Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Medicine

Department of Pharmacology

Potential Anti-Tumor Effect of Dutasteride On Prostate Cancer Cell Line

A thesis

Submitted to the Council of the College of Medicine, the University of Babylon, as a Partial Fulfillment of the Requirements for the Degree of Master in pharmacology/Pharmacology and Toxicology.

By
Zahraa Abdul Hasan jihad Abbas
Pharmacy (2017-2018)

Supervised by

Assist. Prof. Dr.
Majid K. Abbas
MS. Pharmacology

Prof. Dr.
Kaiser N. Madlum
Ph.D. Biology

1445 A.H

2023 A.D

بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ ﴿28﴾

صدق الله العليّ العظيم

(سورة فاطر الآية ٢٨)

Certification

We certify that this thesis entitled **(Potential Anti-Tumor Effect of Dutasteride On Prostate Cancer Cell line)** was prepared by (**Zahraa Abdul Hasan jihad)** under our supervision at the department of pharmacology. College of medicine, university of Babylon (Iraq) in partial fulfillment of the requirements for the master degree of sciences in pharmacology and toxicology.

Assist. Prof. Dr.

Majid K. Abbas

Supervisor

Prof. Dr

Kaiser N. Madlum

Supervisor

The Recommendation of the Head of Department

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

Assist. Prof. Dr.

Selman M. Selman

The Head of Department of Pharmacology
College of Medicine
University of Babylon, Iraq

Examining Committee Certificate

We certify that we have read this thesis entitled "**Potential Anti-Tumor Effect of Dutasteride On Prostate Cancer Cell line"** and as an examining committee examined the student "**Zahraa Abdul Hasan jihad**" in its contents and that in our opinion it meets the standard of a thesis for the degree of Master, in Pharmacology and toxicology.

Prof. Dr.
Najah Raish Hadi
College of Medicine – University of Al- kufa
(Chairman)

Assist.Prof. Dr.
Selman M. Selman
College of Medicine-
University of Babylon
(Member)

Assist.Prof.
Fatima Adnan Ali
College of pharmacy-
University of Babylon
(Member)

Assist.Prof.
Majid K. Abbas
(Member and Supervisor)

Prof.
Kaiser N. Madlum
(Member and Supervisor)

Prof. Dr.
Mohend A. N. Al-shalah
(Dean of College Medicine)

Dedication

Every challenging work needs self-efforts as well as guidance of elders especially those who were very close to our heart.

My humble effort is dedicated to the helpful and supportive Father, Mother, and my brother Ibrahim.

Whose affection, love, encouragement and prays of day and night make me able to get such success and honor.

Along with the hardworking and respected supervisors and all who's inspired me.

Acknowledgment

First and foremost, praises and thanks to the God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I would like to express my deep and sincere gratitude to my research supervisor, Dr. Majid K. Abbas, and Dr. Kaiser N. Madlum for giving me the opportunity to do my research and providing invaluable guidance throughout this research. Their dynamism, vision, sincerity, and motivation have deeply inspired me. They taught me the methodology to carry out the research and to present the research works as clearly as possible. It was a great privilege and honor to work and study under their guidance.

I am extremely grateful to my family for their love, prayers, caring and sacrifices for educating and preparing me for my future.

Finally, special thanks and gratitude to the Department of Pharmacology for the infinite support and cooperation, thanks to all the people who have supported me to complete the research work directly or indirectly.

Zahraa Abdul Hasan jihad

Summary

Prostate cancer is the most often diagnosed male cancer and the fifth highest cause of cancer mortality in men worldwide. In 2020, there were 1,414,249 newly diagnosed cases and 375,000 fatalities globally due to this illness. Prostate cancer is the most prevalent tumor diagnosed globally in more than fifty percent of different countries.

The 5α-reductase inhibitors that are used to treat benign prostatic hyperplasia block the conversion of testosterone to dihydrotestosterone and may reduce the risk of prostate cancer. There are two isoforms of 5α-reductase, type 1 and type 2. Dutasteride is a dual inhibitor that inhibit both isoforms of 5α-reductase.

The aim of this study was to evaluate the effects of dutasteride alone and in combinations with anticancer (doxorubicin and 5-fluorouracil) on cell viability, apoptosis, inflammation and oxidative stress in the LNCap prostate cancer cell line.

The first part of the work was to evaluate the cytotoxicity and determined half inhibitory concentration IC50 of dutasteride, doxorubicin, and 5FU. The LNCap cells were exposed to different concentrations of testosterone, dutasteride, doxorubicin, and 5-fluorouracil for 48 hours at 37C. The best dose of testosterone was (7.8 ng/ml) and the IC50 of other drugs were (75 µg/ml, 3 µg/ml, and 30 µg/ml), respectively. After that, LNCap was exposed to different concentrations of dutasteride starting from its IC50 combined with IC50 of doxorubicin and IC50 of 5FU and the MTT assay was carried after 48hr.

The second part of the work was to evaluate the apoptotic, anti-inflammatory, and antioxidant effects of testosterone, dutasteride alone, and dutasteride -anticancer combinations. Depending on MTT results, LNCap exposed to different concentrations of testosterone, dutasteride alone, and different concentrations of dutasteride combined with IC50 of doxorubicin or 5FU and incubated for 48 hours and apoptotic, anti-inflammatory, anti-oxidant effects were determined by measuring caspase 3, TNF-alpha, and total antioxidant capacity, respectively in the supernatant of cells by using Elisa kits.

The cytotoxicity results for testosterone showed a highly significant ($p < 0.001$) increase in cell viability at concentrations (3.9, 7.8, 15.625) ng/ml with a highly significant ($p < 0.001$) decrease at a concentration (500 ng/ml). The cytotoxicity results of dutasteride alone showed a highly significant ($p < 0.001$) decrease in cell viability at concentrations (50, 25. 12.5, 6.25, 3.125) µg/ml and significant ($p < 0.05$) decrease in cell viability at concentration (1.5625) µg/ml. The cytotoxicity results of 5FU and doxorubicin were highly significant ($p < 0.001$) decrease in cell viability at all concentrations. The results of dutasteride with doxorubicin IC50 or with IC50 of 5FU showed a highly significant ($p < 0.001$) decrease in cell viability at all concentrations compared with positive control (IC50 of doxorubicin or 5FU).

Apoptosis assay results for testosterone were a highly significant ($p < 0.001$) decrease in caspase 3 at concentrations (3.9, 7.8, 62.5) ng/ml with a significant ($p < 0.05$) increase at concentration 500 ng/ml compared with control. And for dutasteride, the results were a highly significant ($p < 0.001$) increase in caspase 3 at all concentrations compared with control.

The results of dutasteride with doxorubicin IC50 were a highly significant ($p < 0.001$) increase of caspase 3 at all concentrations of dutasteride compared with positive (cells treated with IC50 of doxorubicin 3 µg/ml) and negative control (untreated cells). The results of dutasteride with IC50 of 5FU showed a highly significant ($p < 0.001$) decrease at all concentrations compared with positive control (cells treated with IC50 of 5FU 30 µg/ml) and a highly significant ($p < 0.001$) increase at all concentrations compared with negative control (untreated cells).

TNF-alpha results for testosterone showed a highly significant ($p < 0.001$) decrease in TNF-alpha at all concentrations and for dutasteride, the results were highly significant ($p < 0.001$) decrease in TNF-alpha at all concentrations. The results of dutasteride with doxorubicin IC50 showed a highly significant ($p < 0.001$) decrease of TNF-alpha at all concentrations compared with positive control and a highly significant ($p < 0.001$) decrease of TNF-alpha at concentration (75 µg/ml) with significant ($p < 0.05$) decrease of TNF-alpha at concentrations (18.75, 9.37) µg/ml compared with negative control. The results of dutasteride with 5FU were highly significant ($p < 0.001$) decrease of TNF-alpha at all concentrations compared with positive and negative controls.

Total antioxidant capacity assay results for testosterone showed a highly significant ($p < 0.001$) increase at concentrations (500, 62.5) ng/ml and no significant effects at other concentrations compared with control. And for dutasteride showed a highly significant ($p < 0.001$) increase at all concentrations compared with control. The results of dutasteride with doxorubicin IC50 were no significant effects at all concentrations compared with positive control and highly significant ($p < 0.001$) decrease

at all concentrations compared with negative control. The results of dutasteride with 5FU were significant ($p < 0.05$) decrease at concentration (9.37 µg/ml) with no effects at other concentrations compared with positive control.

The results above suggested an anti-proliferative, anti-oxidant, and anti-inflammatory effects of dutasteride on LNCap cells. The combination of dutasteride with doxorubicin improved the effects dutasteride on cell viability, apoptosis, and inflammation but no effects on total antioxidant capacity. Combination of dutasteride with 5FU improved the effects of dutasteride on cell viability and inflammation but decreasing apoptotic effects of dutasteride and no effects on total antioxidant capacity.

Index

List of tables

Lists of Figures

List of abbreviations

Abbreviation	Meaning
17-HSDS	17-hydroxysteroid dehydrogenase
5-FU	5 Fluorouracil
AD	Androstenedione
ADT	Androgen deprivation therapy
AR	Androgen receptor
Bak	Proapoptotic protein
Bax	Bcl-2-associated X protein
Bcl-2	Apoptotic family proteins

Bid	The BH3 interacting-domain a pro-apoptotic member
BPH	Benign prostatic hyperplasia
Cyp450	Cytochrome p450
DHT	Dihydrotestosterone
dTMP	Deoxythymidine monophosphate
ERG	ETS related gene
ETS	E26 transformation-specific
ETV	ETS Variant Transcription Factor
FdUMP	Fluorodeoxyuridine monophosphate
IC50	Median Inhibitory Concentration
IL	Interleukin
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase
Noxa	Proapoptotic protein
p53	Tumor suppressor gene
PCa	Prostate cancer
REDUCE	Reduction by Dutasteride of Prostate Cancer Events
ROS	Reactive oxygen species
RPMI-1640	Roswell park memorial institute
SRD5A2	Steroid-5-reductase 2
T	Testosterone
TNF	Tumor necrosis factor
TS	Thymidylate synthase

Chapter One

Introduction

&

Literature Review

1-1 Introduction

Cancer is caused by the change of normal cells into tumor cells, which occurs in a multistep process that progresses from a precancerous lesion to a malignant tumor. These changes are the result of a person's genetic factors interacting with external influences such as physical carcinogens (e.g., ultraviolet and ionizing radiation), chemical carcinogens (e.g., asbestos, tobacco smoke components, alcohol, aflatoxin, and arsenic), and biological carcinogens (e.g., infections caused by certain viruses, bacteria, or parasites)(Krieg *et al.*, 2022) . Cancer is a serious worldwide public health concern and the second leading cause of death in the United States. In 2022, estimates show that about 609,360 cancer-related deaths and 1,918,030 new cancer cases in the United States with 350 deaths from lung cancer expected daily (Siegel *et al.*, 2022).

Prostate cancer (PCa) is developed as a result of aberrant prostate cell division, which results in abnormal cellular growth and the potential for cancer to spread to other body parts (Packer *et al.*, 2016). In 2018, PCa accounted for 7.1% (1,276,106) of all new cases and 3.8% (358,989) of all male fatalities, making it the second-deadliest cancer in men after lung cancer (Bray *et al.*, 2018). According to the United Kingdom Cancer Research Centre, over than 47,500 men are diagnosed with PCa annually, and in 2030, PCa is anticipated to overtake all other cancers in terms of prevalence, with one in eight males expected to get a lifetime PCa diagnosis (Rawla, 2019)

Androgens (testosterone and dihydrotestosterone) are essential for prostatic development and function as well as for cells’ proliferation and

survival. The most prevalent circulating androgen in males is testosterone, which is produced by the testicular Leydig cells under the direction of the hypothalamus and anterior pituitary gland (Banerjee *et al.*, 2018). The microsomal enzyme 5-alpha reductase facilitates the quick and irreversible conversion of testosterone (T) to dihydrotestosterone (DHT) within the prostate gland. Then, DHT binds to the androgen receptor (AR), which causes the expression of particular genes necessary for the growth of PCa cells (Batista *et al.*, 2022). PCa is prone to develop later in life if high amounts of androgens, particularly DHT, are present.

Depending on the clinical circumstances and disease progression, the primary PCa treatments currently available include surgery, radiation therapy, chemotherapy, cryosurgery, and hormonal therapy (Litwin and Tan, 2017). Another form of treatment has been created that specifically targets the enzyme 5-alpha reductase which is responsible for converting T to DHT since androgen levels, particularly in the early stages, play a crucial role in the growth of prostate cancer. Inhibiting 5-alpha reductase is a good target for reducing the risk of PCa since it will afterward reduce the generation of DHT. Dutasteride is a dual inhibitor that blocks both type I and type II 5-alpha reductase enzymes. It has been used to treat prostatic hyperplasia since it shrinks the prostate and lowers PSA levels (Kim *et al.*, 2018). In the 4-year Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trial, which is a randomized controlled trial, patients between the ages of 50 and 75 with prostate-specific antigen (PSA) levels between 2.5 and 10.0 ng per milliliter tested dutasteride for prostate cancer chemoprevention. Randomized

trials employing dutasteride resulted in a about 25% reduction in the risk of prostate cancer (Andriole *et al.*, 2010). Another meta-analysis study on the impact of 5-alpha reductase inhibitors revealed that these drugs can slow the spread of prostate cancer overall, particularly in patients with localized disease and low Gleason scores of fewer than 7. Additionally, the progression-free survival time was extended, and the percentage of complete therapy responses increased (Deng *et al.*, 2020).

1.2 Aim of the study:

The aim of this study was to evaluate the effects of dutasteride alone and in combinations with anticancer (doxorubicin and 5-fluorouracil) on cell viability, apoptosis, inflammation and oxidative stress in the LNCap prostate cancer cell line.

1.3 Literature Review

1.3.1 Cancer

Cancer is a puzzling and frightening disease or set of diseases. Cancers have afflicted multicellular living beings for more than 200 million years. Unlike infectious diseases, parasites, and many environmental diseases, cancer is not primarily caused by some entity that is foreign to our bodies. Its agents of destruction are human cells that have, as it were, slipped their reins, and have been recruited and to some extent transformed into pathological organisms or the building blocks of tumors(Hausman, 2019).

In 2023, about 1,958,310 new cancer cases and 609,820 cancer deaths are projected to occur in the United States. Cancer incidence increased for prostate cancer by 3% annually from 2014 through 2019 after two decades of decline, translating to an additional 99,000 new cases; otherwise, however, incidence trends were more favorable in men compared to women. For example, lung cancer in women decreased at one half the pace of men (1.1% vs. 2.6% annually) from 2015 through 2019, and breast and uterine corpus cancers continued to increase, as did liver cancer and melanoma, both of which stabilized in men aged 50 years and older and declined in younger men(Siegel *et al.*, 2023).

Multicellular organisms are generally composed of normal cooperating cells, but can also harbor host cells that proliferate abnormally and form masses called tumors or neoplasms (Boutry *et al.*, 2022). Tumors are usually classified into two main categories: benign or malignant. Both types of tumors result from aberrant cell divisions and are composed of abnormal cells. The cells in benign neoplasms are

usually phenotypically similar to normal differentiated cells; nevertheless, they have mutations that affect their growth, function and interactions with the resident tissue and the whole organism. However, benign tumor cells lack the ability to invade surrounding tissues and to spread to other organs (metastasize). Unlike cells in benign tumors, malignant cells do invade surrounding tissues and may also spread to other parts of the body, thereby causing metastatic cancers(Marino-Enriquez and Fletcher, 2014). Because of their ability to spread, it is often assumed that malignant tumors are more life threatening than their benign counterparts. While this is generally true, there are noticeable exceptions. For instance, benign tumors can be detrimental if they press on vital structures or organs, disrupt hormonal balance, and/or become malignant over time (e.g., benign bone tumors, pituitary adenoma, colon adenoma)(Boutry *et al.*, 2022).

1.3.2 Prostate gland

1.3.2.1 Physiology of prostate gland

The prostate gland, a male sex glandular structure, develops from an epithelial bud that originates from the embryonic urogenital sinus (UGS) in the third month of pregnancy. In humans, the urogenital sinus and external genitalia are the locations of the 5 alpha reductase enzyme, which is responsible for the correct development of the prostate gland by converting T to DHT (Cunha *et al.*, 2018). The main role of prostate is to secrete an aqueous liquid representing 10–30% of the seminal fluid volume. In addition to citrate, spermine, proteases, immunoglobulins, prostaglandins, and zinc, prostatic secretion also contains a high concentration of acid phosphatases secreted by prostatic epithelial cells

which formulate ejaculate and maintain sperm viability (Giacomini *et al.*, 2021)

The primary androgen utilized by prostatic cells is testosterone, which is produced by Leydig cells in the testis. Steroidogenic acute regulatory protein (StAR) transports cholesterol to the inner mitochondrial membrane where it is processed by CYP11A (cholesterol side-chain cleavage enzyme) to produce pregnenolone. The first rate-limiting step in the production of testosterone is the conversion of cholesterol to pregnenolone (Miller, 2017). The cytochrome P450 enzyme CYP17A then catalyzes the conversion of pregnenolone to dehydroepiandrosterone (DHEA). Significant levels of 3-hydroxysteroid dehydrogenase 2 (3HSD2), which catalyzes the conversion of DHEA to androstenedione (AD), are expressed in Leydig cells. AD is converted to testosterone by the steroidogenic enzyme 17HSD3, which is mostly expressed in Leydig cells. The prostate gland then receives testosterone that has been released into the bloodstream. Then, testosterone is converted to dihydrotestosterone (DHT) by the enzyme steroid 5- alpha reductase type 2 (SRD5A2) in prostate cells. Because it binds to the AR with a higher affinity and separates from the AR more slowly than testosterone, DHT has greater potency because it persistently activates AR signaling(Ye, Su and Ge, 2011). About 70%–80% of the androgens used by the prostate come from the testis, and after androgen deprivation therapy (ADH), the prostate's androgen concentrations significantly decrease. The adrenal gland is the second primary organ that supplies androgens to prostate cells after the testis (Hou, Huang and Li, 2021). The adrenal gland generates DHEA as a crucial steroidogenic tissue.

DHEA is produced by the zona reticularis cells of the adrenal cortex and is subsequently converted into DHEA-sulfate (DHEAS) by sulfotransferases before being discharged into the blood. DHEAS, in contrast to testosterone, cannot be used directly, nor does DHEA itself trigger AR signaling. Steroid sulfatase hydrolyzes DHEAS back to DHEA in the prostate, and 3HSD1 catalyzes this reaction to produce androstenedione (AD). Based on the conventional steroidogenic route seen in the testis, it is thought that AD is first catalyzed by type 17-hydroxysteroid dehydrogenase (17-HSD5), and subsequently by steroid-5-reductase 2 (SRD5A2) to generate DHT (Elzenaty, Du Toit and Flück, 2022). Steroidogenesis in different tissues is shown in Figure (1.1)

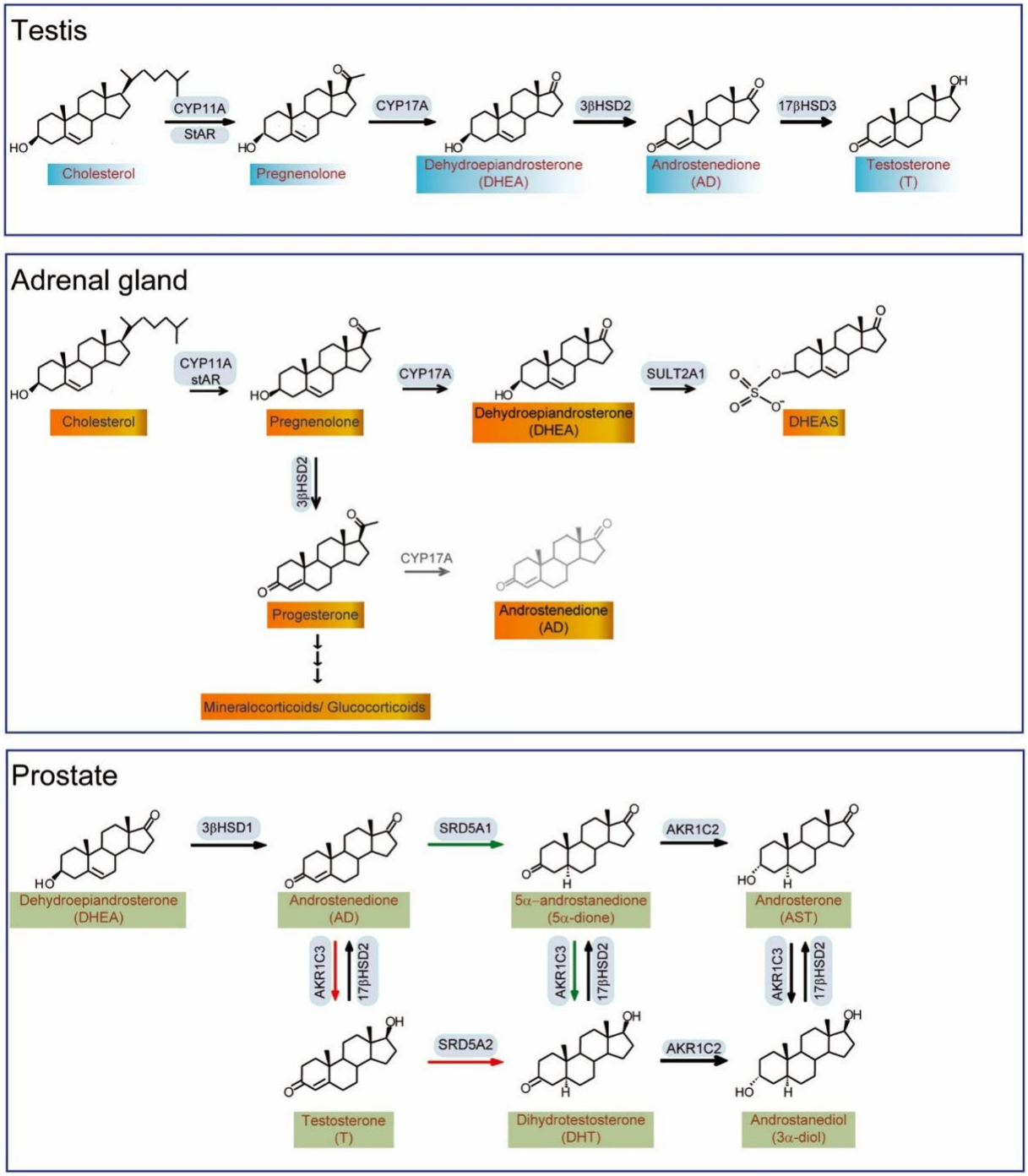


Fig (1.1) Steroidogenesis in different tissues The classic pathway is marked in red and the 5α-androstanedione pathway is marked in green. DHEA, dehydroepiandrosterone; AD, androstenedione; T, testosterone; 5α-dione, 5α-androstanedione; DHT, dihydrotestosterone; AST, androsterone; 3α-diol, androstanediol; DHEAS, DHEA sulfate (Hou, Huang and Li, 2021)

1.3.3 Prostate cancer

1.3.3.1 History

The history of prostate cancer spanned over 200 years. Adams, a surgeon and anatomist presented the first case of prostate cancer identified by histological examination to the London Hospital. In a case that was submitted to the Royal Medical and Chirurgical Society on April 12, 1853, he distinguished for the first time between benign and malignant prostatic hypertrophy. The patient had a rare prostate tumor that had migrated to his pelvic lymph nodes, and he passed away three years after his symptoms first appeared (Goddard, 2019).

1.3.3.2 Epidemiology

Prostate cancer is the second most frequent cancer and the fifth leading cause of cancer death among men worldwide with an estimated almost 1.4 million new cases and 375,000 deaths in 2020 (Sung *et al.*, 2021). In 2022 alone, 268,490 new cases of PC occurred in the US, Also PC makes up around 21% of all cancer cases in males. The disease leads to 345,000 deaths per year; it is the second most common cancer-causing death in the US following lung and bronchus cancer (Rehman *et al.*, 2023). In Europe, prostate cancer is the third diagnostic cancer after breast cancer and colorectal cancer, and the worldwide prostate cancer burden is expected to grow to almost 2.3 million new cases and 740 000 deaths by 2040 simply due to the growth and aging of the population (Bray *et al.*, 2018). Prostate cancer incidence is lower in Arab countries than in Canada, Germany, and the United States, in Iraq, the number of new cases of PCa was 1117. In Saudi Arabia, the number of new cases of PC was 693 (2.5% of all cancers) compared to other Arab countries,

Qatar has the lowest number of new cases (104) followed by Oman and Kuwait with 186 and 255 new cases, respectively. The mortality rate in Middle Eastern, North African, and Asian men was found to have a lower prevalence of prostate cancer than in Europe, America, and Canada, as of 2020, the estimated number of deaths in Iraq is 416, which is higher than that in Saudi Arabia (204 death cases), Qatar (18 death cases), Kuwait (52 deaths), Oman (73 deaths), and Jordan (142 deaths) (Belkahla *et al.*, 2022).

1.3.3.3 Pathogenesis

1) Genetics

Gene mutations are a common cause of cancer in general. Numerous studies have shown that the etiology of prostate cancer has a genetic component, Familial mutations in DNA repair genes such as BRCA2, ATM, CHEK2, and BRCA1 have been discovered by genomic investigations (Pritchard *et al.*, 2016). Also, Prostate cancer is believed to be strongly associated with the accumulation of somatic mutations in the prostate epithelial cell genome over a patient's lifetime. These aberrations can occur in oncogenes or tumor suppressor genes and result in changes in gene transcription and/or translation and functional defects, which lead to deregulated cell homeostasis. Mutations predominantly involve genes that regulate cell growth, DNA damage repair (DDR), cell proliferation, and cell death (Ahmed and Eeles, 2016).

Translocations involving androgen-regulated promoters and the erythroblast transformation specific (ETS) family of transcription factors, including the ERG (ETS- related gene) and the ETV (ETS

variant transcription factor) genes, which are the most frequent genetic changes in prostate cancer (Sizemore *et al.*, 2017). The first translocation was identified by Chinnaiyan as a recurrent gene fusion between the 5′ untranslated region of androgen-regulated gene TMPRSS2 and ERG (TMPRSS2: ERG). This fusion is present in about 50% of localized prostate tumors. About one-third of lethal metastatic castration-resistant prostate cancers (mCRPCs) had ETS2 deletion, which was frequently detected through TMPRSS2: ERG fusions (Wang *et al.*, 2018).

AR signaling has a fundamental impact on how the prostate forms and functions. Most primary and metastatic prostate cancers, according to research, have genetic alterations in the androgen signaling pathway, such as AR amplification or mutations. Additionally, one-third of metastatic castration prostate cancer (mCRPC) had structural rearrangements in the AR genome, which resulted in the aberrant expression of several species of AR variants, devoid of the ligand-binding domain and the ongoing activation of AR signaling, including AR variant 7 (AR-V7), which appears to be in charge of disease progression (Henzler *et al.*, 2016) Notably, recurring mutations in the AR co-operating component FOXA1, which fosters tumor development, have been discovered in 3%–4% of both untreated localized prostate cancer and mCRPC (Grasso *et al.*, 2012).

2**) Precursor of prostate cancer**

Prostatic intraepithelial neoplasia (PIN), which is characterized by the proliferation and anaplasia (the loss of specialized features) of cells lining prostatic ducts and acini, is thought to be a possible precursor

lesion of invasive prostate cancer. PIN occurs more frequently in the peripheral region of the prostate and is more severe there as people get older (Rashid, Shakeel and Mubarak, 2022).

There are two PIN grades: low (LGPIN) and high (HGPIN). For forecasting the development of adenocarcinoma, HGPIN has a strong predictive value. Transrectal ultrasonography (TRUS) and serum PSA levels are not affected by HGPIN, which can only be found with a needle biopsy. Histologically, there are four primary findings: flat, cribriform, tufting, and micropapillary. These features solely have diagnostic significance; their existence or absence does not indicate the aggressiveness of a malignancy. HGPIN is clinically significant, and patients need repeat biopsies for surveillance at intervals of 3-6 months for 2 years, then yearly for the rest of their lives. The presence of HGPIN on a biopsy may call for treatment because numerous studies have shown that it is a strong predictor of the development of prostate cancer (Zhou, 2018).

3) Androgenic regulation of prostate cancer

The binding of testosterone and dihydrotestosterone activates one of the nuclear receptors, the androgen receptor (AR). The four domains that make up the AR receptor are the NH2 terminal transactivation domain (NTD), DNA-binding domain (DBD), hinge region, and ligand binding domain (LBD). Xq11–12 on chromosome X contains the AR gene. The number of glutamine repetitions in the NH2 terminal transactivation domain varies (the majority of males had 19–25 repetitions). The transcriptional activity of AR is associated with shorter

glutamine repeats. Shorter glutamine repeats in males are associated with an increased risk of prostate cancer (Fujita and Nonomura, 2019).

Androgens are essential for the proper growth and operation of the prostate gland, but they also play a crucial role in promoting the development of early-stage PCa. Target genes are activated as a result of androgen activity, which is mediated by androgen receptor signaling. This promotes cancer cell growth and prevents apoptosis. Free testosterone diffuses through the membranes of target cells in the prostatic tissue and acts as a substrate for the steroid 5 alpha-reductase enzyme, this enzyme transforms free testosterone into dihydrotestosterone (DHT), which binds to the LBD of AR in the cytoplasm. After that, AR translocates into the nucleolus, forms a dimer, and binds to the androgen-response element of the promoter and the enhancer of targeted genes through the zinc finger of the DBD. The NTD includes the transcriptional regulatory region, activation function-1 (AF-1), and the LBD includes activation function-2 (AF-2). Upon DNA binding, the AR dimer forms a complex with coactivator and coregulatory proteins at the AF-1 and AF-2 regions that regulate cell processes including growth and survival (Feng and He, 2019).

4) Apoptosis

Apoptosis is the process by which a cell ceases to divide and develop and instead starts a process that leads to the cell's controlled death without the loss of any of its contents into the environment. A distinctive physical pattern of cell death was noticed as cells were destroyed during embryonic development, normal cell turnover in healthy adult tissue, and atrophy with hormone withdrawal. Kerr,

Wyllie, and Currie used the word "apoptosis" for the first time in 1972. 'Cellular suicide' (or, more colloquially, 'programmed cell death') is another name for apoptosis (D'Arcy, 2019).

Under crucial physiological conditions, many endogenous and external cell-damaging chemicals cause programmed cell death in certain cell types. Physical and viral stimuli that operate on most types of cells are examples of exogenous activators of programmed cell death. Radiation, physical trauma, and chemotherapeutic medications are examples of physical agents, while viruses and bacterial toxins are examples of infectious agents. Furthermore, internal imbalances such as growth factor withdrawal can cause apoptosis (Jan, 2019).

