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RESEARCH ARTICLE

THE POSSIBLE PROTECTIVE EFFECT OF SILDENAFIL ON COLCHICINE INDUCED INFERTILITY IN RATS

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Abstract

Sildenafil citrate is a potent and selective inhibitor of cGMP-specific phosphodiesterase type 5 enzymes (PDE5I).it is one of the most common drugs used to treat erectile dysfunction in men. It catalyzes the breakdown of cGMP, one of the primary factors involved in smooth muscle relaxation. A complex cross-talk phenomenon between the cAMP- and the cGMP-generating systems regulating the sperm function occurs in human spermatozoa. This study was thus designed to investigate the role of sildenafil in a model of colchicine induced infertility. Three groups of rats were employed in this study; group 1 served as control.In group 2 rats were treated with colchicine 25 mg/kg/days subcutaneous (s.c.) once daily for 25 successive days. Group 3 (Sildenafil citrate- treated group) was treated as group II and this was done coincidently with oral treatment with sildenafil citrate (8mg/kg) by oral gavage daily orally for the same 25 days. Testicular blood flow (TBF) was estimated by flowmeter. Seminal parameters (progressive motility, sperm cell concentration, epididymal sperm abnormal forms) were estimated. Male sexual behaviour was also evaluated (mounting, intromission, ejaculation, mount and intromission latencies, mount and intromission frequencies, ejaculatory latency, ejaculation frequency and intercopulatery interval).Testicular tissue specimens were histopatholgically examined by hematoxylin & eosin staining. Colchicine administration for 25 days resulted in marked impairment of TBF & seminal parameters and sexual behaviour .All these factors were significantly improved by sildenafil. These results clearly demonstrate the pivotal role of sildenafil in colchicine induced infertility.

INTRODUCTION

Sildenafil citrate inhibits PDE5 , which catalyzes the breakdown of cGMP, one of the primary factors involved in smooth muscle relaxation. Sildenafil induces the upregulation of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS), which generate NO **(Salloum et al.,2003)**.

Sildenafil is used to treat erectile dysfunction in men **(Boolell et al .,1996).** Several studies have shown that sildenafil also offers benefits in pulmonary arterial hypertension and congestive heart failure **(Bocchi et al.,2002).**

A complex cross-talk phenomenon between the cAMP- and the cGMP - generating systems regulating the sperm function occurs in human spermatozoa **(Sofikitis et al ., 1993).** It has been reported that guanylyl cyclase - activating substances (in particular atrial natriuretic peptide and nitric oxide) strongly affect positively sperm motility , capacitation, and acrosomal reactivity. These substances stimulate sperm metabolism and promote the sperm capacity to approach the oocyte, interact with it, and finally fertilize it **(Revelli et al ., 2002).**

As far we know, the effect of sildenafil citrate on the testicular vascular bed was not yet studied . In this study, we have chosen to test the effect of sildenafil (as a potentiator for the effects of cGMP second messenger system) on testicular blood supply and spermatogenesis in a model of colchicine induced infertility. Additionally, the effect of sildenafil on sexual desire and behavior , rather than its well-known erectile benefits, was verified.

1. MATERIALS AND METHODS:

2.1.Drugs and chemicals:

Sildenafil citrate powder (Viagra® , Pfizer) was dissolved (3.5 mg/ml) in water in water . Estradiol benzoate (Folone) (Misr pharmaceutical) was available in one ml ampoule containing 5 mg estradiol benzoate in oily solution. Progesterone caproate (Cidolut-Depot)17 α-hydroxy progesterone-17caproate (CID laboratories Giza-A.R.E under license of SHERING A.G.BERLIN.) was provided as one ml ampoule containing 250 mg progesterone caproate in oily solution.Colchicine was obtained from El-Nasr pharmaceuticals Chemicals Co. ADWIC.It is available as white powder (1g) in dark bottles.

2.2. Animals:

18 Adult male Sprague – Dawley rats previously tested for sexual activity [from the experimental animal breeding farm Helwan ,Cairo], each weighting 120- 150 gm were used. They have acclimatized for one week and were caged (6 / cage) in fully ventilated room and at room temperature at pharmacology department, Benha faculty of medicine. Rats were allowed to free access to water and diet containing cereals and bread. 10 adult female Sprague- Dawley rats were used for evaluation of male sexual behavior.

2.3.Experimental groups:

Rats were divided into 3 groups .Group I : (Control group) (n=6) was given saline p.o. and injected with 0.1ml saline subcutaneously (s.c.) once daily fore 25 successive days. Group II (Colchicine induced infertility group) (n=6) was injected with colchicine 25 mg/kg/day s.c. once daily for 25 successive days **(Almiron and Chemes ,1988).** Group III (Sildenafil citrate- treated group) (n=6) was treated as group II and this was done coincidently with oral treatment with sildenafil citrate (8 mg/kg wt) by oral gavage **(Almiron and Chemes , 1988)** daily orally for the same 25days.

