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Antiproliferative Activity of *Moringa oleifera* Ethanolic Extract and Docetaxel on Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) in-Vitro Study on Prostate Cancer Cell Line (LNCaP)

A thesis

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وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا

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Certification

We certify that this thesis entitled **" Antiproliferative Activity of Moringa oleifera Ethanolic Extract and Docetaxel on Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand (TRAIL) in-Vitro Study on Prostate Cancer Cell Line (LNCaP)**was prepared by (Aymen Ahmed Jawad) 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.

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We certify that we have read this thesis entitled **"Antiproliferative activity of *Moringa oleifera* seeds, leaves ethanolic extract and docetaxel on LNCaP cell line and on the level of tumor necrosis factor-related apoptosis-inducing ligand"** and as an examining committee examined the student "Aymen Ahmed Jawad" in its contents and that in our opinion it meets the standard of a thesis for the degree of Master in Pharmacology and Toxicology.

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Dedication

I dedicate this achievement to my family, especially my dear mother, my wonderful wife, and the little princes (Mustafa and Ali).

Aymen

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To begin, praise and thanks to Allah, the Almighty, for His showers of blessings throughout my research work, which enabled me to successfully complete the research.

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Aymen Ahmed Jawad

Summary

Cancer is abnormal cells growth causing disease that is expected to be the leading cause of death worldwide. Prostate cancer is the second most common type of cancer in men (after lung cancer) and the fifth leading cause of death worldwide. Differences in incidence rates reflect differences in diagnostic testing use around the world. Prostate cancer incidence and mortality rates are strongly related to age, with the highest incidence seen in elderly men over the age of 65. While some types of prostate cancer grow slowly and may require little or no treatment, others are aggressive and spread quickly.

Nature has a hopeful role in the development of many compounds that can be helpful in the treatment of various diseases such as cancer which has special interest in medicine. This study aimed firstly to evaluate the effect of the ethanolic extracts of leaves and seeds of Moringa oleifera and docetaxel on the growth of LNCaP cell line each separately and in combination, secondly to estimate the impact of these agents on the level of tumor necrosis factor inducing apoptosis ligand (TRAIL) in LNCaP cell line.

This research was carried out in a post-graduate student research laboratory at the University of Babylon's college of medicine from January 2021 to October 2021.

For the performance of the first aim cells of LNCaP cell line were seeded in 96 well of tissue culture plate and treated with different concentrations (1000, 500, 250, 125, 62.5, 31.25 µg/ml) of each ethanolic extract (seeds and leaves) of Moringa oleifera and docetaxel then incubated for 24 hours, same procedure was done but instead the incubation period was 48 hours. In order to assess the effect of Moringa oleifera extracts on the anticancer activity of docetaxel, the half maximal inhibitory concentration (IC50) of the later was measured, then same procedure was done but the LNCaP cell line was treated with combination of different concentrations of each ethanolic extract (seeds and leaves) of Moringa oleifera plus docetaxel IC50 and also incubated for different periods (24 and 48 hours). After that the MTT cytotoxicity assay was done to measure the viability of the cells by using a microplate reader.

For the measurement of the TRAIL level in LNCaP cells after 24 hours of treatment with ethanolic extract of Moringa oleifera (leaves and seeds), docetaxel, and combination of ethanolic plant extract plus docetaxel IC50. The Sandwich- ELISA kit is used.

Results of this study revealed a highly significant ($P \leq 0.001$) reduction in the viability of LNCaP cell line after the treatment with ethanolic extract of Moringa oleifera seed at both period of incubation in a concentration and time-dependent manner and its IC50 was 600µg/ml. While the ethanolic extract of Moringa oleifera leaves reduced the viability of LNCaP cell line significantly ($P \leq 0.01$) and high significantly ($P \leq 0.001$) after 24 and 48 hours of incubation respectively in a concentration and time-dependent manner, and its IC50 was 700 µg/ml. Docetaxel decreased the viability of LNCaP cell line high significantly ($P \leq 0.001$) in a concentration and time-dependent manner after both periods of incubation, and its IC50 was 423 µg/ml.
Results showed that after 24 hours of incubation the combination of different concentrations of *Moringa oleifera* ethanolic seed extract plus docetaxel IC50 result in significant (P 0.05) reduction in the viability of LNCaP cell line. Whereas the combination of different concentrations of *Moringa oleifera* ethanolic leaves extract plus docetaxel IC50 result in highly significant ($P \leq 0.001$) reduction in the viability of LNCaP cell line .whereas after 48 hours of incubation the combination of different concentrations of *Moringa oleifera* ethanolic seed extract plus docetaxel IC50 result in highly significant ($P \leq 0.001$) .However the combination of different concentrations of Moringa oleifera ethanolic leaves extract plus docetaxel IC50 result in significant ($P \leq 0.01$) reduction in the viability of LNCaP cell line.

In Comparism between *Moringa oleifera* ethanolic extract (leaves & seeds) with docetaxel on LNCaP cell viability for (24 – 48 hrs.) incubation period results showed that after 24 hours of exposure there was insignificant difference ($p \geq 0.05$) between the effect of ethanolic extract of *Moringa oleifera* seeds and docetaxel on the viability of LNCaP cell line, but the ethanolic extract of *Moringa oleifera* leaves show significant difference in effect on LNCaP cell viability , although there was a highly significant ($P \leq 0.001$) difference in the

reduction of the LNCaP cell line viability after 48 hours of exposure to each of the tested agents (leaves , seeds and docetaxel)at all concentrations except 1000 µg/ml at which the difference was insignificant ($p \geq 0.05$).

Regarding the level of TRAIL result showed a highly significant $P \leq 0.001$ and concentration dependent increase in the TRAIL level after 24 hours of LNCaP cell line treatment with ethanolic extracts of *Moringa oleifera* (seeds and leaves) and docetaxel each separately and in combination. In conclusion both seeds and leaves ethanolic extracts of *Moringa oleifera* have antiproliferative and apoptotic (increase TRAIL level) effects against LNCaP cell line, although seeds extract has more activity than leaves extract. The antiproliferative effects of *Moringa oleifera* seeds and leaves ethanolic extracts against LNCaP cell line are less than that of docetaxel.

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List of abbreviations

Abbreviations	Meaning
LNCaP	androgen-sensitive human prostate adenocarcinoma cells
TRAIL	Human tumor necrosis factor related apoptosis inducing ligand
TNF	Tumor necrosis factor
DR5	Death receptor 5
ASR	Age sensitive rate
DHT	Dihydrotestosterone
TGFα	transforming growth factors alpha
TGFβ	transforming growth factors beta
EGF α	epidermal growth factors alpha
EGFβ	epidermal growth factors beta
IGF-I	insulin-like growth factors 1
IGF-II	insulin-like growth factors 2
FGF	fibroblast growth factor
HGPIN	high-grade prostatic intra-epithelial neoplasia
PSA	prostate-specific antigen
SNPs	single nucleotide polymorphisms
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
ACTH	adrenocorticotropic hormone
CRPC	castrate-resistant prostate cancer
DES	Diethylstilbestrol
BCL-2	Anti-apoptotic gen
BAX	Proapoptotic gen
BAK	Proapoptotic gen
P53	a tumor suppressor gene
MOMP	mitochondrial outer membrane permeabilization
SMAC	second mitochondria-derived activator

APAF-1	apoptotic protease-activating factor-1
FADD	Fas-associated death domain
TRADD	TNF-receptor-associated death domain
DISC	death-inducing signaling complex
DED	death effector domain
c-FLIP	anti-apoptotic regulator and resistance factor
IVF	Invitro fertilization
EDTA	Ethylenediamine tetra-acetic acid
ROS	reactive oxygen species
pigo	1 gm = 10, 00000000000 pigo
µg	Micro gram
Hela	Cancer cell line named after Henrietta lacks
g	Gram
mg	Milligram
M.oleifera	*Moringa* oleifera plant
IC50	Half inhibitor concentration
P53	Tumor suppressor gen
BCL2	Atni apoptotic protein

CHAPTER ONE
INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Cancer is abnormal cell growth leading to developed uncontrolled diseases are responsible for the majority of global deaths and cancer is expected to rank as the leading cause of death and the single most important barrier to increasing life expectancy in every country of the world in the 21st century. according to the estimates from the World Health Organization (WHO) in 2015, cancer is the first or second leading cause of death before age 70 years in 91 of 172 countries, and it ranks third or fourth in an additional 22 countries.(Bray, F., Ferlay, J., *et al* (2018).

Many of the naturally derived anti-cancer drugs in current use such as paclitaxel, vincristine, and vinblastine are naturally derived agents. Several wild edible plant species in Thailand have played a prominent role in traditional medicine. *Moringa oleifera* Lam. is one of the most common plants found in Southeast Asia that has been widely used. It is also a valuable plant because it is rich in vitamins, protein, carbohydrate, fatty acid, fiber and phytochemical components (Tragulpak seerojn *et al*., 2017).

1.2 Human tumor necrosis factor related apoptosis inducing ligand (TRAIL).

It is a TNF family member that induces apoptosis in a variety of cancer cells. The TNF family is made up of at least 13 homologous proteins that play important roles in a variety of biological processes such as apoptosis, immunity, inflammation, and development. TRAIL is expressed by a variety of cell types, including natural killer cells, monocytes, and T-cells, as are many other members of this family. TRAIL appears to have very limited cytotoxicity to normal cells, with the exception of activated T-cells, human hepatocytes, human brain cells, and thymocytes (Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) selectively induces apoptosis of tumor cells but not most normal cells) (Lamhamedi-Cherradi *et al*., 2003).

Death receptor 5 (DR5/TRAIL-R2) is an apoptosis-inducing membrane receptor for tumor necrosis factor–related apoptosis–inducing ligand (TRAIL/Apo2L)(Shiraishi *et al*., 2005).

In present study, was showed that ethanolic extract of *Moringa oleifera* (leaves, seeds) and docetaxel, is a potent enhancer of TRAIL-induced apoptosis.

1.3 Aim of Study

1. Study the effects of ethanolic extract of *Moringa oleifera* (leaves , seeds) and docetaxel each separately and in combinations on LNCaP cell line (androgen-sensitive human prostate adenocarcinoma cells) .
2. Study the effect of ethanolic extract of *Moringa oleifera* (leaves, seeds) and docetaxel on the level of tumor necrosis factor related apoptosis induced ligand (TRAIL).

1.4 Study Design

Experimental study (in vitro study on human cancer cell) (LNCaP cells line).

1.5 Prostate cancer

Prostate cancer is the second most common cancer in men and the fifth leading cause of death globally. Early-stage prostate cancer may be asymptomatic and have an indolent course that requires only active surveillance. According to GLOBOCAN 2020 estimates (global cancer statistics of incidence and mortality worldwide for 36 cancers in 185 countries), 1,408,900 new cases of prostate cancer were reported globally, with developed countries having a higher prevalence. Differences in incidence rates around the world reflect differences in diagnostic testing use. The incidence and mortality rates of prostate cancer are strongly related to age, with the highest incidence seen in elderly men over the age of 65. (Rawla, 2019). However, while some types of prostate cancer grow slowly and may need minimal or even no treatment, other types are aggressive and can spread quickly.

1.6 Epidemiology

In recent years, the global incidence of prostate cancer has increased. The prevalence of prostate cancer varies by region and population. In 2020, there were 1,408,900 new cases of prostate cancer worldwide, accounting for 7.3 percent of all cancers cases (Sung H, Ferlay J, Siegel RL, *et.al* 2021). Globally, the incidence of prostate cancer varies greatly. The rate based on age (ASR). Even in Asian countries where the incidence was previously low, this trend has been increasing. However, the accuracy of data on prostate cancer incidence and mortality in some Asian countries is limited. The reasons for this rising trend are multifaceted. Changes in lifestyle as a result of more Western influence diets are one possible explanation. The prevalence of cancer is also statistically skewed due to the widespread implementation of early detection systems and the accuracy of national cancer registration systems, both of which are still in their infancy in most Asian countries. Since the 1990s, there has been a decrease in mortality rates in Australia, New Zealand, and Japan, which could be attributed to advancements in treatment and/or early detection efforts. However, in the majority of other Asian countries, this rate is rising. Less biased information is provided by studies of latent and incidental prostate cancer. The prevalence of latent and incidental prostate cancer in modern Japan and Korea is comparable to that in Western countries, indicating that lifestyle changes have an impact on carcinogenesis. Many studies have found evidence of both congenital and acquired risk factors for prostate cancer carcinogenesis. Recently acquired risk factors may be linked to the rising prevalence of prostate cancer in Asian countries. This trend may continue, particularly in developing Asian countries (Kimura and Egawa, 2018).

