

LEBANESE AMERICAN UNIVERSITY

CUCURBITA PEPO INHIBITS BENIGN PROSTATIC
HYPERPLASIA INDUCED BY TESTOSTERONE IN
SPRAGUE–DAWLEY RATS

By

TANIA M. NAWFAL

A thesis
Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Molecular Biology

School of Arts and Sciences
January 2011

Thesis approval Form (Annex III)

Student Name: **Tania M. Nawfal** I.D. #: 200600636

Thesis Title : **Cucurbita Pepo Inhibits Benign Prostatic Hyperplasia Induced by Testosterone in Sprague Dawley Rats**

Program : M.S. in Molecular Biology

Division/Dept : Natural Sciences Department

School : **School of Arts and Sciences**

Approved by:

Thesis Advisor: Dr. George Baroody

Member : Dr. Mohamad Mroueh

Member : Dr. Costantine Daher

Date: January 2011

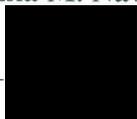
THESIS RELEASE FORM

LEBANESE AMERICAN UNIVERSITY

By signing and submitting this license, I Tania M. Nawfal grant the Lebanese American University (LAU) the non-exclusive right to reproduce, translate (as defined below), and/or distribute my submission (including the abstract) worldwide in print and electronic format and in any medium, including but not limited to audio or video. I agree that LAU may, without changing the content, translate the submission to any medium or format for the purpose of preservation. I also agree that LAU may keep more than one copy of this submission for purposes of security, backup and preservation. I represent that the submission is my original work, and that I have the right to grant the rights contained in this license. I also represent that my submission does not, to the best of my knowledge, infringe upon anyone's copyright. If the submission contains material for which I do not hold copyright, I represent that I have obtained the unrestricted permission of the copyright owner to grant LAU the rights required by this license, and that such third-party owned material is clearly identified and acknowledged within the text or content of the submission. IF THE SUBMISSION IS BASED UPON WORK THAT HAS BEEN SPONSORED OR SUPPORTED BY AN AGENCY OR ORGANIZATION OTHER THAN LAU, I REPRESENT THAT I HAVE FULFILLED ANY RIGHT OF REVIEW OR OTHER OBLIGATIONS REQUIRED BY SUCH CONTRACT OR AGREEMENT. LAU will clearly identify my name(s) as the author(s) or owner(s) of the submission, and will not make any alteration, other than as allowed by this license, to my submission.

Name: **Tania M. Nawfal**

Signature: _____



Date: 28-1-2011.

PLAGIARISM POLICY COMPLIANCE STATEMENT

I certify that I have read and understood LAU's Plagiarism Policy. I understand that failure to comply with this Policy can lead to academic and disciplinary actions against me.

This work is substantially my own, and to the extent that any part of this work is not my own I have indicated that by acknowledging its sources.

Name: **Tania M. Nawfal**

Signature: _____



Date: **28-1-2011**

ACKNOWLEDGMENTS

This research would not have been possible without the help and assistance of many persons.

First I would like to express my gratitude to my supervisor **Dr. George Baroody** whose support and guidance made my thesis work possible. He has always been available to advise me. I am very proud for his motivation and patience to guide me throughout all my graduate studies.

I am also deeply grateful to the jury members **Dr. Costantine Daher & Dr. Mohamad Mroueh** for their great ideas and suggestions. Special thanks to **Dr. Daher** who guided me to finish my project by his professional help and advice.

I would like to thank **Dr. Nasser** who helped me finish the histological parts. He was very friendly and cooperative.

Thanks go also to Mr. **Jean Karam and Mr. Neeman Saade** for their assistance in feeding and maintaining the animals in perfect conditions for this project.

I would also to thank Miss **karmen Baroody** for her help and cooperation.

Special thanks go to my **husband Walid Dagher**, for his endless love, support and encouragement to finish what I have started with my parents because without him it would be impossible for me to accomplish what I have achieved.

Exceptional thanks to **my father Marwan, my mother Samia**, and my sisters: **Paula, Sonia, Katia, and Rania** with **their husbands** for their moral support and love.

Finally I dedicate this work to the **Lebanese American University** and to everyone who contributed to finish this work

Last and not least, I would like to thank our **Lord Jesus** for His graces.

*To my loving parents
and
My supportive husband*

Cucurbita Pepo Inhibits Benign Prostatic Hyperplasia Induced by Testosterone In Sprague–Dawley Rats

Tania M. Nawfal

Abstract

The Cucurbita pepo (pumpkin) seeds are used to treat benign prostatic hyperplasia (BPH). This study examines the effects of pumpkin seeds on dihydrotestosterone produced from testosterone that induces the hyperplasia of the prostate gland of male rats. Hyperplasia was induced by a subcutaneous injection of testosterone that was given in the form of Sustanon 3.568 mg/kg body weight. When the increase in the size of the prostate was achieved in 30 days, simultaneous oral administration of pumpkin seeds with rat chow was given at doses of 10% w/w, 20% w/w, 30% w/w, 60% w/w and 30% w/w + Xatral 1 mg/kg body weight. Animals were weighed before dissection and the influence of testosterone and pumpkin seed on the weight gain of the rats were observed.

Rats were sacrificed at days 12, 24, and 36. The prostate was removed, and weighed immediately. Then prostate weight to body weight ratio were calculated. Blood samples were collected to examine the effects of pumpkin seeds on blood lipid profile and liver enzymes. Results show that pumpkin seeds affect some of the blood lipid test and have no significant effect on the amount of sGOT-AST enzyme and ALP, but there is an increase in the activity of sGPT-AST enzyme for the groups treated with 60% and 30% Xatral. There is no significant influence on the weight gain of rats treated with testosterone and pumpkin seeds. Whereas testosterone increases the weight of the prostate ($p < 0.05$), pumpkin seeds at 30% w/w inhibit hyperplasia and has no effect on the blood profile test. This result is confirmed by the histopathological studies that show that this group has the same appearance of the negative group. There is abundant stroma between glandular cells. In addition there is lack of papillary projections into the lumen of the glands. In conclusion, pumpkin seeds at 30% w/w can inhibit prostate hyperplasia induced by testosterone, and improve the histology of the prostate.

Keywords: Cucurbita pepo, Sustanon, benign prostatic hyperplasia (BPH), Xatral.

TABLE OF CONTENTS

Chapter	Page
I- Introduction and Literature Review	1-15
1.1- The Prostate Gland and Benign Prostatic Hyperplasia.....	1
1.2- Causes of BPH.....	3
1.3- Symptoms of BPH.....	4
1.4- Diagnosis of BPH.....	4
1.4.1- Digital Rectal Examination (DRE).....	4
1.4.2- Rectal Ultrasound and Prostate Biopsy	5
1.4.3- Urine Flow Study	5
1.4.4- Cystoscopy	5
1.4.5- Prostate-Specific Antigen (PSA) Blood Test	5
1.4.5.1- Elevation of the PSA.....	6
1.4.5.2- Total PSA Measurement	6
1.4.5.3- Free PSA	6
1.4.5.4- PSA Density	7
1.5- Treatment	7
1.5.1- Pharmacological treatment	7
A) 5 α -Reductase Inhibitors.....	8
B) Alpha Blockers	8
B.1- Xatral or Alfuzosin hydrochloride	9
C) Combination therapy	10
1.5.2- Phytotherapeutic treatment.....	11
1.5.2.1- Pumpkin or Cucurbita Pepo	11
1.5.2.2- Phytochemistry.....	12
1.5.2.3- Uses of Pumpkin or Cucurbita Pepo	13
1.5.3- Surgical Treatments.....	14
1.5.3.1- Transurethral Resection of the Prostate (TURP).....	14
1.5.3.2- Transurethral Incision of the Prostate (TUIP).....	14
1.5.3.3- Open Surgery	14
1.5.3.4- Laser Prostatectomy	15
II- Materials and Methods	16-25
2.1- Animals Preparation.....	16
2.2- Sources of pumpkin seeds	16
2.3- Animals groups.....	16
2.4- Inducing BPH with Sustanon	17
2.5- Dilution of Sustanon.....	17
2.6- Injection of Sustanon and treatment	17

2.7-	Sample collection and Blood study	18
2.8-	Prostate weight to body weight ratio	18
2.9-	Calculation of the Percentage of inhibition	19
	Serum Assays	19
2.10-	Determination of serum Glucose	19
	Principle	19
	Procedure.....	19
	Calculation	19
2.11-	Determination of Serum TAG	20
	Principle	20
	Procedure.....	20
	Calculation	20
2.12-	Determination of Serum Cholesterol.....	21
	Principle	21
	Procedure.....	21
	Calculation	21
2.13-	Determination of Serum HDL-Cholesterol	22
	Principle	22
	Procedure.....	22
	Calculation	22
2.14-	Determination of serum LDL-Cholesterol	22
2.15-	Determination of Serum GOT-AST	22
	Principle	22
	Procedure.....	23
	Calculation	23
2.16-	Determination of Serum GPT-AST.....	23
	Principle	23
	Procedure.....	23
	Calculation	24
2.17-	Determination of Serum ALP.....	24
	Principle	24
	Procedure.....	24
	Calculation	24
2.18-	Histology	24
2.19-	Statistical Analysis	25

III- Results..... 26-44

3.1-	Serum Lipid Profile	26
	3.1.1- Serum Glucose	26
	3.1. 2- Serum Triglyceride.....	27
	3.1. 3- Serum Cholesterol.....	28
	3.1. 4- Serum HDL-Cholesterol	29
	3.1. 5- Serum LDL-Cholesterol.....	30
3.2-	Serum Enzymes	31
	3.2.1- Serum sGOT-AST	31
	3.2.2- Serum sGPT-AST	32

3.2.3-	Serum ALP	33
3.3-	Evaluation of prostate enlargement	34
3.3.1-	Prostate weights	34
3.3.2-	Prostate weights to body weights ratio	34
3.3.3-	Body weights changes	34
3.3.4-	Histopathology of prostate	41
IV-	Discussion and Conclusion.....	45-49
	Conclusion	49
V-	BIBLIOGRAPHY.....	50-57

LIST OF TABLES

Table	Table Title	Page
Table 3.1.1	Glucose concentrations in serum of treated rats	26
Table 3.1.2	Triglyceride concentrations in serum of treated rats	27
Table 3.1.3	Cholesterol concentrations in serum of treated rats.....	28
Table 3.1.4	HDL-Cholesterol levels in serum of treated rats	29
Table 3.1.5	LDL-Cholesterol levels in serum of treated rats.....	30
Table 3.2.1	sGOT-AST activity in serum of treated rats	31
Table 3.2.2	sGPT- AST activity in serum of treated rats.....	32
Table 3.2.3	ALP activity in serum of treated rats	33
Table 3.3.1	Effect of pumpkin seeds and Xatral on body weights and prostate enlargements in testosterone treated rats during the first twelve days.....	35
Table 3.3.2	Effect of pumpkin seeds and xatral on body weights and prostate enlargements in testosterone treated rats during twenty four days.	37
Table 3.3.3	Effect of pumpkin seeds and xatral on body weights and prostate enlargements in testosterone treated rats during thirty six days.....	39

LIST OF FIGURES

Figure	Figure Title	Page
Figure 1.1	Anatomy of male reproductive system	1
Figure 1.2	Location of the Prostate Gland.....	1
Figure 1.3	Anatomy of Prostate Gland.....	2
Figure 1.4	Structural formula of Alfuzosin hydrochloride.....	9
Figure 1.5	Pumpkin or Cucurbita pepo	11
Figure 1.6	Flower and Leave of Cucurbita Pepo.....	12
Figure 1.7	Seeds of Cucurbita Pepo	12
Figure 3.1	Negative control showing no histopathological signs.....	41
Figure 3.2	Positive group having BPH.....	42
Figure 3.3	Group treated with 30% pepo. Glands with abundant stroma in between. Lack of papillary projections into the lumen of glands.....	43
Figure 3.4	A: negative control, B: positive control, C: testosterone + 30% pepo	44

LIST OF CHARTS

Chart	Chart Title	Page
Chart 3.1.1	Ratio of prostate weight over body weight during the first twelve days.....	35
Chart 3.1.2	Percentage inhibition during the first twelve days	36
Chart 3.2.1	Ratio of prostate weight over body weight during the twenty four days	37
Chart 3.2.2	Percentage inhibition during the twenty four days	38
Chart 3.3.1	Ratio of prostate weight over body weight during the thirty six days	39
Chart 3.3.2	Percentage inhibition during the thirty six days.....	40

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1- The Prostate Gland and Benign Prostatic Hyperplasia

The male reproductive gland or prostate is the gland that secretes part of the semen which is the fluid that carries sperm during ejaculation (Cohen & Levy, 2002). It is located below the urinary bladder and surrounds the proximal portion of the urethra, which is the tube through which urine and semen pass out of the body (Gossel-Williams, Davis & O'Connor, 2006).

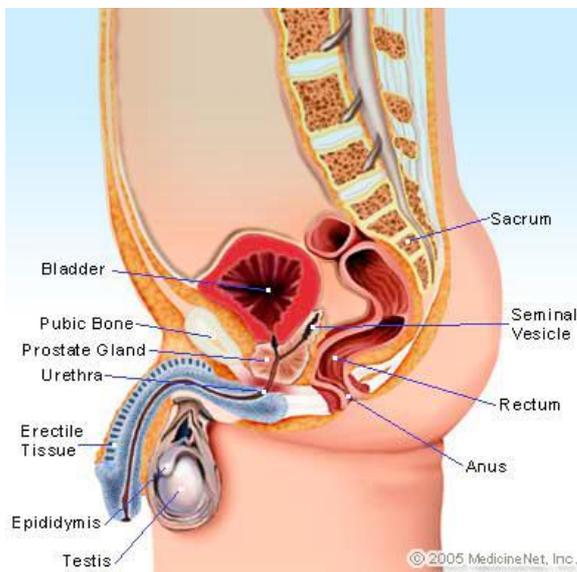


Figure 1.1 Anatomy of male reproductive system (Gerber, 2011).

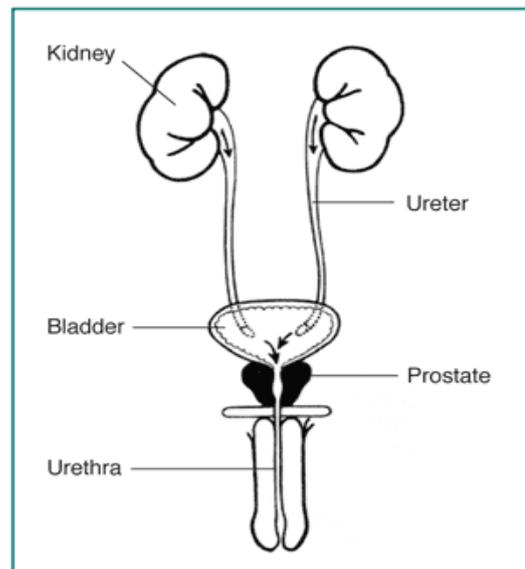


Figure 1.2 Location of the Prostate Gland (National Kidney and Urologic Diseases Information Clearing house, 2006).

The prostate plays an important function during ejaculation because this gland releases an alkaline fluid into the urethra where it is mixed with the seminal fluid, and secreted from the seminal vesicles. This fluid nourishes and carries sperm from the testes and out of the penis (Cohen et al., 2002). McNeal (1988), divided the prostate gland into four glandular zones: peripheral, central, transition, and periurethral.