2.3. Evaluation of male sexual behavior:

24 hours before sacrificing the animals, male copulatory behavior was evaluated . The female rats were made highly receptive to encourage and seduce the male partner by estradiol injections (25 ug s.c. in sesame oil) 52 hours before the time of the test and progesterone (1 mg s.c. in sesame oil) 4 hours before the time of the test for sexual behavior. Females were screened with sexually experienced males ,only those exhibiting good receptivity by showing lordosis behavior in response to mounting and no rejection behavior were used **(Agmo and Paredes. 1988;Clark et al.,1988).** All experiments were performed between 11.0 AM and 3.0 PM in a sound attenuated air conditioned room . Male rats were transferred singly to a glass observation cage into which after 3 minutes adaptation period, a receptive female was introduced. Male copulatory behavior was evaluated according to **(Giuliani and Ferrari ,1996).**

The parameters considered were:

2.3.1. Mounting : Clasping the flanks of the female and performing pelvic thrusts **(Meyerson et al., 1988)**.

2.3.2. Intromission : a mount ending with vigorous backward lunge **(Meyerson et al., 1988)**.

2.3.3 Ejaculation : Prolonged mount with intense clasping of female followed by a slow dismount and genital grooming **(Meyerson et al., 1988)**.

2.3.4. Mount and intromission latencies (ML and IL) : The time from introduction of the female until the first mount and intromission respectively ;were calculated from the beginning of the test and expressed in minutes (**Meyerson et al., 1988)**.

2.3.5. Mount and intromission frequencies (MF and IF) : The number of mounts and intromissions preceding the first ejaculation respectively**(Meyerson et al., 1988)**.

2.3.6. Ejaculatory latency(EL): The interval between the first intromission and ejaculation **(Meyerson et al., 1988).**

2.3.7 Ejaculation frequency (EF): Number of ejaculations in asession **(Senbel and Mostafa, 2008)**.

2.3.8. Intercopulatery interval (ICI): Average interval between successive intromissions **(EL/IF) (Senbel and Mostafa,2008)**.

The tests were discontinued when IL more than 15 minutes or EL more than 20 minutes.

2.4. Testicular blood flow assessment:

One hour after the last oral dose of sildenafil citrate , the animals were lightly anesthetized with ether . Midline incision was made in the scrotal skin and subcutaneous tissue to expose the testes and testicular arteries . The flow probe of the flow meter **(Hadeco ES 1000 SPM, Japan)** was placed on top of the right testicular artery for measurement of testicular blood flow **.** Testicular blood flow was also measured in control animals **(Damber et al., 1982)**.

2.5. Seminal parameters:

The epididymal content of each rat was obtained after cutting the tail of epididymis and squeezing it gently in sterile clean watch glass and examined using the following parameters:

2.5.1. Progressive motility:

The progressive motility of sperms was examined according to the method reported by **Bearden and Fluquary (1980),** where a small droplet of semen was added to one drop of sodium citrate solution 2.9% on a warm slide . Several fields were examined and the incidences of progressively motile sperms were estimated and recorded.

2.5.2 Sperm cell concentration:

This was performed according to the technique adopted by **Bearden and Fluquary(1980) ,** using pipette of haemocytometer . The undiluted semen was withdrawn up to the mark 0.1 and the pipette was then filled up to the mark 101 by normal saline stained with eosin .the contents of the pipette were mixed by holding the end of the pipette with thumb and index fingers and shaking it vigorously. The cover slide was placed over the counting chamber and a drop of diluted semen was spread between the haemocytometer slide and its cover . the sperms in 5 large squares (contain 80 small squares) were counted using the high power objective lens .The sperm cell concentration in cubic milliliter was estimated by multiplying the counted number of sperms by 10 (depth) and 1000 (dilution).

2.5.3. Epididymal sperm abnormal forms:

A drop from epididymal content of each rat was mixed with an equal drop of eosin-nigrosin stain .The semen was carefully mixed with the stain , then films were spread on clean slides . A hundred sperms were examined at random per slide under high power objective lens and percentage of abnormal sperms was recorded.

2.6. Histological techniques :

Directly after sacrificing the animals the testes were fixed in Bouin's fluid **(Bouin ,1897)**, routinely processed an embedded in paraffin wax sectioned at 5 microns , cut and stained with haematoxilin and eosin stain **(Harris ,1900)** , and were evaluated histologically to obtain final status of spermatogenesis.

2.7. Statistical Analysis:

All data were expressed as Mean + SEM. Difference between the groups were compared by student T-test with P- value < 0.05 selected as the level of statistical significance **(Hill, 1971)**. SPSS version 16 was used for statistical analysis.

2. RESULTS:

2.1. Sperm parameters changes:

3.1.1 Sperm count changes:

In non-treated infertile rats, there was significant decrease ($p<0.05$) in sperm count compared to control group. In Sildenafil treated group, there was significant increase ($p<0.05$)in sperm count compared to infertile group but it was still at significant lower level ($p<0.05$) if compared to control group.

