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EFFECT OF VARDENAFIL ON FERTILITY IN MALE RATS

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ABSTRACT
It was found that only vardenafil markedly reduced $CaCl_2$-induced contractions in the phenylephrine-treated isolated pulmonary artery indicates that this additional mechanism could be related to blockade of Ca^{2+} channels. The aim of the present study is to evaluate effects of vardinafil on fertility parameters. Thirty male rats were allocated into five groups, control (D.W.) (n=6) and test groups that received (5mg, 10, 20 mg/kg/day) of vardenafil by oral gavage, each one (n=6) and sulfasalazine (500mg/kg) for 30 days. Animal were kept in standard conditions. At day thirty of experiment the blood sample was drawn for measurement of serum testosterone and gonadotrophin hormones then the testis tissue of rats in whole groups were removed and sperms were collected from epididymis and prepared for analysis. Significant decrease in sperm count and motility in rat treated with (20 mg/kg body weight) of vardenafil for 4 weeks, in addition to significant decrease serum testosterone and gonadotrophin horomes. Further histopathological changes in rat testis and epididymis were also observed. In conclusion administration of 20mg/kg/day vardenafil was very efficacious dose that result in disturbing fertility status in male rats. The results of study have provide direct evidence that Ca^{+2} channels blocker vardenafil are effective in causing a significant arrest of developing spermatids and malfunction of leydig cells.

KEY WORDS: vardenafil, fertility, male rats, gonadotrophins, testosterone.

INTRODUCTION

Calcium ion is implicated in diverse cellular functions in both germ cells and somatic cells in the testis, particularly, mediating the responses to endocrine hormones and local regulators in genital tracts [1,2]. A common belief is that the Ca^{2+} influx and efflux should be tightly regulated to maintain the intracellular Ca^{2+} homeostasis, and an alteration in the Ca^{2+} transport across the cell membrane could result in a drastic impact on spermatogenesis and steroidogenesis[3,4]. Sildenafil, tadalafil and vardenafil are all classified as PDE5 inhibitors, but they differ slightly in their selectivity, pharmacokinetics and side effects[5]. Vardenafil, for instance, is considered to be slightly more potent, possibly due to its different chemical structure, which allows a slower dissociation rate from PDE5 compared with sildenafil and tadalafil [6]. It was found that only vardenafil markedly reduced $CaCl_2$- induced contractions in the phenylephrine- treated isolated pulmonary artery indicates that this additional mechanism could be related to blockade of Ca^{2+} channels. Vardenafil, but not sildenafil or tadalafil, has calcium-channel blocking activity in rabbit isolated pulmonary artery and human washed platelets[7]. The aim of the present study is to evaluate effects of vardenafil on fertility parameters including sperm activity, counts and morphology and on hormones LH , FSH and testosterone.

MATERIALS & METHODS

Animals

Sprague-Dawly male rats weighing 250-300 gm and 8 weeks old were obtained from the Animal House of the College of Pharmacy/University of Baghdad. The animals were maintained on normal conditions of temperature, humidity and light/dark cycle. They were fed standard rodent pellet diet and they have free access to water.

2.3. Preparation of vardenafil solution

Vardenafil tablet (60mg) was dissolved in (30ml) distilled water to produce a solution with concentration of 2mg/ml, which is used as a standard solution for the preparation of different doses.

2.4. The study design

Thirty rats were used in the present study, the study groups were divided into 5 groups:

First group: (control) 6 rats were administered distilled water for 30 successive days by oral gavage.

Second group: 6 rats were used for the study of the infertility activity of vardenafil in which (5mg/kg BW) of vardenafil was given for 30 successive days by oral gavage.

Third group: 6 rats were used, for the study of the possible infertility activity of vardenafil in rat model. In This group (10 mg/kg BW) dose of vardenafil were used for 30 successive days by oral gavage.

Fourth group: 6 rats were used for the study of the possible infertility activity of (20 mg/kg BW) vardenafil was used for 30 successive days by oral gavage.

Fifth group: 6 rats were given a dose of (500mg/kg BW) of sulfasalazine for 30 successive days by oral gavage as a positive control (in this group, sulfasalazine represent standard infertility agent.)

Study the effects of vardenafil on pituitary hormones (testosterone, FSH, LH) by using ELISA hormonal analysis equipment.