1.7 Pathophysiology

Prostate cancer has long been regarded as an unpredictable illness that makes creating sound therapeutic decisions and evaluating the outcomes of various types of treatment difficult (Ilic *et al*., 2013). Androgens regulate the growth and differentiation of cells in the prostate. Free testosterone diffuses into the epithelial cells of the prostate, where it is converted to dihydrotestosterone (DHT). Various growth factors, such as transforming growth factors alpha and beta (TGFα and TGFβ), epidermal growth factors (EGF α and EGFβ) insulin-like growth factors (IGF-I and IGF-II), and some fibroblast growth factor (FGF) members (Rne *et al.*, 1996). Are found in stromal cells, and their interaction with epithelial cells can influence cell proliferation and growth. Prostate cancer usually develops slowly and gradually, with cellular structure changing from normal to dysplastic to cancerous. HGPIN (high-grade prostatic intra-epithelial neoplasia) is a precancerous stage of cellular proliferation in prostate cells that can be detected through biopsy. It can be 10 years or more before carcinoma. Although there is no link between prostate cancer and benign prostate hyperplasia, the two conditions can coexist (M. Martinez and M. Satheesh, chapter 48, page 753, Clinical Pharmacy and Therapeutics, n.d.-a).

Cancer metastasis is frequently caused by multiple competing subclones within a single primary tumor. This evolutionary process culminates in the formation of metastases, which account for 90% of cancer-related deaths. Despite its clinical importance, little is known about the principles governing cancer cell dissemination to distant organs. Although it is widely accepted that each metastasis is caused by a single tumor cell (Gundem *et al*., 2015).

1.8 Signs and Symptoms

Prostate cancer can be asymptomatic in the early stages and has an indolent course, requiring little or no treatment. However, the most common complaint are difficulty urinating, increased frequency, and nocturia, all of which can be caused by prostatic hypertrophy. Because the axis skeleton is the most common site of bony metastatic disease, more advanced stages of the disease may present with urinary retention and back pain (Wu and Bratton, 2013).

1.9 Diagnosis

Many prostate cancers are detected on the basis of elevated plasmatic levels of prostate-specific antigen (PSA > 4 ng/mL), a glycoprotein normally expressed by prostate tissue, which plays a critical role in clinical and patient care. However, because men without cancer have been found to have elevated PSA levels, a tissue biopsy is the standard of care for confirming the presence of cancer (Descotes, 2019).

1.10 Risk factors

Age, family history, and genetic susceptibility are all factors to consider.

1.10.1. Age

Clinically detected prostate cancer is uncommon before the age of 40, but after that, the incidence rises faster than that of any other cancer and continues to rise into the ninth decade of life. Histologic evidence of invasive cancer can be found in men's prostates as early as their third decade of life, and its prevalence rises dramatically with age, reaching 50% to 60% by the age of 90. Morbidity and mortality from prostate cancer will impose increasing burdens in developing countries as life expectancy rises around the world (Cancer Principles and Practice of Oncology 11th Ed. 2018, n.d.)

1.10.2 Genetic Susceptibility and Family History

A man's chances of developing prostate cancer are increased if he has a family history of the disease. When a family member is diagnosed with breast or prostate cancer, the risk is similar. When compared to the general population, men with a first-degree relative with prostate cancer have a 2- to 3-fold increased risk, and those with two or more first-degree relatives with prostate cancer have a 5- to 11-fold increased risk. Despite this, familial factors are thought to play a role in only 11% of prostate cancers, although twin studies suggest that inherited factors may be involved, in as many as 42% of all cases Although genome-wide association studies have linked more than 70 risk alleles (single nucleotide polymorphisms [SNPs]) to prostate cancer, only a few are linked to the risk of aggressive or lethal cancer. Many of these SNPs are found in genes that code for PSA or related kallikreins, which are widely used for diagnosis. The increased risk for these SNPs is aiding in the diagnosis of prostate cancer rather than metastasis or death from the disease. (Cancer Principles and Practice of Oncology, 11th Edition, n.d.)

1.11 plan of prostate cancer treatment

The following factors concerning the tumor and the patient must be considered in order to make an informed treatment decision:

1.Tumor staging findings.

2.Histology.

3.PSA concentration and Gleason score

4.At the time of diagnosis, the patient was of a certain age.

5.The presence of co-morbidities such as cardiovascular disease, chronic obstructive pulmonary disease, and diabetes, as well as life expectancy.

1.11.1 Staging and Grading

The Gleason score is the most commonly used histopathological scoring system for prostate cancer. Grade 1 tissue is well-differentiated, low grade dysplastic tissue, whereas Grade 5 tissue is the most abnormal, dysplastic tissue. The sum of the scores from the most representative pathological samples yields a total score. While scores ranging from 2 to 10 are possible, scores lower than 6 are uncommon. The Gleason score is then used to categorize prostate cancers as low-grade (six), intermediate-grade (seven), or high-grade (eight) (8-10). The risk is then stratified based on grade group, PSA, and clinical stage (Tumor Node Metastasis staging) (Barsouk *et al.*, 2020).

1.11.2 Active surveillance with a curative intent

(Localized prostate cancer Well-established treatments)

Active surveillance (AS) is celebrated as the priority treatment for low-risk prostate cancer. However, no confirmed clinical tools are immediately present to standardize the frequency of biopsies. Genomic scores and PSA density (level of PSA/volume of prostate) are risk factors for biopsy upgrading within 3 years of starting AS (Lu *et al.*, 2019).

1.11.3 Surgical therapy

- **Radical Prostatectomy**

  The preferred treatment is radical prostatectomy, which involves the complete removal of the prostate, seminal vesicle, distal vasa, ejaculatory ducts, and prostatic urethra. Blood loss, postoperative thromboembolism, urinary incontinence, impotence, and rectal injury are the most common complications of prostatectomy (Yaxley *et al*., 2016).

- **Brachytherapy in the interstitial space**

  Interstitial brachytherapy involves the placement of radioactive isotope seeds in the prostate under ultrasound or fluoroscopic guidance. These implants, which can be temporary or permanent, can emit low-energy radiation over a period of several weeks. This treatment may have the advantage of causing less erectile dysfunction than alternative therapies (Hannoun-Lévi, 2017).

- **Cryotherapy**

  Prostate cryosurgical ablation (CSAP). Cryosurgical ablation involves freezing the prostate, which causes cell death through protein denaturation, membrane rupture, and apoptosis(Carter *et al*., 2013).

1.11.4 Radiotherapy

- **External-beam radiotherapy**

  Radiotherapy is used to treat diseases that are contained locally and, more recently, to treat disease states that are locally advanced. The prostate is targeted with high-energy photons produced by a linear accelerator in this treatment. Occasionally, special particles such as protons and heavy ions are used because they have a dose distribution advantage over photons. Impotence, genitourinary stricture, rectal bleeding, hematuria, and incontinence are all possible side effects of radiotherapy.

- **Ablation of interstitial tumors with radiofrequency (RITA).**

A needle electrode is inserted into the prostate and radiofrequency waves are used to heat the tissue to 100 degrees Celsius, resulting in necrosis (Parker *et al*., 2018).

1.11.5 Hormone therapy/androgen deprivation therapy for prostate cancer that has progressed locally

There is an increased risk of relapse and lymph node metastasis following prostatectomy in locally advanced disease involving areas outside the capsule. The testes (95 percent) produce androgens in response to pituitary gland stimulation of luteinizing hormone (LH) and luteinizing hormone-releasing hormone (LHRH), while the adrenal glands (5 percent) produce androgens in response to adrenocorticotropic hormone (ACTH). Androgens are hormone precursors

produced by the adrenal gland. They are enzymatically converted to testosterone and dihydrotestosterone in prostatic and peripheral tissue. Because testosterone is a well-established etiologic factor in prostate cancer, testosterone deprivation can be used as part of treatment. This can be accomplished in practice through surgical or medical castration, with response times ranging from a few months to years. Numerous drugs can be used to reduce testosterone levels during medical castration. These medications include the following:

- **Agonists for the release of luteinizing hormone (Medical Castration)**
    - leuprolide (Lupron, Eligard)
    - goserelin (Zoladex)
    - Triptorelin (Trelstar)
    - Histrelin (Vantas)

    The hypothalamus releases LHRH, which stimulates the secretion of LH by binding to LHRH receptors in the pituitary. Normally, this LHRH receptor complex is degraded by enzymes, releasing LH and releasing the receptors for further LHRH binding. LHRH agonists are synthetic analogues that bind to the LHRH receptor and form an enzymatically inactive complex. As a result, they maintain a constant presence on the receptor and render the pituitary gland insensitive to hypothalamic regulation in comparison to normal individuals' pulsatile release. Continuous administration of LHRH agonists results in a biphasic response, with an initial increase in LH and testosterone release dubbed a "tumour flare up," followed by a decline over the next 1–2 weeks due to LHRH receptor downregulation. Patients with metastatic prostate cancer typically respond well to treatment with LHRH

agonists, which are used in the same manner as described above for locally advanced disease(Ryan *et al*., 2013).

- **Antagonists of luteinizing hormone-releasing hormone.**

LHRH antagonists include the following:

- abarelix
- Degarelix

The LHRH antagonist works by competitively binding to LHRH receptors in the pituitary, inhibiting LH release and resulting in a rapid and sustained decline in testosterone levels. Unlike LHRH agonists, they do not cause an initial spike in testosterone levels, and thus do not result in a "tumour flare-up" or the need for short-term anti-androgen therapy. Abarelix is administered through an induction regimen followed by monthly injections, and its use has been linked to anaphylactic reactions. Degarelix is administered as a monthly depot injection; the most frequently encountered adverse reaction is a local reaction at the injection site, such as swelling and erythema. Degarelix may be used in patients with high-risk prostate cancer who are experiencing acute symptoms of spinal cord compression, ureteric obstruction, or urinary retention. Degarelix decreases testosterone, LH, and PSA levels more rapidly than LHRH agonists and inhibits testosterone microsurges. In patients with metastasis and imminent spinal cord compression, the European Association of Urology (EAU) prostate cancer guidelines recommend the use of LHRH antagonists(Heidenreich *et al*., 2001) .

- **Androgen antagonists**

Anti-androgens are drugs that bind to androgen receptors and inhibit androgen binding, thereby promoting tumor growth. Anti-androgens are occasionally referred to as androgen receptor antagonists (Student *et al*., 2020).

Anti-androgenic medications include the following:

- flutamide (Eulexin)
- Bicalutamide (Casodex)
- Nilutamide(Nilandron)

They are frequently taken as pills on a daily basis (Dorff & Crawford, 2013). Enzalutamide (Xtandi), apalutamide (Erleada), and darolutamide are newer anti-androgens (Nubeqa). These medications may be beneficial for men who have non-metastatic prostate cancer but are no longer responding to other forms of hormone therapy (commonly referred to as non-metastatic castrate-resistant prostate cancer) (CRPC). The FDA has approved enzalutamide for the treatment of metastatic castration-resistant or castration-sensitive prostate cancer (Ritch and Cookson, 2016). Apalutamide is approved for the treatment of metastatic castrate-sensitive prostate cancer. Each day, these medications are taken as pills (Student *et al*., 2020).

- **Adjunctive palliative care**

Prostate cancer that is resistant to castration Androgen deprivation therapy may fail to slow the progression of prostate cancer. The reason for this is unknown,

but it could be explained by the clonal selection or adaptation hypotheses. According to the clonal selection hypothesis, the basal cells are the prostate's stem cells and generate secretory epithelial cells. While secretory epithelial cells go through apoptosis in response to androgen deprivation. With basal or stromal cells, this is not the case. As a result, these androgen-independent cells survive preferentially within the tumor. According to the adaptation hypothesis, androgen independence may be an inherent but dormant property of some prostate cells that is activated during androgen deprivation. Whatever the reason, when first-line therapy fails, a single or combination therapy that includes a second-line hormone treatment (oestrogens), corticosteroids, ketoconazole, or a combination of these medications can improve quality of life.

1.11.6 Chemotherapy and bisphosphonates for a palliative response in the short term

- **Oestrogen** Diethylstilboestrol (DES) is a synthetic oestrogen that exerts a negative feedback effect on the hypothalamus and anterior pituitary, inhibiting the secretion of LHRH and thus testosterone production.Diethylstilboestrol is the least expensive of the synthetic oestrogens and is associated with fewer hot flushes and psychological trauma than surgical oestrogen replacement. Gynecomastia, loss of libido and potency, oedema, nausea, and vomiting are all possible side effects of DES, as is an increased risk of thromboembolism. It is typically administered once daily at a dose of 1 mg to minimize the risk of cardiovascular side effects, as it has been associated with an increase

in cardiovascular mortality (M. Martinez and M. Satheesh, chapter 48, page 762, (*Clinical Pharmacy and Therapeutics*, n.d.).

- **Bisphosphonates:**

Skeletal involvement in prostate cancer can occur as a result of the disease or as a side effect of androgen depletion therapy. Bisphosphonates are pyrophosphates that prevent and treat bone lesions by inhibiting osteoclast activity in bones. Pamidronate , denosumab are prevents bone loss, but zolendronate not only increases bone mass during androgen depletion therapy, but also reduces skeletal complications in patients with prostate cancer-related bone metastasis. Bisphosphonates are also recommended for pain relief in patients who have failed to respond to analgesics and palliative radiotherapy to the bone. Prior to initiating treatment with a bisphosphonate, a dental examination should be performed due to the risk of developing osteonecrosis of the jaw in those with a history of dental trauma, infection, or surgery(Saad & Schulman, 2004).

- **Corticosteroids**

Both prednisolone and hydrocortisone are anti-inflammatory medications that can alter the body's immune response to a variety of stimuli. They are frequently used in conjunction with other treatments (Dorff and Crawford, 2013).

