TAMSULOSIN HYDROCHLORIDE (FLOMAX) EFFECTS ON FERTILITY OF ALBION MALE MICE
Sabah N. Alwachi and Dina K. Husain
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

ABSTRACT
Aim: To evaluate the side effects of Tamsulosin hydrochloride in fertility of experimental rats.

Materials and methods: three groups of mice were used. First and second groups were injected [intraperitoneal (I.P.)] daily for 42 with 8 and 16 µg /kg mouse body weight (kg.b.wt) of Tamsulosin hydrochloride, respectively. Third group was injected with PBS (control). Several biological and histopathological studies were conducted on rat groups.

Results: Significant decrease in number, motility and viability of epididymal sperm post injection with 16 µg /kg.b.wt, while injection with 8 µg /kg.b.wt reduced significantly, percentage of viability of sperm as compared with the control group. High percentage of abnormal sperm was observed in mice that injected (I.P.) with 8 and 16 µg/kg.b.wt versus control group. The injection with both concentrations (8 and 16 µg/kg.b.wt) of Tamsulosin hydrochloride reduced the levels of testosterone (P <0.05), body weight, testes weight, diameter of seminiferous tubules (DST), diameter of primary spermatocyte (DPS), diameter of spermatids and number of Leydig's cells cluster significantly. However, same concentrations of Tamsulosin hydrochloride increased the interstitial space and number of abnormal Leydig's cells cluster (P<0.05). Necrosis and edema was observed clearly in testes of mice that injected with Tamsulosin hydrochloride.

Conclusion: Current study proved clearly, the negative effect of Tamsulosin hydrochloride on sperm activity and number. Moreover, both studied concentrations of Tamsulosin hydrochloride affect negatively on testes structure and testosterone level.

RESEARCH ARTICLE

INTRODUCTION
Tamsulosin hydrochloride is an alpha blocker works by blocking nerve ending called alpha receptors. This will relax the smooth muscles of the prostate and bladder. Tamsulosin hydrochloride is used also to treat benign prostate hyperplasia (BPH) in men that can be recognized by enlargement of prostate and also known as an enlarged prostate that causes difficulties with urination (1). It was discovered by Toichi Takenaka in 1980 as a potential anti-hypertension drug (2). Later it was used to treat several disease such as; benign prostate hyperplasia (BPH), lower urinary tract symptoms associated with BPH and urine retention (3). The chemical structure of Tamsulosin hydrochloride is composed of methoxy- benzene sulfonamide, which, differs from other alpha-blockers such as Alfuzosin, Terazosin and Doxazosin, which are quinazolin derivatives (4). Comparing to others α-antagonists, Tamsulosin hydrochloride has greater specificity for α1 receptors in the human prostate, which do not affect on blood vessels (5). It is the most frequently prescribed medication for the treatment of lower urinary tract symptoms (5). α1-adrenoceptor blockade has a potent anti-fertility effect in male rats, because libido and mating performance remained essentially uninhibited. On the other hand, the anti-fertility effect was accompanied by significant impairment in ejaculatory (6). α-receptor blockers eg. phenoxybenzamine, Prazasin and Tamsulosin that interfere with seminal emission cause sperm retention in the tail of the epididymis. These drugs can either decrease vaginal sperm numbers or cause infertility of an ejaculation (7). In current study, we attempt to investigate the role of Tamsulosin hydrochloride on the fertility of albino male mice in terms of sperm motility, number and the morphological changes. Moreover, the effect of this drug on testes structure and level of testosterone was evaluated in present study.

MATERIALS AND METHODS

Animals
Thirty albino Swiss male mice (Mus musculus) of 8-10 week-old, weighing 30–35 gram were procured from the animal house of Al-kindy company for vaccines, Baghdad, Iraq. Animals were kept in clean polypropylene cages and fed on a standard antibiotic-free diet. The study was conducted following approval from the animal ethics committee of Baghdad University.

