Effect of Tamsulosin drug (flomax) on fertility in Albino male mice

A Thesis Submitted

By

Dina Khudhair Husain Ali

B.SC in Biology/Baghdad university 2006

To the College of Science, University of Baghdad, in partial fulfillment of the requirements for degree of master of science in

Biology /zoology

Supervised by

Prof. Dr. Sabah Naser Alwachi

February 2012A.D.                                                     Rabia alawal
1433A.H.
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمٌ لَّنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ

صَدَقَ الله العظيم
سُورَةَ البقرة (32)

Declaration
I declare that this thesis was prepared by Dina Khudhair Husain Ali, under my supervision at the Department of Biology, College of Science, University of Baghdad, in partial fulfillment of the requirements for degree of M.SC of Science in Biology/Zoology

Signature

Supervisor: Dr. Sabah N. Alwachi
Scientific Degree: Professor
Date: / / 

In view of the available recommendation, I forward this thesis for debate by the examination committee.

Signature

Name: Dr. Hayfa H. Hassani
Scientific Degree: Professor
Head of Department Biology
Date: / / 


Dedication

This work is dedicated to:

My father ......................who brightened my road to success

My mother..........................the source of kindness

My husband......................for his love and encouragement

My sisters and brothers......................for their supports

Dina
Acknowledgements

In the name of the God , the first who deserve all thanks and appreciation for granting me with well , strength and helps with which this research had been accomplished .

I would also like to express my special thanks to my respectful supervisor Prof. Dr. Sabah N. Alwachi for his patience , advise and encouragement throughout the period of the research .

I would like also to express my sincere gratitude & appreciation to the head of Department Biology , Collage of Science in University Baghdad and the her staff for their support and cooperation.

I would like to express my special thanks and debts to Dr. Jabar H. Al-Hilfy, Dr. Hind Husain , Majida H. Mehdy and Sura M. for their assistants during my research period.

I would also like to thank Dr. Mohammed joied from Pathology Department in College of Veterinary Medicine for his assistance.

Deep appreciation and thanks for my friends Nusaiba Amir , Shaman Nasser , Zainab Khudhair, Noor Jaafer , Israa Salem and Maha Nidal from Biology Department for their support and cooperation.

Finally , I would like to express my deep thanks and appreciation to my family especially my parents and husband Mohammed for their love and encouragement throughout my study .
Dina
Summary

The present study was conducted to investigate the effect of Tamsulosin hydrochloride on testicular function and fertility in albino male mice (*Mus musculus*). Thirty mature male mice with average body weight of 25-30g and 8-10 weeks of age were randomly divided in to three group (10 mice / group).

The first group was injected intraperitoneally (I.P) with distilled water as a control group and the other groups (second and third) were also injected I.P with two concentrations of Tamsulosin hydrochloride (8 , 16 µg /kg.b.wt) daily for a period of 42 days.

The results showed a significant (p<0.05) decrease in the body weights and rate of testes weights in male mice after the treatment with Tamsulosin hydrochloride for two concentrations compared with control group.

The treatment with Tamsulosin hydrochloride also showed a significant (p<0.05) decrease in percentage of sperm motility, viability and sperm concentrations in the epididymis at dose 16 µg /kg.b.wt compared with the control group but there was no significant (p<0.05) decrease in the percentage of sperm motility and concentrations at dose 8 µg /kg.b.wt, while there was a significant (p<0.05) decrease in the percentage of viability of sperm at the same dose (8 µg /kg.b.wt) compared with the control group. There was also a significant increase (p<0.05) in the percentage of morphologically abnormal sperm for two concentrations (8 , 16 µg /kg.b.wt) compared with the control group.

Histological examination of testes indicated that the treatment with the drug induced a significant (p<0.05) decrease in seminiferous tubules, primary spermatocytes, spermatid and decrease in number of the leydig’s cells.
clusters, while there was a significant (p<0.05) increase in the interstitial space between tubules comparing with the control group.

The histological examination also revealed an existence of some changes in the testes such as necrosis in the tubule and oedema in the interstitial spaces.

The treatment with all concentrations also caused a significant (p<0.05) decrease in the testosterone levels in blood serum comparing with the control group.

It was concluded from this investigation that Tamsulosin has a negative effect on testicular function (fertility).
# List of contents

<table>
<thead>
<tr>
<th>No.</th>
<th>Contents</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Summary</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>List of Contents</td>
<td>III</td>
</tr>
<tr>
<td></td>
<td>List of Figures</td>
<td>VI</td>
</tr>
<tr>
<td></td>
<td>List of Tables</td>
<td>VIII</td>
</tr>
<tr>
<td></td>
<td>List of Abbreviations</td>
<td>IX</td>
</tr>
<tr>
<td>1</td>
<td>Chapter one: Introduction and literatures review</td>
<td></td>
</tr>
<tr>
<td>1.1</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Literature Review</td>
<td>3</td>
</tr>
<tr>
<td>1-2-1</td>
<td>Alpha adrenergic antagonist (alpha blockers)</td>
<td>3</td>
</tr>
<tr>
<td>1-2-2</td>
<td>Tamsulosin Hydrochloride</td>
<td>4</td>
</tr>
<tr>
<td>1-2-2-1</td>
<td>Tamsulosin hydrochloride description</td>
<td>4</td>
</tr>
<tr>
<td>1-2-2-2</td>
<td>Trade names of Tamsulosin hydrochloride</td>
<td>7</td>
</tr>
<tr>
<td>1-3</td>
<td>Route of Tamsulosin Hydrochloride within the body</td>
<td>7</td>
</tr>
<tr>
<td>1-3-1</td>
<td>Absorption</td>
<td>7</td>
</tr>
<tr>
<td>1-3-2</td>
<td>Distribution</td>
<td>7</td>
</tr>
<tr>
<td>1-3-3</td>
<td>Metabolism</td>
<td>8</td>
</tr>
<tr>
<td>1-3-4</td>
<td>Interaction</td>
<td>8</td>
</tr>
<tr>
<td>1-3-5</td>
<td>Excretion</td>
<td>9</td>
</tr>
<tr>
<td>1-4</td>
<td>Side effect of Tamsulosin hydrochloride</td>
<td>9</td>
</tr>
<tr>
<td>1-5</td>
<td>Male reproductive system</td>
<td>11</td>
</tr>
<tr>
<td>1-6</td>
<td>Characteristics of the sperm</td>
<td>13</td>
</tr>
<tr>
<td>1-6-1</td>
<td>Sperm morphology</td>
<td>13</td>
</tr>
<tr>
<td>1-6-2</td>
<td>Sperm concentration</td>
<td>15</td>
</tr>
<tr>
<td>1-6-3</td>
<td>Sperm viability</td>
<td>15</td>
</tr>
<tr>
<td>1-6-4</td>
<td>Sperm motility</td>
<td>16</td>
</tr>
<tr>
<td>1-7</td>
<td>Spermatogenesis</td>
<td>17</td>
</tr>
<tr>
<td>1-7-1</td>
<td>Spermatocytogenesis (mitosis)</td>
<td>18</td>
</tr>
<tr>
<td>1-7-2</td>
<td>Spermatidogenesis (meiosis)</td>
<td>18</td>
</tr>
<tr>
<td>1-7-3</td>
<td>Spermigenesis</td>
<td>18</td>
</tr>
</tbody>
</table>
1-8 | Role of sertoli cell in spermatogenesis  | 19  
1-9 | Hormonal control of spermatogenesis  | 21  
1-10 | Testosterone hormone  | 23  
1-10-1 | Chemistry and biosynthesis of testosterone  | 23  
1-13-2 | Transport and metabolism of testosterone  | 26  
1-13-3 | Action of testosterone  | 27  
1-13-4 | The role of testosterone in spermatogenesis  | 28  

**Chapter two: Materials and Methods**

2 | Materials and Methods  | 29  
2-1 | Materials  | 29  
2-1-1 | Equipments and Apparatus  | 29  
2-1-2 | Chemicals  | 31  
2-2 | Methods  | 32  
2-1-1 | Preparation of solutions  | 32  
2-2-1 | Preparation of solutions  | 32  
2-2-1-1 | Preparation of histological solutions  | 32  
2-2-1-2 | Sperm count solutions  | 33  
2-2-1-3 | Preparation of Tamsulosin hydrochloride (Flomax) solution  | 33  
2-2-2 | Experimental Animals  | 33  
2-2-3 | Blood collection  | 34  
2-2-4 | Killing of the animals  | 34  
2-2-5 | Collection and Preparation of Sperms  | 34  
2-2-6 | Sperms parameters  | 35  
2-2-6-1 | Percentage of sperm motility  | 35  
2-2-6-2 | Sperm concentration  | 35  
2-2-6-3 | Percentage of sperm viability  | 35  
2-2-6-4 | Percentage of morphologically abnormal sperm  | 36  
2-2-7 | Histological study  | 36  
2-2-8 | Microscopic Examination  | 38  
2-2-9 | Statistical analysis  | 38  

**Chapter three: Results and discussion**

3-1 | Testosterone concentration in serum  | 39  
3-2 | Changes in testes and body weights  | 41  
3-3 | Sperm parameters  | 43
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-3-1</td>
<td>Sperm concentration in the epididymis</td>
<td>43</td>
</tr>
<tr>
<td>3-3-2</td>
<td>Percentage of sperm motility in the epididymis</td>
<td>47</td>
</tr>
<tr>
<td>3-3-3</td>
<td>Percentage of dead sperms in the epididymis</td>
<td>48</td>
</tr>
<tr>
<td>3-3-4</td>
<td>Percentage of morphologically abnormal sperms in the epididymis</td>
<td>50</td>
</tr>
<tr>
<td>3-4</td>
<td>The Histological studies</td>
<td>52</td>
</tr>
<tr>
<td>3-4-1</td>
<td>The diameter of seminiferous tubules Interstitial Space</td>
<td>52</td>
</tr>
<tr>
<td>3-4-2</td>
<td>Interstitial Space</td>
<td>54</td>
</tr>
<tr>
<td>3-4-3</td>
<td>Diameter of Leydig’s cells</td>
<td>56</td>
</tr>
<tr>
<td>3-4-4</td>
<td>primary spermatocytes</td>
<td>58</td>
</tr>
<tr>
<td>3-4-5</td>
<td>Spermatid</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Conclusions &amp; Recommendations</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>61</td>
</tr>
</tbody>
</table>
### List of Figures

