

Histological study of the effect of sildenafil citrate on ovulation induction in mature female mice

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دراسة نسيجية لتأثير عقار سترات السلدينافيل على حث الإباضة في إناث الفئران الناضجة

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المستخلص

تهدف الدراسة الحالية إلى معرفة تأثير التجريع الفموي لعقار سترات السلدينافيل (sildenafil citrate) بمختلف التراكيز على بعض المعايير التكاثرية مثل الحث على الإباضة في إناث الفئران. تضمن العمل التجريبي، تقسيم (30) أنثى فأر سليمة بعمر (10-12) أسبوعاً ووزن (32-52) غراماً اعتماداً على الجرعة المختلفة من سترات السلدينافيل (0.5، 1 ملغم/كغم) وعلى طول فترة التجريع (6) يوماً. المعايير المقيمة تتضمن، دراسة التغيرات النسيجية في المبايض فيما يتعلق بقياس كلا من: الحويصلات المبيضية الابتدائية، الحويصلات المبيضية النامية، الحويصلات المبيضية الناضجة، والاجسام الصفراء. باستخدام صبغات (hematoxyline and eosin stains).

أظهرت نتائج الدراسة الحالية تحسن معنوي ($P < 0.05$) في المعايير التكاثرية المدروسة وبأستعمال كلتا الجرعتين (0.5، 1 ملغم/كغم) مقارنة مع مجموعة السيطرة فيما يتعلق بكل من (الحويصلات المبيضية الابتدائية، الحويصلات المبيضية النامية، الحويصلات المبيضية الناضجة، والاجسام الصفراء). كما أظهرت نتائج التجريع الفموي لإناث الفئران تحسن في المعايير المذكورة سلفاً بأستخدام الجرعة الأعلى (1 ملغم/كغم) من نفس العقار مقارنة بالجرعة الأدنى ومجموعة السيطرة. نستنتج من ذلك أن التجهيز مع السلدينافيل ربما يمتلك دور مهم على حث الإباضة في إناث الفئران.

Abstract

The aim of the present study is to investigate the effects of oral administration with different concentrations of sildenafil citrate (SC) on some of the reproductive parameters as ovulation induction in the female mice. In the experimental work, thirty of healthy female mice at the age (10-12) weeks with weight (23-25) gram were included in this study. They were divided in three groups (10 mature females' mice/group) including one group as control according to

different doses of sildenafil citrate (0.5 mg/kg, 1 mg/kg) throughout administration period for 6 days. Parameters were assessed involving study the histological changes in ovary as, number each of primary follicles, growing follicles, graafian follicles, evaluated using hematoxyline and eosin stains. The results of the present study showed a significant improvement ($P < 0.05$) in the reproductive parameters using both concentrations (0.5 and 1mg/ kg) of the sildenafil citrate when compared with the control group as; number each of primary follicles, growing follicles and grafian follicles. Moreover, high concentration of (1mg/kg), sildenafil citrate showed a significant enhancement in the reproductive parameters which were mentioned previously compare with low dose of sildenafil citrate (0.5mg/kg) and control group .Therefore, it was concluded that the sildenafil citrate supplementation may have important role on the ovulation induction in females mice.

Introduction

Ovulation is the apex of follicular development, which begins with the activation of primordial follicles and culminates with the release of a mature oocyte capable of being fertilized, and with the formation of a corpus luteum (1). Ovulation occurs when a mature egg is released from the ovary, pushed down the fallopian tube, and it is available to be fertilized. The lining of the uterus has thickened to prepare for a fertilized egg. If no conception occurs, the uterine lining as well as blood will be shed. The shedding of an unfertilized egg and the uterine wall is the time of menstruation. Ovulation is triggered by the pituitary LH surge, which initiates a series of ovarian events. The understanding of the ovulatory process is essential to elucidate some of the problems associated to female fertility as recurrent miscarriage, and to improve the technologies applied (2). Nitric oxide (NO) is a major paracrine mediator of various biological processes, including vascular functions and inflammation. Indeed, nitric oxide relaxes vascular smooth muscles through a pathway mediated by cyclic guanosine monophosphate (cGMP). Sildenafil citrate, a phosphodiesterase type 5 (PDE5) inhibitor, elevates the vasodilatory effects of NO by preventing the degradation of cGMP (3).

