



Histological Alterations in the Rabbit Epididymis Induced by Sildenafil Overdosing

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Author's contribution

This whole work was carried out by the author BMJ.

Original Research Article

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ABSTRACT

Background: Sildenafil is used for the treatment of erectile dysfunction and is helping millions of men around the world to achieve and maintain a long lasting erection. The histological alterations in the genital system epithelial lining due to sildenafil overdoses intoxication has not yet been well documented.

Aim: The present study was carried out to investigate the histological alterations induced by sildenafil overdoses in the epididymal epithelial lining.

Methodology: Fifty adult male rabbits (*Oryctolagus cuniculus*) were subjected to sildenafil (0, 1, 3, 6, 9 mg/kg/day) for 5 days per week for 7 weeks. Samples from left and right proximal epididymis were applied to conventional histological techniques and subjected to histological examination.

Results: Exposure to overdoses of sildenafil had provoked histological alterations in the epididymal tissue in the form of epididymal hyperplasia and dilated lumina. Mature spermatozoa were less frequent in the lumen of the epididymis than the control ones while spermatogenic cells, cellular debris and spermatid giant cell were seen in the lumen of the epididymis.

Conclusion: The results of the present study confirms that sildenafil provoked alterations in the epididymal epithelial lining together with partial arresting spermatogenesis and impairing the spermatid cells differentiation towards maturation. The findings together might indicate an affect on male fertility induced by sildenafil overdoses.

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1. INTRODUCTION

Sildenafil is used for the treatment of erectile dysfunction and is helping millions of men around the world to achieve and maintain a long lasting erection [1]. This drug acts as a selective inhibitor of phosphodiesterase-5 that causes release of nitric oxide from the penile nerve ending leading to the relaxation of corpus cavernosum smooth muscles, increasing inflow of blood into the spongy tissue of the penis and then causing an erection [2]. Sildenafil was reported to be supportive to men with erectile dysfunction, including those suffer from diabetes, hypertension, spinal cord injuries, multiple sclerosis, depression, schizophrenia and men after prostatectomy [3-9]. In addition, sildenafil showed an efficacy in treatment of pulmonary hypertension in children where standard oral treatment is limited [10-11]. Some studies have provided promising evidences that this drug can be used to tolerate benign prostatic hyperplasia [12].

Studies on experimental animals showed that sildenafil attenuated renal injury in cisplatin-induced nephrotoxicity, increased antinociception of morphine, inhibited carbonic anhydrase activity in red blood cells and elevated total serum testosterone [13-19]. It was also reported that sildenafil altered the ultrastructure of Leydig cells, reduced pulmonary fibrosis and augmented histological alteration of the myocardial cells induced by hypertension or amlodipine [19-23].

Sildenafil overdoses uptake is mainly seen among men suffer from erectile dysfunction where the stigma of this disease surrounds them and their partners. Clinically, sildenafil is an effective drug in elderly men but had a lower efficacy rate with increasing age [24-25]. Also, sildenafil popularity is increasing with young adults due to the belief that the drug increases libido, improves sexual performance and increases penis size. Research studies also indicate that sildenafil is widely used by body builders and athletes and so far is legal in the world of sports [26]. On the other hand, sildenafil overdoses fatality is on the rise and have reached crisis levels in certain countries [27].

Case studies showed that sildenafil overdoses may result in facial flushing, hearing impairment, nose bleeding, nose stuffiness, hypotension, chest pain, priapism, tachycardia and arrhythmia [28-32]. Moreover, sildenafil overdose was reported to be fatal due to its arrhythmogenic potential [33]. Exposure of male rabbits to overdoses of sildenafil had provoked tubular and interstitial testicular histological alterations including spermatocytes karyopyknosis, spermatocytes degeneration, and spermatogenesis arrest [34]. Also, sildenafil high doses caused cellular degenerative changes and intercellular vacuolation in the stroma of the medial geniculate body [35].

The histological alterations in the epithelial lining of the genital system due to sildenafil overdoses intoxication has not yet been well documented. The present study is an attempt to characterize these alterations in the epididymal epithelial lining following experimental sildenafil overdoses.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Fifty male rabbits (*Oryctolagus cuniculus*), weighing 1050–1100 g and of similar age were obtained from the Animal House, College of Applied Medical Sciences, Al Jouf University. The animals were housed at $24 \pm 1^\circ\text{C}$, and 12 h light-12 h dark cycle. The rabbits were randomly assigned to a control group (n = 10) and other four test groups of ten rabbits each were separately caged.