<table>
<thead>
<tr>
<th>No</th>
<th>subjects</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Chemical structure of Tamsulosin hydrochloride (Patel &amp; Patel, 2010).</td>
<td>5</td>
</tr>
<tr>
<td>1.2</td>
<td>The intratesticular steroidogenic pathway for synthesis of testosterone (Rwerdlott Ronald et al., 2010).</td>
<td>25</td>
</tr>
<tr>
<td>3-1</td>
<td>Sperm morphology from mice treated with Tamsulosin Hydrochloride showing (A) normal sperm , (B) abnormal tail (bent tail) , (C) abnormal head (hummer head ) (E stain) 40X.</td>
<td>51</td>
</tr>
<tr>
<td>3.2</td>
<td>Section in mouse testes (control group), showing normal structure of seminiferous tubules ,diameter of seminiferous tubules (ST), interstitial space (IS) ,spermatide and primary spermatocyte (prim. Sp.) (H and E) X 10 for figure (A) ,40Xfor figure (B).</td>
<td>60</td>
</tr>
<tr>
<td>3.3</td>
<td>Section in mouse testes (treated group 8µg/KgB.W for A ,16 µg/KgB.W for B.) showing abnormal structure of seminiferous tubules. diameter of seminiferous tubules (DST), interstitial space (IS), oedema (E) and necrosis (N) (H and E) X 10.</td>
<td>60</td>
</tr>
<tr>
<td>3.4</td>
<td>Section in mouse testis (control group) showing normal structure of Leydig’s cell clusters (H &amp; E X 40)</td>
<td>61</td>
</tr>
<tr>
<td>3.5</td>
<td>Section in mouse testis (treated group with A 8µg /kg.b.wt. , B 16 µg /kg.b.wt) showing abnormal structure of Leydig’s cell (H &amp; E X 40).</td>
<td>61</td>
</tr>
<tr>
<td>NO.</td>
<td>Subjects</td>
<td>Page</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.1</td>
<td>The equipments and apparatus used .</td>
<td>29</td>
</tr>
<tr>
<td>2.2</td>
<td>Chemicals used</td>
<td>31</td>
</tr>
<tr>
<td>3.1</td>
<td>Effect of Tamsulosin hydrochloride (8µg /kg.b.wt. and 16µg/kg.b.wt.) on serum Testosterone level (ng/ml) in male mice</td>
<td>39</td>
</tr>
<tr>
<td>3.2</td>
<td>Effect of different concentrations of Tamsulosin HCl (8µg/KgB.W and 16µg/Kg B.W ) on the rate of testes and body weights</td>
<td>43</td>
</tr>
<tr>
<td>3.3</td>
<td>Effect of Tamsulosin (µg /kg.b.wt. ) on sperms parameters in male mice.</td>
<td>46</td>
</tr>
<tr>
<td>3.4</td>
<td>Effect of Tamsulosin (µg /kg.b.wt. ) on diameter of seminiferous tubules and interstitial space in male mice .</td>
<td>55</td>
</tr>
<tr>
<td>3.5</td>
<td>Effect of Tamsulosin 8µg/KgB.W and 16 µg /kg.b.wt. on diameters of primary spermatocyte ,spermatids .</td>
<td>57</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>AAG</td>
<td>Primarley Alpha -1-acid Glycoprotein</td>
<td></td>
</tr>
<tr>
<td>AR</td>
<td>Adrenoceptor</td>
<td></td>
</tr>
<tr>
<td>ABP</td>
<td>Androgen Binding Protein</td>
<td></td>
</tr>
<tr>
<td>BPH</td>
<td>Benign Prostate Hyperplasia</td>
<td></td>
</tr>
<tr>
<td>CPPS</td>
<td>Chronic Pelvic Pain Syndrome</td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>Chronic Prostatitis</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>De-oxy Ribo Nucleic Acid</td>
<td></td>
</tr>
<tr>
<td>DHT</td>
<td>Dihydrotestosterone</td>
<td></td>
</tr>
<tr>
<td>DHEA</td>
<td>De Hydro Epi Androsterone</td>
<td></td>
</tr>
<tr>
<td>E₂</td>
<td>Oestadiol</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>Follicle Stimulating Hormone</td>
<td></td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotrophin Releasing Hormone</td>
<td></td>
</tr>
<tr>
<td>GBG</td>
<td>Gonadal steroid Binding Globulin</td>
<td></td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric Acid</td>
<td></td>
</tr>
<tr>
<td>IP</td>
<td>Intraperitonealy</td>
<td></td>
</tr>
<tr>
<td>IU</td>
<td>International Unit</td>
<td></td>
</tr>
<tr>
<td>ICSH</td>
<td>Interstitial Cell Stimulating Hormone</td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
<td></td>
</tr>
<tr>
<td>LUTS</td>
<td>Lower Urinary Tract Symptoms</td>
<td></td>
</tr>
<tr>
<td>In</td>
<td>Half chromosome number</td>
<td></td>
</tr>
<tr>
<td>PSA</td>
<td>Prostate - Specific Antigen</td>
<td></td>
</tr>
<tr>
<td>RE</td>
<td>Retrograde Ejaculation</td>
<td></td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>Roswell Park Memorial Institute-1640</td>
<td></td>
</tr>
<tr>
<td>STAR</td>
<td>Steroidogenic a cute Regulatory protein</td>
<td></td>
</tr>
<tr>
<td>SPR</td>
<td>Stone Passage Rates</td>
<td></td>
</tr>
<tr>
<td>S.T.</td>
<td>Seminiferous Tubules</td>
<td></td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
<td></td>
</tr>
</tbody>
</table>
Chapter One

1: Introduction and Literatures Review

1-1: Introduction

Alpha blockers represent one of the most common therapies for chronic pelvic pain syndrome / chronic prostatitis (CPPS/CP). The rationale is based on presence of varied receptors in the lower genitourinary tract. Starting in the early 1970s, reports suggested that certain patients might benefit from treatment with alpha-blockers. Recently, alpha blockers had side effects that severely limited their utility (Lee et al., 2008).

Tamsulosin hydrochloride, an alpha blocker, works by blocking nerve ending called alpha receptors. This will relax the smooth muscles of the prostate and bladder. Tamsulosin is used also to treat benign prostate hyperplasia (BPH) in men which can be recognized by enlargement of prostate and also known as an enlarged prostate which causes difficulties with urination (Sonnberg, 2003). It was discovered by research team led by Toichi Takenaka in 1980 at first as a potential anti-hypertension drug. Later it was used to treat several disease cases, benign prostate hyperplasia (BPH), lower urinary tract symptoms associated with BPH and urine retention (Hara, 2003).

The chemical structure of Tamsulosin hydrochloride is composed of methoxy - benzenesulfonamide which differs from other alpha blockers such as Alfuzosin, Terazosin and Doxazosin which are quinazolin derivatives (Schulman, 1996).

The abnormal ejaculation of semen is a typical but infrequent side effect of some α1-adrenoceptor antagonists, particularly those with selectivity for α1A-adrenoceptors such as Silodosin and Tamsulosin. Recent clinical studies suggested that this represents a relative ejaculation rather
than a retrograde ejaculation. The reduced ejaculation and related male infertility is shown to be caused by an impaired function of the vas deferens rather than by alterations in sperm formation number or function (Michel, 2007).

Comparing to other $\alpha$-antagonists, Tamsulosin hydrochloride has greater specificity for $\alpha$-1 receptors in the human prostate which do not affect receptors on blood vessels. It is the most frequently prescribed medication for the treatment of lower urinary tract symptoms (Bari et al., 2011).

Tamsulosin has also affected the sexual function in men, which can cause males to experience retrograde ejaculation. In males, retrograde ejaculation occurs when the fluid to be ejaculated, which would normally exit the body via the urethra, is redirected to the urinary bladder. Normally, the sphincter of the bladder contracts and the ejaculate goes to the urethra, the area of least pressure. In retrograde ejaculation, this sphincter does not function properly (Roehrborn et al., 2008)

$\alpha$1-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 (Ratensooria and Wadsworth, 2009).

$\alpha$-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 and cause infertility as a results of an ejaculation (Nieschlag and Behre, 2009).

**Aims of the study:**

Studying The physiological and the histological effects of Tamsulosin hydrochloride on testicular functions of albino male mice (fertility).
1-2: Literature Review

1-2-1: Alpha adrenergic antagonist (alpha blockers).

Adrenergic receptors were originally divided into alpha and beta subtypes, with alpha further classified into alpha-1 and alpha-2 receptors. Currently, there are three subtypes in the alpha1 subfamily: 1a, 1b, 1d. Differences in receptor amino acid sequence among these subtypes alter the binding properties of specific agonist and antagonist. In human prostate cells, the alpha-1a receptor type is the most prevalent type, making the targeting of this subtype therapeutically useful (Blanchad and Abu Baker, 2010).

Docherrty (2010) has been pointed that alpha1-Adrenoceptors (AR) can be divided into alpha 1a, alpha 1b and alpha 1d adrenoceptors. A fourth alpha 1-adrenoceptor, the alpha 1L-receptor, represents a functional phenotype of the alpha 1a-adrenoceptor.

Alpha receptors were further grouped into α1 and α2 subtypes. The α1 receptors are located primarily in the smooth muscle of the prostate gland and bladder neck but also to the lesser degree in the vascular system (Tewari and Naryan, 1999). Molecular studies showed that the α1 subtypes represent 70% of α1 receptors in the prostate gland (Chen et al., 2000).

The first generation of alpha adrenergic antagonists (i.e. phenoxybenzamine) blocked both alpha 1 and alpha 2 receptors in the prostate and vasculature (Lee, 2003).

Second generation of alpha-adrenergic antagonist have greater affinity for alpha 1 receptors than for alpha 2 receptors, leading to an improved side effect profile. These second generation agents include Prazocin, Terazocin, Doxazocin and Alfuzocin (Debruyne, 2000).

Tamsulosin hydrochloride, the third generation alpha adrenergic antagonists, was selective adrenergic receptor blocker with high binding affinity for the alpha 1d receptor (Nobile et al., 1997 and Kuritzky, 2005).
Tamsulosin is selective α1- adrenergic receptor blocker with a tenfold greater affinity for α1a and α1d receptors than for α1b adrenergic receptors. This distinguishes Tamsulosin from other blockers Terazocin, Doxazocin and Alfuzocin that all have similar affinity for all three α1-receptor subtypes. Study has shown that alpha (α1a AR) selective antagonists relieve bladder based irritability symptoms, in contrast blockade of the alpha 1b- adrenergic receptors leads to orthostatic hypotension; a side effect associated with non select alpha blockers (Zelefsky and Stephen, 2010).

1-2-2:Tamsulosin Hydrochloride
1-2-2-1: Description of Tamsulosin Hydrochloride

Tamsulosin hydrochloride is an antagonists of alpha 1a adrenoceptors in the prostate. Tamsulosin hydrochloride is (R)-5-[[2-(2-ethoxyphenoxy) ethyl] amino] propyl]-2-methoxybenzenesulfonamide, monohydrochloride. Tamsulosin HCl. found as a white crystals that melt with decomposition at approximately 230°C. it is sparingly soluble in water and in methanol and practically insoluble in ether (Patel & Patel, 2010).

The empirical formula of Tamsulosin HCl. is C20H28N2O5S.HCl and the molecular weight it HCl. is 444.98. and its structural formula is:
Tamsulosin hydrochloride belongs to a class of alpha-1a receptor antagonists that have performed selectivity for prostate smooth muscle, although it does have some binding to vascular smooth muscle receptors as well. Tamsulosin binding is remarkable for its significantly lower degree of non-specific binding compared to other alpha-receptor antagonists (Naryan et al., 2000).

The symptoms associated with BPH are related to bladder outlet obstruction, which is comprised of two underlying components: static and dynamic.

The static component is related to an increased in prostate size caused in part, by proliferation of smooth muscle cells in the prostate stroma. The dynamic component is a function of an increase in smooth muscle tone in the prostate and bladder neck leading to constriction of the bladder outlet. Smooth muscle tone is mediated by the sympathetic nervous stimulation of alpha-1 adrenoceptor, which are abundant in the prostate, prostatic capsule, prostatic urethra, and bladder neck (Boheringer Ingelheim, 2009).
The combined uses of Longbishu and Tamsulosin hydrochloride is effective and safe in the treatment of chronic a bacterial prostatitis (Huil and Hai-qiu, 2007).

Tamsulosin treatment is recommended for patients with the stone diameter smaller than 8mm because of its feasibility and effectiveness (MengYuan et al., 2009).

Alpha-1 adrenocepter antagonists were considered the first line of treatment for managing lower urinary tract symptoms associated with BPH (Grinwan et al., 2010).

Tamsulosin was superior to placebo in providing symptomatic relief in men with chronic prostatitis or chronic pelvic pain syndrome particularly in those with more sever symptoms (Nickel et al., 2004 and Yamanishi et al., 2009).

Tamsulosin therapy for un complicated distal urethral calculi augments stone passage rates (SPR) shortens passage time and decrease need for analgesia. Particularly, Tamsulosin shortens the passage time for smaller stones and augments the passage rate for larger stones (Abdel-Meguid et al., 2010).