Nitric oxide has an important role in reproductive processes such as ovulation, implantation, and embryo development by the control of the ovarian and uterine blood flow (4), which play important role in promoting fertilization process by protect the ovum before and after fertilization against the damage by oxygen free radical because NO has been shown to regulate contractibility of the fallopian tube (5).

It is well known that FSH and LH stimulate the ovarian follicular development and ovulation (6). With more than one explanation, FSH rescues several primary follicles from a pool of these follicles in ovary in each cycle and stimulates maturation of these follicles, while LH stimulates final maturation of these follicles and causes rupture and ovulation (7). According to previous literatures, it was mentioned that the action of SC is beneficial for increasing of the number of the mature and fertilized oocytes (8). Expression of eNOS increase during the late secretory phase, whereas iNOS express during all menstruation cycle for non pregnant and during pregnancy period, that is agreed with (9). Therefore, the present study was designed to investigate the effects of different doses of SC on: numbers each of primary, secondary, grafian follicles, and corpus luteum.

Materials and methods

Experimental animals

In the present work, thirty mature female mice were divided into three groups included one group as control (each group include 10 mice), depending on different doses of sildenafil citrate namely low dose(0.5 mg/kg/day) and high dose (1mg/kg/day) for 6 days of treatment.

The groups were divided according to doses of sildenafil citrate and as the following:

1. Control group (G1): This group was orally administrated normal saline during (6 days).
2. Treated group (G2): This group was orally administrated (0.5mg/kg/day) of sildenafil citrate during the (6 days) period of the experiment.
3. Treated group (G3): This group was orally administrated (1mg/kg/day) of sildenafil citrate during the (6 days) period of the experiment.

Preparation and administration of sildenafil citrate solution

Sildenafil citrate solution was prepared by dissolving completely a crushed one tablet (50mg) in 10mL of normal saline to obtain different doses of sildenafil citrate (0.5mg/kg/day and 1mg/kg/day). Each dose administered for limited group of the female mice. All females in divided groups were orally route of administration was used for treatment with sildenafil citrate.

Histological study

Reproductive organs (ovaries) of freshly scarified mice were fixed with formalin 10% for 12hr (10), and dehydrated through progressive increasing concentrations of ethanol alcohol, then cleared with xylene for 30 minutes, then replaced by other paraffin over night in

oven. Sections were made from paraffin block (serial sections), then stained with alum haematoxylin and eosin stain. Slides were examined with light microscope using (4X). The parameter, number each of primary follicles, growing follicles, grafian follicle, and corpus luteum (11). Statistical analysis was performed using SPSS (Statistical Package for Social Science; Version 15.0). Crude data analysis was done using student's t-test so called paired sample t-test for tables with mean and standard error of mean (S.E.M.) to compare between pre-and post- treatment for all groups (12).

Results

The results of different doses of sildenafil citrate (SC) on the number of primary follicles are shown in the table (1). In this table, a highly significant differences ($P < 0.01$) in the number of primary follicle were assessed after 6 days of treatment with low (0.5 mg/kg) sildenafil citrate (SC) dose as compared to control group, also high significant increases ($P < 0.001$) in the number of primary follicle appeared with high (1mg/kg) SC dose after compared to control group. Moreover, significant elevation ($P < 0.05$) was noticed in the number of primary follicle between low and high dose for 6 days of treatment (Figures 1,2).

Table (1): Effects of orally administrated of different doses of sildenafil citrate (SC) on number of primary follicles female mice

Period of SC administration for 6 days	Control group treated with normal saline	Low dose group treated with (0.5mg/kg) of sildenafil	High dose group treated with (1mg/kg) of sildenafil
	2.666 ^A ±0.547	7.333 ^{Ba} ±1.201	8.662 ^{Bb} ±0.871

Values mean ± S.E.M.

Number of female mice per group = 10.

Similar capital letters means non-significant differences within groups compare to the control.

Different capital letters means significant differences within groups compare to the control.

Different small letters means significant differences between low and high doses within two groups.