2.2 Drugs and Chemicals

Sildenafil tablets (Fluka, Switzerland), containing sildenafil citrate equivalent to 50 mg of sildenafil were used in the present study.

2.3 Experimental Protocol

Following a period of stabilization (7 days), members of the treated groups were exposed to intraperitoneal (i.p.) injection with a daily single dose of sildenafil (0, 1, 3, 6, 9 mg/kg body weight respectively) for 5 days per week for 7 weeks. The selection of these doses and the route of administration was based on data from previous works [36-37]. Sildenafil has optimum pH at 4.5 [38]. Accordingly, the drug was dissolved in sterile normal saline (0.45% sodium chloride injection, pH at (4.35 -4.5) at 37 °C immediately before use. Drug solutions were prepared so that the necessary dose could be administered i.p. in a volume of one ml. Each member of the control group received daily a single i.p. injection of the same volume of sterile 0.45% sodium chloride.

2.4 Histological Examination

Fresh segments of the right and left proximal epididymis, from each rabbit of all groups (n = 50) were cut out rapidly, fixed in 10% neutral buffered formalin and Bouin's fixatives then dehydrated with ascending grades of ethanol (70, 80, 90, 95 and 100%). Dehydration was then followed by clearing the tissue samples in 2 changes of chloroform before being impregnated with 2 changes of melted paraffin wax, embedded and blocked out. Sections (4-5 μm) were stained according to Pearse [39], Bancroft and Stevens [40] and Kiernan [41]. Stained sections of control and treated rabbits were examined for alterations in the epididymal tissues.

3. RESULTS

The control epididymis appeared in normal structure where the epididymal epithelial lining consisted of ciliated pseudostratified columnar epithelium with mature sperms fill epididymal lumina (Figs. 1 & 2).

Exposure to overdoses of sildenafil had provoked the following histological alterations in the epididymal epithelium:

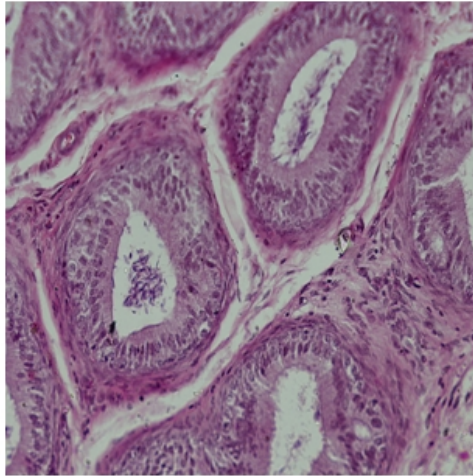


Fig. 1. Normal epididymal tubules of control rabbit. Note mature sperms in the lumen of the epididymal tubules. H&E stain.

Normal epididymal tubules of control rabbit. Note mature sperms in the lumen of the epididymal tubules. H&E stain.

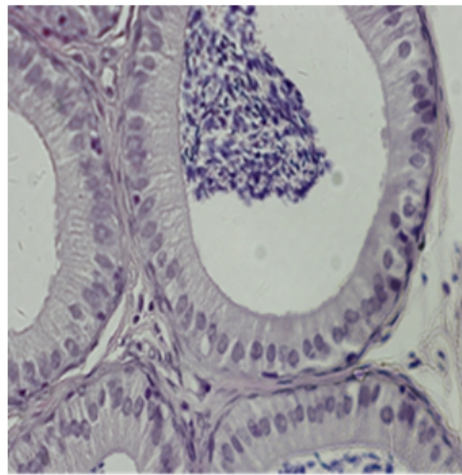


Fig. 2. Epididymal tubule of control rabbit lined by ciliated pseudostratified columnar epithelium with numerous sperms in the lumen. H&E stain.

Epididymal tubule of control rabbit lined by ciliated pseudostratified columnar epithelium with numerous sperms in the lumen. H&E stain.

3.1 Epididymal Tubular Dilatation

Some of the epididymal tubules of sildenafil treated rabbits were highly dilated without atypia or mitotic activity. This alterations was seen in the epididymis of rabbits exposed to sildenafil 9mg/kg/day or 6mg/kg/day and to lesser extent in those received 3mg/kg/day (Figs. 3 & 4).