Experiment
Stock solution (400 µg/ml) of Tamsulosin hydrochloride was prepared by dissolving 40 mg of Tamsulosin hydrochloride in 100 ml of distilled water. Experimental group consisted of 30 BALB/c mice, divided into three subgroups depending on the Tamsulosin hydrochloride (Flomax) dose (8.9).

Group a (n:10): mice injected intraperitoneal (I.P.) with 8 µg/kg.b.wt daily for 42 days.
**Group b (n:10):** mice injected (I.P.) with 16 µg/kg.b.wt daily for 42 days.

**Group c (control group, n:10):** mice injected (I.P.) with 50 µl of PBS (0.1 M, 7.2 pH) daily for 42 days.

**Data and sample collection**

Animals' weight was checked before and after injection with different concentrations of Tamsulosin hydrochloride. Blood was sampled after 42-day injection with Tamsulosin hydrochloride. Immediately, after blood collection, animals were killed by cervical dislocation. Abdominal cavity was opened aseptically. Right male reproductive organ was sampled (epididymis). The tail of the epididymis was utilized to collect sperms for further studies. The caudal of epididymis were isolated and placed in Petri dish containing 1ml of standard tissue culture medium of RPMI-1640 that composed of fetal bovine serum (10 gm), Penicillin (1000000 IU), Streptomycin (1gm), Heps (4 gm) and Sodium bicarbonate (1%) to adjust the pH of medium to 7.2. The standard tissue culture medium was kept at 37°C for 2 h prior to experiment to prevent cold shocks. Epididymis was minced using microsurgical scissor and forceps to release the sperms from the tail of the epididymis (10). Left male reproductive organ was sampled for histopathological study.

**Percentage of sperm motility**

Sperm motility was assessed by calculation of percentage of sperm motility. The standard method of Silverberg and Turner (2001) was followed to determined the percentage of sperm motility (11). Briefly, a drop of sperm suspension was placed on a warmed 37 °C slide and examined under the light compound microscope. Sperm motility percentage was assessed according to the following equation:

Motility (%) = (number of motile sperms / total number of sperms) x 100.

**Sperm concentration**

The total sperm count in seminal specimens was measured using a haemocytometer (12).

**Percentage of sperm viability and abnormality**

A drop of sperm suspension was mixed with a drop of eosin stain (1%) (1 gm of eosin stain in 100 ml of 3% sodium citrate) and two drops of nigrosin stain (5 gm of Nigrosin stain in 100 ml of 3% sodium citrate) (13). Then a thin smear of previous mixture was made and left to dry at room temperature. The slide was examined under light microscope. The dead sperms took a pink color while, the viable sperm took a blue color. The sperm viability were estimated according to the following particular equation [percentage of dead sperm = (number of dead sperm / total number of sperm) x 100] (14).

Moreover, in the same slides the percentage of abnormal sperms that collected from test and control groups was measured. The changes in the head and tail of sperm were focused to estimate the morphological abnormality of examined sperms. The especial equation was followed for this purpose [percentage of abnormality = (number of abnormal sperms / total sperm number) x 100] (15).

**Histopathological examination**

Testes were kept in Bouin’s solution (75 ml of saturated alcoholic picric acid mixed with 25 ml of 40 % formaldehde) for 24 h and then, washed with 70% ethyl alcohol for several times until removing the yellow color. The method of Bancroft and Stevens (16) was followed to prepare the testes sections.

**Microscopic Examination**

Compound light microscope was used to study the histological changes in seminiferous tubules, interstitial spaces, spermatid and Leydig’s cells clusters. Diameter were assessed in each testis using previously calibrated micrometer (Ocular micrometer, stage micrometer, Precision Scientific Instruments Corporation, Delhi, India). The diameters of 20 seminiferous tubules were measured in four fields (5 seminiferous tubules per field). In same way, diameter of spermatids and spermatocyte were measured in four fields and the mean and standard deviation values for each case were calculated. Intersitial space was measured between two consecutive seminiferous tubules using the ocular micrometer. Light microscope with digital camera (Olympus, Japan) was used to take several photos to visualize the histological changes in testes of mice that injected with either PBS or Tamsulosin hydrochloride.