Table (2) showed the results of the effect of sildenafil citrate (SC) administration on the number of growing follicle. After 6 days of administration with low dose (0.5 mg/kg), a high significant elevation ($P < 0.001$) were assessed in the number of growing follicle, and same results were noticed with high SC dose (1mg/kg) for same period of administration compared

to the control group. Moreover, significant increases ($P<0.05$) were showed when compared between low and high dose of SC for 6 days of treatment (Figures1, 3).

Table (2): Effects of orally administrated of different doses of sildenafil citrate (SC) on growing follicles of female mice

Period of SC administration for 6 days	Control group treated with normal saline	Low dose group treated with (0.5mg/kg) of sildenafil	High dose group treated with (1mg/kg) of sildenafil
	2.767 ^A ±0.780	7.767 ^{Ba} ±0.666	9.143 ^{Bb} ±0.243

Values mean ± S.E.M

Number of female mice per group = 10.

Similar capital letters means non-significant differences within groups compare to the control.

Different capital letters means significant differences within groups compare to the control.

Different small letters means significant differences between low and high doses within two groups.

A high significant increases ($P<0.001$) in the number of graafian follicles were assessed after 6 days treatment in treated groups with low dose (0.5mg/kg) and high dose (1mg/kg) of SC when compared to control group. As well as, a significant differences ($P<0.05$) were noticed between low and high dose of SC for 6 days of treatment (Figures1,4).

Table (3): Effects of orally administrated of different doses of sildenafil citrate (SC) on graafian follicles of female mice

Period of SC administration For 6 days	Control group treated with normal saline	Low dose group treated with (0.5mg/kg) of sildenafil	High dose group treated with (1mg/kg) of sildenafil
	2.485 ^A ±0.792	7.566 ^{Ba} ±0.425	8.630 ^{Bb} ±0.975

Values mean ± S.E.M

Number of female mice per group = 10.

Similar capital letters means non-significant differences within groups compare to the control.

Different capital letters means significant differences within groups compare to the control.

Different small letters means significant differences between low and high doses within two groups.

Table (4) showed the results of the effect of sildenafil citrate (SC) administration on number of corpora lutea. After 6 days of administration with low dose (0.5mg/kg), a significant elevation ($P<0.01$) were showed in the number of corpus lutea, and high significant increment ($P<0.001$) in the number of the corpus lutea was assessed with high dose (1mg/kg) of

sildenafil citrate (SC) as compared to control group. Moreover, significant increases ($P < 0.05$) were showed when compared between low and high doses of SC for 6 days of treatment (Figures 1,5).

Table (4): Effects of different doses of sildenafil citrate (SC) on the number of corpora lutea

Period of SC administration For 6 days	Control group treated with normal saline	Low dose group treated with (0.5mg/kg) of sildenafil	High dose group treated with (1mg/kg) of sildenafil
	4.910 ^A ± 0.013	8.900 ^{Ba} ± 0.500	10.801 ^{Bb} ± 0.450

Values mean ± S.E.M.

Number of female mice per group = 10.

Similar capital letters means non- significant differences within groups compare to the control.

Different capital letters means significant differences within groups compare to the control.

Different small letters means significant differences between low and high doses within two group