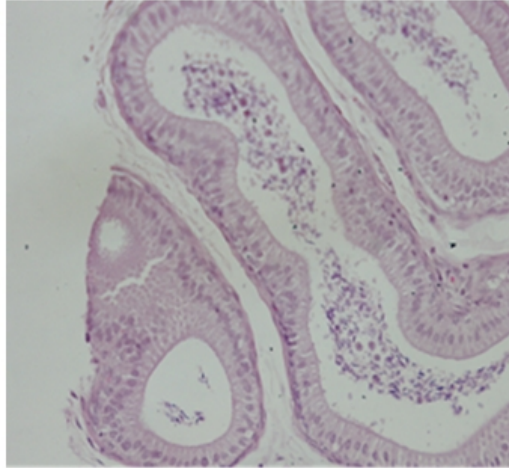


Fig.3. Marked dilatation of the epididymal tubules of rabbit received 9mg/kg sildenafil for 7 weeks. H&E stain.

Marked dilatation of the epididymal tubules of rabbit received 9mg/kg sildenafil for 7 weeks. H&E stain.

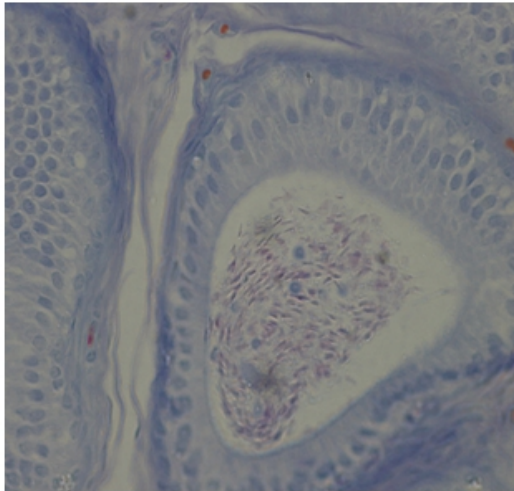


Fig. 4. Epididymal tubule of control rabbit demonstrating normal epididymal structures. Trichrome stain.

Epididymal tubule of control rabbit. Trichrome stain.

3.2 Epididymal Hyperplasia

Segmented hyperplasia in the form of folded pleated epithelium was mainly seen the epididymis of rabbits exposed to sildenafil 9mg/kg/day and to lesser extent in those received 6mg/kg/day but not seen in members of other groups (Figs. 5-6).

The folded epididymal segments were ovoid circle in shape rather than circular as seen in the control ones. Moreover, most papillary projections of the pleated epididymal epithelia showed shorter columnar cells with basal nuclei in comparison with the epididymal epithelial flat portions.

No epididymal hyperplasia or dilatation were observed in the epididymis of members of all groups prior to 3 weeks of sildenafil exposure. Also no local or diffuse loss of cilia was observed in the epididymal epithelial flattening.

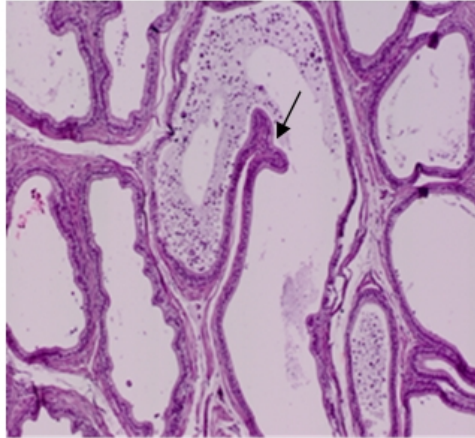


Fig. 5. Segmented epididymal hyperplasia of rabbit received 9mg/kg sildenafil for 7 Weeks (arrow). H&E stain.

Segmented epididymal hyperplasia of rabbit received 9mg/kg sildenafil for 7 Weeks (arrow). H&E stain.

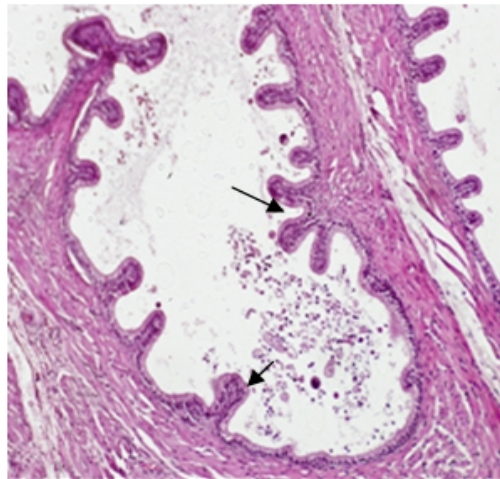


Fig. 6. Epididymal tubule with hyperplasia of rabbit received 9mg/kg sildenafil for 7 weeks. Note the folded pleated epithelial lining of the tubule (arrows). H&E stain.

