Influence of different concentrations of Sildenafil Citrate on histological parameters of mice epididymis

تأثير تراكيز متعددة لسترات السلدينافيل على المتغيرات النسجية لبربخ الفئران

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Abstract

Sildenafil citrate (SC) is widely used for the treatment of erectile dysfunction and maintains a sufficient erection for satisfactory sexual performance. Very limited literatures were published about effects of SC on histological changes of epididymes in human or laboratory animals. The aim of this study was to investigate the influence of different concentrations of SC for different administration periods on some histological parameters of mice epididymes. Seventy five male mice (7-8) weeks old, weighing (23-25)g were used in the present study. The possible effect of SC was tested through the treatment groups of mice with different doses of SC (2, 4, 8, 16)mg/Kg body weight, and for different periods (1, 3, 5) weeks. Histological examination of epididymes were carried out and compared with those belong to untreated group. Results revealed no significant differences (P>0.05) in epididymal weight, tubules diameters and thickness of epithelial layer between treated groups and control group. Conclusion in this study, SC has no effect on epididymis histology.

المستخلص

أستخدم عقار سترات السلدينافيل (الفياغرا) بشكل واسع لعلاج فشل الانتصاب لدى الذكر وكذلك ادامة الانتصاب للحفاظ على الكفاءة الجنسية . فقد نشر عدد قليل جداً من المصادر حول تأثيرات سترات السلدينافيل على التغيرات النسجية للبرابخ في الانسان والحيوانات المختبرية . لذلك هدفت هذه الدراسة الى التحقق من تأثير تراكيز مختلفة لسترات السلدينافيل لفترات تجريع مختلفة على بعض المتغيرات النسجية لبرابخ الفئران . شملت هذه الدراسة خمس وسبعون ذكر فأر بعمر (7 8) اسابيع وبوزن (23 25)غم ولمعرفة تأثير سترات السلدينافيل التي أختبرت خلال المجاميع المعاملة للفئران تم تقسيم الذكور الى مجاميع اعتماداً على تراكيز سترات السلدينافيل المختلفة (2 ، 4 ، 8 ، 16)ملغم/كلغم ولفترات مختلفة (الاسابيع الأول والثالث والخامس) . وبانتهاء فترة التجريع أخذت البرابخ خارج جسم الحيوان وتم تحضيرها للمقاطع النسيجية . أظهرت نتائج الدراسة الحالية فروقات غير معنوية في أوزان البرابخ وأقطار نبيبات البرابخ وفي سمك طبقة الخلايا الطلائية لنبيب البربخ بين مجموعة السيطرة وجميع مجاميع المعاملة . نستنتج من نتائج هذه الدراسة ان التجريع بسترات السلدينافيل لم يعطي تأثيرات على انسجة بربخ الفئران .

Introduction

Sildenafil citrate (SC) is a cyclic nucleotide phosphodiesterase-5 (PDE5) inhibitor and it causes intracellular accumulation of cyclic guanosine monophosphate [1], through action of the endogenous nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway. Also it acts as corpus cavernosum vasodilator, increased blood flow and enhanced erectile function in human [2, 3] and murine [4]. It was revealed that the SC can act by relaxing the arterial wall due to the presence of PDE5 in the arterial wall

Key words: Sildenafil citrate (Viagra)®, epididymis histology.

The epididymis consists of three main parts; the head, body and tail (cauda), the head is composed of a dozen of so coiled ductuli efferents arising from the rete testes while the body and cauda are consist of the coiled ductus epididymis [6]. The epididymis is lined up with a psudostratified columnar epithelium tissue composed of rounded basal cells and tall columnar cells, bearing nemours microvilli, these cells are supported on a long basal lamina and surrounded by smooth muscles, who are their contraction help in the sperm movement along the duct [7].

The epididymis is considered as an androgen-dependent tissue, the epithelial cells lining the adult male epididymis contain the enzyme of 5α-reductase and abundant androgen receptors, which prefentially binds to Dihydrotestosterone (DHT) [8]. One of the major epididymal functions is maturation of spermatozoa which involves morphological, histochemical, physiological, biophysical and metabolic changes [9].

In the last years, very limited information was mentioned in the literatures about the effect of sildenafil citrate on histological changes of epididymes in human or laboratory animals. Therefore, the aim of this study was to investigate the influence of different concentrations of SC for different administration periods on some histological parameters of epididymes in mice.

Material and Methods

1. Animals

Seventy five healthy mature male mice of Swiss albino strain at an age (7-8) weeks old weighing (23-25)g were obtained from the animal house at the Institute of Embryo Research and Infertility Treatment/ Al-Nahrain University. The animals were housed in plastic cages (4 mice/ cage) measuring about (29X15X12) cm.

Animals were kept in air conditioned room with an optimum temperature of 24±2 ºC and exposed to about (12-14) hours/ day light program. Water and food were locally prepared and the consisted of available constituents that fulfill the mouse dietary requirements.

2. Dose preparation and experimental design

Sildenafil citrate solution was prepared by complete dissolving a crushed tablet 100mg in 2mL of normal saline to obtain different doses of sildenafil citrate (2, 4, 8, 16) mg/ Kg in 10µL twice per week during 5 weeks.

