

Effects of Sildenafil Citrate on the Structure of the Testis and the Possible Protective Role of Selenium in Adult Albino Rats: An Electron Microscopic Study

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Abstract

Background: Sildenafil citrate used for treatment of erectile dysfunction and was reported to be supportive to men with erectile dysfunction including who suffer from diabetes, hypertension, spinal cord injuries, multiple sclerosis, depression, schizophrenia and men after prostatectomy. Selenium has a considerable attention as an essential micronutrient for both animal and human being. It is a potentially antioxidant agent.

Aim of Study: This work aimed to study the possible protective effects of selenium on the testes of the adult albino rats treated with sildenafil citrate by transmission electron microscope.

Material and Methods: Thirty adult albino rats were used and divided into three equal groups: Group I (control group), group II; received sildenafil citrate orally in a dose of 10mg/kg body weight for 8 weeks, group III: Received selenium in a dose of 10mg/kg orally before the treatment with sildenafil citrate. At the end of the experiment, the testes were obtained, and subjected to the transmission electron microscopic studies.

Results: After treatment with sildenafil citrate spermatogonia appeared with shrunken nucleus and vacuolated cytoplasm. Sertoli cells appeared dislocated with ill-defined cell membrane. The primary spermatocytes and spermatids appeared with irregular shaped nuclei and abnormal acrosomal cape. Leydig cells appeared with irregular nuclei and vacuolated cytoplasm. Spermatozoa appeared distorted with swollen mitochondrial sheath. Selenium showed some improvement in the ultrastructure of the testis with spermatogonia appeared with large nucleus and less vacuolated cytoplasm. Sertoli cells appeared at their normal sites with indented euchromatic nucleus. Primary spermatocytes and spermatids appeared with normal shape. Leydig cells appeared with less vacuolation and euchromatic nuclei. Most of spermatozoa appeared with intact form.

Conclusion: Sildenafil citrate had a toxic effect on all of the germinal cells and administration of selenium partially improved these toxic effects.

Key Words: Testis – Sildenafil citrate – Selenium.

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Introduction

IN recent times, erectile dysfunction has become a common and multifactorial disease that strongly impairs the quality of life in man [1].

Sildenafil citrate is a water soluble citrate salt that was firstly synthesized in United Kingdom to treat hypertension and angina pectoris. This drug exhibited a different pharmacological effect & a marked penile erection [2]. It is a cyclic nucleotide inhibitor and it causes intracellular monophosphate inhibition through action of the endogenous Nitric Oxide-Cyclic Guanosine Monophosphate (NO-cGMP) pathway [3].

Sildenafil popularity is increasing with young adults due to the beliefs that it increases libido, improves sexual performance and increases the size of the penis, it also widely used by body builders and athletes [4].

Sildenafil had provoked tubular and interstitial histological alteration of the seminiferous tubules, increased Leydig cellularity, tubular degeneration, which finally might lead to complete arrest of spermatogenesis [5].

Selenium is used as a nutritional supplement and it is involved in many essential biological processes as immune functions, free radicals counteraction, DNA protection and repair & production of normal spermatozoa [6].

Selenium is an essential trace nutrient for humans and animals [7]. It is required for normal testicular development and spermatogenesis in rats. Serum selenium is reported to be lower in men with oligospermia and azospermia [8].

Aim of the work:

The aim of the work was to study the possible protective effects of selenium on the structure of the testis of adult albino rats treated with sildenafil citrate by transmission electron microscopic examination.

Material and Methods*Animals used:*

Thirty adult male albino rats, weighing 200-250gm. were used; the animals were isolated and housed in the Animal House at Faculty of Medicine, Assuit University during 2017. They were kept in wire-floored cages under standard laboratory conditions of a 12:12 hour light/dark cycle at 25°C with free access to food and water. All animal procedures were in accordance with the recommendations of the Canadian Committee for Care and Use of Animals and were approved by the local Institutional Animal Ethical Committee. All the experimental procedures were performed from 8 to 10 A.M. [9].

The animals were divided into three equal groups; each of them consists of 10 rats:

- 1- Group I: A control group.
- 2- Group II: The rats were treated with sildenafil citrate.
- 3- Group III: The rats were treated with selenium before the treatment with sildenafil citrate.

Drug, dosage and administration:

- 1- Sildenafil citrate was given daily by oral tube in a dose of 10mg/kg body weight for 8 weeks [10].
- 2- Selenium was given daily by oral tube in a dose of 10 microgram/kg body weight for 8 weeks before the treatment with sildenafil citrate [11].