Currently, Sasaki et al., (2008) also found that α1a adrenocepter antagonists could become useful medication for stone passage in urolithiasis patients. In fact, Tzortzis et al., (2009) noted that "medical expulsive therapy using α1-adrenoceptor antagonists such as Tamsulosin, augments the stone expulsion rate compared to standard therapy for moderately sized distal urethral stones."
1-2-2-2: Trade names of Tamsulosin hydrochloride

Tamsulosin is launched in Japan in August 1993 under the trade name of Harnal (Hara, 2003). Tamsulosin trade names are Alna, Amsulosin hydrochloride, Expras, Flomax, Omic, Omix, Omnic, Pradif, Scotex and Urolosin (Miline, 2005).

1-3: Route of Tamsulosin Hydrochloride within the body

1-3-1: Absorption

Tamsulosin is absorbed in the intestine and almost completely bioavailable. Both the rate and extent of absorption of Tamsulosin were reduced when taken within 30 minutes of a meal.

Tamsulosin shows dose proportional plasma exposure after single dose of it in the fasted state, plasma concentration of Tamsulosin peak at around 6 hours and in the steady state, which is reached by day 5 of multiple dosing (Anonymous, 2010).

After oral administration, more than 90% of Tamsulosin is absorbed. However, its administration with food decreases bioavailability by 30% (Brophy et al., 2010).

The absorption of the drug is also variable and the onset of action is 1-2 hours (Bryant and Kathleen, 2011). After oral administration, peak plasma concentration is reached in 5 days (Narayan et al., 2000).

Absorption of Tamsulosin hydrochloride (Flomax) capsules 0.4 mg is essentially complete (>90%) following oral administration under fasting conditions, the apparent half-life of Tamsulosin hydrochloride is approximately 9 to 13 hours in healthy volunteers and 14 to 15 hours in the unwell healthy man (Chong Kim, 2009).

1-3-2: Distribution

After administration of tamsulosin in healthy male adults, it distributed to extra cellular fluid in the body.
In addition, whole body studies in mice, rat and dogs indicate that Tamsulosin is widely distributed to most tissue including kidney, prostate, liver, gall bladder, heart, aorta, brown fat and minimally distributed to the brain, spinal cord and testes.

Tamsulosin is extensively bound to human plasma protein (94% to 99%), primarily alpha-1-acid glycoprotein (AAG) in human, with linear binding over a with concentration range (20 to 600 ng/ml). The results of in vitro study indicate that the binding of Tamsulosin to human plasma protein is not affected by amitriptyline and diclofenic (Kim et al., 2001).

1-3-3: Metabolism:

Tamsulosin hydrochloride is extensively metabolized by cytochrome p450 enzyme in the liver and less than 10% of the dose is excreted in urine unchanged. Inhibition of hepatic drug-metabolizing enzyme may lead to increase exposure to Tamsulosin.

The metabolites of Tamsulosin HCl. undergo extensive conjugation to glucuronide or sulfate prior to renal excretion. However, results of the in vitro testing of the Tamsulosin HCl. interaction with diclofenac and warfarin were equivocal (Brophy et al., 2010).

1-3-4: Interaction

Kluwer (2011) noticed that there are potential drug interactions with Tamsulosin HCl.
1) Alpha blockers: additive effect of both drugs.
2) Cimitidin: risk of decreased Tamsulosin clearance.
3) CYP2D6 inhibitors: such as Fluoxetine, Paroxetine and Terbinafine
4) CYP3A4 inhibitors: such as Crythromycin, Ketoconazole
5) Phosphodiesterase inhibitors increased risk of hypotension
1-3-5: Excretion

Approximately 10% of the drug is excreted unchanged in the urine (Brophy et al., 2010). Bryant and Knights (2011) shows that the excretion of Tamsulosin occurs via urine & feces. Hepatic metabolism with metabolite excreted in urine (Narayan et al., 2000).

The administration of Tamsulosin hydrochloride to 4 healthy volunteers showed that 97% of the administered was recorded with urine (76%) representing, the primary route of excretion compared to feces (21%) over 168 hours. The elimination half life of Tamsulosin HCl in plasma ranged from 5 to 7 hours. Because of absorption rate controlled pharmacokinetics with Flomax capsule (Boehringer Ingelheim, 2009).

1-4: Side effect of Tamsulosin hydrochloride

A starting point of the mice studies was the observation that α1a, α1b α1d adrenergic receptor reduced fertility in mice; experiment with different combination of male and female wild-type of rat indicated that the impaired ability to induce pregnancy resulted from male infertility (Michel, 2007).

In the rat, the cauda epididymis contain post functional α1-but not α2 adrenoceptor, whereas in the guinea pig, the cauda epididymis contain both α1 and α2 adrenoceptor (Queriroz et al., 2001).

Studies by Bhathal et al., (1974) and Ratensooria & Wadsworth, (2009) have shown that the rats epididymis is signifcically affected by α1a adrenoceptrers blockade. In vivo treatment α1a adrenoceptrers antagonist induced a decrease in ejaculatory capacity associated with reduction in the fertilization ability of the sperm, suggesting a role for the sympathetic nervous system infertility maintenance via α1A adrenoceptrers (Griwan et al., 2010). However, dizziness, rhinitis, general weakness and fatigue have been reported with Tamsulosin (Lowe, 2005 and Kaplan, 2005).
Tamsulosin does not disturb homodynamic in patient with benign prostate enlargement (Nieminen et al., 2007). α1-adrenoceptor blockade has a potent anti fertility effect in male rats (Ratensooria and Wadsworth, 2009).

One of the adverse side effect commonly reported with different α1 blockers was sexual dysfunction. This sexual dysfunction has been related to changes in ejaculation (either retrograde or diminished ejaculation). Moreover, α1 blockers differ in their likhood of causing abnormal ejaculation. In long term open label extension study, 30% of patient treated with Tamsulosin reported abnormal ejaculation. In contrast, incidence of abnormal ejaculation non related to the use of nonselective α1-blockers such as Doxazosin, Terazocin or Alfuzosin generally were lower than 1.5% (Kaplan, 2009).

In another study, Hellstrom and Sikkasc (2006) examined the effect of Tamsulosin and the non selective α-blocker Alfuzosin on ejaculatory function in healthy volunteers and found that Tamsulosin 0.8 mg/day caused markedly reduced ejaculate volume in 90% of patients and an ejaculate in 35% of participants. These ejaculatory disorders with Tamsulosin were not attributed to retrograde ejaculation. In contrast, an ejaculation was not observed in any subjects in the Alfuzosin or placebo groups.

However, analysis of post climatic urine samples showed no increase in sperm counts, suggesting that retrograde ejaculation (RE) did not occur. In addition, others have theorized that α1A-selective blockers may results in reduced or absent seminal emission via inhibition of smooth muscle contraction (Michel, 2007). Also postural hypotension may be caused by Tamsulosin (Naryan et al., 2000).

Tamsulosin has been implicated in decreased contractility of seminal vesicles, vas deferens as well as acting of central nervous system and theoretically affecting neurostimulasion of ejaculation (Poch and Sigman,
2010). Also Intraoperative floppy iris syndrome occurred in 67% of the patients treated with Tamsulosin (Bidaguren et al., 2007).
1-5: Male reproductive system

The human male reproductive system consists of testes, epididymis, ductus deferences, urethra, scrotum and penis as well as the accessory glands: seminal vesicles, prostate and bulb urethral or cowper's gland (Lingappa and Farey, 2000).

The testes are two small avoid organs reside in the scrotum outside the body. Each testicle is surrounded by three layers of tissues: tunica vaginalis, the tunica albuginea and the tunica vasculosa (Wangh and Grant, 2001).

After puberty, testes produce male sex cells called spermatozoa or sperms and a male sex hormone called testosterone (Brichfored, 2009).

There are 2000-3000 lobules in each testis, each lobule consists of 1-4 convoluted seminiferous tubules composed of germinal epithelial and Leydig's cells that secrete testosterone into the bloodstream (Ganong, 2003; Alwachi, 2008).

The seminiferous tubule was lined by two types of cells. The first type is called spermatogenic cell (spermatagonia) which divide by meiosis to produce immature sperms or spermatides. Each spermatid contains a haploid number of chromosomes while the second type called sertoli cells, which secrete oestrogen and nutrient which prolong the survival of the sperms (Aspinall et al., 2009).

Each epididymis is a tightly coiled, thread like tube about 6 meters long, it emerges from the top of the testis and then courses upward to become vas deferens (Shier et al., 2000). The epididymis is an organ in which sperms undergo final maturation and storage prior to ejaculation (Queiróz et al., 2002).

The epididymis is divided into three main regions a head (caput), a body (corpus) and long tail (cauda) (Romer et al., 1977 and Seely et al., 1998). Spermatozoa are capable of surviving longer in the epididymis than in any other segment of the reproductive tract and the cauda is the major site of
storage spermatozoa in the duct system (La Barbera, 1996). The development of the epididymis and accessory gland are under the control of androgens (Kocak et al., 2001).

Each vas deference also, called ductus deferences, is muscular tube about 45 cm long (Shier et al., 2000). The enlarge part of vas deference called ampulla. The ampulla of the ductus deferences with the proximal region of the seminal vesicle to form the terminal portion of the reproductive duct system called ejaculatory duct (Mckinley and O’loughlin, 2006).

Accessory gland produce little fluid to transport the sperms from the testes to the female tract and to aid their survival (Aspinall et al., 2009).

The alveoli of the seminal vesicles are lined with a pseudostratified epithelium whose cells contain numerous granules and clumps of yellow pigment. Some of the epithelial cells have flagella. The secretion of the seminal vesicles is yellow, visicous liquid containg globulin and fructose. This secretion provides the majority of the ejaculate volume (Linda and Schust, 2010).

The prostate gland is a complete encapsulated a walnut measuring a approximately 2 cm by 3 cm. This gland includes submucosal gland that produce mucin and more than 30 tubuloaleveolar gland that open directly through numerous duct in to the prostatic urethra (Mckinley and O'loughlin, 2006). The function of it is to store and secrete a slightly alkaline fluid, milky or white in appearance (Huggins et al., 1942). Usually, the prostate fluid contributed 37–44% of the whole ejaculate and the seminal vesicle contributed 55–61% (Ndovi et al., 2007).

The bulbuorethral gland (also called cowper's gland) is small, paired bulb or membranous urethral glands that may be mistaken for prostatic carcinoma in biopsy specimens. They are composed of closely packed uniform acine lined by cytologically benign cells with a abundant apical
mucinous cytoplasm campers embedded in smooth muscle, mimicking the infiltrative pattern of prostate cancer (Bostwick and Cheng, 2008).

Each gland produces a clear, viscous secretion. This fluid helps to lubricate the urethra for spermatozoa to pass through, neutralizing traces of acidic urine in urethra and helps flush out any residual urine or foreign matter. It is possible for this fluid to pick up sperm, remaining, in the urethral bulb from previous ejaculation and carry them out prior to the next ejaculation. The cowper’s gland also produces some amount of Prostate - Specific Antigen (PSA) and cowper's tumors may increase PSA level that makes prostate cancer suspected (Chughtai et al., 2005).

1-6: Characteristics of the sperm
1-6-1:Sperm morphology

Mammalian spermatozoa are highly differentiated cells. Their particular structural organization results from complex morphogenetic changes during spermiogenesis. These include:

(I) The formation of the acrosome derived from Golgi vesicles.
(II) The formation of an axoneme from the distal centriole, surrounded by periaxonemal structures, which together form the flagellum.
(III) The migration and development of mitochondria in a helicoidal sheath around the axoneme in the midpiece.
(IV) The formation of an anisotropic sperm head (with a ellipsoid face and a pear-shaped profile) containing a highly condensed nucleus.
(V) The disappearance of the vast majority of the cytoplasm (Auger, 2010).