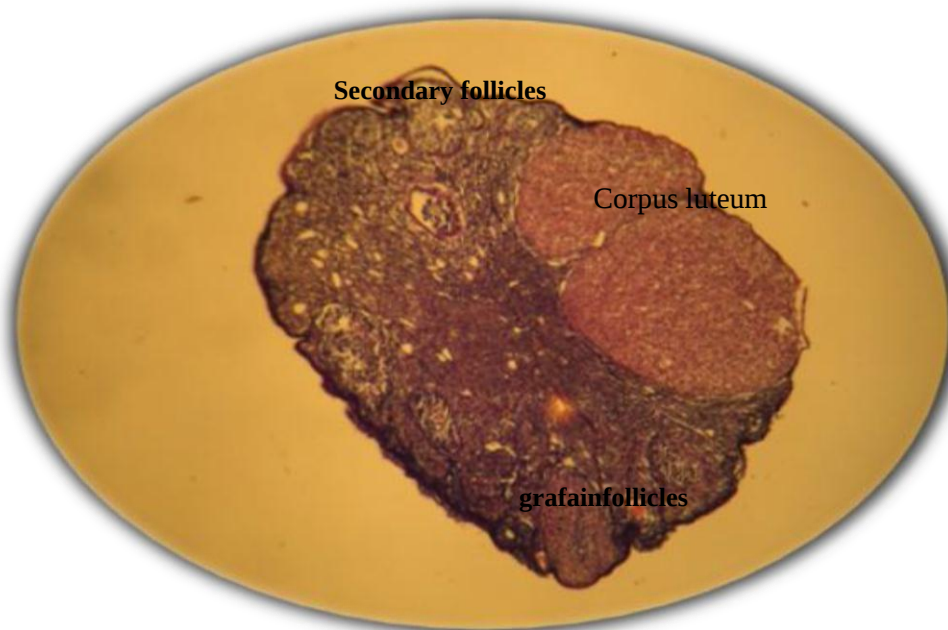


Figure (1): Ovarian sections of female mouse (control group) (H&E,4X)

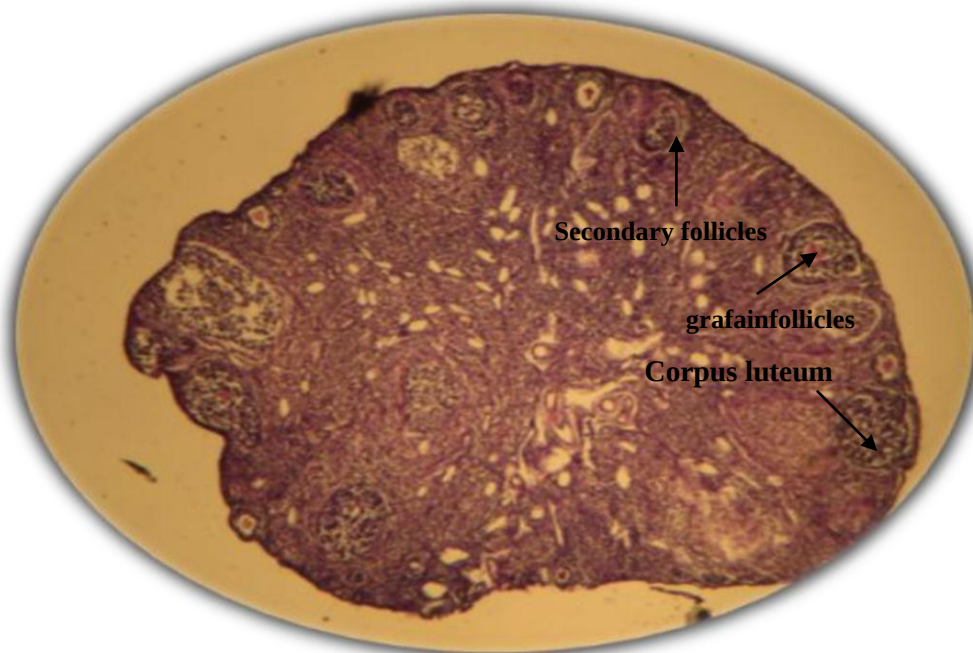


Figure (2): Ovarian sections of female mouse, at 6 days treatment, treated with (0.5mg/kg) SC, showing increasing in the numbers of primary, secondary, and grafainfollicles (H&E,4X)