Determination of serum luteinizing hormone (LH) concentration:

Serum concentrations of luteotropic hormone (LH) were measured by ELISA reader using commercial assay kits according to the manufacturer's protocols. **(CSB-E12654r)**[8].

Determination of serum Follicle-Stimulating (FSH) concentration

Serum concentrations of Follicle-Stimulating Hormone (FSH) were measured by ELISA reader using commercial assay kits according to the manufacturer's protocols. (**CSB-E06869r)**[9].

Determination of serum testosterone concentration.

Serum concentrations of testosterone were measured by ELISA reader using commercial assay kits according to the manufacturer's protocols. (**CSB-E05100r**)[10].

Determination of sperm parameters (sperm activity morphology and counts)

The concentration of spermatozoa in the epididymis was investigated in the Sprague- Dawley albino rat by means of a micropuncture technique[11].

2.7.1. Determination of Sperm morphology and viability

Sperm were removed from the epididymis by internal rinsing with 5mL normal saline[12]. Different staining procedures are being used accordingly for the assessment of sperm morphology and viability. Two slides were prepared from each sample for morphological assessment. A specimen of 10µl was placed on a slide and smeared with another slide and the preparation was left to dry. The dried specimens were stained with eosin (1% Eosin and 10% Nigrosin) stain.

This stain can pass through the sperm membrane. If the membrane is intact, as in the case of viable spermatozoa, dye passage is prevented. If the membrane is broken as often the case with dead or dying spermatozoa, the dye will pass into the sperm's cytoplasm[13].

Sperm viability was determined by preparing an eosin nigrosin smear (37°C) and assessing at least 100 sperm under bright-field microscopy (1000X)[14].

Determination of Sperm activity (motility):

Sperm motion was analyzed. The left cauda epididymis was dissected and incubated in warmed medium (37°C) to release spermatozoa into the medium.

The incubation medium consisted 5ml normal saline (N.S) with, sperm motion was recorded. The sample was loaded into a Neubauer haemocytometer chamber and placed on a microscope stage warmed to 37°C, and the sperm was observed with a light microscope.

Motility assessment was performed by counting at least 100 sperm from 5 different areas (sequares) according Percentage motile sperm, motility percentage sperm out of motile sperm, and percentage of sperm out of all sperm, were calculated.

2.7.3. Determination of Sperm counts:

Briefly, sperm counts was assessed by placing 10µl of diluted sperm and allowed to stand or settle for 5 minutes on Neubauer haemocytometer slide for microscopic evaluation. Counting was done under a light microscope at 40X magnification and expressed as million/ml of suspension [15].

RESULTS

The suppressive effect of different doses of vardenafil and dose of the standard infertility causing agent (sulfasalazine) on sperm counts in rats was shown in table 1. Vardenafil at dose of 5g/kg and 20mg/kg and 500mg/kg of sulfasalazine significantly (P<0.05) decreased the count numbers of sperms compared to all groups. Meanwhile, vardenafil at dose of 10mg/kg non-significantly decrease sperms count compared to control group and vardenafil group at dose of and 20mg/kg., the sperm count decreased significantly compared to vardenafil at dose of 5mg/kg and 10mg/kg.

TABLE 1.Effects of different doses of vardenafil on sperm counts in rats

Groups	Mean±SD x 10^5
Control	81.28±1.33
Vardenafil (5mgL/Kg)	72.67±3.22* a
Vardenafil(10mg/Kg)	81.72±2.23 c
Vardenafil (20mg/Kg)	51.58±2.58 * b
Sulfasalazine (500mg/Kg)	76.26±3.89* a

* Significant compared with control (P<0.05)

a,b and c values with different letters are Significant (P<0.05)

Table 2. Demonstrated that vardenafil at minimum doses of 5mg/kg and maximum dose at 20mg/kg caused significant increase in percentage of sperm abnormality compared to control and vardenafil group at dose of 10mg/kg. Sulfasalazine caused significant increase (P<0.05) in percentage of sperm abnormality compared to control and vardenafil at dose of 10mg/kg and 20mg/kg.