The normal sperms head should be oval in shape. In normal sperm there must be no defect in neck, mide piece and tail and no cytoplasmic droplet, more than one –third the size of a normal sperm is the head (Mendeluk et al., 1997).
Normal spermatozoa sought in the ejaculate are those that are biologically selected by reaching endocervical mucus. These spermatozoa recovered and stained in air dried fixed smears, exhibit no defect of the head or tail (Garrett et al., 1997).

Sperms have regular oval shaped head with an intact and slender mid piece and principal piece displaying no breaks. The acrosome is clearly visible and covers 40-70% of the sperm area. The vacuoles do not exceed 20% of the acrosomal area and not present in the post acrosomal region. The cytoplasm occupying <30%. The men were considered normal when produce few potentially fertilizing spermatozoa which bind to zona pellucida (Liue et al., 2003).

The morphologic examination of spermatozoa is a sensitive indicator of the quality of spermatogenesis and fertility (Kruger et al., 1988).

The defect in the process of spermatogenesis lead to abnormalities known as the primary abnormalities that may occur in the sperm head , including the massive head (Macrocephalic), small head (Microcephalic), double head and round head (WHO, 1992).

1-6-2: Sperm concentration

This term should be used instead of sperm density to a void confusion with the specific gravity of spermatozoa (Brinsden, 2005).

The concentration or the total number of sperm is a marker of the integrity of testicular function. If the number of sperms are small by usual fertility standard, but their motility and morphology are excellent, then fertility might be normal. But if the number of sperm are large but their motility and morphology are poor, the person might be infertile (WHO, 1980). In general, sperms concentration in normal men ranges from about 20X10^6 / ml to 200X 10^6 / ml. There is a large variation among and within people (WHO, 1987).
Normal sperm concentration have been reported to range between 20-250 million per milliliter. Azoospermia is a complete absence of sperm. Sperm count less than normal may be due to chromosomal disorders, ductal obstruction, drugs, gonadotropin deficiencies maturation arrest, pituitary gland disorders, radiation, renal failure and hormonal abnormality (Mundt and Shanahan, 2011).

1-6-3: Sperm viability

Determination whether non motile sperms are viable or non viable is important in establishing a cause for infertility in males. The membranes of dead sperm are damaged and can easily take up eosin stain. The membranes of viable sperm remain intact and do not allow eosin stain to penetrate leaving the sperm colorless (they will appear white). Eosin stain can be used alone or in conjugation with nigrosin stain, which provide dark back ground against which the red colored dead sperm and white colorless sperms can be visualized. Sperms that are non motile may be a live but may have effects of the tail piece. However, the proportion of viable sperms, dead sperms can not demonstrate motility. Normally >75% of sperm are viable (Mundt and Shanahan, 2011).

Reduced percentage of motility with high percentage of viable sperms may reflect structural or metabolic abnormalities of sperms that are derived from abnormalities in testicular function or anti motility factor in the seminal plasma (Siegel, 1993).

There are two general approaches to viability testing, traditional staining versus the hypo osmotic swell test. The advantage of this approach is that the sperms remain viable and can be used for intracytoplasmic sperm injection (Hsiao and Schlegal, 2011).
1-6-4: Sperm motility

Motility is the amount of flagellar motion and forward motion that a sperm makes. The most commonly system of motility grades used as follow:

1- Zero -0.4 indication no motility
2- One denotes sluggish motion with no forward progression.
3- Two denotes slow forward progression.
4- Three Indicates sperm moving in a straight line at great speed. If motility is low (<30%) a viability stain should be performed (Hsiao and Schlegal, 2011).

Fertilization of ovum is dependent on the ability of sperm to reach and unite with it. Motility should be evaluated within one hour of specimen collection, because motility will decrease over time. The movement of sperm is evaluated and may be subjectively estimated or counted in to three categories. These categories may be called high motile, low motile, non motile (Mundt and Shanahan, 2011).

The presence of progressively motile sperm in the ejaculate is critical to ensure an adequate sperm transport and fertilization. Sperm motility is considered as compromised if the percentage of forward progressive sperm falls bellow 50% within 60 min of sample collection (Agarwal and Said, 2011).

The presence of low sperm motility asthenozoospermia could occur as a result of prolonged time to processing of collected samples. Samples container may be toxic to the sperm and sample exposure to extreme temperature or sun light may result in decreased sperm motility. Long period of abstinence also proved to be a cause of a asthenozoospermia include sperm axonemal deformities, excessive leukocyte and unknown factors. Asthenozoospermia is also most commonly seen with anti sperm antibodies. The observation of sperm clumping combined with low sperm motility is a further indication of the presence of anti sperm antibodies (WHO, 1999).
Sperm motility is usually rated in two ways: the fraction or percentage of all sperm that are moving and the quality of sperm movement which include active motile, low motile and non motile (Turek, 2000).

1-7: Spermatogenesis

Spermatogenesis is a process of generating mature sperms with halve the number of chromosomes (haploid) produced from germ cell precursors with full number of chromosomes (diploid) (Wang et al., 2009).

The efficacy of spermatogenesis is being reflected by daily sperm production in adulthood is known to be determined by the total number of functional sertoli cell (Sharp, 1994).

The duration of spermatogenesis is considered to be constant and it is vary considerably in species thus, it is measured about 64 days in man (Heller and Clermont 1963) and about 35 days in mice (Berne and Levy, 1993).

Spermatogenesis happened within several structures of the male reproductive system. The initial stages occur within the testes and progress to the epididymis where the developing gametes mature and are stored until ejaculation. The seminiferous tubule of the testes is the starting point for this process (Middendorff et al., 2002).

Spermatogenesis process may be divided in to three phases: spermatocyto- -genesis (mitosis) , spermatidogenesis (meiosis) and spermiogenesis (Schlegel and Chang, 1998).

1-7-1: Spermatocytogenesis (mitosis)

Spermatocytogenesis is the process of which male spermatogonia become progressively modified and enlarged to form large primary spermatocytes (Guyton and Hall, 2006).
1-7-2: Spermatidogenesis (meiosis)

In this phase, each primary spermatocyte undergoes a reduction division (first meiotic division) to yield two haploid secondary spermatocytes which bear the (1n) version chromosome number (Guyton and Hall, 2006).

Each secondary spermatocyte then undergo the second meiotic division to form two haploid rounded spermatids which are located near the lumen of seminiferous tubules, after that no divisions occur and spermatides enters the phases of morphological changes (Berne and Levy, 1993).

The special type of nuclear division, which forms haploids gametes, is termed meiosis. The mitotic phase terminates at the primary spermatocytes, which at first resembles the cytological characteristics of spermatogonia from which they a rise (Gupta, 2005).

1-7-3: Spermiogenesis

The final phase of differentiation of male germ cells prior to their release from seminiferous tubule. During this phase the round spermatides which are relatively less an differentiated haploid germ cell, undergo complex morphological, biochemical and physiological changes that result in the formation of a symmetrical flagellated spermatozoa (Gupta, 2005 and de Rooij, 2001).

During spermiogenesis, the spermatides begin to grow a tail and develop a thickened mid-piece, where the mitochondria gather and form an axoneme. Spermatide DNA also undergoes packaging, becoming highly condensed. The Golgi apparatus surrounded the condensed nucleus, becoming the acrosome, one of the centrioles of the cell elongates to become the tail of the sperm (de Rooij, 2001).

The end result of spermiogenesis phases, two mature spermatozoa per each transformed spermatide, then released in the lumen of seminiferous tubules by the process of spermiation which is managed by sertoli cells, that
many studies had shown the differentiation of spermatides into spermatozoa occur within the cytoplasm of these cells (Berne and Levy, 1993).

Maintenance of spermatogenesis requires testosterone androgen production, in turn, is regulated by the luteinizing hormone (LH). Follicle stimulating hormone (FSH) required for development of the seminiferous epithelium and initiation and maintenance of the mitotic phases of spermatogenesis (La Barbera, 1996).

1-8: Role of Sertoli cell in spermatogenesis

Sertoli cell has served important roles in spermatogenesis support and nutrition of the developing germ cells; compartmentalization of the seminiferous tubules by right junction, which provides a protected and specialized environment for the developing cells; controlled release of mature spermatides into the tubular lumen (spermiation); secretion of fluid, protein and several growth factor and phagocytosis of the degenerating cells and phagocytosis of the excess cytoplasm (residual body) that remain from released sperm (Cheng, 2008).

Sertoli cell also mediates the action of FSH and LH stimulated testosterone production in the testes. FSH induce sertoli cell to produce nutrient for sperm, LH induce Leydig cell to produce T which has important role in maintaining normal spermatogenesis (Sharp, 1994).

Sertoli cells provide physical support for the germ cells is a previous from their topographic relationship. Sertoli cell show both configurational relationship and specialized contact with the developing germ cells. The configurational relationship and contacts suggest that sertoli cell originates four to five different germ cell generation in one tubule, so that they all evolve synchronously to form the next stage. They also suggest facilitation of germ cell movement upward as each layer of cells matures (Barth and Oka, 1989).
Although it is suggested that FSH plays a major role initiation, maintaining quantitatively normal spermatogenesis, whereas FSH play qualitative role and is not strictly necessary for fertility (Sharp, 1994).

Sertoli cell may control the temporal and special organization of spermatogenesis these observation on spermatogenesis imply a remarkable degree of temporal and spatial organization among the spermatogenic cells. The cytoplasm of an adjacent sertoli cells are in continuity with one another via extensive gap junctional contact. In addition, each sertoli cell spans the tubule from per tubular basement membrane to lumen for communication (Johnson and Everit, 2007).

Sertoli cell works to regulate the proliferation of primary germ cells primordial (germ cell) and continuity of the spermatogenesis where sertoli cells processed testicular blood barrier by strong connected junctions and are thus equipped with a small environment to facilitate the spermatogenesis (Haywood et al., 2004).

1-9: Hormonal control of spermatogenesis

Spermatogenesis in mammals requires the actions of a complex assortment of peptide and steroid hormones, each of which plays an important role in the normal functioning of the seminiferous epithelium. These hormonal messengers are critical not only for regulation of male germ cell development but also for the proliferation and function of the somatic cell types required for proper development of the testes (Holdcraft and Braun, 2004).

These include the interstitial steroidogenic leydig’s cells whose primary function appears to be the production of testosterone. The myoid cells that surround the seminiferous tubules provide physical support and contractile motion to these structures and the sertoli cells, whose direct contact with proliferating and differentiating germ cells, within the seminiferous tubules,
makes them essential for providing both physical and nutritional support for spermatogenesis (Meng et al., 2005).

The spermatogenesis process and all other aspects of male reproductive function depend on the presence of reproductive hormones produced by the hypothalamus, anterior pituitary and testes (Tilbrook and Clarke, 2001).

Hormonal regulation of spermatogenesis is organized as a control circuit with a negative feed-back mechanism involving the hypothalamus, pituitary gland and testes (Brehm and Stegar, 2005).

Selected neuron in the nucleus of hypothalamus secrete the peptide gonadotropin releasing hormone (GnRH) in to the pituitary portal blood stream. The GnRH travels to the anterior pituitary, where specialized cells termed gonadotrops initiate signaling in response to GnRH binding. In response to GnRH ,triggered signaling gonadotrops synthesized and secrete LH and FSH (Lingappa and Farey, 2000).

Baker et al., (2003) pointed that the loss of GnRH hormones in the mice causes the pool of fatty droplets in the leydig’s cells and secretion of steroidal hormones.

The spermatogenesis in mammals required an act common to all peptide and steroid hormones ; each of them has a key role in the normal functioning of the seminal lining in regulating the growth and development of germ cells and the proliferation of somatic cells important in the development of the testes cell such as leydig’s cells , sertoli cells and muscle cells that surround the seminiferous tubules (Holdcraft and Braun, 2004).