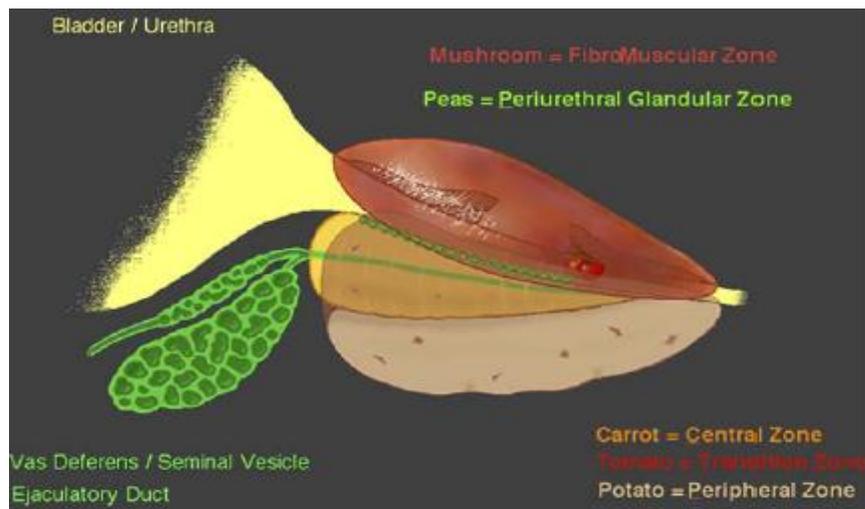


Figure 1.3 Anatomy of Prostate Gland (Wasserman, 2006).

The uncontrolled growth of this gland is referred to as benign prostatic hyperplasia (BPH) (Gossel-Williams et al., 2006). It represents the noncancerous form of the abnormal growth of the cells of the prostate (Veeresh Babu, Veeresh, Patil & Warke, 2010). In man, this overgrowth occurs in the transition and periurethral zones (Lepor, 2004).

1.2- Causes of BPH

Benign prostatic hyperplasia (BPH) is the most common disease that affects older men (Edwards, 2008). Early development occurs usually after age of 40 years. Its prevalence is greater than 50% by age of 60 years, and by age of 85 years. It is as much as 90% (Wasserman, 2006).

Histological BPH is characterized by the presence of discrete nodules in the prostate gland that occurs in the periurethral zone. Clinical manifestations of BPH are caused by the squeezing of the prostatic urethra leading to a difficulty in urination and inability to completely void the bladder, which in turn cause its distension with hypertrophy. The major characteristics on which the clinical BPH is identified are: bladder outflow obstruction, lower urinary tract symptoms (LUTS), and prostatic enlargement (Edwards, 2008). The development of BPH is associated with aging and hormonal changes of men (Lepor, 2004). In the prostate gland, testosterone which is produced by the testicular interstitial cells is converted to dihydrotestosterone. Androgens have been identified as the major cause of the disease process. The inhibition of the growth of the prostate gland can be reached by the inhibition of the production of dihydrotestosterone (Gossel-Williams et al., 2006). It has been identified by other studies that there are factors other than androgens that might be responsible for the enlargement of the prostate and development of BPH (Lepor, 2004). Some studies have demonstrated that many factors such as obesity, hypertension, and diabetes that are associated with cardiovascular diseases can be considered as risk factors for BPH. For example, the non-insulin dependent diabetes mellitus (NIDDM), which involves excessive insulin levels, play a direct role in the growth of the prostate (Hammarsten & Hogstedt, 2001). So this type of hormone affects positively the development of BPH (Dharmananda, 2001).

Moreover, The National Kidney and Urologic Diseases Information Clearing house states that men produce both testosterone and small amount of the female hormone named estrogen throughout their lives. By age the amount of active testosterone in the blood decreases, whereas the amount of estrogen increases in the prostate gland. This will lead to an increase in the activity of substances, such as dihydrotestosterone, that promote cell growth which in turn promotes BPH (NKUDIC, 2006).

On the other hand, some hormones lead to the progression of BPH. It has been proven by Dharmananda (2001), that men have a lower incidence of BPH if they are affected with liver cirrhosis, compared to those who have normal liver function. This could be due to the reduction in the metabolism of hormones to compounds that influence BPH. Both exercise and moderate alcohol consumption appear to be protective for men affected with cardiovascular disease, and BPH (Dharmananda, 2001).

1.3- Symptoms of BPH

There are two obstructive mechanisms leading to the appearance of BPH. The first is the mechanical obstruction of the urethra, which is due to an overgrowth of epithelial tissues in the transitional zone of the prostate. The second is the dynamic obstruction, which is due to an excess of sympathetic tone in the prostate and bladder neck (Mcpartland & Pruitt, 2000).

The symptoms of the lower urinary tract are many such as: hesitancy, interrupted, or weak urine stream caused by decreased force, feeling that the bladder has not emptied completely after urination, urgent need to urinate, hematuria or the blood in the urine and nocturia (Wilt et al., 1998). There are also many complications of BPH such as the urinary tract infections (UTIs) or bladder stones. In addition, bladder catheterization is needed if acute urinary retention or complete inability to void occurs (Edwards, 2008).

1.4- Diagnosis of BPH

The person may notice by himself one of the symptoms of BPH mentioned above or his doctor may find during a routine checkup that the prostate is enlarged. Several tests were also done to identify the problem and decide whether surgery is needed. The most common are the following: (NKUDIC, 2006).

1.4.1-Digital Rectal Examination (DRE)

In this examination the doctor inserts a gloved finger into the rectum and feels the part of the prostate. According to this test, the doctor can have an idea of the size of the gland (NKUDIC, 2006). So prostate volume predicts whether the male suffer from BPH. The normal prostate volume is 20 to 30 ml, if the prostate volume is greater than 40 ml; this means that the male might have a certain problem (Edwards, 2008).

1.4.2-Rectal Ultrasound and Prostate Biopsy

Rectal ultrasound is recommended if there is a suspicion of prostate cancer. In this procedure, the doctor inserts a probe in the rectum, which directs sound waves at the prostate. These sound waves will form on a display screen an image of this gland. Then the doctor uses the probe and the ultrasound images to guide a biopsy needle to the suspected tumor to determine whether the cells are cancerous. A few pieces of the prostate tissues are collected with this needle for examination with a microscope (NKUDIC, 2006).

1.4.3-Urine Flow Study

The urination into a special device can be used to measure how quickly the urine is flowing. BPH was suggested if there is a reduction in the flow of the urine (NKUDIC, 2006).

1.4.4-Cystoscopy

In this examination, a small tube is inserted into the penis through the opening of the urethra. This procedure is done after the introduction of a solution inside the penis that arrests all sensation. The cystoscope tube contains a lens and a light system that help the doctor see the inside of the bladder and the urethra. Then the size of the prostate is determined, and the degree and location of obstruction is identified (NKUDIC, 2006).

1.4.5-Prostate-Specific Antigen (PSA) Blood Test

The PSA test is a simple blood test that measures the amount of PSA (prostate specific antigen) in the bloodstream of the individual. It is the most conclusive test used for the early detection of prostate cancer (Strax, 2006).

Hara et al. (1971), were first who discovered the prostate specific antigen PSA and named it γ -seminoprotein. Then Sensabaugh (1978), found that PSA is a protein, having a molecular weight of 30 kDa. Recent studies show that PSA is an organ-specific enzyme, or protease, produced by the prostate gland and having a molecular weight of 38 kDa. This molecule is the most important protein, which can be found in semen and the function is to liquefy it (Miteva, Yotov, Georgiev & Fasulkov, 2006).

PSA is immunologically specific for prostatic tissue; it is present in normal, benign hyperplastic, and malignant prostatic tissue, in metastatic prostatic carcinoma, and also in prostatic fluid and seminal plasma. Elevated serum PSA concentrations have been reported in patients with prostate cancer and benign prostatic hypertrophy. It has been shown that PSA diffused into the bloodstream from prostate cancer tissue at a much higher concentration than from benign prostatic tissue. Previously, digital rectal examination (DRE) was used to diagnose most prostate cancer (Loeb & Catalona, 2008).

1.4.5.1- Elevation of the PSA

The PSA test should be done before the digital rectal examination (DRE) because PSA level can be elevated if prostate gland is touched. Also there are other changes in the prostate that can raise the amount of PSA. The most common are: sexual intercourse or any ejaculation within 24 hours before the test. Also prostate biopsy increases the amount of PSA and keeps this value high for one week. For PSA to return to its normal value, it takes a month or more after biopsy (Strax, 2006).

1.4.5.2- Total PSA Measurement

PSA is used to detect prostate cancer, using a threshold of 4ng/ml as the upper limit of normal. If the amount of PSA is greater than 4 ng/ml, further examination is recommended such as digital rectal examination DRE, rectal ultrasound and prostate biopsy, because this level can indicate a problem with the prostate, such as inflammation, infection, enlargement or even cancer if it exceeds 10 ng/ml (Loeb et al., 2008).

1.4.5.3- Free PSA

PSA circulates in the bloodstream in two different forms: free (or unbound) form and complexed (cPSA) form that is bound to a protein such as α -1-antichymotrypsin. BPH can be distinguished from prostate cancer by measuring the proportion or the percent of PSA circulating in the free form. Catalona et al. (2000), showed that men had prostate cancer if their free PSA was less than 10%. Free PSA must be measured in order to detect prostate cancer if the total PSA level is between 4-10 ng/ml (Loeb et al., 2008).

1.4.5.4- PSA Density

PSA is produced by prostatic epithelial cells; this means that larger prostate glands produce more PSA. In order to differentiate between prostate cancer and benign enlargement, PSA level should be evaluated in relation to the size of the prostate. The calculation of PSA density (PSAD) is obtained by dividing the PSA level to the prostate volume (Loeb et al., 2008). Benson et al. (1992), emphasized that men having prostate cancer have PSAD higher than men having BPH.

1.5- Treatment

When a diagnosis of BPH is determined, the person should be treated. Results have shown that early treatment for men having mildly enlarged gland is not recommended because the symptoms of BPH can be cleared up immediately, just a regular checkups is needed to detect early problems. On the other hand, treatment is recommended urgently for men having severe symptoms to avoid complicated problems to patient's health (NKUDIC, 2006). Before starting the treatment of BPH, patient must take antibiotics to remove any infection in the urinary tract because one of the symptoms of BPH is the urinary tract infections (NKUDIC, 2006).

There are many ways such as the medical and surgical treatment to relieve symptoms of BPH. Treatment options include medical treatments such as the pharmacological and phytotherapeutic therapies, minimally invasive treatments and surgical treatments (Edwards, 2008).

1.5.1- Pharmacological treatment

The first line to treat men having BPH is the pharmacological treatment (Nix & Carson, 2007). Several medications are available, the most common are: 5alpha-reductase inhibitors and alpha1 blockers or (alpha1-adrenergic antagonists) (Mcpartland et al., 2000).

A) 5 α -Reductase Inhibitors

In the prostate gland, the conversion of testosterone to dihydrotestosterone by the enzyme 5 α -reductase promotes the growth of the prostate (Edwards, 2008). Reducing the androgenic stimulation of the prostate gland is considered as one of the pharmacological treatment of BPH (Matzkin, Chayen, Goldfarb, Gilad & Braf, 1992). The most common 5 α -reductase inhibitors are Finasteride (Proscar) and dutasteride (Avodart) which suppress prostate growth by inhibiting the conversion of testosterone to dihydrotestosterone. They are beneficial if the prostate volume is greater than 40 ml (Edwards, 2008). Matzkin et al. (1992), showed that the 5 α - and 5 β -reductases metabolize the two hormones testosterone and androstenedione to their conjugates androsterone (A) and etiocholanolone (E), that are excreted in the urine. They showed, that in normal men the ratio of A/E is 1.5 due to the high activity of α -reductase, but in men suffering from BPH and treated with 5 α -reductase inhibitor, the ratio of A/E decreases to less than 0.5. To achieve clinical benefit, the 5-alpha reductase inhibitors are recommended for six to 12 months because they do not relieve symptoms immediately (Edwards, 2008). Moreover, it was shown that, 5-alpha reductase inhibitors reduce the risk of acute urinary retention (Edwards, 2008), but at the same time, they caused adverse effect for men such as decreasing ejaculate volume, libido, and sexual erectile dysfunction (Nix et al., 2007).

Andriole et al. (1998), showed that patients treated using 5-alpha reductase inhibitors, have a high risk of prostate cancer because they do lower PSA blood level; which is a substance used to detect prostate cancer. To correct this effect, we should double the value of the measured PSA when screening for prostate cancer.

B) Alpha1 Blockers

The constriction of the prostatic urethra is due to the contraction of the smooth muscles of the prostate gland which is stimulated by alpha1-adrenergic receptor. To promote the relaxation of the smooth muscle of the prostate and urethra, alpha blockers or alpha1-receptor antagonists is the most effective to relieve the symptoms of lower urinary tract for men having moderate to severe BPH (Edwards, 2008). Therefore they work by relaxing the smooth muscle and not by reducing the size of the prostate (Blanchard &

Abu-Baker, 2010). Thus the mechanism that leads to the relaxation of this smooth muscle is to inhibit norepinephrine from binding to alpha1-adrenergic receptor, by inhibiting alpha1-adrenergic receptors (Roehrborn & Schwinn, 2004). The alpha1-adrenergic antagonists are: prazosin, terazosin, doxazosin, alfuzosin, and tamsulosin (Blanchard et al., 2010). Three of them, such as prazosin, terazosin, doxazosin are used to treat hypertension. They act on vascular smooth muscles thereby lowering blood pressure. On the other hand, alfuzosin and tamsulosin have minimal or no effect on blood pressure, so they are more selective to treat the constriction of the smooth muscles of the prostate and urethra (Edwards, 2008). In addition Nix et al. (2007), showed that alpha blockers are more selective than other agents, especially for men who need to preserve the sexual function. They demonstrated that alfuzosin preserves the function of ejaculation and tamsulosin has the effect to induce ejaculation.

B.1 Xatral or Alfuzosin hydrochloride

Chemical Name: (R, S)-N-[3-[(4-amino-6, 7-dimethoxy-2-quinazoliny] methylamino]propyl] tetrahydro-2-furancarboxamide hydrochloride

Molecular Formula: C₁₉H₂₇N₅O₄•HCl

Structural formula:

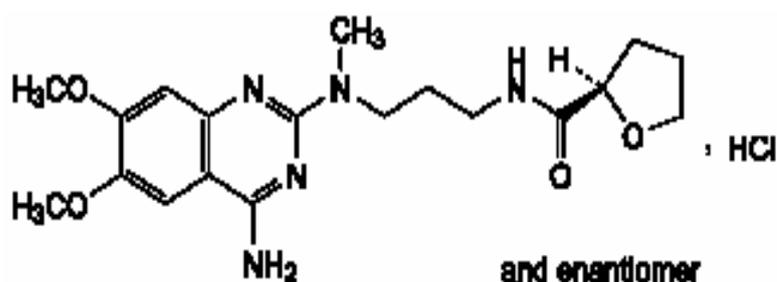


Figure 1.4 Structural formula of Alfuzosin hydrochloride (Roehrborn, Van Kerrebroeck & Nordling, 2003).