At the end of the experiment the animals were anaesthetized with ether and perfused with saline to wash blood out of the circulatory system then with the appropriate fixative (Formalin 10%). The Scrotums were opened and the testis of the control and treated animals were extracted, cut, and processed for transmission electron microscopic studies. Specimens were cut in small pieces and fixed in 2.5% glutaraldehyde for 24 hours, semithin sections were stained with toluidine blue. Ultrathin sections were examined by transmission electron microscope (Jeol-1010) in Assiut University EM Unit.

Morphometric and statistical analysis:

Estimation of the maximum diameters of the nucleus of spermatogonia and middle piece of the spermatozoa in control and treated animals was done. Measurements were performed on the testes sections (2-3 sections per animal). In each section five measurements were obtained using image analysis system (digimizer version 3.7. 2005-2010) MedCalc statistical software in the Anatomy Department at Sohag University. Statistical analysis were done using SPSS software Version 16. Variables were represented by mean \pm SD (mean \pm standard deviation of mean). Student *t*-test was used to compare the means of these variables between different groups. Finally the significance was considered according to the level of *p*-value significance as follows:

- $p > 0.05$ non significant.
- $p \leq 0.05$ significant*.
- $p \leq 0.01$ highly significant**.
- $p \leq 0.001$ very high significant difference***.

Results*Group I:*

Ultrastructurally, the base of the seminiferous tubules appeared formed by the basement membrane. The spermatogonium appeared resting on the basement membrane with large rounded nucleus and condensed chromatin, a rim of cytoplasm around appeared with organelles. The primary spermatocyte appeared inner to the spermatogonia with large rounded nucleus and a thin rim of cytoplasm filled with mitochondria Fig. (1). Sertoli cell appeared resting on the basement membrane with large oval nucleus and prominent nucleolus, cytoplasm around the nucleus were filled with organelles Figs. (1,2). Early spermatids appeared as oval cells next to the Sertoli cells. Their cytoplasm showed peripherally located mitochondria and small oval nucleus Fig. (2). The spermatids appeared as oval large cells with large oval nuclei. The acrosomal cap appeared fitted to one pole of the nucleus. Their nuclei were surrounded by multiple peripherally located mitochondria Fig. (3). Interstitial cells of Leydig appeared in-between the seminiferous tubules with large irregular euchromatic nucleus and prominent nucleolus. The cytoplasm contained numerous mitochondria Fig. (4).

Cross sections in the middle pieces of the spermatozoa consisted of central axoneme surrounded by nine doublets of microtubules and mitochondrial sheath. Terminal end pieces were formed of central axoneme surrounded by cell membrane Fig. (5).

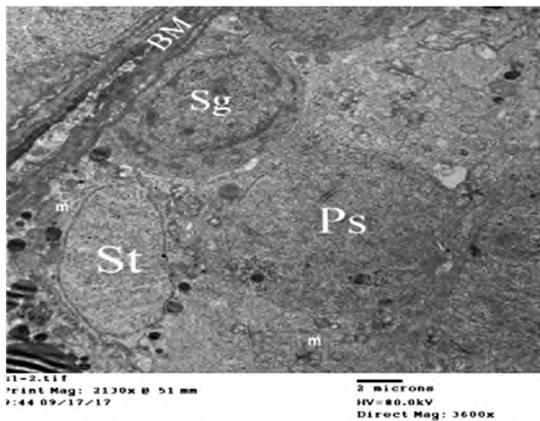


Fig. (1): An electron micrograph from the testis of a control adult albino rat showing spermatogonium (Sg) resting on the basement membrane (BM). The primary spermatocyte (Ps) appears with large rounded nucleus and cytoplasm filled with mitochondria (m), Sertoli cell (St) adjacent to the basement membrane with large faintly stained nucleus and multiple mitochondria (m). (TEM X3600).

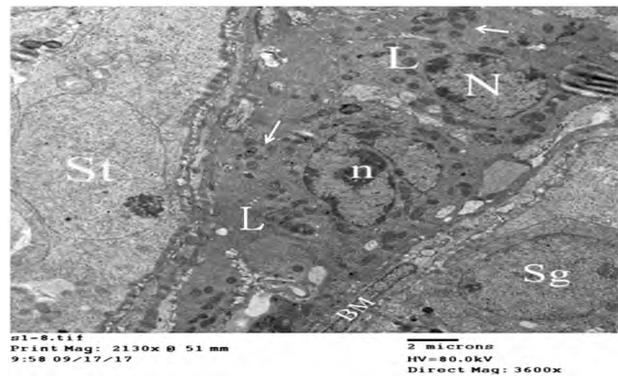


Fig. (4): An electron micrograph from the testis of a control adult albino rat showing two interstitial cells of Leydig (L) with large euchromatic nucleus (N) and prominent nucleolus (n). The cytoplasm contains numerous amounts of mitochondria (arrows). Sertoli cell (St) and spermatogonium (Sg) resting on the basement membrane (BM). (TEM X3600).