Luteinizing hormone, T and FSH are the prime regulators, which control spermatogenesis. However, androgens are indispensable for initiation and maintenance of spermatogenesis , although testosterone feeds back on both gonadotrophic hormone , an additional feed back loop exists between the testes and the brain for FSH . Inhibin , activin and follistatin are involved in
this regulatory system. While inhibin functions to suppress FSH secretion, (Gupta, 2005).

Follicle stimulating hormone stimulate the proliferation of the spermatogonia and formation of the primary spermatocytes, while androgens are involved in bringing about the meiosis division of the primary spermatocyte and their final conversion in to the spermatide. Follicle stimulating hormone is also influences the secretion of sertoli cells, secretion of androgen binding protein (ABP) is under the control of FSH. However, LH control the synthesis of androgens by the Leydig cells clusters (Negi, 2009). Follicle stimulating hormone and testosterone act through the sertoli cell since the receptors for those hormone are located on these cells and not on the germ cells (Verhoeven et al., 2007).

Follicle stimulating hormone stimulates the production of androgen binding protein by sertoli cells. ABP is essential to concentrating testosterone in levels high enough to initiate and maintain spermatogenesis, which can be 20-50 times higher than the concentration found in blood. The hormone inhibin acts to decrease the levels of FSH. Studies from rodent models suggest that gonadotropin hormone (LH and FSH) suppresing the proapoptotic signals and therefore promote spermatogenic cell survival (Pareek et al., 2007).

Within the testes, LH causes synthesis of testosterone by intertubular leydig’s cells, which negatively influences hormone release in the hypothalamus and pituitary by contrast, FSH acts on sertoli cells. It induces the production of ABP by means of which testosterone can pass the sertoli – sertoli junction complexes and also inducing the production of activin and inhibin by sertoli cells which both influence hormone release in the hypothalamus and pituitary (Brehm and Stegar, 2005).
1-10: Testosterone hormone

1-10-1: Chemistry and biosynthesis of testosterone

Testosterone is the major hormone of testes, it is synthesized from cholesterol and is also formed from androstenedione secreted by the Leydig’s cells (Negi, 2009).

It transformed to dihydrotestosterone in the cytoplasm of the prostate gland cells and other accessory sexual gland cells. The interstitial cell of the testes, known as, leydig’s cell is the main source of testosterone. The activity of these cells mediated by the interstitial cell stimulating hormone (ICSH) which is produced by the pituitary gland (Alwachi, 2008).

The first steps of T production and processing occur in mitochondria or smooth endoplasmic reticulum of leydig’s cells, cholesterol is the main and started compound in the manufacturing process (Beamer et al., 1983).

As summarized in figure (1.1) Testosterone is synthesized in the leydig’s cell of the interstitial tissue of the testes by an enzymatic sequence steps from cholesterol which predominantly formed by synthesis from acetate. Also contribute the first and rate-limiting step in gonadel and adrenal steroid genesis in the transfer of steroidogenic substrate cholesterol from the outer mitochondrial membrane to the inner membrane mediated by the cholesterol transport protein steroidogenic a cute regulatory protein (STAR) (Swerdloff et al., 2010).

After testosterone form from the testes, testosterone is bound by plasma protein in the circulation about 45% of plasma testosterone. In adult men bound with high affinity to sex binding globulin (SBG), 50% is losing bound to albumin 9.1-2% to cortisol-binding globulin and less than 4% is free (not protein bound) (Winters and Clark, 2003).
Figure (1.2) The intratesticular steroidogenic pathway for synthesis of testosterone (Swerdloff Ronald et al., 2010).
1-13-2. **Transport and metabolism of testosterone**

The normal range of Testosterone is 2.8-8.2 ng /ml testosterone secretion rate is 4.9% in normal adult males. Small amount of testosterone are also secreted in females, probably from the ovary, but possibly from the adrenal as well. ninety eight percent of the testosterone in plasma is bound to protein; 65% is bound to a B-globulin called gonadal steroid binding globulin (GBG) or sex steroid binding globulin, and 33 percent to albumin. (Ganong , 2005) . 70% of testosterone hormone turned to oestradiol hormone through the process of aromatization, small quantities of it secreted by the adrenal gland, and about 30% secreted by the testes, as part of it produces by leydig’s cells ( Ganong,1985).

The testosterone hormone is also metabolized to oestradiole (E2) , this metabolism occur due to aromatize in the brain , testes and adipose tissue , in the normal men , the rate between estradiol hormone levels are compared with the total testosterone is known and between the levels of DHT and testosterone hormone in the plasma is about the ratios 1:200 and 1:10 respectively (Winters et al., 1999).

Steroid hormone are diffused freely and rapidly a cross cell membrane as following its synthesis , such as testosterone leave leydig’s cells to enter the testicular interstitial compartment and diffuse across the capillary endothelium in to the circulation (Winters and Clark, 2003).
1-13-3. **Action of testosterone**

In addition to their action, during development, testosterone and other androgens exert an inhibitory feedback effect on pituitary LH secretion; develop and maintain the male secondary sex characteristics; exert an important protein—anabolic, growth promoting effect and along with FSH, maintain spermatogenesis increase the synthesis and decrease the breakdown of protein and thus, results in increase in the rate of growth (Ganong, 2005).

Testosterone contributes to the development of seminal vesicles and epididymis, while it seems that the development of the testicle and scrotum and penis stimulates by the dihydrotestosterone (Alwachi, 2008).

Testosterone deficiency in men is manifested typically by symptoms of hypogonadism, including decreases in erectile function and libido. Testosterone also has an important role in the regulation of normal growth, bone metabolism and body composition. Specifically, T deficiency is an important risk factor for osteoporosis and fractures in men. Men with testosterone deficiency have significant decreases in bone density, particularly in the trabecular bone compartment (Laurence, 2000).

Normal levels of testosterone in males are required to maintain secondary sexual characteristics, fertility, muscle mass, hair and sexual function as part of natural male aging. There is generally a decrease in testosterone levels secondary to diminished gonadal function. The effect of reduced testosterone levels varies among subjects and has become known as androgen decline in the aging male. In conjunction with testosterone reduction in men, a decline in erectile function is often seen. This is important such as in the metabolic syndrome in which the hypogonadal state is frequently accompanied by erectile and sexual dysfunction (Jones, 2009).
1-13-4. The role of testosterone in spermatogenesis

Testosterone leaves the testes through the blood stream and 95% of circulating testosterone in the adult man is derived from testicular secretion; the remainder arises from metabolic conversion of precursor steroids. Predominantly secreted by the adrenal cortex such as dehydroepiandrosterone (DHEA) (Ishimaru et al., 1978).

Moreover, T is essential for spermatogenesis, fertility and maintenance of the male phenotype (Pakarainen et al., 2005).

Testosterone produced by the leydig’s cells under the stimulation of LH is not only secreted in the system circulation but diffuse from leydig’s cells in the interstitial space into the seminiferous tubule where it enters sertoli cells. Which can convert testosterone to DHT and leaves sertoli cells to enter the testicular fluid around the germ cells. Resulting in high intra-testicular levels (Jones and López, 2006 and Swerdloff et al., 2010).

Leydig’s cells secrete the androgen (T), especially under contact of Luteinizing Hormone (LH) that secreted by the pituitary gland which is known as interstitial cell stimulating hormone (ICSH) in males; also it may be helped by Follicle Stimulating Hormone (FSH) to stimulated sertoli cells to production Androgen Binding Protein (ABP) (Alwachi, 2008).

The testosterone act as a synergistic with FSH to regulate the division of spermatogonia and the continuation of the spermatogenesis (Singh and Handelsman, 1996).

It is testosterone and possibly DHT that stimulate certain phases of spermatogenesis, not LH directly, for example testosterone in the rat stimulate the first meiotic division during which diploid primary spermatocytes are converted to haploid secondary spermatocytes. Thus, LH causes this division only indirectly by causing the secretion of testosterone from the leydig’s cells (Jones and López, 2006).
# Chapter Two

## 2: Materials and Methods

### 2-1: Materials

#### 2-1-1: Equipments and Apparatus

The equipments and apparatus which were used through the study are listed in Table 2-1.

(Table 2-1): The equipments and apparatus used.

<table>
<thead>
<tr>
<th>Instruments and Equipments</th>
<th>Suppliers</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eppendorf Centrifuge</td>
<td>Olympus</td>
<td>(Japan)</td>
</tr>
<tr>
<td>autoclave</td>
<td>Webeco gmbh</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Electrical Oven</td>
<td>Memmert</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Eppendorf tubes</td>
<td>Espif</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Dissecting microscope</td>
<td>Sartorius</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Hot plate</td>
<td>Heidolph</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Incubator</td>
<td>Eppendorf</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Light microscope</td>
<td>Novex</td>
<td>(Holland)</td>
</tr>
<tr>
<td>Mini-VIDAS</td>
<td>Bio meieusvitek</td>
<td>(USA)</td>
</tr>
<tr>
<td>Petri dish</td>
<td>Localy made</td>
<td>(plastic)</td>
</tr>
<tr>
<td>Digital Camera</td>
<td>Sony- Japan</td>
<td></td>
</tr>
<tr>
<td>Rotary microtom</td>
<td>Gallenkamp</td>
<td>(England)</td>
</tr>
<tr>
<td>Slides and cover glasses</td>
<td>Memmert</td>
<td>(Germany)</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Water bath</strong></td>
<td>Memmert (Germany)</td>
<td></td>
</tr>
<tr>
<td><strong>Electric balance</strong></td>
<td>Sartorius (Germany)</td>
<td></td>
</tr>
</tbody>
</table>
2-1-2: Chemicals

Chemicals which were used throughout the study are listed in table 2-2 (Table 2-2)

Table (2-2): Chemicals used.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol 70%</td>
<td>Switzerland, fluka</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>Locally prepared</td>
</tr>
<tr>
<td>Bouin’s fixative</td>
<td>Switzerland</td>
</tr>
<tr>
<td>Zylene</td>
<td>Gainal and Chemical, USA</td>
</tr>
<tr>
<td>Paraffin wax</td>
<td>fluka, Switzerland</td>
</tr>
<tr>
<td>Glycerine</td>
<td>fluka, Switzerland</td>
</tr>
<tr>
<td>Hematoxylin Stain</td>
<td>BDH, England</td>
</tr>
<tr>
<td>Eosin stain</td>
<td>Reidle, Germany</td>
</tr>
<tr>
<td>Nigrosin</td>
<td>BDH, England</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
</tr>
<tr>
<td>Canada Balsam</td>
<td>BDH, England</td>
</tr>
<tr>
<td>RPMI-1640</td>
<td>BDH, England</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>BDH, England</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>BDH, England</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td>Fluka-switzerland</td>
</tr>
</tbody>
</table>
2-2 :Methods

2-2-1 :Preparation of solutions:

2-2-1-1: Preparation of histological solutions :

- **Bouin’s fixative** prepared by mixing the following:

  1- Saturated alcoholic picric acid solution (75 ml)
  2- Formaldehyde 40% (25ml)
  3- Glacial acetic acid (5ml) (Galigher & kozloff, 1946).

- **Eosin stain**: it was prepared by mixing:

  1- Eosin pigment (1 gm)
  2- Distilled water (30ml)
  3- Ethyl alcohol (70 ml) (Luna & Lee, 1968)

- **Harris –Hematoxylin Stain**: prepared as a mixture of

  1- Hematoxylin Stain (1.5 gm)
  2- Absolut ethyl alcohol (15 ml)
  3- Potassium Alum (30 gm)
  4- Red mercury oxide (1,5 gm)
  5- Glacial acetic acid (12ml)
  6- Distilled water (25ml) (Luna and Lee, 1968).