3.1.2. Sperm viability changes:

In non-treated infertile rats, there was significant decrease ($p<0.05$) in sperm viability compared to control group. In Sildenafil treated group, there was significant increase ($p<0.05$) in sperm viability compared to infertile group and it was at non-significant lower level ($p>0.05$) if compared to control group.

3.1.2 Sperm motility changes:

In non-treated infertile rats, there was significant decrease ($p<0.05$) in sperm motility compared to control group. In Sildenafil treated group, there was significant increase ($p<0.05$) in sperm motility compared to infertile group and it was at non-significant higher level ($p>0.05$) if compared to control group.

3.1.4. Sperm abnormal forms changes:

In non-treated infertile rats, there was significant increase ($p<0.05$) in sperm abnormal forms compared to control group. In Sildenafil treated group, there was non-significant increase ($p>0.05$) in sperm abnormal forms compared to infertile group and it was at significant higher level ($p<0.05$) if compared to control group.

3.2.Testicular blood flow changes:

In non-treated infertile rats, there was non-significant decrease ($p>0.05$) in testicular blood flow compared to control group. In Sildenafil treated group, there was significant increase ($p<0.05$) in testicular blood flow compared to infertile group and it was also at significant higher level ($p<0.05$) when compared to control group

3.3. Sexual behavior changes:

In non-treated infertile rats, there was not any significant changes ($p>0.05$) in ML, MF, IL

Table (1): Effect of treatment with sildenafil (8 mg/kg p.o.) for 25 days on sperm parameters and testicular blood flow in experimentally induced male infertility by colchicine (25mg/kg s.c.) for the same 25 days.

Groups	Parameters				
	Sperm count Millions/ Mm^3	Sperm Viability %	Sperm motility %	Sperm abnormal forms %	Testicular blood flow Ml/min
I	79.60 ±2.25	85.00±2.94	78.5 ±3.25	12.5 ±0.92	4.2 ± 0.23
II	52.4 [a] ±2.43 (*↓34%)	65.5 [a] ±1.60 *↓23%	56.00 [a] ±2.59 *↓29%	17.16 [a]±1.24 *↑37%	3.8 ±0.18 *↓10%
III	64[a,b] ±1.58 *↓20% **↑22%	77.00 [b] ±1.70 *↓9% **↑18%	70.00 [b] ±2.23 *↓11%**↑25%	21[a] ±1.41 *↑68% **↑22%	7.60[a,b] ±0.47 *↑81% **>100%

a: Significant difference versus control at $p<0.05$

b: Significant difference versus chronic male infertile group at $p<0.05$

N.B: % change is calculated in relation to control group (*) and chronic male infertile group(**).

Table (2): Effect of treatment with sildenafil (8 mg/kg p.o) for 25 days on mount latency (ML) , mount frequency (MF) , intromission latency (IL) and Intromission frequency (IF) in experimentally induced male infertility by colchicine (25mg/kg s.c.) for the same 25 day.

Groups	Parameters Mount latency (ML) Minutes	Mount frequency (MF)	Intromission latency (IL) Minutes	Intromission frequency (IF)
I	1.5 ±0.08	6.50 ±0.42	3.07 ±0.1	14.33 ±0.88
II	1.8 ±0.14 *↑20%	8 ±0.57 *↑23%	3.34 ±0.08 *↑9%	15.5 ±0.56 *↑8%
III	$0.99^{a,b}$±0.04 *↓34% **↓45%	$4.5^{a,b}$±0.42 *↓31% **↓44%	$1.88^{a,b}$±0.07 *↓39%**↓44%	$8^{a,b}$±0.57 *↓44% **↓48%

a: Significant difference versus control at p<0.05
b: Significant difference versus chronic male infertile group at p<0.05
N.B: % change is calculated in relation to control group (*) and chronic male infertile group (**).

Table (3): Effect of treatment with sildenafil (8 mg/kg p.o.) for 25 days on ejaculation latency(EL) , ejaculation frequency (EF) , post- ejaculatory interval (PEI) and inter- copulatory interval (ICI) in experimentally induced male infertility by colchicine (25mg/kg s.c.) for the same 25 days.

Groups	Parameters Ejaculation Latency EL Minutes	Ejaculation frequency EF	Post- ejaculatory interval PEI Minutes	Inter- copulatory interval ICI Minutes
I	15.12 ±0.73	1±0	15.45±0.55	1.060±0.019
II	15.53 ±0.67 *↑3%	1±0 *0%	17.41±1.12 *↑13%	1.000^{a}±0.017 *↓6%
III	$6.58^{a,b}$±0.59 *↓56% **↓58%	$1.83^{a,b}$±0.16 *↑83% **↑83%	$10.37^{a,b}$±0.24 *↓33% **↓40%	$0.818^{a,b}$±0.020 *↓23% **↓18%

a: Significant difference versus control at p<0.05
b: Significant difference versus chronic male infertile group at p<0.05
N.B: % change is calculated in relation to control group (*) and chronic male infertile group(**).