**Testosterone measurement**

Mini VIDAS radioimmunoassay (Biomerieux, USA) was carried out to measure the levels of sera testosterone for test and control groups of mice. Manufacture's instructions of Biomerieux Testosterone Mini Vidas Kit (Atlantic Medical Solutions, USA) were followed.

**Statistical analysis**

All values have been used to give a mean value and the standard deviation calculated. The differences were analyzed using Student’s t-test, and one-way ANOVA test (followed by Tukey test) with Origin version 8.0 software. A value of P < 0.05 was considered to be statistically significant.

**RESULT**

**Sperm concentration in the epididymis**

The results showed that a significant (P < 0.05) decrease in the sperm concentration (sperm/ml) in a group of mice that injected (I.P.) with 16 µg/kg.b.wt. of Tamsulosin hydrochloride as compared with the control group.

**Table 1 Effect of different concentrations of Tamsulosin on sperms numbers, motility, viability and abnormality. Asterisks indicate a significant difference from the control group**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sperm Concentration (x106/ml)</th>
<th>Motility of sperms (%) (mean±SD)</th>
<th>Dead sperms (%) (mean±SD)</th>
<th>Abnormalities of sperms (%) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>30.61±3.43</td>
<td>76.40±5.56</td>
<td>18.90±1.70</td>
<td>14.70±1.60</td>
</tr>
<tr>
<td>Tamsulosin (8µg/Kg, b.w.)</td>
<td>26.94±4.22*</td>
<td>70.00±5.7*</td>
<td>24.70±3.2*</td>
<td>20.90±1.30*</td>
</tr>
<tr>
<td>Tamsulosin (16µg/Kg, b.w.)</td>
<td>21.34±4.31*</td>
<td>57.90±4.8*</td>
<td>30.70±1.8*</td>
<td>29.70±2.90*</td>
</tr>
</tbody>
</table>
While, no significant (P < 0.05) decrease in the sperm concentration in the group of mice that injected (I.P.) with 8µg /kg.b.wt. of Tamsulosin hydrochloride (Table 1). Similar finding was observed when motility of sperms was measured (Table 1). Significant increase (P < 0.05) in percentage of dead sperms was found when mice injected with both concentrations of Tamsulosin hydrochloride (8 and 16 µg/kg.b.wt). The results of counting of abnormal sperms showed significant increase in percentage of abnormal sperms in testes of mice that injected with Tamsulosin hydrochloride as compared with control group (mice injected with PBS) (Table 1).

Percentage of dead sperms and abnormal sperms considered as an important criteria in the assessment of sperm function. Thus, the current study proved strongly, the negative effect of Tamsulosin hydrochloride on the sperm function and this effect was in a dose dependent manner. Figure 1 presented the morphological changes in the sperms that collected from mice post injected with Tamsulosin hydrochloride for 42 days. The changes were seen in sperm tail (bent tail) and in the sperm head (hummer head).

**Level of testosterone**

In present study, the effect of Tamsulosin HCl on the levels of testosterone was evaluated. Figure 2 showed the significant decrease in level of testosterone in sera of mice that injected with both concentrations of Tamsulosin hydrochloride (8 and 16 µg/kg.b.wt). The lowest level of testosterone was found in mice that injected with highest concentration of Tamsulosin hydrochloride. That is why; the effect of Tamsulosin hydrochloride on levels of testosterone was in a dose dependent manner.

