EFFECTS OF TADALAFIL AND/OR SILYMARIN ON GONADAL FUNCTION IN ADULTS MALE ALBINO RATS

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ABSTRACT

Taldalafil is a long acting phosphodiesterase-5 inhibitor widely used in the treatment of erectile dysfunction. The abuse of tadalafil, which is a worldwide problem, is associated with many side effects. Sylimarin considered as a herbal drug, its active constituents are silybin, isosilybin, silydianin and silychristin. A controversy exist with regard to the effects of tadalafil and silymarin on gonadal function in animal species. The present study aimed to investigate the effects of tadalafil and/or sylimarin on gonadal function adult male rates. Twenty four male rats are used in the study, they divided into 4 groups (n=6) and gavaged daily for 1 month as follows; 1-control(0.5ml distilled water), 2-tadalafil (10mg tadalafil/kg), 3-silymarin (100 mg silymarin/ kg) and 4-tadalafil + silymarin (10 mg tadalafil and 100 silymarin mg/ kg). Tadalafil significantly increases serum testosterone and decreases LH and FSH, it adversely affect the process of spermatogenesis (sperm count and percentage of motile, dead, and abnormal sperms). While, silymarin significantly increases serum testosterone, LH and FSH and improve spermatogenesis. Serum testosterone remains significantly higher in tadalafil+silymarin group compared with control group, while FSH and LH and parameters of spermatogenesis became insignificantly different from those in control group.

INTRODUCTION

Tadalafil is a long acting phosphodiesterase-5 inhibitor widely used in the treatment of erectile dysfunction (1) and recently approved for the treatment of pulmonary artery hypertension (2, 3, 4). The antioxidant activity of tadalafil has been observed in systemic circulation (5, 6) as well as in local tissues (7, 8). The abuse of PDE5 is a worldwide problem which is associated with many side effects. Tadalafil administration in rats may leads to irreversible hepatotoxicity (9), inhibition of erythropoiesis (10) and alteration in lipid profile (11). Khalaf *et al*. 2012 reported a deterioration in testicular function and structure of rats chronically administered tadalafil (12). Moreover, increased serum testosterone and decreased serum gonadotropic hormones with hyperprolactinemia was observed in rats chronically administered tadalafil (13). In man, tadalafil used in patients with metabolic syndrome resulted in increased serum testosterone and decreased LH (14), while an increased in testosterone/estrogen ratio without change in serum testosterone level has been recorded after 12 months of tadalafil treatment (15).

Silymarin is extracted from the seeds and fruits of annulal plant (*Silybum marianum*). It is main active constituents are silybin, isosilybin, silydianin and silychristin (16). Silymarin considered as a herbal drug, the use of sylimarin in liver disorders as a hepatoprotective agent (17, 18) is attributed to its powerful antioxidant activity (19. The positive effect of symilarin on serum cholesterol lipoproteins results from inhibition of intestinal absorption of cholesterol (20) and enhancement of LDL removal by the liver (21). Silymarin stimulates dopamine (D2) receptors (22), this effect was associated with raised plasma prolactin level (22, 23). Increased serum testosterone, LH and FSH and improvement in spermatogenesis have been observed in male rats administered silymarin (24), Khalil, (2002) mentioned similar effect of silymarin on serum testosterone and LH (25).

MATERIALS AND METHODS

The study was carried out in College of Pharmacy, University of Basrah during the period from 1st of Jan to 1st of Jun 2017. Twenty four male albino rats (*Rattus norvegicus*), 12 ± 2 weeks old and weighing 200 ± 15 g were used in this study. Rats were obtained from the Animals House of the College of Pharmacy, University of