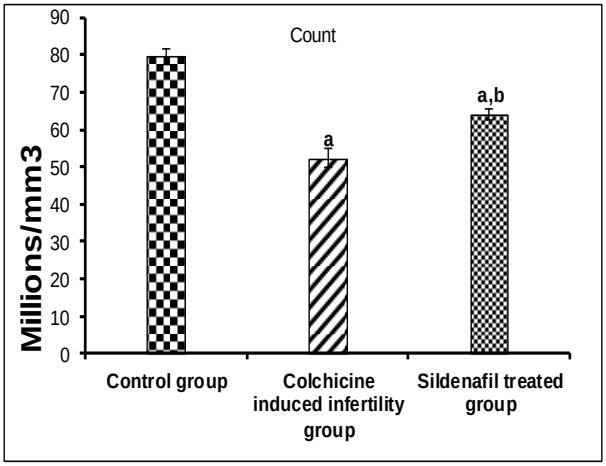

Figure (1): Effect of treatment with sildenafil (8 mg/kg p.o.) for 25 days on sperm count in experimentally induced chronic male infertility by colchicine (25 mg/kg s.c.) for the same 25 days.

Data are presented as mean ± SEM .

a: Significant difference versus control group at $p<0.05$.

b: Significant difference versus chronic infertile group at $p<0.05$.

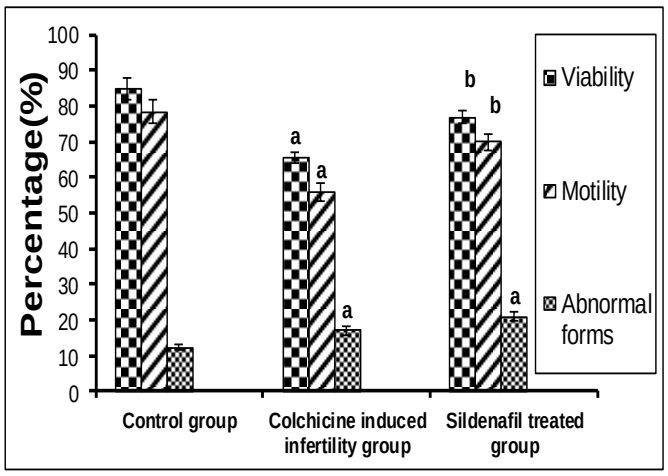

Figure (2): Effect of treatment with sildenafil (8 mg/kg p.o.) for 25 days on sperm motility, viability and abnormal forms in experimentally induced chronic male infertility by colchicine (25 mg/kg s.c.) for the same 25 days. .

Data are presented as mean ± SEM .

a: Significant difference versus control group at $p<0.05$.

b: Significant difference versus chronic infertile group at $p<0.05$.

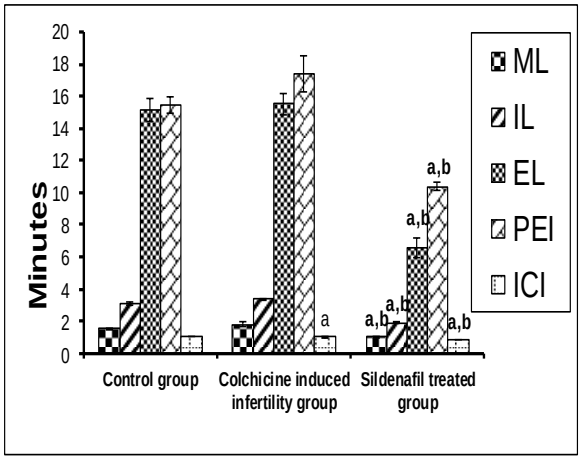

Figure (4): Effect of treatment with sildenafil (8 mg/kg p.o.) for 25 days on mount latency (ML) , intromission latency (IL) , ejaculation latency (EL) , post ejaculatory interval (PEI) and intercopulatory interval (ICI) in experimentally induced chronic male infertility by colchicine (25 mg/kg s.c.) for the same 25 days.

Data are presented as mean ± SEM .
a: Significant difference versus control group at $p<0.05$.
b: Significant difference versus chronic infertile group at $p<0.05$.

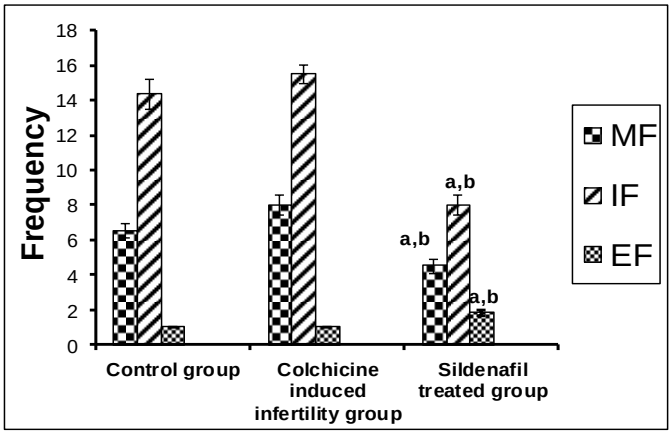

Figure (5): Effect of treatment with sildenafil (8 mg/kg p.o.) for 25 days on mount frequency (MF) , intromission frequency (IF) and ejaculation frequency (EF) in experimentally induced chronic male infertility by colchicine (25 mg/kg s.c.) for the same 25 days.