Epididymal tubule with hyperplasia of rabbit received 9mg/kg sildenafil for 7 weeks. Note the folded pleated epithelial lining of the tubule (arrows). H&E stain.

3.3 Luminal Epididymal Spermatozoa

Few or no spermatozoans were seen in some of the epididymal lumina of rabbits exposed to sildenafil 9mg/kg/day in comparison with the control ones (Figs. 7). Some epididymal tubular lumens were mainly occupied by spermatogenic cells and intraluminal cellular exudates (Figs. 8 & 9). This picture was seen in rabbits received sildenafil 6mg/kg/day or more for 3 weeks or more.

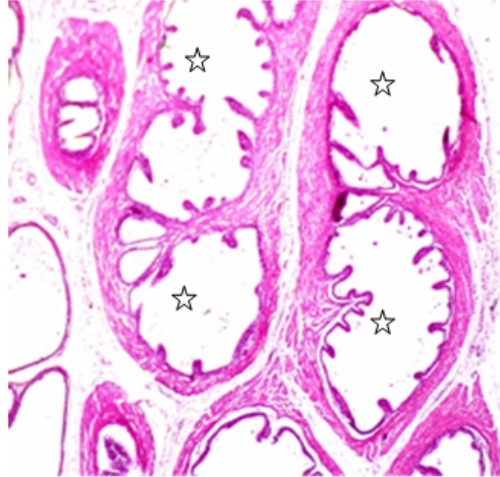


Fig. 7. Cross section of rabbit epididymis with total absence of mature sperm (stars).

Cross section of rabbit epididymis with total absence of mature sperm (stars).

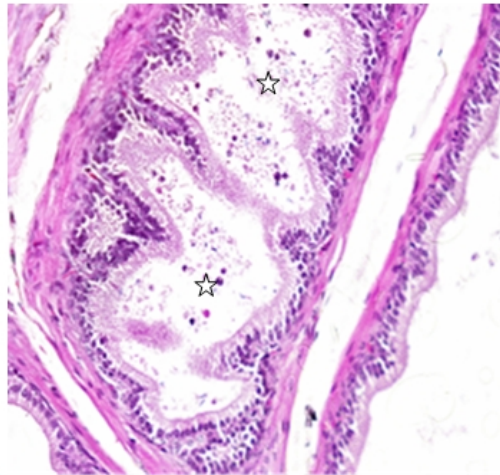


Fig. 8. Epididymal tubule of rabbit received 9mg/kg sildenafil for 7 weeks filled mainly with spermatogenic cells and cellular debris (stars). H&E stain.

Epididymal tubule of rabbit received 9mg/kg sildenafil for 7 weeks filled mainly with spermatogenic cells and cellular debris (stars). H&E stain.

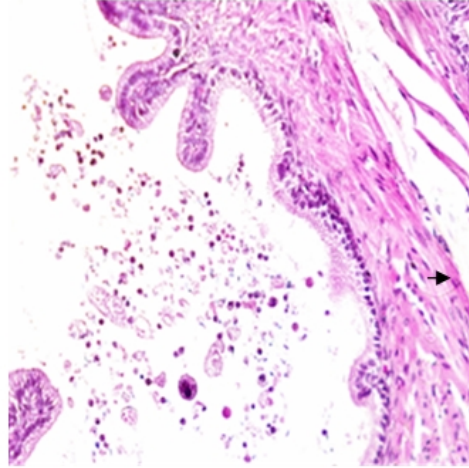


Fig. 9. Hyperplased epididymal epithelial lining of rabbit received 9mg/kg sildenafil for 7 weeks. Note spermatozoa and cellular debris occupying the lumen. H&E stain.

Hyperplased epididymal epithelial lining of rabbit received 9mg/kg sildenafil for 7 weeks. Note spermatozoa and cellular debris occupying the lumen. H&E stain.

3.4 Spermatid Giant Cells

Multinucleated spermatid giant cells were seen occasionally in the epididymal lumens of rabbits received sildenafil 9mg/kg/day for 7 weeks (Figs. 10).