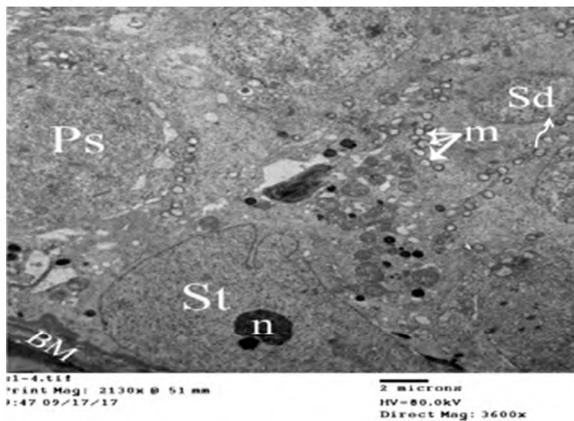


Fig. (2): An electron micrograph from the testis of a control adult albino rat showing Sertoli cell (St) adjacent to the basement membrane (BM) with large oval nucleus and prominent nucleolus (n). The primary spermatocytes (Ps) appear with large nucleus. The early spermatids (Sd) appear oval in shape with small nucleus with intact acrosomal cap (irregular arrow) surrounded by peripherally located mitochondria (m). (TEM X3600).

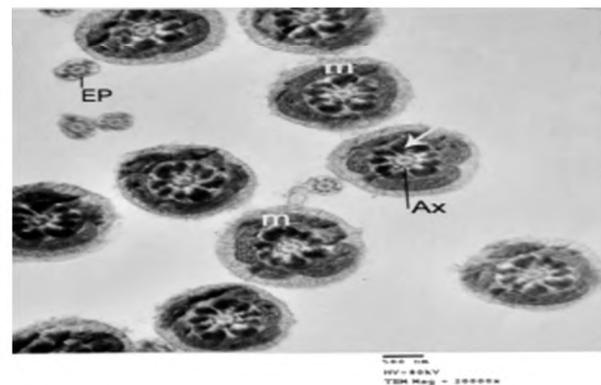


Fig. (5): An electron micrograph from the testis of a control adult albino rat showing cross section in spermatozoa. The middle piece of the spermatozoa consists of central axoneme (Ax) surrounded by nine outer dense fibers (arrow) and mitochondrial sheath (m). Terminal end piece (EP) is formed of central axoneme surrounded by a cell membrane. (TEM X20000).

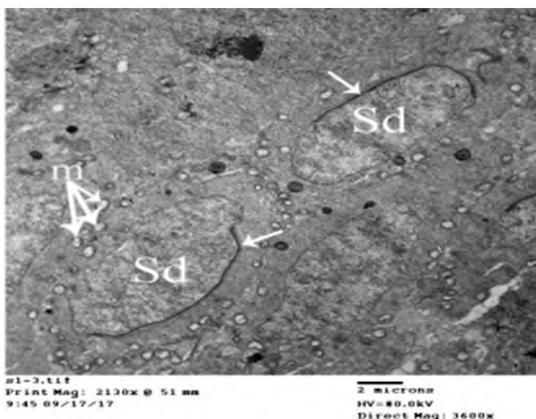


Fig. (3): An electron micrograph from the testis of a control adult albino rat showing two spermatids (Sd) with large oval nuclei. Their cytoplasm shows peripherally located mitochondria (m). The acrosomal cap appears fitted to one side of the nucleus (thin arrow). (TEM X3600).

Group II:

After treatment with the sildenafil citrate the following changes appeared by electron microscopic examination:

Spermatogonia appeared ballooned with multiple vacuoles in the cytoplasm, nucleus appeared condensed and smaller in size. Some cells are replaced by large sized vacuoles Fig. (6). The Sertoli cell appeared detached from their sites with large irregular nucleus and ill-defined cell membrane Fig. (6). The primary spermatocytes appeared degenerated with pyknotic shrunken nucleus and vacuolated cytoplasm and destructed organelles with a large number of lipid droplets and ill-defined cell membrane Figs. (7,8). The Early spermatids appeared with an irregular nucleus and acrosomal cap with ill-defined cell membrane Fig. (8). The spermatid appeared with large irregular oval nucleus. The acrosomal cap appeared irregular or incomplete Fig. (9). The interstitial Leydig cells

appeared with irregular nuclear membrane and peripheral chromatin condensation, some showed destructed nucleus and multiple vacuoles within the cytoplasm Fig. (10).