Basrah. They were housed in plastic cages (2 rats per cage), maintained in a controlled temperature (20±5°C) and 12 h photoperiod. Free access to standard rat pellets and tap water was allowed. Rats acclimatized for 2 weeks before the starting the experiment. Tadalafil (Pfizer, India) and ethanolic extract of silymarin (26) were used in this study. Rats were randomly divided into 4 groups (n=6) as follows: control, tadalafil, silymarin, and tadalafil + silymarin groups. Tadalafil group given tadalafil (10mg/kg) (11), silymarin group given silymarin (100mg/kg) (27), tadalafil + silymarin group given 10 mg tadalafil and 100 silymarin mg/ kg. Tadalafil and silymarin are administered daily for 1 month by oral route as a suspension in 0.5 ml distilled water. Control and treated rats were scarified after 1 month. Blood sample was obtained from posterior vena cava by disposable 5 ml syringe (28). Serum level of testosterone, FSH and LH were measured using ELISA kits (abbexa, UK). Calculation of sperm count, motility, percentage of dead and abnormal sperm was done as described by Evans and Maxwell (29). Data were analyzed by computerized SPSS (V.20) program, and the results were expressed as mean ±SD. Least significant difference (LSD) test was used to test the difference between group means, a significant difference considered when the $P \leq 0.05$.

RESULTS

The results in table (1) showed that serum level of free testosterone was increased significantly in all treated groups (tadalafil, silymarin, and tadalafil+silymarin groups) compared with control group. FSH and LH are significantly reduced in tadalafil group compared with control group, whereas they are significantly elevated in silymarin group. There was no significant effect on FSH and LH in tadalafil + silymarin group when compared with control group.

Table (2) revealed a significant reduction in sperm count and motility in tadalafil group compared with control group. Moreover, the percentage of dead and abnormal sperm are significantly elevated. Sperm count and motility are increased by silymarin treatment. They became significantly higher than those in tadalafil and control groups. The percentage of dead and abnormal sperms also significantly reduced compared with tadalafil group, but it is not significantly different from that in control group. No

significant difference has been observed in sperm count and percentage of motile, dead and abnormal sperm in tadalafil + silymarin treated rats compared with control.

Table 1: Serum level of free testosterone, FSH and LH in adult male rats administered tadalafil and/or silymarin for 1 month (n=6)

Group	Free testosterone (pg/ml)	FSH (IU/L)	LH (IU/L)
Control	124.05 ± 5.2 b	50.87 ± 3.6 b	148.90 ± 3.7 b
Tadalafil (10mg/kg)	134.00 ± 5.7 a	41.93 ± 2.8 c	141.75 ± 4.0 c
Silymarin (100mg/kg)	132.03 ± 6.9 a	57.83 ± 2.3 a	156.37 ± 5.1 a
Tadalafil(10mg/kg) + silymarin (100mg/kg)	135.01 ± 6.5 a	51.87 ± 4.1 ab	150.20 ± 5.8 b
LSD	7.98	6.96	6.16

Different letters indicates significant difference (P ≤ 0.05)