Each Xatral tablet contains 2.5 mg of the active ingredient alfuzosin hydrochloride. Alfuzosin hydrochloride is a crystalline powder having a white to off-white color and melts at 240°C. It is readily soluble in water (Nordling, 2005). In addition this tablet contains microcrystalline cellulose, lactose, povidone, sodium, starch glycollate,

magnesium stearate, hypromellose, Macrogol 400, and titanium dioxide (Roehrborn et al., 2003). Alfuzosin is used for the treatment of moderate to severe BPH. It removes the lower urinary tract symptoms and has no effect on blood pressure and the ejaculatory function. It is classified as an alpha 1-adrenergic antagonist (Lee, 2003). The contraction of the smooth muscle is due to the presence of alpha-1 receptors. There are three subtypes of alpha-1 receptors: alpha-1a, alpha-1b, and alpha-1d. The contraction of the smooth muscle of the prostate is mediated by alpha-1a receptors while the others are involved in the contraction of the vascular muscle. Alfuzosin is an agent specific that is involved in the inhibition of alpha-1a receptors; this will lead to the relaxation of the smooth muscle in the prostate, bladder neck, and urethra (Langer, 1999). Alfuzosin is recommended twice or thrice daily when using the immediate-release (IR) administration. When using the extended-release (ER) administration, it is recommended once- or twice-daily. This formulation produces small differences between peak and in the level of the drug in serum because the administration of the drug is continuous over the time. This will explain why a fewer frequency of cardiovascular adverse effects occur when using ER versus IR alfuzosin. For the management of benign prostatic hyperplasia (BPH), the recommended adult dosage of alfuzosin hydrochloride is 10 mg once daily which is safe and effective even if it is administered to patients over the age of 65 years. No titration is needed for this dosage and within few days of treatment its onset of peak action begins (Lee, 2003).

In addition, Alfuzosin has many side effects such as: headache, dizziness, fatigue and upper respiratory tract infection (Roehrborn et al., 2004). Alfuzosin should not be used in patients with renal insufficiency, and liver failure (Van Kerrebroeck, Jardin, Laval & Van Cangh, 2000). As conclusion, alfuzosin 10 mg is recommended for men with lower urinary tract symptoms to improve the erectile function while preserving the ejaculatory function (Rosen, Seftel & Roehrborn, 2007).

C) Combination therapy

The combination of the two drugs, the 5 α -Reductase inhibitors and the alpha-adrenergic antagonists increase the efficacy to remove the symptoms of BPH instead of using each drug alone, or doing the surgery (McConnell et al, 2003).

1.5.2- Phytotherapeutic treatment

In the 15th century, the Egyptians were the first people who use the plant extract to treat BPH. This type of treatment is called phytotherapeutic treatment. After that, it was known in Europe, and the western hemisphere. In Italy, phytotherapy represents half the medications to treat BPH, compared to 5 α -reductase inhibitor and alpha blockers that represent 10%. In the United States, phytotherapies can be used as dietary supplements without prescription to treat BPH. So the phytotherapeutical agents are used to treat mild to moderate symptomatic BPH (Wilt et al., 1998). The most common herbal preparations used to treat BPH are: *Serenoa repens*, Saw palmetto, *Pygeum africanum*, Tadenan, Cernilton, and *hypoxis rooperi*, (Dedhia, 2008). In addition, coconut oil, *urtica dioica radix* (nettle root), *pinus pinea*, *cucurbita pepo* (pumpkin), and *secale cereale* are used. The combination extracts of two or more plants can also be used (Dreikorn, 2007).

1.5.2.1- Pumpkin or Cucurbita Pepo

In developing countries, people use dietary plants and herbal preparations as a traditional medicine. Pumpkin is one of the plants, which are used as medicine or functional food to treat many diseases.



Figure 1.5 Pumpkin or Cucurbita pepo (Winters, 2011).

The family of pumpkin is Cucurbitaceae. It is divided into *Cucurbita pepo*, *C. moschata*, *C. maxima*, *C. mixta*, *C. ficifolia* and *C. telfairia*.

Cucurbita pepo, *C. maxima*, and *C. moschata*, are the most important species because they have a great productivity which make them worldwide cultivated (Caili, Huan & Quanhong, 2006). The pumpkin plant belongs to the dicotyledonous seed vegetable. It is an annual herb or vine. It is made of a flexible stem with trifoliate leaves. When pumpkin matures, yellow flowers then large fruits appear. Each fruits contain numerous seeds (Caili et al., 2006). Those seeds have an oval flat shape, and a greenish dark color (Abdel-Rahman, 2006).



Figure 1.6 Flower and Leave of Cucurbita Pepo (Danos, 1989).



Figure 1.7 Seeds of Cucurbita Pepo (Danos, 1989).

1.5.2.2- Phytochemistry

Pumpkin seeds contain the biologically active components such as: polysaccharides, para-aminobenzoic acid, γ -aminobutyric acid and carotenoid (Caili et al., 2006). They are considered as a good source of vitamins A, B, and E (Gossel-Williams et al., 2006). Four isomers of vitamin E are found: γ - and α -tocopherol which are present at high amount and β - and δ -tocopherol which are present at low amount (Murkovic, Hillebrand, Draxl, Pfannhauser & Winkler, 1999). It has been identified, that vitamin E inhibits the growth of the prostate cell by regulating the cell cycle mechanisms and apoptosis (Uzzo, et al. 2004). Moreover pumpkin seeds have a physiological effect due to the presence of proteins, antioxidants, oil and fiber which is found in the cell wall of the seeds (Kostalova, Hromadkova & Ebringerova, 2009). Those seeds are considered also as a high source of energy due to the presence of 40 to 50 % lipids and 30 to 37% proteins of the dry material (Caili et al., 2006). Four fatty acids are found in the pumpkin seeds.

The unsaturated fatty acids are dominant over the saturated one. Those acids are distributed in the following percentage: (8.0%) stearic acid C18:0, (13.3%) palmitic acid C16:0, (29.0%) oleic acid C18:1 and (47.0%) linoleic acid C18:2. These four fatty acids represent around 98 % of the total amount. The rest of the fatty acids are found at a level below 0.5 % (Younis, Ghirmay & Al-Shihry, 2000). Pumpkin seeds are also rich in phytosterols, which is of special interest because it is responsible to lower the serum cholesterol. Of those sterols, Δ^7 - sterols is the main one which is rare in other plant seeds, and Δ^5 - sterols and Δ^8 - sterols are also found (Murkovic, Piironen, Lampi, Kraushofer & Sontag, 2004). In addition, pumpkin seeds contain the chemical elements such as potassium, phosphorus, calcium, chromium, copper, magnesium, manganese, selenium, molybdenum, sodium, iron and zinc which is the most useful element in treating the enlargement of the prostate (Glew et al., 2006). Zinc inhibits the overgrowth of the prostate, by inhibiting NF-kB which is the transcriptional factor that is responsible for the survival of the cell (Uzzo et al., 2004).

1.5.2.3- Uses of Pumpkin or Cucurbita Pepo

Pumpkin is used as antidiabetic, antihypertensive, antibacterial, antitumor, antihypercholesterolemia (regulate cholesterol level), intestinal antiparasitia (fight against intestinal worms and parasites), anticancer, antiinflammation and immunomodulation (Caili et al., 2006). Pumpkin seeds are also used as anti-depressing agent, due to the presence of L-tryptophan (Eagles, 1990). Pumpkin seeds can lower the risk of bladder-stone disease due to the presence of high amount of phosphorus (Suphakarn, Yarnnon & Ngunboonsri, 1987). Studies show that the pumpkin seed oil is used to treat BPH by inhibiting the production or the action of dihydrotestosterone produced from testosterone that induces the hyperplasia of the prostate gland (Gossel-Williams et al., 2006). Moreover, the Δ^7 - phytosterols together with their glycosides, which are found in the seeds, inhibit the action of the enzyme 5 α -reductase due to their similarity to testosterone. Thus Δ^7 - phytosterols relieve the symptoms of BPH by reducing the concentrations of dihydrotestosterone (Mandl, Reich & Linder, 1999). Another study shows that, phytosterols interfere with the actions of dihydrotestosterone thus blocking the androgen receptor. Thus, pumpkin seed oil improves urinary function and relieves the symptoms of BPH because it has a direct influence on the prostate size

(Gossel-Williams et al., 2006). Tsai et al. (2006), show that pumpkin seed oil combined with Phytosterol-F can block the testosterone prazosin-induced (T-P) increases in the ratio of prostate weight to body weight.

1.5.3-Surgical Treatments

If medical treatments fail, surgical treatment is needed to remove the obstruction and incomplete voiding caused by BPH. There are many types of surgery such as the transurethral resection of the prostate, transurethral incision of the prostate, open surgery, and laser prostatectomy (Edwards, 2008).

1.5.3.1 Transurethral Resection of the Prostate (TURP)

It is used to reduce the pressure on the urethra, due to the enlargement of the prostate gland. It is performed under anesthesia. The urologist inserts through the urethra an instrument called a resectoscope that contains an electrical loop to remove pieces of prostate tissue (NKUDIC, 2006). There are many complications of TURP such as sexual dysfunction where the semen flow backward, hemorrhage, and strictures (NKUDIC, 2006).

1.5.3.2 Transurethral Incision of the Prostate (TUIP)

This technique is used to widen the urethra by reducing the pressure caused by the prostate. In this procedure, the doctor makes a cut in the neck of the bladder and in the prostate instead of removing the tissue such as TURP. It has less complication on the retrograde ejaculation (NKUDIC, 2006).

1.5.3.3 Open Surgery

When the prostate gland is large, open surgery is made to remove the inner core of the prostate by making an incision in the skin of the lower abdomen (NKUDIC, 2006).

1.5.3.4 Laser Prostatectomy

Several studies show that Holmium laser enucleation of prostate (HoLEP) is more effective and safe than TURP for BPH patients. It has no role when the prostate has a large volume (Naval, Saurabh & Aneesh, 2006). It relieves the symptoms of BPH by the energy released from the laser that destroys the prostate tissue (NKUDIC, 2006).

CHAPTER TWO

MATERIALS AND METHODS

2.1- Animals Preparation

All experiments were performed on adult Male Sprague-Dawley rats bred in the animal room of the biology department at the Lebanese American University. The rats were housed in a controlled environment in stainless steel wire- bottom cages at a temperature of 21 ± 2 °C, and humidity of 40-60% with 12 hours photoperiod, during the whole study. Standard rat chow food (Laboratory rodent starter diet no. 1, Hawa Chicken Co., Lebanon) and tap water were given *ad libitums*.

2.2- Sources of pumpkin seeds

The pumpkin seeds were purchased from local Lebanese roastery. The seeds were plain without shells. No heating treatment was applied to the seeds.

2.3- Animals groups

One hundred twenty (120) Sprague Dawley rats were used in this experiment. Rats were divided into eight groups:

- 1) 15 rats as negative control, were kept growing normally, during the period of the study, without being injected with Sustanon (testosterone).
- 2) 15 rats as positive control were injected, every ten days, with Sustanon 3.568 mg/kg body weight and fed on chow diet.
- 3) 15 rats were injected with sustanon, and then treated with Xatral 1 mg/kg body weight.

The other groups were also injected, every ten days, with Sustanon 3.568 mg/kg body weight. Before each injection, two rats from the negative control and the positive control were removed and weighed. After sacrificing, prostates were also removed and weighed. Prostate weight over body weight was calculated in order to compare the two different groups. After 30 days, we realized that the increase in size of the prostate was achieved; treatment begins using different dosages of pumpkin seeds.

- 4) 15 rats were treated with 10% w/w of food pepo.
- 5) 15 rats were treated with 20% w/w of food pepo.

- 6) 15 rats were treated with 30% w/w of food pepo.
- 7) 15 rats were treated simultaneously with both 30% w/w of food pepo and Xatral.
- 8) 15 rats were treated with 60% w/w of food pepo.

2.4- Inducing BPH with Sustanon

Hyperplasia of the rat prostate was induced by a subcutaneous injection of testosterone which was given in the form of Sustanon 3.568 mg/kg body weight, dissolved in ordinary olive oil. It is an oil-based injectable containing four different testosterone combinations which are: testosterone propionate, 30 mg; testosterone phenylpropionate, 60 mg; testosterone isocaproate, 60mg; and testosterone decanoate, 100 mg (Roberts, 2010).

2.5- Dilution of Sustanon

Put 149 ml of olive oil in a 300 ml Erlenmeyer flask. Seal the tube with cotton. Fill a 500 ml beaker with tap water and then heat it. After that, put the tube in the beaker that contains the boiling water. Allow sterilization of Olive oil for 15-20 minutes. Finally allow the tube to cool. Then add the 1ml Sustanon 250 mg/ml to the sterilized olive oil and vortex them. Always work near a flame (Aseptic conditions).

2.6- Injection of Sustanon and treatment

The diluted appropriate volume was injected to rats *intraperitoneally*: the administration of materials into the peritoneal cavity should be done into the space surrounding the abdominal organs, avoiding the injection directly into an organ. Rats should be restrained with their abdomen exposed and their head held downwards, this causes the freely moveable abdominal organs to move towards the animal's diaphragm making accidental puncture of organs less likely.

Treatments with pepo were done for 12, 24 and 36 days. It was given simultaneously with their foods. For the group treated with Xatral, rats were fed on their chow diet and Xatral 1mg/kg body weight was included in the drinking water. On the day of each treatment with pepo, all the rats of each group were weighed. Calculation was also done for the amount of each dose and percentage that must be given for each group according to the average body weights knowing that each rat consumes 7g/100g body weight.

2.7- Sample collection and Blood study

Each twelve days, five out of fifteen rats from the above groups were sacrificed, after sedating them with ether. Anesthesia was maintained by the use of a nose cone. Stretch the rat out in the supine position in the dissection tray. Make a midline incision through the skin from the pubic region to the tip of the lower jaw. Make a midline incision through the abdominal wall musculature from the pubic symphysis to the lower edge of the sternum. Proceed by isolating the following organs: prostate and liver. Livers were removed to be sure that the treatment doesn't affect it. Prostate glands were also removed and weighed. Then calculation of the prostate gland over body weight was done.

While dissecting, 10 ml of blood samples were aspirated with a syringe from the inferior vena cavae of the rats. To collect the serum, tubes were incubated at room temperature for 30 minutes to clot then centrifuged at a speed of 5000X (rpm) for 20 minutes. Some of the serum was placed in Eppendorf tubes for direct liver enzyme analysis: GOT-AST, GPT- AST, and ALP. The remaining samples were collected in Eppendorf tubes and stored in deep freeze for subsequent analyses. Samples stored were later used to assess triglycerides, glucose, cholesterol, and HDL-Cholesterol, using appropriate SPINREACT kit.

2.8- Prostate weight to body weight ratio

Animals were weighted before dissection. After scarifying, prostates were removed and weighed immediately after blotting them dry of excess fluids. Then prostate weight to body weight ratio were calculated.

2.9- Calculation of the Percentage of inhibition

- a) Prostate weight
- b) Prostate weight/ body weight

Then percentage of inhibition was calculated as follows:

$$100 - \left(\frac{\text{treated group} - \text{negative control}}{\text{positive control} - \text{negative control}} \times 100 \right)$$

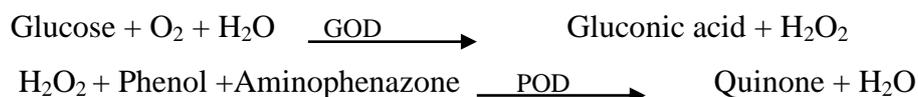
Serum Assays

2.10- Determination of serum Glucose

Principle

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxidase formed reacts under catalysis of peroxidase with phenol and 4-aminophenazone to form a colored compound.

Glucose in serum is measured based on the following enzymatic reactions:



The intensity of the red-violet dye formed at the end of the reaction is proportional to the glucose concentration and is measured by spectrophotometry at a wavelength at 505 nm.