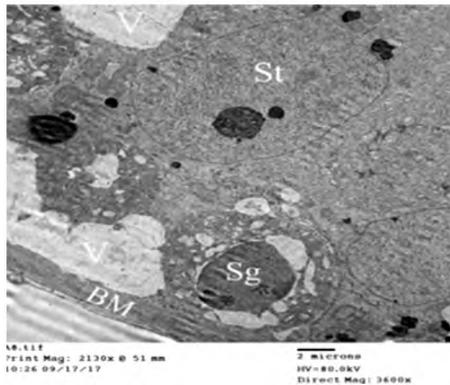


Fig. (6): An electron micrograph from the testis of group II showing the spermatogonium (Sg) close to the basement membrane (BM). The cell appears ballooned with small nucleus with abnormal chromatin distribution. The Sertoli cell (St) appears mislocated with ill-defined cell membrane. The other cells appear replaced by large sized vacuoles. (TEM X3600).

A cross section in the middle pieces of the spermatozoa showed distorted ballooned surrounding microtubules and swollen mitochondrial sheath Fig. (11).

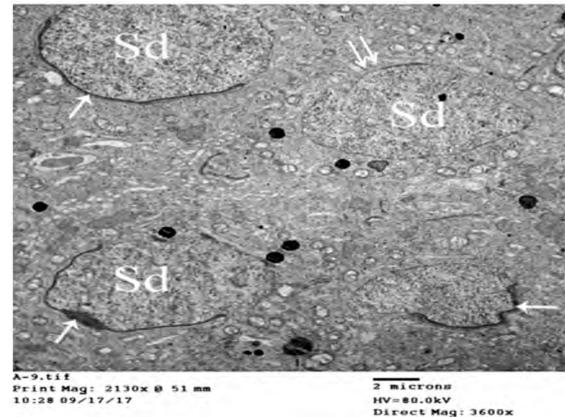


Fig. (9): An electron micrograph from the testis of group showing spermatids (Sd) with pyknotic large irregular oval nuclei. The acrosomal cap appears irregular (T) or incomplete (TT). (TEM X3600).

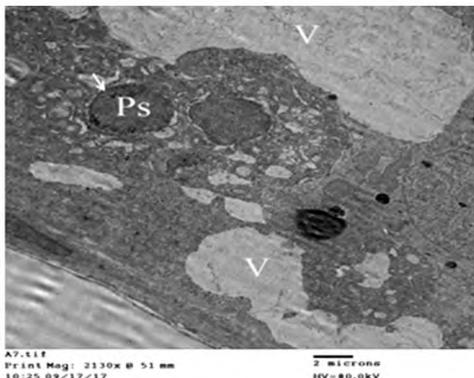


Fig. (7): An electron micrograph from the testis of group II showing the primary spermatocytes (Ps) appears with pyknotic shrunken nucleus (arrow) and vacuolated cytoplasm and destructed organelles, multiple vacuoles appear around (V). (TEM X3600)

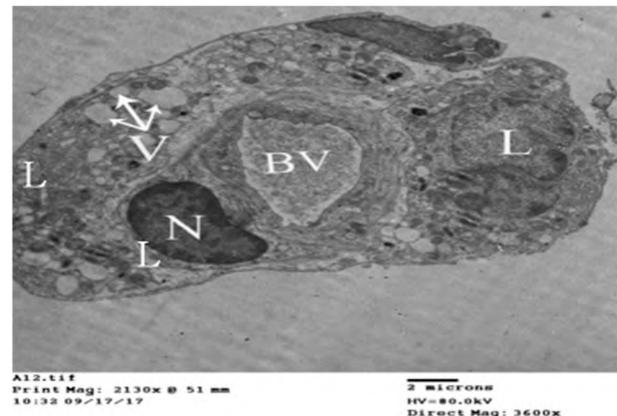


Fig. (10): An electron micrograph from the testis of group II showing multiple Leydig cells (L) with irregular densely packed heterochromatic nucleus (N), vacuolated cytoplasm (V). Note the dilated blood vessels (BV). (TEM X3600).

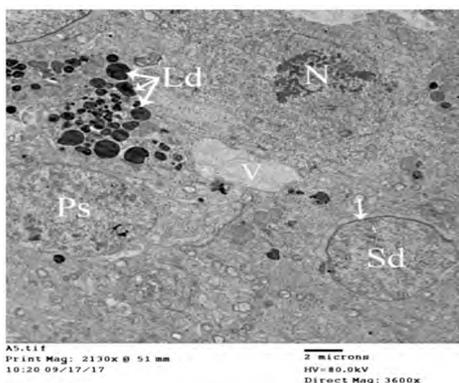


Fig. (8): An electron micrograph from the testis of group II showing two primary spermatocytes (Ps) appears with irregular shaped, destructed nucleus (N) and ill-defined cell membrane with a large number of lipid droplets (Ld) and vacuolated cytoplasm (V). The Early spermatids (Sd) appears with an irregular nucleus and acrosomal cap (T) and ill-defined cell membrane. (TEM X3600).