- **Chemoprevention**

Chemoprevention is defined as the use of micronutrients, dietary supplements, or pharmaceutical agents to prevent or delay the progression of prostate cancer.

- **Diet**

Numerous studies have been conducted on a variety of products. Soy-containing foods, green tea, pomegranates, and omega-3 fatty acids are currently popular in the diet. Selenium and vitamin E are no longer regarded as beneficial. Similarly, vitamin D and lycopene, which are found in tomatoes, have fallen out of favor due to a dearth of beneficial effects(Yendapally and Sikazwe, 2019)

- **Drugs**

Five-Reductase inhibitors, which are currently used to reduce the size of the prostate in benign prostate hyperplasia due to their inhibitory effect on the production of dihydrotestosterone, are thought to have chemopreventive potential. Dutasteride, on the other hand, appears to increase the risk of cardiac failure and is therefore not routinely used in chemoprevention(Rittmaster *et al*., 2008). Dutasteride has been shown in studies to reduce the risk of prostate cancer and improve outcomes in patients with benign prostate hyperplasia (Schmidt & Tindall, 2011).

- **Docetaxel**

Docetaxel is a semisynthetic taxane derived from the European yew tree (Trevor *et al*., n.d.). Docetaxel has been the mainstay of chemotherapy that has been used for prostate cancer with cabazitaxel as second-line therapy. Varying combinations with docetaxel have been attempted but not found to be successful. Changes in the treatment landscape with establishment of docetaxel earlier in the disease course have made it the default standard of care for metastatic hormone-sensitive or castration-sensitive prostate cancer along with androgen deprivation therapy (*Enhanced Reader*, n.d.).It is utilized with prednisone or prednisolone when the malignant growth cannot be treated by decreasing the formation of testosterone (castration-resistant prostate cancer). Also, it can be utilized in

conjunction with androgen-deprivation treatment (hormone-sensitive prostate cancer) (Mukherji *et al*., 2020).

The cytotoxic activity of docetaxel exerted by promoting and stabilizing microtubule assembly leading to a significant decrease in free tubulin, needed for microtubule formation, which results in inhibition of mitotic cell division between metaphase and anaphase of cancer cell thus preventing further cancer cell progeny (Lyseng-Williamson *et al*., 2005). Because microtubules do not disassemble in the presence of docetaxel they accumulate inside the cell and cause initiation of apoptosis. LNCaP cell line is highly sensitivity to docetaxel than another type of prostate cancer cell lines like DU145 and PC3 cells line(Liu *et al*., 2013).

Chemical structure

Docetaxel is a clinically well-established anti-mitotic chemotherapy medication used mainly for the treatment of breast, ovarian, prostate cancer and non-small cell lung cancer. Docetaxel reversibly binds to tubulin with high affinity in a 1:1 stoichiometric ratio(Wishart *et al*., 2018).

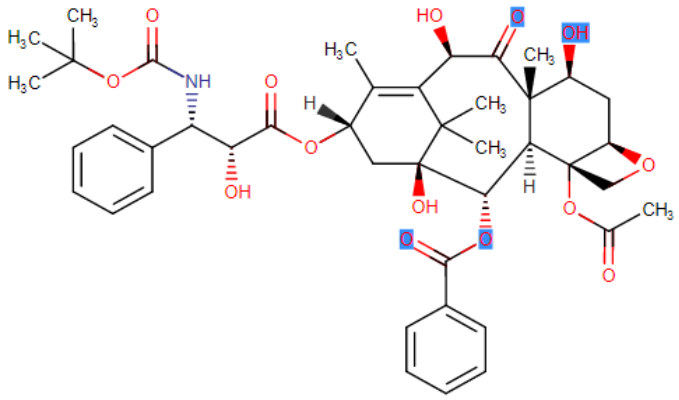

Figure (1.1) Chemical structure of docetaxel Weight Average: 807.8792 Monoisotopic: 807. 346605409, **Chemical Formula** $C_{43}H_{53}NO_{14}$ (Wishart *et al*., 2018)

- **Cabazitaxel**

Cabazitaxel is a semi-synthetic taxane produced from yew needles (Yew "Taxus baccata" an ornamental tree, the taxine alkaloids contained in yew berries, needles or bark are poisonous). Cabazitaxel attached to tubulin and stabilizes microtubules, it is sensitive to docetaxel-sensitive and docetaxel resistant tumors. Cabazitaxel lipophilic in nature so it is poorly absorbed orally, it is cross blood brain barrier easily, extensively metabolized by the liver, and eliminated mainly with feces.

It is used for the treatment of patients with castration resistant (hormone refractory) metastatic prostate cancer in combination with prednisone or prednisolone who have been previously treated with a docetaxel containing regimen (Muñoz-Rodríguez *et al*., 2019).

1.12 Apoptosis

Apoptosis, the cell's natural mechanism for death, is a highly regulated process that serves to eliminate any unnecessary or unwanted cells while also playing a critical role in development and homeostasis within the body. Apoptosis is thought to be a promising target for anticancer therapy. It is made up of two paths (intrinsic & extrinsic pathway) Caspases are used in both of these pathways to carry out apoptosis by cleaving hundreds of proteins.

The apoptotic pathway is typically inhibited in cancer through a variety of mechanisms, including overexpression of antiapoptotic proteins and underexpression of proapoptotic proteins, which can result in intrinsic resistance to the most commonly used anticancer drugs. To combat this resistance, new anticancer plant-derived compounds have been developed. These compounds exhibit anticancer activity by activating the apoptotic pathway (Pfeffer and Singh, 2018).

1.12.1 Intrinsic Pathway

The mitochondria and mitochondrial proteins are used in the intrinsic mechanism of apoptosis (Figure 2). This pathway can be activated by cells with damaged DNA or oncogenes that are overexpressed. Other stimuli for this pathway include:

Growth factor deficiency, excess Ca2+, oxidants, and microtubule-targeting drugs. The BCL-2 protein family regulates the entire pathway. Various apoptotic stimuli cause an increase in the expression of BH3-only proteins, which then activate both BAX and BAK. BAX is controlled by p53 (a tumor suppressor gene). BAX and BAK oligomerize after activation, resulting in mitochondrial outer membrane permeabilization (MOMP). MOMP is the defining event of intrinsic apoptosis and the point of no return. Permeabilization allows for the release of intermembranous proteins such as cytochrome c, the second mitochondria-derived activator of caspase (SMAC), and Omi. The apoptosome is formed from cytochrome c, apoptotic protease-activating factor-1 (APAF-1), dATP, and procaspase-9 after cytochrome c is released. Procaspase-9 is converted within the apoptosome into caspase-9, which activates the executioner caspases-3 and 7. Executioner caspases rapidly begin to degrade proteins, resulting in cell death (Pfeffer and Singh, 2018).

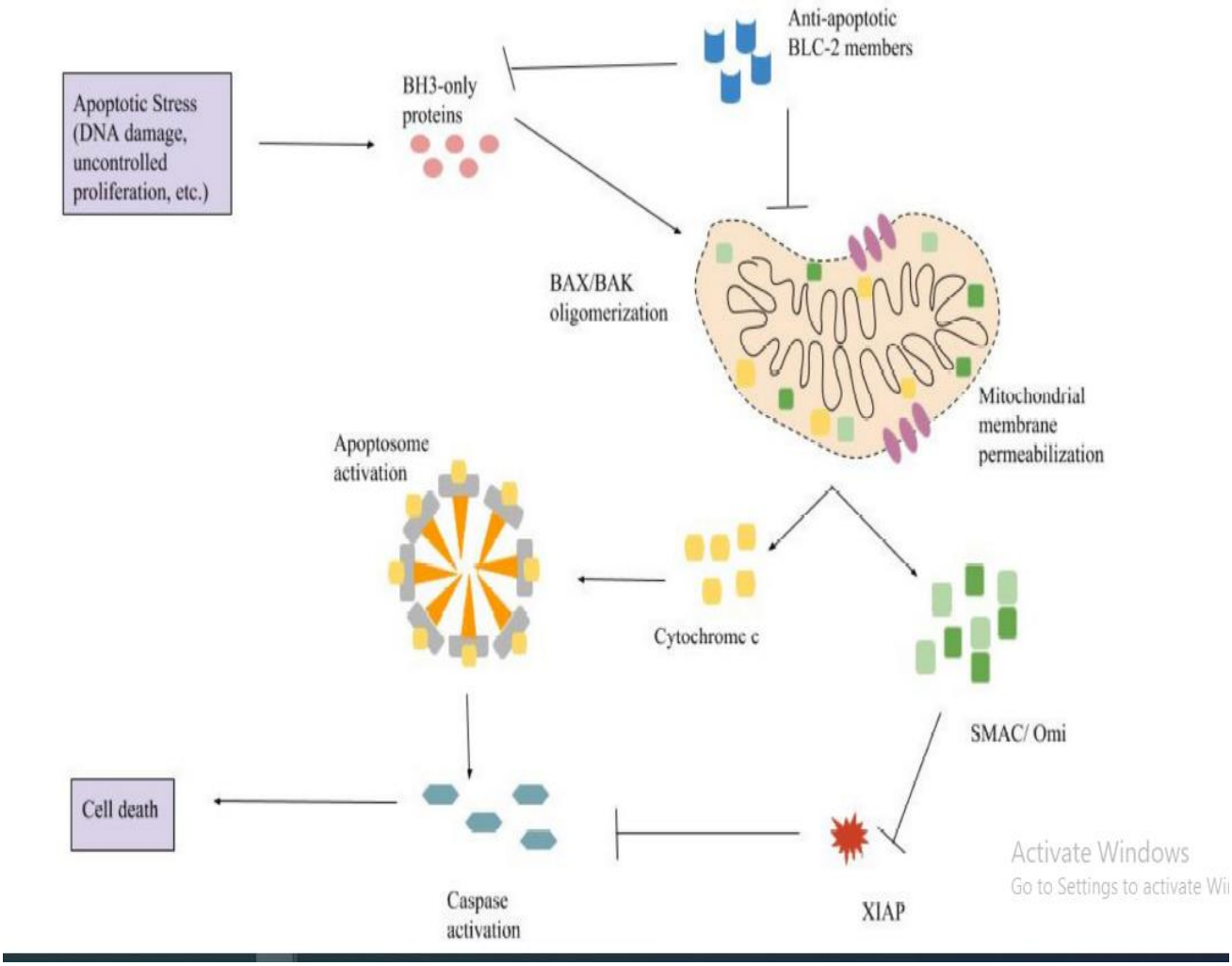

Figure 1.2. In response to apoptosis, the pathway of intrinsic apoptosis BH3-only proteins is upregulated.

apoptotic stress They cause BAX (BCL-2-associated X protein) and BAK (BCL-2 homologous antagonist killer) to oligomerize and permeabilize the mitochondrial membrane. Procaspase-9, dATP, cytochrome c, and APAF-1 are released, and the apoptosome is formed from procaspase-9, dATP, cytochrome c, and APAF-1. Caspases are then activated, and cellular proteins are cleaved, resulting in apoptosis. The activation is represented by arrows, while the inhibition is represented by T bars.

1.12.2 Extrinsic Pathway

Extracellular signals are used by the extrinsic pathway to induce apoptosis (Figure 3). Cell death signals, also known as death ligands, bind to death receptors of the tumor necrosis factor (TNF) family (Zaman *et al*., 2014).

Among the death ligands are

TNF-related apoptosis-inducing ligand (TRAIL), Fas ligand (Fas-L), Tumor necrosis factor (TNF).

The death receptor makes use of an adaptor protein. Among the adaptor proteins are TNF receptor-associated death domain (TRADD) Fas-associated death domain (FADD) .

The death-inducing signaling complex is formed when initiator procaspases-8 and -10 bind to the adaptor protein (DISC). Procaspases contain a death effector domain (DED) that binds to the adaptor protein's DED. DISC activates procaspases-8 and -10. Executioner caspases-3,-6, and -7 are then activated, causing protein and cytoskeleton cleavage and cell death. The inhibitor c-FLIP, which is homologous to caspase-8 but lacks caspase activity, regulates DISC (Pfeffer and Singh, 2018). (c-FLIP) is a master anti-apoptotic regulator and resistance factor that inhibits tumor necrosis factor- (TNF-), Fas-L, and TNF-related apoptosis-inducing ligand (TRAIL)-induced apoptosis in malignant cells, as well as chemotherapy-induced apoptosis (Safa, A.R.,2012).

