in agreement with Salwa et al.[11]; Sofikitis et al.[17]; and Sivasankaran et al.[18] who reported that spermatogonia appeared with shrunken nucleus and vacuolated cytoplasm. The Sertoli cells seemed to be displaced, with a hazy cell membrane. The nuclei of the main spermatocytes and spermatids were irregularly shaped, and the acrosomal cape was aberrant. Spermatozoa appeared distorted and have a swollen mitochondrial sheath. On the other hand, Uslu et al.[16] was against the results of this study as he reported that non-diabetic rats treated with sildenafil had no significant change when compared to control group.

The present study also revealed that, the electron microscopic examination of rat testicles in the diabetic group treated with low-dose sildenafil (9 mg) showed seminiferous tubules with scattered primary spermatocytes showing small nucleus showing clumped chromatin in the periphery, small rounded mitochondria and large cytoplasmic vacuoles, many rounded and elongated spermatids with less-formed acrosomal cap, and portions of well-formed spermatozoa with well-formed acrosomal cap directed towards Sertoli cells with numerous rounded mitochondria and numerous lysosomes.

Salwa et al.[11]; Uslu et al.[16]; Sofikitis et al.[17]; and Sivasankaran et al.[18] were in agreement with the results of the present study. They owed these changes to the deleterious effects of hyperglycemia on the different tissues -especially testes- and free radical formation is synergistic to the hazardous effect of sildenafil on the testes; resulting in clearly observed pathological changes on the ultrastructural level of the testis.

The present study also revealed that, the electron microscopic examination of rat testicles in the diabetic group treated with high-dose sildenafil (13.5 mg) showed seminiferous tubules with spermatogonia resting on thick tunica propria with myoid cells, nucleus with dispersed chromatin and swollen mitochondria, primary spermatocytes with

nucleus showing dispersed chromatin and small cytoplasmic vacuoles, Sertoli cells and small rounded mitochondria, many elongated spermatids with well-formed acrosomal cap, few lysosomes, and well-formed spermatozoa with well-formed acrosomal cap. These results were in agreement with Salwa et al.[11]; Uslu et al.[16]; Sofikitis et al.[17]; and Sivasankaran et al.[18].

On the other hand, sildenafil was found to have great protective and/or therapeutic effects on the testes in other situations such as testicular torsion[19], testicular trauma[20], and testosterone deficiency[21].

CONCLUSION

This study proved histologically that sildenafil citrate has a toxic effect on the testes of adult albino rats that was more severe with the toxic dose of sildenafil citrate. Also, the changes were mild in non-diabetic and moderate to severe in diabetic rats.

Conflict of interest : none

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