GOD: glucose oxidase; POD: Peroxidase

Procedure

Samples, run in duplicates, were mixed with the working reagent, and incubated for 10 minutes at 37°C. The absorbance (A) of the samples and the standard are measured against the reagent blank at $\lambda = 505$ nm (Helios- γ spectrophotometer, UVG 101103. UK)

Calculation

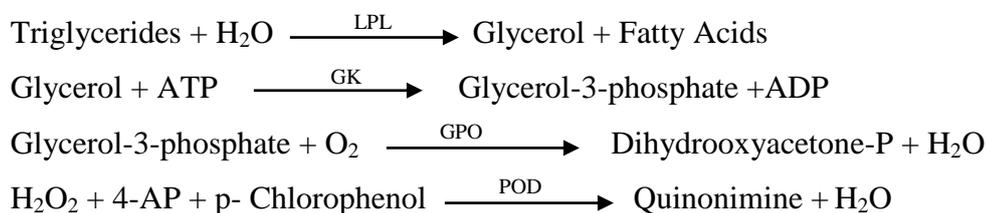
$$\text{Concentration of the sample (mg/ dl)} = \frac{\text{Abs. of the sample}}{\text{Abs. of the standard}} \times \text{Conc. of the standard}$$

Glucose standard is 100 mg/dl concentration according to the manufacturer's leaflet (SPINREACT).

2.11- Determination of Serum TAG

Principle

The TAG assay kit (spinreact), used is based upon a colorimetric method. The TAG are enzymatically hydrolyzed to glycerol and free fatty acids. The glycerol liberated reacts with Glycerol Kinase (GK) and Glycerol-3-Phosphate Oxidase (GPO) yielding H₂O₂. The H₂O₂ concentration is determined through the Trinder's reaction. The enzymatic reaction sequence employed in the assay was as follows:



The intensity of the ended red dye formed is proportional to the concentration of triglycerides in the sample and can be determined by spectrophotometer at a wavelength = 505 nm.

Procedure

Samples, along with standards, were run in duplicates, and were mixed with the working reagent and incubated for 10 minutes at room temperature. The absorbance (A) of the unknown and the standard samples were measured against Blank reagent at $\lambda = 505$ nm (Helios- γ spectrophotometer). Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 minutes.

Calculation

Concentration of triglycerides (mg/ dl) in the sample = $\frac{\text{Abs.of the sample}}{\text{Abs.of the standard}} \times \text{Conc.}$

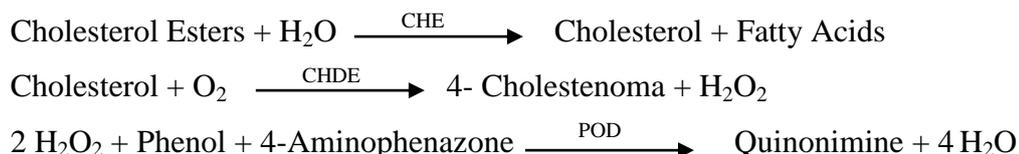
of the standard.

Triglycerides standard is 200 mg/dl concentration according to the manufacturer's leaflet (SPINREACT).

2.12- Determination of Serum Cholesterol

Principle

The cholesterol assay kit (Spinreact, S.A, Spain) used was based upon a colorimetric method. Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters and is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol- oxidase according to the following reactions:



The resulted Hydrogen peroxide forms a red dye. The color intensity is proportional to the cholesterol concentration in the sample that can be determined using the spectrophotometer at a wavelength $\lambda = 505 \text{ nm}$.

Procedure

Samples, along with standards, were run in duplicates, and were mixed with the working reagent and incubated for 10 minutes at room temperature. The absorbances of the samples and the standard samples were measured against Blank reagent at $\lambda = 505 \text{ nm}$ (Helios- γ spectrophotometer). Color of the mixtures was varying from light pink to dark red, and it was stable for about 30 minutes.

Calculation

Concentration of cholesterol (mg/ dl) in the sample = $\frac{\text{Abs.of the sample}}{\text{Abs.of the standard}} \times \text{Conc. of}$

the standard.

Cholesterol standard is 200 mg/dl concentration according to the manufacturer's leaflet (SPINREACT).

2.13- Determination of Serum HDL-Cholesterol

Principle

Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) in the serum are precipitated by phosphotungstate in the presence of magnesium ions and can be removed by centrifugation where high density lipoprotein remain in the supernatant. Determination of HDL cholesterol is performed using the clear supernatant.

Procedure

0.5 ml of the serum sample is mixed with 50 μ l of the precipitating reagent (14 mmol/L phosphotungstic acid with 2 mmol/L magnesium chloride). Then, samples were incubated for 10 minutes at room temperature, followed by centrifugation at 12000 rpm for 4 minutes. The clear supernatant was used for cholesterol determination using the cholesterol assay kit as described in the previous section and the absorbance of the samples is measured by spectrophotometry at $\lambda = 505$ nm.

Calculation

HDL- cholesterol concentration was estimated according to the following equation:

HDL- cholesterol (mg/dl) = Absorbance of the sample \times 320 at $\lambda = 505$ nm.

2.14- Determination of serum LDL-Cholesterol

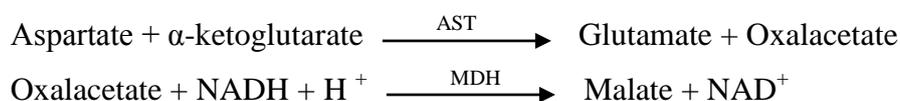
Concentration of LDL cholesterol was determined according to the Friedwald equation:

$$\text{LDL cholesterol} = \text{Total Cholesterol} - (\text{TAG}/5) - \text{HDL Cholesterol}$$

2.15- Determination of Serum GOT-AST

Principle

Aspartate aminotransferase (AST) or glutamate oxaloacetate (GOT) is one of the liver enzymes that catalyses the reversible transfer of an amino group from aspartate to α -ketoglutarate. Then the oxalacetate formed will be reduced to malate by malate dehydrogenase (MDH) and NADH as follows:



Using the spectrophotometer, the rate of decrease in concentration of NADH is measured at a wavelength = 340 nm. This decrease in the concentration of NADH is proportional to the catalytic concentration of AST found in the sample.

Procedure

100 µl of each sample are mixed with 1 ml of the working reagent and incubated at a temperature of 25 °C -30°C for 1 minute, after which the extinction decrease (ΔA) is measured at $\lambda = 340$ nm (Helios- γ spectrophotometer) against distilled water every 1 to 3 minutes.

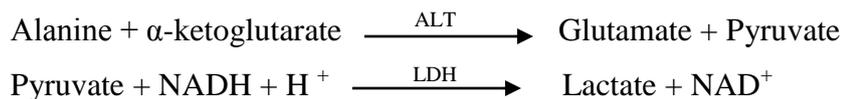
Calculation

$$\text{sGOT activity (U/L)} = \frac{\Delta A}{(\text{min}) \times 1750}$$

2.16- Determination of Serum GPT-AST

Principle

Alanine aminotransferase (ALT) or glutamate pyruvate transaminase (GPT) is another liver enzyme that catalyses the conversion of amino acids to the corresponding α -keto acids through the transfer of an amino group. GPT is determined based on the following enzymatic reactions:



Using the spectrophotometer, the rate of decrease in concentration of NADH is measured at a wavelength = 340 nm. This decrease in the concentration of NADH is proportional to the catalytic concentration of ALT found in the sample.

* LDH: Lactate dehydrogenase.

Procedure

100 µl of each sample are mixed with 1 ml of the working reagent and incubated at a temperature of 25 °C -30°C for 1 minute, after which the extinction decrease (ΔA) is measured at $\lambda = 340$ nm (Helios- γ spectrophotometer) against distilled water every 1 to 3 minutes.

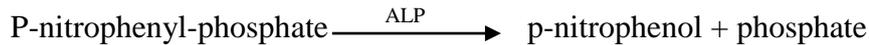
Calculation

$$\text{sGOT activity (U/L)} = \frac{\Delta A}{(\text{min}) \times 1750}$$

2.17- Determination of Serum ALP

Principle

Alkaline Phosphatase is measured based on the following reaction:



Procedure

20 μl of each sample are mixed with 1.2 ml of the working reagent and incubated at a temperature of 25 °C –30°C for 1 minute, after which the extinction decrease (ΔA) is measured at $\lambda = 405 \text{ nm}$ (Helios- γ spectrophotometer) against distilled water every 1 to 3 minutes.

Calculation

$$\text{ALP activity (U/L)} = \frac{\Delta A}{(\text{min}) \times 3300}$$

2.18- Histology

The tissues of the prostate glands were microscopically examined, after being fixed and preserved in 10% neutral formalin, processed and trimmed, embedded in paraffin, sectioned with a paraffin microtome to produce tissue sections of about 4-10 micrometers in thickness. Those sections which are colorless were stained to distinguish the histological elements of the tissue. Hematoxylin is a basic dye which is used to stains acidic structures purplish blue. So the nucleus, ribosomes, and rough endoplasmic reticulum are stained blue. In contrast eosin is an acidic dye which stains basic structures red or pink. The cytoplasmic proteins are basic, so they are stained with eosin. Periodic Acid Shicff (PAS) is used to stain basement membrane, and glycoprotein. To produce permanent slides, tissue sections were covered by a natural or synthetic mounting medium named balsam. This is followed by covering the slides with

a clean cover slip that is laid down on the slide and is left to dry. A complete histopathologic examination was performed, and then images of all slides were classified to be presented in the results.

2.19- Statistical Analysis

All results were expressed as mean \pm S.E.M. Statistical Analysis was performed using student t-test to compare any two group together. A (p) value of less than 0.05 was considered to be significant.

CHAPTER THREE

RESULTS

3.1- Serum Lipid Profile

The tables below summarize the mean serum concentration values in mg/dl of glucose, triglycerides, cholesterol, HDL-Cholesterol, and LDL- Cholesterol, in positive control and groups treated with pumpkin seeds for the three different dissections.

3.1.1- Serum Glucose

There was an increase in the concentration of serum glucose of the groups Xatral, 10%, 20% and 30% pepo in the first and second dissection. There was also an increase in the groups 60% pepo, and 30% pepo + Xatral in the second and third dissection with respect to the negative group. On the other hand, there was a significance increase in the concentration of serum glucose of the groups 20% pepo and 30% pepo (trird dissection), whereas there was a decrease in the concentration of the groups 60% pepo, and 30% pepo + Xatral with respect to the positive group.

Table 3.1.1 Glucose concentrations in serum after 12, 24 and 36 days for testosterone treated rats consuming different doses of pepo and Xatral. Values denote mean \pm S.E.M. (n = 5)

Glucose	12 Days	24 Days	36 Days
Negative control	85.72 \pm 6.48	86.4 \pm 8.43	84.86 \pm 11.32
Positive control	227.49 \pm 25.83	193.37 \pm 18.31	75.98 \pm 5.51
Xatral	169.57 \pm 16.96 ^b	146.75 \pm 16.20 ^b	91.71 \pm 12.20
10% pepo	200.63 \pm 13.61 ^b	157.57 \pm 5.51 ^b	94.74 \pm 8.32
20% pepo	221.06 \pm 20.48 ^b	146.92 \pm 11.80 ^b	111.41 \pm 14.23 ^a
30% pepo	194.89 \pm 11.96 ^b	168.11 \pm 10.34 ^b	97.62 \pm 11.56 ^a
60% pepo	88.04 \pm 4.90 ^a	98.24 \pm 6.34 ^a	136.97 \pm 16.72 ^{a b}
30% pepo + Xatral	87.05 \pm 6.01 ^a	186.86 \pm 44.50 ^b	124.83 \pm 10.64 ^{a b}

^a Significant difference (p < 0.05) with respect to the positive control

^b Significant difference (p < 0.05) with respect to the negative control

3.1. 2- Serum Triglyceride

Results showed an increase in the concentrations of the circulating TAG of the groups Xatral (first dissection), 20% (second and third dissection), 30% pepo (second dissection) with respect to the negative group. There was also an increase in the second and third dissection for the groups 60% pepo (first dissection), and 30% pepo +Xatral (first and second dissection).

Whereas a significant decrease in the concentrations of the circulating TAG was observed in the groups: 10% pepo (first dissection), 30% pepo, and 30% pepo + Xatral with respect to the positive group. There was no significant difference among the other groups.

Table 3.1.2 Triglyceride concentrations in serum after 12, 24 and 36 days for testosterone treated rats consuming different doses of pepo and Xatral. Values denote mean \pm S.E.M. (n = 5)

Triglyceride	12 Days	24 Days	36 Days
Negative control	62.5 \pm 8.51	62.67 \pm 9.30	62.76 \pm 10.87
Positive control	137.10 \pm 14.71	47.18 \pm 8.22	78.47 \pm 9.18
Xatral	91.40 \pm 26.75 ^b	43.06 \pm 3.73	57.94 \pm 7.22
10% pepo	66.28 \pm 15.67 ^a	54.35 \pm 7.37	68.87 \pm 9.61
20% pepo	99.00 \pm 27.95 ^b	61.85 \pm 11.44	94.78 \pm 8.36 ^b
30% pepo	54.67 \pm 11.72 ^a	91.93 \pm 10.84 ^{a b}	78.15 \pm 7.54
60% pepo	126.12 \pm 21.60 ^b	66.33 \pm 10.43	71.19 \pm 0.65
30% pepo + Xatral	102.37 \pm 24.7 ^b	104.17 \pm 8.46 ^{a b}	40.32 \pm 0.01 ^a

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

3.1. 3- Serum Cholesterol

There was a significant decrease in the concentrations of circulating cholesterol of the groups 60% pepo, and 30% pepo + Xatral with respect to the negative group.

Also with respect to the positive group, there was a significant decrease of the groups: 20% pepo (first dissection), 30% pepo (first dissection), 60% pepo, and the 30% pepo + Xatral. No significant difference was observed among the other groups.

Table 3.1.3 Cholesterol concentrations in serum after 12, 24 and 36 days for testosterone treated rats consuming different doses of pepo and Xatral. Values denote mean \pm S.E.M. (n = 5).

Cholesterol	12 Days	24 Days	36 Days
Negative control	85.90 \pm 6.15	84.34 \pm 6.81	83.09 \pm 7.98
Positive control	92.08 \pm 4.20	87.27 \pm 15.25	86.98 \pm 5.99
Xatral	98.06 \pm 8.52	95.13 \pm 13.55	76.77 \pm 9.90
10% pepo	79.41 \pm 7.98	98.65 \pm 11.67	77.95 \pm 4.53
20% pepo	66.16 \pm 5.62 ^a	66.52 \pm 9.84	77.17 \pm 10.72
30% pepo	67.92 \pm 7.64 ^a	88.68 \pm 6.84	73.35 \pm 1.64
60% pepo	55.84 \pm 8.83 ^{a,b}	39.94 \pm 8.06 ^{a,b}	47.65 \pm 6.42 ^{a,b}
30% pepo + Xatral	65.92 \pm 8.78 ^{a,b}	58.59 \pm 5.34 ^b	56.01 \pm 2.72 ^{a,b}

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

3.1. 4- Serum HDL-Cholesterol

Compared to the negative group, there was a significant increase in the concentrations of circulating HDL-Cholesterol among the groups: Xatral (third dissection), 60% pepo and 30% pepo + Xatral (first dissection). In addition, there was a decrease of the groups 20% pepo (third dissection), 60% pepo and 30% pepo + Xatral (second and third dissection).

More a decrease was observed in the concentrations of circulating cholesterol of the groups: 10% and 20% pepo (third dissection), 30% and 60% pepo (second and third dissection), and the 30% pepo + Xatral (third dissection) with respect to the positive group. No significant difference was observed among the positive group and Xatral group compared to the negative group.