TABLE 2. Effects of different doses of vardinafil on the mean percentage of abnormal sperms in rats

Groups	Mean±SD
Control	2.7±0.45
Vardenafil (5mg/Kg)	3.56±0.27* a
Vardenafil (10mg/Kg)	2.88±0.13 b
Vardenafil (20mg/Kg)	9.88±0.95* c
Sulfasalazine (500mg/Kg)	3.56±0.23* a

* Significant compared with control (P<0.05)

a, b and c values with different letters are Significant (P<0.05)

Table 3, showed that all doses of vardenafil showed non – significant change in sperm motility except at dose (20mg/kg) which caused decrease in percentage of motility of sperms significantly (P<0.05) compared to control and vardenafil at lower doses 5mg/kg and 10mg/kg. Sulfasalazine caused significant change in sperm motility compared to control.

TABLE 3. Effect of different doses of vardenafil and sulfasalazine on percentage of sperms motility

Groups	Mean±SD
Control	89.52±1.56
Vardenafil (5mg/Kg)	90.58±1.45a
Vardenafil (10mg/Kg)	90.36±1.35a
Vardenafil (20mg/Kg)	79.68±1.38*b
Sulfasalazine (500mg/Kg)	86.66±2.8*a,b

*Significant compared with control (P<0.05)
a, b values with different letters are Significant (P<0.05)

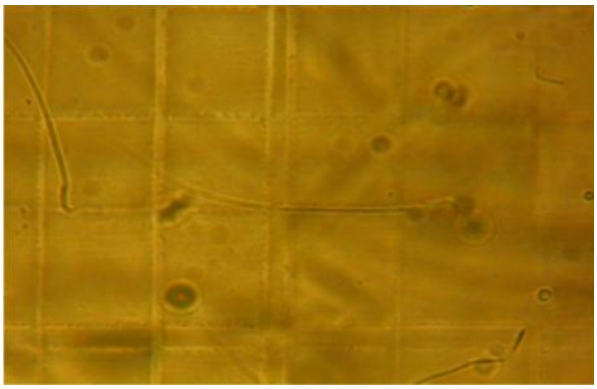

FIGURE 1: Light microphotograph of spermatozoa treated with vardenafil low dose (5) mg/kg b.w. for (30) days showing decrease numbers and with less anomalies of sperms.

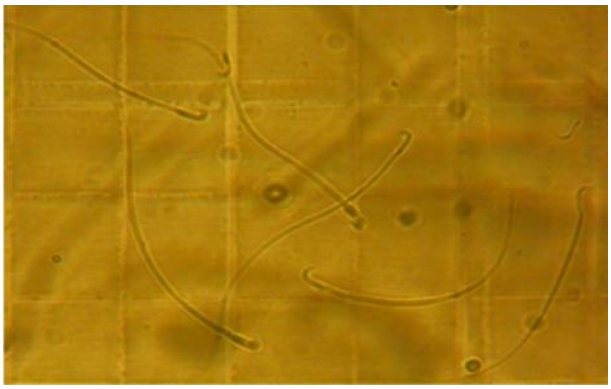

FIGURE 2: Light microphotograph of spermatozoa treated with vardenafil medium dose (10) mg/kg b.w. for (30) days showing increase numbers and with out anomalies of sperms

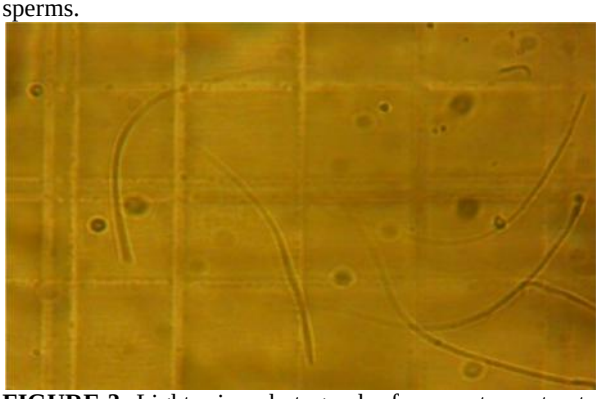

FIGURE 3: Light microphotograph of spermatozoa treated with vardenafil high dose (20)mg/kg b.w. for (30) days showing decrease numbers and with showing detached head of sperms.