**Effect of Tamsulosin hydrochloride on body and testis weight**

The current study was conducted to test whether the injection mice with different concentrations of Tamsulosin hydrochloride was associated with body and testis weight. The experimental animals were injected daily for 42 days with two concentrations of Tamsulosin hydrochloride (8 and 16 µg/kg.b.wt). Figure 3a showed a significant decrease in body weight of mice that injected with Tamsulosin hydrochloride as compared with control group (P <0.05). The lowest animal weight was observed in mice group that injected with highest concentration of Tamsulosin hydrochloride (16 µg/kg.b.wt.). The result of weight post injection with Tamsulosin hydrochloride was similar to results of body weight (Fig. 3b).

**Diameters of seminiferous tubules (DST) and interstitial space (IS)**

Ocular micrometer (stage micrometer) was used to measure the diameters of seminiferous tubules and interstitial space for test and control groups. Figure 4 showed the mean of DST and IS for mice that injected with two concentrations of Tamsulosin hydrochloride (8 and 16 µg/kg b.wt). The significant decrease (P < 0.05) was observed in DST in testes of mice that injected with Tamsulosin hydrochloride (8 and 16 µg/Kg b.wt) as compared with control group. In contrast, Figure 4b showed the significant increase in IS of testes of mice that injected with Tamsulosin hydrochloride (8 and 16 µg/Kg B.W) as compared with control.
The effect of Tamsulosin hydrochloride was dependent on the concentration. The highest effect of Tamsulosin hydrochloride was found in mice group that injected with the highest concentration. Histological study proved the significant difference between DST and IS of mice injected with PBS (control group) (Fig. 5 a and b) and DST and IS of mice injected with Tamsulosin hydrochloride (test group) (Fig. 5 c and d).

The lowest DST was found in testes of mice that injected with highest concentration of Tamsulosin hydrochloride (16 µg /kg.b.wt). Other histopathological remarks were found in testes of mice that injected with Tamsulosin hydrochloride, such as edema and necrosis (Fig. 5 c and d).

**DISCUSSION**

Tamsulosin hydrochloride is one of very important drug in reducing the enlargement of prostate in man (1). In this study, the side effects of this drug on number, motility, viability and morphological changes of sperm abnormality in testes of animal model post injection with Tamsulosin hydrochloride were studied. The sperms were collected from different groups of mice that injected (I.P.) daily for 42 days with different concentrations of Tamsulosin hydrochloride. We found that the high dose (16 µg/kg.b.wt.) of Tamsulosin hydrochloride yielded a significant reduction in number, motility and viability of sperm. While, mice that administrated with low dose (8 µg/kg.b.wt.) of Tamsulosin sperm showed only decrease in sperm viability. Both doses of Tamsulosin hydrochloride resulted significant increase in the

**Leydig’s cells clusters**

In current study, number and morphological changes of leydig’s cells clusters in animals that injected with either PBS (control group) or Tamsulosin hydrochloride (test group) were evaluated. The histological study helped in evaluating those items. Significant decrease in number of leydig’s cells clusters was observed in testes of mice that injected with two concentrations of Tamsulosin hydrochloride (8 and 16 µg/kg.b.wt.) as compared to control group. Significant presence of abnormal leydig’s cells clusters was found in testes of mice that injected with Tamsulosin hydrochloride (8 and 16 µg/Kg. B.W) versus to control group. Figure 7 a and b showed the high power field of testes that collected from control mice while, Figure 7 a and b represents testis of mouse that injected with PBS (control) [bars, 50 µm and 100 µm in (a) and (b), respectively], c, section in mouse testis collected from mouse injected with 16 µg/kg.b.wt. of Tamsulosin hydrochloride (bar, 200 µm). d, section in mouse testis collected from mouse after injecting with 8 µg/kg.b.wt. of Tamsulosin hydrochloride (bar, 100 µm). Arrows pointed at Leydig’s cell clusters. Abnormal structure of Leydig’s cell clusters was observed in mice groups that injected with different concentrations of Tamsulosin hydrochloride. Testes sections were prepared and stained for histological analysis with haematoxylin and eosin.