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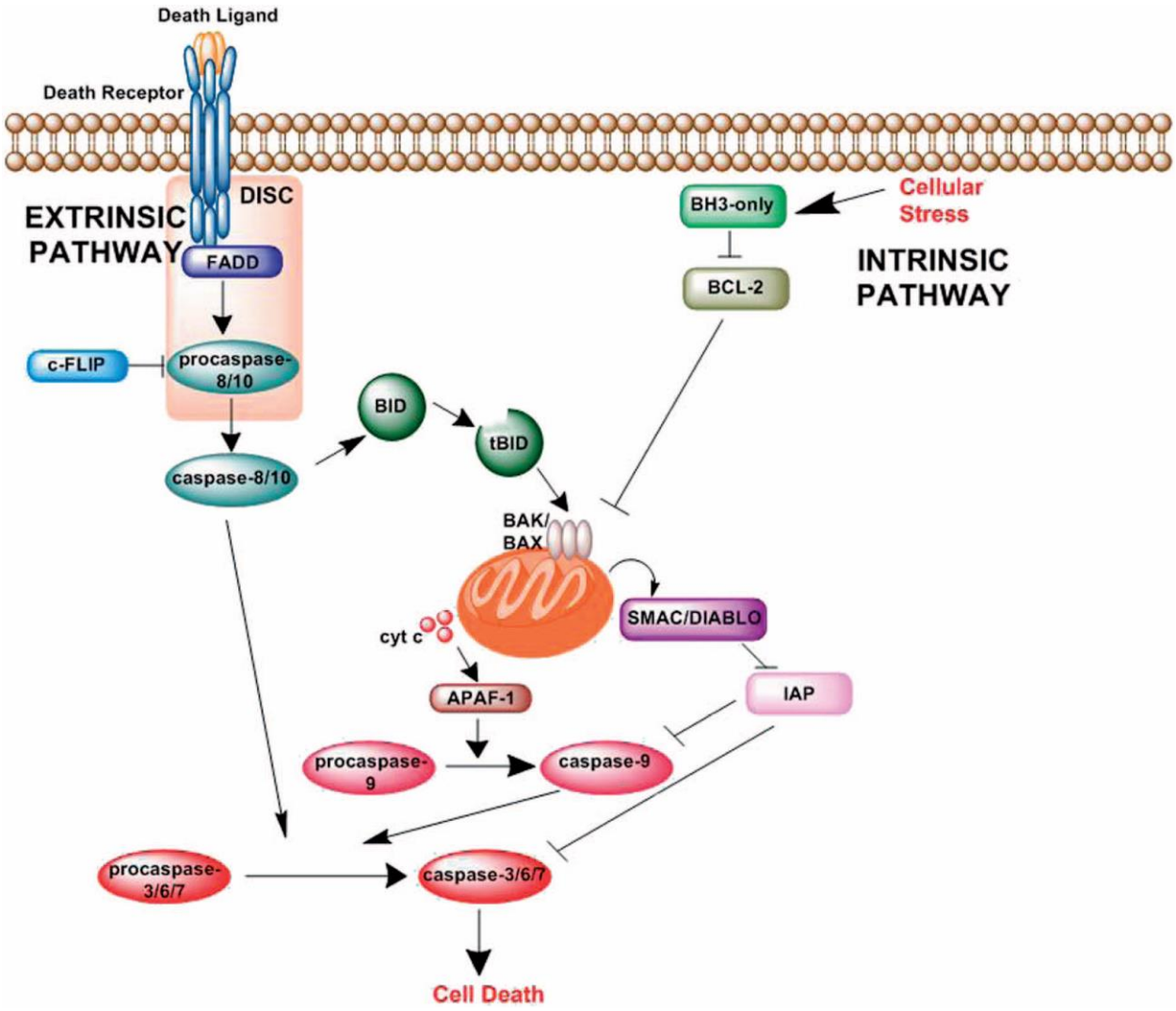


Figure1.3. Apoptosis is a cell-death process. There are two types of apoptosis: intrinsic and extrinsic. The intrinsic pathway is activated by cellular stress.

When activated, BH3-only proteins inhibit antiapoptotic BCL-2 proteins while inducing BAK/BAX oligomerization, mitochondrial permeabilization, and the release of cytochrome c (cyt c) and SMAC/DIABLO. Cytochrome c forms a complex with caspase-9 and APAF-1, resulting in caspase-9 activation. Caspase-9 causes cell death by activating the executioner caspases (caspases-3,-6, and-7). Death signals, which are mediated by death ligands, activate the extrinsic pathway. The death ligand activates the death receptor, which forms the deathinducing signaling complex (DISC) and sends a signal to caspase-8 and -10. Caspase-8 and Caspase-10 both activate Caspase-3. The extrinsic pathway communicates with the intrinsic pathway in some cells via caspase-8-mediated truncation of BID to tBID. Through the mitochondrial pathway, tBID activates BAK/BAX oligomerization and induces apoptosis.

1.13 Apoptotic Changes in Cancer

A variety of mechanisms are used by cancer cells to avoid apoptosis. Deviation from the normal pathways can result in either prosurvival or proapoptotic adjustment. Regulation prosurvival genes, while not classified as such, are potentially oncogenic and can be mutated to increase their expression. Proapoptotic genes, on the other hand, may function as tumor suppressors. All of the inhibitors and activators were discovered in cancer cell lines outside of their normal range of expression. BCL-2 expression, for example, is elevated in nearly half of all human cancers. To kill cancer cells, the vast majority of traditional anticancer drugs dependent mechanisms (Yip & Reed, 2008). If this mechanism is disrupted or altered in any way, drugs fail and develop intrinsic chemoresistance. Furthermore, the chemotherapy cut-off.

Resistance to chemotherapy or radiotherapy as a result of apoptosis defects. Because the immune system depends on apoptosis, altered apoptotic signaling pathways promote immune system resistance (Hassan *et al.,* 2014).

1.14 Proapoptotic Regulation in Tumor Cells

Despite their usual evasion of apoptosis, cancer cells can produce apoptotic signals. Cancer cells are "predisposed to apoptosis," meaning they are more likely than normal cells to initiate it. Apoptotic signals are more sensitive in primed cells (Lopez and Tait, 2015). The simultaneous upregulation of proapoptotic and antiapoptotic proteins causes priming (Elkholi *et al*., 2014). Proapoptotic proteins can cause apoptosis if antiapoptotic proteins are not upregulated.

Using an antiapoptotic protein inhibitor on primed cells may cause apoptosis and tumor cell death. Furthermore, environmental stresses like nutrient deficiency or hypoxia make cancer cells more prone to apoptosis (Lopez & Tait, 2015). In

general, tumor cells are more sensitive to the extrinsic pathway than the intrinsic one, indicating that the extrinsic pathway should be targeted for cancer therapy. Other oncogenes and tumor suppressors may also influence apoptosis, explaining evasion. The tumor suppressor p53 triggers transcription.Anti-apoptotic BCL-2 proteins Apoptotic evasion caused by a tumor suppressor mutation requires a different apoptotic pathway activation. This knowledge can help predict the most effective cancer therapy target mechanism (Elkholi *et al*., 2014).

1.15 Cell line

Cell lines are widely used in both academic and industrial settings for experimental biomedical research. The mouse L cells were the first immortalized cell line to be established in 1943, but for almost everyone, the dawn of the era of cell lines is associated with George Otto Gey's establishment of the HeLa cell line from Henrietta Lacks' cervical tumor in 1951. These cells were considered dangerous because they were obtained from a biopsy taken from a cervix sore as part of the discovery of malignant growth. Despite everything, there is a disagreement about the arrangement of these cells. Since then, the world of cell lines has expanded exponentially, not only in terms of the number of cell lines available, but also in terms of their variability. The discovery of hybridomas, hybrid cell lines that produce monoclonal antibody , by Milstein and Kohler in 1975; the derivation of embryonic stem cell (ESC) lines from mice in 1981 and from humans in 1984; and, finally, the development of induced pluripotent stem cells (iPSCs) by Takahashi and Yamanaka in 2006. All of these advancements have contributed to the increased usefulness of cell lines as reagents in laboratories, and we recently

estimated (unpublished results) that there are approximately 2 million publications that use cell lines

(Bairoch, 2018). Primary culture refers to culture obtained from primary tissue. When a primary culture is transferred to another culture vessel, it transforms into a cell line. Adherent cultures, the cells were isolated and subdivided using a protease, such as trypsin, and a chelating agent, such as EDTA — this procedure was known as passaging. The way of life was incorporated into new culture vessels for cells that will develop in suspension. Under these conditions, specific culture conditions are used, and a moderately uniform population of proliferative cells was chosen within a couple of entries. This population was most likely delegated specialist of the cells that partition when the starting point tissue is endured, and will continue developing until the end of the normal proliferative life expectancy was reached and senescence occurs. To the extent that cells multiply, they exhibit near-zero tissue-explicit separation law. In any case, if the appropriate signs are present, they may be able to recover a useful tissue. Cell lines are cultures obtained from primary subculture, whereas sub clones are obtained from continuous culture or from sections of cell lines.

(Athukoralalage *et al*., 2019).

1.15.1 Cell culture

Animal and human cell cultures are now important tools used in many fields of life science. Cell culture variants are used in disease modeling, IVF technology, stem cell and cancer research, monoclonal antibody production, regenerative medicine, and therapeutic protein production. All of these different scientific approaches would not be possible without some critical discoveries made over the centuries, ranging from Aristotelian spontaneous generation doctrine to Pasteur's experiments and Carrel's cell culture, to largescale cultures for therapeutic protein production

and a vision of the future of regenerative medicine and in situ bioprinting of wounds. The conditions and physical-chemical properties of the environment for the growth and maintenance of human and animal cell cultures were established based on various experiments on cells cultured in vitro to obtain a typical environment for cell growth that mimics the normal environment and conditions in the cell's original site(Jedrzejczak-Silicka, 2017).

1.15.2 Types of cells or cell line classification

Finite cell lines are those that lose their ability to isolate after a certain period of time. These cell lines, for example, have a limited lifespan. Normally, finite cell lines contain cells that can separate 20–100 times before losing their ability to separate (for example, the population multiplies by 20–100 times). The degree of population multiplying is affected by a number of factors, including cell genealogy, cell type, origin, species, culture condition, and so on. Human cell lines have been observed to multiply 50–100 times before eradication, whereas mouse cell lines partition 20–30 times before eradication. Consistent subculturing of cells in free culture or treatment of cells with cancer-causing agents (synthetics), oncogenic infections, and so on causes changes in phenotypic qualities, specifically morphology, which can adjust cells and lead to the advancement of cells that are faster than typical cells. Cell lines derived from these modified cells have an infinite lifespan. Such cell lines are frequently referred to as infinite cell lines. These cell lines, unlike the cell strains from which they were derived, are immortal, altered, and tumorigenic (Bhatia *et al*., 2019).

1.15.3 LNCaP Prostate cancer cell line

The LNCaP cell lines are considered an example of prostate cancer cell lines, where LNCaP cells are a human cell line that is commonly used in oncological research. The LNCaP cells are androgen-sensitive human prostate adenocarcinoma cells isolated from a 50-year-old Caucasian male's left supraclavicular lymph node metastasis in 1977. They are adherent epithelial cells that grow in aggregates as well as single cells(Delafield *et al*., n.d.).

1.16 *Moringa olifera*

Moringa oleifera Lam. is a member of the Moringaceae family of cruciferous plants. *Moringa oleifera* is commonly referred to by locals as horseradish tree, bean oil tree, drumstick, miracle tree, sohanjna, and mother's best friend, and it is a popular staple in various parts of the world. *Moringa oleifera* fruits are pendulous, linear, three-sided pods that range in length from 250 to 450 mm and contain approximately 20 globular seeds. Raw or roasted, the seeds can be added to curries or steeped in tea or other beverages. Prefers sandy or loamy soil with a pH of 6.5-7.5, a temperature range of 25°C-35°C, and 250-300 cm of rainfall. It is frequently recommended as famine food due to its high resistance to drought, which accounts for its tuberous roots (Padayachee and Baijnath, 2012). Along with the pods, the flowers are white in color and can be consumed as vegetables (Chhikara *et al*., 2019) . *Moringa oleifera* is consumed for its nutritional value as well as its medicinal properties. *Moringa oleifera* has been shown to enhance a wide variety of biological functions, including anti-inflammatory, anti-cancer, hepatoprotective, and neuroprotective functions. Additionally, numerous studies have demonstrated its therapeutic value in the treatment of diabetes, rheumatoid arthritis,

atherosclerosis, infertility, pain relief, and depression, as well as diuretic and thyroid regulation (Kou *et al*., 2018).

This pictures for *Moringa oleifera* leaves & seeds used in current study

1.17 Bioactive Components in *Moringa oleifera*

- **vitamins**

M. oleifera's fresh leaves are an excellent source of vitamin A (Michel *et al*., 2008). Vitamin A is well established to play critical roles in vision, reproduction, embryonic development and growth, immune competence, and cell differentiation (lvarez *et al*., 2014). *Moringa oleifera*, leaves are a good source of carotenoids that may act as a provitamin A source (Slimani *et al*., 2007). Additionally, *Moringa oleifera* leaves contain a higher concentration of vitamin C than oranges Likewise, *M. oleifera*, leaves protect the body from the harmful effects of free radicals, pollutants, and toxins by acting as antioxidants (Chambial *et al*., 2013). *Moringa oleifera* fresh leaves contain vitamin E in concentrations comparable to those found in nuts (E *et al*., 2013). This is crucial because vitamin E has been shown to inhibit cell proliferation in addition to acting as an antioxidant (Borel *et al*., 2013).

- **Polyphenols**

Moringa oleifera dried leaves are an excellent source of polyphenol compounds, including flavonoids and phenolic acids.

Moringa oleifera leaves are an excellent source of flavonoids (Pandey2009, n.d.). Flavonoids, which are synthesized by the plant in response to microbial infections, share a common structure with benzo—pyrones (Bovicelli *et al*., 2002; Kumar & Pandey, 2013) It has been demonstrated that flavonoids protect against chronic diseases associated with oxidative stress, such as cardiovascular disease and cancer, when consumed.