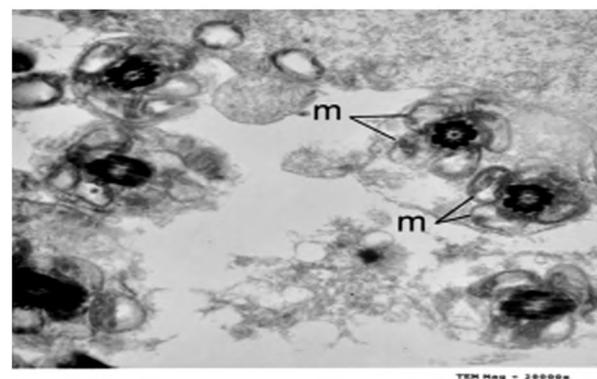


Fig. (11): An electron micrograph from the testis of group II showing a cross section in the middle pieces of spermatozoa which appear distorted with swollen mitochondrial sheath (m). (TEM X20000).

Group III:

Administration of selenium with sildenafil citrate showed the following changes on electron microscope examination:

Spermatogonia appeared resting on the basement membrane with rounded euchromatic large nucleus and less vacuolated cytoplasm Fig. (12). Sertoli cell appeared resting on the basement membrane with indented euchromatic nucleus and well defined cell membrane. Fig. (12). Primary spermatocytes appeared with large oval euchromatic nucleus and less vacuolated cytoplasm with well-defined membrane of the nucleus and the cell Fig. (13). The Early spermatids appeared with large nucleus and incomplete acrosomal cap Fig. (13). Spermatids appeared with euchromatic nuclei that are covered by intact and complete acrosomal caps Fig. (14). The interstitial cells of Leydig appeared normal with euchromatic nucleus and less vacuolated cytoplasm Fig. (15).

A cross section in the spermatozoa shows that the middle piece consisted of intact axoneme surrounded by nine outer dense fibers and mitochondrial sheath. Discontinuous mitochondrial sheath was also noticed in some pieces. Terminal end piece was formed of central axoneme surrounded by a cell membrane Fig. (16).

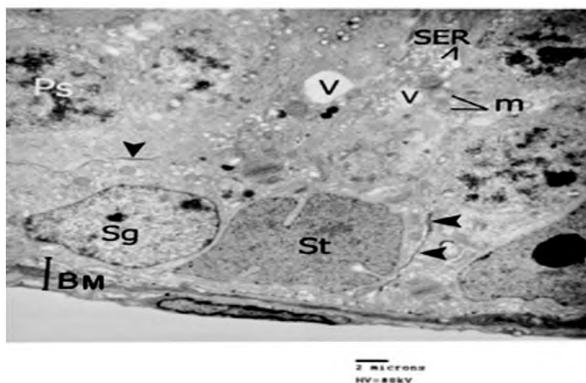


Fig. (12): An electron micrograph from the testis of group III showing the spermatogonium (Sg) resting on the basement membrane (BM) with euchromatic large nucleus and well defined cell membrane (arrow head). The Sertoli cell (St) appears within normal site and with indented euchromatic nucleus and well defined cell membrane (arrow head). Primary spermatocytes (Ps) have oval nucleus and irregular clumps of heterochromatin are observed. Vacuoles (V) in between cells still present, with free organelles mitochondria (m) and smooth endoplasmic reticulum (SER) (TEM X3600).

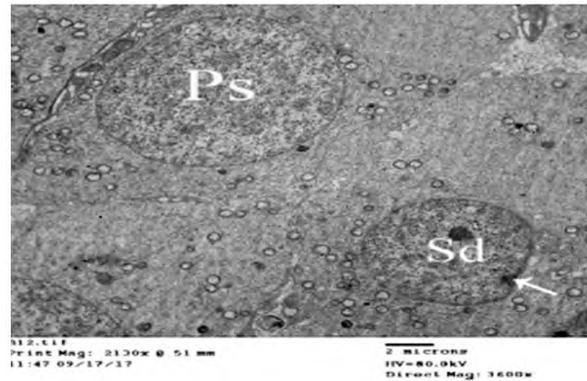


Fig. (13): An electron micrograph from the testis of group III showing the primary spermatocytes (Ps) appear with large rounded euchromatic nucleus with well-defined nuclear and cell membrane. An early spermatid (Sd) appears with an euchromatic large nucleus which is partially covered by acrosomal cap (T). (TEM X3600).