The mechanisms initiating apoptosis include both intrinsic and extrinsic signals, are complex and there is considerable crosstalk between them. The intrinsic pathway of apoptosis, also known as the mitochondrial pathway of apoptosis, this pathway is initiated after DNA damage. Damage of DNA lead to activation of p53 (tumor suppressor protein). Then p53 will activate Bax, Bak, Noxa, and Puma proteins. These proteins are interacting with Bcl2 proteins (Bcl2 are anti-apoptotic proteins that prevent cytochrome c release from mitochondria). This interaction leads to mitochondrial outer membrane permeability and the release of cytochrome c from mitochondria to the cytoplasm of the cell. Then cytochrome c interacts with apoptotic protease activating factor 1(Apaf-1) and forms apoptosome. Apoptosome converts procaspase 9 to caspase 9 which activate caspase 3 which leads to the demolition of cells and ends up in apoptosis (Cavalcante *et al.*, 2019). The extrinsic pathway of apoptosis, the death

receptor pathway, involves a subset of TNF receptors (TNFR) including DR3 (Death Receptor 3), Fas, TNF-R1, TRAIL-R1 (Tumor necrosis Factor-Related Apoptosis-Inducing Ligand -Receptor 1), and TRAIL-R2. These death receptors, upon ligand engagement, lead to activation of the intracellular signaling intermediaries that ultimately trigger activation of the initiator caspase 8. Caspase 8 activates caspase 3 by proteolytic cleavage, or activates the Bid, leading to the generation of a mitochondrial-permeabilizing (Jan, 2019). Both the extrinsic and intrinsic pathways meet at the same point (the execution phase). The execution phase is the final stage of apoptosis. Caspases 8 and 9 are initiator caspases, whereas caspases 3, 6, and 7, as well as Caspase-10, are effector or executioner caspases. Caspase 3 is a protease that is frequently activated during apoptosis. Caspase-3 is important in mediating nuclear apoptosis in several cell types. Caspase-3 is essential for some of the common nuclear and morphological changes associated with apoptosis, such as cell shrinkage, chromatin condensation, and DNA fragmentation (Jan, 2019).

5) Inflammation

Inflammation has been linked in a huge body of research to cancer, Inflammation is commonly seen in the prostate microenvironment and has been theorized to play a role in the development and spread of prostate cancer (Sfanos *et al.*, 2018). The nuclear factor- kappa B (NF-κB) transcription factors are important participants in both inflammation and cancer. Several preclinical findings have demonstrated that NF-κB contributes to the development, survival, angiogenesis, and metastatic spread of prostate cancer (Staal

and Beyaert, 2018). TNF- is a major modulator of cancer and inflammation, according to mounting data. Many malignant tumors produce TNF- continuously from the tumor microenvironment, and this trait is frequently linked to a bad prognosis. TNF- can directly aid in the development of cancer by regulating the proliferation and survival of neoplastic cells. It can also indirectly affect the development of cancer by exerting its effects through endothelial cells and other inflammatory cells present in the tumor microenvironment which include macrophages, dendritic cells, and fibroblasts that produce IL-1, IL-6, and TNF-alpha to promote the growth and survival of genetically altered tumor cells, also these cytokines draw and recruit additional inflammatory cells to the tumor microenvironment. Additionally, the inflammatory characteristics of the tumor microenvironment might result in additional genetic alterations in malignancy-related cells (Josephs *et al.*, 2018).

TNF alpha is one of the NF-*κ*B pathway activators. By binding to TNF-R1 (TNF receptor), an adaptor protein called TNF-R1-associated death domain protein (TRADD), which binds to a particular death domain (DD) in the cytoplasmic domain of TNF-R1, is recruited as a result of TNF-R1 ligation, which also causes receptor trimerization. Additionally, TNF-R1-associated death domain protein attracts TNF receptor-associated factor (TRAF2) and releases IκB kinase (IKK) via receptor-interacting protein (RIP). The RIP1 is required for TNF-R1-induced IKK and NF-B activation and is distributed in a TRAF2-dependent way throughout this process. Multiple features of oncogenesis, including enhancing cancer cell proliferation, blocking

apoptosis in drug resistance, and increasing tumor angiogenesis and metastasis, have been linked to sustained activation of NF-B (Van Quickelberghe *et al.*, 2018).

6) Oxidative stress regulation

Increased risk of prostate cancer has been linked to elevated levels of cellular reactive oxygen species (ROS) and compromised defensive systems (Miller, 2019). Through immunological activity, oxidative metabolism, and mitochondrial bioenergetics, the body continually produces ROS. Superoxide anion, hydrogen peroxide, singlet oxygen, hypochlorite, hydroxyl radicals, and lipid peroxides are examples of ROS that are often present and are formed throughout the development, growth, death, and differentiation of cells (Tan, Norhaizan and Liew, 2018). They are capable of attaching to proteins, membrane lipids, nucleic acids, enzymes, and other small molecules. Prostate cancer growth and progression have been linked to increased oxidative stress, which has been recognized as a key risk factor (Ahmed Amar *et al.*, 2019). Animal models and cell culture experiments have reported the mechanisms that implicate prostate cancer are complex and involve many cell signaling pathways (Long *et al.*, 2019). Oxidative free radicals are caused by several factors including regulating androgens, delaying the recruitment of p53, and inflammation. Specifically, it has been suggested that serum androgens increase ROS accumulation and production in prostate cancer cells (Tan and Norhaizan, 2021).

Atypical prostatic growth is linked to changes in the ratios of androgenic hormones, as well as paracrine/autocrine growth stimulatory substances such as insulin growth factor (IGF) binding proteins.

Surprisingly, physiological activation of the androgen receptor has been shown to increase ROS generation (Gonthier, Poluri and Audet-Walsh, 2019). Because aging is linked to reduced free radical scavenging enzymes and intracellular antioxidant levels, and androgen stimulation in prostate cancer cells; thereby, disrupting the balance of antioxidant-pro oxidant levels. Such a finding implies the androgen activation existing in prostate cancer cells could be attributed to mitochondrial DNA mutation and aging (Kowluru and Mishra, 2015).

1.3.3.6 Treatment

The first step in managing prostate cancer is deciding whether or not to take therapy. Prostate cancer, particularly low-grade tumors, generally grow so slowly that therapy is frequently unnecessary, especially in the elderly and those with comorbidities that would fairly lower life expectancy to 10 years or less.

1) Hormonal therapy

A. Androgen deprivation therapy (ADT)

Prostate glands would atrophy and prostate cancer would recede after androgen deprivation (castration), according to urologist Charles Huggins of the University of Chicago, who made this discovery in 1941. This discovery, which forms the cornerstone of all hormonal (testosterone deprivation-based) therapy used to treat prostate cancer, earned him the Nobel Prize in medicine in 1966(Choi *et al.*, 2022). Androgen deprivation therapy is primarily used to treat metastatic prostate cancer, although it can also be used in conjunction with radiation to treat intermediate or high-risk illnesses. In individuals with low-risk or very low-risk illnesses, either ADT alone or with adjuvant

radiation did not improve survival (May *et al.*, 2019). In general, androgen deprivation produces in remission in 80-90% of men with advanced PCa with a median progression-free survival of 12 to 33 months (Student *et al.*, 2020).

Androgen deprivation therapy causes hypoandrogenism in males, which manifests as exhaustion, and vasomotor symptoms such as hot flashes, reduced libido, erectile dysfunction, and gynecomastia. ADT can also effect on cardiovascular, bone, and muscular health, as well as induce cognitive/psychiatric abnormalities. Low testosterone levels have been linked to insulin resistance, central obesity, hypercholesterolemia and hypertriglyceridemia, and other precursors of metabolic syndrome, according to research (Pan and McKay, 2021). Androgen deprivation therapy uses medicines or Orchiectomy (surgical castration) to reduce the amount of androgens generated by the testicles.

- **Gonadotropin-releasing hormone (GnRH agonists)**

Long-acting GnRH agonists (Histrelin, Goserelin, Leuprolide, and Triptorelin) function by activating LHRH receptors in the hypothalamus, which initially increases FSH and LH and subsequently increases testosterone synthesis. The pituitary gland becomes desensitized to the effects of LHRH as a result of continuous stimulation, which disrupts the normal pulsatile pattern of LHRH receptors. Thus, FSH and LH production is suppressed, and gonadal testosterone production drops sharply to castrate levels as a result (Gründker and Emons, 2017). Tumor flare from the initial testosterone spike, which can occur in up to 63% of patients with advanced prostate cancer, is a drawback of GnRH agonists. In more severe situations, complications from tumor flare

might result in urethral blockage, pain that gets worse, and cord compression, to reduce this flare phenomenon, antiandrogens are typically used before GnRH agonist dosing (Pan and McKay, 2021).

❖ **Gonadotropin-releasing hormone antagonists**

These agents are analogs of GnRH that competitively inhibit GnRH receptors. GnRH antagonists offered an alternative route to testosterone suppression via direct inhibition of GnRH receptors found in the anterior pituitary gland. This mechanism of action avoided the initial surge of FSH and LH and thus the initial testosterone rise and clinical flare associated with GnRH agonists (Fontana *et al.*, 2020). The key clinical difference between the novel GnRH antagonist and the traditional agonist is the instantaneous blockage of pituitary GnRH receptors, which results in a fast fall of blood testosterone to castrate levels in less than 24 hours against the classic agonist, which produced this decline over a month (Schweizer *et al.*, 2018).

The FDA has finally approved and made Relugolix, an oral GnRH antagonist, it is a direct GnRH antagonist and induces a very quick drop in serum testosterone, just like injectable degarelix does. It appears to have fewer significant cardiovascular events and is extremely effective since it caused maintained castrate levels of testosterone in 97% of the assessed men. Similar to degarelix, it is approved for individuals with biochemical recurrence and locally progressed, castrate-resistant, or metastatic prostate cancer (Shore *et al.*, 2020).

B. Androgen receptor blockers

These drugs blockade the ligand-binding domain of the androgen receptor AR to prevent the binding of T and DHT.

❖ First generation: Anilide derivatives flutamide, nilutamide, and bicalutamide exhibit comparable efficacy but have various half-lives (6 to 8 hours, two days, and seven days, respectively). These medicines have relatively low affinity for AR which consider a serious drawback due to 5–10% of DHT is left unblocked and activating the AR and promoting the proliferation of PCa cells, the potential side effects of this group which important to take into account including: Flutamide, nilutamide, and bicalutamide all still resulted in hepatic toxicity, in severe circumstances lead to cirrhosis and liver failure. Additionally, they promoted gynecomastia, raised estradiol levels, decreased libido, and raised blood pressure. They also increased the risk of arrhythmia. The fact that flutamide, nilutamide, and bicalutamide exhibit a partial agonist action and stimulate the AR, resulting in an elevated PSA level, is a significant issue. This generation of anti-androgens is also ineffective in the treatment of castration-resistant prostate cancer CRPC (Student *et al.*, 2020).

❖ Second generation (Enzalutamide, Apalutamide, Daralutamide) With a 5- to 8-fold higher binding affinity than bicalutamide, enzalutamide is a strong AR inhibitor. The medication also prevents AR from binding to DNA and coactivators, translocation to the nucleus, and other processes. Enzalutamide is currently FDA-approved for the treatment of CRPC and metastatic sensitive prostate cancer mCSPC, either as first- or second-line medication depending on the clinical situation. Enzalutamide was first approved for use after docetaxel. Enzalutamide lowers PSA levels, prolongs progression-free survival,

enhances the quality of life, and postpones unfavorable bone occurrences (Desai, McManus and Sharifi, 2021).

Enzalutamide has a variety of side effects that can include fatigue, diarrhea, hot flashes, headaches, soreness in the bones and muscles, and issues with the central nervous system, the medication is typically well tolerated, especially when compared to first-generation hormonal medicines. Enzalutamide medication is not advised for individuals who have a higher risk of epileptic seizures (Student *et al.*, 2020).

C. Drugs that lower androgen levels from other parts of the body

Although LHRH agonists and antagonists can decrease androgen synthesis in the testicles, cells in other parts of the body, particularly the adrenal glands, can continue to generate male hormones that encourage cancer growth. Some drugs can inhibit the production of androgens by these cells.

❖ Abiraterone: It was initially approved by the FDA in 2011 for the treatment of patients with metastatic, castration-resistant prostate cancer (with or without initial chemotherapy) and in 2018 for high-risk metastatic, castration-sensitive prostate cancer. Cytochrome P17A is inhibited by abiraterone acetate. Pregnenolone is changed into dehydroepiandrosterone (DHEA) by this enzyme. The main androgens thought to be related to prostate cancer are testosterone and dihydrotestosterone, both of which will be decreased as a result of inhibiting this enzyme. By simultaneously inhibiting CYP17A's 17-hydroxylase activities, abiraterone inhibits CYP17A. Both the production of cortisol from corticosterone and the synthesis of DHEA depend on the CYP17A enzyme's 17-hydroxylase activity. As a result,

when abiraterone is administered, plasma cortisol is decreased and adrenocorticotropic hormone (ACTH) is increased. Hypertension, hypokalemia, and fluid overload are the negative effects brought on by the up-regulated ACTH, which also promotes excess mineralocorticoids. Prednisone is consequently given along with Abiraterone to compensate for the loss of cortisol (Hou, Huang and Li, 2021). Patients with locally advanced or metastatic PCa who received ADT in combination with abiraterone acetate and prednisolone (1000 mg and 5 mg daily, respectively) had significantly better rates of overall survival and failure-free survival when compared to patients who received just ADT (Werutsky *et al.*, 2019).

2) Chemotherapy (Taxan family)

Docetaxel (DTX), a member of the Taxanes family, was initially created by Pierre Potier while he was focusing on taxol derivatives. It is a semi-synthetic chemical made from 10-deacetyl-bacattin III, a non-cytotoxic precursor substance that was obtained from the needles of the European yew tree Taxus baccata L (Zhang *et al.*, 2019).

The most widely acknowledged mode of action of DTX in the treatment of prostate cancer involves its binding to b tubulin, which encourages polymerization and stabilizes microtubules. Under normal circumstances, these microtubules only polymerize when guanosine triphosphate GTP, which is in charge of the association with b-tubulin, and microtubule-related proteins are present. However, DTX can bind to b-tubulin preferentially, leading to microtubule assembly even in the absence of GTP and cofactor proteins. DTX also prevents microtubule disassembly once they are attached. Through the disruption of the

regular mitotic process and cell cycle arrests in the G and M phases, this static polymerization eventually leads to apoptosis (da Silva *et al.*, 2018).

Docetaxel and cabazitaxel are the chemotherapies for PCa that the FDA has approved. Patients with metastatic hormone-sensitive prostate cancer (mHSPC) and metastatic castration-resistant prostate cancer (mCRPC) are treated with docetaxel as the first-line treatment. When patients do not tolerate or progress during or after docetaxel, capaztaxel is used as second-line chemotherapy. Additional chemotherapies such as carboplatin, etoposide, or cisplatin may be taken into account. Fatigue, nausea, vomiting, taste changes, mouth sores, nail changes, liver damage, alopecia, renal toxicity, neutropenia, anemia, thrombocytopenia, and fluid retention/edema are common side effects of these chemotherapies (Tsang, 2021).

3) Immunotherapy

Sipuleucel-T is currently the only form of immunotherapy approved for the treatment of mCRPC, and the first therapeutic cancer vaccine for prostate cancer to be approved by FDA in 2010, Sipuleucel-T is an autologous dendritic cell vaccine prepared by taking a patient's peripheral blood mononuclear cells, including antigen-presenting cells, initially extracted by leukapheresis, cellular activation and then treated with a fusion protein consisting of Granulocyte-macrophage colony-stimulating factor (GMCSF) and the antigen prostate acid phosphatase (PAP) associated with prostate tumors. These cells, once injected back into the patients can then present antigens to prime T cells into becoming PAP antigen-specific cytotoxic T cells directed against the

patients' tumors, this process is repeated every two weeks for a total of three doses (Rizzo *et al.*, 2020). Median survival in the sipuleucel-T-treated patients was 25.8 months, compared to 21.7 months in placebo-treated patients. There was no difference observed in time to the progression of the disease. The most common adverse events seen in the sipuleucel-T were fevers, chills, headache, influenza-like illness, myalgias, hypertension, and groin pain (Fay and Graff, 2020).

1.3.4 Alpha reductase enzyme inhibitors

Family of 5-alpha reductase enzymes are responsible for conversion of testosterone to DHT. In conjunction with the cofactor nicotinamide adenine dinucleotide phosphate (NADPH), isoenzymes of the 5-alpha reductase enzyme family catalyze the irreversible break of the double bond between carbons 4 and 5 of the testosterone molecule, resulting in conversion to the DHT, which is essential for the pathogenesis, development, and function of the prostate (Azzouni *et al.*, 2011). Fig (1.2) Conversion of testosterone to dihydrotestosterone by 5-alpha reductase enzyme.

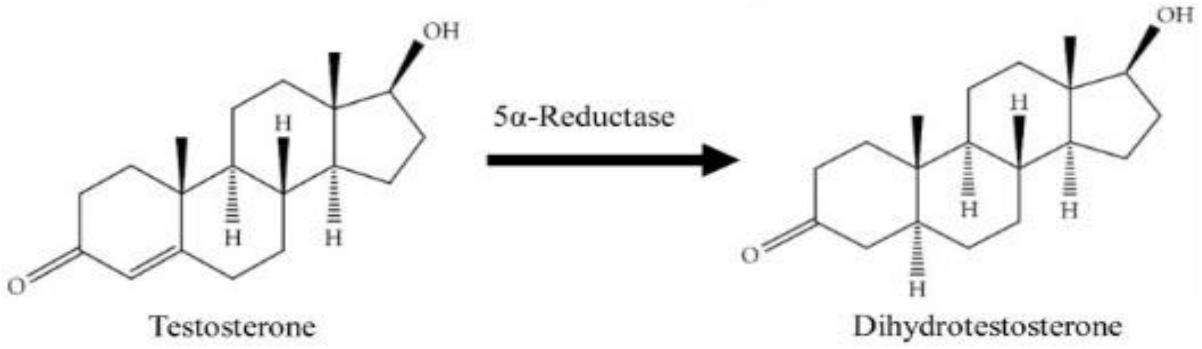


Fig (1.2) Conversion of testosterone to dihydrotestosterone by 5- alpha reductase enzyme (Azizi, Mumin and Shafqat, 2021)

Three types of 5'-reductase that are currently recognized: type 1, which is primarily found in the skin and liver but is also found in the prostate, type 2, which is primarily found in the prostate and male genital tissues; and type 3, which was just recently discovered and is also expressed in many tissues, including the prostate (Lorenzo *et al.*, 2020).

Isoenzymes 1 and 2 control the conversion of testosterone to DHT to a large extent. The proliferative and apoptotic processes involved in the onset and development of both benign prostatic hyperplasia (BPH) and PCa are known to be regulated by DHT (Chughtai *et al.*, 2016). Additionally, BPH tissue has higher levels of type 1 and type 2 of 5'-reductase expression, with type 2 being the predominant form. Both 5α-reductase isoenzymes 1 and 2 are also present in PCa, with increasing levels in more aggressive tumors. However, 5'-reductase type 1 is more prevalent in PCa and is present at greater levels than in BPH and healthy tissue, whereas type 2 is present at lower or comparable levels (Lorenzo *et al.*, 2020). To reduce the synthesis of DHT, inhibiting 5'-reductase isoenzymes become a frequent goal in the therapeutic therapy of BPH and a viable method for chemoprevention of PCa. Finasteride is a synthetic 4-azasteroid and is the first 5'-reductase inhibitor authorized for the treatment of male pattern baldness (MPB) and benign prostatic hyperplasia (BPH). Finasteride inhibits 5'-reductase type 1 less efficiently than 5'-reductase type 2 (Azizi, Mumin and Shafqat, 2021). After 24 weeks of usage, finasteride reduces the average blood level of DHT by 71% (Azzouni *et al.*, 2011).

1.3.4.1 Dutasteride

1.3.4.1.1 Chemical structure

Dutasteride, chemically known as N-[2,5-Bis(trifluoromethyl)phenyl]-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide, belongs to a class of dual 5-alpha reductase inhibitors (Choi *et al.*, 2018). Dutasteride is classified as a biopharmaceutics classification system class II drug with low solubility (0.038 ng/mL) and high permeability Despite its high pKa value of 13.5, the compound is non-ionic and its solubility remains similar at low level from pH 1 to pH 12. The drug is reported to degrade under heat treatment, and under acidic, basic, photolytic, and oxidative conditions. The marketed product Avodart® (GlaxoSmithKline, Brentford, UK) is commercially available as a soft gelatin capsule (softgel) (Hwang *et al.*, 2022). Softgels are known to have risks of rupture during storage as the moisture from the atmosphere and from the fill of the softgel is reported to weaken hardness of the shell (Gullapalli, 2010). Tablets, compared to softgel, are more robust against deformation at high temperature and moisture, which may mitigate the risks associated with teratogenicity in pregnant women for dutasteride. Additionally, utilizing a readily-absorbed technology (such as self-emulsifying formulation) may improve the PK property of the original softgel product, wherein its bioavailability and inter-individual variance are shown to have a scope for improvement (Hwang *et al.*, 2022). Figure (1.3) The chemical structure of dutasteride.

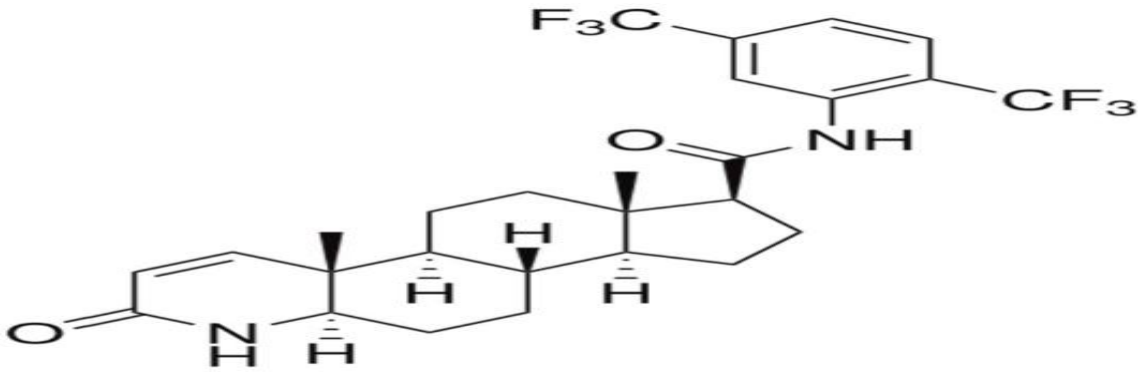


Figure (1.3) The chemical structure of dutasteride (Kim *et al.*, 2015).

1.3.4.1.2 Mechanism and pharmacological effects

Dutasteride (DUT) is a synthetic analogue of testosterone and a specific and selective inhibitor of both 5α-reductase isoforms of type 1 and 2, which convert testosterone to dihydrotestosterone. Dutasteride used in the management and treatment of benign prostatic hyperplasia (BPH) and androgenic alopecia (male pattern hair loss) (Gniazdowska *et al.*, 2021). Dutasteride is 45 times more potent than finasteride in inhibiting 5'-reductase type 1 and 2 activity (Azizi, Mumin and Shafqat, 2021). At 24 weeks, dutasteride lowered mean blood DHT levels more effectively than finasteride (94.7% suppression) (Clark *et al.*, 2004) and in males with PCa treated with 5 mg/d for 6–10 weeks resulted in a 97% decrease in intraprostatic DHT levels (Andriole *et al.*, 2004). Before radical prostatectomy, a different study found that using dutasteride 3.5 mg/d for 4 months reduced intraprostatic DHT by 99% (Gleave *et al.*, 2006). Additionally, it has been noted that dutasteride inhibits T and DHT-induced LNCaP cell proliferation by targeting the 5aRs activity and doing so more effectively than finasteride does (Lazier *et al.*, 2004). Furthermore, there are claims that dutasteride, particularly in low-risk prostate cancer, might enhance the prognosis for people with the

disease. Finasteride can also raise the chance of high-grade Gleason prostate cancers, although the Prostate Cancer Prevention Trial (PCPT) showed that it had equivalent effects to dutasteride in terms of lowering the incidence of prostate cancer (Yu *et al.*, 2020).

1.3.4.1.3 Pharmacokinetics of Dutasteride

Dutasteride is available as a soft capsule containing a 0.5mg dose administered by oral route. It presents 60% oral bioavailability, and its median time to reach maximum plasma concentration (t_{max}) is around 3 h (1–10 h range) after the administration of a 0.5 mg single dose. Dutasteride shows a volume of distribution (Vd) of 300–500 L and a high plasma protein binding (>99.5%). Its elimination is dose-dependent. At single doses lower than 5 mg, dutasteride clearance is rapidly performed, with a shorter half-life ($t_{1/2}$) of 3–9 days. However, at 0.5 mg daily doses, the elimination is slower, reaching a $t_{1/2}$ of 3–5 weeks. It is extensively metabolized by cytochrome P450 (*CYP*) isoforms CYP3A4 and CYP3A5 into its metabolites: Two minor metabolites (6,4′- dihydroxydutasteride and 15-hydroxydutasteride) and three major metabolites (4′-hydroxydutasteride, 1,2- dihydrodutasteride, and 6-hydroxydutasteride) are identified in the human serum. Of these metabolites, only 6-beta-hydroxydutasteride maintains activity comparable to dutasteride(Pohlman, Pohlman and Crawford, 2011). They are primarily excreted in stools and marginally in urine; only between 1 and 15.4% of the dutasteride dose is excreted unmetabolized in feces (Villapalos-García *et al.*, 2021).

According to in vitro data, blood concentrations of dutasteride might increase in the presence of CYP3A4 inhibitors such as ritonavir,

ketoconazole, verapamil, diltiazem, cimetidine, and ciprofloxacin. Nevertheless, care should be taken when administering dutasteride to patients taking potent, chronic CYP3A4 inhibitors, such as ritonavir (Marihart, Harik and Djavan, 2005).

1.3.4.1.4 Contraindications and adverse effects of Dutasteride

Dutasteride should be administered with caution to patients with mild to moderate hepatic impairment and is contraindicated in patients with hypersensitivity to dutasteride or other 5'-reductase inhibitors. Dutasteride is also contraindicated in women, adolescents, and children, who must void skin contact with the drug, the skin should immediately be washed because dutasteride is absorbed by the skin (Evans and Goa, 2003)

The most common general adverse events reported by patients receiving dutasteride were ear, nose, and throat infection; malaise; headache; dizziness; and musculoskeletal pain. No differences in the rates of these adverse effects were found when compared with the placebo-treated group(Dolder, 2006). Other adverse effects associated with dutasteride that may effected on treatment continuation including increased risk of adverse sexual effects, especially erectile dysfunction, decreased libido and impotence (Lee *et al.*, 2019), cognitive/ psychological dysfunction (Saengmearnuparp *et al.*, 2021), gynecomastia (Trinchieri *et al.*, 2021)), and cardiovascular events (Ayodele *et al.*, 2021).

1.3.5 Anthracyclines

One of the best chemotherapy medications for solid tumors is doxorubicin, a naturally occurring anthracycline antibiotic, which is used to treat several cancer types. Although the exact mechanism is unknown, it is thought that it interacts with DNA by intercalation as other anthracyclines do. The intercalation of doxorubicin into DNA and subsequent interruption of DNA repair, which would be mediated by topoisomerase II, is one of the theories put forth to explain the action of doxorubicin on malignant cells (Taymaz-Nikerel *et al.*, 2018). Topoisomerase II overwinds DNA during transcription, and doxorubicin inhibits it. This prevents the double strand of DNA from recombining, which halts DNA replication. The production of free radicals is a different hypothesis that has been put out. These radicals may damage DNA and cause cell death (van der Zanden *et al.*,2021).

Doxorubicin (dox, Adriamycin) is a water-soluble, orange to red coloured (at neutral pH), photosensitive chemotherapeutic drug, Since its FDA approval in 1974, dox alone or in combination with other chemotherapeutic drugs, is used widely as the first-line therapy for a myriad of solid and metastatic tumors: acute lymphoblastic/myeloblastic leukemia, neuroblastoma, breast, small cell lung, ovarian, bladder, gastric, thyroid, osteogenic bone tumors, Wilm´s tumor, Hodgkins and cutaneous T cell lymphoma. Nanoformulations of dox have also been approved by FDA for its therapeutic use: Doxil for treating Kaposi's syndrome, ovarian cancer, breast cancer, and multiple myeloma (with Velcade); Myocet for breast cancer (with cyclophosphamide); Lipo-dox for breast and ovarian cancer(Sritharan and Sivalingam, 2021)

Cardiotoxicity being the most well-known and most comprehensively studied adverse effect related to doxorubicin Also, alopeci, nephrotoxicity, and haematological suppression limit the usage of doxorubicin, despite the fact that it is powerful in the treatment of cancer(Kullenberg *et al.*, 2021). Activation of ROS-scavenging mechanisms reduces ROS levels and lessens DNA damage, resulting in enhancement of chemo resistance(Kim *et al.*, 2019). The failure of treatment with mono-administration of doxorubicin is mostly attributable to the severe adverse effects. Despite the fact that doxorubicin treatment has demonstrated major potential in delaying the progression of disease over the years, clinically utilized doses exhibit insufficient anti-tumour activity, while larger doses frequently cause systemic toxicity in patients (Kullenberg *et al*., 2021).

1.3.6 Antimetabolites drugs

5-Fluorouracil (5-FU) is one of the anti-metabolite drugs in which the hydrogen is replaced with fluorine at the C5 position of uracil. 5-FU was one of the first chemotherapeutic drugs was reported to have anticancer activity and was synthesized by Heidelberger (Ishiba *et al.*, 2018). The moment 5-FU enters cancer cells, it changed into fluorodeoxyuridine monophosphate (FdUMP). Through the creation of a ternary complex including FdUMP, thymidylate synthase, and 5,10-methylene tetrahydrofolate in tumor cells, FdUMP inhibits the activity of the enzyme thymidylate synthase (TS). Deoxythymidine monophosphate (dTMP) synthesis was decreased as a result of this enzyme's inhibition. DNA replication and repair depend on dTMP, and its depletion causes an imbalance in intracellular nucleotides, which

allows the enzyme endonuclease to cause double-stranded breaks in the DNA (Kobuchi and Ito, 2020). 5-FU is a chemotherapeutic medicine that is frequently used to treat various malignant tumors, such as head and neck, breast, pancreatic, skin, stomach, and esophageal cancers (Vodenkova *et al.*, 2020). When alternative treatment options are not viable, 5-FU is used topically to treat a variety of dermatologic diseases, such as numerous actinic or solar keratoses and superficial basal cell carcinomas (Reinehr and Bakos, 2020). When used systemically, 5-FU causes a wide range of negative pharmacological effects. Diarrhea was the most often reported adverse reaction in individuals receiving systemic 5-FU therapy. Dehydration, nausea, and vomiting are among the more typical side effects. Neutropenia, pyrexia, pulmonary embolism, thrombocytopenia, and leukopenia are more serious side effects that need to be watched in individuals taking systemic 5-FU treatment. Leukopenia can cause secondary pneumonia or sepsis in people because it impairs their immune system (Kadoyama *et al.*, 2012).