Data are presented as mean ± SEM .
a: Significant difference versus control group at $p<0.05$.
b: Significant difference versus chronic infertile group at $p<0.05$.

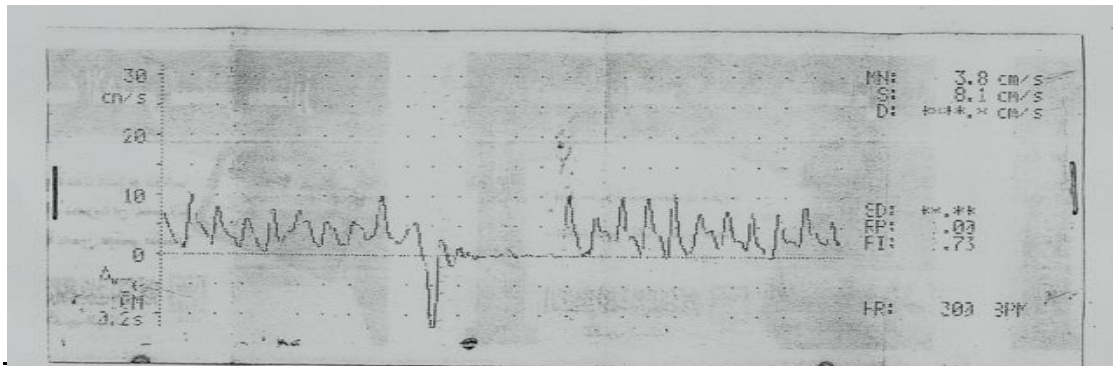

Figure (6): Doppler ultrasound record of testicular blood flow in normal control group.

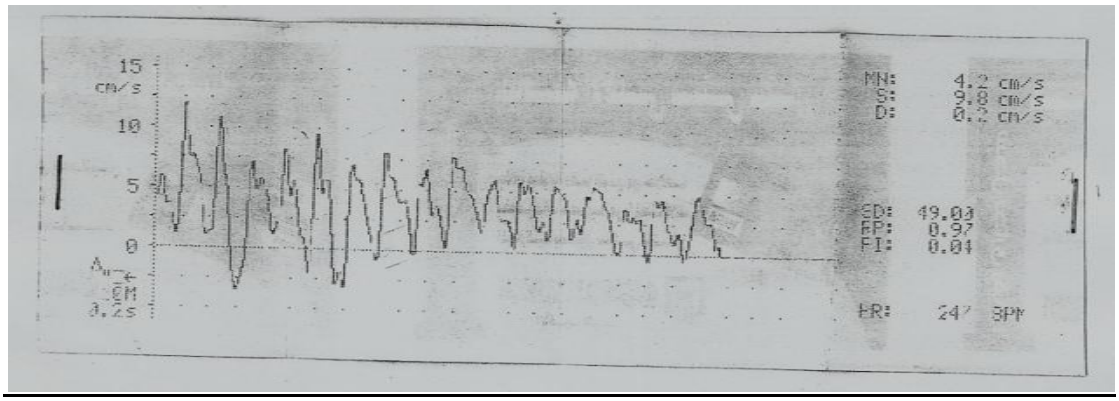

Figure (7): Doppler ultrasound record of testicular blood flow in colchicine induced infertility group.

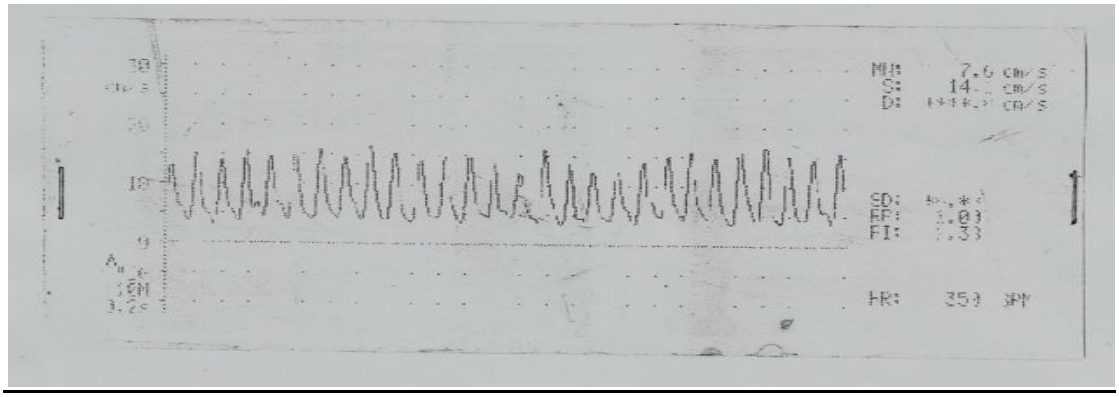

Figure (8): Doppler ultrasound record of testicular blood flow in sildenafil treated group