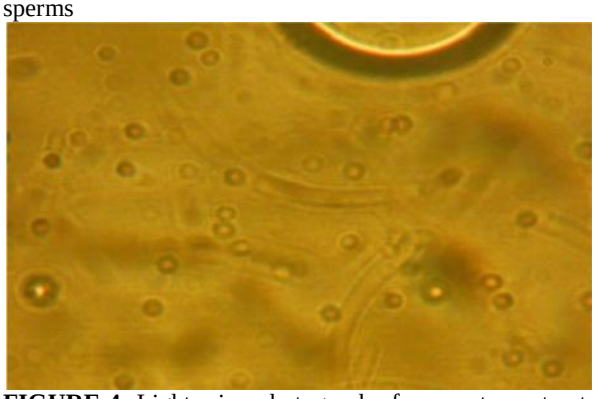

FIGURE 4: Light microphotograph of spermatozoa treated with sulfasalazine at dose (500)mg/kg b.w. for (30) days showing decrease numbers without anomalies of sperms.

Table 4. revealed that only vardenafil at a dose (20mg/kg) and sulfasalazine cause significant decline in FSH (P<0.05) compared to control.

TABLE 4. Effect of different doses of vardenafil and sulfasalazine on FSH in male rats

Groups	Mean±SD
Control	0.268±0.11
Vardenafil (5mg/Kg)	0.202±0.009 a
Vardenafil (10mg/Kg)	0.172±0.069a
Vardenafil (20mg/Kg)	0.112±0.013*a
Sulfasalazine (500mg/Kg)	0.128±0.016*a

* significant compared with control (P<0.05)
a,b and c values with different letters are Significant (P<0.05)

Table-5 showed that vardenafil at maximum dose (20mg/kg) and sulfasalazine cause significant change (P<0.05) in LH compared to control in male rats.

TABLE 5. Effect different doses of vardenafil and sulfasalazine on LH in male rats

Groups	Mean±SD
Control	0.496±0.312
Vardenafil (5mg/Kg)	0.37±0.229a
Vardenafil (10mg/Kg)	0.166±0.069a
Vardenafil (20mg/Kg)	0.144*±0.29a
Sulfasalazine (500mg/Kg)	0.222*±0.211a

* significant compared with control (P<0.05)
a,b and c values with different letters are Significant (P<0.05)

Table-6 revealed a Significant decline (P<0.05) in serum testosterone levels by sulfasalazine and vardenafil at dose maximum dose of 20 mg/kg compared to control and vardenafil at dose of 5mg/kg and 10mg/kg .

TABLE 6. Effect of different doses of vardenafil and sulfasalazine on testosterone in male rats

Groups	Mean±SD
Control	1.914±2.86
Vardenafil (5mg/Kg)	1.738±2.69a
Vardenafil (10mg/Kg)	0.232±0.12a
Vardenafil (20mg/Kg)	0.116*±0.16 b
Sulfasalazine (500mg/Kg)	0.12*±0.18 b

* Significant compared with control (P<0.05)
a, b and c values with different letters are Significant (P<0.05)

Effect of vardenafil on histological structural elements s of rat testis

After 30 days of treatment with vardenafil with different doses (5, 10, and 20 mg/kg body weight), it is remarked that:

First group (control)

Fig 5. Revealed the presence of mature spermatozoa with normal heads and tails with mild differences in the size of seminiferous tubules also no signs of tissue inflammation in addition to presence of sertoli and ledige cells with normal morphology.

Second group (5mg/kg) B.W. vardenafil)

Fig 6. Showed hyperplasia of the spermatogonia and sertoli cells. The spermatozoa and spermatid filled the lumen of the seminiferous tubules, while other section showed mature spermazoa filled the lumen of seminiferous tubules, also there is congestion blood vessels in the tunica vasculaosa.

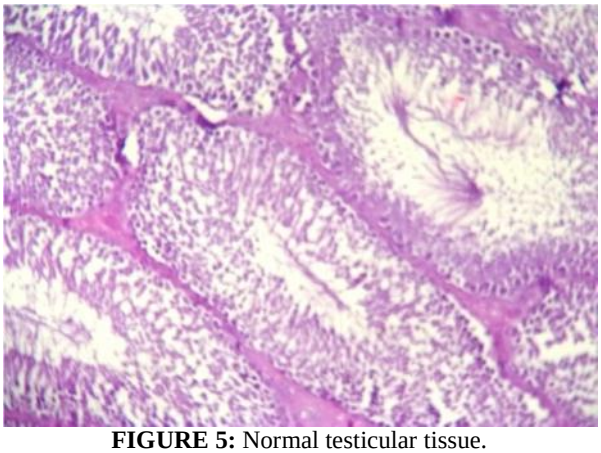

FIGURE 5: Normal testicular tissue.