In the present work, seventy five male mice were divided into five major groups (included one group as control) depending on different doses of sildenafil citrate (15 animals each). The period of the experiment was unified for each major group during 5 weeks. Oral route of administration was used for treatment with sildenafil citrate. Each major group was subdivided into three minor groups, (5animals each) according to periods of orally-administered SC for one, three and five weeks.

3. Histological sections and study

At the end of administration period, males were killed and both epididymes were obtained and cleaned from adipose tissues. Then, both epididymes were weighted and fixed in 10% formalin processed routinely, embedded in paraffin and stained with hematoxyllin and eosin for histological examination as mentioned by [10]. Diameter

of epididymal tubule and thickness of epithelial cells layer were assessed for all groups of male mice as mentioned in details by [11].

4. Statistical analysis

Means and standard error of mean (mean ± SEM) were determined using descriptive statistical methods. The data were statistically analyzed by multiple analysis of variance (MANOVA) to compare among different means of groups using special statistical computerized package SPSS (statistical Package of Social Science version 14).

Results

Table (1) showed that mice epididymal weights were increased non significantly (P>0.05) for all treated groups after one week administration of SC as compared to the control group. Similarly, non significant differences (P>0.05) in the weights of epididymes were reported for control and treated groups after three and five weeks administration period Table(1).

Table(1): Effects of different doses of sildenafil citrate on epididymal weight (gm) for different orally administration periods to male mice compared to control group.

Administration periods	Male mice groups				
	G – 1 control	G – 2 2 mg/Kg	G – 3 4 mg/Kg	G – 4 8 mg/Kg	G – 5 16 mg/Kg
One week treatment	0.307 ± 0.234	0.309 ± 0.237	0.313 ± 0.24	0.312 ± 0.239	0.311 ±0.238
Three weeks treatment	0.31 ± 0.238	0.312 ± 0.24	0.313 ± 0.242	0.312 ± 0.24	0.311 ± 0.239
Five weeks treatment	0.314 ± 0.238	0.316 ± 0.24	0.316 ± 0.24	0.315 ± 0.239	0.313 ± 0.237

In Table (2), diameter of epididymal tubules were increased non significantly (P>0.05) for different treated groups as compared to control group after one week period of SC administration. After three weeks of SC treatment with different concentrations, no significant differences (P>0.05) in the diameter of epididymal tubules were noticed between the control and all treated groups. In this study, administration of (2, 4)mg/Kg to male mice for five weeks causes non significant increase (P>0.05) in the diameter of epididymal tubules when compared to the control. While same parameter was reduced non significantly (P>0.05) in male mice administered (8, 16) mg/Kg for 5 weeks. Table (2), which also showed no significant differences (P>0.05) among different treated groups within each administration period.

Table (2): Effects of different doses of sildenafil citrate on diameter of epididymal tubule (μ) for different orally administration periods to male mice compared to control group.

Administration periods	Male mice groups				
	G – 1 control	G – 2 2 mg/Kg	G – 3 4 mg/Kg	G – 4 8 mg/Kg	G – 5 16 mg/Kg
One week treatment	349.20 ± 8.79	361.83 ± 10.04	357.16 ± 10.18	358.09 ± 9.72	348.94 ± 9.83
Three weeks treatment	362.18 ± 9.28	363.29 ± 11.08	351.91 ± 8.38	354.24 ± 9.94	350.03 ± 10.09
Five weeks treatment	356.84 ± 11.52	359.21 ± 10.83	359.97 ± 9.86	351.37 ± 10.32	354.62 ± 9.78

After one week, assessment of thickness of epithelial cells layer revealed no significant differences (P>0.05) between both control and treated groups, and also among treated groups Table (3). However, after three weeks treatment with different concentrations of SC no significant differences (P>0.05) in the thickness of epithelial cells layer were observed between the control and treated groups. As compared to the control group, no significant changes (P>0.05) in the thickness of epithelial cells layer were evaluated between control and treated groups, as well as, among different treated groups after five weeks of SC administration. Furthermore, the thickness of epithelial cell layers were non significantly different (P>0.05) among all treated groups Table (3).

Table (3): Effects of different doses of sildenafil citrate on thickness of epithelial cells layer (µ) for different orally administration periods to male mice compared to control group.

Administration periods	Male mice groups				
	G – 1 control	G – 2 2 mg/Kg	G – 3 4 mg/Kg	G – 4 8 mg/Kg	G – 5 16 mg/Kg
One week treatment	168.81 ± 8.32	170.52 ± 9.11	172.26 ± 8.53	167.84 ± 9.72	169.46 ± 9.61
Three weeks treatment	170.21 ± 7.97	172.11 ± 8.42	169.27 ± 7.94	171.76 ± 8.52	176.58 ± 9.72
Five weeks treatment	163.71 ± 7.89	161.48 ± 8.27	167.89 ± 8.18	163.79 ± 8.68	168.26 ± 8.41

In general, histological sections of mouse epididymes revealed no significant histological changes for all SC doses administered at different periods. Figure (1- a, b, c, d, e) show different epididymal sections of male mice treated with different SC doses.