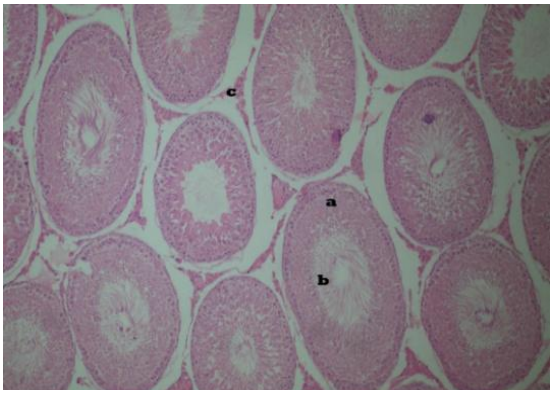

Figure (9): A photomicrograph of a cut section in the testis of a control rat (group I)
showing normal histological structure of seminiferous tubules :(a) lining with normal layers of germinal cells (b) lumen filled with sperms and spermatids (c)normal spaces between seminiferous tubules with normal stroma. (H&Ex20).

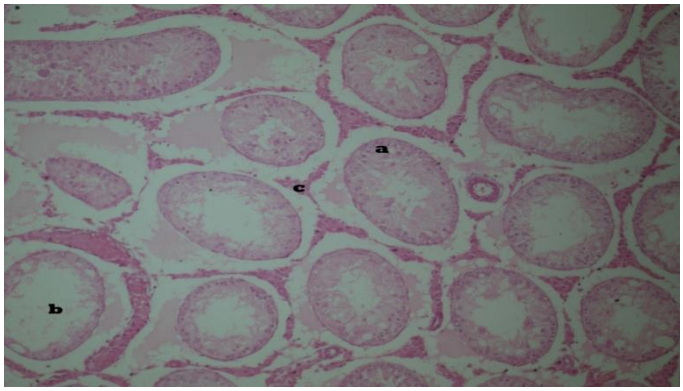

Figure (10): A photomicrograph of a cut section in the testis of a rat with colchicine induced infertility (group II) showing :(a) moderate spermatogenic arrest (b) decreased in number of sperms and spermatids in seminiferous tubules (c) normal spaces between seminiferous tubules with normal stroma. (H&Ex20).

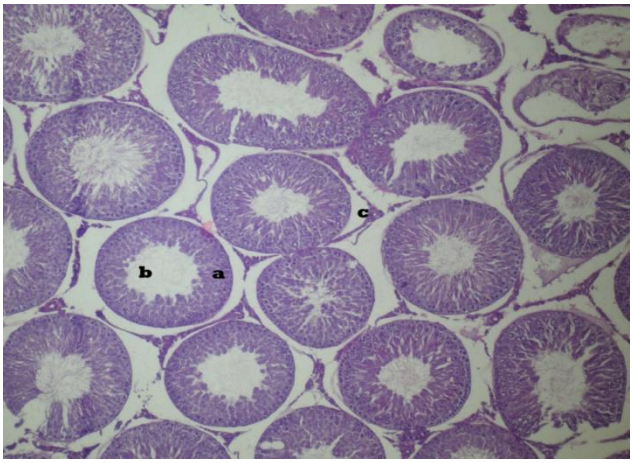

Figure (11): A photomicrograph of a cut section in the testis of a sildenafil treated rat (group I) showing certain improvement of histological findings :(a) increased growing of spermatogenic cells (b) many spermatozoa in seminiferous tubules (c) normal spaces between seminiferous tubules with normal stroma. (H&Ex20).

, IF ,IL ,EL ,EF or PEI when compared to control group but there was significant decrease ($p<0.05$) in ICI.

In sildenafil treated group, there was significant decrease ($p<0.05$) in ML, MF, IL, IF, PEI, EL and ICI compared to infertile and control group . On the other hand there was significant increase ($p<0.05$) in EF when compared to both control and infertile groups.

3.4.Histopathological examination:

3.4.1.Control group : showed normal histological structure of seminiferous tubules lined with normal layers of germinal cells and sertoli cells .Many sperms and spermatids were seen in the lumen of seminiferous tubules. The spaces between seminiferous tubules were normal without clubbing or other degenerative changes in interstitial Leydig cells

3.4.2.Infertile group : Showed moderate degenerative changes in some spermatogenic cells and necrosis of some seminiferous tubules. Moderate spermatogenic arrest at secondary spermatocyte level was observed . Also sertoli cells showed degeneration with significant decrease in the number of spermatids and sperms inside the seminiferous tubules

3.4.3.Sildenafil treated group: showed improvement of testicular histology compared to group II and nearly normal germinal epithelial layers with preservation of sertoli cells .There was also increased growing of spermatogenic cells with many spermatozoa in the lumen of seminiferous tubules

4.Discussion :