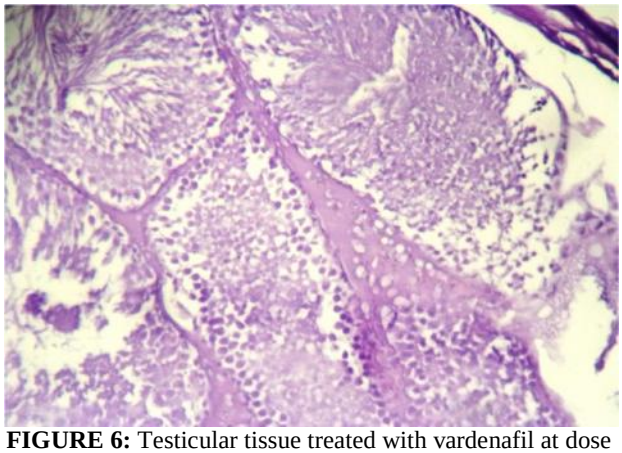

FIGURE 6: Testicular tissue treated with vardenafil at dose of 10mg/kg.

Third group (10mg/kg B.W vardenafil)

Fig 7. Demonstrated normal testes but few seminiferous tubules filled with large number of degenerative spermatids or spermatocytes .

Fourth group (20 mg/kg B.W. vardenafil)

Fig 8. Showed severe hyperplasia in the spermatogonia, primary and secondary spermatocytes and Sertoli cells in the seminiferous tubul. Other section showed that the seminiferous tubules filled with necrotic cells.

Fifth group (500mg/kg B.W. sulfasalazine)

Fig 9. demonstrated severe destruction in the seminiferous tubules due to severe degenerative and necrotic changes in wall of seminiferous tubules. In addition, other section showed desquamation and sloughing of the degenerative seprmatogonia, spermatocytes in the lumen of the seminiferous tubule. Also there is severe damage in the interstitial tissue including Leydic cells

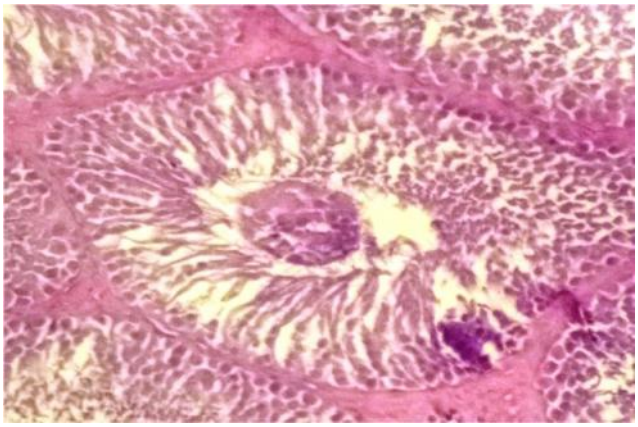

FIGURE 7: Testicular tissue treated with vardenafil at dose of 20mg/kg.

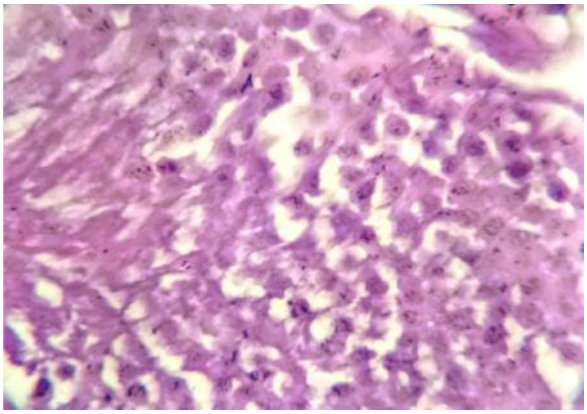

FIGURE 8: Testicular tissue treated with vardenafil at dose 20mg/kg.