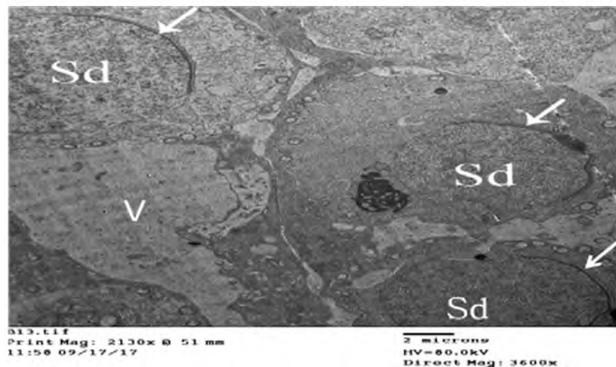


Fig. (14): An electron micrograph from the testis of group III showing the Spermatids (Sd) appear with euchromatic nuclei that are covered by intact and complete acrosomal caps (arrows), Vacuoles (V) still present in-between cells (TEM X3600).

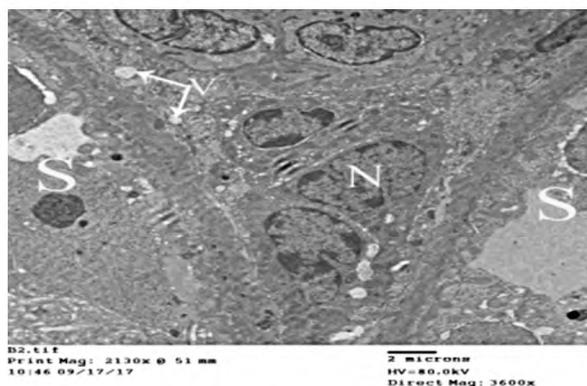


Fig. (15): An electron micrograph from the testis of group III showing multiple Leydig cells with less vacuolation (V), with euchromatic nuclei (N). The area is surrounded on both sides by an area of seminiferous tubules (S). (TEM X3600).

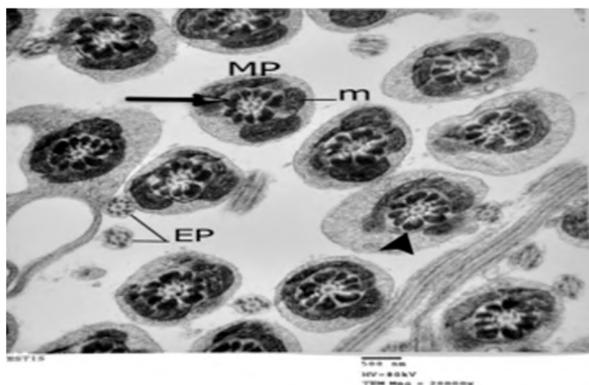


Fig. (16): An electron micrograph from the testis of group III showing cross section in spermatozoa. The middle piece (MP) consists of intact axoneme surrounded by nine outer dense fibers (arrow) and mitochondrial sheath (m). Discontinuous mitochondrial sheath is also noticed in some pieces (arrow head). Terminal end piece (EP) is formed of central axoneme surrounded by a cell membrane. (TEM X20000).

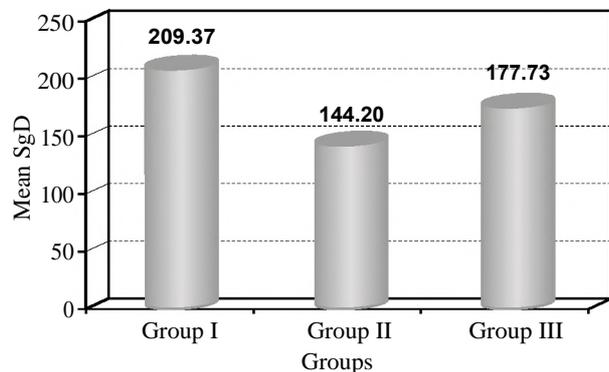
Morphometric results:

1- *Diameter of the nuclei of spermatogonia:* The mean diameter of the nuclei of spermatogonia of control group is (209±20) showed significant increase in comparison to group II (144±8.8) with *p*-value=0.000 and group III (177±6.8) with *p*-value=0.000 (Table 1), Graph (1).