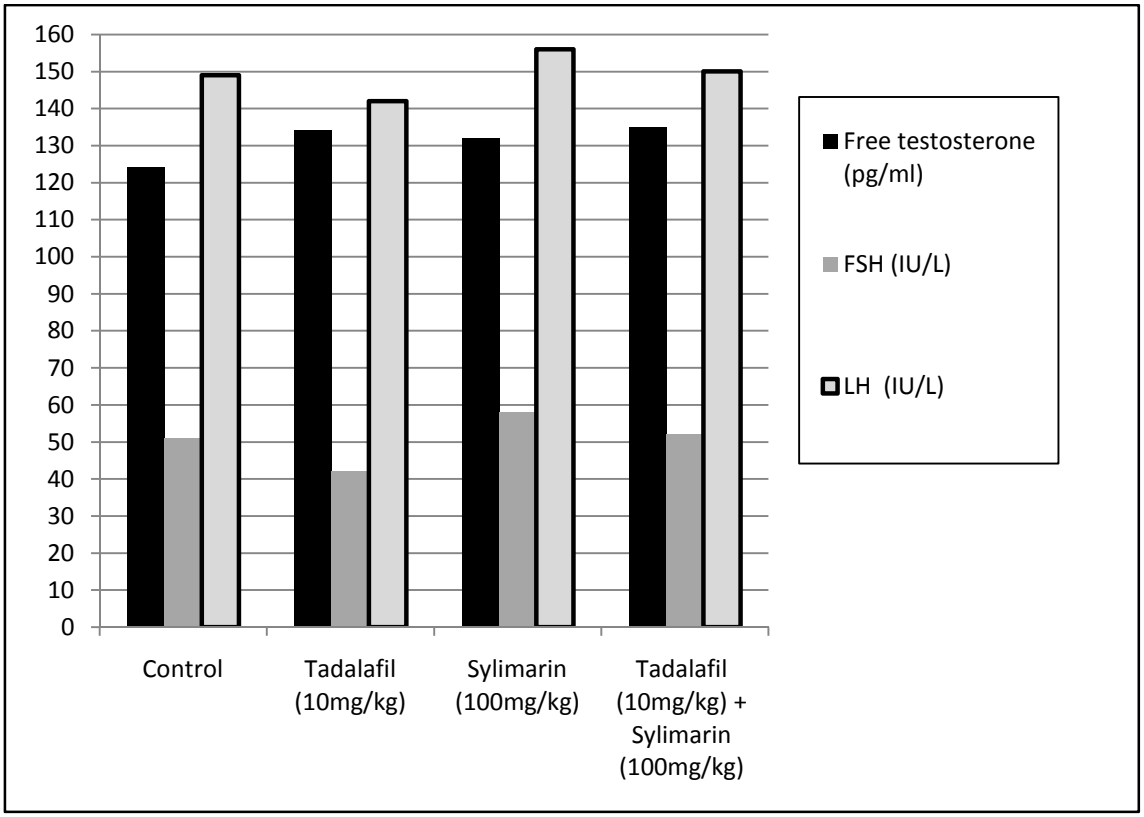

Figure 1: Serum level of free testosterone, FSH and LH in adult male rats administered tadalafil and/or silymarin for 1 month (n=6)

Table 2: Sperm count and percentage of motile, dead and abnormal sperms in adult male rats administered tadalafil and/or silymarin for 1 month (n=6)

Group	Sperm count ($\times 10^6/\mu L$)	Sperm motility (%)	Dead sperm (%)	Abnormal sperm (%)
Control	192.83 ± 9.5 b	83.7 ± 4.5 b	7.5 ± 1.9 b	7.8±1.5 b
Tadalafil (10mg/kg)	176.17 ± 12.8 c	75.3 ± 5.5 c	11.5 ± 1.6 a	11.2±2.1 a
Silymarin (100mg/kg)	204.33 ± 5.5 a	90.5 ± 5.7 a	8.2 ± 2.5 b	8.0±1.7 b
Tadalafil(10mg/kg) + silymarin (100mg/kg)	188.67 ± 7.1 b	84.3 ± 3.1 b	7.2 ± 1.2 b	7.0±0.6 b
LSD	11.5	6.16	3.3	3.1

Different letters indicates significant difference ($P \leq 0.05$)