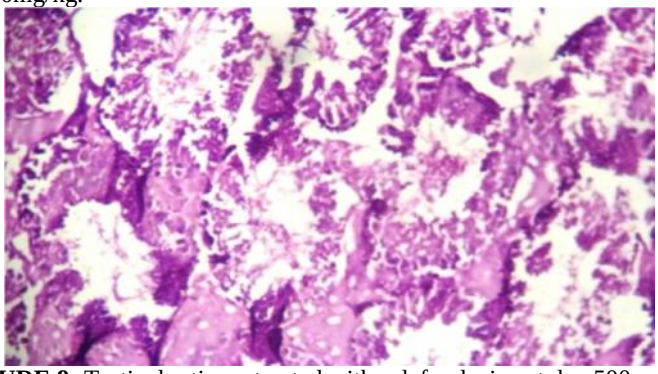

FIGURE 9: Testicular tissue treated with sulafasalazine at doe 500mg/kg.

DISCUSSION

Results of this study also revealed significant drop ($p<0.05$) in sperm counts in treated groups with vardenafil at lower dose (5mk/kg) and maximum dose (20mg/kg) resulted in decrease sperm counts to 72.67 x 10^5 and 51.58x10^5/ml respectively compared to the control (table 1, fig.1, 2, and 3) . The decline in spermatogenesis (sperm production) may be attributed to the effect of vardenafil on the pituitary gonadal axis (PGA) and hence on gonadal function. Also the effects evoked by vardenafil on sperm counts profile might be strongly linked with status of FSH and LH (tables 4 and 5) which also reduced at dose of vardenafil (20mg/kg) and greatly affect sertoli cells functions in the testes specially sperms production. Sulfasalazine suppress the sperm count significantly ($P<0.05$) may be due to ability to decrease the gonadotrophin hormones. Regarding effects of vardenafil on the percentage of normal and abnormal sperms, the results of this study showed an increase in the percentage of abnormal sperms to be reached to its maximum effect (9.88%) at the dose of 20mg/kg compared to the controls and more offensive than sulfasalazine treated group(table-2, fig1, 2 and 3). This increment accompanied by decrease in the percentage of normal sperms (table-2) and this rise suggesting that vardenafil by affecting Ca^{+2} influx through Ca^{+2} channels[7], might results in different sperms malformations, which include sperm heads loss ,double heads, loss of tails. However these findings also could be attributed to effects of vardenafil on pituitary hormones which necessary for both sperms production and maturation. The inhibitory effect elucidated by vardenafil targeting sperm motility was much tenser than corresponding effect evoked by standard infertility agent sulfasalazine, which decrease sperms motility to 79.68% (table-3, fig. 4) compared to the controls. Of the various voltage gated channel sub units, Cav 2.3 may play a role in sperm motility. The expression of Cav 2.3 was detected along the dorsal ventral sides of proximal segments of the principle piece of rodents sperms suggesting that Cav 2.3 may control Ca^{+2} influx necessary for asymmetric flagella movement of sperm[16]. Moreover, the utilization of Ca^{+2} channel blocker vardenafil by affecting Ca^{+2} influx through these channels in sperms support this results. On the other hand, several studies indicated that Ca^{+2} not only play a role in sperm motility but also it is a key regulator in the initiation and maturation of sperm hyper activation [17,18]. Sulfasalazine induced reduction of the percentage of progressively motile sperm might be related to the infertility. Sulfasalazine might affect CD59 protein, which is one of the complement regulatory proteins that are important for the reproductive function of sperm. This protein is also known to be located in the acrosomal region of the sperm head and seems to protect the sperm from complement mediated damage in the female reproductive tract[19]. The present study also showed that treatment of male rats with different doses of vardenafil produce a significant reduction in the serum conc. of FSH at dose of (20mg/kg) of vardenafil compared to control (table -4). The inhibitory effect of vardenafil on serum level of FSH might be attributed to the intervention of Ca^{+2} ions in the signal transduction pathways responsible for FSH secretion from pituitary gland.