1.4 Cell Culture

Cell culture is a laboratory procedure that enables the isolation of cells from various sources and placement of those cells in a suitable medium for growth and division. The cell culture technique was initially created in the early 20th century as a way of investigating animal cell behavior in vitro. When Roux, an embryologist, used warm saline to keep a chicken embryo alive for several days, he discovered the tissue culture principle (Hudu *et al.*, 2016). The first benefit of these systems is that they let researchers keep an eye on the circumstances in which the cells are, the second benefit to make the researchers know how can grow

the cells in an environment free from contamination and ensure their safety, and the third benefit is there are too numerous fields that can benefit from studying normal cellular processes in cell culture systems, including toxicological research, medication development, and therapy approaches (Segeritz and Vallier, 2017). Cell culture studies need special working conditions. A cell culture laboratory must be a sterile environment. Contamination is the most common problem in cell culture studies. When it cannot be resolved, it might cause serious losses. Two significant factors that limit the reproduction of the cells in culture, the first is the free space in the culture vessel and the other is the decrease in the nutrition support that the medium provides. In this condition, cell passaging is required. Criteria of the passage needs can be put in order as cell concentration, pH of the environment, a period that has passed since the last passaging, and special conditions that the study brings (Segeritz and Vallier, 2017)

1.4.1 Primary cell culture

Primary culture is the cell culture system that is formed by culture cells directly obtained from tissue. A primary culture starts with the biopsy (about 1 cm^3) of tissue or organ via dissection. In tissue organization, cells have intercellular and cell basal membrane or cell-matrix connections. For cells to be cultured, first of all, they need to get rid of these connections (single-cell suspension). In the separation of cells, there are enzymatic or chemical digestion methods in which various proteolytic enzymes are used such as trypsin or collagenase, and mechanic separation methods like splitting the tissue (mincing of the tissue) with surgical knives. (Uysal *et al.*, 2018). The majority of the

cells in this culture are heterogeneous cells. Most of them divided only for short period but they resemble their predecessors very much. The benefits of primary cultures include their direct derivation from the tissue of interest and the absence of any genetic or epigenetic alterations. They typically display the primary tissue phenotype from which they were obtained(Freudenrich and Shafer, 2020)

1.4.2 Cell line

A primary culture that has been subcultured or passed through multiple cultures is called a cell line. The number of passages is the total number of subcultures that the cells have undergone. It should be monitored and kept from rising too high to avoid genetic modifications and other variants to the cells (Phelan and May, 2017). Continuous cell lines are those that continue to develop unabatedly during repeated subcultures, whereas finite cell lines eventually die after numerous subcultures. Continuous cell lines are typically produced from embryonic tissues or malignancies (Coecke *et al.*, 2005).

1.4.2.1 Applications of cell line

1. Very good modeling systems for studying normal cell physiology and biochemistry (e.g., metabolic studies, aging).
2. Test of the toxicity to study how new drugs effect
3. The characteristics of cancer cells and how they are affected by radiation, viruses, and different substances.
4. Production of pharmaceutical drugs, monoclonal antibodies (mABs), and vaccines (Verma *et al.*,2020).

1.4.2.2 Advantages and disadvantages of cell line

Advantages include:

1. Physiochemical and physiological state: The role and impact of pH, temperature, O2/CO2 concentration, and osmotic pressure of the culture media can be varied to evaluate their impacts on the cell culture.

2. Cytotoxic assay: It is possible to study how different substances or medications affect particular cell types, such as liver cells.

3. Consistency in results: The ability to get consistent results while using a single type or clonal population.

4. Cell type identification is possible through the use of karyotyping or by looking for markers like molecules.

5. Ethics: It is possible to avoid moral, legal, and ethical complications while using animals in research (Verma *et al.*,2020).

While disadvantages involve:

1. Cost and skill: This is a specialist method that needs aseptic settings, skilled workers, and costly equipment.

2. De-differentiation: Cell features can alter following a period of continuous cell development in cultures, resulting in differentiated qualities compared to the original strain.

3. Low production: The extremely low production levels of monoclonal antibodies and recombinant protein, along with the subsequent downstream processing necessary to recover pure products, greatly raise costs.

4. Contamination: Viral infections and mycoplasma are extremely infectious and difficult to detect (Verma *et al.*,2020).

1.4.2.3 LNCap prostate cancer cell line

A metastatic lymph node lesion of a Caucasian patient with metastatic prostate cancer gave rise to the malignant cell line known as LNCap. The LNCap cell line requires androgens for its growth due to it being positive for androgen receptors (AR) (Abate-Shen and Nunes de Almeida, 2022).

Chapter Two

Materials

&

Methods

2 Materials and Methods

The experimental work was performed in the Postgraduate lab /Department of Pharmacology at the College of Medicine\ University of Babylon from January - May 2023.

2.1 Materials

2.1.1 Chemicals

The chemicals used in this study were listed in (Table 2.1) with their suppliers.

Table 2. 1 chemicals used in the study.

Chemical	Company	Country
Alcohol liquid 99.9% Ethanol solution	FranceAlcools	France
Alcohol spray (ethanol 70%)	Aljoud	Iraq
Dimethyl sulfoxide	Sigma Aldrich	Germany
Fetal bovine serum (FBS)	Capricorn	Germany
MTT(3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder	Roth	Germany
Penicillin-Streptomycin 1%	Capricorn	Germany
Phosphate buffer saline packets	BioPLUS chemicals	USA
RPMI 1640 medium w/L-glutamine, 10mM HEPES	HI MEDIA	India

buffer without phenol red and sodium bicarbonate (powder)		
Sodium bicarbonate powder	Ludeco	Belgium
Trypsin-Ethylenediaminetetraacetic acid (EDTA) powder	US biological	USA

2.1.2 Instruments and Tools

The instruments and tools used in the study were listed in (Table 2.2) with their suppliers.

Table 2. 2 List of Instruments and Tools Used in the Study

Instrument/ tool	Company	Country
Autoclave	Jeiotech	Korea
Cell culture flask (25ml)	SPL	Korea
Cell culture plate (48-wells)	SPL	Korea
Cell culture plate (96-wells)	SPL	Korea
Centrifuge	Rotanta	Germany
Distiller	ROWA	Germany
Double distillation water stills	GFL	Germany
Electric oven	Memmert	Germany

ELISA Reader	Bio Tek 800 TS	Korea
Incubator	Memmert	Germany
Inverted microscope	T.C Meiji techno	Japan
Laminar air flow cabinet	Labtech	Korea
magnetic stirrer	Scotech	Germany
Micropipettes (different sizes)	Dragon-Med	India
Millipore filter(0.45, 0.22µm)	Biofil	Australia
Refrigerator	Arcelik	Turkey
Sensitive Balance	Labtech	Korea
Water bath	Memmert	Germany

2.1.3 ELISA Immunoassay kits

The assay kits used in this study include:

Table 2.3 list of ELISA assay kits used in the present study

ELISA kit Caspase 3	Elabscience	USA
ELISA kit Total anti-oxidant	Elabscience	USA
ELISA kit TNF-alpha	Elabscience	USA

❖ **Kit contents include the following:**

Table 2.4 List of contents of the ELISA assay kit

Micro ELISA Plate (Dismountable)	96T:8wells×12strips 48T: 8 wells ×6 strips
Reference Standard	96T:2vials 48T: 1 vial
Concentrated Biotinylated Detection Ab (100×)	96T:1vial,120µL 48T: 1 vial, 60 µL
Concentrated HRP Conjugate (100×)	96T:1vial,120µL 48T: 1 vial, 60 µL
Reference Standard & Sample Diluent	1 vial, 20 mL
Biotinylated Detection Ab Diluent	1 vial, 14 mL
HRP Conjugate Diluent	1 vial, 14 mL
Concentrated Wash Buffer (25×)	1 vial, 30 mL
Substrate Reagent	1 vial, 10 mL
Stop Solution	1 vial, 10 mL
Plate Sealer	5 pieces
Manual	1 copy
Certificate of Analysis	1 copy

2.1.4 Drugs

Table 2.5 List of drugs

Dutasteride powder	MedChem Express	USA
5FU vial (1000mg/20 ml)	Kocak pharma	Turkish
Doxorubicin vial (50mg/25ml)	Pfizer	USA
Testosterone ampoule (250mg /ml)	Galenika	Serbian

2.2 Methods

2.2.1 Preparation of Reagents and Solutions

A) Phosphate Buffer Saline (PBS):

According to the manufacturer's instructions, one tablet of PBS was dissolved in 500 ml of deionized distilled water (DDW) and stirred by a magnetic stirrer at room temperature, the pH of the PBS solution was adjusted to 7.4 and then sterilized for 20 minutes at 120˚C in autoclave and keeping it sterile until it was utilized

B) Trypsin-(EDTA) Solution:

About 10.1 grams of trypsin-EDTA powder was dissolved in 900 ml of double distilled water (DDW) with continuous mixing at room temperature, the PH was adjusted to value of 7.2 and the volume was completed to 1 Liter by DDW. Then, the solution was sterilized using Millipore filters of 0.45 and 0.22 micron, respectively, and the solution was stored at (- 20C).

C) Culture media preparation

Liquid RPMI-1640 phenol-free medium was made from RPMI-1640 phenol-free powder as follows:

About 15.4 grams in 900ml ddH2O, stirring gently until completely dissolved. Then 2g sodium bicarbonate was added and the volume was completed to 1L. The media was filtrated with a 0.22-micron membrane and transferred to sterilized containers.

The whole media were prepared by adding 1% Penicillin-Streptomycin solution and 10% fetal bovine serum to RPMI and filtered with a 0.22-micron membrane filter.

D) Freezing media preparation

A proximally 10 mL of freezing media was made up of the following ingredients: 6 mL of serum-free medium (RPMI-1640), 3 mL fetal bovine serum, and 1 mL dimethyl sulfoxide (DMSO) was added. The medium was kept at -20C until use.

2.2.2 Preparation of drugs stock solutions

A) Preparation of testosterone stock solution

Testosterone ampoule 250mg/ml has been used in this study. The ampoule was stored at room temperature and considered a stock solution. four dilutions were prepared from original ampoule (10 mg/ml, 0.5 mg/ml, 30 µg/ml, 1000 ng/ml), then the serial concentrations of testosterone started from (500 ng/ml) down to (3.9 ng/ml) were prepared by adding 1ml of dilution 4 to 1ml of media.

B) Preparation of dutasteride stock solution

Dutasteride powder has been used in this study. About 10 mg of dutasteride has been dissolved in 1 ml DMSO to obtain a stock solution of 10 mg/ml and stored at -20C and used in the experiments. Different concentrations were prepared from stock solution. The first concentration was 50 µg/ml was prepared by adding about 10 µl of dutasteride stock solution and the volume was completed to 2ml with media and the serial dilutions from first concentration was used to prepared the other concentrations (25, 12.5, 6.25. 3.125, 1.5625) µg/ml.

C) Preparation of Doxorubicin stock solution

Doxorubicin vial (2mg/ml) has been used in this study. This vial was stored at temperature below 25 C and considered a stock solution The first concentration was 25 µg/ml and prepared by adding 25 µl of stock

solution and the volume was completed to 2ml with media and the serial dilution was used to prepared other concentrations (12.5, 6.25, 3.125, 1.5625) µg⁄ml.

D) Preparation of 5FU stock solution

A vial of 5FU (50mg/ml) has been used in this study. This vial was stored at room temperature and considered a stock solution. Different concentrations of 5FU were prepared. The first concentration was 100 µg/ml and prepared by adding 4 µl of stock solution and the volume was completed to 2ml with media and the serial dilution was used to prepared other concentrations (50. 25, 12.5, 3.125) µg⁄ml.

2.2.3 Preparation of LNCap cell line

A) LNCap Cell line

Human prostate cancer (LNCap) cell line in frozen vials was obtained from the Tissue Culture Laboratory /College of Medicine / University of Babylon.

B) Thawing of LNCap cell line

One of the LNCap cryovials is taken from a liquid nitrogen container with caution and placed into sterile pre-warmed water (37°C), then the vial is removed from the water before the ice is completely dissolved. The vial is sterilized with 70% ethanol. Inside the laminar flow cabinet, the cell suspension is transferred by sterilized pipette into a 15 ml tube containing 10 ml of serum-free medium, then the tube is centrifuged at 800- 1000 rpm for 5-10 minutes to precipitate the cells in the bottom of the tube. After centrifugation, the supernatant was discarded and the cells pellet was re-suspended in 5 ml medium with 10% serum. After that, the cells were

transferred into a 25 ml size culture flask and incubated at 37°C. At next day the medium is replaced with a new one (Luhur *et al.*, 2019).

C) Sub-culturing of cells

1- The cell flasks were examined under an inverted microscope to determine the degree of confluence of cells and ensure that the cells are free from contamination.

2- After the cells reached 80-90 % confluence, the cell flask is taken and sterilized with 70% ethanol and inserted into a laminar flow cabinet

3- Inside the cabinet, the medium was removed and the cells were washed with phosphate buffer saline to ensure that a medium is completely removed.

4- After washing, about 1ml of trypsin/EDTA solution is added to the flask and incubated at 37 c° for (2-10 min) to permit the cells to detach from the surface of the culture flask.

5- The cells after that examined by using an inverted microscope to ensure that all cells have been detached and been in a suspension state. If there are any remaining cells, gently taping on the flask sides by the palm of the hand is used to release the attached cells.

6- An appropriate volume of serum-containing media is added to stop the trypsin activity and avoid cell degradation.

7- Then cell suspension is divided into two flasks and marked each flask with the date, passage number, and cell line name.

8- In two flasks or on a culture plate, the cells were sub-cultured (Uysal *et al.*, 2018).

D) Freezing the cells

The following approach was used to maintain cell lines at -180 C^0 in liquid nitrogen:

1- Tissue culture flask with a monolayer near the exponential phase was taken and washed twice with 5 ml of PBS, and then 3 ml of warm trypsin EDTA was added.

2- The flask was incubated at 37C° until the cell layer detached and the cells was aided to disaggregate into single cells by gentle rocking on the flask sides.

3- The flask content was transferred into 15 ml sterile plastic centrifuge tube; centrifugation was done at 800 rpm for 10 minutes.

4- The supernatant was decanted and the cell pellet was re-suspended with 1 ml of the freezing media and transferred into 1.5 ml sterile freezing vial.

5- The vial kept for 10 minutes at room temperature and transferred to – 80C° deep freezer for 24 h and then stored for a long time in the liquid nitrogen freezer (Uysal *et al.*, 2018)**.**

2.3 Experiments

2.3.1 Cytotoxicity assay (MTT assay) Experiments.

2.3.1.1 Testosterone effects on LNCap cell line

LNCap cells were cultured in 96 well plates with phenol free RPMI-1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin and maintained at 37˚C. After 24hr incubation the medium was removed and the cell was exposed to different concentrations of testosterone (500, 250, 125, 62.5, 31.25. 15.125, 7.8, 3.9) ng/ml in three replicates for each concentration and then incubate for 48 hours. After incubation, the cell viability was assessed by MTT assay.

2.3.1.2 Dutasteride effects on LNCap cell line

LNCap cells were cultured in 96 well plates with phenol free RPMI-1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin and incubated for 24hr. After incubation the medium was decanted and replaced with another containing (7.8 ng/ml) of testosterone (depending on the testosterone experiment, a concentration of (7.8 ng/ml) gives the best proliferation degree for LNCap cells) and the cells exposed to different concentrations of dutasteride (50, 25, 12.5, 6.25, 3.125, 1.5625) µg⁄ml in three replicate for each concentration and then incubate for 48hr. After incubation, the cytotoxic effects were assessed by MTT assay.

2.3.1.3 Doxorubicin effects on LNCap cell line

LNCap cells were cultured in 96 well plates with phenol free RPMI-1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin and incubated for 24hr. After incubation, the medium was decanted and the cells were exposed to different concentrations of doxorubicin (25, 12.5, 6.25, 3.125, 1.5625) µg⁄ml in three replicates for each concentration and incubated for 48 hr. After incubation, the cytotoxic effects were assessed by MTT assay.

2.3.1.4 Fluorouracil effects on LNCap cell line

LNCap cells were cultured in 96 well plates with phenol free RPMI-1640 supplemented with 10% FBS, 1% Penicillin-Streptomycin and incubated for 24hr. After incubation, the medium was decanted and the cells were exposed to different concentrations of 5FU (100, 50. 25, 12.5, 3.125) µg⁄ml in three replicates for each concentration and incubated for 48 hr. After incubation, the cytotoxic effects were assessed by MTT assay.

- The half maximal inhibitory concentration (IC50) is a measure of the potency of a substance in inhibiting a specific biological or biochemical function. The simplest estimate of IC50 is to plot x-y and fit the data with a straight line (linear regression). The IC50 value is then estimated using the fitted line, according to the following equation:

  Y = a * X + b,

  IC50 = (50 - b)/a.
- The IC50 of dutasteride, 5FU, and doxorubicin that calculated from above experiments were (75, 30, and 3) µg⁄ml respectively.
- Depending on the IC50 of dutasteride different concentrations of dutasteride were prepared starting from its IC50 (75, 37.5, 18.75, 9.37, 4.68, 2.34) µg/ml.

2.3.1.5 Effects of different concentrations of dutasteride with doxorubicin IC50 on LNCap cell line

LNCap cells were cultured in 96 well plates with phenol free RPMI-1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin and incubated for 24hr. After incubation, the medium was decanted and replaced with another containing (7.8 ng⁄ml) of testosterone. Then, the cells were exposed to different concentrations of dutasteride starting from its IC50 with a constant concentration of doxorubicin (3 µg⁄ml) (75:3, 37.5:3, 18.75:3, 9.375:3, 4.6875:3, 2.34:3) µg⁄ml in three replicates for each concentration with three replicates for the positive control (IC50 only) and three replicates for negative control and incubated for 48 hr. After incubation, the cytotoxic effects were assessed by MTT assay.

2.3.1.6 Effects of different concentrations of dutasteride with IC50 of 5FU in LNCap line

LNCap cells were cultured in 96 well plates with phenol free RPMI-1640 supplemented with 10% FBS and 1% Penicillin-Streptomycin and incubated for 24hr. After incubation, the medium was decanted and replaced with another containing (7.8ng⁄ml) of testosterone. Then, the cells were exposed to different concentrations of dutasteride starting from its IC50 with a constant concentration of 5FU (30µg⁄ml): (75:30, 37.5:30, 18.75:30, 9.375:30, 4.6875:30, 2.34:30) µg⁄ml in three replicates for each concentration with three replicates for the positive control (IC50 only) and three replicates for negative control and incubated for 48 hr. After incubation, the cytotoxic effects were assessed by MTT assay.

2.3.2 ELISA Immunoassay Experiments

2.3.2.1 Apoptotic, Anti-inflammatory, and Antioxidant effects of Testosterone in LNCAP cell line.

LNCap were incubated in 48 well plates with phenol free RPMI-1640 supplemented with 1% Penicillin-Streptomycin and 10% FBS and incubated for 24hr. after incubation, the media was removed and the cells were exposed to different concentrations of testosterone (500, 62.5, 7.8, 3.9) ng/ml which choosing depending on MTT results with three replicates for each concentration and three replicates for the control and incubated for 48 hours at 37C. After incubation, the Apoptotic, Anti-inflammatory, and antioxidant effects of testosterone were assessed by measuring caspase 3, TNF-alpha, and total anti-oxidant capacity, respectively by using Elisa assay kits.

2.3.2.2 Apoptotic, Anti-inflammatory, and Antioxidant effects of dutasteride in LNCap

LNCap were incubated in 48 well plates with phenol free RPMI-1640 supplemented with 1% Penicillin-Streptomycin and 10% FBS and incubated for 24hr. after incubation, the media was removed and replaced with another containing (7.8 ng⁄ml) of testosterone. Then, the cells were exposed to different concentrations of dutasteride (50, 25, 6.25, 3.125) µg/ml which choosing depending on MTT results with three replicates for each concentration and three replicates for the control and incubated for 48 hours at 37C. After incubation, the Apoptotic, Anti-inflammatory, and antioxidant effects of dutasteride were assessed by measuring caspase 3, TNF-alpha, and total anti-oxidant capacity, respectively by using Elisa assay kits.

2.3.2.3 Apoptotic, Anti-inflammatory, and Antioxidant effects of different concentrations of dutasteride combined with doxorubicin IC50 in LNCap cell line

LNCap were incubated in 48 well plates with phenol free RPMI-1640 supplemented with 1% Penicillin-Streptomycin and 10% FBS and incubated for 24hr. after incubation, the media was removed and replaced with another containing (7.8 ng⁄ml) of testosterone. Then, the cells were exposed to different concentrations of dutasteride starting from its IC50 with doxorubicin IC50 (75:3, 18.75:3, 9.37:3) µg/ml which choosing depending on MTT results with three replicates for each concentration and three replicates for the positive control and negative control and incubated for 48 hours at 37C. After incubation, the Apoptotic, Anti-inflammatory,

and antioxidant effects were assessed by measuring caspase3, TNF-alpha, and total anti-oxidant capacity, respectively by using Elisa assay kits.

2.3.2.4 Apoptotic, Anti-inflammatory, and Antioxidant effects of different concentrations of dutasteride combined with IC50 of 5FU in LNCap cell line

LNCap were incubated in 48 well plates with phenol free RPMI-1640 supplemented with 1% Penicillin-Streptomycin and 10% FBS and incubated for 24hr. after incubation, the media was removed and replaced with another containing (7.8 ng⁄ml) of testosterone. Then, the cells were exposed to different concentrations of dutasteride starting from its IC50 with IC50 of 5FU (75:30, 18.75:30, 9.37:30) µg/ml depending on MTT results with three replicates for each concentration and three replicates for the positive control and negative control and incubated for 48 hours at 37C. After incubation, the Apoptotic, Anti-inflammatory, and antioxidant effects were assessed by measuring caspase3, TNF-alpha, and total anti-oxidant capacity, respectively by using Elisa assay kits.

2.4 MTT assay

The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) is a mono-tetrazolium salt made up of three aromatic rings, comprising two phenyl moieties and one thiazolyl ring, around a positively charged quaternary tetrazole ring core. The core tetrazole ring is broken when MTT is reduced, which causes the creation of the violet-blue, water-insoluble molecule formazan. The MTT reagent is transformed to formazan by metabolically active cells because it has a lipophilic nature that allows it to pass through both the mitochondrial inner membrane and the cell membrane of live cells. At several points along the glycolytic

pathways to the mitochondrial electron transport chain, oxidoreductase and dehydrogenase enzymes, as well as electron donors—mostly NAD(P)H—, can induce MTT reduction intracellularly (Ghasemi *et al.*, 2021)

After incubating cells with MTT for a few hours, the MTT test is normally carried out. Following that, a solvent such as dimethyl sulfoxide (DMSO) is used to dissolve the generated water-insoluble formazan. The optical density (OD) of the homogenized MTT-formazan solution is then determined using a microplate reader at a wavelength where MTT-derived formazan absorbs the maximum light, which is about 570 nm. This measurement is done to determine how much the homogenized MTT-formazan solution reduces light transmission through absorbance and other processes. Based on the obtained OD values, it is presumed that formazan concentration and, subsequently, the intracellular reduction of MTT, are representations of the two variables. This is the rationale for the nearly four decades of widespread use of the MTT test as a tool to assess cell viability and proliferation, drug cytotoxicity, and metabolic and mitochondrial activities (Stockert *et al.*, 2018). Figure 2.1: Principle of MTT Assay.

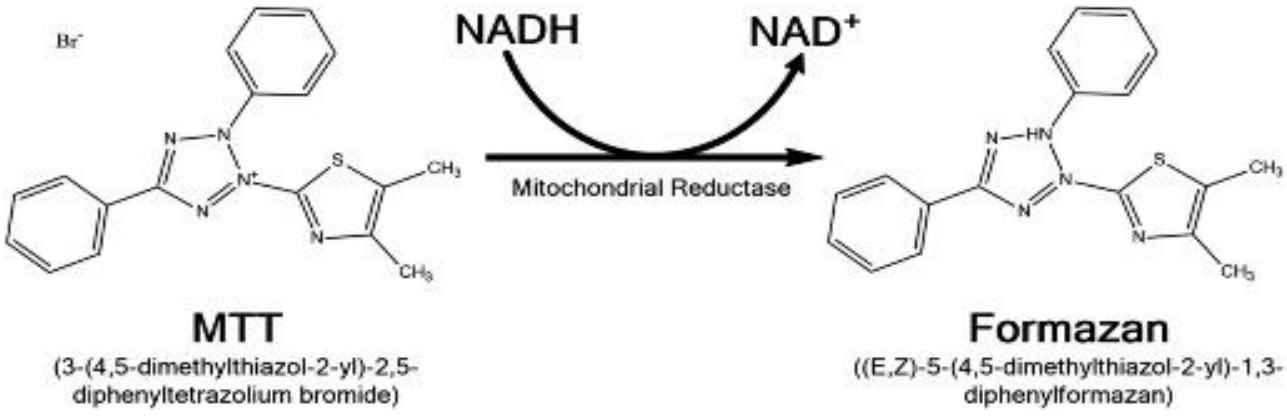


Figure 2.1: Principle of MTT Assay(Kamiloglu *et al.*, 2020).

- **MTT solution preparation**

1. MTT stock solution. 5 mg/mL MTT reagent (Sigma Aldrich, Spain) was dissolved in PBS and sterilized by filtration through a 0.22 μm filter and stored at 4 °C in the dark.

2. MTT incubation media. 1 mL of MTT stock solution was added to 10 mL of serum-free culture medium (Tolosa, Donato and Gómez-Lechón, 2015)

- **MTT Assay protocol:**

1- The medium was removed carefully, and the cells were rinsed very carefully once with 100 μL pre-warmed PBS. The rinsing solution was removed by gently blotting on paper towels.

2- About 100 μL MTT incubation medium was added to each well and incubated at 37 °C for 2-4 h).

3- After incubation, the MTT incubation medium was removed and the excess medium in wells was decanted by gently blotting on paper towels.

4- Exactly 100 μL dimethyl sulfoxide (DMSO) was added to all wells.

5- Then, the plate gently shakes with a microplate shaker for 20–45 s to extract formazan from the cells and to form a homogeneous solution.

6- The plates are left for at least 10 min at room temperature and protected from light by using a cover (Tolosa, Donato and Gómez-Lechón, 2015).

The absorbance readings from the test samples must then be divided by those from the control and multiplied by 100 to calculate the percentage of cell viability or proliferation. Higher absorbance values indicate cell proliferation, while lower values indicate cell death or

suppression of proliferation. The following formula was used to compute the percentage of cell viability or inhibition:

% viability = (AT) / (AC) × 100%

Where, AT = Absorbance of treated cells (drug).

AC = Absorbance of control (untreated).

% Inhibition = 100 – % viability

2.5 TNF –alpha and caspase 3 ELISA kits assays

The Sandwich-ELISA method is employed by this ELISA kit. This kit's micro ELISA plate has been pre-coated with a human caspase 3 or TNF-alpha-specific antibody. The micro ELISA plate wells are filled with samples (or standards) and the particular antibody. After that, each microplate well receives a sequential addition of an Avidin-Horseradish Peroxidase (HRP) combination and a detection antibody that has been biotinylated and is specific for either human caspase 3 or TNF-alpha. Free parts are removed during washing. To each well, the substrate solution is applied. Only the wells that also contain a biotinylated detection antibody, human caspase 3 or TNF-alpha, and an Avidin-HRP conjugate will show blue color. The addition of a stop solution stops the enzyme-substrate reaction, and the color changes to yellow. At a wavelength of 450 2 nm, the optical density (OD) is determined spectrophotometrically. The Human Caspase 3 or TNF-alpha concentrations are inversely correlated with the OD value. By comparing the OD of the samples to the standard curve, it was possible to determine the amount of human caspase 3 or TNF-alpha present in the samples. The assay procedure is: -

1. Wells for diluted standard and sample were determined and 100 µL of each dilution of standard and sample were added into the appropriate

wells (all samples and standards be assayed in triple replicate). The plate was covered with the sealer provided in the kit and incubated for 90 min at 37℃.

2. The liquid from each well was decanted and 100 µL of Biotinylated Detection Ab working solution was added to each well immediately and without washing and the plate was covered with a new sealer and then incubated for 1 hour at 37°C.

3. The solution from each well was decanted and 350 µL of wash buffer was added to each well and Soaked for 1 min. Then, the solution was aspirated or decanted from each well and patted to dry against clean absorbent paper. This wash step was repeated 3 times.

4. About 100 µL of HRP Conjugate working solution was added to each well and the plate was covered with a new sealer and incubated for 30 min at 37°C.

5. The solution was removed from each well and the wells were washed five times as conducted in step 3.

6. After washing 90 µL of Substrate Reagent was added to each well and the plate was covered with a new sealer and incubated for about 15 min at 37°C for 30 min and was protected from light.

The Microplate Reader was preheated for about 15 min before OD measurement.

7. About 50 µL of Stop Solution was added to each well in the same order as the substrate solution.

8. Then the optical density (OD value) was determined of each well at once with a micro-plate reader set to 450 nm. Figure (2.2). standard curve of caspase3 and TNF-alpha.

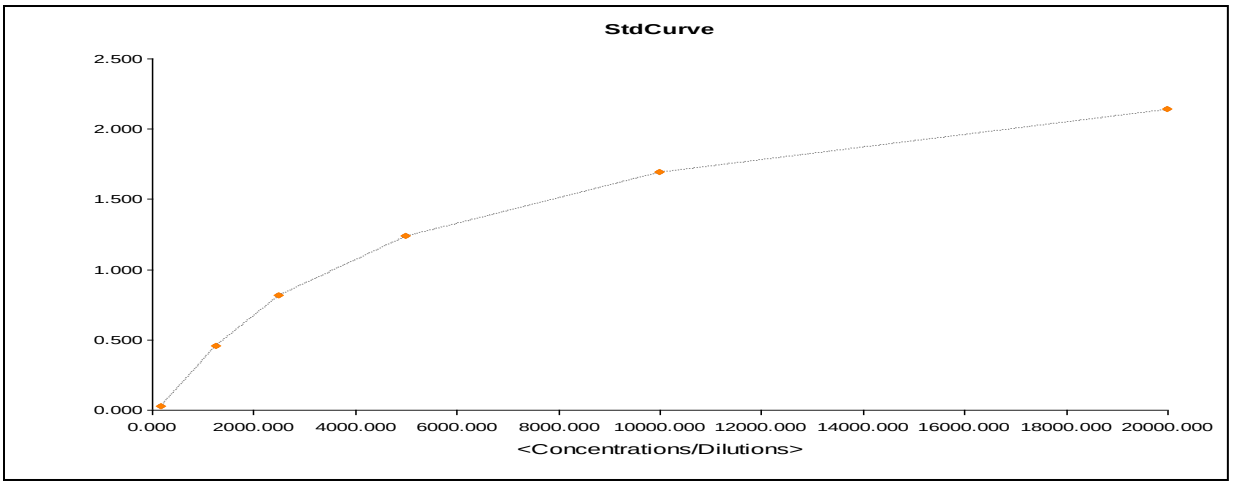


Figure (2.2): standard curve of caspase3 and TNF-alpha.