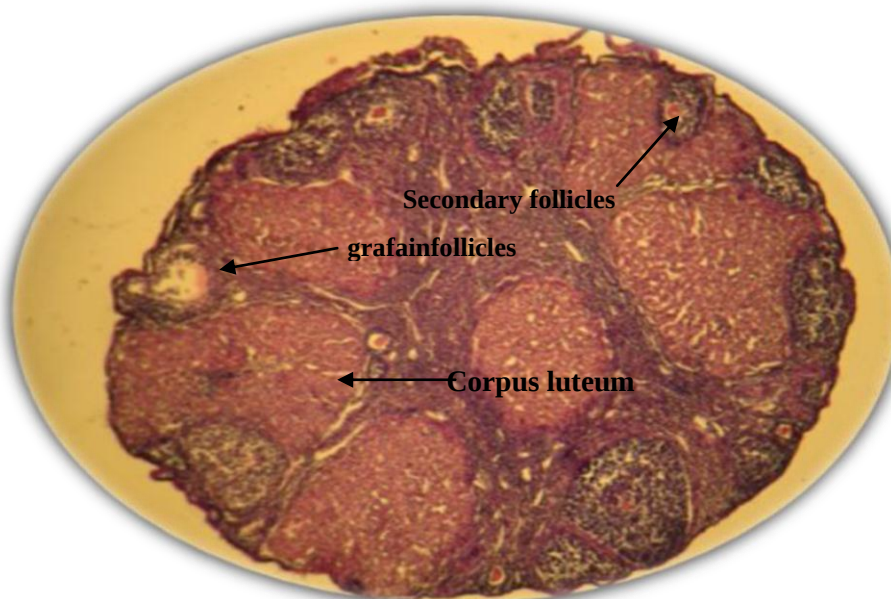


Figure (3): Ovarian sections of female mouse, at 6 days treatment, treated with (0.5mg/kg) SC, showing increasing in the numbers of primary, secondary, and grafainfollicles (H&E,4X)

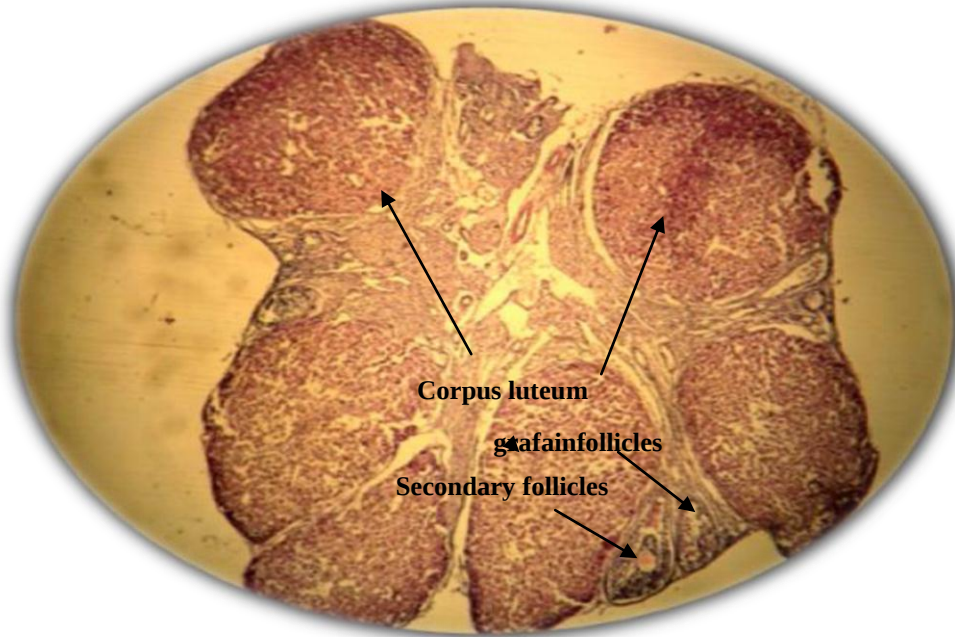


Figure (4):Ovarian sections of female mouse, at 6 days treatment, treated with (1mg/kg) SC, showing increasing in the numbers of primary, secondary ,grafian follicles, and corpus luteum (H&E,4X)