Vardenafil was effective in attenuating LH serum concentration as showed in (table-5). LH serum levels

were reduced in treated groups with different doses of vardenafil but significantly decline was at dose of 20mg/kg compared to the control. GnRH regulates the gonadotrophins release by means of rapid increase in [Ca^{+2}] ions. After its binding to GnRHr through G-protein coupled signal transduction, GnRH rapidly activates PLC, which produce IP3and DAG from PIP2 which in turn rapidly mobilize transient intracellular Ca^{+2} to trigger burst initiation of exocytosis causing rapid LH secretion within 10 seconds and lasting for 100 seconds[20-24]. It is acknowledge that PKC and Ca^{+2} ions influx differentially control the expression of LH and FSH subunit genes. The mechanism by which GnRH causes such control within gonadotropes is unknown but may involve the activation of different transcription factors and second messengers including Ca. Intracellular Ca^{+2} directly modulate IP3 receptor activity and may also act through modulation of IP3 binding[25]. The decrease in testosterone serum levels caused by corresponding rise in the dose of vardenafil (table-6) to reach its maximum effect at the higher dose of vardenafil (20mg/kg) compared to control; these results indicate that the administration of vardenafil has negative effect on the sexual reproduction function in the male rats. Histological evaluations of testis rat tissues have important role in male reproductive risk assessment. In this study there were significant biologically histopathological changes in treated rat's testis tissue in contrast to control group (fig.5) .Since the use of vardenafil greatly affect serum levels of testosterone hormone and at the same time both of FSH and LH it would be expected to disrupt the testicular histoarchitecture. The histopathological changes in the testes due to vardenafil treatment were characterized by degeneration of spermatocytes associated with marked decrease in the process of spermatogenesis.These alterations were very slight at the first group and became more offensive as the dose of vardenafil was increased to be end with hyperplasia of seminiferous tubules (fig. 6, 7 and 8) with presence of edematous tissue and loss of all stages of spermatogenesis. In this study serum level of testosterone was decreased by vardenafil treatments and at the same time serum levels of LH and FSH were also reduced these finding suggest that the alteration observed in this study are mediated at least in part by testosterone deficiency. Also hormonal control of spermatogenesis governed by pituitary gonadotropins FSH and LH and by poorly defined paracrine mechanisms. Sertoli cells possesses receptors for FSH so it is likely that this hormone exert its stimulating effects on sertoli cells which in turn result in stimulation of intra tubular factors for survival of germ cells[26-28].

CONCLUSION

Vardenafil was effective in attenuating steriodogensis and testosterone production.It appears to be with antifertility effects on male rats through the inhibition of pituitary gonadal axis hormones. Administration of 20mg/kg/day of vardenafil was considered a very efficacious dose that cause infertility effects in male rats.

REFERENCES

[1]. Steele, G. L., Leung, P. C. (1992) Intragonadal signalling mechanisms in the control of steroid hormone production. *J Steroid Biochem MolBiol*.; 41:515–22.

[2]. Berridge, M.J., Bootman, M.D., Roderick, H.L. (2003) Calcium signalling: dynamics, homeostasis and remodeling. Nat Rev Mol CellBiol., 4:517–29.

[3]. Li, L.H., Wine, R.N., Miller, D.S., Reece, J.M., Smith, M., Chapin, R.E. (1947) Protection against methoxyacetic-acid-induced spermatocyte apoptosis with calcium channel blockers in cultured rat seminiferous tubules: possible mechanisms. *Toxicol Appl Pharmacol.* (1997); 144:105–19.

[4]. Yamaguchi, M. (2005) Role of regucalcin in maintaining cell homeostasis and function. *Int J Mol Med*., 15:371–89.

[5]. Rosen, R.C., Kostis, J.B. (2003) Overview of phosphor diesterase 5 inhibition in erectile dysfunction. Am J Cardiol., 92:9M–18M.

[6]. Blount, M.A., Beasley, A., Zoraghi, R., Sekhar, K.R., Bessay, E.P., Francis, S.H. (2004) Binding of tritiated sildenafil, tadalafil, or vardenafil to the phosphodiesterase-5 catalytic site displays potency, specificity, heterogeneity, and cGMP stimulation. Mol Pharmacol. 66:144–152.

[7]. Toque, H. A., Teixeira, C. E., Priviero, F. B. M., Morganti, R. P., Antunes, E. and De Nucci, G. (2008) Vardenafil, but not sildenafil or tadalafil, has calcium-channel blocking activity in rabbit isolated pulmonary artery and human washed platelets. Br J Pharmacol. 2008 June; 154(4): 787–796.

[8]. Rat leutrophic hormone (LH) ELISA Kit, Catalog No. CSB-E12654r.