2.6 Total antioxidant capacity ELISA assay

The basic workings of ABTS are as follows: a suitable oxidant converts ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) to green ABTS+, which can be blocked if antioxidants are present. The absorbance of ABTS+ at 414 nm or 734 nm can be used to calculate and quantify the sample's T-AOC. The antioxidant activity of Trolox, an analog of vitamin E, is comparable to that of VE. As a benchmark for other antioxidants, trolox is used. The antioxidant capacity of another chemical at the same concentration is shown, for instance, by the ratio of its antioxidant capacity to that of Trolox, where the T-AOC of Trolox is 1. The test's methodology was:

1. Standard well: 10 µl of standard was added with different concentrations to the wells.
2. Sample well: 10 µl of the sample was added to wells.
3. About 20 µl of reagent 4 application solution was added to each well of step 1.
4. A proximally 170 µl of ABTS working solution was added to each well of step 2.

5. Mix fully and stand for 6 min at room temperature. The OD values of each well was measured at 414nm with a Microplate reader. Figure (2.3): standard curve of total antioxidant capacity.

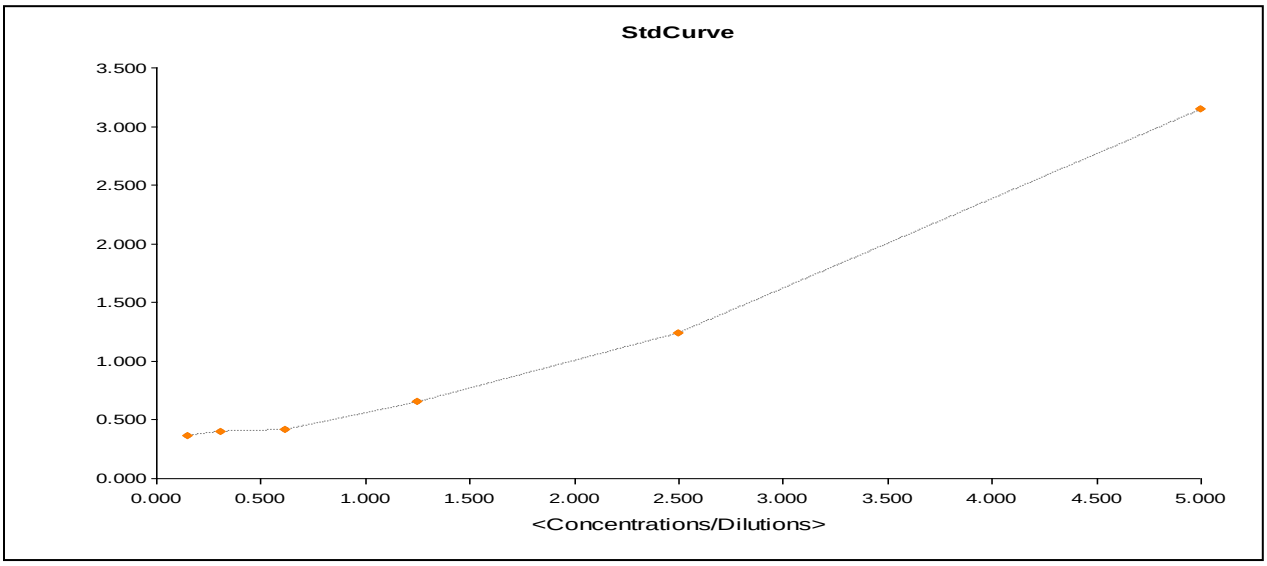
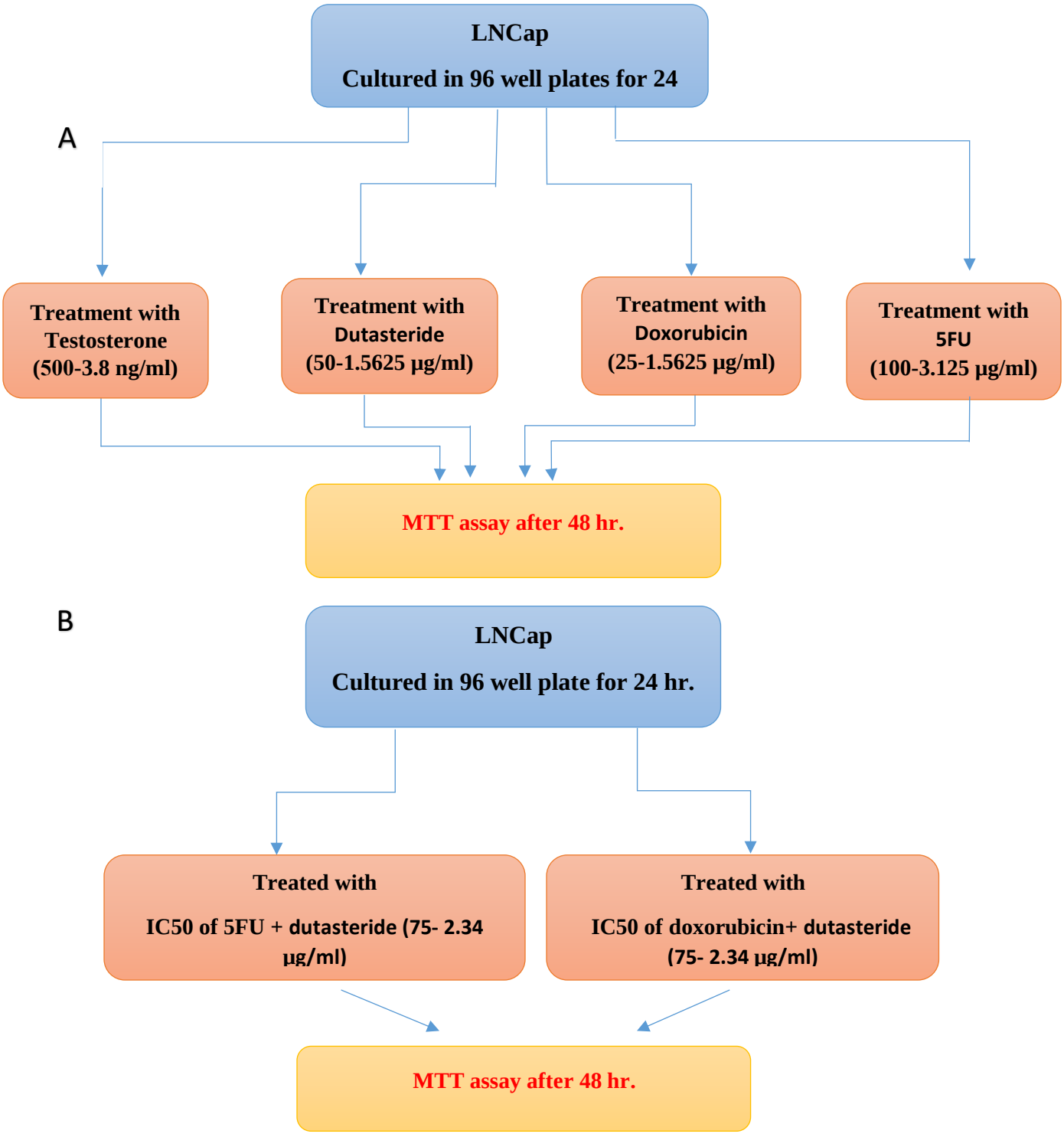
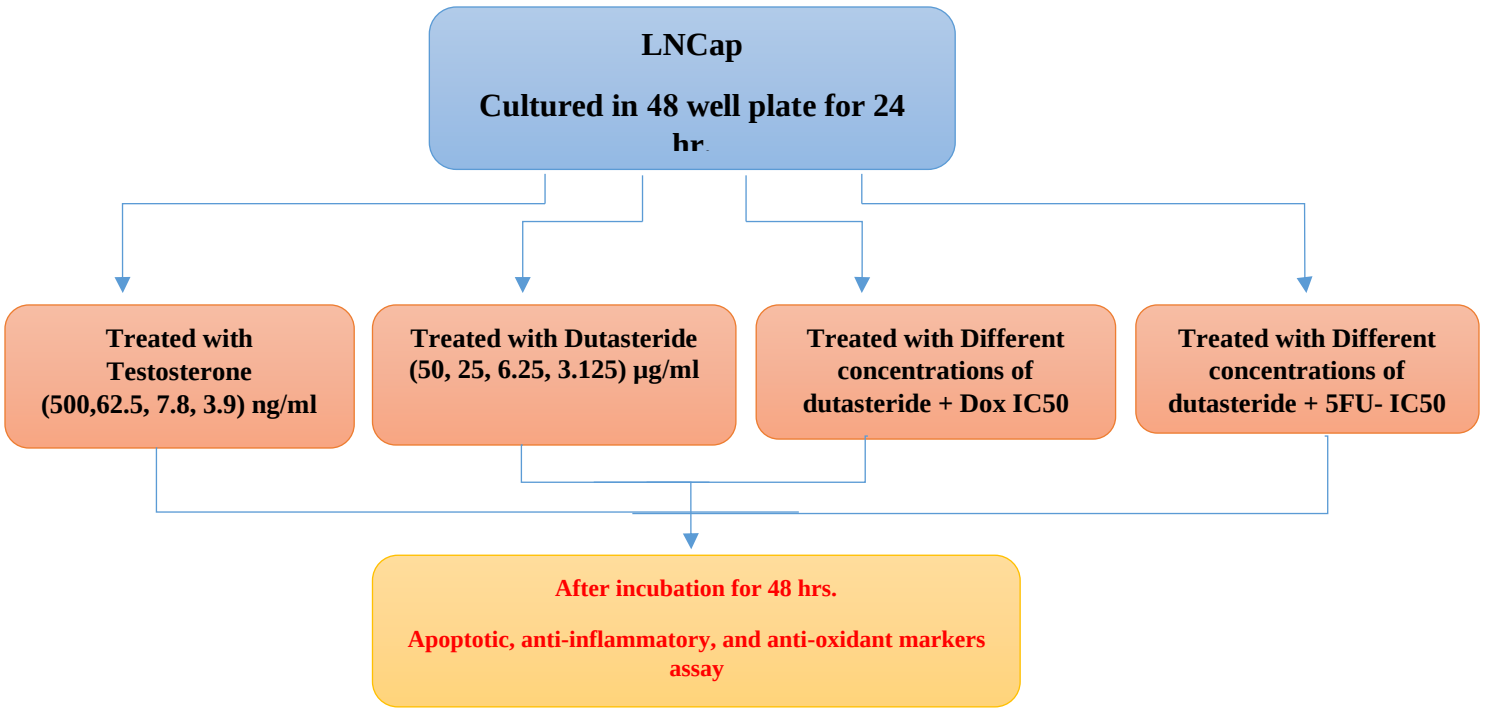


Figure (2.3): standard curve of total antioxidant capacity.

2.7 study plan: Part 1 Cytotoxicity assay



Part 2: ELISA Immunoassay



2.8 Statistical Analysis

Sigma Plot version 12.5 and Microsoft Office Excel 2016 were used to collect and analyze all the data. The significance of variations between the data means was evaluated using the ANOVA one-way test. Statistics differences with P-values (0.001 and 0.05) were considered highly significant and significant, respectively.

Chapter Three
Results

3.1 Cytotoxicity (MTT assay) results

3.1.1 Effects of testosterone on the viability of LNCap prostate cancer cells

LNCap cells were treated with serial concentrations (3.9, 7.8, 15.625, 31.25, 62.5, 125, 500) ng/ml of testosterone and incubated for 48 hours at 37 C, then cell viability was assessed using MTT assay. Results in figure (3.1) showed a highly significant ($p < 0.001$) increase in cells viability at lower concentrations (15.625, 7.8, 3.9) ng/ml and highly significant ($p < 0.001$) decrease in cell viability at high concentration (500 ng/ml) compared to control group (untreated cells). Also, the results showed a highly significant ($p < 0.001$) increase in cells viability at concentration (7.8 ng/ml) compared with other concentrations (15.625, 3.9) ng/ml.

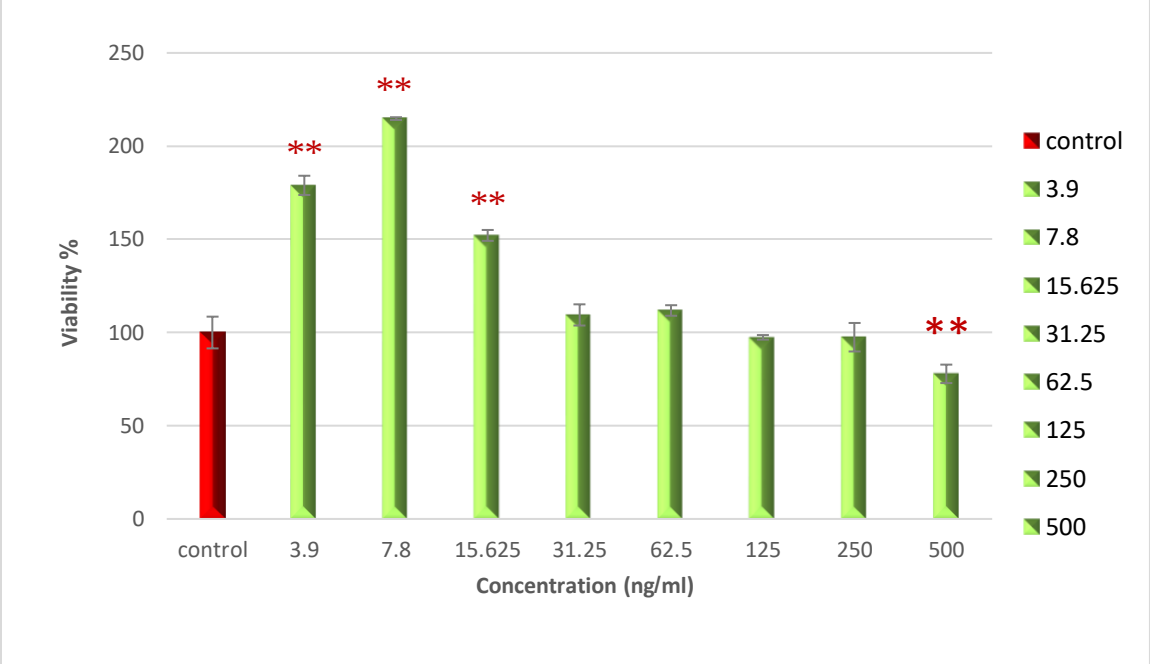


Figure 3.1. The effects of testosterone on the viability of LNCap cell line. ** means ($P<0.001$) for highly significant difference between concentrations. control (untreated cells)

3.1.2 Effects of dutasteride on cell viability in LNCap cell line

LNCap cells were treated with serial concentrations (50, 25, 12.5, 6.25, 3.125, 1.5625) µg/ml of dutasteride in the presence constant concentration of testosterone (7.8 ng/ml), then the cells were incubated for 48 hours at 37C. The effects of dutasteride on cell viability were determined by MTT assay. Results showed a highly significant ($p < 0.001$) decrease in cell viability at concentrations (50, 25, 12,5, 6.25, 3.125) µg/ml of dutasteride with significant ($p < 0.05$) decrease at concentration 1.5625 µg/ml compared to control group as results shown in figure (3.a). Depending on MTT results, the IC50 of dutasteride was estimated by using the excel software and was (75 µg/ml) as shown in fig (3.5b).

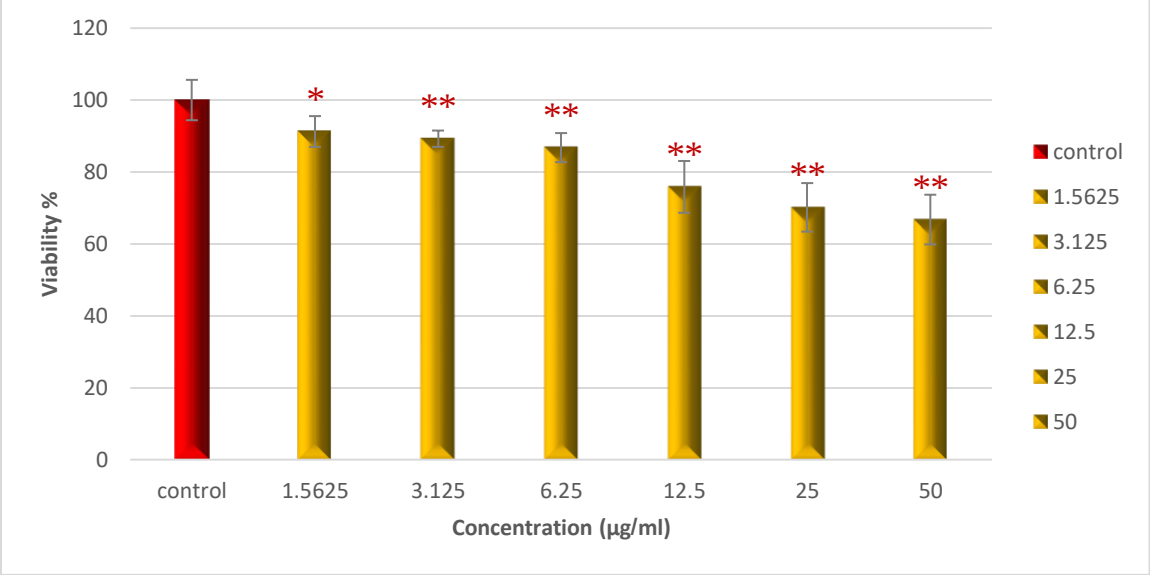


Figure 3.2a. The effects of dutasteride on cell viability of LNCap cell line. * means ($P<0.05$) and ** means ($P<0.001$) for significant and highly significant difference between concentrations. control (untreated cells)

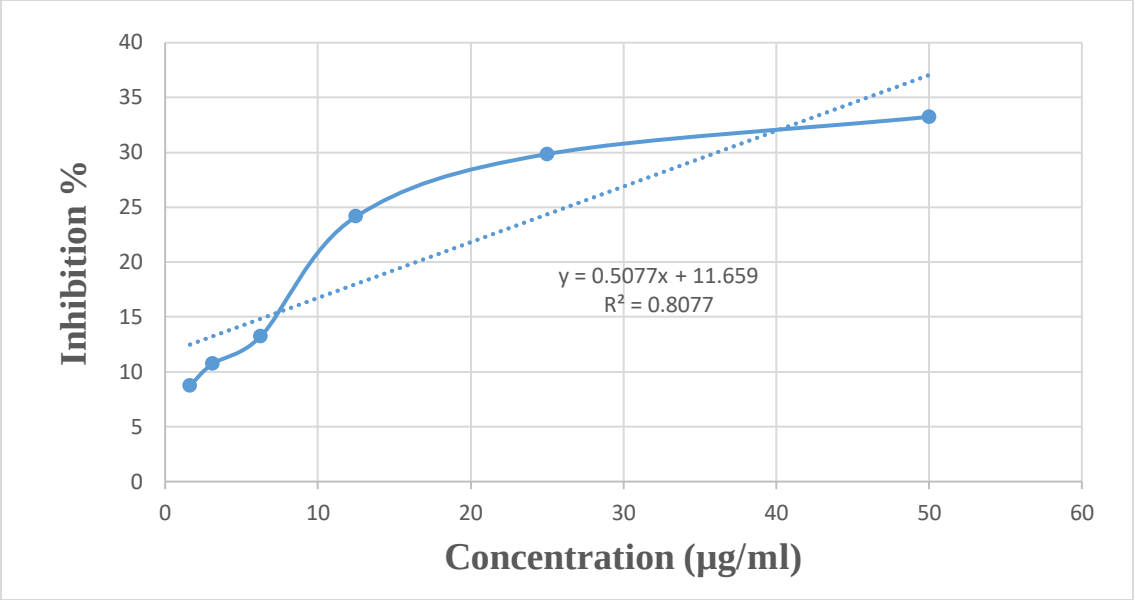


Figure 3.2b. IC50 calculation of dutasteride in LNCap cell line.

3.1.3 Effects of doxorubicin on cell viability in LNCap prostate cancer cells

LNCap cells were treated with serial concentrations (25, 12.5, 6.25, 3.125, 1.5625) µg/ml of doxorubicin, then the cells were incubated for 48 hours at 37C. The effects of doxorubicin on cell viability were determined by MTT assay. Results in fig (3.9a) showed a highly significant ($p < 0.001$) decrease in cell viability at all concentrations of doxorubicin compared to control group. Depending on MTT results, the IC50 of doxorubicin was estimated by using the excel software and was (3 µg/ml) as shown in fig (3.9b).

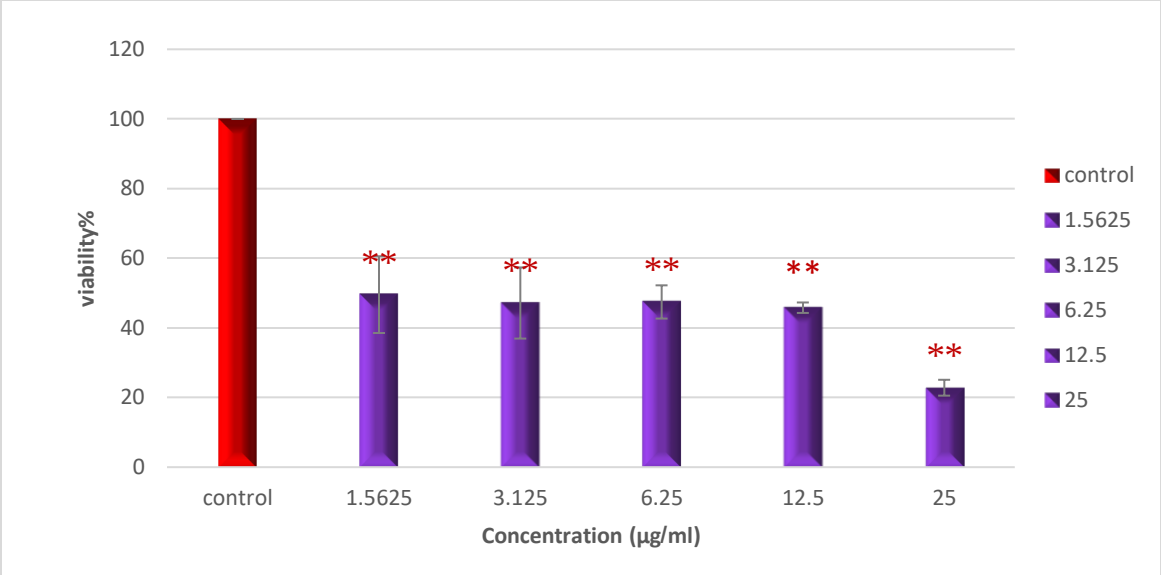


Figure 3.3a. The effects of doxorubicin on cell viability of LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. control (untreated cells)

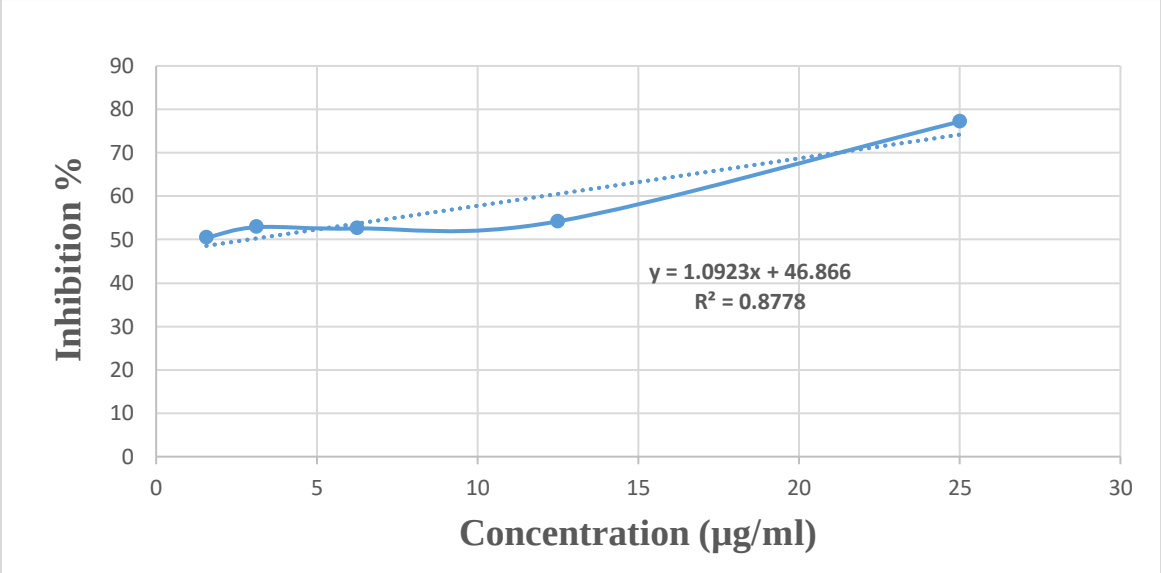


Figure 3.3b. IC50 calculation of doxorubicin in LNCap cell line.

3.1.4 Effects of 5FU on cell viability in LNCap prostate cancer cells

LNCap cells were treated with serial concentrations (100, 50, 25, 12.5, 3.125) µg/ml of 5FU, then the cells were incubated for 48 hours at 37C. The effects of 5FU on cell viability were determined by MTT assay. Results in fig (3.10a) showed a highly significant ($p < 0.001$) decrease in cell viability at all concentrations of 5FU compared to control group. Depending on MTT results, the IC50 of 5FU was estimated by using the excel software and was (30 µg/ml) as shown in fig (3.10b).

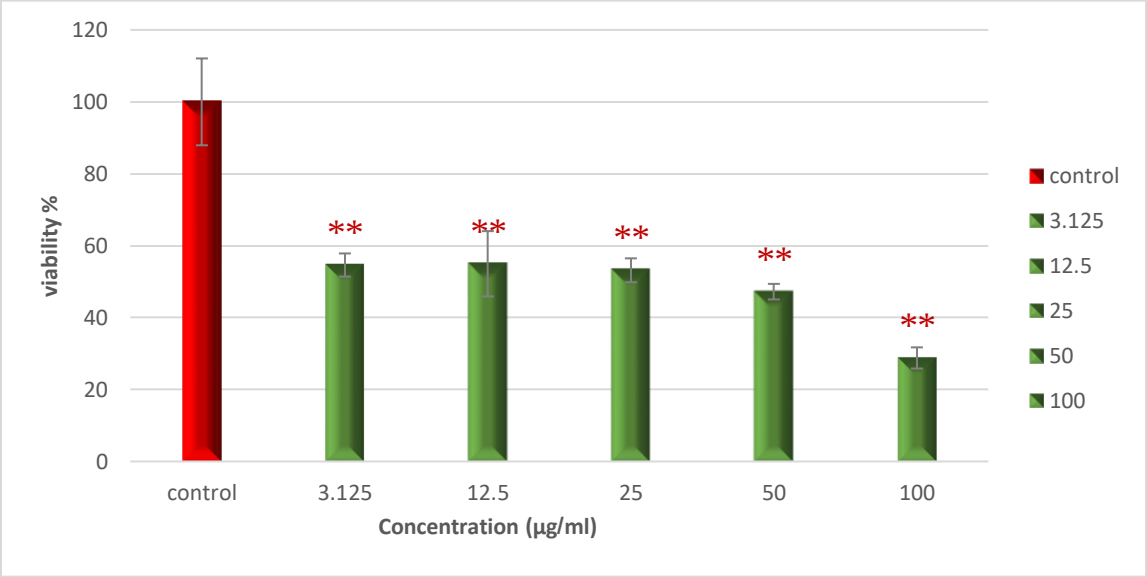


Figure 3.4a. The effects of 5FU on cell viability of LNCap cell line. ** means ($P<0.001$) for highly significant difference between concentrations. control (untreated cells)

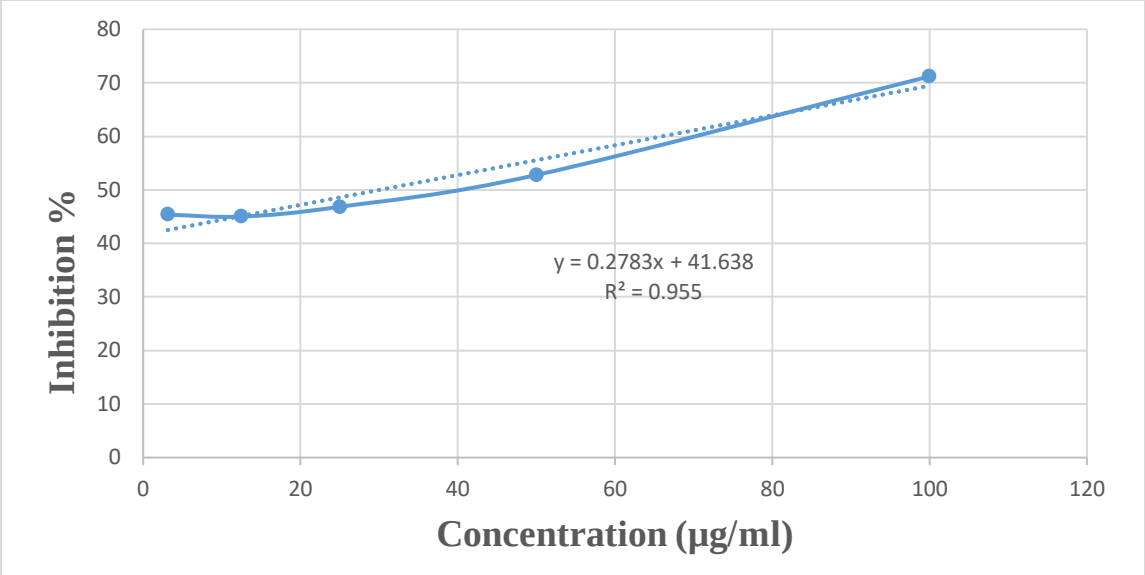


Figure 3.4b. IC50 calculation of 5FU in LNCap cell line.

3.1.5 Effects of (dutasteride-doxorubicin) combination on cell viability in LNCap cell line

Estimated IC50 of dutasteride and doxorubicin was calculated depending on MTT results. It was 75µg/ml for dutasteride and 3 µg/ml for doxorubicin. In order to investigate the co-effects of dutasteride-doxorubicin, different concentrations of dutasteride were used starting from its IC50 (75 µg/ml) down to (2.34 µg/ml) in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (3 µg/ml) of doxorubicin. Cells were incubated for 48 hours at 37 C, then the effects of dutasteride with doxorubicin on cell viability were assessed using MTT assay. Results in figure (3.11) are shown a highly significant ($p < 0.001$) decrease in cell viability at all concentrations compared to positive (IC50 concentration 3 µg/ml of doxorubicin) and negative control group.