A complex cross-talk phenomenon between the cAMP- and the cGMP - generating systems regulating the sperm function occurs in human spermatozoa **(Sofikitis et al ., 1993).** It has been reported that guanylyl cyclase - activating substances (in particular atrial natriuretic peptide and nitric oxide) strongly affect positively sperm motility , capacitation, and acrosomal reactivity. These substances stimulate sperm metabolism and promote the sperm capacity to approach the oocyte, interact with it, and finally fertilize it **(Revelli et al ., 2002).** In this study, we have chosen to test the effect of sildenafil (as a potentiator for the effects of cGMP second messenger system) on testicular blood supply and spermatogenesis in a model of colchicine induced infertility. Additionally, the effect of sildenafil on sexual desire and behavior , rather than its well-known erectile benefits, was verified

Microtubules are ubiquitous cytoskeletal elements, with numerous structural and functional roles, including nuclear division, movement, cell shape, and secretion **(Dustin, 1984).** They are polymers of the core protein tubulin, a 100 kDa a-b heterodimer, with attached microtubule associated proteins **(Dustin, 1984).** Sertoli cells produce a nurturing environment for germ cells within the seminiferous epithelium by forming inter Sertoli cell junctions, known as the blood-testis barrier. This special environment is maintained structurally by microtubules that are highly concentrated in radially oriented cytoplasmic trunks of the Sertoli cells. Exposure to microtubule disrupters which are known testicular toxicants (as colchicine) consistently results in impaired Sertoli cell function and germ cell loss, indicating that Sertoli cell microtubules are critical to normal testicular homeostasis **(Boekelheide et al., 2003).**

Colchicine , a well described microtubule disrupter , causes testicular damage in rats ,including slouphing ,similar to testicular damage caused by carbendazim (another microtubule disruptor used as a broad spectrum fungicide) **(Russel et al ., 1981; Allard et al ., 1993) .**

The induction of infertility in male rats by colchicine (25 mg /kg /day s.c. injection) for 25 days resulted in significant decrease in sperm count, motility, viability and increase in sperm abnormal forms . There were significant histological changes in the testicular tissue . On the other hand , it did not affect sexual behavior or testicular blood flow in the studied rat groups .

The obtained effects were in line with previous studies on the effect of colchicine on seminal parameters **Ben-Chetrit et al .(1993) and Haimov-Kochman and Ben-Chetrit .(1998)** who concluded that colchicine decreases sperm count and motility respectively .

These effects were also in line with **Handel .(1979)**who concluded that colchicine increases sperm abnormal forms.

The administration of colchicine in rats did not affect sexual behavior and this result may be attributed to negligible passage of colchicine through the blood brain barrier that was proved by **Steven et al. (1989).**

Oral administration of sildenafil caused significant increase in sperm count , motility and viability. At the same time it caused non-significant increase in abnormal forms compared to colchicine group. These results agreed with **Alp et al.(2012)** who concluded that sildenafil has positive effects on spermatogenesis and semen production and quality, and it increases fertility and that sidenafil prevents the sperm head defects induced by streptomycin (STR) and can decrease the testicular toxicity induced by isoniazid (INH).

A positive effect of sildenafil on sperm kinematics was proven in a prospective double-blind, placebo-controlled, crossover, two-period-administration, clinical investing-ation by **Du Plessis et al. (2004)**.

In addition ,a concentration-dependent stimulatory effect of sildenafil on sperm motility was also demonstrated by **Mostafa. (2007)** when 85 semen specimens from asthenozoospermic patients were exposed to different five concentrations of sildenafil (4.0 mg/mL, 2.0 mg/mL,1.0 mg/mL, 0.5 mg/mL, 0.1 mg/mL). However, the evaluation of sperm motility in this study was only 3 hours after the spermatozoa exposure to the medicine.

Cyclic AMP appears to be involved in the signaling pathways that regulate sperm motility **(Visconti et al .,1995; Leclerc et al ., 1996)** .In fact increased levels of intracyt-osolic cAMP have been demonstrated to enhance sperm motility and viability **(Zhung and Zheng,1996; Lewis et al ., 1996)** by a) increasing the rate of glycolysis and fructolysis and b) enhancing the oxidation of lactate or pyruvate to CO2 **(Rees et al .,1990)**.

Yanagimachi .(1994) reported that the asymmetrical, high amplitude beats of the sperm flagellum (referred to as "hyperact-ivated motility") and the capacitation process are dependent on the intracellular cAMP levels. These findings were consistent with those of **MacLeod et al. (1991)** who demonstrated that the majority of the cAMP-dependent protein kinases in the rat spermatozoa are located within the flagellum. cGMP may also cross-activate cAMP pathways by binding to cAMP-binding sites on cAMP receptors such as cAMP-dependent protein kinases (PKA) **(Jiang et al .,1992).**

Lefièvre et al.(2000) investigated whether PDE5 is present in human spermatozoa and whether sildenafil affects sperm function. In semen samples incubated with increasing concentrations of sildenafil, the authors noted a dose dependent increase in intracellular cAMP levels The authors have suggested that sildenafil might act on PDEs other than type 5. They have also suggested that sildenafil in high concentrations as high as 30, 100 and 200 μmol/L acts no longer as type-5 specific and probably partially inhibits other PDEs present in spermatozoa such as PDE1 and PDE4 which have high affinity for cAMP .