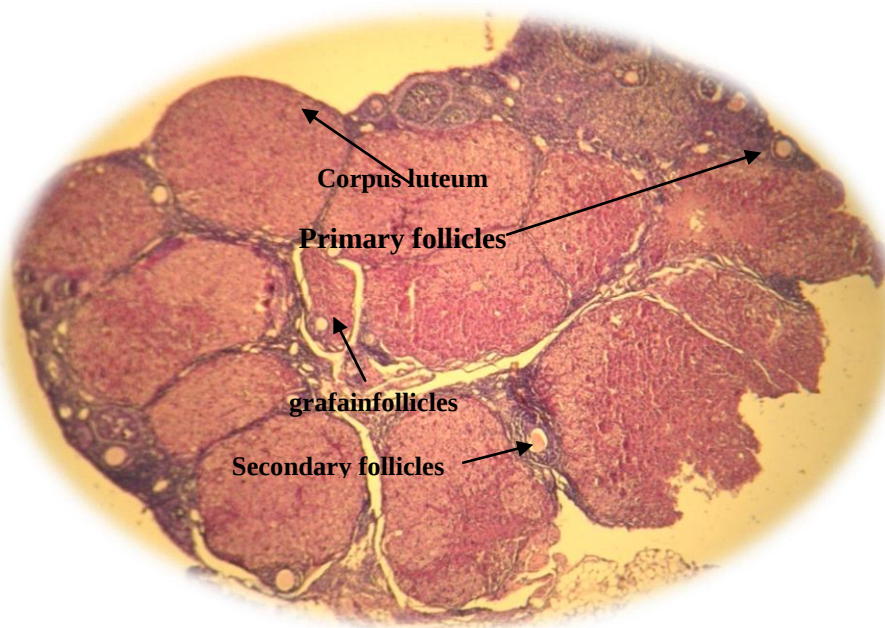


Figure (5):Ovarian sections of female mouse, at 6 days treatment, treated with (1mg/kg) SC, showing increasing in the numbers of primary, secondary ,grafian follicles, and corpus luteum (H&E,4X)

Discussion

Sildenafil citrate (SC) is the first useful therapy that used widely as oral treatment for patients with erectile dysfunction with low adverse effects (13), and recently used for treatment of several diseases such as pulmonaryhypertension (14).

Phosphodiesterase type -5 (PDE5) belong to an important family of proteins that regulate the intracellular levels of cyclic guanosin monophosphate (cGMP) (15). Phosphodiesterase type-5 is the primary enzyme with cGMP- hydrolyzing activation (16). Therefore, the molecular structure for sildenafil citrate is very similar to cGMP (17, 18). As well as, low quantities of nitric oxide (19), which play important role in promoting fertilization process by protect the ovum before and after fertilization against the damage by oxygen free radical because nitric oxide has been shown to regulate contractibility of the fallopian tube (5).

It is well known that FSH and LH stimulate the ovarian follicular development and ovulation (6). With more than one explanation, FSH rescues several primary follicles from a pool of these follicles in ovary in each cycle and stimulates maturation of these Follicles, while LH stimulates final maturation of these follicles and causes rupture and ovulation (7).

Shahpar, 2007 (20) observed that, nitric oxide by same mechanisms induces releasing of gonadotropin releasing hormone (GnRH) from the hypothalamus, thus stimulate the pituitary gland function to produce and release of follicular stimulating hormone (FSH) and luteinizing hormone (LH). According to previous literatures, it was mentioned that the action of SC is beneficial for increasing of the number of the mature and fertilized oocytes (21).Expression of nitric oxide increase during the late secretery phase, also during all menstruation cycle for non pregnant and during pregnancy period, that is agreement with (9).

The action of SC on the ovary is similar to that in uterus, by the same cGMP/PDE-5 pathway SC causing inhibition of PDE-5 to prevent cGMP damage by enhancing nitric oxide vasodilation action. Kazuo,2001 (22) who stated that SC improve ovarian blood flow in the vasodilated arteries leading to an increase blood and nutrients supply and then increase ovarian weight and growth of the corpus luteum. This result in agreement with Tamura, 2008 (23) who observed that corpus luteum blood flow is closely associated with luteal phase function and SC improve blood flow that elevating secretion levels of estrogen and progesterone from corpus luteum. The continued production of progesterone by the corpus luteum stimulates the proliferation and differentiation of stromal cells (24). Kukreja, 2003 (2)

investigated that role of nitric oxide in the control ovulation was regulated locally by ovarian cells, as well as nitric oxide generated within the ovarian vasculature. Subsequently, elevation nitric oxide levels that induced by SC treatment resulting in the increased production of both FSH and LH response to (GnRH) leading to increase numbers of primary, secondary ,and graafianfollicles and estrogen levels by acts of FSH (25,26). Also, stimulate the corpus luteum to increase progesterone secretion by LH, which maintain pregnancy (27).

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