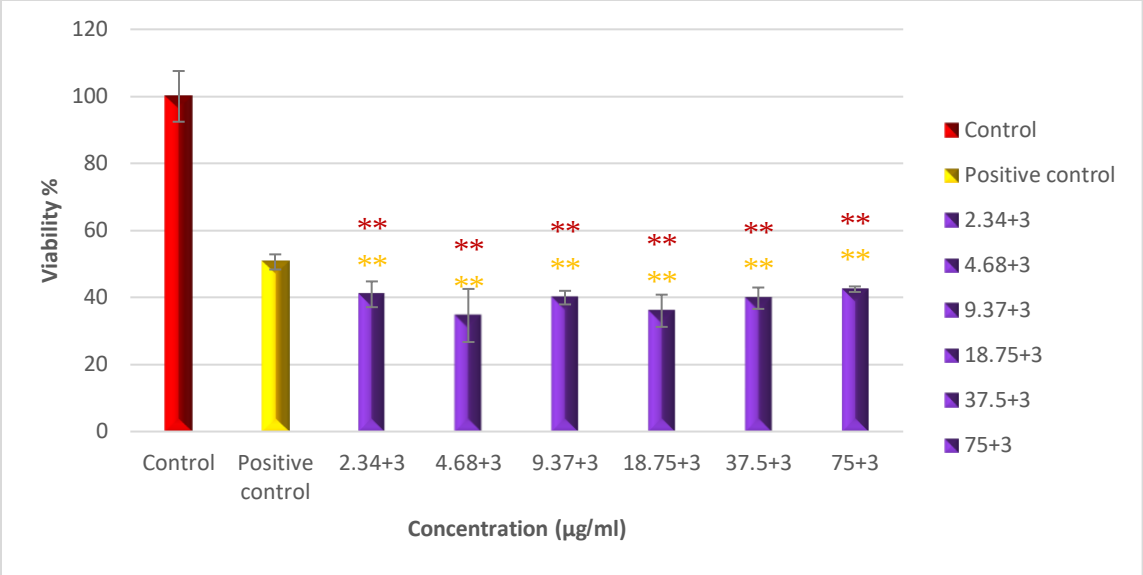


Figure 3.5. Effects of (dutasteride-doxorubicin) Combination on cell viability of LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. Positive control (cells treated with doxorubicin IC50 3µg/ml), control (untreated cells)

3.1.6 Effects of (dutasteride-5FU) combination on cell viability in LNCap cell line

Estimated IC50 of dutasteride and 5FU was calculated depending on MTT results. It was 75µg/ml for dutasteride and 30 µg/ml for 5FU. In order to investigate the co-effects of dutasteride-5FU, different concentrations of dutasteride were used starting from its IC50 (75 µg/ml) down to (2.34 µg/ml) in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (30 µg/ml) of 5FU. Cells were incubated for 48 hours at 37 C, then the effects of dutasteride with 5FU on cell viability were assessed using MTT assay. Results in figure (3.15) are shown a highly significant ($p < 0.001$) decrease in cell viability at all concentrations compared to positive control group (30 µg/ml).

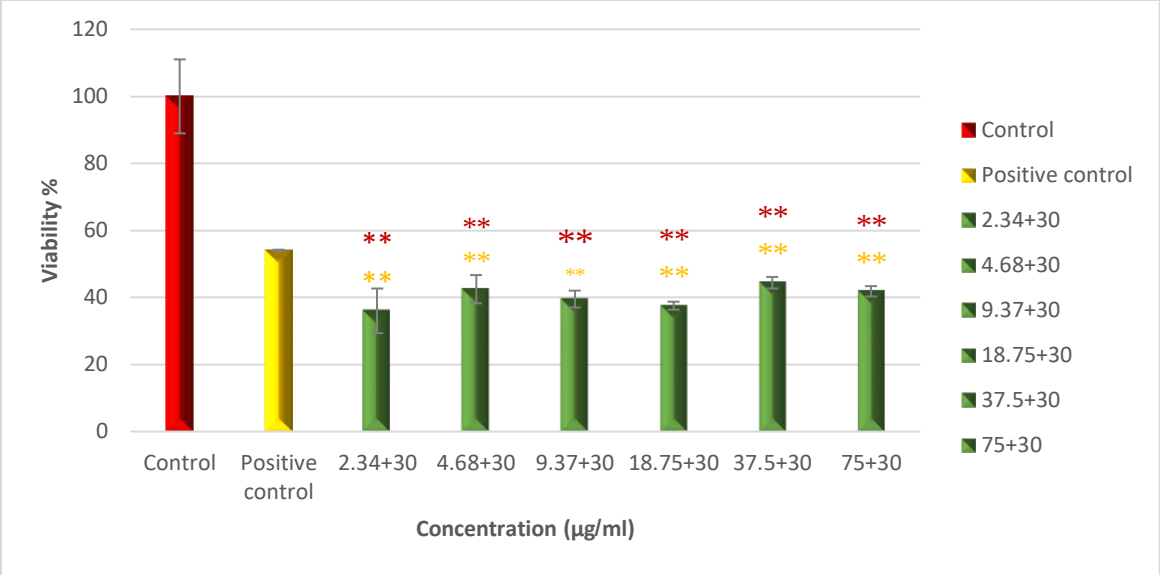


Figure 3.6. Effects of (dutasteride-5FU) Combination on cell viability of LNCap cell line. ** means (*P*<0.001) for highly significant difference between concentrations. Positive control (cells treated with IC50 of 5FU 30µg/ml), control (untreated cells)

3.2 ELASIA Immunoassay results

3.2.1 Apoptotic, Anti-inflammatory, and Antioxidant effects of testosterone in LNCAP cell line.

3.2.1.1 Effects of testosterone on caspase 3 marker in LNCap cells

LNCap cells were treated with serial concentrations (3.9, 7.8, 62.5, 500) ng/ml of testosterone and incubated for 48 hours at 37 C, then the effects of testosterone on caspase 3 level were determined. Results shown in fig (3.2) revealed a highly significant ($p < 0.001$) decrease in caspase 3 at concentrations (62.5, 7.8, 3.9) ng/ml with significant ($p < 0.05$) increase in concentration 500 ng/ml compared to control group.

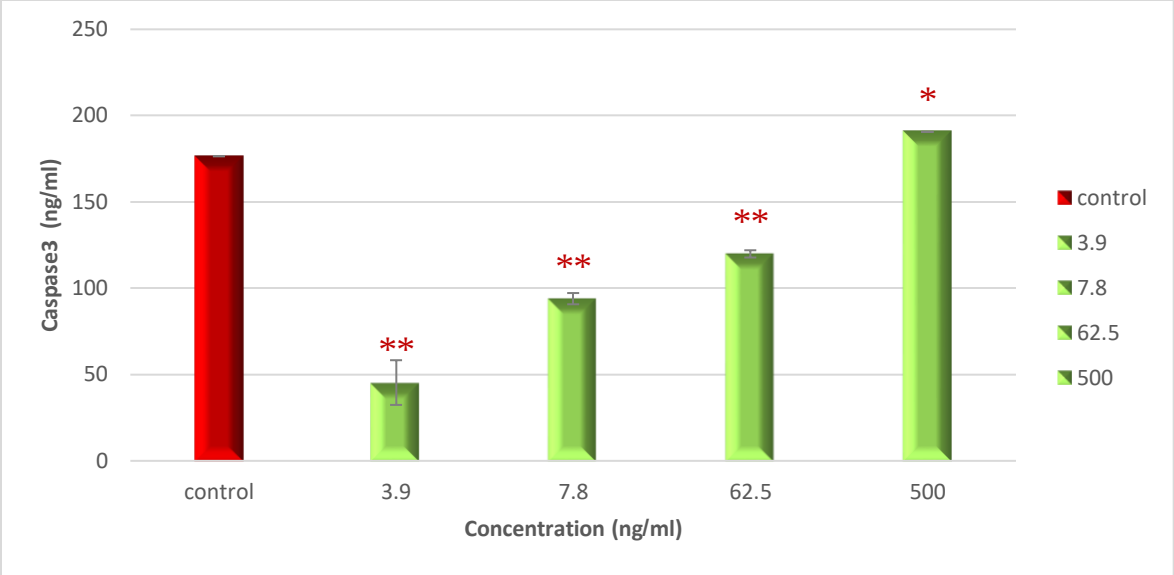


Figure 3.7. The effects of testosterone on caspase 3 in LNCap cell line. * means (P<0.05) and ** means (*P*<0.001) for significant and highly significant difference between concentrations. control (untreated cells)

3.2.1.2 Effects of testosterone on TNF- alpha in LNCap cells

LNCap cells were treated with serial concentrations (3.9, 7.8, 62.5, 500) ng/ml of testosterone and incubated for 48 hours at 37 C, then the effects of testosterone on TNF-alpha were determined Results in figure (3.3) showed a highly significant ($p < 0.001$) decrease in TNF-alpha at all concentrations of testosterone compared to control group.

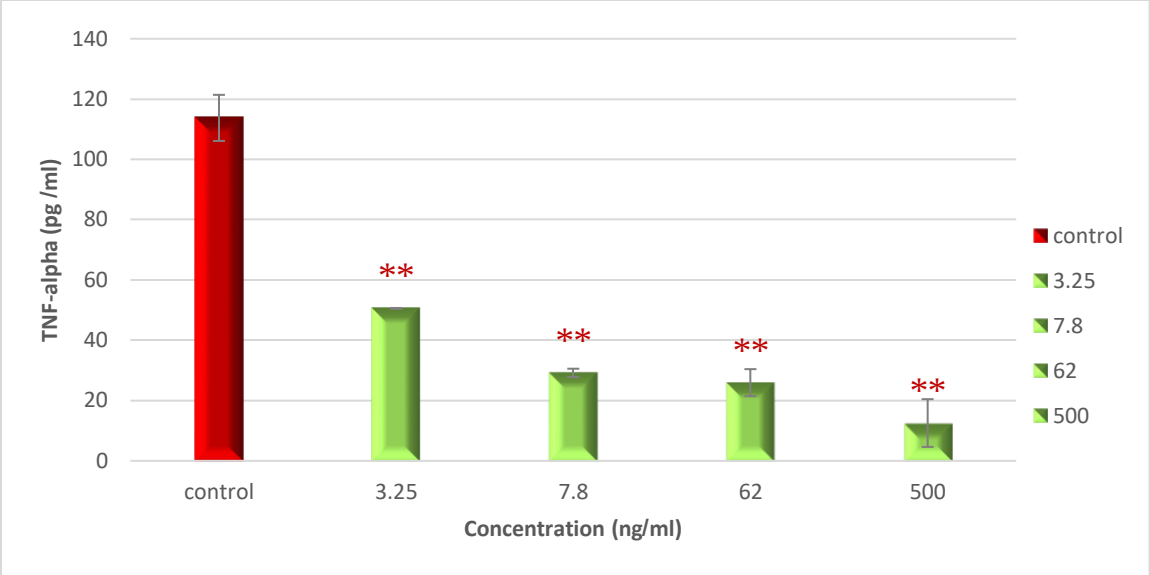


Figure 3.8. The effects of testosterone on TNF-alpha in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. control (untreated cells)

3.2.1.3 Effects of testosterone on total anti-oxidant capacity in LNCap cells

The cells were treated with serial concentrations (3.9, 7.8, 62.5, 500) ng/ml of testosterone and incubated for 48 hours at 37 C, then the effects of testosterone on total antioxidant capacity TAC were determined.

Results showed a highly significant ($p < 0.001$) increase in total antioxidant capacity (TAC) at concentrations (500, 62.5) ng/ml of testosterone with no significant effects at concentrations (7.8, 3.9) compared to control group. Results are shown in figure (3.4).

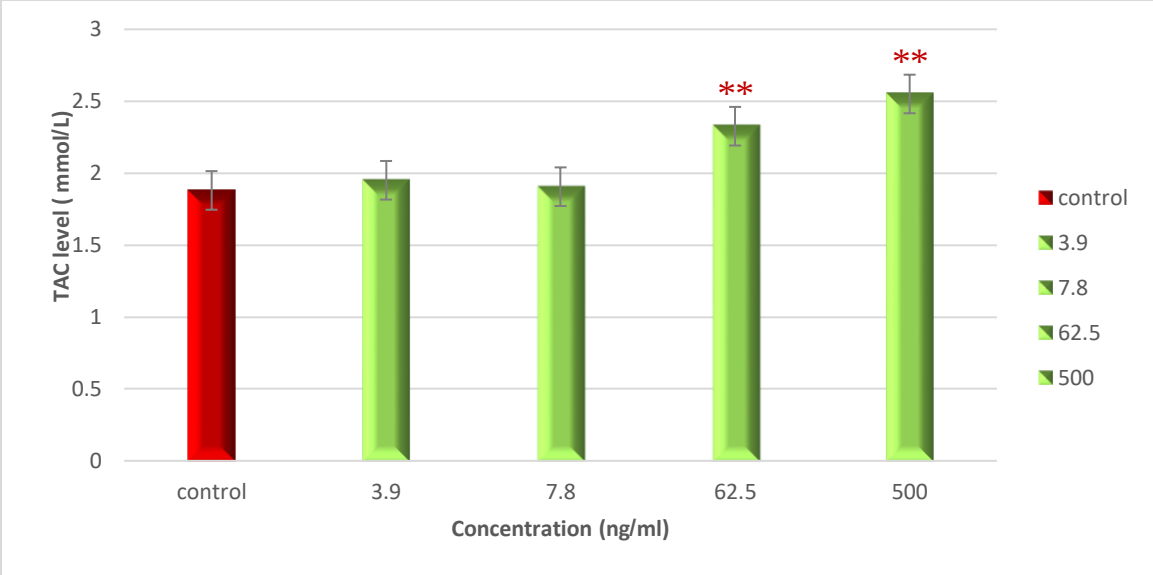


Figure 3.9. The effects of Testosterone on total anti-oxidant capacity (TAC) in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. control (untreated cells)

3.2.2 Apoptotic, Anti-inflammatory, and Antioxidant effects of dutasteride in LNCAP cell line.

3.2.2.1 Effects of dutasteride on caspase 3 marker in LNCap cell line

The cells were treated with serial concentrations (3.125, 6.25, 25, 50) µg/ml of dutasteride in the presence constant concentration of testosterone (7.8 ng/ml) and incubated for 48 hours at 37 C, then the effects of dutasteride on caspase 3 level were determined. Results showed a highly significant ($p < 0.001$) increase in caspase 3 at all concentrations compared to control group. Results are shown in figure (3.6).

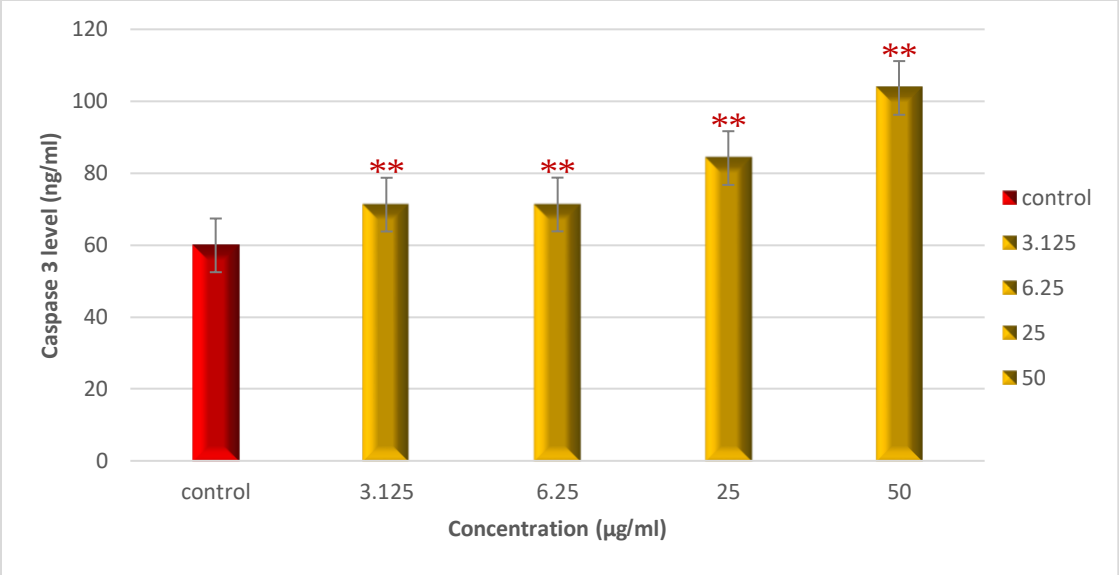


Figure 3.10. The effects of dutasteride on caspase 3 in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. control (untreated cells)

3.2.2.2 Effects of dutasteride on TNF- alpha in LNCap cell line

LNCap cells were treated with serial concentrations (3.125, 6.25, 25, 50) µg/ml of dutasteride in the presence constant concentration of testosterone (7.8 ng/ml) and incubated for 48 hours at 37 C, then the effects of dutasteride on TNF-alpha were determined. Results showed a highly significant ($p < 0.001$) decrease in TNF-alpha at all concentrations compared to control group. Results are shown in figure (3.7).

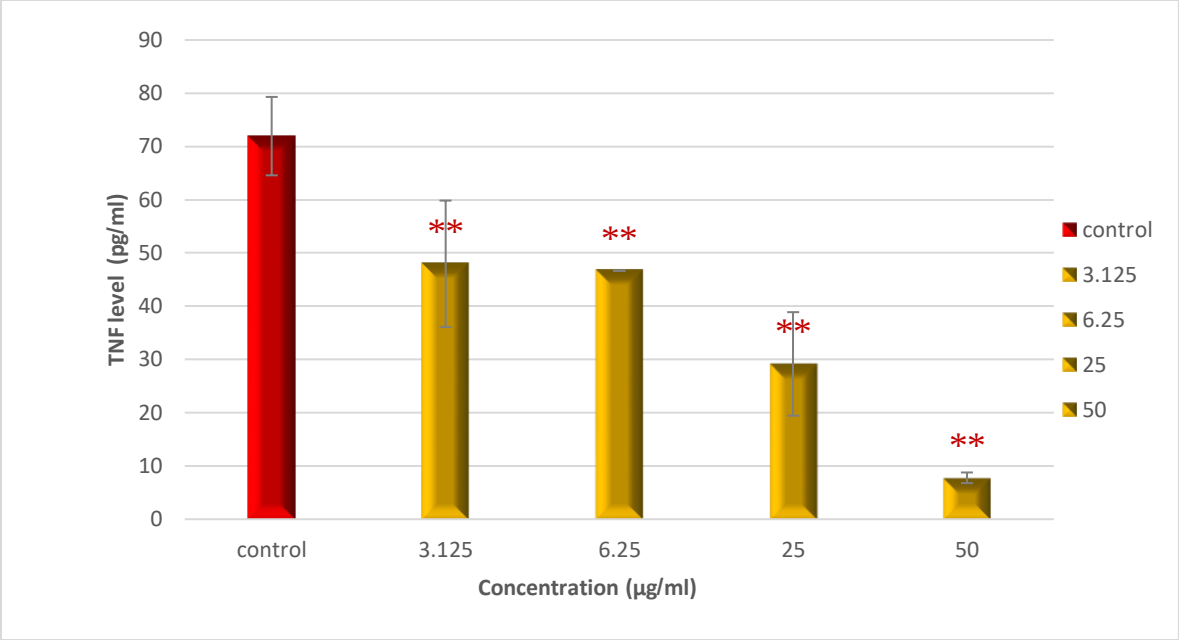


Figure 3.11. The effects of dutasteride on TNF-alpha in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. control (untreated cells)

3.2.2.3 Effects of dutasteride on total anti-oxidant capacity in LNCap cell line

The cells were treated with serial concentrations (3.125, 6.25, 25, 50) µg/ml of dutasteride in the presence constant concentration of testosterone (7.8 ng/ml) and incubated for 48 hours at 37 C, then the effects of dutasteride on TAC level were determined. Results showed a highly significant ($p < 0.001$) increase in total antioxidant capacity (TAC) at all concentrations of dutasteride compared to control group. Results are shown in figure (3.8).

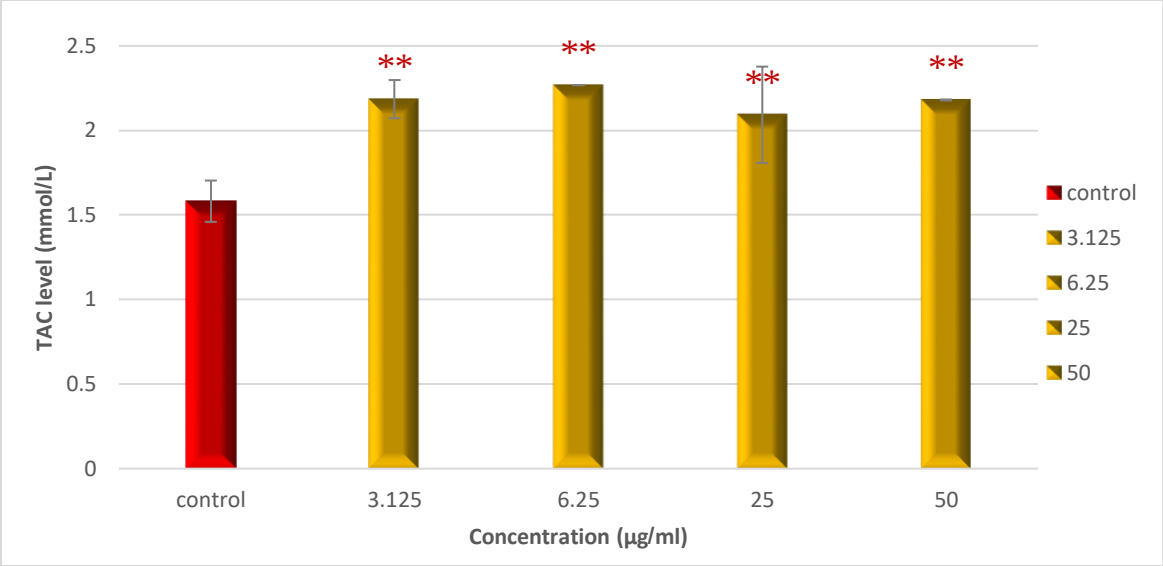


Figure 3.12. The effects of dutasteride on Total antioxidant capacity (TAC) in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. control (untreated cells)

3.2.3 Apoptotic, Anti-inflammatory, and Antioxidant effects of different concentrations of dutasteride combined with doxorubicin IC50 in LNCap cell line

3.2.3.1 Effects of (dutasteride-doxorubicin) combination on caspase3 marker in LNCap cell line

Depending on MTT results that mentioned in fig (3.11), the LNCap cells were treated with serial concentrations of dutasteride (75, 18.75, 9.37) µg/ml in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (3 µg/ml) of doxorubicin for 48 hours at 37 C, then the supernatants were withdrawn from each well and used for detection caspase 3 using Elisa kit. Results in figure (3.12) are shown a highly significant ($p < 0.001$) increase in caspase 3 at all concentrations compared to positive and negative control group.

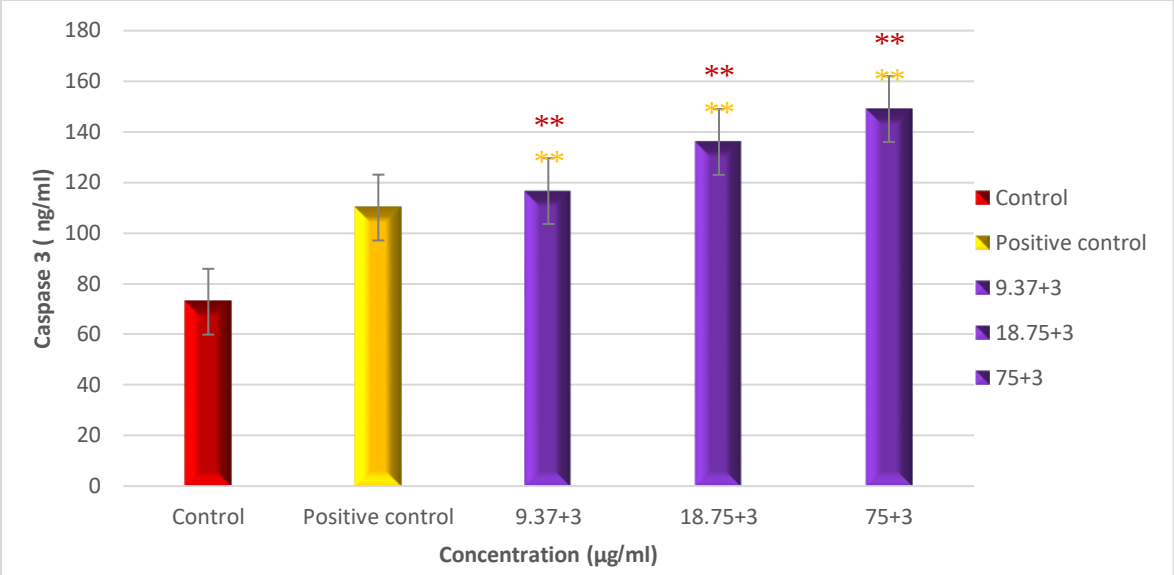


Figure 3.13. Effects of (dutasteride-doxorubicin) Combination on caspase 3 in LNCap cell line. ** means (*P*<0.001) for highly significant difference between concentrations. Positive control (cells treated with doxorubicin IC50 3µg/ml), control (untreated cells)

3.2.3.2 Effects of (dutasteride-doxorubicin) combination on TNF- alpha in LNCap cell line

Depending on MTT results that mentioned in fig (3.11), the LNCap cells were treated with serial concentrations of dutasteride (75, 18.75, 9.37) µg/ml in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (3 µg/ml) of doxorubicin for 48 hours at 37 C, then the supernatants were withdrawn from each well and used for detection TNF – alpha using Elisa kit. Results in figure (3.13) are showed a highly significant ($p < 0.001$) decrease in TNF-alpha at all concentrations compared to positive control group (3 µg/ml). Results also showed a highly significant ($p < 0.001$) decrease in TNF-alpha at concentration 75:3 with significant ($p < 0.05$) decrease at other concentrations compared with negative control.

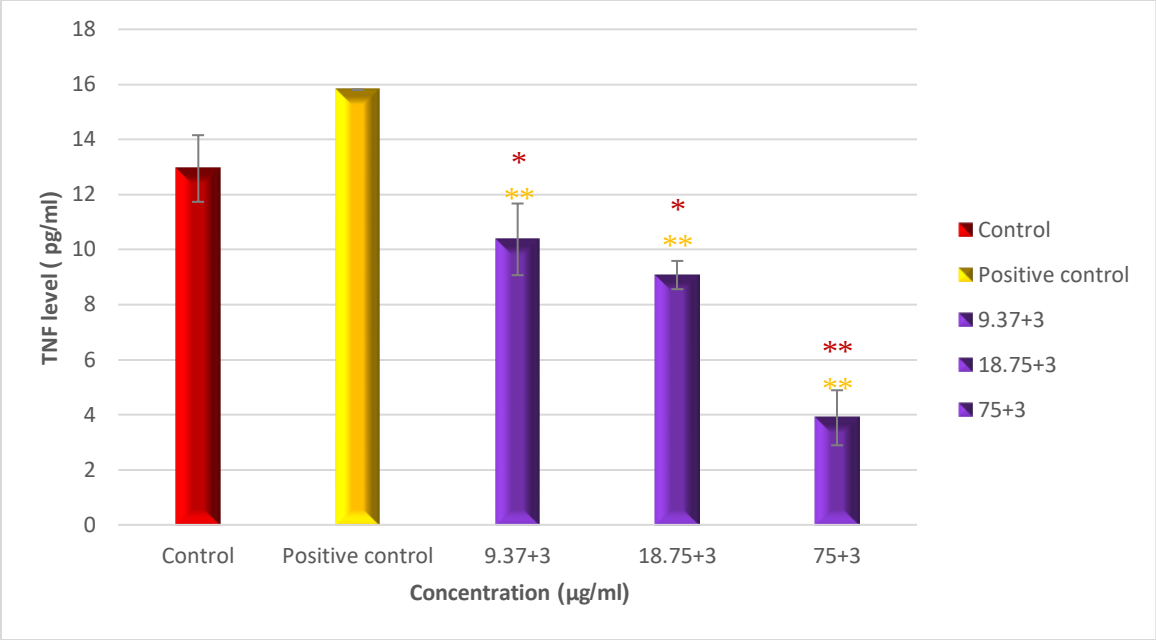


Figure 3.14. Effects of (dutasteride-doxorubicin) Combination on TNF-alpha in LNCap cell line. * means (P<0.05) and ** means (*P*<0.001) for significant and highly significant difference between concentrations. Positive control (cells treated with doxorubicin IC50 3µg/ml), control (untreated cells)

3.2.3.3 Effects of (dutasteride-doxorubicin) combination on total anti-oxidant capacity in LNCap cell line

Depending on MTT results that mentioned in fig (3.11), the LNCap cells were treated with serial concentrations of dutasteride (75, 18.75, 9.37) µg/ml in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (3 µg/ml) of doxorubicin for 48 hours at 37 C, then the supernatants were withdrawn from each well and used for detection total anti-oxidant capacity (TAC) using Elisa kit. Results in figure (3.14) are showed no significant effects on TAC level at all concentrations compared to positive control group (3 µg/ml). Results also showed a highly significant ($p < 0.001$) decrease in TAC at all concentrations compared with negative control.

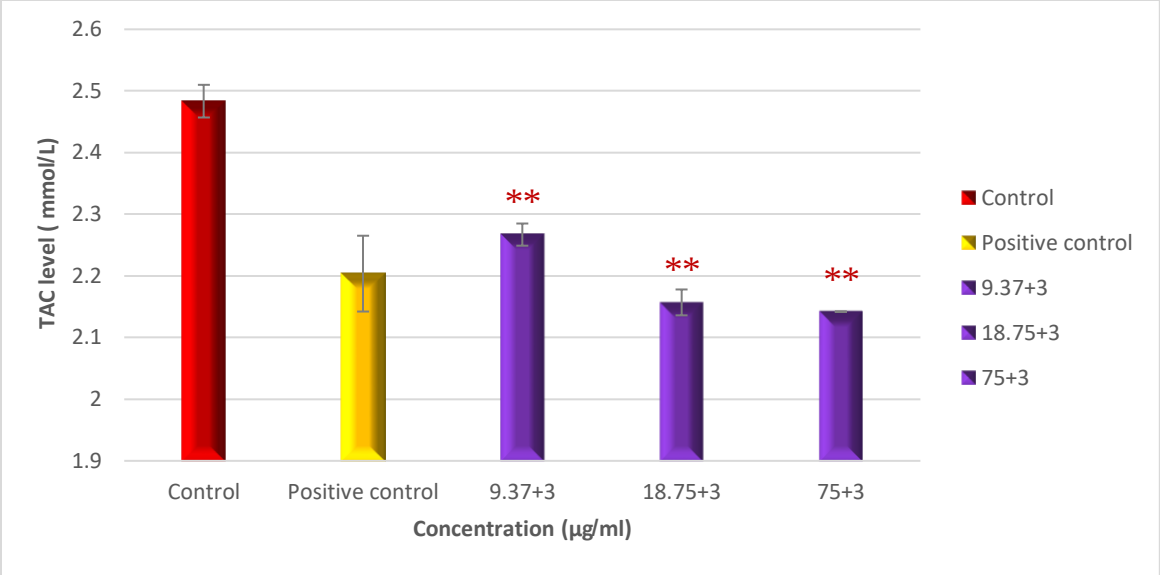


Figure 3.15. Effects of (dutasteride-doxorubicin) Combination on Total antioxidant capacity (TAC) in LNCap cell line ** means (P<0.001) for highly significant difference between concentrations. Positive control (cells treated with doxorubicin IC50 3µg/ml), control (untreated cells)

3.2.4 Apoptotic, Anti-inflammatory, and Antioxidant effects of different concentrations of dutasteride combined with IC50 of 5FU in LNCap cell line

3.2.4.1 Effects of (dutasteride-5FU) combination on caspase 3 marker in LNCap cell line

Depending on MTT results that mentioned in fig (3.15), the LNCap cells were treated with serial concentrations of dutasteride (75, 18.75, 9.37) µg/ml in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (30 µg/ml) of 5FU for 48 hours at 37 C, then the supernatants were withdrawn from each well and used for detection caspase 3 using Elisa kit. Results in figure (3.16) are shown a highly significant ($p < 0.001$) decrease in caspase 3 at all concentration compared to positive control group (30 µg/ml). Results also showed ($p < 0.001$)

significant increase in caspase 3 at all concentrations compared with negative control.