**Mayer's Adhesive**: It was prepared by mixing:

  1- Albumin (50 ml)  
  2- Glycerol (50 ml)
2-2-1-2: Sperm count solutions:

Eosin – Nigrosin stain: It was prepared from:

1- (1 gm) of Eosin stain soluble in (100 ml) of 3% sodium citrate.

2- (5gm) of Nigrosin stain soluble in (100 ml) of 3% sodium citrate (Hancook, 1951)

2-2-1-3: Preparation of Tamsulosin hydrochloride (Flomax) solution

Tamsulosin hydrochloride (Flomax) was obtained from (Astellas pharma Europe B.V. Leiderdorp, The Netherlands) at concentration (0.4mg), The Tamsulosin hydrochloride doses (8, 16µg /kg.b.wt) was prepared by dissolving one capsule of the drug (400µg/ml) in 100ml of distilled water to prepare a (8µg /kg.b.wt).

Same procedure was followed using one capsule of the drug and dissolved in 200 ml of distilled water to prepare the second dose (16µg /kg.b.wt). (Patel and Patel., 2010).

2-2-2: Experimental Animals:

Thirty albino Swiss male mice (Mus musculus) aged 8-10 weeks were obtained from the animal house of Al-kindy company for vaccines. The weight range was 35-30gm. The animals were housed in plastic caged. The caged were embedded with wooden shelves, under natural 10 hr light and 12 hr dark, The animals were caged at lab temperature of 23 – 25°C, and the animals were feed ad libitum. They were divided into three groups, 10 animals/group.

Group 1: as control, animals were treated with (0.1ml) of distilled water.

Group 2: Animal were given 8 µg /kg.b.wt/day of Flomax for each mouse.

Group 3: Animal were given 16 µg /kg.b.wt/day (0.4 µg) of Flomax for each mouse.

The doses were daily injected intraperitonially for 42 successive days (Tambaro. et al., 2005)
2-2-3: Blood collection

Animals weight has been recorded before and after the injection by using electrical balance. Blood samples (0.5-1.0 ml) were obtained (immediately after sacrificing) by heart puncture and placed into eppendorf tubes and allowed to clot. Serum was separated by centrifugation for 10 minutes at 3000 rpm. The isolated serum was kept at 4°C below zero to determine the testosterone hormone level. Mini VIDAS radioimmunoassay for serum testosterone was carried out with a testosterone kit.

2-2-4: Killing of the animals

Animals were killed by cervical dislocation. Immediately after killing the abdominal cavity was opened in overturned (T) shape, and then the male reproductive organs were extirpated (right testes & right testes epididymis). The adipose tissues were removed. The right testes were placed on a filter paper to be weighted with an electrical balance. The tail of the Epididymis was utilized to study sperm parameters. The testes were fixed in Bouin’s fixative for 24 hr then transferred to 70% ethyl alcohol for storage, and then it was submitted to microscopic examination for histological study.

2-2-5: Collection and Preparation of sperms

Soon after killing and dissection, the caudal of right epididymis of were isolated and placed in Petri dish containing 1ml of RPMI-1640 Medium which is nourishing media and composed of Fetal bovine serum (10g), Penicillin (1000000 IU), Streptomycin (1g), Heps (4g) and Sodium bicarbonate (1%). Which maintained at 37°C to prevent cold shocks, and minced by using microsurgical scissor and forceps to release sperm from the tail of the epididymis (Nara and McCulloch, 1985).
2-2-6: Sperms parameters:

2-2-6-1: Percentage of sperm motility:

Sperm motility represents the quantitative parameter of motile sperms expressed as a percentage (Silverage and Turner, 2001). A drop of semen was placed on a warmed 37°C slide and examined under the power of 400X. Active sperm motility percentage was assessed according to the following equation

\[
\text{Motility (\%) = \frac{\text{No. of motile sperm}}{\text{Total N. of sperms}} \times 100}
\]

2-2-6-2: sperm concentration:

A drop of spermatozoa was placed on a slide and covered with a cover slide. Concentration of spermatozoa (sperm/ml) was calculated from the mean number of sperm in five high powers microscopically fields under magnification of 400X. This number multiplied by a factor of one million (x10^6/ml) (Smith and Mayer, 1955).

Sperm concentration = No. of spermatozoa X multiplication factor

2-2-6-3: percentage of sperm viability:

A drop of sperm suspension was mixed with a drop of eosin stain (1%) and two drops of nigrosin stain. Then a thin smear of semen – eosin - nigrosin mixture was made using other slide which used to make a thin smear in a third slide and the third slide left to dry at room temperature, the slides was examined under light microscope at (400x). The dead sperms appear pink colour while the normal ones show a blue colour. also the morphology abnormal sperm were determined by this stain.
The sperm viability were estimated according to the following equation (Hafez, 1987)

\[
\text{Percentage of dead sperm} \% = \left( \frac{\text{No. of dead sperm}}{\text{Total sperm No.}} \right) \times 100
\]

2-2-6-4: Percentage of morphologically abnormal sperm:

Spermatozoa were examined for normal and abnormal morphology of head or tail or both. To calculate the abnormality of sperm by using the same slide which was used for calculating live and dead sperm according to Dale and Edler, 1997.

\[
\text{Abnormality} \% = \left( \frac{\text{No. of abnormal sperms}}{\text{Total N. of sperms}} \right) \times 100
\]

2-2-7 Histological study:

Testes were kept in Bouin’s solution for 24 hr then they are washed by 70% ethyl alcohol for several times until the yellow colour was removed. Testes were kept in ethyl alcohol 70% of until use.

Dehydration, clearing, infiltration, embedding, sectioning, staining and mounting were respectively done to prepare the slides for histological examination according to Bancroft and Stevens (1982).
1) Dehydration

Water was withdrawn from the tissue by passing specimens in a series of progressive concentrations of ethanol (80%, 90%, 100%, and 100%) and for 45 min for each concentration.

2) Clearing

The specimens were placed in xylene for 45 min. for clearance.

3) Infiltration

Samples were set with a mixture of xylene and melted paraffin wax (58-5°C) in (1:1) ratio for 30 minutes in an electric oven, and then the samples were transferred to a new melted wax for only three phase and one hour for each phase.

4) Embedding

The specimens were placed in a metal template filled with melted paraffin and left until wax is solidified. The paraffin blocks were removed from the template and submerged in cold water for 2-3 hours.

5) Sectioning

Sectioning was done by a microtome. At a thickness of 5µm, and the sections were placed on clean slide put in water bath (45-50°C) for two min there after they were fixed on the slide by using Mayer’s albumin.

6) De waxing

The section were putted in xylene at two phases, for 30 minutes each phase.

7) Staining

The slide passed in descending serial concentration of ethyl alcohol: 100%, 95%, 90%, 80%, 70% for two minutes in each concentration, then washed with
distilled water for two minutes. Sections were stained with Haematoxilin Harris for
two minutes and then washed with tap water. Then the slides were stained with
alcoholic eosin for two minutes and then passed ascending concentration of ethanol is:
70%, 80%, 90%, 95%, 100%, about twice for each concentration and then the
slides were transferred to xylene for clearing in two stages in a period of 15 minutes

8) Mounting

Sections were mounted with canada balsam then covered with cover slide.

2-2-8 :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 testes using previously calibrated micrometer (Ocular
micrometer, stage micrometer). The diameter of 20 seminiferous tubules was
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 value of
each was calculated. The interstitial space measured between two consecutive
seminiferous tubules by using the ocular micrometer. Photos were taken to visualize
some of results using a light microscope supplied with Sony camera

2-2-9: Statistical analysis

Data were statistically analyzed by 1-way analysis of variance with ANOVA-test. Data are presented as means ± SD. The level of significance of P < 0.05 was used
for analysis of variance test (ANOVA) (Al-Mohammed et al., 1986).
Chapter Three
Results and Discussion

3-1: Testosterone concentration in serum:

The obtained results demonstrated that there was a significant (p<0.05) decrease in the T. level in the serum of the groups administered with 8µg/kg.b.wt. and 16µg/kg.b.wt. of Tamsulosin hydrochloride. There was also a significant decrease (p<0.05) in the T level between treatment Table 3-1.

Table (3-1): Effect of Tamsulosin hydrochloride (8µg/kg.b.wt. and 16µg/kg.b.wt.) on serum Testosterone level (ng/ml) in male mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Testosterone ng/ml (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 2.86±0.23</td>
</tr>
<tr>
<td>Tamsulosin 8µg/KgB.W</td>
<td>B 1.61±0.12</td>
</tr>
<tr>
<td>Tamsulosin 16µg/KgB.W</td>
<td>C 0.91±0.09</td>
</tr>
</tbody>
</table>

- A, B, C representing a significant (P<0.05) differences in comparison to each other.
These results were in agreement with that of Mocktary et al., (2007) who noticed that the level of testosterone decreases through either the effect on steroidogenesis enzymes in testes, or its inactivation properties on adrenergic systems involved in steroidogenesis.

This effect can be explained by the anti androgenic action as well as the stimulation of steroidal anti androgen to the negative feedback inhibition of the hypothalamus which resulted in lowering the concentration of plasma testosterone. However, it is well known that in non steroidal anti androgens, the androgenic actions were blocked in both hypothalamus and target tissues. Therefore, negative feedback signals were inhibited and production of testosterone was increased in the testes (Akakura et al., 1998). There is a great number of steroids like testosterone which metabolized by CYP3A enzyme. This enzyme increases these steroids clearance from plasma. Also, CYP3A induction would produce some metabolites which have endocrine disrupting function (Guillette, 2006).

These decreases in testosterone level can also be explained by necrosis induced in the seminiferous tubule as noticed by Hibi et al., (1995). The significant decrease in interstitial cells number lead to decreased testosterone secretion from interstitial cells.

Serova et al., (1994) pointed that Adrenoceptor blocking drugs such as phentolamine (alpha receptor blocker) or obsidan (beta blocker) caused a decrease in testosterone levels in mice.
3-2: Changes in testes and body weights

There was a significant (p<0.05) decrease in the rate of testes weights of animals treated with Tamsulosin hydrochloride at the concentration 8µg/kg.b.wt. and 16µg/kg.b.wt. compared to control group. There was also a significant (p<0.05) difference in testes weights between treatments (group I and group II) Table 3-2.

The effects of the drug on testes weight was in consistence with Mocktary et al., (2007) who noticed that alpha blockers caused reduction in testicular weight. This resulted from the large reduction of spermatogenic cell number as evidenced by the examination of stained testes sections (Giuliano, 2006). Moreover the reduction in testes weight may be attributed to an impairment of spermatogenesis process that normally stimulated by FSH and testosterone (Haywood et al., 2003).

The physiologic concentrations of T, LH and FSH play an important role in spermatogenesis (Zitzmann, 2008), so a significant decrease of these hormones decreases the number and function of germinal cells of testes followed by testes weight reduction.

Mhaouty-Kodja et al., (2007) concluded from their work that alpha blocker can cause infertility with reduction in testicular weight.

The results also showed a significant decrease (p<0.05) in body weight after treatment with concentrations of 8µg/kg.b.wt. and 16µg/kg.b.wt. of the drug compared to control group. There was also a significant (p<0.05) decrease in body weights between treatment Table 3-2.

This decrease in body weight may be attributed to many factors such as; the deficiency in testosterone level, as suggested by Rolf et al., (2002). Testosterone plays a key role in the development of male
reproductive tissues such as the testes and prostate as well as promoting secondary sexual characteristics such as increased muscle, bone mass and growth of body-hair. (Alwachi, 2008; Sherwood, 1991; Mooradian et al., 1987). In addition, testosterone is essential for health and well-being (Bassil et al., 2009) as well as the prevention of osteoporosis (Tuck and Francis, 2009).