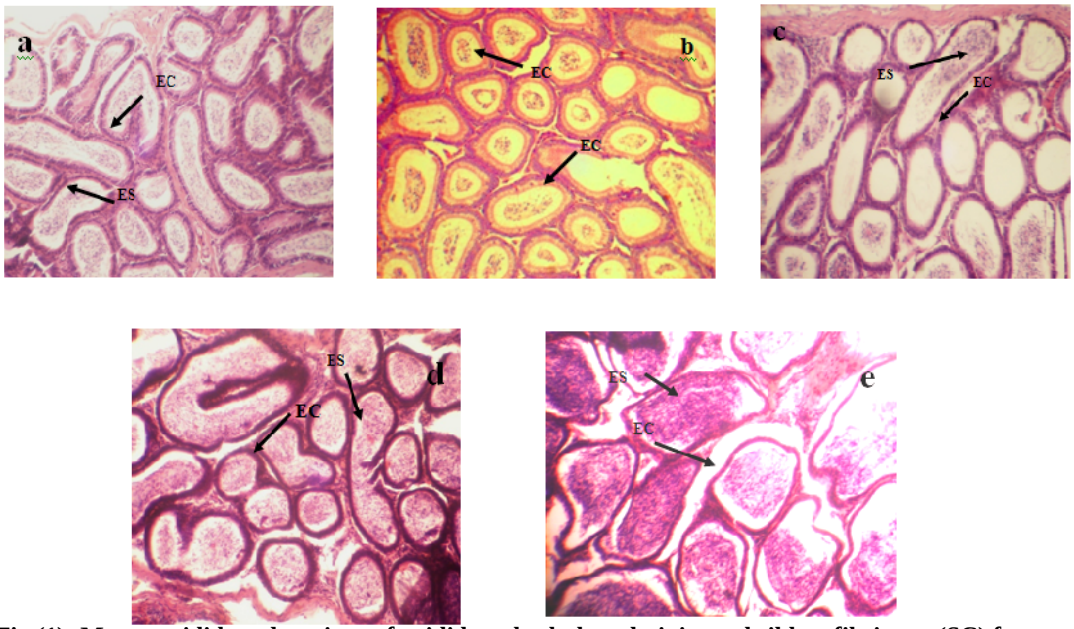

Fig (1): Mouse epididymal sections of epididymal tubules administered sildenafil citrate (SC) for five weeks and different doses:
G-1 (control; 0 mg/Kg) G-2 (2 mg/Kg) G-3 (4mg/Kg) G-4 (8 mg/Kg) G-5 (16 mg/Kg).
(H & E stain, X170). EC; Epithelial cell layer, ES; Epididymal spermatozoa.

Discussion

The results in this work revealed no significant increased (P>0.05) in the mouse epididymal weight, diameter of the epididymal tubules and thickness of epithelial cells layer among control and different treated groups of sildenafil citrate (SC) concentrations after different orally administration periods (one, three, five) weeks.

Sildenafil citrate has a strong systemic vasodilator effect that decreases systemic blood pressure. Subsequently, SC activity increases blood flow in the vasodilated arteries [12]. Therefore, SC was used in the treatment of coronary heart disease, hypertension and angina pectoris [13, 14].

The vascular wall is composed of three basic structural constituents: endothelium, muscular tissue and connective tissue [15]. In addition, structural plan of the blood vessel consist of tunica intima, tunica media and tunica adventitia [16]. On the other hand, the distinction between different types of vessels is often not clear-cut because the transition from one type of vessel to another is gradual [17]. Same reference [17] stated "as result of presence differences in the size and type blood vessels such as: vascular smooth muscle tissue is present in all vessels except capillaries, the elastic laminae of large arteries are stretched and in medium arteries the tunica media may contain up to 40 layers of smooth muscle cells (depending on the size of the vessel). Therefore, according to histological architecture of the epididymis may be not affected by SC treatment.

It was mentioned that prolonged use of SC may enhance fibrosis of the rat penile tissue and corpus cavernosum [18]. After use of SC, smooth muscle cell dilates temporarily and recovery to the normal physiological status. Subsequently, no real increased in the diameter or thickness of epididymal tissue.

The biochemical pathway for SC action was through Nitric oxide (NO)/ cyclic guanosine monophosphate (cGMP) pathway [19]. NO binds to soluble guanylate cyclase (sGC) and the conversion of guanosine triphosphate (GTP) to cyclic guanosine 3, 5-monophosphate (cGMP) will be facilitated. The increase in cGMP concentration results in diminished levels of intracellular calcium and alterations in protein phosphorylation, thereby causing relaxation of smooth muscle cells [20]. In rabbit, it was observed that selective PDE5 inhibitors influence (inhibit) the nerve-induced release of NO, probably via cGMP mediated negative feedback [21]. This action may be clarified why no significant differences in the epididymes seen after treatment with SC.

In other study, mouse sperm parameters enhanced when SC administered for short period and at low doses [22]. Therefore, SC has no effects on physiology and histology of mouse epididymis. From the results of the present study, SC administration to male mice has no effects on epididymis in mice.

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