Cuadra et al .(2000) conducted a similar study on human semen samples and concluded that cGMP directly opens cyclic nucleotide-gated channels for calcium entry into the spermatozoa, initiating the acrosome reaction. In the same way cGMP regulates calcium entry into microdomains along the sperm flagellum affecting sperm motility. since PDE5 hydrolyzes cGMP, inhibition of PDE5 by sildenafil citrate enhances the effects of cGMP on sperm motility and sperm acrosome reaction. The data provided by the authors suggests a dual mechanism for PDE5 inhibition with a stimulatory effect on sperm motility when PDE5 is moderately inhibited; however, extensive inhibition of PDE5 leads to decreased sperm motility.

The increase in sperm count reported in our study with sildenafil administration was consistent with results obtained by **Alp et al.(2012)** who reported the positive effect of sildenafil on sperm concentration. The authors showed that sildenafil administration in rats caused elevation in serum FSH and testosterone levels and supposed that this may be the cause of improvement in sperm parameters by sildenafil administration . Furthermore ,The conversion of spermatogonia to spermatocytes and the conversion of spermatocytes to round spermatids depend on the synergistic action of both FSH and testosterone. However, the effect of FSH is greatest on the conversion of spermatocytes to spermatids, i.e. meiosis **(Sun et al .,1990).**

In a previous study, protective effects of the sildenafil administration on testicular torsion/detorsion damage in rats were investigated and it was found that apoptosis was significantly reduced with sildenafil treatment in testicular torsion/detorsion **(Beheshtian et al ., 2008)**.This may be a possible mechanism by which prolonged sildenafil treatment in oligozoospermic rats caused preservation of testicular spermatogenic and Sertoli cells and increased sperm viability.

Sildenafil may exert a positive influence on spermiogenic epithelium by enhancing testicular blood flow that was observed in our work. In addition , the drug induced inhibition of phosphodiesterase in Leydig and Sertoli cells might increase their responsiveness to gonadotropin stimulation .In this way ,the local production of testosterone by

leydig cells and androgen binding protein by sertoli cells ,both known to be involved in the maintenance of normal spermatogenesis , might be increased.

In contrast to our work **Khalaf et al . (2012)** concluded that tadalafil(the long acting PDE 5 inhibitor) cuased negative effects on sperm parameters and testicular histology when administered for three months and six months (1.8 mg /kg/day). Sperm count showed a significant time-dependent decrease. Sperm motility decreased significantly in both durations with higher effect in six months duration. The incidence of abnormal forms increased in both durations. Histological examination revealed mild and moderate changes in three and six months durations respectively in the form of loosely packed connective stroma around seminiferous tubules, reduction in number of spermatogenic cells with sloughing of many spermatocytes within the lumen of some tubules. The researchers concluded that chronic daily use of tadalafil produces detrimental effects on the structure and function of the testes of old male albino rats which are duration dependent .That difference between our results and these results may be attributed to the long duration of action of tadalafil and the longer duration of treatment . Also their experiment was done on normal aged rats .

In Sildenafil treated group, there was significant decrease in mount latency (ML), mount frequency (MF), intromission latency (IL), ejaculation latency (EL) , ejaculation frequency (EF), post ejaculatory interval (PEI) and intercopulatory interval (ICI) compared to group I and group II.

The obtained results are in line with those by **Senbel and Mostafa . (2008)** who reported that sildenafil succeeded to significantly reduce IF , IL and EL and also significantly reduced ML ,IL and EL and increased number of ejaculations per session especially when combined with yohimbine .

Guiliani et al .(1993) reported that sildenafil acts not only peripherally but also centrally since oral administration of sildenafil (1mg kg $^{1-}$) in rats modified both sexual and ejaculatory mechanisms of copulation . However ,it was demonstrated that sildenafil did not improve sexual function in men without erectile dysfunction and it did not induce erection in young healthy men **(Gemalmaz et al ., 2001)** .

The moderate results of sildenafil on matting parameters in our work are consistent with the low expression of PDE5 in the brain , as the most widely expressed PDE isozymes in the brain are PDE1 and PDE2, which are related to memory **(Baevo ,1995)** .Some investigators reported that sildenafil enhanced object recognition memory and that vardenafil increased cGMP concentrations in neural fibers of the hippocampal region in rats **(Prickaerts et al .,2002)** .

In addition, the inhibition of PDE activity during prolonged sildenafil treatment increased serum testosterone level and Leydig cells' steroidogenic capacity by coordinated stimulatory action on cAMP and cGMP signaling pathway **(Andric et al .,2010).** That may explain the enhancement of sexual mating parameters in our work.

From previous data one may assume that sildenafil has positive effects on spermatogenesis and semen production and quality , and it increases fertility .These effects of sildenafil are attributed to its vasodilator effects (positive effects on renal and testicular blood flow) of NO-cGMP signal pathway . We also confirmed the positive effects of sildenafil on sexual desire and behavior.

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