The main flavonoids found in MO leaves are myrecytin, quercetin and kaempferol, in concentrations of 5.8, 0.207 and 7.57 mg/g, respectively (Coppin *et al*., 2013; Sultana & Anwar, 2008)

Quercetin-3-O- -d glucoside (iso-quercetin or isotrifolin) is found in dried *M.oleifera* leaves at a concentration of 100 mg/100 g. (Atawodi *et al*., n.d.). Quercetin is a strong antioxidant, with multiple therapeutic properties (Bischoff, 2008). In obese Zucker rats with metabolic syndrome, it has hypolipidemic, hypotensive, and anti-diabetic properties. It can protect insulin-producing pancreatic cells from oxidative stress and apoptosis induced by streptozotocin (STZ) in rats(Rivera *et al*., 2008).

Phenolic acids are a class of phenolic compounds that are derived from hydroxybenzoic acid and hydroxycinnamic acid. They are found naturally in plants and exhibit antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (El-Seedi *et al*., 2012).

- **Alkaloids, Glucosinolates and Isothiocyonates**

Alkaloids are a class of chemical compounds that are predominantly composed of basic nitrogen atoms. Several of these compounds have been isolated from *Moringa oleifera* leaves, including N, -L-rhamnopyranosyl vincosamide, phenylacetonitrile pyrrolemarumine, 40-hydroxyphenylethanamide- -L-rhamnopyranoside, and its glucopyranosyl derivative(Sahakitpichan *et al*., 2011). Antioxidants In plants, glucosinolates are a class of secondary metabolites (Förster *et al*., 2015). Both glucosinolates and isothiocyanates have been shown to possess significant pro-health properties (Dinkova-Kostova and Kostov, 2012).

- **Tannins**

Tannins are phenolic compounds that precipitate alkaloids, gelatin, and other proteins when exposed to water. Their concentrations in dried leaves range between 13.2 and 20.6 g tannin/kg (Teixeira *et al*., 2014). with freeze-dried leaves having slightly higher concentrations (Richter *et al*., n.d.).Tannins have been shown to be anti-cancer, anti-atherosclerotic, anti-inflammatory, and anti-hepatotoxic(Vergara-Jimenez *et al*., 2017).

- **Saponins**

Additionally, *M.oleifera* leaves contain saponins, which are natural compounds composed of an isoprenoidal-derived aglycone covalently linked to one or more sugar moieties ,Saponin concentrations in freeze-dried leaves of *M.oleifera* range between 64 and 81 g/kg dry weight] Saponins are anti-cancer in nature (Augustin *et al*., 2011).

1.18. *Moringa oleifera* seeds contain the following

- **protein**

Proteins provide the majority of the nitrogen required for human nutrition. the essential amino acids required for the body's construction and renewal afflicted (Biesalski and Grimm, 2010). The seeds of *Moringa oleifera* are a source of protein. and they are the seeds' second major component. following lipids. Recent studies indicate that the protein content varies significantly between18% (Kawo *et al*., 2009) and 37.2 % (Bridgemohan, Bridgemohan, and 2014; Mohamed). However, the defatted M. oleifera seed analysis Protein content ranged from 32% to 62.8 percent (Anwar and Rashid,

Governardhan Singh *et al*., 2007; Govardhan Singh *et al*., 2011). However, the composition of the proteins of the seeds can meet only a portion of the requirements in a few critical and for humans, semi-essential amino acids (histidine, threonine, tyrosine, and tryptophan(Saa *et al*., 2019a).

- **Carbohydrates**

Carbohydrate content of *M. oleifera* seeds ranges between 9.17 and 53.36 percent. Fibers, which are carbohydrates that are unavailable to the organism, account for approximately 24% of the dry weight of fresh seed and 3% of dehulled seed (Bridgemohan *et al*., 2014). M. oleifera seeds have a low glucose (2.57 g/100 g dw), fructose (0.03 g/100 g dw), and sucrose (2.91 g/100 g dw) content when compared to other medicinal plants.Thus, *M. oleifera* seeds have the potential to be used in diabetic food (Saa *et al*., 2019).

- **Vitamins and minerals**

(Mbah *et al*. 2012) discovered that *M. oleifera* seeds contain provitamin A (2.04 percent) and vitamin B group, specifically vitamin B1 or thiamin (0.94 percent). Vitamin A is critical for vision and possesses antioxidant properties, it presents in form of β-carotene.

Moringa oleifera seed oil has been found to contain vitamin E , in form of alphatocopherol, gammatocopherol, and delta-tocopherol.

Moringa oleifera seeds are mineral-dense. such as potassium, phosphorus, sodium, zinc, magnesium, and calcium are the major minerals.

Moringa oleifera seeds had a Ca/Potassium ratio greater than one, whereas the sodium/potassium ratio is greater than the recommended value (0.60). However,

the mineralogy Seeds of M. oleifera vary significantly from region to region (Saa *et al*., 2019a)

- **Lipids**

The level of lipids in *M. oleifera* seeds was reported between 14% and 46%). The lipids are low in monounsaturated and saturated fatty acids but higher in polyunsaturated fatty acids representing up to75%–79%. However, the oil is a source of some minor compounds (phytosterols and tocopherols). Several studies investigated the role of *M. oleifera* seed oil in human nutrition, and this includes physicochemical characteristics of the oil and its biological value(Saa *et al*., 2019b) .

1.19 Pharmacological effect of *Moringa oleifera*

Moringa oleifera has long been perceived in the Ayurveda and all plant parts have been used in the treatment of different illnesses. Phytochemicals are nonnutritive plant chemicals that possess defensive properties against various infections. Plant secrets these chemicals for self-defense but recent research revealed that these phytochemicals can protect from various human diseases (Chhikara *et al*., 2019). In general, these phytochemicals protect plants from environmental pollution, stress, drought, ultraviolet exposure and various pathogenic attacks. They also contribute to the flavor, texture, smell and color of the plant (Chhikara *et al*., 2021a). *Moringa oleifera* contains many bioactive compounds such as polyphenols, flavonoids, ascorbic acid and carotenoids. The highest quantity of total polyphenols presents in leaves. Several phytochemicals are found in *Moringa oleifera* leaves are rich in simple sugar, rhamnose and other compounds called glucosinolates and isothiocyanates.Intake of flavonoids from M. oleifera protects

from oxidative stress-induced different chronic diseases including cardiovascular problems and cancer . *M. oleifera* leaves and seeds contain an abundant amount of flavonoids. Total flavonoids present in the highest amount in seeds followed by fresh leaves. The maximum amount of ascorbic acid (871.28 mg/100 g) is present in pods and minimum amount in dried leaves (67.84mg/100 g). Content of b - carotene is not available in large amounts and is negligible in pods (0.97mg/100 g) and seeds (0.65 mg/100 g) but it is present more in (28.36mg/100 g) fresh leaves. Carotenoids and total antioxidants were largely present in dried leaves (147.42mg/100 g) and fresh leaves (149.75 mg/100 g) (el -Massry *et al*., n.d.).

Some pharmacological effects of M. oleifera reported by previous studies are:

- **Anti-diabetic effect**
- **Antihypertensive**

The Juice of *Moringa oleifera* leaves has a stabilizing effect on blood pressure (Chhikara *et al*., 2021b). Systolic and diastolic blood pressure level of hypertensive male albino rats was significantly reduced upon feeding with *M. oleifera* leaves powder (Adefegha *et al*., 2019).

- **Antimicrobial activity**

Moringa oleifera roots act as an antimicrobial agent because of the presence of pterygospermin and 4-a-L-rhamnosyloxy benzyl isothiocyanate (Farooq *et al*., 2012).

- **Hepatoprotective and antispasmodic behavior**

The ethanolic extract of M. oleifera showed a hepatoprotective effect against antitubercular drugs (rifampicin, isoniazid and pyrazinamide) in rats, this effect may be related to presence of the quercetin, flavonoid (Chhikara *et al*., 2019) (Jahan *et al*., 2014) .

- **Antitumor**

Anti-proliferative activity of *M. oleifera* depend on it is ability to scavenge reactive oxygen species (ROS) or free radical in the cancer cells, which enhanced cell apoptosis process. The soluble cold distilled water extract (4°C; concentration, 300mg/mL) from leaves have significant ability to enhance cell apoptosis, thus it will inhibit tumor cell growth and lowered the level of internal ROS in several types of cancerous cells, suggesting that the treatment of cancer cells with *M. oleifera* leaves significantly declined cancer cell proliferation and incursion. Compounds such as O-ethyl-4-(a-L-rhamnosyloxy) benzyl carbamate, 4(a-L-rhamnosyloxy)-benzyl isothiocyanate, niazimicin and 3-O-(6'-O-oleoyl-b -D-glucopyranosyl)-b -sitosterol have been checked for their antitumor promoting activity in laboratory analysis and which showed inhibitory effects on Epstein–Barr virus antigen. Niazimicin has been proposed to be strong chemo preventive agent in chemical carcinogenesis. Seed extracts have been observed effectual in hepatic cancer-causing metabolizing enzymes and skin papillomagenesis in mice (Chhikara *et al*., 2021c).The aqueous extract of *M. oleifera* seeds and leaves has massive potential to inhibit the tumor progression without affecting the normal physiology and functioning of the experimental animal (mice) body and thus can be used as a cancer therapeutic agent(Barhoi et *al*., 2021).

CHAPTER TWO

MATERIAL S & METHODS

2.1 Materials and methods

This chapter includes a detailed description of all materials and equipment used in the present research work. The experimental work was performed in the cancer research laboratory at College of Medicine\ University of Babylon during the period from January 2021 to October 2021. This invitro study was done to investigate the antiproliferative effect of ethanolic extract to (*Moringa oleifera* leaves and seeds) on prostate cancer cell line in comparison to docetaxel.

2.2 Equipment

The equipment employed in the present work and their origin are listed in table below.

No.	Equipment	Manufacturers/country(Origin)
1.	Autoclave	Prestige medical, England
2.	Centrifuge	Hettich, Germany
3.	Distiller	Griffin, England
4.	Electric oven	Lab.tech, Korea
5.	Freezer -20 °C	Mettler, Switzerland
6.	Incubator	Memmert, Germany
7.	Inverted microscope	T.C Meiji techno, Japan
8.	Laminar air flow cabinet	Labtech, Korea
9.	Magnatic stirrer	Scotech, Germany
10.	Refrigerator	Arcelik, Turky

11.	Shaker	Denely, England
12.	Sensitive Balance	Labtech, Korea
13.	Ultrasonic	Binder, Germany
14.	Vortex	Kottermann, Germany
15.	Water bath	Minilyotrap, England
16.	Warring Blender	National, Japan
17.	Nalgene filters 0.45 and 0.22 µM Millipore	Sigma, USA
18.	Elisa microplate reader	Model/Aspan
19.	Flow cytometry	Beckman Coulter Life Sciences
20.	Gas chromatography-Mass spectrometry	USA
21	Microplate reader	800 TS / BIOTEK
22	Millipore filter (0.45, 0.22µm)	Biofil / Australia
23	Whatman filter paper	Merck/ Germany
24	pH Meter	WTW / Germany

Table 2-1 equipment used in this study

2.3 Chemicals and drugs

Chemicals and drugs used in this study with their origin and companies are listed in table (2-2).

Chemicals	Company	Country
Alcohol spray (ethanol 99%)	**Iraq**	**SDI**
Dimethyl sulfoxide (DMSO)	**Sigma Aldrich**	**Germany**
Fetal bovine serum (FBS)	**Capricorn scientific Germany**	**USA**
Penicillin -Streptomycin	**Elabscience**	**USA_China**
Docetaxel 80 mg/3ml vial	**GLS**	**India**
RPMI-1640 Media -Liquid with L-glutamine +25% Hepes	**Capricorn scientific**	**Germany**
Hepes Buffer	**Capricorn scientific**	**Germany**
MTT(3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide) dye powder	**Roth**	**Germany**
Phosphate buffer saline powder	**Bio-Word**	**USA**
Sodium bicarbonate powder	**Ludeco**	**Belgium**
Trypsin Ethylenediaminetetraacetic acid (EDTA) powder	**Elabscience**	**USA-china**

table (2-2)

2.4 Cell lines

Frozen vials of human prostate cancer LNCaP(cells was developed from a needle aspiration tissue biopsy of a supraclavicular lymph node lesion in a 50- years-old Caucasian male , diagnosed with stage one D1 prostate cancer 1 year prior to admission) ; (animal models for the study of Human Disease ,2013) , obtain from the laboratory in the College of Medicine / University of Babylon.