Table 3.1.4 HDL-Cholesterol levels in serum after 12, 24 and 36 days consumption of different doses of pepo and Xatral in testosterone treated rats. Values denote mean \pm S.E.M. (n = 5)

HDL – Cholesterol	12 Days	24 Days	36 Days
Negative control	32.17 \pm 2.89	31.65 \pm 2.39	30.05 \pm 3.07
Positive control	32.10 \pm 8.91	24.70 \pm 3.71	50.94 \pm 3.35
Xatral	23.68 \pm 0.29	31.42 \pm 3.15	46.98 \pm 0.41 ^b
10% pepo	36.80 \pm 2.11	32.32 \pm 4.65	27.94 \pm 2.18 ^a
20% pepo	23.74 \pm 2.51	30.14 \pm 4.32	17.63 \pm 1.62 ^{a,b}
30% pepo	28.93 \pm 3.13	43.20 \pm 4.76 ^a	25.06 \pm 4.83 ^a
60% pepo	43.68 \pm 7.79 ^b	13.57 \pm 2.51 ^{a,b}	17.50 \pm 1.44 ^{a,b}
30% pepo + Xatral	63.46 \pm 7.37 ^b	16.58 \pm 1.64 ^b	23.14 \pm 1.99 ^a

^a Significant difference (p < 0.05) with respect to the positive control

^b Significant difference (p < 0.05) with respect to the negative control

3.1. 5- Serum LDL-Cholesterol

The groups: Xatral (third dissection), 10% pepo (first dissection), 20% pepo, 30% pepo, 60% pepo, and 30% pepo + Xatral (first, second and third dissection) showed a significant decrease in the concentrations of circulating LDL-Cholesterol compared to the negative control group. Whereas the groups: Xatral (first and second dissection), and 10% pepo (second dissection) showed an increase in the concentrations with respect also to the negative group.

On the other hand, there was an increase in the circulating LDL-Cholesterol of the groups: Xatral (first dissection), 10% pepo, 20% pepo and 30% pepo (third dissection) and a decrease in the groups: 20% pepo and 30% pepo (second dissection), 60% pepo, and 30% pepo + Xatral (first, second dissection) compared to the positive group.

Table 3.1.5 LDL-Cholesterol levels in serum after 12, 24 and 36 days consumption of different doses of pepo and Xatral in testosterone treated rats. Values denote mean \pm S.E.M (n = 5)

LDL – Cholesterol	12 Days	24 Days	36 Days
Negative Control	41.23 \pm 1.56	40.16 \pm 2.56	40.49 \pm 2.74
Positive Control	32.56 \pm 0.35	53.13 \pm 9.90	20.35 \pm 0.80
Xatral	56.10 \pm 2.88 ^{a b}	55.10 \pm 9.65 ^b	18.20 \pm 8.05 ^b
10% Pepo	29.35 \pm 2.74 ^b	55.46 \pm 5.55 ^b	36.24 \pm 0.43 ^a
20% Pepo	22.62 \pm 1.52 ^b	24.01 \pm 3.23 ^{a b}	40.58 \pm 7.43 ^a
30% Pepo	28.06 \pm 2.17 ^b	27.09 \pm 0.91 ^{a b}	32.66 \pm 0.30 ^a
60% Pepo	3.94 \pm 2.72 ^{a b}	13.10 \pm 3.46 ^{a b}	15.91 \pm 4.85 ^b
30% Pepo + Xatral	1.99 \pm 2.47 ^{a b}	21.18 \pm 2.01 ^{a b}	24.81 \pm 0.73 ^b

^a Significant difference (p < 0.05) with respect to the positive control

^b Significant difference (p < 0.05) with respect to the negative control

3.2- Serum Enzymes

Tables 3.2.(1, 2, 3) summarizes the serum activities of sGOT-AST, sGPT- AST, and ALP in the positive control and groups treated with pumpkin seeds and Xatral for the three periods 12, 24, and 36 days.

3.2.1-Serum sGOT-AST

No significant difference was observed among the negative control groups and all the other groups. When compared with the positive control group, there was a significant increase in the concentrations of serum GOT between the control group and the groups: xatral (first dissection), 60% pepo (third dissection), and the 30% pepo +Xatral (second dissection).

Table 3.2.1 sGOT-AST activity in serum after 12, 24 and 36 days consumption of different doses of pepo and Xatral in testosterone treated rats. Values denote mean \pm S.E.M. (n = 5).

GOT	12 Days	24 Days	36 Days
Negative control	9.43 \pm 0.51	9.68 \pm 0.59	9.72 \pm 0.52
Positive control	10.11 \pm 0.44	8.28 \pm 0.42	7.78 \pm 1.21
Xatral	14.27 \pm 1.38 ^a	11.32 \pm 2.75	10.58 \pm 1.22
10% pepo	12.25 \pm 1.14	7.43 \pm 0.67	8.91 \pm 0.86
20% pepo	10.95 \pm 1.18	8.59 \pm 0.94	8.98 \pm 0.97
30% pepo	11.74 \pm 0.76	7.08 \pm 0.39	10.23 \pm 0.86
60% pepo	12.21 \pm 1.35	11.01 \pm 1.16	11.47 \pm 1.09 ^a
30% pepo + Xatral	11.67 \pm 1.17	11.32 \pm 1.25 ^a	10.27 \pm 1.45

^a Significant difference (p < 0.05) with respect to the positive control

^b Significant difference (p < 0.05) with respect to the negative control

3.2.2-Serum sGPT-AST

There was a significant difference in the groups: Xatral (third dissection), 10% pepo (second dissection), 30% pepo (first dissection), 60% pepo and 30% pepo + Xatral compared to the negative group.

When compared to the positive group, a significant increase in the concentration of serum GPT was observed in the groups: Xatral (third dissection), 20% pepo (second dissection), and 60% pepo (second dissection); and a decrease was observed in the groups 60% pepo and 30% pepo +Xatral.

Table 3.2.2 sGPT- AST activity in serum after 12, 24 and 36 days consumption of different doses of pepo and Xatral in testosterone treated rats. Values denote mean \pm S.E.M. (n = 5)

GPT	12 Days	24 Days	36 Days
Negative control	5.21 \pm 0.19	5.37 \pm 0.24	5.41 \pm 0.28
Positive control	8.13 \pm 0.67	3.23 \pm 0.52 ^b	5.48 \pm 0.95
Xatral	7.16 \pm 0.58	6.11 \pm 1.37	11.20 \pm 2.07 ^{a b}
10% pepo	6.27 \pm 0.92	4.08 \pm 0.74 ^b	5.18 \pm 1.19
20% pepo	6.29 \pm 0.23	5.95 \pm 0.72 ^a	7.12 \pm 0.73
30% pepo	8.05 \pm 0.83 ^b	4.63 \pm 0.62	5.95 \pm 0.55
60% pepo	4.95 \pm 0.48 ^{a b}	7.66 \pm 1.20 ^{a b}	6.77 \pm 0.96
30% pepo + Xatral	5.72 \pm 0.72 ^a	8.94 \pm 0.58 ^{a b}	7.27 \pm 0.71 ^b

^a Significant difference (p < 0.05) with respect to the positive control

^b Significant difference (p < 0.05) with respect to the negative control

3.2.3-Serum ALP

A significant difference was observed in the groups: Xatral (first and second dissection), 10% pepo (third dissection), 20%, 30% and 60% pepo (second and third dissection) and 30% pepo + Xatral (third dissection) compared to the negative group.

There was also a significant decrease in the concentration of serum ALP the Xatral group (third dissection), 20% pepo (first dissection), 60% pepo and 30% pepo + Xatral (first and third dissection) compared to the positive group.

Table 3.2.3 ALP activity in serum after 12, 24 and 36 days consumption of different doses of pepo and Xatral in testosterone treated rats. Values denote mean \pm S.E.M. (n = 5)

ALP	12 Days	24 Days	36 Days
Negative control	50.52 \pm 1.72	51.17 \pm 1.62	49.50 \pm 1.94
Positive control	36.96 \pm 3.38	50.38 \pm 8.49	29.63 \pm 3.46
Xatral	36.74 \pm 2.29 ^b	39.75 \pm 4.44 ^b	42.46 \pm 3.62 ^a
10% pepo	45.17 \pm 2.43	48.69 \pm 5.91	33.00 \pm 3.96 ^b
20% pepo	50.20 \pm 4.47 ^a	68.27 \pm 11.31 ^b	44.29 \pm 6.36
30% pepo	45.32 \pm 4.25	69.67 \pm 9.92 ^b	39.09 \pm 4.41 ^b
60% pepo	51.55 \pm 2.59 ^a	41.73 \pm 6.69 ^b	53.97 \pm 5.95 ^a
30% pepo + Xatral	58.08 \pm 1.44 ^a	48.69 \pm 5.72	41.87 \pm 1.35 ^{a,b}

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

3.3- Evaluation of prostate enlargement

3.3.1-Prostate weights

Significant elevation in prostate weights was found with testosterone treatment when compared with negative control rats. On the other hand there was significant reduction in elevation of prostate weights of groups treated with pumpkin seeds in testosterone treated rats.

3.3.2-Prostate weights to body weights ratio

Testosterone significantly elevated the prostate weights/body weights ratio when compared with negative control rats. But significant reduction in elevation of prostate weights/body weights ratio was found by pumpkin seeds treatment in testosterone treated rats.

During the first 12 days, the groups VI (30% pepo), VII (60% pepo), and VIII (30% pepo + Xatral) showed the highest % of inhibition especially the group VI which was inhibited 86.7%.

During the second dissection (24 days), the same results were observed especially the group 30% pepo that was also inhibited 98%.

The same observation was noticed in the final dissection after 36 days where group VI (30% pepo) was inhibited 98.4%. In addition group 10% showed 84.91% of inhibition.

3.3.3-Body weights changes

There were no significant differences in body weights of rats before and after testosterone treatment among groups.

Table 3.3.1 Effect of pumpkin seeds and Xatral on body weights and prostate enlargements in testosterone treated rats during the first twelve days. Values denote mean \pm SEM (n=5)

Groups	BW(g)	PW(g)	PW/BW ratio($\times 10^{-3}$)	% inhibition
Group I	374.66 \pm 19.61	0.800 \pm 0.063	2.135 \pm 0.000138 ^b	
Group II	300.00 \pm 9.24	0.493 \pm 0.013	1.652 \pm 0.000086 ^a	
Group III	338.22 \pm 16.29	0.707 \pm 0.021	2.100 \pm 0.000057 ^b	7.18% ^b
Group IV	342.14 \pm 25.06	0.687 \pm 0.036	2.048 \pm 0.000181 ^b	18.02% ^b
Group V	361.82 \pm 26.24	0.728 \pm 0.050	2.025 \pm 0.000114 ^b	22.71% ^b
Group VI	387.00 \pm 10.87	0.664 \pm 0.050	1.716 \pm 0.000119 ^a	86.70% ^a
Group VII	355.80 \pm 8.67	0.635 \pm 0.065	1.783 \pm 0.000163 ^a	72.84% ^a
Group VIII	312.00 \pm 13.47	0.566 \pm 0.009	1.826 \pm 0.000067 ^a	63.90% ^a

- PW: Prostate weight, BW: Body weight, Group I: Positive control, Group II: Negative Control, Group III: Xatral, Group IV: 10% Pepo, Group V: 20% Pepo, Group VI: 30% Pepo, Group VII: 60% Pepo, Group VIII: 30% + Xatral. Values are expressed as mean \pm S.E.M.

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

*

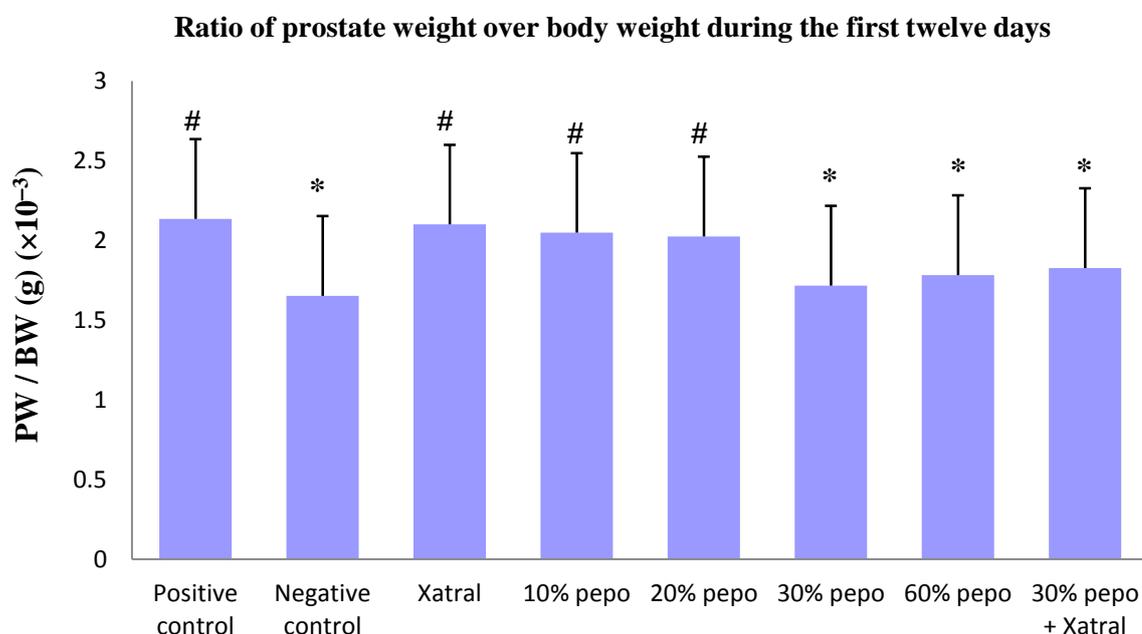


Chart 3.1.1 Ratio of prostate weight over body weight during the first twelve days.

*Significant difference ($p < 0.05$) with respect to the positive control

Significant difference ($p < 0.05$) with respect to the negative control

% inhibition during the first twelve days

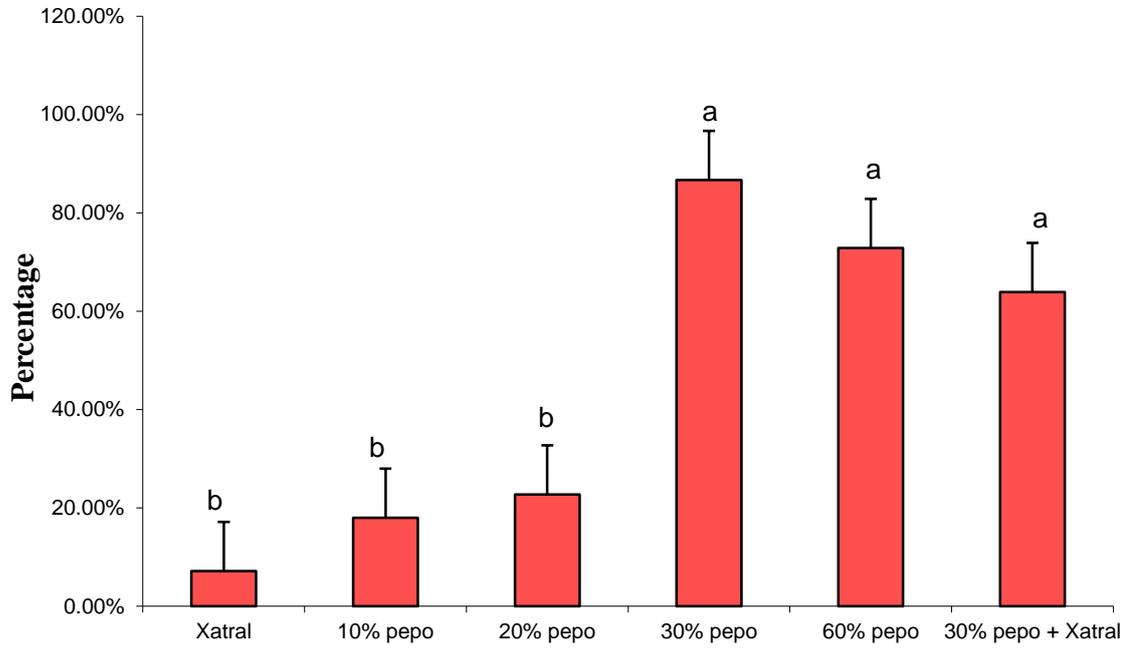


Chart 3.1.2 Percentage inhibition during the first twelve days.