**Ocular micrometer (stage micrometer)** was used to measure the diameters of primary spermatocytes (DPS) and spermatids (DS) for test and control groups. Figure 6a showed a significant decrease in DPS of testes that collected from mice post injection daily for 42 day with Tamsulosin hydrochloride as compared with testes that collected from mice that injected with PBS (P < 0.05).

Both concentrations of Tamsulosin hydrochloride gave similar effect on DPS. Data of the effect of two concentrations of Tamsulosin hydrochloride on DS was presented in figure 6b. Significant decrease (P < 0.05) in DS was observed in testes of mice that injected with Tamsulosin hydrochloride (8 and 16 µg/kg.b.wt.). In current study, the effect of Tamsulosin hydrochloride on DS was in a dose dependent manner.
percentage of abnormal sperm. In current study, the negative effect of this drug on the sperm activity and fertility was highlighted. The effect of Tamsulosin hydrochloride on the histological changes of animal testes and level of testosterone in animal serum post injection with this drug was evaluated in current study. It was found that the negative effect of Tamsulosin hydrochloride on testosterone level and histological structure of testes. The injection animals with Tamsulosin hydrochloride reduced the body and testes weight. Another effect of this drug was reducing the DST and leydig’s cells number. In contrast, the injection with this drug increased interstitial spaces (IS).

Previous studies reported that Tamsulosin hydrochloride distributed easily to sexual organs such as testes and effect negatively on the number of sperm, semen volume, semen viscosity, transportation of sperm from the testes, size of the seminiferous tubules and ejaculation (17-21). It is well accepted, that the low sperm production was in relation with the reduction in size of the seminiferous tubules (21). Thus, all these reasons may explained the reduction in the numbers of sperms after treating with Tamsulosin hydrochloride. Naturally, the sexual hormones play a central role in decreasing spermatogenesis and the number of testes germinal cells (22). The process of spermatogenesis is highly sensitive to fluctuations in the environment, particularly hormones and temperature. Testosterone is required in large local concentrations to maintain the process, which is achieved via the binding of testosterone by androgen binding protein present in the seminiferous tubules (23).

The low percentage of motile sperm or sperm forward progression, or both, may be due to spermatozoon structure defect (24). The function of seminal vesicle is important for fertility parameters such as sperm motility, sperm chromatin stability, and immune-protection and may be changed in case of its hypo-function (25). The prostatic secretion makes spermatozoon motile and helps to neutralize vaginal acidity (26). The seminal vesicles and the accessory sex glands secrete fructose, which acts as a donor of energy to the spermatozoon. It is the major carbohydrate found in seminal plasma, and appears essential for normal sperm motility (27). This may be attributed to effect of the drug on leydig’s cells as well as on the T hormone as core hormone in the spermatogenesis and maturity of sperm. Decline in T hormone is causing the small number of living sperm as well as affects on the function of the epididymis and its tissues that lead to mature sperm and thus the effect leads to a negative impact on the percentage of living sperm (28). Sertoli cells comprise the main structural component of the seminiferous tubules. They are responsible for the structural support for germ cell development (29). Thus, any negative response of sertoli cells leads to the production of few sperm and lost its vitality (28).

Lenzi et al., (1998) demonstrated that the altered sperm morphology might reflect disturbances during spermiogenesis, spermiation and sperm passage through epididymis (30). It was accepted that sperm morphology is a sensitive indicator of overall testicular health because the sperm morphologic characteristics are determined during spermatogenesis (31). It was also found that any defect in spermatogenesis process might affect the sperms and lead to the production of abnormal or deformed sperm. This can also happen during the passage of sperm in the epididymis (32). That is why; the drug that studied in current study plays an important role in infertility of host in terms of reducing the activity and number of sperms. The negative effect of this drug was serious because it makes a serious histological damage in testes and reduces the levels of very important sexual hormones such as testosterone. Thus, the present study suggested that using this drug in spite of the positive effect on the enlargement of prostate it plays a serious role in reducing the fertility of host thus, this drug should be used in very limited cases and in very special status that specified by specialist physician.

References

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