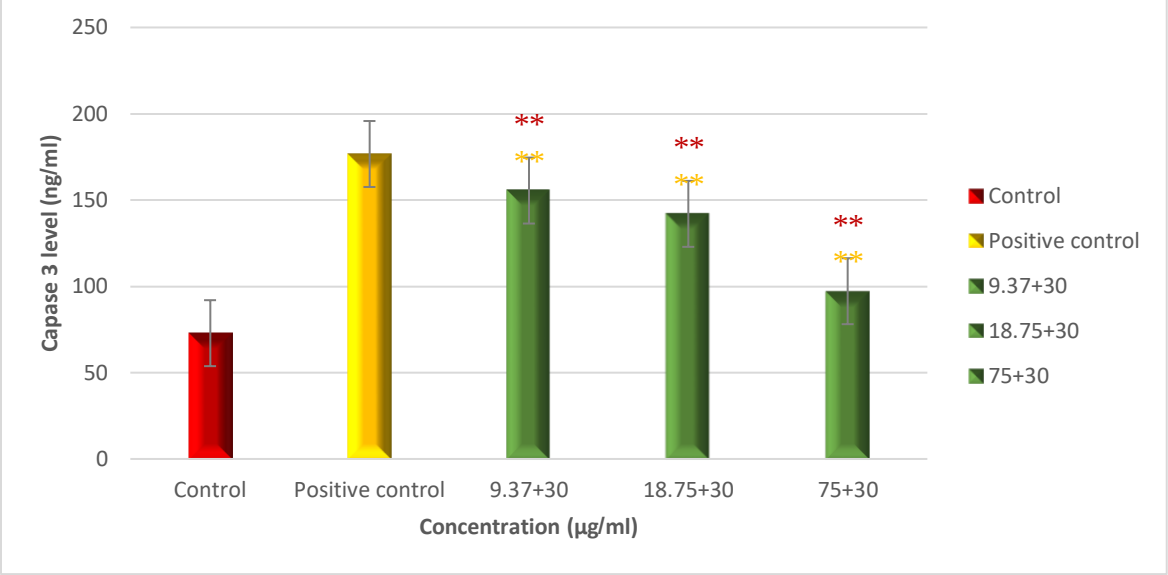


Figure 3.16. Effects of (dutasteride-5FU) on caspase 3 in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. Positive control (cells treated with IC50 of 5FU 30µg/ml), control (untreated cells)

3.2.4.2 Effects of (dutasteride-5FU) combination on TNF-alpha in LNCap cell line

Depending on MTT results that mentioned in fig (3.15), the LNCap cells were treated with serial concentrations of dutasteride (75, 18.75, 9.37) µg/ml in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (30 µg/ml) of 5FU for 48 hours at 37 C, then the supernatants were withdrawn from each well and used for detection TNF – alpha using Elisa kit. Results in figure (3.17) are shown a highly significant ($p < 0.001$) decrease in TNF-alpha at all concentrations compared to positive control group (30 µg/ml). Results also showed a highly significant (p

< 0.001) decrease in TNF-alpha at all concentrations of dutasteride and 5FU compared with negative control.

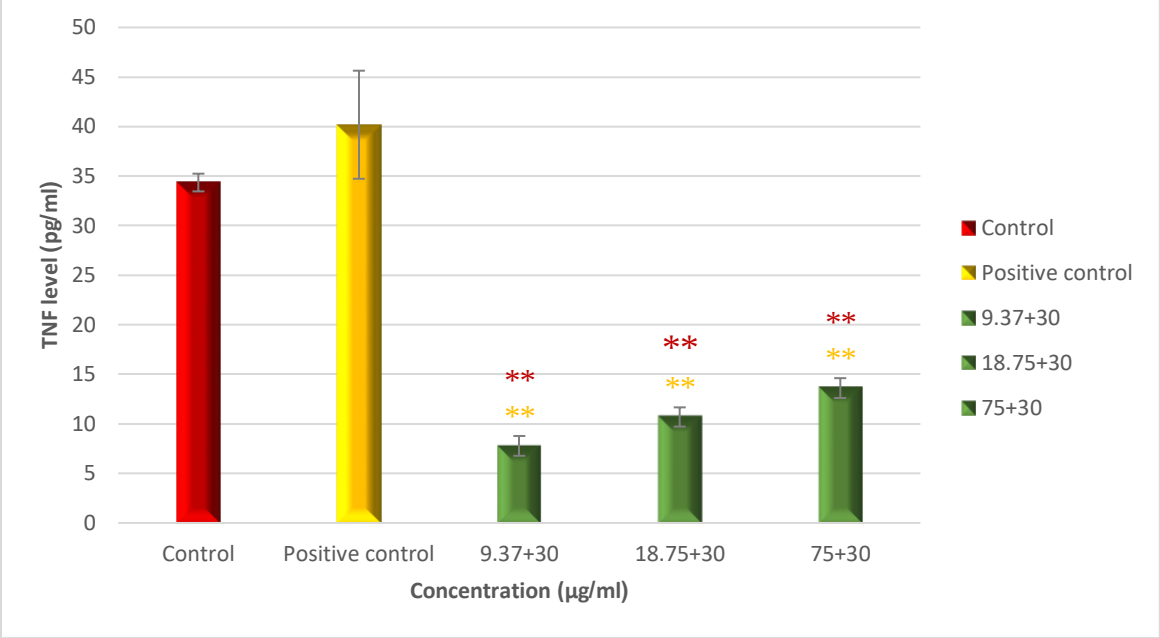


Figure 3.17. Effects of (dutasteride-5FU) on TNF- alpha in LNCap cell line. ** means (P<0.001) for highly significant difference between concentrations. Positive control (cells treated with IC50 of 5FU 30µg/ml), control (untreated cells)

3.2.4.3 Effects of (dutasteride-5FU) combination on total anti-oxidant capacity in LNCap cell line

Depending on MTT results that mentioned in fig (3.15), the LNCap cells were treated with serial concentrations of dutasteride (75, 18.75, 9.37) µg/ml in the presence of constant concentration of testosterone (7.8ng/ml) and constant IC50 concentration (30 µg/ml) of 5FU for 48 hours at 37 C, then the supernatants were withdrawn from each well and used for detection total anti-oxidant capacity (TAC) using Elisa kit. Results in figure (3.18) are shown a significant (p < 0.05) decrease in TAC at concentration 9.37 µg/ml with no significant effects at other concentrations compared to

positive control group (30 µg/ml). Results also showed a highly significant ($p < 0.001$) decrease in TAC at all concentrations of dutasteride and 5FU compared with negative control.

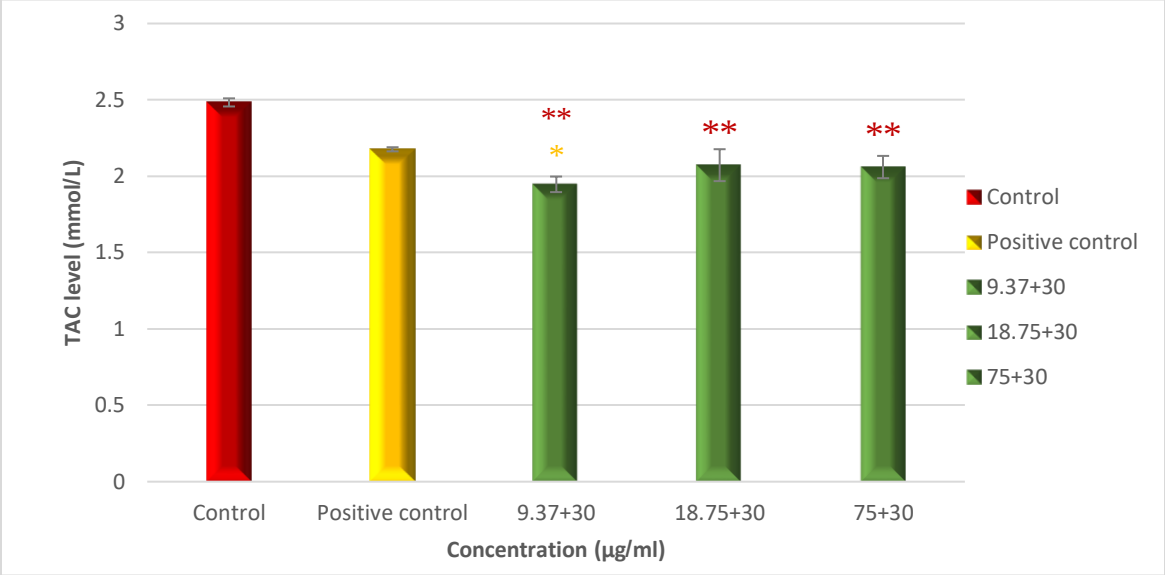


Figure 3.18. Effects of (dutasteride-5FU) on total antioxidant capacity (TAC) in LNCap cell line. * means ($P<0.05$) and ** means ($P<0.001$) for significant and highly significant difference between concentrations. Positive control (cells treated with IC50 of 5FU 30µg/ml), control (untreated cells)

Chapter Four

Discussion

4.1 Testosterone effects on LNCap prostate cancer cells

A) Effects on cell viability and apoptosis of LNCap cells

According to many reports, various illnesses, including prostate cancer, depends heavily on androgen receptor signaling. As a result, this study tried to figure out how higher and lower levels of androgen affect AR signaling. The results in Fig (3.1) showed multiphasic effects of testosterone on the viability of the cells, which was significantly increasing at low concentrations and declined at higher concentrations no effects in between. These results may have been explained depending on the androgen saturation model theory. AR in human prostate tissue is saturated when it approaches 120 ng/dl (or 12 ng/ml) in vitro, which is comparable to roughly 240-250 ng/dl (or 24-25 ng/ml) in vivo, according to Traish et al's findings, which demonstrated that the maximal amount of testosterone binding to AR occurs at a relatively low androgen concentration (Traish *et al.*, 1988). Morgentaler's research on androgen saturation complements Traish's work on testosterone. It claims that a narrow range of testosterone levels can affect PCa in this situation because PCa is sensitive to low testosterone levels and excessive testosterone cannot access the nucleus to encourage cell development after ARs have become completely occupied (Morgentaler, 2006).

Another study by (Siciliano *et al.*, 2022) showed that exposing the LNCap to different concentrations of R1881 (a potent synthetic androgen) increased cell viability in a concentration-dependent manner, with the best cell viability observed at low concentrations and the lowest cell viability at a high concentration. Also, another

study found that while normal and high levels of testosterone can suppress the proliferation of PCa cells, physiologically low amounts are necessary for PCa cell growth (Song and Khera, 2014). This explains why androgen deprivation treatment patients may experience a cancer remission followed by a recurrence (Chuu *et al.*, 2011).

Different studies have linked low and high levels of testosterone to the AR transcriptional activity and the target genes. At low concentration, testosterone stimulate the androgen receptor signaling pathway in LNCap cells which then activate transcriptional gens leading to an increase the cell division. This stimulation is dose-dependent and stopped when androgen levels were increased. (Nyquist *et al.*, 2019). On the other hand, some studies showed that the supraphysiological level of testosterone induces cell growth inhibition due to several mechanisms involved: Cell cycle arrest, disruption of AR-mediated DNA licensing, DNA damage, transcriptional reprogramming, transcriptional repression of AR, cellular senescence or quiescence, activation of apoptosis, and transcriptional repression of A (Chatterjee *et al.*, 2019). In the present study high level of testosterone was associated with a significant increase in caspase 3 level at high concentrations and a highly significant decreasing in caspase 3 levels at low concentrations. This may be another mechanism by which testosterone decreases the LNCap cells' proliferation by inducing apoptosis. Androgens could trigger apoptosis and Apoptosis can be sensitized or triggered in prostate cancer cells by upregulating the proapoptotic Bcl-2 family protein Bax or downregulating the

antiapoptotic Bcl-2 family members Bcl-2 and Bcl-xL. In the intrinsic apoptotic pathway, the multidomain proapoptotic protein Bax is essential. According to Lin et al study androgen receptors may promote endogenous Bax's translocation to mitochondria and contribute to cell death (Lin *et al.*, 2006). Therefore, testosterone in the present study may activate androgen receptor and causes apoptosis according to Lin et al study.

On the other hand, Lorenzo and Saatcioglu demonstrated that testosterone may guard the LNCap against apoptosis by inhibiting c-Jun N-terminal kinase activation. In eukaryotic cells, one of the most common signal transduction routes is mediated by mitogen-activated protein kinases (MAPKs). JNK, also known as stress-activated protein kinase, is one of the major MAPKs and is recognized to be essential for a variety of cellular processes, including cell proliferation and programmed cell death (Lorenzo and Saatcioglu, 2008). In Lorenzo and Saatcioglu study, the LNCap was exposed to different proapoptotic stimuli that induce apoptosis due to activation of the JNK pathway. After that, the cells were exposed to R1881 a synthetic androgen. Treatment with low doses of R1881 reduced the mortality of LNCaP cells by almost 50%. Because R1881 did not alter cell proliferation in these experimental settings, the increase in cell viability in the presence of R1881 was instead caused by a decrease in cell death. Additionally, these findings were supported by the observation that the anti-androgen bicalutamide prevented the androgen-mediated suppression of JNK activation, demonstrating the necessity of AR-dependent transcriptional activation for this function. According to these findings, androgen receptor activation

may prevent cell death, particularly in low doses, and improve cell survival in LNCap.

B) Effects of testosterone on proinflammatory cytokines (TNF alpha)

Inflammation continues to be a key factor in prostatic disorders and may help raise the scales in favor of tumor cell proliferation. In our study, the results in Fig (3.3) showed a highly significant decrease in TNF-alpha compared with the control. Some studies showed that testosterone treatment lowers these biomarker levels. According to Kalinchenko et al., testosterone treatment for 30 weeks in 113 hypogonadal males reduced IL-1, TNF- alpha, and C-reactive protein (CRP) levels, but did not affect IL-6 and IL-10 levels (Kalinchenko *et al.*, 2010). Another study by Malkin et al. demonstrated that testosterone treatment in hypogonadal men resulted in reductions in TNF-α and IL-1β, and an increase in IL-10 (Malkin *et al.*, 2004). These studies accepted with our study but the specific mechanism by which testosterone decreased TNF alpha is still unknown and further investigations are needed.

C) Effects of testosterone on total antioxidant capacity(TAC)

In our study, testosterone showed a highly significant increase in total antioxidant capacity at high concentrations of testosterone with no significant effects at low concentrations. Some studies linked testosterone with total antioxidant capacity. Demirbag, Yilmaz, and Erel assessed TAC in men with low and normal testosterone levels, as well as in menopausal and premenopausal women, and discovered that the male study group had greater levels of TAC than the female control group did. TAC levels were also

lower in the male group with reduced testosterone than in the male control group. In the lower testosterone male and female menopausal groups, there were no discernible variations in TAC levels. In male groups, there was shown to be a significant positive association between TAC and total testosterone (Demirbag, Yilmaz and Erel, 2005). These studies are compatible with our study where TAC was elevated at high concentrations of testosterone and no significant effects at low concentrations.

Another study by (Chodari *et al.*, 2019) examined the effects of testosterone and voluntary exercise, either separately or in combination, on oxidative stress in diabetic rats' blood and heart. superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) activities were considerably elevated in the groups treated with either testosterone or exercise as a result of these treatments. The molecular processes behind the rise in phosphorylation and activation of Nrf-2 (nuclear factor erythroid 2-related factor 2, an emerging regulator of cellular resistance to oxidants), an emerging regulator of SOD, GPX, and CAT enzyme activity, may be directly connected. To trigger gene transcription, activated Nrf2 interacts with the antioxidant response elements (ARE). Interestingly, the activity of the SIRT1 gene (silent mating type information regulation homolog), which directly controls the cellular stress response, was raised by both testosterone and exercise. Additionally, it enhanced the ability of antioxidants and stopped the cell cycle to aid in DNA repair caused by oxidative stress. Depending on this study, testosterone in high concentrations may activate the Nrf-2 in LNCap cells and increase the production of

anti-oxidants that lead to increase the level of total anti-oxidant capacity.

4.2 Dutasteride effects on LNCap prostate cancer cells

A) Effects of dutasteride on cell viability and apoptosis in LNCap

Dutasteride showed a dose-dependent inhibition of cell viability with a highly significant decrease at high concentrations. These results agreed with Lazier et al who showed that dutasteride can inhibit the conversion of testosterone to dihydrotestosterone by more than 99% in LNCap cells (Lazier *et al.*, 2004) by blocking both types of 5-alpha reductase enzyme which is already present in LNCap cells (Zhu *et al.*, 2003). Also, these results were agreed with the study of (Schmidt, Murillo and Tindall, 2004). In this study, LNCap were exposed to different concentrations of dutasteride, and the inhibition was noted after 48 hrs in the presence of DHT. Schmidt et al in their study showed that dutasteride was inhibit the proliferation of LNCap not only by preventing T from becoming DHT but also dutasteride was up-regulated 2 genes UGT2B15 and UGT2B17, these UDP-glucuronosyltransferases can inactivate the DHT or its metabolites and this may be considered another mechanism by which dutasteride has inhibited the proliferation of cells and these effects were consistent with our results due to dutasteride was inhibit the proliferation of cells in spite of the presence of testosterone in media, also these effects consistent with (Lazier *et al.*, 2004) where the proliferation rate of LNCap cells was stilled dramatically decreased after exposure to dutasteride combined with exogenous DHT. Depending on these results, dutasteride may

have a benefit in prostate cancer, especially in earlier stages when cancer is still sensitive to androgens.

Apoptosis is one of the important mechanisms by which can kill cancer cells. Several treatments were developed to target this pathway. In this study, dutasteride showed a decrease in the viability of LNCap cells and this accompanied with a highly significant increasing in caspase 3 at all concentrations of dutasteride. This indicated that dutasteride may interfere with apoptotic pathways of LNCap cells and lead to caspase 3 elevation. This results agree with (Golbano *et al.*, 2008) study. In this study, Golbano *et al* used finasteride which is another alpha- reductase inhibitor and, also blocks both types of the enzyme but with more selectivity to type1. LNcap cells in this study were incubated with different concentrations of finasteride for 4 days. After incubation, the procaspase 3 (which is an inactive form of caspase 3 and converted to caspase 3 after proteolytical activation) was measured. Finasteride was showed a significant increase in caspase 3. Therefore, finasteride induces apoptosis in LNCaP cells by activating caspases. Also, finasteride showed a significant descent in expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL with a significant increase in the expression of pro-apoptotic proteins Bax ratios and this may be indicated that finasteride may increase caspase3 due to the intrinsic pathway of apoptosis. Regarding our study, dutasteride showed a significant increase in caspase 3 after only 48 hr. We suggest that dutasteride may increase caspase 3 in mechanism similar to finasteride but in time less than finasteride because dutasteride was considered more potent and highly selective to both

types of alpha redutase than finasteride. Also, some studies showed that DHT may inhibit apoptosis in LNCap cells (Berchem *et al.*, 1995)(Rokhlin *et al.*, 2005). DHT may shield LNCap cells against radiation- and etoposide-induced apoptosis, according to prior investigations (Coffey *et al.*, 2002). In these experiments, LNCaP was pre-treated with DHT and decreased levels of several apoptosis regulators including caspase-3 were found. Depending on these studies and depending on dutasteride is dual 5-alpha reductase inhibitor and prevent the conversion of T to DHT which leads to decreased intracellular amounts of DHT, we suggest that dutasteride may induce apoptosis by affecting DHT- apoptosis inhibition activity.

B) Effects of dutasteride on TNF alpha in LNCap

Several studies showed that TNF alpha was increased in PCa patients (Ali, Al-Rubaei et al., 2020). Likewise, Pro-inflammatory cytokines (especially TNF alpha) levels were correlated with prostate cancer. TNF alpha may be connected to disease progression, with levels being lowest in males in good health, greatest in individuals with bulky locally advanced PCa, and highest in those with metastatic disease, according to some research. (Michalaki *et al.*, 2004). TNF was found to inhibit dihydrotestosterone (DHT)-induced proliferation of LNCaP cells and dose-dependent reduce the expression of the androgen receptor (AR), suggesting that TNF may play a role in the beginning of an androgen-independent state in these cells (Mizokami *et al.*, 2000).

Depending on these studies, we suggested that any drug may have a role in decreasing TNF alpha and may be contributed in

reducing or even limiting the progression and initiation of prostate cancer. In the present study, dutasteride showed a significant decrease in TNF levels at all concentrations of dutasteride. This indicated that dutasteride has anti-inflammatory effects on LNCap by reducing TNF levels. The definitive mechanism by which dutasteride has lowered the TNF alpha was unclear in other studies and the recent study was the first study that examined dutasteride as anti-inflammatory on LNCap cells. But regarding to study of Zhao et al we explained this effect. In this study, they made a surgical prostatic wound in experimental canines and focused on the effects of androgens and inflammation in the presence of finasteride. They found after 1-2 weeks the macrophages and concentration of TNF alpha in the testosterone group more than the finasteride group at the site of the wound. Also after adding a small dose of DHT the TNF alpha was increased. They explained that DHT can upregulate the TNF alpha during activation AR on activated macrophage (Zhao *et al.*, 2018). Based on these results, DHT may act on AR that is present in LNCap cells and increase TNF alpha from cells (as we know TNF alpha is released from cancer cells, macrophages, and other cells of the immune system). Therefore, blocking alpha-reductase enzyme by dutasteride and reducing DHT in the prostate may be contributed to decreasing the level of TNF which consider one of the important activators of the NF-κB pathway that is already activated in cancer cells. Also, these results were confirmed by (Sudeep *et al.*, 2019) who found subcutaneous testosterone administration in mice activated the NF-κB pathway in prostate cells and the levels of TNF with other cytokines were elevated.

C) Effects of dutasteride on oxidative stress in LNCap

Many therapies may possess antioxidant properties or cause oxidative stress in cancer cells. In this study, dutasteride showed a highly significant increase in TAC at all concentrations compared with the control. These results mean that dutasteride may have antioxidant activity in these cells. No previous studies showed the effects of dutasteride on this type of the cells. But we can explaine these results depending on the study of (Ripple *et al.*, 1997). In this study, the LNCaP cells and DU145 cells were treated with 5α-dihydrotestosterone (DHT) and R1881 and the effect of androgens on oxidative state parameters such as hydroxyl radicals and hydrogen peroxide production, oxygen consumption, and lipid peroxidation were assessed, also antioxidant parameters such as catalase and glutathione were measured. The results were DHT and R1881 effectively induced oxidative stress in LNCaP cells. DHT and R1881, in contrast, showed no impact on oxidative stress in DU145 cells. DHT considerably increased the levels of mitochondrial activity in LNCaP cells over those of the untreated controls, as determined by MTT. Based on these findings, it was shown that androgen-responsive LNCaP prostate cancer cells are susceptible to increased oxidative stress caused by physiological amounts of androgens. The research indicates that enhanced mitochondrial activity is partially responsible for this outcome. The activity of certain detoxification enzymes, such as glutamyl transpeptidase, which is crucial for maintaining the cellular prooxidant-antioxidant balance, as well as intracellular glutathione levels are also affected by androgens. Depending on this study we can predict that

dutasteride may reverse these effects of androgens in LNCap by blocking the 5-alpha reductase enzyme and preventing the conversion of T to DHT which leads to reduced intraprostatic DHT and decreases its ability to produce oxidative stress in this type of cells. On the other hand, we can suggest that dutasteride blocks the conversion of T to DHT and this may lead to increasing in testosterone level in media. Testosterone during our study showed increasing in TAC level Fig (3.4) in LNCap cells by the mechanism previously explained. This suggestion might be another mechanism that explained the anti-oxidant effects of dutasteride.

4.3 Effects of (Dutasteride- doxorubicin) combination on LNCap prostate cancer cells

A) Effects of Dutasteride- doxorubicin on cell viability and apoptosis in LNCap

The results showed a highly significant decrease in cell viability compared with positive control and negative control. Doxorubicin and other anthracyclines primarily work by inducing a DNA damage response (DDR), which then initiates an apoptotic pathway to destroy proliferating cells like cancer cells. Lin et al showed in their study that doxorubicin caused p53 overexpression in LNCap cells. In general, when DNA is exposed to any type of damage the p53 will activate and tried to repair this damage. If the repairing process failed, the p53 will trigger the cell to apoptosis by activating the intrinsic apoptotic mechanisms that leads to activating caspase cascades which eventually activate caspase 3 and induce cell death (Lin *et al.*, 2018). Other study by (Yang *et al.*, 2016) evaluated

the apoptotic response to doxorubicin between LNCaP and PC3 cells. Based on the minimal dose of doxorubicin required to activate caspase 3, the outcomes showed that doxorubicin promotes apoptosis in LNCaP cells at very low concentrations. In PC3 cells, doxorubicin can similarly activate caspase 3, but it does so at a 4- to 8-fold greater dose than it can in LNCaP. They were informed that Bcl-xl, an anti-apoptotic protein, is present in PC3 cells at a level that is around five times greater than that of LNCaP cells and the primary distinction between the two cells was this elevation. Additionally, they demonstrated that the sole protein capable of inhibiting Bcl-xl activity in response to doxorubicin-induced apoptosis in LNCaP cells is Bim (pro-apoptotic protein). Depending on these studies, dutasteride (which also increased caspase 3 in LNCap by mechanisms shown above) was combined with doxorubicin and give synergistic effects on LNCap cells viability due to apoptotic pathway.

B) Effects of dutasteride -doxorubicin on TNF-alpha in LNCap

In a current study, dutasteride showed a highly significant decrease in TNF-alpha at all concentrations compared to the positive control group. Several studies demonstrated that chemotherapy activated p38 mitogen-activated protein kinase (p38 MAPK) in cells. Activation of p38 MAPK mediated release of pro-inflammatory cytokines which is associated with fatigue similar to sickness behavior that occurs during chemotherapy use (Wood, 2007). Also, chemotherapy can activate p38 MAPK in tumor cells as a part of its cytotoxic activity on these cells (Boldt, Weidle and Kolch, 2002).

Elsea *et al* showed that doxorubicin and other chemotherapies activate p38 MAPK in model cell systems. This activation was associated with increasing IL-6, TNF alpha, and other cytokines. On other hand inhibition of p38 MAPK caused IL-6, TNF alpha and other cytokines to lower without affecting on cytotoxic effects of chemotherapies (Elsea *et al.*, 2008). Depending on these studies we can suggest that doxorubicin may activate p38 MAPK in LNCap as a part of its cytotoxic effects and result in TNF alpha elevation. But when combined with dutasteride which showed anti-inflamatory effects during this study by decreasing TNF alpha, this resulted in decreasing of inflammatory effects of doxorubicin in dose-dependent manner without effect on doxorubicin cytotoxicity (caspase 3 was high and cell viability was low). Therefore, combination dutasteride with doxorubicin might contribut in decrease the inflammatory effects of doxorubicin depending on our results.

C) Effects of dutasteride- doxorubicin on oxidative stress in LNCap

The outcomes showed no considerable differences in TAC level at all concentrations compared with the positive control, also the findings revealed a very large drop in TAC at all concentrations of dutasteride compared with negative control. According to certain researches, anthracycline anti-cancer medications may increase the amount of hydrogen peroxide produced inside the cells. Doxorubicin converted to a semiquinone by metabolic reductive activation increases the one-electron reduction of oxygen to produce superoxide. Superoxide dismutase (SOD) enzymes or spontaneous dismutation of the resultant superoxide produces H2O2, which may

be the species causing the cytotoxicity of doxorubicin as illustrated below(Kalyanaraman, 2020).

$$\text{Doxorubicin(Dox)} \rightarrow \text{semiquinone}^{\bullet -} \quad (1)$$

$$\text{semiquinone}^{\cdot -} + O_2 \rightarrow \text{Dox} + O_2^{\cdot -} \quad (2)$$

$$O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 \quad (3)$$

Also, study by Wagner *et al* demonstrated an increase in intracellular hydrogen peroxide is a particular oxidative event related to anthracycline exposure in PC3 human prostate cancer cells (Wagner *et al.*, 2005). Our results accepted with this study when doxorubicin (positive control) showed decreasing in TAC level compared with negative control this is due to the ability of doxorubicin to produce free radicals inside cells. Also, recent studies showed that cardiotoxicity associated with doxorubicin resulted from doxorubicin's ability to produce free radicals (Kong *et al.*, 2022). During our study, a combination of dutasteride (which was shown strong anti-oxidant activity by increasing TAC level) with doxorubicin didn’t show any significant effects on the oxidative effects of doxorubicin. On the other hand, the combination of doxorubicin with dutasteride decreases the anti-oxidant effects of dutasteride when compared with negative control. This may be indicated that doxorubicin is a potent oxidative agent and this makes it a potent anticancer agent.

4.4 Effects of (dutasteride -5FU) combination on LNCap prostate cancer cells

A) Effects of different concentrations of dutasteride with 5FU on cell viability and apoptosis in LNCap

Kawabata et al demonstrated in their research that prostate cancer cells may employ IGF1 (insulin-like growth factor 1) instead of DHT to grow, but upregulation of IGF-binding protein 3 (IGFBP3) was shown to prevent these effects. By using the prostate cancer cell lines LNCaP, 22Rv1, DU145, and PC3, they looked into the synergistic mechanism behind the combination anti-tumor action of anti-androgens (bicalutamide) and 5-FU and found that the addition of 5-FU was boosted IGF-binding protein 3 (IGFBP3) expression. These findings imply that the 5-FU-induced up-regulation of IGFBP3 is crucial in enhancing the anticancer effects of 5-FU in combination with anti-androgens (Kawabata *et al.*, 2011). These results were accepted with our study due to we used dutasteride which is a potent and irreversible 5-alpha-reductase inhibitor and stop the conversion of T to DHT and was reduced the viability of LNCap cells (which are androgen-sensitive) when used alone during this study. But when dutasteride was combined with 5FU there was an addative effect on LNCap viability. Depending on these effects we can suggest that 5FU may potentiate the effects of dutasteride in two ways: first, by acting as anti-tumor and preventimg the functioning of thymidylate synthase, which thereby prevents the production of DNA and at the same time alters the normal processing and function of RNA when it is integrated into

DNA and RNA. Second, by up-regulate IGF-binding protein 3 (IGFBP3) in LNCap cells and prevent the action of IGF1 and inhibits cell growth and potentiated anti androgenic effects of dutasteride depending on Kawabata *et a*l study.