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

Table 3.3.2 Effect of pumpkin seeds and xatral on body weights and prostate enlargements in testosterone treated rats during twenty four days. Values denote mean \pm SEM (n=5)

Groups	BW(g)	PW(g)	PW/BW ratio($\times 10^{-3}$)	% inhibition
Group I	325.00 \pm 7.96	0.805 \pm 0.027	2.477 \pm 0.198 ^b	
Group II	322.80 \pm 20.66	0.550 \pm 0.037	1.711 \pm 0.086 ^a	
Group III	307.00 \pm 21.07	0.744 \pm 0.023	2.475 \pm 0.198 ^b	0.25% ^b
Group IV	292.40 \pm 29.60	0.641 \pm 0.031	2.250 \pm 0.171 ^b	29.59% ^b
Group V	344.20 \pm 13.11	0.680 \pm 0.025	1.981 \pm 0.073 ^b	64.76% ^b
Group VI	400.20 \pm 13.31	0.690 \pm 0.059	1.726 \pm 0.146 ^a	98.00% ^a
Group VII	331.00 \pm 7.05	0.571 \pm 0.021	1.728 \pm 0.064 ^a	97.74% ^a
Group VIII	329.20 \pm 9.14	0.569 \pm 0.031	1.727 \pm 0.064 ^a	97.88% ^a

- PW: Prostate weight, BW: Body weight, Group I: Positive control, Group II: Negative Control, Group III: Xatral, Group IV: 10% Pepo, Group V: 20% Pepo, Group VI: 30% Pepo, Group VII: 60% Pepo, Group VIII: 30% + Xatral. Values are expressed as mean \pm S.E.M

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

Ratio of prostate weight over body weight during the twenty four days.

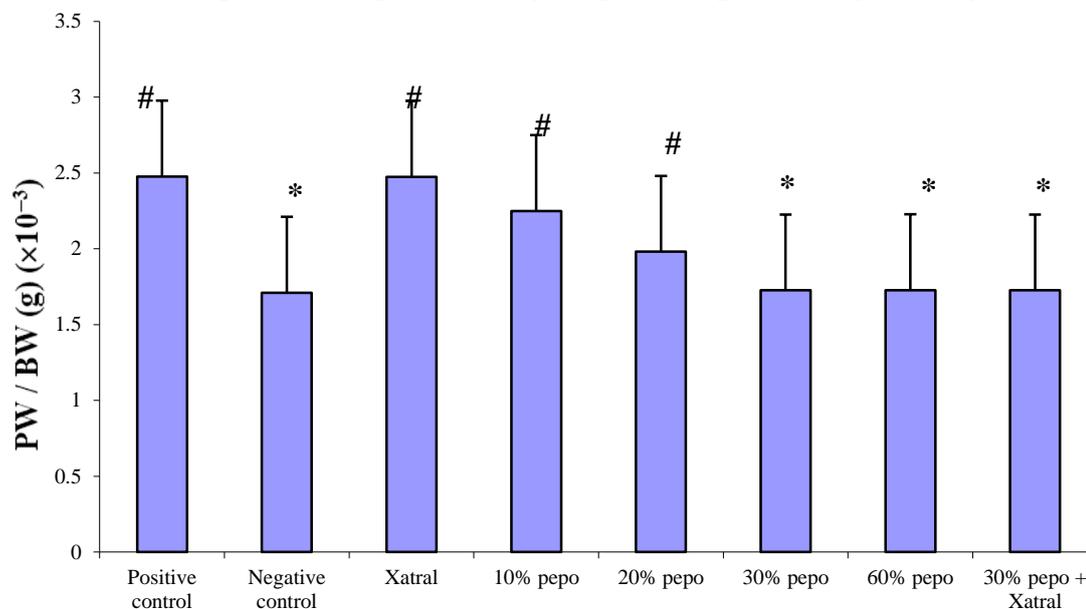


Chart 3.2.1 Ratio of prostate weight over body weight during the twenty four days.

* Significant difference ($p < 0.05$) with respect to the positive control

Significant difference ($p < 0.05$) with respect to the negative control

% inhibition during the twenty four days

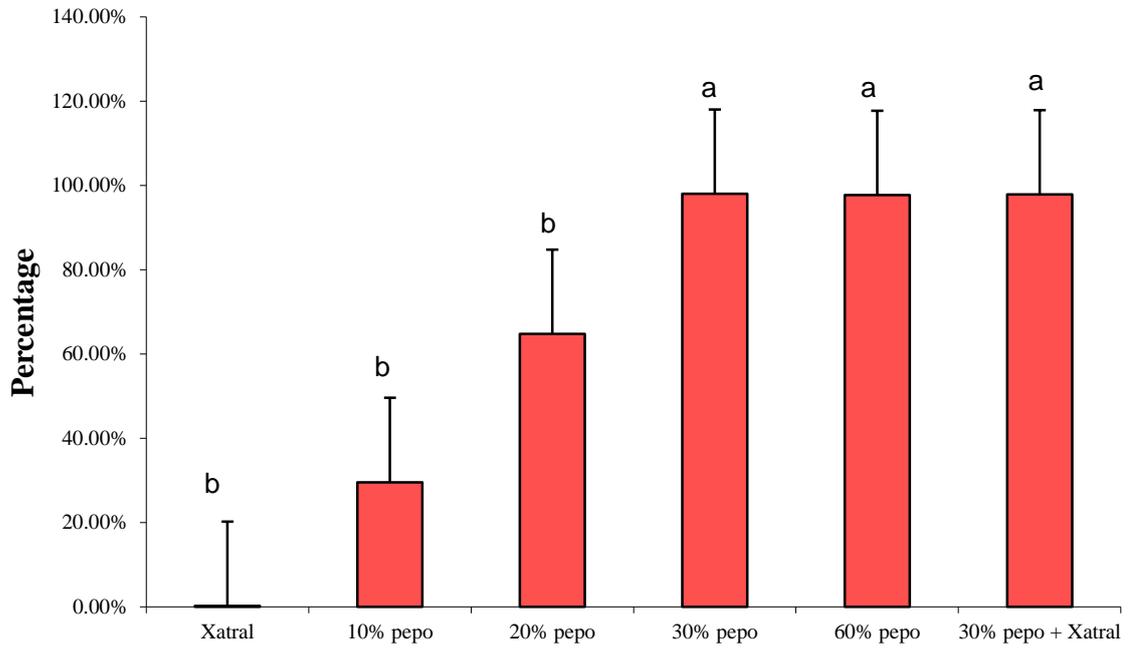


Chart 3.2.2 Percentage inhibition during the twenty four days.

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

Table 3.3.3 Effect of pumpkin seeds and xatral on body weights and prostate enlargements in testosterone treated rats during thirty six days. Values denote mean \pm SEM (n=5).

Groups	BW(g)	PW(g)	PW/BW ratio($\times 10^{-3}$)	% inhibition
Group I	322.20 \pm 23.18	0.749 \pm 0.053	2.338 \pm 0.070 ^b	
Group II	297.00 \pm 14.90	0.543 \pm 0.026	1.833 \pm 0.064 ^a	
Group III	285.20 \pm 10.02	0.655 \pm 0.022	2.301 \pm 0.070 ^b	7.32% ^b
Group IV	370.20 \pm 16.97	0.705 \pm 0.052	1.909 \pm 0.129 ^a	84.91% ^a
Group V	339.20 \pm 29.83	0.693 \pm 0.074	2.049 \pm 0.178 ^b	57.25% ^b
Group VI	322.20 \pm 16.85	0.590 \pm 0.018	1.841 \pm 0.060 ^a	98.43% ^a
Group VII	372.60 \pm 17.19	0.686 \pm 0.041	1.843 \pm 0.091 ^a	97.85% ^a
Group VIII	360.20 \pm 19.24	0.658 \pm 0.016	1.843 \pm 0.086 ^a	98.02% ^a

- PW: Prostate weight, BW: Body weight, Group I: Positive control, Group II: Negative Control, Group III: Xatral, Group IV: 10% Pepo, Group V: 20% Pepo, Group VI: 30% Pepo, Group VII: 60% Pepo, Group VIII: 30% + Xatral. Values are expressed as mean \pm S.E.M

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

Ratio of prostate weight over body weight during the thirty six days.

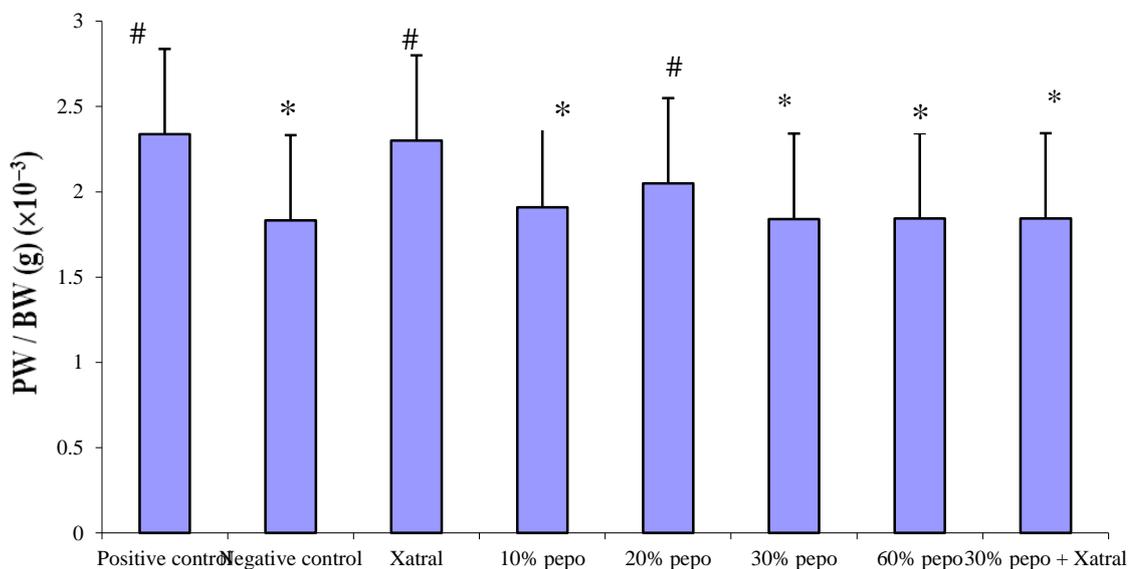


Chart 3.3.1 Ratio of prostate weight over body weight during the thirty six days.

*Significant difference ($p < 0.05$) with respect to the positive control

Significant difference ($p < 0.05$) with respect to the negative control

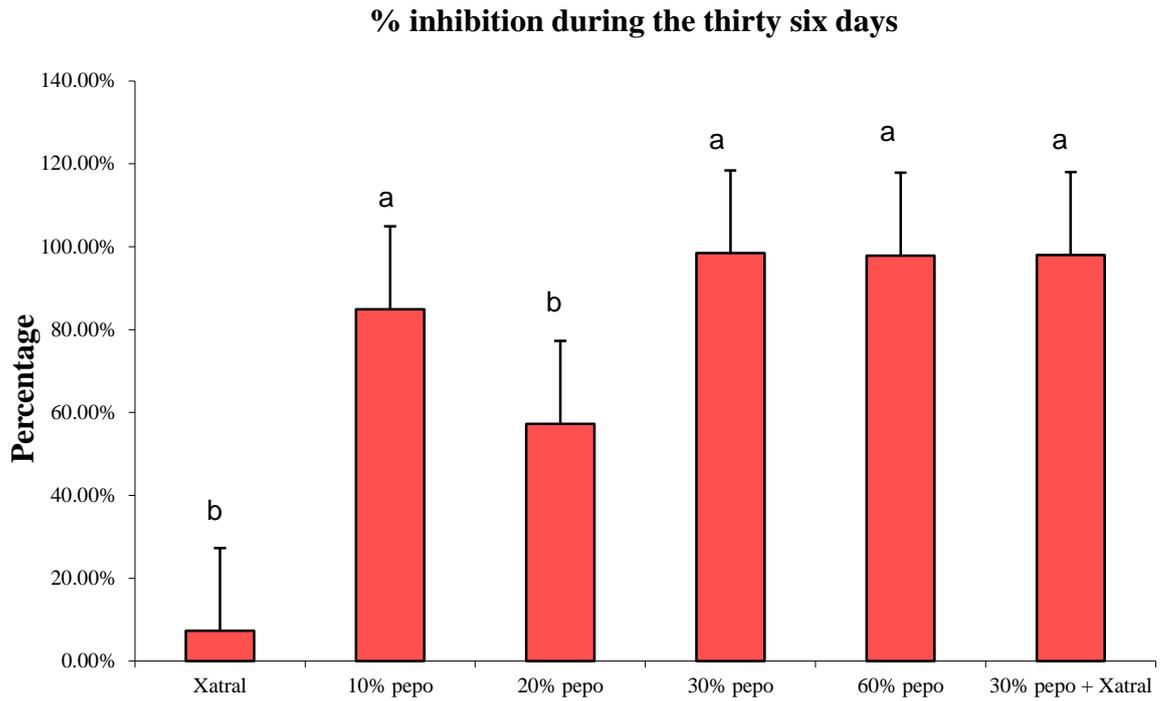


Chart 3.3.2 Percentage inhibition during the thirty six days.

^a Significant difference ($p < 0.05$) with respect to the positive control

^b Significant difference ($p < 0.05$) with respect to the negative control

3.3.4-Histopathology of prostate

The prostate glands were removed and preserved in 10% formaldehyde. Biopsies were later processed to end up with slides representing all the samples. Slides were then stained to highlight the differences between the control and treated groups. Figure 3.4 shows the prostate gland of a rat that had no histopathological signs. The glandular cells are lined by epithelial cells that show mild convolutions. So the tissues were tightly packed; epithelium was cuboidal and regular in size. There is abundant stroma in between glands.

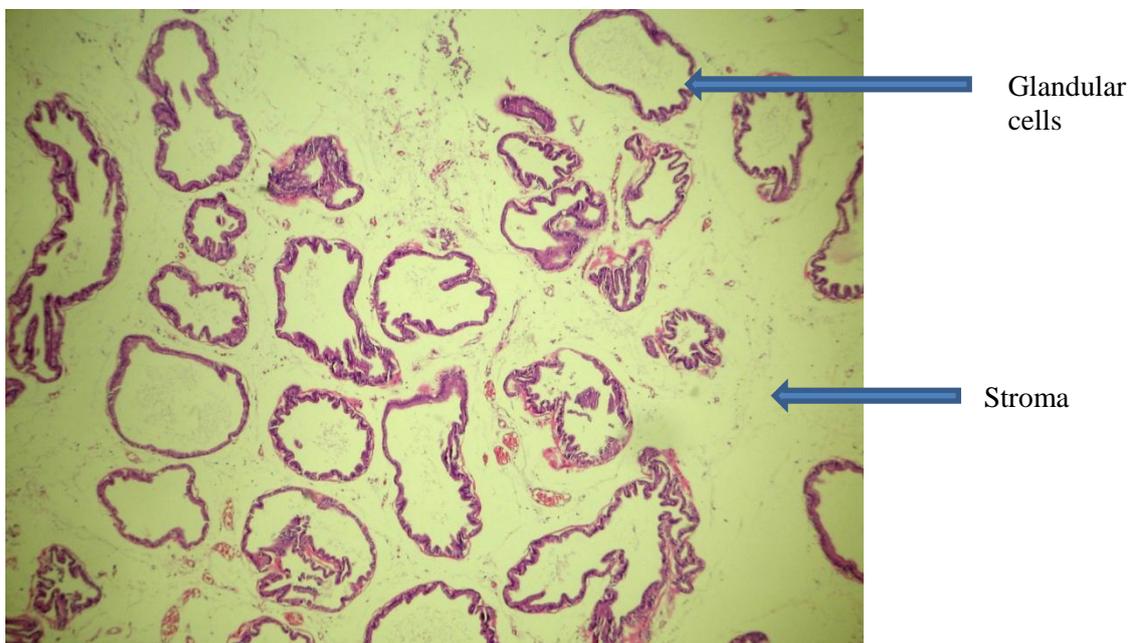


Figure 3.1 Negative group showing no histopathological signs (10x).