[9]. Rat follicle stimulating hormone (FSH) ELISA Kit, Catalog No. CSB-E06869r.

[10]. Rat Testosterone (T) ELISA Kit, Catalog No. CSB-E05100r.

[11]. Turner, T.T., Hartmann, P.K. & Howards, S.S. (1977) In vivo sodium, potassium and sperm concentrations in the rat epididymis. *Fertility and Sterility*. 28, 191-194.

[12]. Filler, R., Chapin, R.E., Heindel, J.H. (1993) Methods for evaluation of rats epididymal sperm morphology. *Male reproductive toxicology*. *Press*. p. 334–43.

[13]. Blom, E. (1950) The Evaluation of Bull Semen, with Special Reference to its Use in Artificial Insemination. Copenhagen: A/S Carl Fr Mortensen. *Abstract in Anim Breed Abstr*., 19:648, 1951).

[14]. Morakinyo, A.O., Iranloye, B.O., Adegoke, O.A. (2009) Antireproductive effect of calcium channel blockers on male rats. *Reprod Med Biol*., 8(3):97-102.

[15]. Lowe, D.G. and Teffrey, I.M. (eds): (1990) Surgery Pathology Technique 1st edition.

[16]. Wennemuth, G., Westenbroek, R.E., Xu, T., Hille, B., Babcock, D.F. (2000) CaV2.2 and CaV2.3 (N- and R-type) Ca21 channels in depolarization-evoked entry of Ca21 into mouse sperm. *J Biol Chem.* (2000); 275:21210–21217.

[17]. Yanagimachi, R. (1982)Requirement of extracellular calcium ions for various stages of fertilization and fertilization-related phenomena in the hamster. *Gamate Res*. (1982); 5:323–344.

[18]. Suarez, S.S., Vincenti, L., Ceglia, M.W. (1987) Hyperactivated motility induced in mouse sperm by calcium ionophore A23187 is reversible. *J Exp Zool*., 244:331–336.

[19]. Simpson, K. L. and Holmes, C. H. (1994) Differential expression of complement regulatory proteins decay-accelerating factor (CD55), membrane cofactor protein (CD46) and CD59 during human spermatogenesis. *Immunology 81*.(1994), 452–461.

[20]. Naor, Z. and Catt, K. J. (1981) Mechanism of action of gonadotropin releasing hormone. Involvement of phospholipid turnover in luteinizing hormone release. *J. Biol. Chem*., 256, 2226–2229.

[21]. Leong, D. A., and Thorner, M. O. (1991) A potential code of luteinizing hormone-releasing hormone-induced calcium ion responses in the regulation of luteinizing hormone secretion among individual gonadotropes. *J. Biol. Chem.*, 266, 9016–9022.

[22]. Thomas, P., Mellon, P. L., Turgeon, J. L., and Waring, D. W. (1996) The LβT2 clonal gonadotrope: a model for single cell studies of endocrine cell secretion. *Endocrinology*., 137,2979–2989.

[23]. Smith, C. E., Wakefield, I., King, J. A., Naor, Z., Millar, R. P. and Davidson, J. S. (1987) The initial phase of GnRHstimulated LH release from pituitary cells is independent of calcium entry through voltage-gated channels. *FEBS Lett.*, 225, 247–250.

[24]. Tse, A., Tse, F. W., Almers, W., and Hille, B. (1993) Rhythmic exocytosis stimulated by GnRH-induced calcium oscillations in rat gonadotropes. *Science*. (1993), 260, 82–84.

[25]. Pietri, F., Hilly, M., and Mauger, J. P. (1990) Calcium mediates the interconversion between two states of the liver inositol 1, 4, 5-trisphosphate receptor. *J. Biol. Chem.* 265,17478–17485.

[26]. Jegou, B. (1993) the Sertoli-germ cell communication network in mammals. Internatonal. *Review of Cytology* (1993), 147:25–96.

[27]. Parvinen, M. (1993) Cyclic function of Sertoli cells. In: Russell LD and Griswold MD (eds.) *the Sertoli Cell*,pp. 331–347. Cache Clearwater, FL: River Press.

[28]. Pescovitz, O.H., Srivastava, C.H., Breyer, P.R. and Monts, B. A. (1994) Paracrine control of spermatogenesis. *TEM*, 5: 126–131.