Furthermore, dutasteride when combined with 5FU there was significant decrease in caspase 3 at all concentrations of dutasteride compared with both negative and positive control. These results mean that the viability of LNCap cells was decreasing a manner independent on caspase 3. On the other hand, dutasteride increased caspase 3 when used alone during this study but when combined with 5FU the level of caspase 3 was decreased and this means that 5FU might antagonize the apoptotic effects of dutasteride but potentiated anti-androgenic effects of dutasteride by mechanisms that explained above.

B) Effects of different concentrations of dutasteride with 5FU on TNF alpha in LNCap

The outcomes revealed a highly significant decline in TNF alpha at all concentrations of dutasteride compared with both positive and negative control and 5FU significantly increased TNF alpha as compared with negative control.

Elsea *et al* showed that 5FU and other chemotherapies activate p38 MAPK in model cell systems. And this activation was associated with increasing TNF, IL-6, and other cytokines. On the other hand, inhibition of p38 MAPK caused TNF, IL-6, and other cytokines to lower without affecting on cytotoxic effects of chemotherapies (Elsea *et al.*, 2008). Depending on this study we can

suggest that 5FU may activate p38 MAPK in LNCap as a part of its cytotoxic effects on these cells (Boldt *et al.*, 2002) and result in TNF alpha elevation (positive control). But when combined with dutasteride which showed anti-inflamatory effects during this study by decreasing TNF alpha, this resulted in decreasing of inflammatory effects of 5FU in dose-dependent manner without effect on 5FU cytotoxicity (cell viability was low). Therefore combination dutasteride with 5FU might contribut in decrease the inflammatory effects of 5FU and further investigations should be applied to confirm these effects because no previous studies were used these two drugs on this type of cells.

C) Effects of different concentrations of dutasteride with 5FU on Oxidative stress in LNCap

The outcomes didn't reveal any significant differences in TAC level at concentrations (75 . 18.75) µg⁄ml with a siginficant decrease at concentration 9.37 µg⁄ml compared with positive control. Also, results showed a significant decrease in TAC level at positive control as compared with negative control. 5FU is a conventional drug that is widely investigated and it is the first line treatment of choice for patients with colon cancer (Peng *et al.*, 2020). One of the mechanisms of anticancer action of 5FU is the generation of ROS such as $HO^{\bullet}$ and $O^{2\bullet-}$ which attach cancer cells in several different ways. Furthermore, these surge in intracellular ROS generation by 5FU is the main fator responsible for the major side effects associated with 5FU therapy such as cardiotoxicity, hepatotoxicity and nephrotoxicity (Elghareeb *et al.*, 2021). Also, 5FU and doxorubicin are the most chemotherapies associated with

cardiotoxicity because these agents formed free radicals as a part of their cytotoxic effects that are toxic to cancers cells and other cells such as cardiac cells (Focaccetti *et al.*, 2015). Other studies focusing on damage caused at the cellular level have postulated the oxidative stress theory, demonstrating increased levels of reactive oxygen species such as superoxide anions in rat cardiomyocytes after treatment with 5-FU (Sara *et al.*, 2018). These effects were the cause of limiting the use of these drugs. To decrease these effects on normal cells, Several studies have investigated the use of antioxidants, specifically dietary antioxidants, to suppress the side effects of 5FU (Rtibi *et al.*, 2021). Also, recent studies showed that the use antioxidants with 5FU may be helped in decreasing these effects of 5FU without affecting on its anticancer activity (Diba *et al.*, 2021). In the present study, 5FU decreased total antioxidant capacity by inducing free radical formation in LNCap cells and when combined with dutasteride there were no significant effects on TAC levels compared with positive control. In contrast, the levels of TAC were decreased at concentration (9.37 µg⁄ml) of dutasteride which combined with IC50 of 5FU (30 µg⁄ml) and that mean dutasteride unable to reverse or decrease the free radicals of 5FU at this concentration but in high concentrations dutasteride equalized the effects of 5FU on TAC level. Depending on these results, dutasteride in high concentration may play a role in reduce the side effects of 5FU on normal cells by reducing the free radicals of 5FU. Further investigations should be applied to confirm these effects because no previous studies were used these two drugs on this type of cells.

Conclusions
&
Recommendations

Conclusions

1- Dutasteride has apoptotic effects on LNCap by increasing caspase-3 levels., anti-inflammatory effects by decreasing the levels of TNF alpha, and antioxidant effects by increasing total anti-oxidant capacity with the best effects at high concentrations.

2- Combination of dutasteride with doxorubicin showed additive effects on cell viability, apoptosis, and inflammation but no effects on total antioxidant capacity.

3- Combination of dutasteride with 5FU showed addative effects on cell viability and inflammation, decreasing the apoptotic effects of dutasteride and no effects on total anti-oxidant capacity.

4- The best combination was dutasteride with IC50 of doxorubicin because doxorubicin gives addative effects on apoptotic effects of dutasteride. On the other hand, 5FU was decreased these effects.

Recommendations

1- Study the biological effects of dutasteride on other types of cell lines such as bladder cell line since several recent studies investigate the use of this drug for bladder cancer treatment.

2- Study the effects of dutasteride on other apoptotic pathways (such as other caspases) and other inflammatory biomarkers.

3- Since dutasteride increased total antioxidant capacity, further investigations are required to determine its effect on individual antioxidants (such as glutathione, Catalase or Vitamins).

4- Study other combinations of dutasteride with other anticancer drugs that have a different mechanism of action.

References

References

- Abate-Shen C, Nunes de Almeida F. Establishment of the LNCaP Cell Line - The Dawn of an Era for Prostate Cancer Research. Cancer Res. 2022 May 3;82(9):1689-1691. doi: 10.1158/0008-5472.CAN-22-1065. PMID: 35502546; PMCID: PMC9153264.
- Ahmed Amar SA, Eryilmaz R, Demir H, Aykan S, Demir C. Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. Aging Male. 2019 Sep;22(3):198-206. doi: 10.1080/13685538.2018.1488955. Epub 2018 Oct 15. PMID: 30322333.
- Ahmed M, Eeles R. Germline genetic profiling in prostate cancer: latest developments and potential clinical applications. Future Sci OA. 2015 Dec 18;2(1):FSO87. doi: 10.4155/fso.15.87. PMID: 28031937; PMCID: PMC5137984.
- Ali, M.S., Al-Rubaei, S.H. and Ahmed, N.S. (2020) 'Tumor necrosis factor alpha as a potential marker for prostate cancer', *Journal of Biotechnology Research Center*, 14(1), pp. 5–9.
- Andriole GL, Humphrey P, Ray P, Gleave ME, Trachtenberg J, Thomas LN, Lazier CB, Rittmaster RS. Effect of the dual 5alpha-reductase inhibitor dutasteride on markers of tumor regression in prostate cancer. J Urol. 2004 Sep;172(3):915-9. doi: 10.1097/01.ju.0000136430.37245.b9. PMID: 15310997.
- Andriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, Pettaway CA, Tammela TL, Teloken C, Tindall DJ, Somerville MC, Wilson TH, Fowler IL, Rittmaster RS; REDUCE Study Group. Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 2010 Apr 1;362(13):1192-202. doi: 10.1056/NEJMoa0908127. PMID: 20357281.
- Ayodele O, Cabral HJ, McManus D, Jick S. The Risk of Venous Thromboembolism (VTE) in Men with Benign Prostatic Hyperplasia Treated with 5-Alpha Reductase Inhibitors (5ARIs). Clin Epidemiol. 2021 Aug 3;13:661-673. doi: 10.2147/CLEP.S317019. PMID: 34377032; PMCID: PMC8349190.
- Azizi A, Mumin NH, Shafqat N. Phytochemicals With Anti 5-alpha-reductase Activity: A Prospective For Prostate Cancer Treatment. F1000Res. 2021 Jul 6;10:221. doi: 10.12688/f1000research.51066.3. PMCID: PMC8276191.
- Azzouni F, Godoy A, Li Y, Mohler J. The 5 alpha-reductase isozyme family: a review of basic biology and their role in human diseases. Adv Urol. 2012;2012:530121. doi: 10.1155/2012/530121. Epub 2011 Dec 25. PMID: 22235201; PMCID: PMC3253436.

- Banerjee PP, Banerjee S, Brown TR, Zirkin BR. Androgen action in prostate function and disease. Am J Clin Exp Urol. 2018 Apr 1;6(2):62-77. PMID: 29666834; PMCID: PMC5902724.
- Batista RL, Mendonca BB. The Molecular Basis of 5α-Reductase Type 2 Deficiency. Sex Dev. 2022;16(2-3):171-183. doi: 10.1159/000525119. Epub 2022 Jul 6. PMID: 35793650.
- Belkahla S, Nahvi I, Biswas S, Nahvi I, Ben Amor N. Advances and development of prostate cancer, treatment, and strategies: A systemic review. Front Cell Dev Biol. 2022 Sep 9;10:991330. doi: 10.3389/fcell.2022.991330. PMID: 36158198; PMCID: PMC9501970.
- Berchem GJ, Bosseler M, Sugars LY, Voeller HJ, Zeitlin S, Gelmann EP. Androgens induce resistance to bcl-2-mediated apoptosis in LNCaP prostate cancer cells. Cancer Res. 1995 Feb 15;55(4):735-8. PMID: 7850782.
- Boldt S, Weidle UH, Kolch W. The role of MAPK pathways in the action of chemotherapeutic drugs. Carcinogenesis. 2002 Nov;23(11):1831-8. doi: 10.1093/carcin/23.11.1831. PMID: 12419831.
- Boutry J, Tissot S, Ujvari B, Capp JP, Giraudeau M, Nedelcu AM, Thomas F. The evolution and ecology of benign tumors. Biochim Biophys Acta Rev Cancer. 2022 Jan;1877(1):188643. doi: 10.1016/j.bbcan.2021.188643. Epub 2021 Oct 27. PMID: 34715267.
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
- Cavalcante GC, Schaan AP, Cabral GF, Santana-da-Silva MN, Pinto P, Vidal AF, Ribeiro-Dos-Santos Â. A Cell's Fate: An Overview of the Molecular Biology and Genetics of Apoptosis. Int J Mol Sci. 2019 Aug 24;20(17):4133. doi: 10.3390/ijms20174133. PMID: 31450613; PMCID: PMC6747454.
- Chatterjee P, Schweizer MT, Lucas JM, Coleman I, Nyquist MD, Frank SB, Tharakan R, Mostaghel E, Luo J, Pritchard CC, Lam HM, Corey E, Antonarakis ES, Denmeade SR, Nelson PS. Supraphysiological androgens suppress prostate cancer growth through androgen receptor-mediated DNA damage. J Clin Invest. 2019 Jul 16;129(10):4245-4260. doi: 10.1172/JCI127613. PMID: 31310591; PMCID: PMC6763228.
- Chodari L, Smailnejad S, Fallahi M, Khalaji N, Ghorbanzadeh V. OXIDATIVE STRESS IS MARKEDLY REDUCED BY COMBINED VOLUNTARY EXERCISE AND TESTOSTERONE IN THE HEART

OF DIABETIC RATS. Acta Endocrinol (Buchar). 2019 Apr-Jun;15(2):173-181. doi: 10.4183/aeb.2019.173. PMID: 31508173; PMCID: PMC6711632.

- Choi E, Buie J, Camacho J, Sharma P, de Riese WTW. Evolution of Androgen Deprivation Therapy (ADT) and Its New Emerging Modalities in Prostate Cancer: An Update for Practicing Urologists, Clinicians and Medical Providers. Res Rep Urol. 2022 Mar 30;14:87-108. doi: 10.2147/RRU.S303215. PMID: 35386270; PMCID: PMC8977476.
- Choi JS, Lee SE, Jang WS, Byeon JC, Park JS. Solid dispersion of dutasteride using the solvent evaporation method: Approaches to improve dissolution rate and oral bioavailability in rats. Mater Sci Eng C Mater Biol Appl. 2018 Sep 1;90:387-396. doi: 10.1016/j.msec.2018.04.074. Epub 2018 Apr 30. PMID: 29853105.
- Chughtai B, Forde JC, Thomas DD, Laor L, Hossack T, Woo HH, Te AE, Kaplan SA. Benign prostatic hyperplasia. Nat Rev Dis Primers. 2016 May 5;2:16031. doi: 10.1038/nrdp.2016.31. PMID: 27147135.
- Chuu CP, Kokontis JM, Hiipakka RA, Fukuchi J, Lin HP, Lin CY, Huo C, Su LC. Androgens as therapy for androgen receptor-positive castration-resistant prostate cancer. J Biomed Sci. 2011 Aug 23;18(1):63. doi: 10.1186/1423-0127-18-63. PMID: 21859492; PMCID: PMC3170584.
- Clark RV, Hermann DJ, Cunningham GR, Wilson TH, Morrill BB, Hobbs S. Marked suppression of dihydrotestosterone in men with benign prostatic hyperplasia by dutasteride, a dual 5alpha-reductase inhibitor. J Clin Endocrinol Metab. 2004 May;89(5):2179-84. doi: 10.1210/jc.2003-030330. PMID: 15126539.
- Coecke S, Balls M, Bowe G, Davis J, Gstraunthaler G, Hartung T, Hay R, Merten OW, Price A, Schechtman L, Stacey G, Stokes W; Second ECVAM Task Force on Good Cell Culture Practice. Guidance on good cell culture practice. a report of the second ECVAM task force on good cell culture practice. Altern Lab Anim. 2005 Jun;33(3):261-87. doi: 10.1177/026119290503300313. PMID: 16180980.
- Coffey RN, Watson RW, O'Neill AJ, Mc Eleny K, Fitzpatrick JM. Androgen-mediated resistance to apoptosis. Prostate. 2002 Dec 1;53(4):300-9. doi: 10.1002/pros.10159. PMID: 12430141.
- Cunha GR, Vezina CM, Isaacson D, Ricke WA, Timms BG, Cao M, Franco O, Baskin LS. Development of the human prostate. Differentiation. 2018 Sep-Oct;103:24-45. doi: 10.1016/j.diff.2018.08.005. Epub 2018 Sep 4. PMID: 30224091; PMCID: PMC6234090.

- D'Arcy MS. Cell death: a review of the major forms of apoptosis, necrosis and autophagy. Cell Biol Int. 2019 Jun;43(6):582-592. doi: 10.1002/cbin.11137. Epub 2019 Apr 25. PMID: 30958602.
- Demirbag R, Yilmaz R, Erel O. The association of total antioxidant capacity with sex hormones. Scand Cardiovasc J. 2005 Jul;39(3):172-6. doi: 10.1080/14017430510035862. PMID: 16146980.
- Deng T, Lin X, Duan X, He Z, Zhao Z, Zeng G. Prostate cancer patients can benefit from 5-alpha-reductase inhibitor treatment: a meta-analysis. PeerJ. 2020 Jun 1;8:e9282. doi: 10.7717/peerj.9282. PMID: 32547884; PMCID: PMC7271889.
- Desai K, McManus JM, Sharifi N. Hormonal Therapy for Prostate Cancer. Endocr Rev. 2021 May 25;42(3):354-373. doi: 10.1210/endrev/bnab002. PMID: 33480983; PMCID: PMC8152444.
- Diba M, Seghatoleslam A, Namavari M, Assadi S, Vakili SN, Babaei Z, Akmali M. Potential Protective Role of Cyrtopodion Scabrum in Antioxidant Parameters in Serum and Liver of Rats with 5-FU-Induced Oxidative Damage. Arch Razi Inst. 2021 Mar;76(1):95-105. doi: 10.22092/ari.2019.126702.1356. Epub 2021 Mar 1. PMID: 33818962; PMCID: PMC8410198.
- Dolder CR. Dutasteride: a dual 5-alpha reductase inhibitor for the treatment of symptomatic benign prostatic hyperplasia. Ann Pharmacother. 2006 Apr;40(4):658-65. doi: 10.1345/aph.1G540. Epub 2006 Mar 28. PMID: 16569804.
- Elghareeb MM, Elshopakey GE, Hendam BM, Rezk S, Lashen S. Synergistic effects of Ficus Carica extract and extra virgin olive oil against oxidative injury, cytokine liberation, and inflammation mediated by 5-Fluorouracil in cardiac and renal tissues of male albino rats. Environ Sci Pollut Res Int. 2021 Jan;28(4):4558-4572. doi: 10.1007/s11356-020-10778-0. Epub 2020 Sep 18. PMID: 32946057.
- Elsea CR, Roberts DA, Druker BJ, Wood LJ. Inhibition of p38 MAPK suppresses inflammatory cytokine induction by etoposide, 5-fluorouracil, and doxorubicin without affecting tumoricidal activity. PLoS One. 2008 Jun 4;3(6):e2355. doi: 10.1371/journal.pone.0002355. PMID: 18523641; PMCID: PMC2396285.
- Naamneh Elzenaty R, du Toit T, Flück CE. Basics of androgen synthesis and action. Best Pract Res Clin Endocrinol Metab. 2022 Jul;36(4):101665. doi: 10.1016/j.beem.2022.101665. Epub 2022 May 6. PMID: 35595638.Evans HC, Goa KL. Dutasteride. Drugs Aging. 2003;20(12):905-16; discussion 917-8. doi: 10.2165/00002512-200320120-00005. PMID: 14565784.

- Fay EK, Graff JN. Immunotherapy in Prostate Cancer. Cancers (Basel). 2020 Jul 1;12(7):1752. doi: 10.3390/cancers12071752. PMID: 32630247; PMCID: PMC7409298.
- Feng Q, He B. Androgen Receptor Signaling in the Development of Castration-Resistant Prostate Cancer. Front Oncol. 2019 Sep 4;9:858. doi: 10.3389/fonc.2019.00858. PMID: 31552182; PMCID: PMC6738163.
- Focaccetti C, Bruno A, Magnani E, Bartolini D, Principi E, Dallaglio K, Bucci EO, Finzi G, Sessa F, Noonan DM, Albini A. Effects of 5-fluorouracil on morphology, cell cycle, proliferation, apoptosis, autophagy and ROS production in endothelial cells and cardiomyocytes. PLoS One. 2015 Feb 11;10(2):e0115686. doi: 10.1371/journal.pone.0115686. PMID: 25671635; PMCID: PMC4324934.
- Fontana F, Marzagalli M, Montagnani Marelli M, Raimondi M, Moretti RM, Limonta P. Gonadotropin-Releasing Hormone Receptors in Prostate Cancer: Molecular Aspects and Biological Functions. Int J Mol Sci. 2020 Dec 14;21(24):9511. doi: 10.3390/ijms21249511. PMID: 33327545; PMCID: PMC7765031.
- Freudenrich, T.M. and Shafer, T.J. (2020) 'Mammalian cell culture models', in *An Introduction to Interdisciplinary Toxicology*. Elsevier, pp. 463–475.
- Fujita K, Nonomura N. Role of Androgen Receptor in Prostate Cancer: A Review. World J Mens Health. 2019 Sep;37(3):288-295. doi: 10.5534/wjmh.180040. Epub 2018 Sep 10. PMID: 30209899; PMCID: PMC6704300.
- Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. Int J Mol Sci. 2021 Nov 26;22(23):12827. doi: 10.3390/ijms222312827. PMID: 34884632; PMCID: PMC8657538.
- Giacomini A, Grillo E, Rezzola S, Ribatti D, Rusnati M, Ronca R, Presta M. The FGF/FGFR system in the physiopathology of the prostate gland. Physiol Rev. 2021 Apr 1;101(2):569-610. doi: 10.1152/physrev.00005.2020. Epub 2020 Jul 30. PMID: 32730114.
- Gleave M, Qian J, Andreou C, Pommerville P, Chin J, Casey R, Steinhoff G, Fleshner N, Bostwick D, Thomas L, Rittmaster R. The effects of the dual 5alpha-reductase inhibitor dutasteride on localized prostate cancer--results from a 4-month pre-radical prostatectomy study. Prostate. 2006 Nov 1;66(15):1674-85. doi: 10.1002/pros.20499. PMID: 16927304.
- Gniazdowska E, Kaza M, Buś-Kwaśnik K, Giebułtowicz J. LC-MS/MS

determination of dutasteride and its major metabolites in human plasma. J Pharm Biomed Anal. 2021 Nov 30;206:114362. doi: 10.1016/j.jpba.2021.114362. Epub 2021 Sep 3. PMID: 34562803.

- Goddard, J.C. (2019) 'History of the prostate, part two: the cause of urinary symptoms', *Trends in Urology & Men's Health*, 10(3), pp. 31–34.
- Golbano JM, Lóppez-Aparicio P, Recio MN, Pérez-Albarsanz MA. Finasteride induces apoptosis via Bcl-2, Bcl-xL, Bax and caspase-3 proteins in LNCaP human prostate cancer cell line. Int J Oncol. 2008 Apr;32(4):919-24. PMID: 18360719.
- Gonthier K, Poluri RTK, Audet-Walsh É. Functional genomic studies reveal the androgen receptor as a master regulator of cellular energy metabolism in prostate cancer. J Steroid Biochem Mol Biol. 2019 Jul;191:105367. doi: 10.1016/j.jsbmb.2019.04.016. Epub 2019 Apr 30. PMID: 31051242.
- Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M, Mehra R, Prensner JR, Palanisamy N, Ryslik GA, Vandin F, Raphael BJ, Kunju LP, Rhodes DR, Pienta KJ, Chinnaiyan AM, Tomlins SA. The mutational landscape of lethal castration-resistant prostate cancer. Nature. 2012 Jul 12;487(7406):239-43. doi: 10.1038/nature11125. PMID: 22722839; PMCID: PMC3396711.
- Gründker C, Emons G. The Role of Gonadotropin-Releasing Hormone in Cancer Cell Proliferation and Metastasis. Front Endocrinol (Lausanne). 2017 Aug 4;8:187. doi: 10.3389/fendo.2017.00187. PMID: 28824547; PMCID: PMC5543040.
- Gullapalli RP. Soft gelatin capsules (softgels). J Pharm Sci. 2010 Oct;99(10):4107-48. doi: 10.1002/jps.22151. PMID: 20737624.
- Hausman DM. What Is Cancer? Perspect Biol Med. 2019;62(4):778-784. doi: 10.1353/pbm.2019.0046. PMID: 31761807.
- Henzler C, Li Y, Yang R, McBride T, Ho Y, Sprenger C, Liu G, Coleman I, Lakely B, Li R, Ma S, Landman SR, Kumar V, Hwang TH, Raj GV, Higano CS, Morrissey C, Nelson PS, Plymate SR, Dehm SM. Truncation and constitutive activation of the androgen receptor by diverse genomic rearrangements in prostate cancer. Nat Commun. 2016 Nov 29;7:13668. doi: 10.1038/ncomms13668. PMID: 27897170; PMCID: PMC5141345.
- Hou Z, Huang S, Li Z. Androgens in prostate cancer: A tale that never ends. Cancer Lett. 2021 Sep 28;516:1-12. doi: 10.1016/j.canlet.2021.04.010. Epub 2021 May 28. PMID: 34052327.

- Hudu SA, Alshrari AS, Syahida A, Sekawi Z. Cell Culture, Technology: Enhancing the Culture of Diagnosing Human Diseases. J Clin Diagn Res. 2016 Mar;10(3):DE01-5. doi: 10.7860/JCDR/2016/15837.7460. Epub 2016 Mar 1. PMID: 27134874; PMCID: PMC4843260.
- Hwang KM, Choi MS, Seok SH, Park ES. Development of self-microemulsifying tablets containing dutasteride for enhanced dissolution and pharmacokinetic profile. Int J Pharm. 2022 Apr 25;618:121660. doi: 10.1016/j.ijpharm.2022.121660. Epub 2022 Mar 12. PMID: 35292395.
- Ishiba T, Hoffmann AC, Usher J, Elshimali Y, Sturdevant T, Dang M, Jaimes Y, Tyagi R, Gonzales R, Grino M, Pinski JK, Barzi A, Raez LE, Eberhardt WE, Theegarten D, Lenz HJ, Uetake H, Danenberg PV, Danenberg K. Frequencies and expression levels of programmed death ligand 1 (PD-L1) in circulating tumor RNA (ctRNA) in various cancer types. Biochem Biophys Res Commun. 2018 Jun 7;500(3):621-625. doi: 10.1016/j.bbrc.2018.04.120. Epub 2018 Apr 30. PMID: 29679564; PMCID: PMC9165692.
- Jan R, Chaudhry GE. Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. Adv Pharm Bull. 2019 Jun;9(2):205-218. doi: 10.15171/apb.2019.024. Epub 2019 Jun 1. PMID: 31380246; PMCID: PMC6664112.
- Josephs SF, Ichim TE, Prince SM, Kesari S, Marincola FM, Escobedo AR, Jafri A. Unleashing endogenous TNF-alpha as a cancer immunotherapeutic. J Transl Med. 2018 Aug 31;16(1):242. doi: 10.1186/s12967-018-1611-7. PMID: 30170620; PMCID: PMC6119315.
- Kadoyama K, Miki I, Tamura T, Brown JB, Sakaeda T, Okuno Y. Adverse event profiles of 5-fluorouracil and capecitabine: data mining of the public version of the FDA Adverse Event Reporting System, AERS, and reproducibility of clinical observations. Int J Med Sci. 2012;9(1):33-9. doi: 10.7150/ijms.9.33. Epub 2011 Nov 17. PMID: 22211087; PMCID: PMC3222088.
- Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ, Saad F. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. Clin Endocrinol (Oxf). 2010 Nov;73(5):602-12. doi: 10.1111/j.1365-2265.2010.03845.x. Erratum in: Clin Endocrinol (Oxf). 2011 Aug;75(2):275. PMID: 20718771.
- Kalyanaraman B. Teaching the basics of the mechanism of doxorubicin-induced cardiotoxicity: Have we been barking up the wrong tree? Redox Biol. 2020 Jan;29:101394. doi: 10.1016/j.redox.2019.101394. Epub 2019

Nov 26. PMID: 31790851; PMCID: PMC6909145.

- Kamiloglu, S., Sari, G., Ozdal, T., & Capanoglu, E. (2020). Guidelines for cell viability assays. *Food Frontiers*, *1*(3), 332-349. https://doi.org/10.1002/fft2.44
- Kawabata R, Oie S, Takahashi M, Kanayama H, Oka T, Itoh K. Up-regulation of insulin-like growth factor-binding protein 3 by 5-fluorouracil (5-FU) leads to the potent anti-proliferative effect of androgen deprivation therapy combined with 5-FU in human prostate cancer cell lines. Int J Oncol. 2011 Jun;38(6):1489-500. doi: 10.3892/ijo.2011.991. Epub 2011 Mar 30. PMID: 21455575.
- Kim EK, Jang M, Song MJ, Kim D, Kim Y, Jang HH. Redox-Mediated Mechanism of Chemoresistance in Cancer Cells. Antioxidants (Basel). 2019 Oct 10;8(10):471. doi: 10.3390/antiox8100471. PMID: 31658599; PMCID: PMC6826977.
- Kim EH, Brockman JA, Andriole GL. The use of 5-alpha reductase inhibitors in the treatment of benign prostatic hyperplasia. Asian J Urol. 2018 Jan;5(1):28-32. doi: 10.1016/j.ajur.2017.11.005. Epub 2017 Nov 26. PMID: 29379733; PMCID: PMC5780290.
- Kim MS, Ha ES, Choo GH, Baek IH. Preparation and in vivo evaluation of a dutasteride-loaded solid-supersaturatable self-microemulsifying drug delivery system. Int J Mol Sci. 2015 May 13;16(5):10821-33. doi: 10.3390/ijms160510821. PMID: 25984604; PMCID: PMC4463677.
- Kobuchi S, Ito Y. Application of Pharmacometrics of 5-Fluorouracil to Personalized Medicine: A Tool for Predicting Pharmacokinetic-Pharmacodynamic/Toxicodynamic Responses. Anticancer Res. 2020 Dec;40(12):6585-6597. doi: 10.21873/anticanres.14683. Epub 2020 Dec 7. PMID: 33288553.
- Kong CY, Guo Z, Song P, Zhang X, Yuan YP, Teng T, Yan L, Tang QZ. Underlying the Mechanisms of Doxorubicin-Induced Acute Cardiotoxicity: Oxidative Stress and Cell Death. Int J Biol Sci. 2022 Jan 1;18(2):760-770. doi: 10.7150/ijbs.65258. PMID: 35002523; PMCID: PMC8741835.
- Kowluru RA, Mishra M. Oxidative stress, mitochondrial damage and diabetic retinopathy. Biochim Biophys Acta. 2015 Nov;1852(11):2474-83. doi: 10.1016/j.bbadis.2015.08.001. Epub 2015 Aug 4. PMID: 26248057.
- Krieg S, Roderburg C, Krieg A, Luedde T, Loosen SH, Kostev K. The association between body height and cancer: a retrospective analysis of 784,192 outpatients in Germany. J Cancer Res Clin Oncol. 2023 Jul;149(8):4275-4282. doi: 10.1007/s00432-022-04335-0. Epub 2022

Sep 6. PMID: 36066621; PMCID: PMC10349780.
- Kullenberg F, Degerstedt O, Calitz C, Pavlović N, Balgoma D, Gråsjö J, Sjögren E, Hedeland M, Heindryckx F, Lennernäs H. In Vitro Cell Toxicity and Intracellular Uptake of Doxorubicin Exposed as a Solution or Liposomes: Implications for Treatment of Hepatocellular Carcinoma. Cells. 2021 Jul 6;10(7):1717. doi: 10.3390/cells10071717. PMID: 34359887; PMCID: PMC8306283.
- Lazier CB, Thomas LN, Douglas RC, Vessey JP, Rittmaster RS. Dutasteride, the dual 5alpha-reductase inhibitor, inhibits androgen action and promotes cell death in the LNCaP prostate cancer cell line. Prostate. 2004 Feb 1;58(2):130-44. doi: 10.1002/pros.10340. PMID: 14716738.
- Lee S, Lee YB, Choe SJ, Lee WS. Adverse Sexual Effects of Treatment with Finasteride or Dutasteride for Male Androgenetic Alopecia: A Systematic Review and Meta-analysis. Acta Derm Venereol. 2019 Jan 1;99(1):12-17. doi: 10.2340/00015555-3035. PMID: 30206635.
- Lin RW, Ho CJ, Chen HW, Pao YH, Chen LE, Yang MC, Huang SB, Wang S, Chen CH, Wang C. P53 enhances apoptosis induced by doxorubicin only under conditions of severe DNA damage. Cell Cycle. 2018;17(17):2175-2186. doi: 10.1080/15384101.2018.1520565. Epub 2018 Sep 22. PMID: 30198376; PMCID: PMC6226221.
- Lin Y, Kokontis J, Tang F, Godfrey B, Liao S, Lin A, Chen Y, Xiang J. Androgen and its receptor promote Bax-mediated apoptosis. Mol Cell Biol. 2006 Mar;26(5):1908-16. doi: 10.1128/MCB.26.5.1908-1916.2006. PMID: 16479009; PMCID: PMC1430231.
- Litwin MS, Tan HJ. The Diagnosis and Treatment of Prostate Cancer: A Review. JAMA. 2017 Jun 27;317(24):2532-2542. doi: 10.1001/jama.2017.7248. PMID: 28655021.
- Long MD, Singh PK, Russell JR, Llimos G, Rosario S, Rizvi A, van den Berg PR, Kirk J, Sucheston-Campbell LE, Smiraglia DJ, Campbell MJ. The miR-96 and RARγ signaling axis governs androgen signaling and prostate cancer progression. Oncogene. 2019 Jan;38(3):421-444. doi: 10.1038/s41388-018-0450-6. Epub 2018 Aug 17. PMID: 30120411; PMCID: PMC6336686.
- Lorenzo G, HughesTJR, Reali A. *et al.* (2020) 'A numerical simulation study of the dual role of 5α-reductase inhibitors on tumor growth in prostates enlarged by benign prostatic hyperplasia via stress relaxation and apoptosis upregulation', *Computer Methods in Applied Mechanics and Engineering*, 362, p. 112843.
- Lorenzo PI, Saatcioglu F. Inhibition of apoptosis in prostate cancer cells by androgens is mediated through downregulation of c-Jun N-terminal

kinase activation. Neoplasia. 2008 May;10(5):418-28. doi: 10.1593/neo.07985. PMID: 18472959; PMCID: PMC2373916.