Figure 3.5 shows the prostate gland of the group having BPH. There was disruption in the histoarchitecture of the prostate tissue. Here the glandular cells are crowded with only little intervening stroma. In addition those cells are lined by high convoluted epithelium showing intraluminal papillary projections.

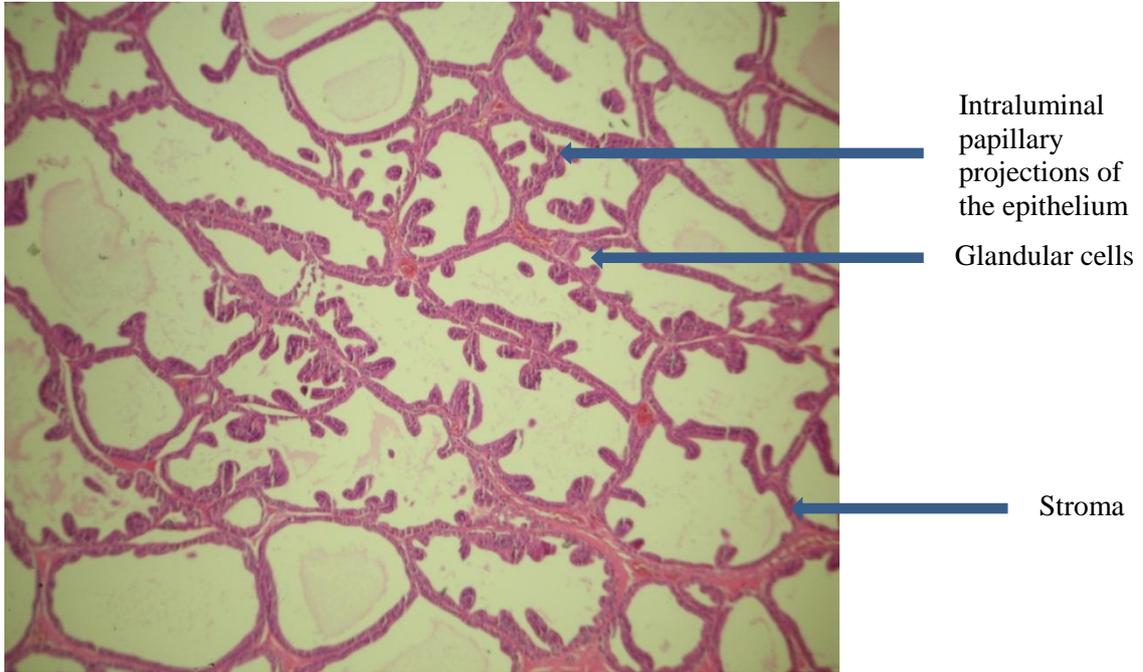


Figure 3.2 Positive group having BPH (10x).

This figure shows the group that has BPH and treated with 30% w/w pepo. It shows a reduction in histoarchitecture disruption of the prostate compared to the positive control group. So the appearance is similar to the negative control group. There is abundant stroma between the glandular cells. In additions there is a lack of papillary projections into the lumen of those glands.

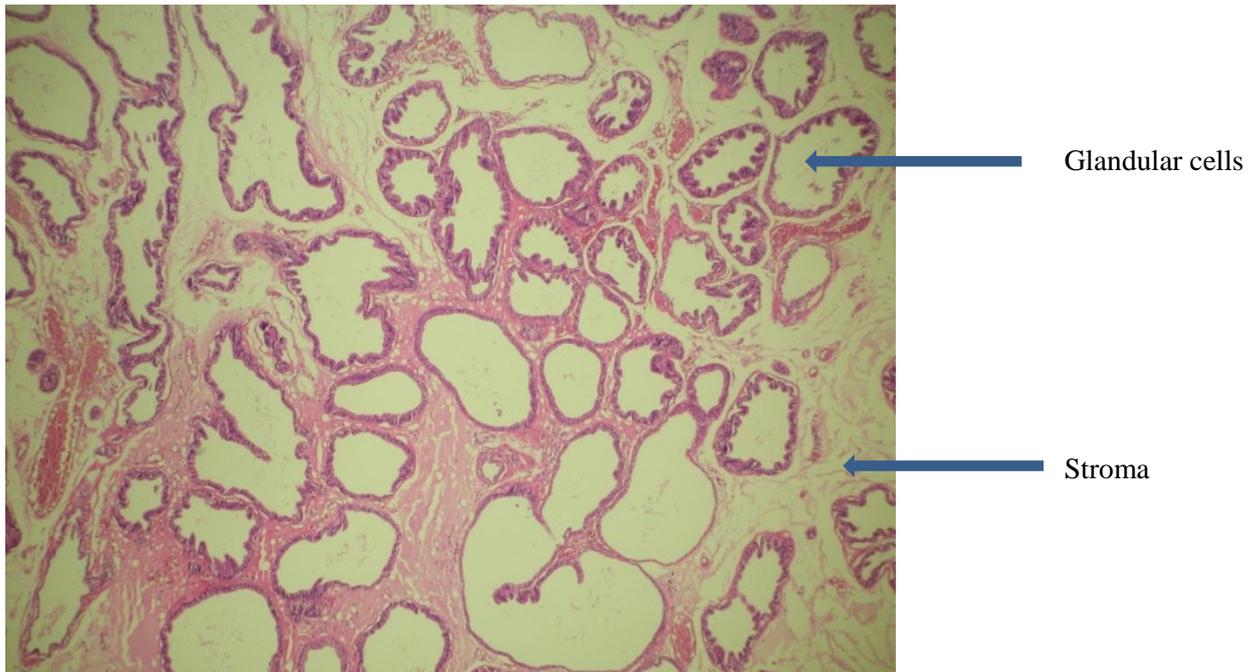
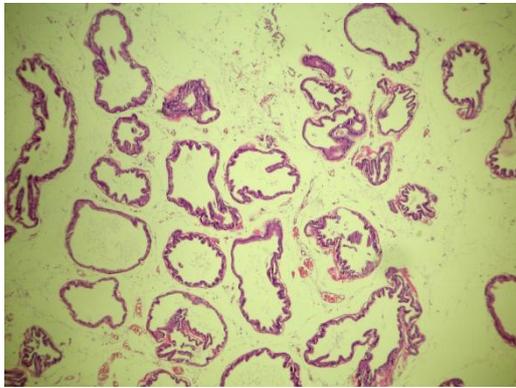
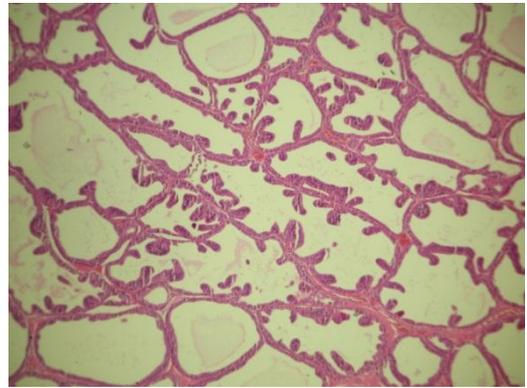


Figure 3.3 Group treated with 30% w/w pepo (10x). Glands with abundant stroma in between. Lack of papillary projections into the lumen of glands.

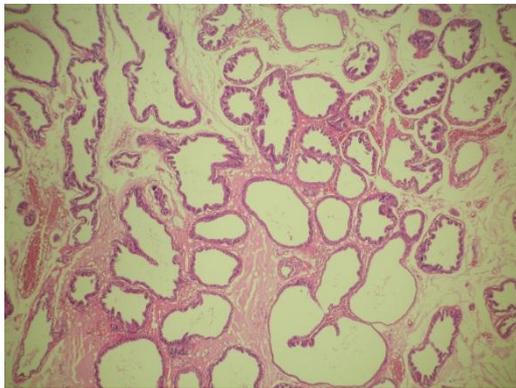
All slides representing the experimental groups are presented in figure 3.7 to facilitate the comparison between the different groups concerning the morphological features discussed before.



A



B



C

Figure 3.4 A: negative control, B: positive control, C: testosterone + 30% pepo.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

The present study focuses on assessing the effects of pumpkin seeds on benign prostatic hyperplasia which is induced by testosterone. The decrease in prostate enlargement was shown by a reduction in prostate weight to body weight ratio compared to normal rat. Lipid profile test (glucose, triglycerides, cholesterol and HDL-cholesterol) and liver enzymes test, GOT-AST, GPT-AST; and ALP) were determined in order to assess possible secondary effects of the pumpkin seeds. The present study was conducted on the male rats in order to avoid any possible toxic effect in human. Chronic human studies are conflicted by differences such as liver function, nutritional factors, smoking, physical activity, and other confounding factors. Thus, animals' studies are easier to control.

In this study, pumpkin seeds appear to have a significant effect on blood lipid profile. First serum glucose concentrations were increased in all groups injected with testosterone ($p < 0.05$). This increase in blood sugar level is due to testosterone treatment as previously shown in several investigations (Caili et al., 2006). This hyperglycemic level was not reduced by ungerminated pumpkin seeds except for the group 20% pepo, and 30% pepo (third dissection). Studies show that proteins from germinated pumpkin seeds after 4 days of germination reduce hyperglycemia by increasing the level of blood insulin; whereas the ungerminated seed didn't possess any effect (Caili et al., 2006).

On the other hand there is a decrease in the concentrations of the circulating TAG compared to the positive control. Whereas no important significant difference was observed compared to the negative controls. So all groups returned to the normal criteria range during treatment with pepo after the first dissection.

Similarly the concentration of the total cholesterol and HDL cholesterol were also kept in the normal range such as the value of the negative control; whereas the groups 60% pepo and 30% pepo+Xatral, show a decrease in the concentration more than the normal value, so we can conclude that those doses affect negatively the amount of total and HDL cholesterol. As for the decrease that appears for the group 20% pepo (third dissection), this could be related to the long term treatment.

Concerning the concentration of LDL-Cholesterol, results showed that pumpkin seeds

reduce the concentration more than the normal value. On the other hand, Xatral increased the concentration of LDL-Cholesterol for short term treatment. In addition, the groups 60% and 30% Xatral showed a high decrease in the amount of circulating LDL-cholesterol. So as the concentration of pumpkin seeds increase the concentration of LDL-cholesterol decrease more and more. This is also confirmed by a study that show that foods containing high concentrations of unsaturated fatty acids such as linoleic acid and oleic acid and plants sterols decrease serum LDL cholesterol by increasing the excretion of cholesterol from the bowl and reducing cholesterol absorption. So plants sterols reduce serum cholesterol and are protective for individuals having heart diseases (Ellegard, Andersson, Normen & Andersson, 2007).

On the other hand, there was no significant difference in the amount of sGOT-AST enzyme when treating with the different doses of pumpkin seeds. Whereas for SGPT-AST enzyme that is produced by the liver the abnormal value attained by the positive control returned to the normal value when treating with the different percentage of pumpkin seeds except for the groups 60% and 30% Xatral that show also some toxicity on the Liver enzyme. The concentration of this enzyme in those groups increase more than the normal value. Concerning ALP, the decrease attained by the positive control returned to the normal value for the groups treated with the different doses of pumpkin seeds whereas for the groups treated with Xatral the amount remain as the positive control. It was important to examine the differences in the gained weights between the groups. It was shown from all the groups tested that neither testosterone nor pumpkin seeds administration had any influence on the weight gain of the rats, so the weight gain between the groups and for the three different dissections was similar. Hyperplasia of the prostate rats was induced with testosterone. This is due to the fact that the hormone is responsible for the development of BPH. Then the value of the prostate size of these rats was compared to the value of the negative control group. It was established that the enzyme 5 α -reductase that is found in the epithelial cells of the prostate and more precisely in the nuclear membrane, converts testosterone to dihydrotestosterone (DHT) which is responsible for the development of BPH. A reduction in the formation of the enzyme 5 α -reductase will induce a decrease in the concentration of DHT. This inhibition will not affect the sexual function of the body since they only inhibit the formation of DHT (Veeresh Babu, Veeresh, Patil & Warke, 2010). Moreover, the

protein cucurbitacins that are found in pumpkin seeds inhibit the activity of 5 α -reductase (Thomas, 1999). It was also shown in the same study that many plants such as saw palmetto, coconut, *Serenoa repens* and pumpkin seeds are responsible for the inhibition of BHP due to the presence of fatty acids in their active ingredients (Veeresh Babu et al., 2010). Another study showed that the extract of saw palmetto is similar in action to finasteride in the improvement of the urinary tract symptoms and the flow of the urine for short term treatment (Wilt et al., 1998). Moreover, pumpkin seeds in conjunction with saw palmetto seeds extract are used against benign prostatic hyperplasia (Abdel-Rahman, 2006).

The ratio of prostate weight over body weight has been used as the main marker for the treatment. This treatment had no effect on the body weight gain but it had a high effect on the prostate weight over body weight gain. It was noticed that BPH was less in rats given simultaneously testosterone with pumpkin seeds, and this protection is significant when the pumpkin seeds dose was 30% and 30% + Xatral in the first 12 days of treatment. After 24 days, the inhibitions increase more and more and it reached 98% for the group 30% pepo. For long term treatment, or after 36 days, a reduction appeared in the group 10% and 30% pepo. This result is also confirmed by the histopathological studies that showed that the group treated with 30% w/w pepo had the same appearance as the negative control. There is abundant stoma between glandular cells. In addition there is lack of papillary projections into the lumen of the glands.

Also during all period of treatment a decrease in PW/BW ratio appeared also for the group 60% and 30% + Xatral. But those percentages showed some negative effects on the lipid profile and liver enzyme. That's why we can consider that the 30% pumpkin seeds is the suitable dose for short treatment. For long term treatment we have the effectiveness of the groups' 10% and 30% w/w pepo.

A study done by Abdel-Rahman (2006), showed also that pumpkin seeds at 10% inhibited BPH by decreasing the level of protein binding prostate (PBP); and decreasing the prostate weight. He noticed that pumpkin seeds, that are rich in omega 3, 6 and 9 that are unsaturated fatty acids and their high protein content, reduce protein binding prostate tissues, and this will lead to a reduction of BPH. Moreover, he established that pumpkin seeds are not only effective in reducing the symptoms of BPH in its early stage but there are no significant side effects. However, in the present study, the dose 10%

appears to reduce prostate weight after long term treatment but not as much as the dose 30%. The histopathological study didn't confirm this result; the appearance of the prostate treated with group 10% remains similar to the positive control whereas the group treated with 30% pepo is similar to the negative control group.