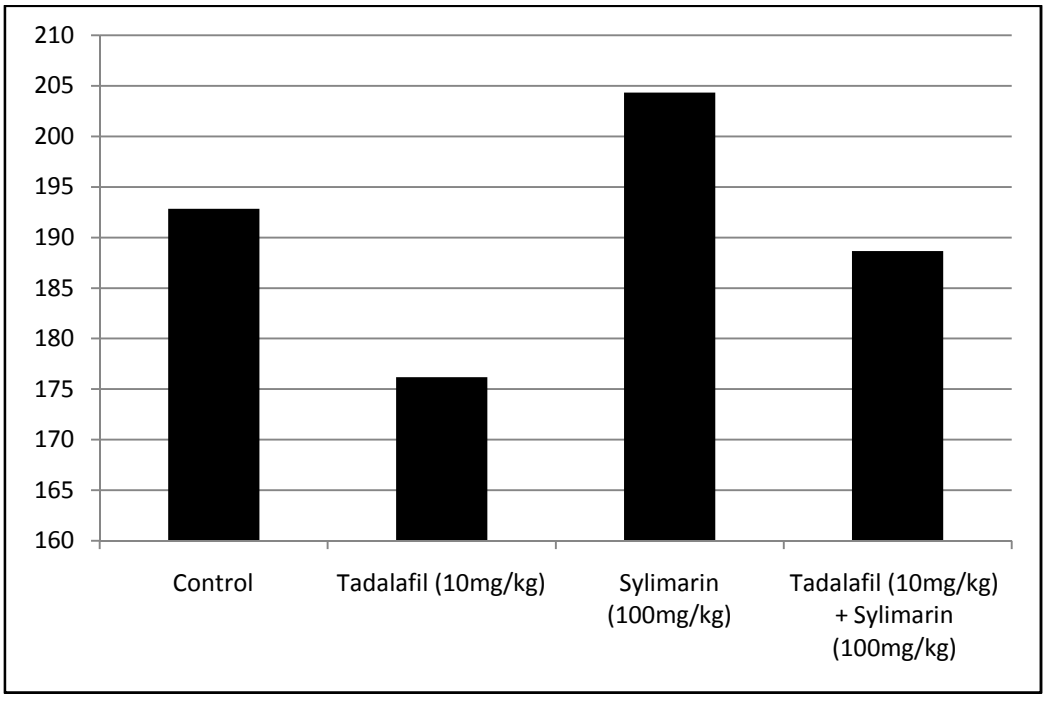

Figure 2: Sperm count ($\times 10^6$) in adult male rats administered tadalafil and/or silymarin for 1 month (n=6)