Many studies have proven that testosterone has a direct effect on protein synthesis in all tissues and organs of the body and can increase bone and muscle mass in males (Wade et al., 1968). So the decreased testosterone could lead to body weight loss via decreasing protein synthesis and bone/muscle mass.

Tamsulosin hydrochloride also found to cause many disorders such as asthenia, dizziness, drowsiness, headache, insomnia, syncope, vertigo, chest pain orthostatic hypotension, pharyngitis, diarrhea, general weakness and nausea (Kluwer, 2011). All of these may be the cause of decrease in body weights of treated animal.
Table (3-2): Effect of different concentrations of Tamsulosin HCl (8µg/KgB.W and 16µg/Kg B.W) on the rate of testes and body weights.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Final Body Weight (gm) (mean±SD)</th>
<th>Testes weight (gm)/100gm (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 32.50±1.30</td>
<td>A 0.20±0.01</td>
</tr>
<tr>
<td>Tamsulosin 8µg/KgB.W</td>
<td>B 28.200±0.90</td>
<td>B 0.18±0.02</td>
</tr>
<tr>
<td>Tamsulosin 16µg/KgB.W</td>
<td>C 25.20±0.90</td>
<td>C 0.14±0.01</td>
</tr>
</tbody>
</table>

A, B, C representing a significant (P<0.05) differences in comparison to each other.

3-3: Sperm parameters:

3-3-1: sperm concentration in the epididymis

The results showed that there was a significant (p<0.05) decrease in sperm concentration of the group administered with Tamsulosin HCl at a concentration 16µg/kg.b.wt. of Tamsulosin compared with the control group but there was no significant (p<0.05) decrease in sperm concentration of the group administered with Tamsulosin hydrochloride in concentration 8µg/kg.b.wt. of the drug and there was a significant (p<0.05) difference between treatments Table 3-3).
Erin et al., (2006) found that Tamsulosin decreases sperm concentration and total sperm count, semen volume, motility and normal morphology.

The clinical findings studied by Michel (2007) suggested that the drug Tamsulosin have an effect on semen formation, transport and the cause of the abnormal ejaculation. Moreover, The abnormal ejaculation of semen is a typical but infrequent side effect of some 1-adrenoceptor antagonists, particularly those with selectivity for 1A-adrenoceptors such as Silodosin and Tamsulosin. Recent clinical studies suggest that this represents a relative an ejaculation rather than a retrograde ejaculation (Van Dijk et al., 2006; Giuliano, 2006 and Michel, 2007).

Other researchers proposed that the nature of the ejaculation caused by short-term (5-day) Tamsulosin treatment was reduced the volume of ejaculation rather than retrograde ejaculation. This could be caused by a decrease in the contractions of the seminal vesicle, a decrease in the production of seminal fluid, or both (Hellstrom and Sikka, 2007).

It was found that 0.8 mg Tamsulosin can cause a marked decreased in ejaculate volume in almost 90% of men and an ejaculation in approximately 35% of men (Hellstrom and Sikka, 2007).

α1A-adrenoceptors was also found to be widely distributed in all the organs participating in the emission phase (epididymis, vas deferens, seminal vesicle, prostate gland, prostatic urethra and bladder neck). This means that α1A-adrenoceptors plays a role in the emission phase of ejaculation. Tamsulosin is the only α1-adrenoceptor blocker showing some α1A selectivity and may affect the first phase of ejaculation (Giuliano et al., 2004). However, the decreased capacity of contraction of the seminal vesicles is proposed as the cause of the ejaculatory disorder induced by alpha-1 blockers (Hisasue et al., 2006).
The treatment with Tamsulosin was also found to induce retrograde ejaculation but not other ejaculatory disorder due to abnormal sperm progression (Grasso et al., 2006). The impaired transportation of sperm from the testes to the vas deferens was found to be the potential mechanism of ejaculation dysfunction in mice after treatment with Tamsulosin (Sanbe, 2009). Also, the administration of Tamsulosin (3 μg/kg i.v.) also caused significant reduction in the contractions evoked by electrical pulses in the epididymal portion, (Tambaro et al., 2004).

It is shown that the decreasing LH, FSH, and testosterone level could be effective in decreasing spermatogenesis and the number of testes germinal cells (Shariati et al., 2008). 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 (Harrison and Weiner, 1949).

It is well accepted that the low sperm production was in relation to the reduced size of the seminiferous tubules (Ratensooria & Wadsworth, 1994) and this study confirm this result. All of these reasons may explained the reduction in the concentration of sperm after treatment.
Table 3.3: Effect of Tamsulosin (µg /kg.b.wt. ) on sperms parameters in male mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Sperm Concentration (×10⁶/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>A 30.61±3.43</td>
<td>A 76.40±5.56</td>
<td>A 18.90±1.70</td>
<td>A 14.70±1.60</td>
</tr>
<tr>
<td>Tamsulosin 8µg/KgB.W</td>
<td>A 26.94±4.22</td>
<td>A 70.00±5.77</td>
<td>B 24.70±3.20</td>
<td>B 20.90±1.30</td>
</tr>
<tr>
<td>Tamsulosin 16µg/KgB.W</td>
<td>B 21.34±4.81</td>
<td>B 57.90±4.88</td>
<td>C 30.70±1.80</td>
<td>C 29.70±2.90</td>
</tr>
</tbody>
</table>

A, B, C representing a significant (P<0.05) differences in comparison to each other.
3-3-2: Percentage of sperm motility in the epididymis

The statistical analysis showed a significant (p<0.05) decrease in sperm motility of the group administered with Tamsulosin 16µg/kg.b.wt. compared with control group while the results of other treated group, 8µg/kg.b.wt, demonstrated that there was no (p<0.05) significant decrease (p<0.05) in sperm motility compared with control group and there was a significant difference (p<0.05) in sperm motility between treatment Table 3-3.

The low percentage of motile sperm or sperm forward progression, or both, may be due to spermatozoa structure defect (Sigman and Howards, 1998). On the other hand the treatment with Tamsulosin may induce retrograde ejaculation but not other ejaculatory disorder due to abnormal sperm progression (Grasso et al., 2006).

The function of seminal vesicle is important for fertility parameters as sperm motility, sperm chromatin stability, and immune-protection and may be changed in case of its hypo function (Gonzales, 2001). The prostatic secretion makes spermatozoa motile and helps to neutralize vaginal acidity (Mann et al., 2006). The seminal vesicles and the accessory sex glands secrete fructose, which acts as a donor of energy to the spermatozoa. It is the major carbohydrate found in seminal plasma, and appears essential for normal sperm motility (Ahmed et al., 2010).
3-3-3: Percentage of dead sperms in the epididymis

Determination of the percentage of dead sperm is one of the important criteria in the assessment of sperm functions. The statistical analysis revealed that there was a significant increase in the percentage of dead sperms of both treated groups 8µg / kg.b.wt. and 16µg/kg.b.wt. compared with control group. There was a significant difference (p<0.05) in dead sperm between treatments. Table 3-3.

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 is causing the small number of living sperm as well as affect 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. (Joshi & Ambaye, 1968).

Sertoli cells comprise the main structural component of the seminiferous tubules. They are responsible for the structural support for germ cell development (Vogl et al., 2000). So, any negative response of sertoli cells leads to the production of few sperm and had lost its vitality as it reach dead to the epididymis, because the essential function of sertoli cell is nutrition and support to the cells responsible for the formation of sperm and then the negative impact of these cells would have an effect on sperm (Joshi & Ambaye, 1968).

Other researchers proposed that sertoli cell facilitate germ cell movement and mature germ cell release (Mruk and Cheng, 2004) and secretion of diverse functional glycoprotein and peptides to nourish germ cells (Skinner, 1993) as well as, maintenance of the blood-testis barrier, and secretion of seminiferous tubular fluid (Waites and Gladwell, 1982).
It is well accepted that the glycoprotein hormones, FSH and LH, are involved in the process of spermatogenesis (Sharpe, 1994). However, sertoli cells express functional receptors for FSH (Krishnamurthy et al., 2000).
3-3-4: Percentage of abnormal morphology of sperms in the epididymis

Table 3-3 show that there was a significant (p<0.05) increase in the percentage of morphologically abnormal sperm in animals administered with Tamsulosin 8µg/kg.b.wt. and 16µg/kg.b.wt. compared with control groups. There was also a significant (p<0.05) difference (p<0.05) in sperm abnormalities between treatments. This may be explained by many proposals demonstrated by many other workers.

Lenzi et al., (1998) demonstrated that, altered sperm morphology might reflect disturbances during spermiogenesis, spermiation and sperm passage through epididymis. It is accepted that sperm morphology is a sensitive indicator of overall testicular health because the sperm morphologic characteristics are determined during spermatogenesis (Turk, 2000).

It was also found, that any defect in spermatogenesis process may 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 (Alwachi, 2008).

The other reason may be the decrease in the level of testosterone. However, T is necessary for normal sperm development and promote differentiation of spermatogonia (Mehta et al., 2008). So this may explain the sperm abnormalities in treated mice Fig 3-1.
Figure (3-1): Sperm morphology from mice treated with Tamsulosin Hydrochloride showing (A) normal sperm, (B) abnormal tail (bent tail), (C) abnormal head (hummer head) (E stain) 40X.
3-4: The Histological studies
3-4-1: The diameter of Seminiferous tubules

Tissue examination of normal testes of mice showed that the seminiferous tubule are spherical or ovoid shape, lined by germinal epithelium consisting primarily of spermatogenic cells which are in progressive stages of spermatogenesis (primary spermatocytes, secondary spermatocytes, spermatide and sperms in the lumen of seminiferous tubule), between seminiferous tubule there are interstitial space with group of steroid producing interstitial leydig’s cells (Fig 3-2).

The results of seminiferous tubule showed there was a significant (p<0.05) decrease in the diameter of the seminiferous tubule in the treated groups 8µg/kg.b.wt. and 16µg/kg.b.wt. compared to control group. There was also a significant difference (p<0.05) in diameter of the seminiferous tubule between treatment.

The histological examination in this study showed decrease in the diameter of seminiferous tubule and thickness of epithelium. Also an increase in the diameter of interstitial spaces with oedema in interstitial tissues accompanied with low testosterone level Fig 3-3.

The reduction in the diameter of seminiferous tubules could be referred to the reduction in testosterone hormone level, since testosterone has an important role in the development and growth of male reproductive ducts and epithelial cells of epididymal tubules (Umezu et al., 2004).

Other researchers proposed that these reduction may be due to the destruction of gap junctions (between sertoli and germ cells) in mice which may impact on both spermatogenesis and T production because the important role of these junctions in the regulation of cells growth and
differentiation by controlling the transport of small molecules, including secondary messengers between adjacent cells and a cross the epithelium toward the lumen (St-Pierre et al., 2003). However, the reduction in tubular diameter may be due to severe germ-cell impairment, although the distribution is irregular (Guarch, 1992).

It is well accepted that testosterone stimulates the epithelium of the seminiferous tubule to produce sperm and control the process of spermatogenesis (Alwachi, 2008).

This observation is in consistency to that reported by Back et al., (1977) and Mausle et al., (1982) and who found that the significant decrease in the diameter of seminiferous tubule caused by different antiandrogens may be referred to the changes that happens in spermatogenesis. Also Mhaouty-Kodja (2007) pointed that these changes mainly resulted from the large reduction of spermatogenic cell number.

The reason of degeneration and necrosis changes in seminiferous tubules in mice group treated with Tamsulosin may be due to the reduced number of Leydig’s cells which lead to the reduction in level of T hormone which are responsible of active division of spermatogenic cells in spermatogenesis therefore, the reduction in level of T hormone leads to the damage of these cells and then damage in the seminiferous tubules (Gyton & Hill, 2000 and Sigman and Howards, 1998) Fig 3-3
3-4-2: Interstitial Space

The histological examination also proved that the treated mice groups (8µg/kg.b.wt. and 16µg/kg.b.wt.) showed a significant (p<0.05) increase in the interstitial space compared to the control groups. There was also a significant difference (p<0.05) in interstitial space between treatment Table-3.4.