Many studies show that the active ingredients of pumpkin seeds interrupt the multiplication of the prostate cell induced by testosterone. First, it is shown that the Δ^7 -phytosterols together with their glycosides that is found in the pumpkin seed oil inhibit the action of the enzyme 5 α -reductase because there is a conformational similarity between testosterone and Δ^7 -phytosterols. As a result the symptoms of prostate hypertrophy are reduced due to the competition between both at the same receptor (Mandl et al., 1999).

Also the vitamins that are found in pumpkin seeds play an important role in reducing BPH. Among them vitamin E succinate inhibits the proliferation of several cancer cells by arresting the synthesis of the DNA of cancerous cells or by the stimulation of the transforming growth factor beta (TGF- β) (Zhang et al., 2002).

Since the amount of Zinc decreases in prostatitis and prostate cancer, pumpkin seeds that contain Zinc reduce the size of the developed prostate (Lagiou et al., 1999). Leake et al (1984), showed that Zinc has also a modulator role on the activity of 5 α -reductase of prostate gland of the human. They showed that Zinc play a physiological role in the regulation of testosterone metabolism. This hormone which is converted to DHT by the NADPH dependent enzyme 5 α -reductase is an important step in the prostate gland to express the androgenic responses. It was shown that Zinc regulates this conversion, but it remained unknown whether this metal acts on the 5 α -reductase directly or on the generation of the cofactor NADPH.

CONCLUSION

The oral administration of pumpkin seed is useful in the treatment of benign prostatic hyperplasia (BPH) induced by testosterone in male Sprague-Dawley rats. Pumpkin seeds at 30% w/w can alleviate the signs of BPH such as decrease in the weight of the prostate, and improvement of the histology of this gland. In addition, this dose is beneficial because there are no negative effects concerning the lipid profile tests and liver enzyme tests.

Further experimental studies are necessary to verify the present study to be able to decide whether this dose is meaningful to human having benign prostatic hyperplasia without any complication as it was shown. In addition further recommendations are required to identify the relation between pumpkin seed and prostate cancer.

GLOSSARY

A: Androsterone
Abs: Absorbance
Ad lib: Ad libitum
ALP: Alkaline phosphatase
ALT: Alanine aminotransferase
AST: Aspartate aminotransferase
BPH: Benign prostatic hyperplasia
BW: Body weight
C. pepo : Cucurbita Pepo
CHE : Cholesterol esterase
Conc : Concentration
cPSA : Complex prostate specific antigen
DHT: Dihydrotestosterone
DRE: Digital rectal examination
E: Etiocholanolone
ER: Extended-release
FA: Fatty acid
GK: Glycerol Kinase
GOD: Glucose oxidase
GOT: Glutamate oxaloacetate
GPO: Glycerol-3-Phosphate Oxidase
GPT: Glutamate pyruvate transaminase
HDL: High density lipoprotein
HoLEP: Holmium laser enucleation of prostate
IR: immediate-release
LDH: Lactate dehydrogenase
LDL: Low Density Lipoprotein
LPL: Lipoprotein lipase
LUTS: Lower urinary tract symptoms
MDH: Malate dehydrogenase
NIDDM : Non-insulin dependent diabetes mellitus

PAS: Periodic Acid Shieff
POD: Peroxidase
PSA: Prostate specific antigen
PSAD: Prostate specific antigen density
PW: Prostate weight
SEM: Standard error from the mean
sGOT: Serum glutamic oxaloacetic transaminase
sGPT: Serum glutamic pyruvic transaminase
TAG: Triacylglycerol
TGF- β : Transforming growth factor beta
T-P: Testosterone prazosin
TUIP: Transurethral incision of the prostate
TURP: Transurethral resection of the prostate
UTI: Urinary tract infections
VLDL: Very low density lipoprotein
5 α -Reductase: 5alpha-reductase

BIBLIOGRAPHY

- Abdel-Rahman, M. (2006). Effect of Pumpkin Seed (*Cucurbita pepo* L.) Diets on Benign Prostatic Hyperplasia (BPH): Chemical and Morphometric Evaluation in Rats. *World Journal of Chemistry*, *1*(1), 33-40.
- Andriole, G., Guess, H., & Epstein, J. (1998). Treatment with finasteride preserves usefulness of prostate-specific antigen in the detection of prostate cancer: Results of a randomized, double-blind, placebo-controlled clinical trial. *Urology*, *52*, 195–201.
- Benson, M. C., Whang, I. S., Olsson, C. A., McMahon, D. J., & Cooner, W. H. (1992). The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum prostate specific antigen. *Journal of Urology*, *147*, 817-821.
- Blanchard, N. R., & Abu Baker, A. M. (2010). The Treatment of Benign Prostatic Hyperplasia. Retrieved from http://www.uspharmacist.com/continuing_education/ceviewtest/lessonid/105803/
- Caili, F., Huan, S., & Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods for Human Nutrition*, *61*(2), 73-80.
- Catalona, W. J., Southwick, P. C., Slawin, K. M., Brawer, M. k., Flanigan, R. C., &

- Patel, A., et al. (2000). Comparison of percent free PSA, PSA density, and age-specific PSA cutoffs for prostate cancer detection and staging. *Urology*, 56 (2), 255–260.
- Cohen, H., & Levy, S. (2002). Newer pharmacotherapeutic approaches to the management of benign prostatic hyperplasia. *United States Pharmacist*, 27, 73-82.
- Danos, B. (1989). Cucurbita Pepo. Retrieved from <http://www.google.com.lb/search?hl=en&q=Cucurbita+Pepo+&aq=f&aqi=&aql=&oq>
- Dedhia, R. C. (2008). Phytotherapy for lower urinary tract symptoms secondary to benign prostatic hyperplasia. *Journal of Urology*, 179(6), 2119-25.
- Dharmananda, S. (2001). Herbal Therapy for BPH. *Institute for Traditional Medicine-European branch*, 1(1), 1-7.
- Dreikon, K. (2007). Phytotherapeutic agents in the treatment of benign prostatic hyperplasia. *Current Urology Reports*, 1(2), 103-109.
- Eagles, J. M. (1990). Treatment of depression with pumpkin seeds. *British Journal of Psychiatry*, 157, 937-8.
- Edwards, J. E. (2008). Diagnosis and Management of Benign Prostatic Hyperplasia. *American Family Physician*, 77(10), 1403-1410.
- Ellegard, L., Andersson, S., Normen, L., & Andersson, H. (2007). Dietary Plant sterols

and Cholesterol Metabolism. *Nutrition in clinical care*, 65(1), 39-45.

Gerber, (2011). Benign Prostatic Hyperplasia (BPH, Enlarged Prostate). Retrieved from

http://www.medicinenet.com/benign_prostatic_hyperplasia/article.htm

Glew, R. H., Glew, R. S., Chuang, L. T., Huang, Y.S., Millson, M., Constans, D., et al.

(2006). Amino Acid, Mineral and Fatty Acid Content of Pumpkin Seeds (*Curcubita spp*) and *cyperus esculentus* Nuts in the republic of Niger. *Plant foods for human nutrition*, 61, 51-56.

Gossel-Williams, M., Davis, A., & O'Connor, N. (2006). Inhibition of Testosterone-

Induced Hyperpalsia of the Prostate of Sprague-Dawley Rats by Pumpkin Seed Oil. *Journal of Medicinal Food*, 9(2), 284-286.

Hammarsten, J., & Hogstedt, B. (2001). Hyperinsulinemia as a risk factor for

developing benign prostatic hyperplasia. *European Eurology*, 39(2), 151-158.

Hara, M., Inorre, T., & Fukuyama, T. (1971). Some physico-chemical characteristics of

gamma-seminoprotein, an antigenic component specific for human seminal plasma. *Japanese Journal Legal Medecine*, 25, 322-326.

Kostalova, Z., Hromadkova, Z., & Ebringerova, A. (2009). Chemical evaluation of

seeded fruit biomass of oil pumpkin. *Chemical Papers*, 63(4), 406-413.

Lagiou, P., Wuu, J., Trichopoulou, A., Hsieh, C., Adami, H., & Trichopoulous, D.

(1999). Diet and Benign Prostatic Hyperplasia: a study in Greece. *Adult Urology*, 54(2), 284-290.

- Langer, S. Z. (1999). History and nomenclature of alpha-1 adrenoceptors. *European Urology*, 36(1), 2-6.
- Leake, A., Chisholm, G. D., & Habib, F. K. (1984). The effect of Zinc on the 5 α – reduction of testosterone by the hyperplastic human prostate gland. *Journal of steroid Biochemistry*, 20(2), 651-655.
- Lee, M. (2003). Alfuzosin hydrochloride for the treatment of benign prostatic hyperplasia. *American Journal of Health-System Pharmacy*, 60(14), 1426-1439.
- Lepor, H. (2004). Pathophysiology, Epidemiology, and Natural History of Benign Prostatic Hyperplasia. *Urology*, 6(9), 3-10.
- Loeb, S., & Catalona, W. J. (2008). What to do with an Abnormal PSA Test. *The Oncologist*, 13(3), 299-305.
- Mandl, A., Reich, G., & Linder, W. (1999). Detection of adulteration of pumpkin seed oil by analysis of content and composition of specific Δ^7 -phytosterols. *Eurology food resource technology*, 209, 400-406.
- Matzkin, H., Chayen, R., Goldfarb, H., Gilad, S., & Braf, Z. (1992). Laboratory Monitoring of Androgenic Activity in Benign Prostate Hypertrophy Treated with a 5 α -Reductase Inhibitor. *Clinical chemistry*, 38(7), 1304-1306.
- McConnell, J., Roehrborn, C., Bautista, O., Andriole, G., Dixon, C., & Kusek, J., et al.

- (2003). The long-term effect of doxazosin, finasteride, and combination therapy on the clinical progression of benign prostatic hyperplasia. *National English Journal of Medicine*, 349(25), 2387-2398.
- McNeal, J. (1988). Normal histology of the prostate. *American Journal of Surgery and Pathology*, 12, 619–633
- Mcpartland, J. M., & Pruitt, P. L. (2000). Benign prostatic hyperplasia treated with saw palmetto: a literature search and an experimental case study. *Joao*, 100(2), 89-91.
- Miteva, R., Yotov, S., Georgiev, P., & Fasulkov, I. (2006). Determination of Species Specificity of Prostate-Specific antigen (PSA) in Semen. *Trakia Journal of sciences*, 4(3), 64-68.
- Murkovic, M., Piironen, V., Lampi, A., Kraushofer, T., & Sontag, G. (2004). Changes in chemical composition of pumpkin seeds during the roasting process for production of pumpkin seed oil (Part 1: non-volatile compounds). *Food Chemistry*, 84, 359-365.
- Naval, K., Saurabh A., & Aneesh S. (2006). Holmium laser enucleation of prostate for patients in large prostatic gland with urinary retention. *Indian Journal of Urology*, 22(2), 164-165.
- Nix, J. W., & Carson, C. C. (2007). Medical management of benign prostatic hypertrophy. *Canadian Journal of Urology*, 1, 53-57.
- Nordling, J. (2005). Efficacy and safety of two doses (10 and 15 mg) of alfuzosin or

tamsulosin (0.4 mg) once daily for treating symptomatic benign prostatic hyperplasia. *British Journal of Urology International*, 95, 1006-1012.

Roberts, (2010). Sustanon 250. Retrieved from <http://www.mesomorphosis.com/steroid-profiles/sustanon-250.htm>

Roehrborn, C. G., & Schwinn, D. A. (2004). Alpha1-adrenergic receptors and their inhibitors in lower urinary tract symptoms and benign prostatic hyperplasia. *Journal of Urology*, 171, 1029-1035.

Roehrborn, C., Van Kerrebroeck, P., & Nordling, J. (2003). Safety and efficacy of alfuzosin 10 mg once-daily in the treatment of lower urinary tract symptoms and clinical benign prostatic hyperplasia: a pooled analysis of three double-blind, placebo-controlled studies. *British Journal of Urology International*, 92(3), 257-61.

Rosen, R., Seftel, A., & Roehrborn, C. (2007). Effects of alfuzosin 10 mg once daily on sexual function in men treated for symptomatic benign prostatic hyperplasia. *International journal of Impotence Research*, 19(5), 480-485.

Sensabaugh, F. (1978). Isolation and characterization of a semen specific protein from human seminal plasma: a potential new marker for semen identification. *Journal of Forensic Science*, 23, 106-115.

Slowik, (2009). Prostate Enlargement (Benign Prostatic Hyperplasia). Retrieved from <http://www.urologychannel.com/prostate/bph/index.shtml>

- Strax, J. (2006). Prostate Cancer Treatment Guidelines for Patients. Retrieved from <http://www.whfoods.com/genpage.php?tname=foodspice&dbid=82>.
- Suphakarn, V., Yarnnon, C., & Ngunboonsri, P. (1987). The effect of pumpkin seeds on oxalcrystalluria and urinary compositions of children in hyperendemic area. *American Society for Clinical Nutrition*, 45, 115-121.
- Thomas, J. (1999). Diet, Micronutrients, and the prostate gland. *Nutrition reviews*, 57(4), 95-103.
- Tsai, Y. S., Tong, Y. C., Cheng, J. T., Lee, C.H., Yang, F. S. & Lee, H. Y. (2006). Pumpkin Seed Oil and Phytosterol-F can Block Testosterone/Prazosin-Induced Prostate Growth in Rats. *Urology international*, 77, 269-274.
- U.S. Department Of Health And Human Services, National Institutes of Diabetes and Digestive, and Kidney Diseases, National Kidney and Urologic Diseases Information Clearinghouse. (2006). Prostate Enlargement: Benign Prostatic Hyperplasia (NIH publication No.07-3012). Retrieved from <http://kidney.niddk.nih.gov/kudiseases/pubs/prostateenlargement/>
- Uzzo, R., Brown, J., Horwitz, E., Hanlon, A., Mazzoni, S., & Konski, A., et al. (2004). Prevalence and patterns of self-initiated nutritional supplementation in men at high risk of prostate cancer. *British Journal of Urology international*, 93(7), 955-960.
- Van Kerrebroeck P., Jardin A., Laval K., & Van Cangh, P. (2000). Efficacy and safety of a new prolonged release formulation of alfuzosin 10 mg once daily versus

alfuzosin 2.5 mg thrice daily and placebo in patients with symptomatic benign prostatic hyperplasia. *European Urology*, 37(3), 306-313.

Veeresh Babu, S.V., Veeresh, B., Patil, A. A., & Warke, Y. B. (2010). Lauric acid and myristic acid prevent testosterone induced prostatic hyperplasia in rats. *European Journal of Pharmacology*, 626, 262-265.

Wasserman, N. F. (2006). Benign prostatic hyperplasia. *Radiologic clinics of North America*, 44(5), 689-710.

Wilt, T., Ishani, A., Stark, G., MacDonald, R., Lau, J., & Mulrow, C. (1998). Saw palmetto extracts for treatment of benign prostatic hyperplasia. *Journal of American Medical Association*, 280(18), 1604-1609.

Winters, K. (2011). Pumpkin. Retrieved from

www.winmarkcom.com/images/pumpkin1.jpg

Younis, Y. M. H., Ghirmay, S., & Al-Shihry, S. S. (2000). African Cucurbita pepo L.: properties of seed and variability in fatty acid composition of seed oil. *Phytochemistry*, 54, 71-75.

Zhang, Y., Ni, J., Messing, E., Chang, E., Yang C., & Yeh, S. (2002). Vitamin E succinate inhibits the function of androgen receptor and the expression of prostate specific antigen in prostate cancer cells. *Academy of sciences of the United States of America*, 99(11), 7408-7413.