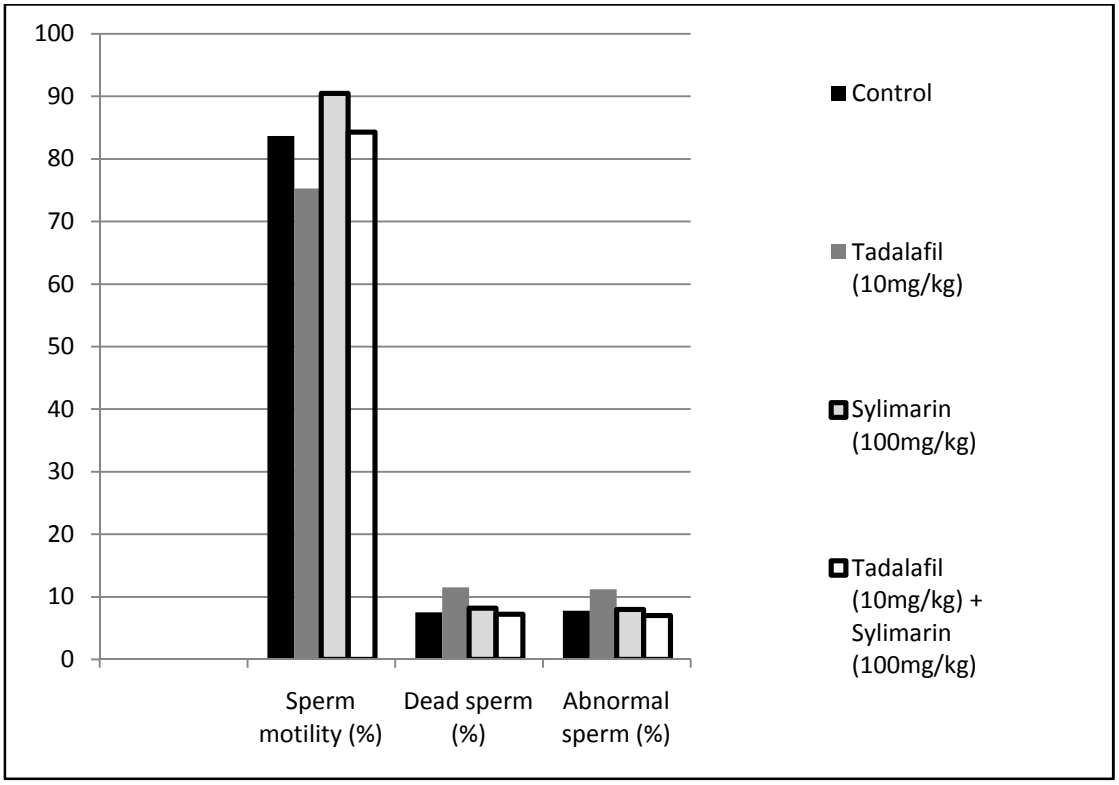

Figure 3: Percentage of motile, dead and abnormal sperms in adult male rats administered tadalafil and/or silymarin for 1 month (n=6)

DISCUSSION

Sexual function is controlled primarily by the hypothalamic secretion of GnRH, which stimulates the secretion of FSH and LH by the anterior pituitary gland. LH stimulates testosterone secretion by testicular Leydig, whereas FSH stimulates spermatogenesis. Testosterone directly inhibits hypothalamic secretion of GnRH by a negative feedback mechanism, which results in decrease secretion of FSH and LH. Testicular androgens, primarily testosterone, are steroid hormones synthesized from cholesterol or directly from acetyl coenzyme. Therefore, serum level of steroid hormones are partly regulated by serum cholesterol level (30). Increase in serum total cholesterol and LDL cholesterol had been observed in rats after eight weeks of tadalafil administration at a dose of 10mg/kg (11). The present study showed a significant increase in serum testosterone and a significant decrease in LH and FSH (Table 1 and Figure 1). The increased serum testosterone may be attributed to the

alteration in serum cholesterol level, whereas the negative feedback effect of testosterone on GnRH secretion may explain the decrease in serum LH and FSH (30).

Prolactin inhibits pituitary secretion of FSH and LH by a direct effect on pituitary gonadotropes (31, 32) as well as by inhibition of hypothalamic secretion of GnRH (33). Hyperprolactinemia with reduced serum LH and FSH has been observed in male rats chronically administered tadalafil (13).

The effects of LH/testosterone and FSH on spermatogenesis are of crucial importance (34). Therefore, altered sperm quality and quantity (decreased sperm count, motility, and increased percentage of abnormal sperm) that are shown in Table 2, Figure 2 and Figure 3 may resulted from alteration in serum level of these hormones.

Hypoxia has a detrimental effect on spermatogenesis (35). It inhibits spermatogenesis by causing apoptosis of primary spermatocytes and spermatogonia (36). The inhibitory effect of tadalafil on erythropoiesis in rats has been previously observed. It has been found that decrease in hemoglobin concentration may resulted from the decreased erythrocytes number or from impairment of heme biosynthesis (10) Tadalafil induced anemic hypoxia is an additional factor which may affect gonadal function.

A positive and negative feedback mechanisms play an important role in control of hypothalamic pituitary gonadal axis (37). The hypothalamic secretion of GnRH in explants of rats basal hypothalamus is stimulated by oxytocin and norepinephrine. The stimulatory effect is mediated through oxytocin receptors and NO release (38). In mice, intraperitoneal injection of high dose of silymarin (250mg/kg) for 5 days resulted in altered regional brain neurotransmitters (39). Stimulation of GnRH secretion by silymarin has been reported by Abedi *et al*, (2016) in rats (24). The increase in serum testosterone and gonadotropic hormones showed in silymarin treated group in this study (Table 1, Figure 1) may be attributed to the effect of silymarin on the hypothalamic pituitary gonadal axis (24).

Flavenoids are extracted from different parts of *Silybum marianum* (40). Flavenoids are aromatase inhibitor that inhibit peripheral conversion of testosterone into estrogen (41). In the present study aromatase inhibitory activity of silymarin is an additional factor which may explain the elevated serum testosterone in silymarin treated rats.