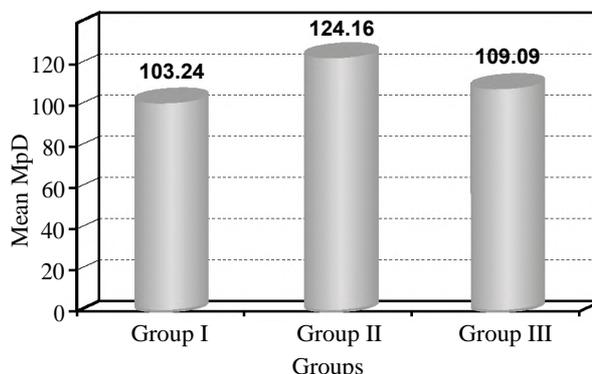
2- *Diameter of the middle piece spermatozoa:* The mean diameter of the middle piece spermatozoa of control group is (103±4.8) showed significant decrease in comparison to group II (124±7) with *p*-value=0.000 and non-significant change with group III (109±10) with *p*-value=0.06 (Table 1), Graph (2).

Table (1): Mean diameters (in pixels) of the nucleus of spermatogonia and the middle piece spermatozoa in control and experimental groups.

	Diameter of the nucleus of spermatogonia (mean ± SD)	Diameter of the middle piece spermatozoa (mean ± SD)
Group I	209±20	103±4.8
Group II	144±8.8***	124±7***
Group III	177±6.8***	109±10



Graph (1): Mean diameters (in pixels) of the nucleus of spermatogonia in control and experimental groups.



Graph (2): Mean diameters (in pixels) of the middle piece spermatozoa in control and experimental groups.

Discussion

Sildenafil citrate is considered a specific inhibitor of cGMP and causes dilatation of peripheral veins and arteries as it enhances nitric oxide release from the penile tissues, leading to the relaxation of corpus cavernosum smooth muscles, increasing blood flow into the spongy tissue of the penis, thus causing erection [12,13].

In the present work EM examination of group (II) revealed that the sildenafil citrate affected the cells of the testis as follows; spermatogonia appeared with shrunken nucleus and vacuolated cytoplasm. The Sertoli cells appeared dislocated with ill-defined cell membrane. The primary spermatocytes and spermatids appeared with irregular shaped nuclei and abnormal acrosomal cape. Spermatozoa appeared distorted and have a swollen mitochondrial sheath.

In this respect previous studies found testicular abnormalities and depressed spermatogenesis of albino rat after sildenafil administration [14,15].

Sildenafil citrate administration induces histological changes in the rat testis in the form of destruction of spermatogenic layer, degeneration in spermatogonia and impaired spermatogenesis, necrosis of seminiferous tubules and interstitial tissue and congested blood vessels [5,16-18].

Sildenafil citrate administration caused testicular oxidative stress which resulted in destruction of seminiferous tubules, affection of spermatogenesis, vacuolation and abnormal spermatozoa morphology [1,10].

Sildenafil citrate decreases number of normal sperms and increases abnormal sperms. It also results in histopathological changes in the testis as vacuolations, necrosis of seminiferous tubules and increases inflammatory cells. These effects

may be due to changes in the expression of various receptors associated cGMP or responsive effect of these receptors in the brain and this will cause damage of testicular tissues and failure in spermatogenesis [19].

Previous studies reported that when albino rats treated with sildenafil citrate, the seminiferous tubules are widely separated, their volume and diameter are decreased. This is due to inter-tubular oedema which disturbs the nutritional supply of the seminiferous epithelium and result in lysis of spermatogenic cells [20].

In the present work Leydig cells appeared with irregular nuclei and vacuolated cytoplasm, previous studies also reported that sildenafil citrate altered the ultrastructure of Leydig cells [21-23].

Vacuolated cytoplasm that was observed in this study can be interpreted by leakage of lysosomal hydrolytic enzymes and peroxidation of polyunsaturated fatty acids induced by sildenafil, which causing cytoplasmic degeneration due to impairment in the cell membrane permeability function [24].

In contrary to the present results previous studies stated that sildenafil have a protective effect on ischemic reperfusion injury to the testes, kidney, brain, spinal cord, ileum and myocardium. Also reported that sildenafil has a positive effect on the spermatogenesis, semen production and quality and increases the fertility [25,26].

Electron microscopic examination of group (III) after addition of selenium showed some improvement in the ultrastructure of the testis; spermatogonia appeared with rounded euchromatic large nucleus and less vacuolated cytoplasm. Sertoli cell appeared resting on the basement membrane with indented euchromatic nucleus. The primary spermatocytes and spermatids appeared with euchromatic nuclei and well defined nuclear and cell membrane. Leydig cells appeared with less vacuolation and euchromatic nuclei. Most of spermatozoa appeared with intact axoneme surrounded by nine outer dense fibers and mitochondrial sheath.

Previous studies reported that the rat testes treated with selenium showed the seminiferous tubules with uniform size and shape and normal spermatogenesis. They were lined by regularly arranged rows of spermatogenic cells in different stages. The spermatogonial and Sertoli cells were observed in normal structure, vacuolation, congestion & oedema were decreased [11,27-29].