2.5 Methods

1. Preparation of reagents and solutions.

a. Phosphate Buffer Saline (PBS)

According to the BioWorld manufacturer manual, the PBS was prepared through dissolving only one packet in 500 ml of deionized distilled water (DDW) with continuous stirring by a magnetic stirrer at room temperature resulting in PH value of 7.45 without need for adjustment. Autoclaving was done to complete sterilization and then the solution was stored in a closed bottle until use to keep sterile.

b. Trypsin-Ethylene Diamine Tetra acetic acid- Solution:

As indicated by US Biological headings, a weight of a 10.1 gm of trypsin-Ethylene Diamine Tetra-acetic acid (EDTA) powder was dissolved in 0.9 liter of double distilled water (DDW) with continuous mixing at room temperature. A7.2 of PH value was reached and the volume was completed to 1 liter by DDW, then the solution was sterilized through using Millipore filters of 0.45 and 0.22 µm respectively, after that, the solution was kept at a temperature (-20C˚).

c. Preparing MTT assay solution

• Principle

The general purpose of the MTT assay is to measure viable cells in relatively high throughput (96-well plates) without the need for elaborate cell counting. Therefore, the most common use is to determine the cytotoxicity of several drugs at different concentrations. The principle of the MTT assay is to detect the cellular mitochondrial activity of the viable and thereby an increase or decrease in the number of viable cells is linearly related to the mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the pale-yellow tetrazolium salt (MTT dye) into dark purple formazan crystals by NADH **(table 2.1)** which can be solubilized for homogenous measurement. Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration that reflected in the measurements of optical density (absorbance) using a plate reader at 570 nm. The darker the solution, the greater the number of viable and metabolically active cells (Sukhramani *et al*., 2011) .

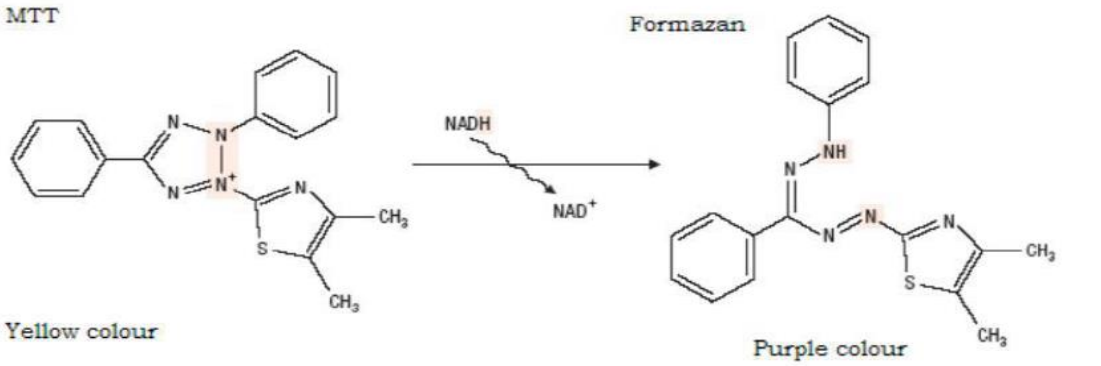


Figure 2.1: Principle of MTT Assay (Sukhramani et al., 2011)

• Procedure (Meerloo *et al*; 2011):

1- After the drug exposure period was completed, the medium was removed from the wells and the cells washed with PBS. To assess unspecific formazan conversion, a blank control was used.

2- To obtain a final concentration of 0.5 mg/ml, 1.2 ml MTT solution (5 mg/ml) was added to 10.8 ml medium. After that, each well was filled with 200µl of the resulting solution.

3- For 3 hours at 37°C, the plate was incubated until intracellular purple formazan crystals were visible under an inverted microscope.

4- After removing the supernatant, 100 µl DMSO was added to each well to dissolve the formed formazan crystals.

5- For 30 minutes at room temperature, the plate was incubated until the cells lysed and the purple crystals dissolved.

6- A microplate reader was used to determine the absorbance at 570 nm.

The absorbance reading of the blank must be subtracted from all samples. Absorbance readings from test samples must then be divided by those of the control and multiplied by 100 to give percentage cell viability or proliferation. Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation. Percentage of cell viability or percentage of inhibition was calculated by the following formula:

% viability = (AT – AB) / (AC – AB) × 100%

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

AB = Absorbance of blank (only medium).

AC = Absorbance of control (untreated).

% Inhibition = 100 – % viability

d. Preparation of tissue culture medium liquid RPMI-1640

The medium was prepared according to US Biologics from RPMI-1640 medium powder as the following:

A 16.353 g of RPMI-1640 powder was dissolved in 900ml of double distilled water without heating, 2 g of sodium bicarbonate was added if required with gentle stirring to adjust pH additional water was added to obtain 1 liter then filtered using 0.22-micron membrane. Penicillin-Streptomycin at 1% was added and 10% fetal bovine serum was also added, then sterilizing filter of

0.22-micron membrane filter was used for sterilization. because the mixture contains heat labile compounds that can be damaged with autoclaving.

The prepared media should be kept at 4°C and used within 48 hours.

e. Preparation of docetaxel stock solution

Docetaxel 80 mg/3ml vial is consider as stock solution and was store at 4 c for use it during the experiment.

f. Preparation of *Moringa oleifera* extract:

Fresh leaves and seeds of *Moringa oleifera* plant were identified by botanist at university of Babylon college of agriculture.

g. Preparation of ethanolic extract of *Moringa oleifera* Leaves.

Fresh leaves of *Moringa oleifera* plant were collected from small plant nursery, in Hilla city- Iraq. The plant leaves were washed with deionized water thoroughly and left to dry for 14 days in dark place at room temperature, then grinded to fine powder and stored in tight dry container for further use.

A 200 g of leaves powder was soaked and macerated in alcoholic (ethanol 70%) in 1 liter of Ethanol and left for extraction at room temperature for 48 hours, where the Maceration with 70% ethanol is the most suitable extraction method of the dried leaves of M. oleifera. It promoted high yield of the crude extract, the highest contents of total phenolics, total flavonoids, major active compounds, and the most potent antioxidant activity.(Vongsak *et al*., 2013).

- Ethanolic mixture was filtered by two layers filter (Whitman filter paper) and then the final Ethanolic extract is then placed in oven at 40 ℃ until drying then collected and stored in refrigerator. (Rodríguez-Pérez *et al*., 2015).

h. Preparation of ethanolic extract of *Moringa Oleifera* Seeds.

Fresh seeds were collected from local herbal shop and then grinded to fine powder and stored in tight dry container for further use.

A 200 g of seeds was soaked and macerated in 1 liter of ethanol 70 % in and left for extraction at room temperature for 48 hours.

- Ethanolic seeds extract was filtered by using Whitman filter paper no 1 and then the final Ethanolic extract was placed in oven at 40 ℃ until drying after that will collected and stored in refrigerator (Rodríguez-Pérez *et al*., 2015) .

2. Preparation of LNCaP cell line

a. Thawing:

1.To minimize cell warming/thawing, a cryogenic vial containing the cell line (LNCaP) was carefully removed from storage (-80°C or liquid nitrogen freezer) and placed immediately in a pre-equilibrated Cool Rack CFT30 tube module (or other thermo conductive rack) resting on dry ice.

2.The cryogenic vials were then immediately transferred to a 37°C water bath. Alternatively, the tube module could be placed on a thermal tray platform that has been preheated to 37 degrees Celsius for 1 to 2 minutes.

3. The vial's exterior was wiped with 70% ethanol before being placed inside a sterilized tissue culture hood.

4. The vial was then opened, and the cells were gently resuspended with a sterile 1-ml pipet and transferred to a sterile 12-ml tube, and a vial with 1 ml

fresh prewarmed medium was added to the 12-ml tube to ensure complete cell transfer.

5. Eight milliliters of prewarmed medium were gradually added (1 to 2 drops at a time, with swirling). Close the tube with the cap and centrifuge for 5 minutes at 1500 rpm (500 ×g) at room temperature with the TH-4 rotor, discarding the supernatant.

6. Re-suspend the cells in medium to the desired concentration and transfer to a tissue culture flask or other culture vessel of choice.
(Yokoyama *et al*., 2012)

b. Sub-culturing of cell culture

1. The cells were checked and examined using an inverted microscope equipped with phase contrast to ensure that they were healthy, sub-confluent, and free of contamination.

2. The laminar flow was sanitized by wiping the working area's surface with 70% ethanol.

3. Using a pipette, the growth medium was removed from the flask, and the monolayer was washed with enough PBS to ensure that all of the media was removed from the flask.

4. The flask was filled with an appropriate volume of trypsin/EDTA solution and incubated at 37 °C for 2–10 minutes to allow the cells to detach from the inside surface of the flask.

5. An inverted microscope was used to ensure that all of the cells were detached and suspended. To free any cells that remained attached, the flask was gently covered with the palm of the hand a few times.

6. By adding an equal volume of serum-containing media to the flask, the trypsin was inactivated.

7. The cell suspension was then separated into two flasks and labeled with the cell line's name, passage number, and date. The cell line was incubated at 37 degrees Celsius for 24 hours (Meleady & O'connor, 2006).

c. Harvesting of cell culture

Harvesting is a technique that uses proteolytic enzymes to detach adherent cells from the surface of a cell culture flask. First, the growth medium in the vessel was aspirated and discarded. PBS was used to wash the cells twice. Afterward, the enzymatic harvesting solution was added to the vessel. After 15 minutes, the proteolytic reaction was neutralized by adding the serum-containing culture medium. The cells in the tissue culture flasks were harvested by using different enzymatic solutions composed of different concentrations of trypsin and Ethylenediaminetetraacetic acid (EDTA)(Viazzi *et al*., 2015) .

2.6 Experiments

1. Effect of docetaxel on LNCaP cells line viability.

In this experiment measure the effect of docetaxel (chemotherapy) on LNCaP cells viability after exposure to different concentration of docetaxel (31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250µg/ml, 500µg/ml, 1000µg/ml) in compare with control.

2. Effect of ethanolic *Moringa oleifera* leaves extract on LNCaP cell viability

In this experiment measure the effect of ethanolic moringa leaves extract on LNCaP cells line viability after exposure to different concentration of extract (31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250µg/ml, 500µg/ml, 1000µg/ml) for incubation period 24 hour and 48 hours in compared with control.

3. Effect of ethanolic *Moringa oleifera* seeds extract of LNCaP cell viability.

In this experiment measure the effect of ethanolic moringa seeds extract on LNCaP cells line viability after exposure to different concentration of extract (31.25 µg/ml, 62.5 µg/ml ,125 µg/ml, 250 µg/ml ,500µg/ml ,1000µg /ml) For incubation period 24 hours and 48 hours in compared with control.

4. Effect of different concentrations of ethanolic extract of *Moringa oleifera* seeds and docetaxel on the viability of LNCaP cell line after (24- 48 hours) of incubation.

In this experiment measure the difference in effect on LNCaP cell viability between ethanolic seeds extract and docetaxel after exposure to different

concentrations (31.25µg/ml to 1000µg/ml) from each one and for two periods of incubation (24-48 hours).

5. Effect of different concentrations of ethanolic extract of *Moringa oleifera* leaves and docetaxel on the viability of LNCaP cell line after (24-48 hours) of incubation.

In this experiment measure the difference in effect on LNCaP cell viability between ethanolic leaves extract and docetaxel after exposure to different concentrations (31.25µg/ml to 1000µg/ml) from each one and for two periods of incubation (24-48 hours).

6. Effect of combination between different concentration of *Moringa oleifera* leaves extract and IC50 of docetaxel on LNCaP cell viability

In this experiment we measure the effect of ethanolic leaves extract in different concentrations (31.25 µg/ml, 62.5 µg/ml ,125 µg/ml, 250 µg/ml ,500µg/ml ,1000µg /ml) and docetaxel IC50 as combination on LNCaP cells viability after occupation period to 24 hours and 48 hours in compared with control.

7. Effect of different concentrations from ethanolic *Moringa oleifera* seeds extract and IC50 of docetaxel on LNCaP cell viability

In this experiment measure the effect of ethanolic seeds extract in different concentration (31.25 µg/ml, 62.5 µg/ml ,125 µg/ml, 250 µg/ml ,500µg/ml ,1000µg /ml) and docetaxel IC50 as combination on LNCaP cells viability after incubation period to 24 hours and 48 hours in compared with control.

8. The half maximal inhibitory concentration (IC50) of docetaxel

Half-maximal inhibitory concentration (IC50) is the most widely used and informative measure of a drug's efficacy. It indicates how much drug is needed to inhibit a biological process by half, thus providing a measure of potency of an antagonist drug in pharmacological research (Sebaugh, 2011).

In this experiment measure the IC50 of docetaxel by use serial concentration (200 , 400, 600,800,1000, 1200 µg/ml) and then use MTT assay to determine the docetaxel concentration which inhibit 50% of LNCaP cells.

9. The half maximal inhibitory concentration (IC50) of *Moringa oleifera* seeds ethanolic extract.

In this experiment measure the IC50 of *Moringa oleifera* seeds ethanolic extract by use serial concentration (200, 400, 600, 800, 1000, 1200 µg/ml) and then use MTT assay to determine the *Moringa oleifera* seeds ethanolic extract IC50 concentration which inhibit 50% of LNCaP cells.

10. The half maximal inhibitory concentration (IC50) of *Moringa oleifera* leaves ethanolic extract.

In this experiment measure the IC50 of *Moringa oleifera* leaves ethanolic extract by use serial concentration (200, 400, 600, 800, 1000, 1200 µg/ml) and then use MTT assay to determine the *Moringa oleifera* leaves ethanolic extract IC50 concentration which inhibit 50% of LNCaP cells.