The high polyunsaturated fatty acid content of mammalian spermatozoa make them susceptible to damage by free radicals in oxidative stress conditions. Fortunately, local antioxidant materials in reproductive tissues protect spermatogenic cells from this damaging effect (42). The antioxidant activity of silymarin has been well studied (19, 40, 43). Silymarin antioxidant activity is attributed to inhibition of free radical formation and their scavenging, enhancement of enzymatic and non enzymatic antioxidant mechanisms, and decreasing the response to inflammatory conditions (44). The powerful antioxidant activity of silymarin make it a herbal drug that inhibits oxidative stress in biological systems. In the current study, the positive effect of silymarin on testicular function (testosterone secretion and spermatogenesis) may be attributed to the antioxidant activity of flavenoids contents of silymarin. Abedi *et al*, (2016) reported a significant increase in FSH, LH, GnRH, and number of spermatids and spermatozoa in silymarin treated rats (24). Improvement in spermatogenesis also observed by Khalil, (2002) in silymarin administered rats (25). In mice, treatment with silibinin (a component of silymarin) causes a significant increase in testosterone and diameter of spermatid (45). Silymarin estrogenic property had been observed in uterine and bone tissues of ovariectomized rats. However, this estrogenic property did not affect the hypothalamo/pituitary axis (46).

The increased serum testosterone in tadalafil + silymarin group (Table 1) may be explained by the previously mentioned mechanisms. However, the decreased level of FSH and LH compared with silymarin treated group may resulted from the negative feedback effect of increased testosterone on hypothalamic pituitary axis.

CONCLUSION

Tadalafil increases serum testosterone and decreases LH and FSH, it adversely affects the process of spermatogenesis. While, silymarin increases serum testosterone, LH and FSH and improves spermatogenesis.

آثار تادالافيل و / أو سيليمارين على وظيفة الغدد التناسلية في ذكور الجرذان البيضاء البالغة

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الخلاصة

التادالافيل هو مانع طويل الامد لانزيم فوسفوديستريس-٥ يستخدم على نطاق واسع في علاج ضعف الانتصاب. سوء استعمال التادالافيل،الذي هو مشكلة في جميع أنحاء العالم،يرتبط مع العديد من الآثار الجانبية. سيليمارين يعتبر من الادويةالعشبية، ومكوناته النشطة هي (silybin, isosilybin, silydianin and silychristin).

وهنالك جدل فيما يتعلق بآثار التادالافيل والسيليمارين على وظيفة الغدد التناسلية في أجناس الحيوانات. الهدف من هذه الدراسة هو التحقيق من آثار تادالافيل و/ أو سيليمارين على وظيفة الغدد التناسلية في ذكور الجرذان البالغة . أستخدم أربعة وعشرون ذكراً من الفئران في الدراسة، وقسمو إلى ٤ مجموعات (٦ جرذان في كل مجموعة) وجرعت يوميا لمدة شهر واحد على النحو التالي: ١- مجموعة السيطرة (٠,٥مل ماء مقطر) ٢- مجموعة التادالافيل (١٠ملغم تادالافيل/ كجم)، ٣-مجموعة السيليمارين (١٠٠ ملغ سيليمارين/ كجم) و ٤- مجموعة التادالافيل + السيليمارين (١٠ملغ تادالافيل/ كجم و ١٠٠ملغ سيليمارين/ كجم). التادالافيل أدى إلى زيادة معنوية في هرمون التستوستيرون ونقصان في FSH و LH، وانه اثرَ سلباً على عملية تكون الحيوانات المنوية (عدد الحيوانات المنوية والنسبة المئوية للحيوانات المنوية المتحركة، الميتة والغير طبيعية). بينما السيليمارين أدى إلى زيادة معنوية في هرمون التستوستيرون ، FSH و LH وحسن عملية تكون الحيوانات المنوية. أما في مجموعة تادالافيل + سيليمارين فأن هرمون تستوستيرون لايزال أعلى معنوياً مقارنة مع مجموعة السيطرة، في حين أن FSH و LH ودلالات تكون الحيوانات المنوية أصبحت غير مختلفة معنونياً مقارنتاً مع تلك الموجودة في مجموعة السيطرة.

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