Animals of the control group and those received sildenafil 1mg/kg/day for 7 weeks showed none of the above observed morphological histological alterations in the epididymal epithelia or evidences of spermatogenic disturbances.

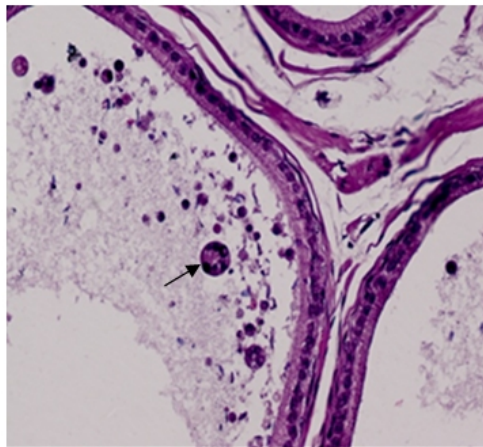


Fig. 10. Spermatid giant cell in the epididymal lumen of rabbits
Spermatid giant cell in the epididymal lumen of rabbits received sildenafil 9mg/kg/day for 6 weeks (arrow). Trichrome stain.

4. DISCUSSION

Sildenafil is phosphodiesterase type 5 inhibitor which leads to the breakdown of guanosine monophosphate and thereby increases blood flow to the penis and enhances penile erection [1-2]. Epididymal histological alterations as presented by the results of the present study might indicate that male reproductive organs are affected by this drug overdoses.

The results of the present investigation showed that sildenafil exposure causes epididymal hyperplasia which is an increase in number of cells most likely resulted from an increased rate of cellular division [42]. This alteration might indicate susceptibility of the reproductive epithelia to sildenafil toxicity and may represent a sort of epididymis response to the injury induced by this drug. Moreover, the seen epididymal dilated lumina might be a sort of secondary change related to progressive pressure on the epididymal tubules induced by sildenafil overdoses. This finding is in agreement with other reports where interstitial testicular proliferation together with partial spermatogenic arrest were induced by sildenafil overdoses [34].

The presence of spermatogenic cells in the epididymal lumina as seen in the results of the present work might be resulted from the loss of adhesion between these cells and/or loss of the normal intracytoplasmic bridges preventing their further maturation. Spermatogenic cells cytoskeleton is testosterone dependent where the junction between spermatogenic cells is dispersed in the absence of testosterone as indicated by some studies where testosterone was declined as a result of sildenafil chronic treatment [13,43]. Low testosterone level could be resulted from Leydig cells disturbance. Jarrar [34] reported that sildenafil overdoses induced Leydig cell proliferation as a compensation action to modulate the action of this drug overdoses on spermatocytes differentiation, a process which is highly controlled by testosterone produced by Leydig cells. However, the appearance of immature germ cells in the epididymal tubular lumen of rabbits exposed to sildenafil overdoses might indicate an effect of this drug on the architecture of the testicular and epididymal epithelial lining integrity and the process of spermatogenesis.

Spermatid giant cells are generally interpreted to be a degenerative lesion associated with a wide variety of agents and circulatory disturbances [44]. The appearance of spermatid giant cells in the epididymal lumen is in line with the results of previous reports where overdoses of the drug under study had provoked testicular damage, arrest of spermatogenesis and the appearance of these multinucleated spermatids in the seminiferous tubules lumen of rabbits exposed to sildenafil overdoses [34]. Spermatid giant cells are known to develop as a result of failure of cytokinesis during meiotic division or might be due to disruption of cytoplasmic bridges connecting spermatocytes clones [45-46].

These epididymal alterations could also indicate an interference of this drug with the antioxidant defense mechanism which may lead to reactive oxygen species generation and enhancement of the seen epididymal histological alterations.

5. CONCLUSION

On the basis of the findings of the present study, one might conclude that chronic exposure to sildenafil overdoses is capable of inducing toxicity on the epididymis which might affect male fertility, particularly with regard to patients at the age and need of reproductiveness.

CONSENT

It is not applicable as no human subjects were used in the present work.

ETHICAL APPROVAL

The experimental protocol was approved by Al Jouf University Local Animal Care and Ethics Committee and the experimental procedures were carried out in accordance with international guidelines for care and use of laboratory animals.

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COMPETING INTERESTS

Author declares that no competing interest exists.

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