2.7 Human TRAIL (Tumor Necrosis Factor Related Apoptosis Inducing Ligand) ELISA Kit

Synonyms: APO2L, Apo2-L, TL2, CD253

Catalog No: E-EL-H1593 96T

1.The intended application

This ELISA kit is used to determine the concentrations of Human TRAIL in serum, plasma, and other biological fluids in vitro.

2. Specification

- 9.38 pg/ml Sensitivity
- 15.63-1000 pg/ml detection range

Human TRAIL is recognized in samples by this kit. There is no evidence of significant cross-reactivity or interference, between Human TRAIL/TNFSF10 and analogues was observed.

- Repeatability: The coefficient of variation is 10%.

3. principle of the test

The Sandwich-ELISA principle is used in this ELISA kit. This kit includes a micro-ELISA plate that has been pre-coated with an antibody specific to Human TRAIL. Standards or samples are mixed with the specific antibody in the micro-ELISA plate wells. Following that, a biotinylated detection antibody specific for Human TRAIL was used.

Each microplate well is incubated with an Avidin-Horseradish Peroxidase (HRP) conjugate in turn. Free the components have been washed away. Each well receives the substrate solution. Only those wells containing Human The color of TRAIL, biotinylated detection antibody, and Avidin-HRP conjugate will be blue. The substrate of the enzyme.

The reaction is stopped by the addition of a stop solution, and the color changes to yellow. The optical density (OD) is defined as measured spectrophotometrically at 450 nm ± 2 nm The OD value is proportional to the amount of TRAIL in humans By comparing the OD of the samples to the standard curve, you can calculate the concentration of Human TRAIL in the samples.

4. Kit components & Storage

An unopened kit can be kept at 2-8°C for one month. If the kit is not used within one month, store the items separately according to the conditions listed below.

5. Item Specifications Storage

Item	Specification	Storage
Micro ELISA Plate (Dismountable)	8 wells ×12 strips	-20℃, 6 months
Reference Standard	2 vials	
Concentrated Biotinylated Detection Ab(100×)	1 vial, 120 μL	
Concentrated HRP Conjugate (100×)	1 vial, 120 μL	-20°C(shading light), 6 months
Reference Standard & Sample Diluent	1 vial, 20 mL	4°C, 6 months
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	4°C (shading light)
Stop Solution	1 vial, 10 mL	4°C
Plate Sealer	5 pieces	

Table 2-3

Note: To prevent evaporation and microbial pollution, all reagent bottle caps must be tightened.

6. Other supplies required

- Microplate reader with 450 nm wavelength filter
- High-precision transfer pipette, EP tubes and disposable pipette tips
- Incubator capable of maintaining 37℃
- Deionized or distilled water
- Absorbent paper
- Loading slot for Wash Buffer

7. Collection of samples

Cell lysates: For adherent cells, gently wash them with a moderate amount of pre-cooled PBS before dissociating them with trypsin. Place the cell suspension in a centrifuge tube and spin for 5 minutes at 1000g.
Remove the medium and wash the cells three times in pre-cooled PBS. To keep the cells suspended, add 150-250 L of pre-cooled PBS for every $1*10^6$ cells.

8. Preparation of reagents

1. Bring all reagents to room temperature (18~25℃) before use. Set up the Microplate reader according to the manual, and preheat it for 15 minutes before measuring optical density (OD).

2. Wash Buffer: To make 750 mL of Wash Buffer, dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water.

If crystals form in the concentrate, warm it in a 40°C water bath and gently mix it until the crystals are completely dissolved.

3. Standard working solution: Centrifuge the standard for 1 minute at 10,000g. Allow it to stand for 10 minutes before gently inverting it several times with 1.0 mL of Reference Standard and Sample Diluent. After it has completely dissolved, thoroughly mix it with a pipette. This reconstitution yields a 1000 pg/mL working solution. Then, as needed, make serial dilutions.

The following dilution gradient is recommended: 1000, 500, 250, 125, 62.5, 31.25, 15.63, 0 pg/mL.

Dilution method: Take 7 EP tubes and fill each with 500uL of Reference Standard and Sample Diluent. 500µL pipette

Add 500 pg/mL of the 1000 pg/mL working solution to the first tube and mix to make a 500 pg/mL working solution. Pipette 500µL of the solution from the first tube into the second, following these steps. The example below is for reference.

Note: the last tube is regarded as a blank. Don't pipette the solution into it from the previous tube.

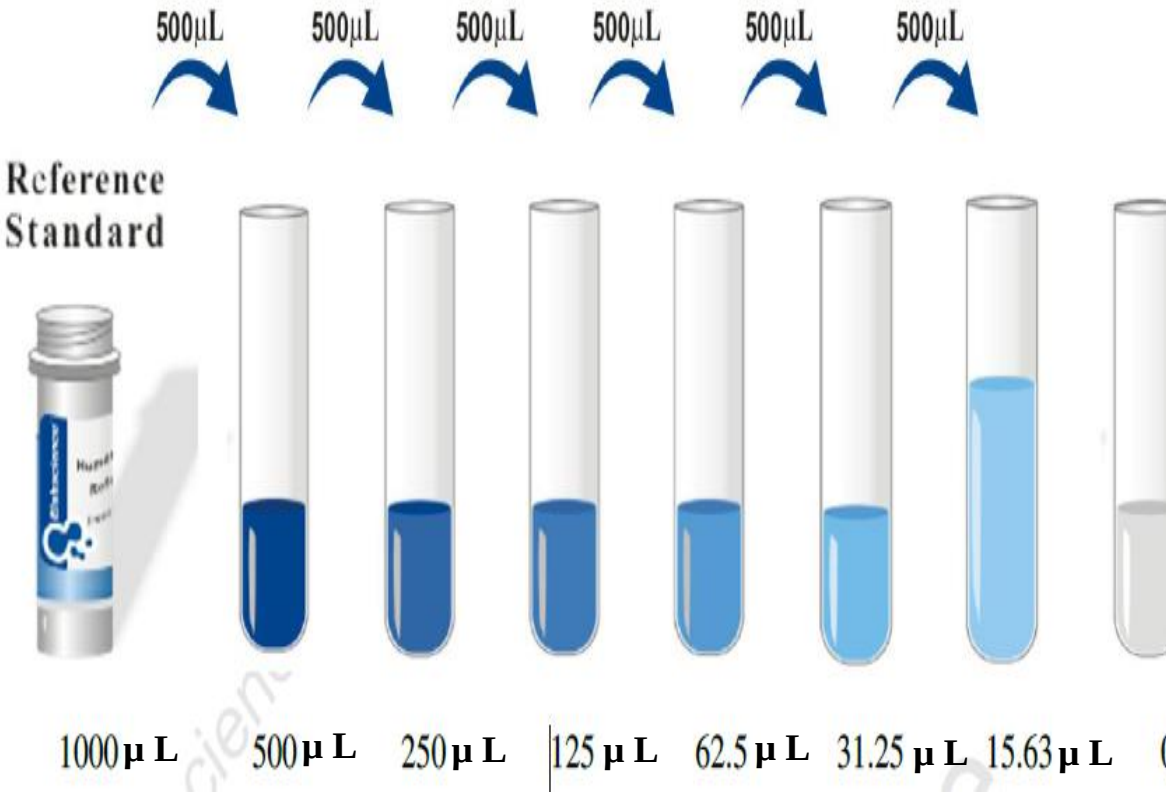


Figure (2.2) reference standard solution serial dilution process

9. Assay method

1. Fill in the first two columns with the Standard working solution: Each concentration of the solution is added in duplicate, to one well each, side by side (100 µl for each well). Fill the remaining wells with the samples (100 µL each). Cover the plate with the sealer that came with the kit. Incubate at 37°C for 90 minutes.

Note: solutions should be added to the bottom of the micro-ELISA plate well as much as possible to avoid touching the inside wall and causing foaming.

2. Remove the liquid from each well without washing. Add 100 µL of Biotinylated Detection Ab working solution to each well right away. Apply the Plate sealer to the top. Gently combine. Incubate at 37°C for 1 hour.

3. After aspirating or decanting the solution from each well, add 350 µL of wash buffer to each well. Soak for 12 minutes before aspirating or decanting the solution from each well and patting it dry with clean absorbent paper. Repeat this wash step 3 times.

Note: a microplate washer can be used in this step and other wash steps.

4. Fill each well with 100 µl of HRP Conjugate working solution. Apply the Plate sealer to the top. Incubate at 37°C for 30 minutes.

5. Aspirate or decant the solution from each well, then repeat the wash process as described in step 3.

6. Fill each well with 90 µl of Substrate Reagent. Apply a new plate sealer on top. Incubate for 15 minutes at 37°C. Keep the plate away from direct sunlight.

Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.

7. Fill each well with 50µL of Stop Solution.

Note: Adding the stop solution should be done in the same order as the substrate solution.

8. Using a microplate reader set to 450 nm, determine the optical density (OD value) of each well at the same time.

10. Result calculation

Average the duplicate readings for each standard and sample, then subtract the average optical density of the zero standard.

On log-log graph paper, draw a four-parameter logistic curve with standard concentration on the x-axis and OD values on the y-axis.

If the samples have been diluted, the standard curve concentration must be multiplied by the dilution factor. If the sample's OD exceeds the upper limit of the standard curve, re-test it with an appropriate dilution. The calculated concentration multiplied by the dilution factor yields the actual concentration.

11. Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(pg/mL)	1000	500	250	125	62.5	31.25	15.63	0
OD	2.395	1.66	0.981	0.472	0.277	0.184	0.134	0.082
Corrected OD	2.313	1.578	0.899	0.39	0.195	0.102	0.052	-

Table 2-4

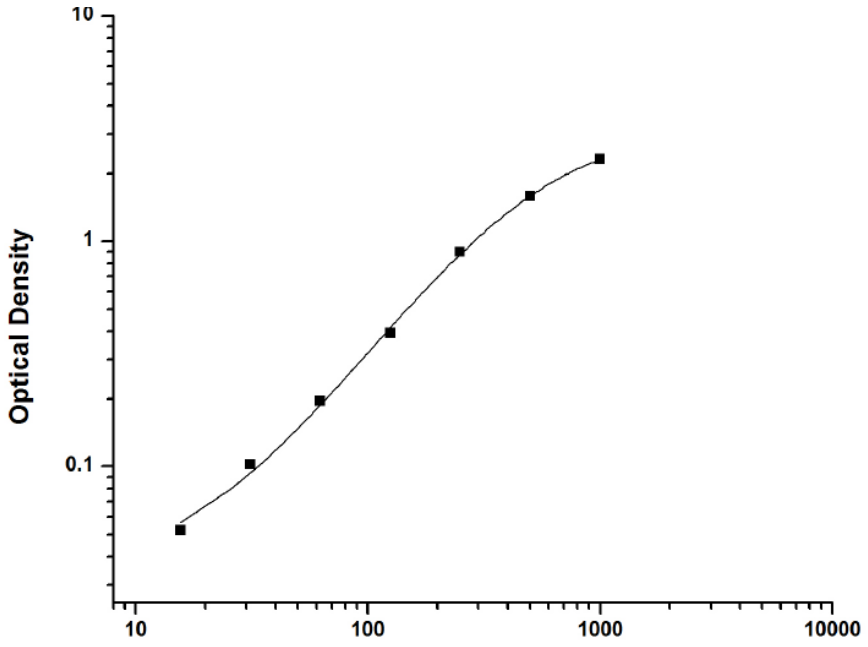


Figure (2.3) standard curve of Human TRAIL/TNFSF10 concentration(pg/mL)

2.8 Statistical Analysis

All data were collected and analyzed by Microsoft Office Excel 2016 and Sigma plot version 12.5 software. ANOVA one way test and T_Test were used to assess significant differences among the means of data. the p-value ($p<0.001$, $p<0.05$) were considered statistically significant

CHAPTER THREE

THE RESULTS

3.1 Effect of ethanolic extract *Moringa oleifera* leaves extract on LNCaP cells.

Results showed that there was a significant reduction (p value ≤ 0.01) in cells viability of LNCaP cell line at concentrations ≥ 500 µg/ml of ethanolic extract of *M. oleifrea* leaves after both 24 hours and 48 hours of incubation in comparison to the control group and this reduction was concentration dependent. Regarding the influence of period of incubation only the concentration 1000µg/ml result in a highly significant reduction (P ≤0.001) in the viability of LNCaP cells after 48 hours of exposure to the extract compared to 24 hours of exposure as shown in figure (3-1).