In the present study the presence of edema in interstitial tissues may be due to accumulation of water in the tissue of testes because of an increase in the spaces between seminiferous tubule resulting from low number in leydig’s cells and this lead to increase diffusion of fluid inside interstitial tissues (Luis et al., 1986).

Therefore as the seminiferous tubules were reduced in diameter, the interstitial spaces were increased. Fig 3-3.
Table (3.4): Effect of Tamsulosin (µg /kg,b.wt.) on diameter of seminiferous tubules and interstitial space in male mice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Diameter of seminiferous tubules (µm) (mean±SD)</th>
<th>Interstitial space (µm) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 208.43±2.88</td>
<td>A 19.15±1.80</td>
</tr>
<tr>
<td>Tamsulosin 8µg/Kg.B.W</td>
<td>B 183.00±2.94</td>
<td>B 28.29±1.78</td>
</tr>
<tr>
<td>Tamsulosin 16µg/Kg.B.W</td>
<td>C 169.43±4.43</td>
<td>C 38.29±2.10</td>
</tr>
</tbody>
</table>

A, B, C representing a significant (P<0.05) differences in comparison to each other.
3-4-3: Leydig’s cells clusters

The result obtained from histological studies demonstrated that there was a significant (p<0.05) decrease in number of leydig’s cells clusters of animal groups treated with Tamsulosin (8µg /kg.b.wt., 16µg/kg.b.wt.) compared to control group. There was a significant difference (p<0.05) in the number of leydig’s cells clusters between treatment Table-3.4.

The reason for the decreases in diameter of leydig’s cells clusters refer to damage in the leydig’s cells leading to low level of T. hormone responsible for effective on the cells divisions during spermatogenesis. Damaging of leydig’s cells and reduce in its number has a direct impact on the level of the testosterone hormone (Baille et al., 1966). Fig 3-5

The leydig’s cells drive spermatogenesis via the secretion of testosterone which acts on the sertoli cells to create an environment which enables normal progression of germ cells through stage VII of the spermatogenic cycle. In addition, testosterone is involved in the control of the vasculature, and hence the formation of testicular interstitial fluid, presumably again via effects on the sertoli when leydig’s cells regenerate and mature, it can be shown that both the rate and the location of regenerating leydig’s cells is determined by an interplay between endocrine (LH and perhaps FSH) and paracrine factors that secrete these hormones (Sharp et al., 1990).
Table 3.5: Effect of Tamsulosin 8µg/KgB.W and 16 µg/kg.b.wt. on diameters of primary spermatocyte, spermatids.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Primary spermatocytes(µm) (mean±SD)</th>
<th>Spermatids(µm) (mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A 6.74±0.37</td>
<td>A 4.35±0.50</td>
</tr>
<tr>
<td>Tamsulosin 8µg/KgB.W</td>
<td>B 4.561±0.29</td>
<td>B 3.36±0.38</td>
</tr>
<tr>
<td>Tamsulosin 16µg/KgB.W</td>
<td>B 4.59±0.45</td>
<td>C 2.60±0.33</td>
</tr>
</tbody>
</table>

A, B, C representing a significant (P<0.05) differences in comparison to each other.
3-4-4: The diameter of Primary spermatocytes

The histological examination of testes proved that the treated mice group with Tamsulosin showed a significant (p<0.05) decrease in the diameter of primary spermatocytes after 42 days of mice exposure to 8µg/kg.b.wt. and 16µg/kg.b.wt. compared to control group Table 3-5.

It is well known that T or its metabolites such as DHT is essential for maintenance of spermatogenesis by stimulation of leydig’s cells with pituitary hormone LH, which binds to specific high affinity receptors on the surface of leydig’s cells, resulting in increased productions of cyclic AMP (Holdcraft and Braun, 2004)

FSH also stimulates sperm production and it is essential to initiate spermatogenesis, while T maintains spermatogenesis (O’Riordan et al., 1988).

Uhler et al., (2003) reported that serum FSH levels has been used as a marker of spermatogenesis in the clinical evaluation of male infertility.

Ganong (1995) proved that conversion of primary spermatocyte into secondary spermatocyte (Meiosis I) are dependent on T hormone and the final step of maturation of spermatids are dependent on FSH. However, decreases FSH level lead to inhibiting the production of ABP from sertoli cells, and then decreasing T hormone level in testes which adversely affect the spermatogenesis (Yin et al., 2007).

Anderson (1993) pointed that pituitary secretion of LH stimulate the leydig’s cells of the testes to produce T. Some of the T produced is delivered to the seminiferous tubules, where it acts on sertoli cells to stimulate spermatogenesis and some enters the blood stream.
3-4-4: The diameter of Spermatid

The results showed that there was a significant (p<0.05) decrease (p<0.05) in the diameter of spermatids after treatment with Tamsulosin 8µg/kg.b.wt. and 16µg/kg.b.wt. compared with the control group. There was also a significant difference (p<0.05) in diameter of the spermatid between treatment Table 3-5.

Research showed that the meiosis of the primary spermatocyte and its conversion to a secondary spermatocyte, requires essentially the presence of the testosterone and follicle-stimulating hormone which is essential for the development of spermatide and its conversion to mature sperm (Alwachi, 2008).

Testosterone has been shown to be essential for normal spermatogenesis, because it stimulates the conversion of round spermatids to elongated spermatids (Maureen Isoken, 2010). Also the decrease in testosterone hormone led to decrease in spermatogenesis (Hammani et al., 2010), While Tamsulosin hydrochloride decreased the level of testosterone hormone (Mokhatary, 2007) and the secretion of testosterone stimulate sperms production and control of spermatogenesis (AL-Alwachi, 2008). So changes in diameter of spermatide occur after treatment with Tamsulosin hydrochloride.

Anthony et al., (1989) reported that, spermatogenic activity requires sufficient T concentration and they indicated that ,T level could be critical for the final steps of spermatogenesis.
Fig(3. 2): Section in mouse testis (control group), showing normal structure of seminiferous tubules, diameter of seminiferous tubules (ST), interstitial space (IS), spermatid and primary spermatocyte (prim. Sp.) (H and E) X 10 for figure (A), 40X for figure (B).

Fig(3. 3): Section in mouse testis (treated group 8µg/KgB.W for A and 16 µg/KgB.W for B.) showing abnormal structure of seminiferous tubules, diameter of seminiferous tubules (DST), interstitial space (IS), oedema (E) and necrosis (N) (H and E) X 10.
Fig(3. 4): Section in mouse testis (control group) showing normal structure and normal number of Leydig’s cell clusters (H & E X 40).

Fig(3. 5): Section in mouse testis (treated group with A 8µg/kg.b.wt., B 16 µg/kg.b.wt) showing abnormal structure and abnormal number of Leydig’s cell clusters (H & E X 40).
Conclusions and Recommendations

Conclusions

From the results of the present study it could be concluded that:

Tamsulosin hydrochloride has negative effect on fertility in male albino mice especially prolonged uses can be important cause of infertility.

Recommendations

The following suggestions may be recommended for further studies:

1- Study the effect of Tamsulosin hydrochloride on accessory gland of male reproductive system such as prostate gland, seminal vesicle.
2- Study of other types of alpha blockers used in benign prostate hyperplasia therapy and make a comparisons between them.
3- Conduct electron microscope study to clarify the exact effects caused by the drug which block the growth of sperm in the seminiferous tubule and vitality in the epididymis.
4- Study the effect of Tamsulosin on other hormones LH, FSH.


References


B


References


References


- **Boehringer ingelheim (2009)** Tamsulosin hydrochloride capsule prescribing information. Ridgefield, CT.


References


References


D


E


References


- Glaxosmith UK limited (2010) Combodart 0.5 mg/0.4 hard capsule http://emc.one click patient.co.uk/Medicine/22943/spc/combodart 0.5 mg/0.4 hard capsule.


References


References


References

I


J


K


References


**M**


human testes is characterized by complex contraction and relaxation activities regulated by cyclic GMP. J.Clin Endocrinol. pp: 87:3486-3499.


References


• **Nieshlag, E.; Behre, H. M. (2009)** Approaches to hormonal male contraception In: Nieshlag, E.; Behre, H. M. and Nieschlag, S. Andrology


References


References


• **Sonnberg, L. (3003)** Genetic and brand name presecrption drugs . Bames and noble publishing .INC, USA . PP: 963-965.
References


T


U


V


W


References


Y


الخلاصة

صممت هذه الدراسة لتحديد تأثير عقار هيدروكلوريد التامسولوسين على وظيفة الخصية والإخصاب في ذكور الفئران البيض. استخدم ثلاثون ذكر بالغ تراوح معدل أوزانها 25-30 غم وأعمارها 10-18 أسبوع وقسمت عشوائيا إلى ثلاث مجموعات (10 ذكور للمجموعة).

حقيق المجموعة الأولى بالماء الم컨تر تحت البريتون واعتبرت مجموعة سيطرة، وبالمجموعات الأخرى (الثانية والثالثة) حفت تحت البريتون بتراكيز مقدارها 8، 16 ميكروغرام/كم موزن الجسم يومياً لفترة 42 يوم.

أظهرت النتائج انخفاض معنوي في أوزان الجسم ومعدل أوزان الخصى لذكور الفئران بعد المعاملة بعقار هيدروكلوريد التامسولوسين للتركيزين مقارنة بمجموعة السيطرة.

كما أظهرت المعاملة بعقار التامسولوسين انخفاض معنوي في النسبة المئوية لحركة النطف والنمذجة المئوية للنطف الحية وتراكيز النطف في ذيل البربخ في الجرعة 16 ميكروغرام/كم من وزن الجسم، بينما ليس هناك انخفاض معنوي في النسبة المئوية لحركه النطف وتركيز النطف في الجرعة 8 ميكروغرام/كم من وزن الجسم، بينما هناك انخفاض معنوي في النسبة المئوية للنطف الحية.

الجرعة مقارنة مع مجموعة السيطرة، وهنا ارتفاع معنوي في النسبة المئوية للتشوهات المورفولوجية في النطف للتركيزين مقارنة بمجموعة السيطرة.

بيني الفحوصات المجهرية لانسجة الخصى للمجموع المعاملة بهذا العقار حدوث انخفاض معنوي في معدل أقطار النبيلات المنوية، الخلايا المولدة للنطف، طالع النطف وانخفاض في اعداد الخلايا المولدة النطف، بينما حصل ارتفاع معنوي في المسافات البدنية مقارنة بمجموعة السيطرة.

ظهرت الفحوصات المجهرية أيضا وجود بعض التغيرات في نسيج الخصبة تضمنت تنخر في نسيج النبيلات المنوية وحدوث الوريق في الفسح البدني.

بينما أظهرت المعالمة ولجميع التراكيز المستخدمة انخفاض معنوي في مستوى هرمون الاستيرويد في بلازما الدم للحيوانات المعالمة مقارنة بمجموعة السيطرة.

نستنتج من هذه الدراسة أن عقار التامسولوسين تأثير سلبي في وظيفة الخصية (الخصوبة).
تأثير عقار التامسولوسين (الفلوماكس) على الخصوبة في ذكور الفئران البيض

رسالة مقدمة
من قبل
دينا خضير حسين علي
بكالوريوس / علوم حياة / جامعه بغداد 2006

إلى كلية العلوم في جامعة بغداد وهي جزء من متطلبات نيل
درجة الماجستير في علوم الحياة / علم الحيوان

بأشراف
أ.د. صباح ناصر العلوجي

شباط
ربيع الثاني
2012 م
1433 هـ