Selenium is a potentially antioxidant agent, it possibly reduces the oxidative stress and apoptosis caused by testicular injury in rats [30]. Its antioxidant property has been able to fight against oxidative stress acting on the nucleus and nucleolus by increasing the anti-oxidative capacity and picking superoxide and hydrogen peroxide active species and reducing lipid peroxidation [24].

Free radicals are expected to induce testicular atrophy, therefore, supplementation of antioxidants can be considered as alternative method for chelating therapy [31]. A previous work reported that selenium has the ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against oxidation injury [6].

The decreased vacuoles in the group treated with selenium can be interpreted by researchers who stated that the selenium prevents formation of vacuoles by reducing the production of free radicals, via preventing DNA damage and decreasing gene responsible for its damage [32].

Selenium attenuate testicular toxicity by restoration of normal diameters of the seminiferous tubules and by decreasing necrosis and desquamation of spermatogenic cells especially oxidative damage of Sertoli and Leydig cells [33].

Diet supplementation with selenium has been associated with beneficial effects on sperm function, decreased abnormal sperms and infertility in rats [34].

Conclusion:

- Sildenafil citrate has a toxic effect on all of the germinal cells.
- Administration of selenium partially prevents these toxic effects.

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تأثيرات عقار السيلدينافيل سترات على تركيب الخصية والتأثير الواقي المحتمل للسيلينيوم في الفئران البيضاء البالغة؛ دراسة بالميكروسكوب الإلكتروني

المقدمة: تستخدم سترات السيلدينافيل لعلاج ضعف الإنتصاب وتفيد التقارير أنها داعمه للرجال الذين يعانون من ضعف الإنتصاب بما في ذلك أولئك الذين يعانون من مرض السكري وإرتفاع ضغط الدم وإصابات النخاع الشوكي والتصلب المتعدد والإكتئاب وإنقاص الشخصية والرجال بعد إستئصال البروستاتا. هناك إهتمام كبير بالسيلينيوم بإعتباره من المغذيات الدقيقة الأساسية لكل من الحيوان والإنسان. وهو يحتمل أن يكون مضاداً للاكسدة.

الهدف من الدراسة: دراسة التأثيرات الوقائية المحتملة للسيلينيوم على خصى الفئران المعالجة بعقار السيلدينافيل بإستخدام الميكروسكوب الإلكتروني النافذ.

- المجموعة الأولى: المجموعة الضابطة.
 - المجموعة الثانية: تم معالجتهم بإستخدام السيلدينافيل سترات بجرعة ١٠مج/كغ لمدة ٨ أسابيع.
 - المجموعة الثالثة: وقد تم إعطاء السيلينيوم عن طريق الفم بجرعة ١٠مج/كغ لمدة ٨ أسابيع قبل جرعة السيلدينافيل سترات.
- وفي نهاية التجربة تم تخدير الفئران وتم إستخراج الخصى وتحضير العينات لفحصها بإستخدام الميكروسكوب الإلكتروني النافذ.

النتائج:

- بإستخدام السيلدينافيل سترات: ظهرت أمهات الخلايا المنوية بإنكماش في الأنوية وفجوات في السيتوبلازم. وقد ظهرت الخلايا الدعامية في غير أماكنها مع وجود فجوات في السيتوبلازم. وبالنسبة للخلايا المنوية الأولية وطلائع الحيوانات المنوية ظهرت بأنوية غير منتظمة مع عدم إنتظام في الغطاء الأكرسومي. وقد ظهرت خلايا ليندنج بأنوية غير منتظمة وفجوات في السيتوبلازم أما بالنسبة للحيوانات المنوية قد لوحظ وجود شكل غير طبيعي مع تضخم في غلاف الميتوكوندريا.
- بإضافة السيلينيوم مع السيلدينافيل سترات: حدث بعض التحسن فقد ظهرت أمهات الخلايا المنوية بنواة كبيرة مع وجود فجوات أقل في السيتوبلازم، الخلايا الدعامية ظهرت في أماكنها وبأنوية طبيعية وبالنسبة للخلايا المنوية الأولية وطلائع الحيوانات المنوية ظهرت بالشكل الطبيعي وقد ظهرت خلايا ليندنج بفجوات أقل في السيتوبلازم وأنوية طبيعية أما بالنسبة للحيوانات المنوية فقد ظهر معظمها بالشكل الطبيعي.

الخلاصة:

- السيلدينافيل سترات لع تأثيرات سامة على كل الخلايا المنوية المنبئة.
- تقل هذه التأثيرات جزئياً مع إضافة السيلينيوم.