- Luhur A, Klueg KM, Roberts J, Zelhof AC. Thawing, Culturing, and Cryopreserving Drosophila Cell Lines. J Vis Exp. 2019 Apr 16;(146):10.3791/59459. doi: 10.3791/59459. PMID: 31058891; PMCID: PMC7032961.
- Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab. 2004 Jul;89(7):3313-8. doi: 10.1210/jc.2003-031069. PMID: 15240608.
- Marihart S, Harik M, Djavan B. Dutasteride: a review of current data on a novel dual inhibitor of 5alpha reductase. Rev Urol. 2005 Fall;7(4):203-10. PMID: 16985831; PMCID: PMC1550785.
- Marino-Enriquez A, Fletcher CD. Shouldn't we care about the biology of benign tumours? Nat Rev Cancer. 2014 Nov;14(11):701-2. doi: 10.1038/nrc3845. PMID: 25688395.
- May A, Henke J, Au D, Raza SJ, Davaro F, Hamilton Z, Siddiqui SA. National Trends in the Utilization of Androgen Deprivation Therapy for Very Low Risk Prostate Cancer. Urology. 2019 Aug;130:79-85. doi: 10.1016/j.urology.2019.02.043. Epub 2019 Apr 29. PMID: 31047912.
- Michalaki V, Syrigos K, Charles P, Waxman J. Serum levels of IL-6 and TNF-alpha correlate with clinicopathological features and patient survival in patients with prostate cancer. Br J Cancer. 2004 Jun 14;90(12):2312-6. doi: 10.1038/sj.bjc.6601814. Erratum in: Br J Cancer. 2004 Sep 13;91(6):1227. PMID: 15150588; PMCID: PMC2409519.
- Miller, D.R. (2019) 'The Role of ROS in the Progression and Treatment of Castration-Resistant Prostate Cancer'.
- Miller WL. Steroidogenesis: Unanswered Questions. Trends Endocrinol Metab. 2017 Nov;28(11):771-793. doi: 10.1016/j.tem.2017.09.002. Epub 2017 Oct 12. PMID: 29031608..
- Mizokami A, Gotoh A, Yamada H, Keller ET, Matsumoto T. Tumor necrosis factor-alpha represses androgen sensitivity in the LNCaP prostate cancer cell line. J Urol. 2000 Sep;164(3 Pt 1):800-5. doi: 10.1097/00005392-200009010-00053. PMID: 10953159.
- Morgentaler A. Testosterone and prostate cancer: an historical perspective on a modern myth. Eur Urol. 2006 Nov;50(5):935-9. doi: 10.1016/j.eururo.2006.06.034. Epub 2006 Jul 27. PMID: 16875775.
- Nyquist MD, Corella A, Mohamad O, Coleman I, Kaipainen A, Kuppers DA, Lucas JM, Paddison PJ, Plymate SR, Nelson PS, Mostaghel EA.

Molecular determinants of response to high-dose androgen therapy in prostate cancer. JCI Insight. 2019 Oct 3;4(19):e129715. doi: 10.1172/jci.insight.129715. PMID: 31503550; PMCID: PMC6795499.

- Packer JR, Maitland NJ. The molecular and cellular origin of human prostate cancer. Biochim Biophys Acta. 2016 Jun;1863(6 Pt A):1238-60. doi: 10.1016/j.bbamcr.2016.02.016. Epub 2016 Feb 26. PMID: 26921821.
- Pan, E. and McKay, R. (2021) 'GNRH agonists vs antagonists in prostate cancer: How to choose', *Urology Times Urologists in Cancer Care*, 10(02).
- Peng W, Zhang H, Tan S, Li Y, Zhou Y, Wang L, Liu C, Li Q, Cen X, Yang S, Zhao Y. Synergistic antitumor effect of 5-fluorouracil with the novel LSD1 inhibitor ZY0511 in colorectal cancer. Ther Adv Med Oncol. 2020 Jul 23;12:1758835920937428. doi: 10.1177/1758835920937428. PMID: 32754230; PMCID: PMC7378962.
- Phelan K, May KM. Mammalian Cell Tissue Culture Techniques. Curr Protoc Mol Biol. 2017 Jan 5;117:A.3F.1-A.3F.23. doi: 10.1002/cpmb.31. PMID: 28060407.
- Pohlman, G.D., Pohlman, E.A. and Crawford, E.D. (2011) 'Dutasteride: a review of its use in the management of prostate disorders', *Clinical Medicine Insights: Therapeutics*, 3, p. CMT-S1956.
- Pritchard CC, Mateo J, Walsh MF, De Sarkar N, Abida W, Beltran H, Garofalo A, and Gulati R.*et al*. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. N Engl J Med. 2016 Aug 4;375(5):443-53. doi: 10.1056/NEJMoa1603144. Epub 2016 Jul 6. PMID: 27433846; PMCID: PMC4986616.
- Van Quickelberghe E, De Sutter D, van Loo G, Eyckerman S, Gevaert K. A protein-protein interaction map of the TNF-induced NF-κB signal transduction pathway. Sci Data. 2018 Dec 18;5:180289. doi: 10.1038/sdata.2018.289. PMID: 30561431; PMCID: PMC6298254.
- Rashid, R., Shakeel, S. and Mubarak, M. (2022) 'Prostate Intraepithelial Neoplasia in Prostate Core Biopsies done for Suspected Prostate Cancer'.
- Rawla P. Epidemiology of Prostate Cancer. World J Oncol. 2019 Apr;10(2):63-89. doi: 10.14740/wjon1191. Epub 2019 Apr 20. PMID: 31068988; PMCID: PMC6497009.
- Rehman LU, Nisar MH, Fatima W, Sarfraz A, Azeem N, Sarfraz Z, Robles-Velasco K, Cherrez-Ojeda I. Immunotherapy for Prostate Cancer: A Current Systematic Review and Patient Centric Perspectives. J Clin Med. 2023 Feb 11;12(4):1446. doi: 10.3390/jcm12041446. PMID: 36835981; PMCID: PMC9966657.

- Reinehr CPH, Bakos RM. Actinic keratoses: review of clinical, dermoscopic, and therapeutic aspects. An Bras Dermatol. 2019 Nov-Dec;94(6):637-657. doi: 10.1016/j.abd.2019.10.004. Epub 2019 Nov 6. PMID: 31789244; PMCID: PMC6939186.
- Ripple MO, Henry WF, Rago RP, Wilding G. Prooxidant-antioxidant shift induced by androgen treatment of human prostate carcinoma cells. J Natl Cancer Inst. 1997 Jan 1;89(1):40-8. doi: 10.1093/jnci/89.1.40. PMID: 8978405.
- Rizzo A, Mollica V, Cimadamore A, Santoni M, Scarpelli M, Giunchi F, Cheng L, Lopez-Beltran A, Fiorentino M, Montironi R, Massari F. Is There a Role for Immunotherapy in Prostate Cancer? Cells. 2020 Sep 8;9(9):2051. doi: 10.3390/cells9092051. PMID: 32911806; PMCID: PMC7564598.
- Rokhlin OW, Taghiyev AF, Guseva NV, Glover RA, Chumakov PM, Kravchenko JE, Cohen MB. Androgen regulates apoptosis induced by TNFR family ligands via multiple signaling pathways in LNCaP. Oncogene. 2005 Oct 13;24(45):6773-84. doi: 10.1038/sj.onc.1208833. PMID: 16007156; PMCID: PMC1361275.
- Rtibi, K., Marzouki, L. and Sebai, H. (2021) 'Oxidative stress due to 5-fluorouracil and dietary antioxidants', in *Toxicology*. Elsevier, pp. 291–295.
- Saengmearnuparp T, Lojanapiwat B, Chattipakorn N, Chattipakorn S. The connection of 5-alpha reductase inhibitors to the development of depression. Biomed Pharmacother. 2021 Nov;143:112100. doi: 10.1016/j.biopha.2021.112100. Epub 2021 Aug 31. PMID: 34479019.
- Sara JD, Kaur J, Khodadadi R, Rehman M, Lobo R, Chakrabarti S, Herrmann J, Lerman A, Grothey A. 5-fluorouracil and cardiotoxicity: a review. Ther Adv Med Oncol. 2018 Jun 18;10:1758835918780140. doi: 10.1177/1758835918780140. PMID: 29977352; PMCID: PMC6024329.
- Schmidt LJ, Murillo H, Tindall DJ. Gene expression in prostate cancer cells treated with the dual 5 alpha-reductase inhibitor dutasteride. J Androl. 2004 Nov-Dec;25(6):944-53. doi: 10.1002/j.1939-4640.2004.tb03166.x. PMID: 15477368.
- Schweizer MT, Hancock ML, Getzenberg RH, Yu EY. Hormone levels following surgical and medical castration: defining optimal androgen suppression. Asian J Androl. 2018 Jul-Aug;20(4):405-406. doi: 10.4103/aja.aja_38_17. PMID: 29111538; PMCID: PMC6038162.
- Segeritz CP, Vallier L. Cell Culture: Growing Cells as Model Systems In Vitro. Basic Science Methods for Clinical Researchers. 2017:151–72. doi: 10.1016/B978-0-12-803077-6.00009-6. Epub 2017 Apr 7. PMCID:

PMC7149418.

- Sfanos KS, Yegnasubramanian S, Nelson WG, De Marzo AM. The inflammatory microenvironment and microbiome in prostate cancer development. Nat Rev Urol. 2018 Jan;15(1):11-24. doi: 10.1038/nrurol.2017.167. Epub 2017 Oct 31. PMID: 29089606.
- Shore ND, Saad F, Cookson MS, George DJ, Saltzstein DR, Tutrone R, Akaza H, Bossi A, van Veenhuyzen DF, Selby B, Fan X, Kang V, Walling J, Tombal B; HERO Study Investigators. Oral Relugolix for Androgen-Deprivation Therapy in Advanced Prostate Cancer. N Engl J Med. 2020 Jun 4;382(23):2187-2196. doi: 10.1056/NEJMoa2004325. Epub 2020 May 29. PMID: 32469183.
- Siciliano T, Sommer U, Beier AK, Stope MB, Borkowetz A, Thomas C, Erb HHH. The Androgen Hormone-Induced Increase in Androgen Receptor Protein Expression Is Caused by the Autoinduction of the Androgen Receptor Translational Activity. Curr Issues Mol Biol. 2022 Jan 25;44(2):597-608. doi: 10.3390/cimb44020041. PMID: 35723327; PMCID: PMC8928990.
- Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022 Jan;72(1):7-33. doi: 10.3322/caac.21708. Epub 2022 Jan 12. PMID: 35020204.
- Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023 Jan;73(1):17-48. doi: 10.3322/caac.21763. PMID: 36633525.
- da Silva GH, Fernandes MA, Trevizan LNF, de Lima FT, Eloy JO, Chorilli M. A Critical Review of Properties and Analytical Methods for the Determination of Docetaxel in Biological and Pharmaceutical Matrices. Crit Rev Anal Chem. 2018 Nov 2;48(6):517-527. doi: 10.1080/10408347.2018.1456315. Epub 2018 Apr 27. PMID: 29701480.
- Sizemore GM, Pitarresi JR, Balakrishnan S, Ostrowski MC. The ETS family of oncogenic transcription factors in solid tumours. Nat Rev Cancer. 2017 Jun;17(6):337-351. doi: 10.1038/nrc.2017.20. Epub 2017 Apr 28. PMID: 28450705.
- Song W, Khera M. Physiological normal levels of androgen inhibit proliferation of prostate cancer cells in vitro. Asian J Androl. 2014 Nov-Dec;16(6):864-8. doi: 10.4103/1008-682X.129132. PMID: 24923459; PMCID: PMC4236330.
- Sritharan S, Sivalingam N. A comprehensive review on time-tested anticancer drug doxorubicin. Life Sci. 2021 Aug 1;278:119527. doi: 10.1016/j.lfs.2021.119527. Epub 2021 Apr 20. PMID: 33887349.
- Staal J, Beyaert R. Inflammation and NF-κB Signaling in Prostate

Cancer: Mechanisms and Clinical Implications. Cells. 2018 Aug 29;7(9):122. doi: 10.3390/cells7090122. PMID: 30158439; PMCID: PMC6162478.

- Stockert JC, Horobin RW, Colombo LL, Blázquez-Castro A. Tetrazolium salts and formazan products in Cell Biology: Viability assessment, fluorescence imaging, and labeling perspectives. Acta Histochem. 2018 Apr;120(3):159-167. doi: 10.1016/j.acthis.2018.02.005. Epub 2018 Feb 26. PMID: 29496266.
- Student S, Hejmo T, Poterała-Hejmo A, Leśniak A, Bułdak R. Anti-androgen hormonal therapy for cancer and other diseases. Eur J Pharmacol. 2020 Jan 5;866:172783. doi: 10.1016/j.ejphar.2019.172783. Epub 2019 Nov 8. PMID: 31712062.
- Sudeep HV, Venkatakrishna K, Amrutharaj B, Anitha, Shyamprasad K. A phytosterol-enriched saw palmetto supercritical CO_2 extract ameliorates testosterone-induced benign prostatic hyperplasia by regulating the inflammatory and apoptotic proteins in a rat model. BMC Complement Altern Med. 2019 Oct 17;19(1):270. doi: 10.1186/s12906-019-2697-z. PMID: 31623582; PMCID: PMC6798398..
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021 May;71(3):209-249. doi: 10.3322/caac.21660. Epub 2021 Feb 4. PMID: 33538338.
- Tan BL, Norhaizan ME. Oxidative Stress, Diet and Prostate Cancer. World J Mens Health. 2021 Apr;39(2):195-207. doi: 10.5534/wjmh.200014. Epub 2020 May 11. PMID: 32648373; PMCID: PMC7994655.
- Tan, B.L., Norhaizan, M.E. and Liew, W.-P.-P. (2018) 'Nutrients and oxidative stress: friend or foe?', *Oxidative medicine and cellular longevity*, 2018. Available at: https://doi.org/https://doi.org/10.1155/2018/9719584.
- Taymaz-Nikerel H, Karabekmez ME, Eraslan S, Kırdar B. Doxorubicin induces an extensive transcriptional and metabolic rewiring in yeast cells. Sci Rep. 2018 Sep 12;8(1):13672. doi: 10.1038/s41598-018-31939-9. PMID: 30209405; PMCID: PMC6135803.
- Tolosa L, Donato MT, Gómez-Lechón MJ. General Cytotoxicity Assessment by Means of the MTT Assay. Methods Mol Biol. 2015;1250:333-48. doi: 10.1007/978-1-4939-2074-7_26. PMID: 26272156.
- Traish AM, Williams DF, Hoffman ND, Wotiz HH. Validation of the

exchange assay for the measurement of androgen receptors in human and dog prostates. Prog Clin Biol Res. 1988;262:145-60. PMID: 3375279.

- Trinchieri A, Perletti G, Magri V, Stamatiou K, Trinchieri M, Montanari E. Drug-induced gynecomastia: A systematic review and meta-analysis of randomized clinical trials. Arch Ital Urol Androl. 2021 Dec 21;93(4):489-496. doi: 10.4081/aiua.2021.4.489. PMID: 34933535.
- Tsang, M.L. (2021) 'Chemotherapy and Prostate Cancer', in *Chemotherapy and Immunotherapy in Urologic Oncology*. Springer, pp. 105–118.
- Uysal, O. *et al.* (2018) 'Cell and tissue culture: the base of biotechnology', in *Omics technologies and bio-engineering*. Elsevier, pp. 391–429.
- Verma A, Verma M, Singh A. Animal tissue culture principles and applications. Animal Biotechnology. 2020:269–93. doi: 10.1016/B978-0-12-811710-1.00012-4. Epub 2020 Jun 26. PMCID: PMC7325846.
- Villapalos-García G, Zubiaur P, Navares-Gómez M, Saiz-Rodríguez M, Mejía-Abril G, Martín-Vílchez S, Román M, Ochoa D, Abad-Santos F. Effects of Cytochrome P450 and Transporter Polymorphisms on the Bioavailability and Safety of Dutasteride and Tamsulosin. Front Pharmacol. 2021 Oct 7;12:718281. doi: 10.3389/fphar.2021.718281. PMID: 34690761; PMCID: PMC8529037.
- Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P, Vymetalkova V. 5-fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. Pharmacol Ther. 2020 Feb;206:107447. doi: 10.1016/j.pharmthera.2019.107447. Epub 2019 Nov 19. PMID: 31756363.
- Wagner BA, Evig CB, Reszka KJ, Buettner GR, Burns CP. Doxorubicin increases intracellular hydrogen peroxide in PC3 prostate cancer cells. Arch Biochem Biophys. 2005 Aug 15;440(2):181-90. doi: 10.1016/j.abb.2005.06.015. PMID: 16054588; PMCID: PMC4538991.
- Wang G, Zhao D, Spring DJ, DePinho RA. Genetics and biology of prostate cancer. Genes Dev. 2018 Sep 1;32(17-18):1105-1140. doi: 10.1101/gad.315739.118. PMID: 30181359; PMCID: PMC6120714.
- Werutsky G, Maluf FC, Cronemberger EH, Carrera Souza V, Dos Santos Martins SP, Peixoto F, Smaletz O, Schutz F, Herchenhorn D, Santos T, Mavignier Carcano F, Queiroz Muniz D, Nunes Filho PRS, Zaffaroni F, Barrios C, Fay A. The LACOG-0415 phase II trial: abiraterone acetate and ADT versus apalutamide versus abiraterone acetate and apalutamide in patients with advanced prostate cancer with non-castration testosterone levels. BMC Cancer. 2019 May 23;19(1):487. doi: 10.1186/s12885-019-

5709-y. PMID: 31122212; PMCID: PMC6533731.

- Wood LJ, Nail LM, Gilster A, Winters KA, Elsea CR. Cancer chemotherapy-related symptoms: evidence to suggest a role for proinflammatory cytokines. Oncol Nurs Forum. 2006 May 3;33(3):535-42. doi: 10.1188/06.ONF.535-542. PMID: 16676010.
- Yang MC, Lin RW, Huang SB, Huang SY, Chen WJ, Wang S, Hong YR, Wang C. Bim directly antagonizes Bcl-xl in doxorubicin-induced prostate cancer cell apoptosis independently of p53. Cell Cycle. 2016;15(3):394-402. doi: 10.1080/15384101.2015.1127470. PMID: 26694174; PMCID: PMC4943702.
- Ye L, Su ZJ, Ge RS. Inhibitors of testosterone biosynthetic and metabolic activation enzymes. Molecules. 2011 Dec 2;16(12):9983-10001. doi: 10.3390/molecules16129983. PMID: 22138857; PMCID: PMC6264586.
- Yu ZJ, Yan HL, Xu FH, Chao HC, Deng LH, Xu XD, Huang JB, Zeng T. Efficacy and Side Effects of Drugs Commonly Used for the Treatment of Lower Urinary Tract Symptoms Associated With Benign Prostatic Hyperplasia. Front Pharmacol. 2020 May 8;11:658. doi: 10.3389/fphar.2020.00658. PMID: 32457631; PMCID: PMC7225336.
- van der Zanden SY, Qiao X, Neefjes J. New insights into the activities and toxicities of the old anticancer drug doxorubicin. FEBS J. 2021 Nov;288(21):6095-6111. doi: 10.1111/febs.15583. Epub 2020 Oct 19. PMID: 33022843; PMCID: PMC8597086.
- Zhang E, Xing R, Liu S, Li P. Current advances in development of new docetaxel formulations. Expert Opin Drug Deliv. 2019 Mar;16(3):301-312. doi: 10.1080/17425247.2019.1583644. Epub 2019 Feb 27. PMID: 30773947.
- Zhao R, Wang X, Jiang C, Shi F, Zhu Y, Yang B, Zhuo J, Jing Y, Luo G, Xia S, Han B. Finasteride accelerates prostate wound healing after thulium laser resection through DHT and AR signalling. Cell Prolif. 2018 Jun;51(3):e12415. doi: 10.1111/cpr.12415. Epub 2017 Nov 30. PMID: 29194865; PMCID: PMC6528864.
- Zhou M. High-grade prostatic intraepithelial neoplasia, PIN-like carcinoma, ductal carcinoma, and intraductal carcinoma of the prostate. Mod Pathol. 2018 Jan;31(S1):S71-79. doi: 10.1038/modpathol.2017.138. PMID: 29297491.
- Zhu YS, Cai LQ, You X, Cordero JJ, Huang Y, Imperato-McGinley J. Androgen-induced prostate-specific antigen gene expression is mediated via dihydrotestosterone in LNCaP cells. J Androl. 2003 Sep-Oct;24(5):681-7. doi: 10.1002/j.1939-4640.2003.tb02727.x. PMID: 12954658.

الخلاصة

سرطان البروستاتا هو أكثر أنواع السرطان التي يتم تشخيصها عند الذكور وخامس أعلى سبب لوفيات السرطان بين الرجال في جميع أنحاء العالم. وفي عام 2020، تم تشخيص 1,414,249 حالة جديدة و375,000 حالة وفاة على مستوى العالم بسبب هذا المرض. يعد سرطان البروستاتا هو الورم الأكثر انتشارًا الذي يتم تشخيصه عالميًا في أكثر من خمسين بالمائة من البلدان المختلفة.

مثبطات اختزال5α المستخدمة لعلاج تضخم البروستاتا الحميد تمنع تحويل التستوستيرون إلى داي هايدروتستوسترون وقد تقلل من خطر الإصابة بسرطان البروستاتا. هناك نوعان من من إنزيم اختزال5α، النوع 1 والنوع 2. دوتاستيرايد هو مثبط مزدوج يثبط كلا النوعين من إنزيم اختزال 5α.

كان الهدف من هذه الدراسة هو تقييم آثاردواء الدوتاستيريد بمفرده وبالاشتراك مع مضاد للسرطان (دوكسوروبيسين و5-فلورويوراسيل) على حيوية الخلية وموت الخلايا LNCap المبرمج والالتهاب والإجهاد التأكسدي في خط خلايا سرطان البروستات

كان الجزء الأول من العمل هو تقييم السمية الخلوية وتحديد التركيز النصف المثبط IC50 للدوتاستيرايد والدوكسوروبيسين و5FU . تم تعريض خلايا LNCap لتركيزات مختلفة من هرمون التستوستيرون، والدوتاستيرايد، والدوكسوروبيسين، و5FU لمدة 48 ساعة عند 37 درجة مئوية. أفضل جرعة من هرمون التستوستيرون كانت (7.8 نانوجرام/مل) و IC50 للأدوية الأخرى كانت (75 ميكروجرام/مل، 3 ميكروجرام/مل، و 30 ميكروجرام/مل)، على التوالي. بعد ذلك، ثم تعريضت LNCap لتركيزات مختلفة من الدوتاستيريد بدءًا من IC50 الخاص به مع IC50 من دوكسوروبيسين و IC50 من 5FU وتم إجراء اختبار MTT بعد 48 ساعة.

كان الجزء الثاني من العمل هو تقييم تأثيرات موت الخلايا المبرمج، والمضادة للالتهابات، ومضادات الأكسدة للتستوستيرون، والدوتاستيرايد وحده، ومجموعات دوتاستيرايد المضادة للسرطان. اعتمادًا على نتائج MTT، تم تعريض LNCap لتركيزات مختلفة من هرمون التستوستيرون، ودوتاستيريدي وحده، وتركيزات مختلفة من دوتاستيريدي مع IC50 من دوكسوروبيسين أو 5FU واحتضانها لمدة 48 ساعة وتم تحديد تأثيرات موت الخلايا المبرمج والمضادة للالتهابات ومضادة للأكسدة عن طريق قياس كاسباس 3. ، TNF-alpha، والقدرة الكلية لمضادات الأكسدة، على التوالي في طاف الخلايا باستخدام مجموعات الاليزا.

أظهرت نتائج السمية الخلوية لهرمون التستوستيرون زيادة معنوية عالية (P <0.001) في حيوية الخلية عند التراكيز (3.9، 7.8، 15.625) نانوغرام/مل مع انخفاض معنوي كبير (P <0.001) عند التركيز (500 نانوغرام/مل). أظهرت نتائج السمية الخلوية للدوتاستيرايد وحده انخفاضًا كبيرًا (P <0.001)في حيوية الخلايا عند التركيزات (50، 25.12.5، 6.25، 3.125) ميكروغرام/مل وانخفاضًا ملحوظًا (P <0.05) في قابلية الخلية للخلايا عند التركيز (1.5625) ميكروجرام/مل. كانت نتائج السمية الخلوية لالدوكسوروبيسين و 5FU انخفاضًا كبيرًا جدًا (P <0.001) في قابلية حيوية الخلية في جميع التركيزات. أظهرت نتائج دوتاستيريد مع دوكسوروبيسين IC50 أو مع IC50 لـ 5FU انخفاضًا معتدًا به للغاية (P <0.001) في قابلية بقاء الخلية عند جميع التراكيز مقارنة بالتحكم الإيجابي(IC50) للدوكسوروبيسين أو5FU

كانت نتائج اختبار موت الخلايا المبرمج لهرمون التستوستيرون انخفاضًا كبيرًا (P <0.001) في الكاسبيز 3 عند التراكيز (3.9، 7.8، 62.5) نانوغرام / مل مع زيادة معنوية (P <0.05) عند التركيز 500 نانوغرام / مل مقارنة مع السيطرة. وبالنسبة لدوتاستيريد، كانت النتائج زيادة معنوية للغاية (P <0.001)في كاسباس 3 في جميع التراكيز مقارنة مع السيطرة. كانت نتائج دوتاستيريد مع دوكسوروبيسين IC50 زيادة كبيرة للغاية (P <0.001) في كاسباس 3 في جميع تراكيز دوتاستيريدي مقارنة مع السيطرة الإيجابية (الخلايا المعالجة بـ IC50 من دوكسوروبيسين 3 ميكروغرام / مل) والسيطرة السلبية (الخلايا غير المعالجة). أظهرت نتائج دوتاستيريد مع IC50 لـ 5FU انخفاضًا معنويًا للغاية (P <0.001) في جميع التراكيز مقارنة بالتحكم الإيجابي (الخلايا المعالجة بـ IC50 من 5FU (30ميكروغرام/مل) وزيادة كبيرة جدًا (P <0.001) في جميع التراكيز. مقارنة مع السيطرة السلبية (الخلايا غير المعالجة).

أظهرت نتائج TNF-alpha للتستوستيرون انخفاضاً معنوياً عالياً (p <0.001) في TNF-alpha في جميع التراكيز وبالنسبة للدوتاستيرايد، كانت النتائج انخفاضاً معنوياً عالياً (p <0.001) في-TNF alphaفي جميع التراكيز. أظهرت نتائج دوتاستيريد مع دوكسوروبيسين IC50 انخفاضاً معنوياً عالياً (p <0.001)في TNF-alpha في جميع التراكيز مقارنة مع السيطرة الإيجابية وانخفاضاً معنوياً عالياً (p <0.001) في TNF-alpha عند التركيز (75 ميكروغرام/مل). مع انخفاض معنوي (P <0.05)في TNF-alpha عند التراكيز (18.75، 9.37) ميكروغرام/مل مقارنة مع السيطرة السلبية.

كانت نتائج دوتاستيريدي مع 5 FUانخفاضًا معنويًا للغاية (P <0.001) في TNF-alpha في جميع التراكيز مقارنة مع الضوابط الإيجابية والسلبية.

أظهرت نتائج فحص القدرة الكلية لمضادات الأكسدة لهرمون التستوستيرون زيادة معنوية عالية (P <0.001) عند التراكيز (500، 62.5) نانوغرام/مل، ولم تظهر أي تأثيرات معنوية عند التراكيز الأخرى مقارنة مع السيطرة. أما بالنسبة للدوتاستيراید فقد أظهرت زيادة معنوية (p<0.001) في جميع التراكيز مقارنة مع السيطرة. لم تظهر نتائج الدوتاستيراید مع دوكسوروبيسين IC50 أي تأثيرات معنوية في جميع التراكيز مقارنة مع السيطرة الإيجابية وانخفاض كبير (P <0.001) في جميع التراكيز مقارنة مع السيطرة السلبية. أظهرت نتائج الدوتاستيراید مع 5FU انخفاضاً معنوياً (P <0.05) عند التركيز (9.37 ميكروغرام/مل) مع عدم وجود تأثيرات عند التراكيز الأخرى مقارنة مع السيطرة الإيجابية.

تشير النتائج المذكورة أعلاه إلى وجود تأثيرات مضادة للتكاثر ومضادة للأكسدة ومضادة للالتهابات للدوتاستيراید على خلايا LNCap. أدى الجمع بين دوتاستيريد مع دوكسوروبيسين إلى تحسين تأثيرات دوتاستيراید على حيوية الخلية وموت الخلايا المبرمج والالتهابات ولكن لم يكن له أي تأثير على إجمالي قدرة مضادات الأكسدة. أدى الجمع بين دوتاستيراید و5FU إلى تحسين تأثيرات دوتاستيراید على حيوية الخلية والالتهابات، لكنه قلل من تأثيرات موت الخلايا المبرمج للدوتاستيراید ولم يؤثر على إجمالي قدرة مضادات الأكسدة.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة بابل/ كلية الطب

التأثير المضاد للورم المحتمل لدوتاستيريد على خط خلايا سرطان البروستات

رســـالـــــــة

مقدمـــــة إلـــى مجلس كليـــة الطـــب / جامعـــة بابـــل

كجزء من متطلبـــات نيـل درجـــة الماجستيـر في الأدوية / الادوية والسموم

من قبل

زهراء عبد الحسن جهاد عباس

(بكالوريوس صيدلة ، ٢٠١٧-٢٠١٨)

إشراف

أ.م ماجد كاظم عباس **أ.د. قيصر نعمة مظلوم**

١٤٤٥هـ ٢٠٢٣م