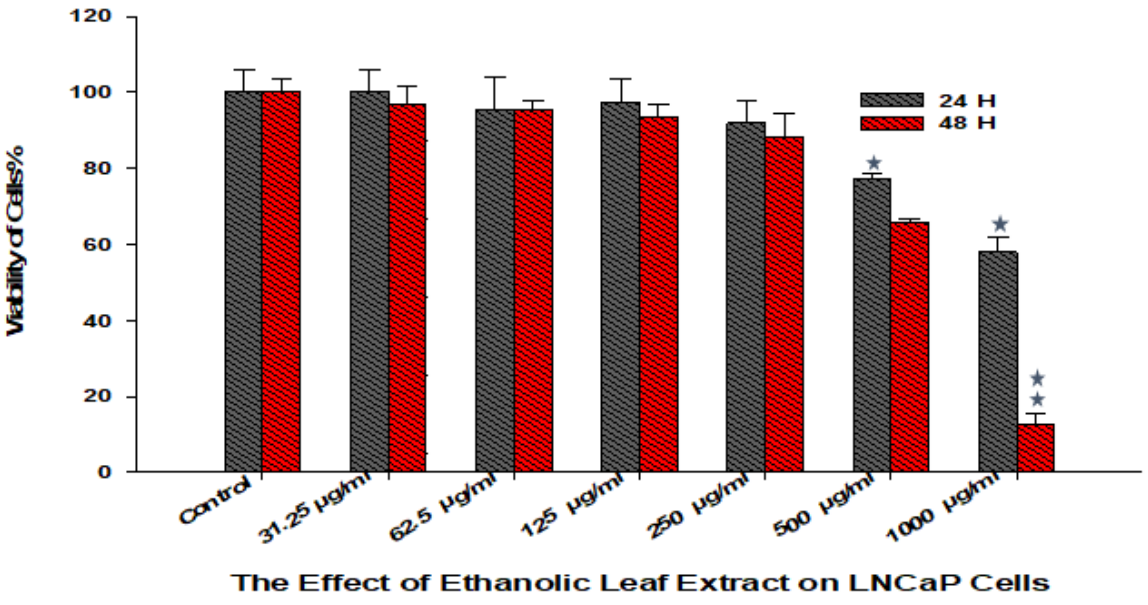
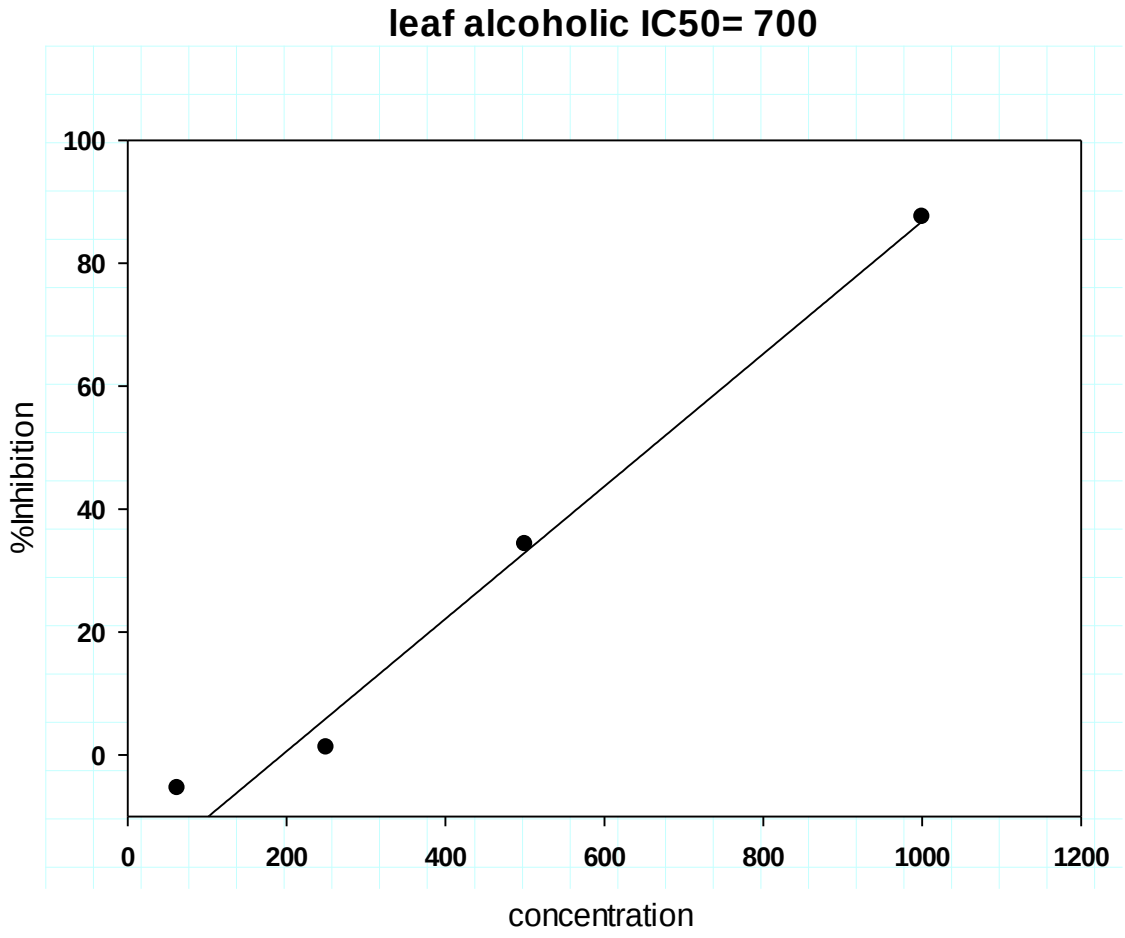


Figure (3-1)

3.2 Half maximal inhibitory concentration of ethanolic *Moringa oleifera* leaves extract.

Result of present study found that the concentration 700µg/ml of ethanolic *Moringa Oleifera* leaves extract inhibit 50% of LNCaP cell growth as showed in figure.



3.3 Effect of docetaxel on LNCaP cells line

Results showed that different concentrations of docetaxel (31.25 µg/ml, 62.5 Mg/ml, 125 Mg/ml, 250Mg/ml, 500Mg/ml, 1000Mg/ml) cause significant reduction (P value ≤0.001) in the viability of LNCaP cells in comparison to the control group, and this reduction was directly proportion to the concentration of docetaxel and duration of exposure (24 or 48 hour) especially at low concentrations as show in figure 3-3.

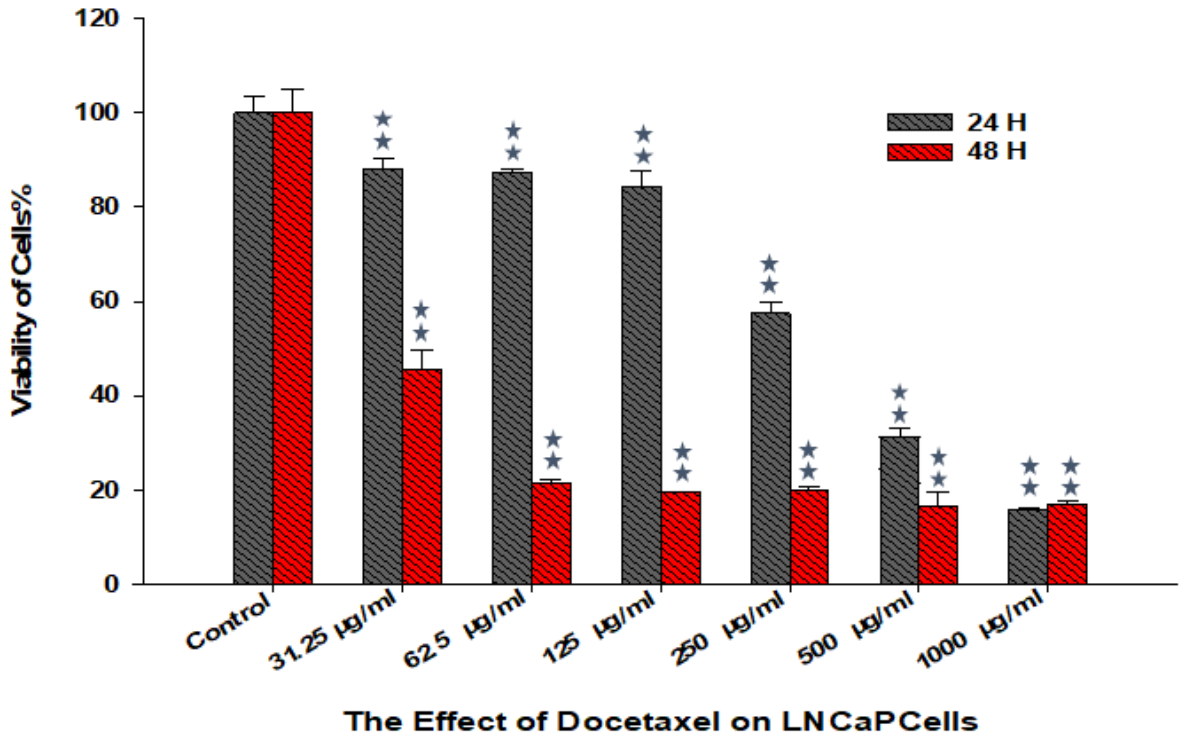


Figure (3-3)

3.4 Half maximal inhibitory concentration (IC50) of docetaxel

Result of present study found that the concentration 423 µg/ml of docetaxel inhibit 50% of LNCaP cell as showed in figure (3-4).

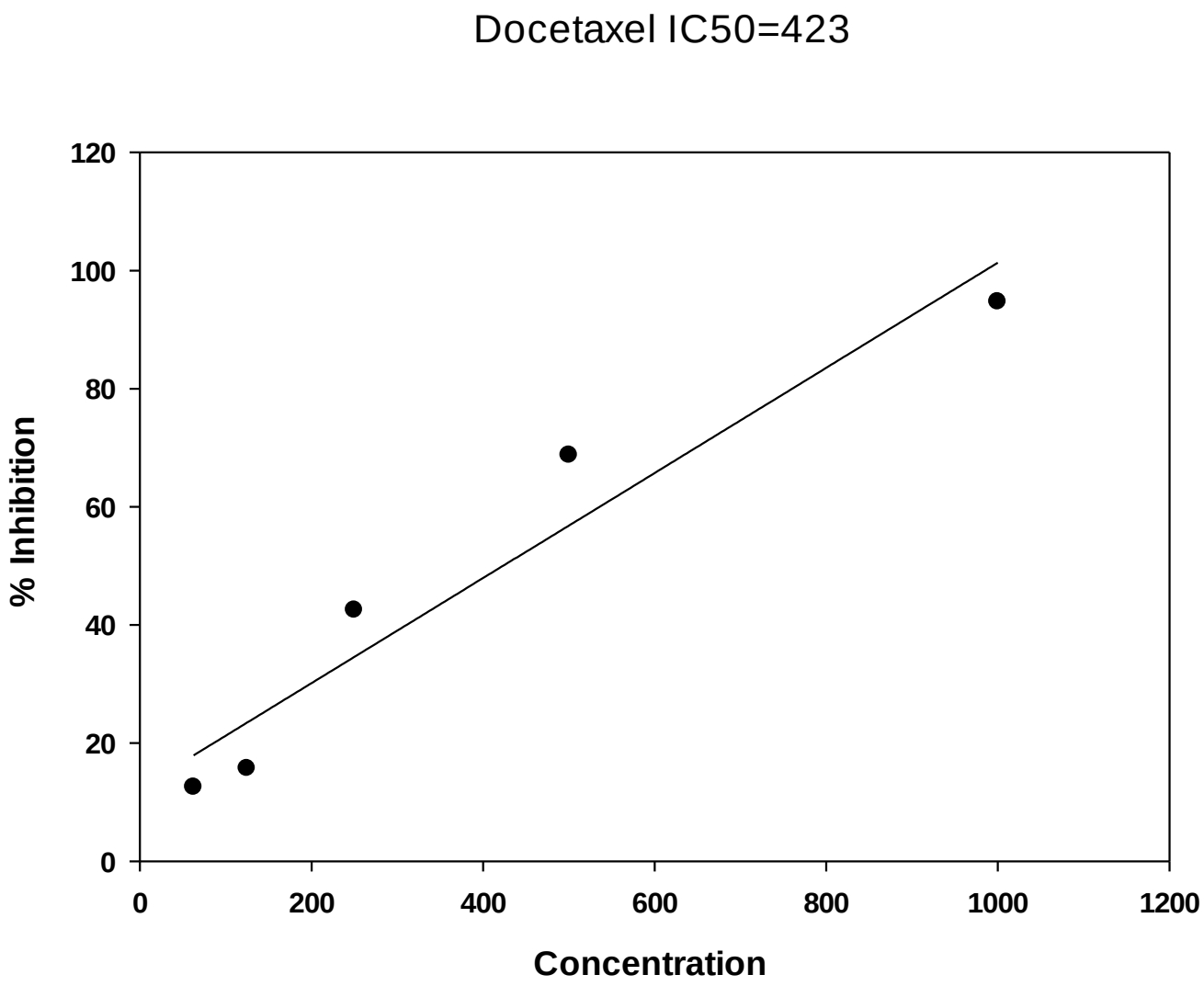


Figure (3-4)

3.5 Effect of the combination of ethanolic extract to *Moringa oleifera* leaves plus docetaxel IC50 on LNCaP Cell Line

Results showed that the combination of different concentrations of ethanolic extract *M. Oleifera* leaves (31.25 µg/ml, 62.5 µg/ml ,125 µg/ml, 250 µg/ml ,500µg/ml ,1000µg /ml) plus the IC50 of docetaxel (423 µg/ml) cause highly significant reduction ($p \leq 0.001$) in the viability of LNCaP cells after 24 hour of incubation at the concentrations ≥ 250 µg/ml, whereas after 48 hour of incubation all concentrations of the extract cause significant reduction ($P \leq 0.01$) in the viability of LNCaP cells as shown in figure (3-5) .

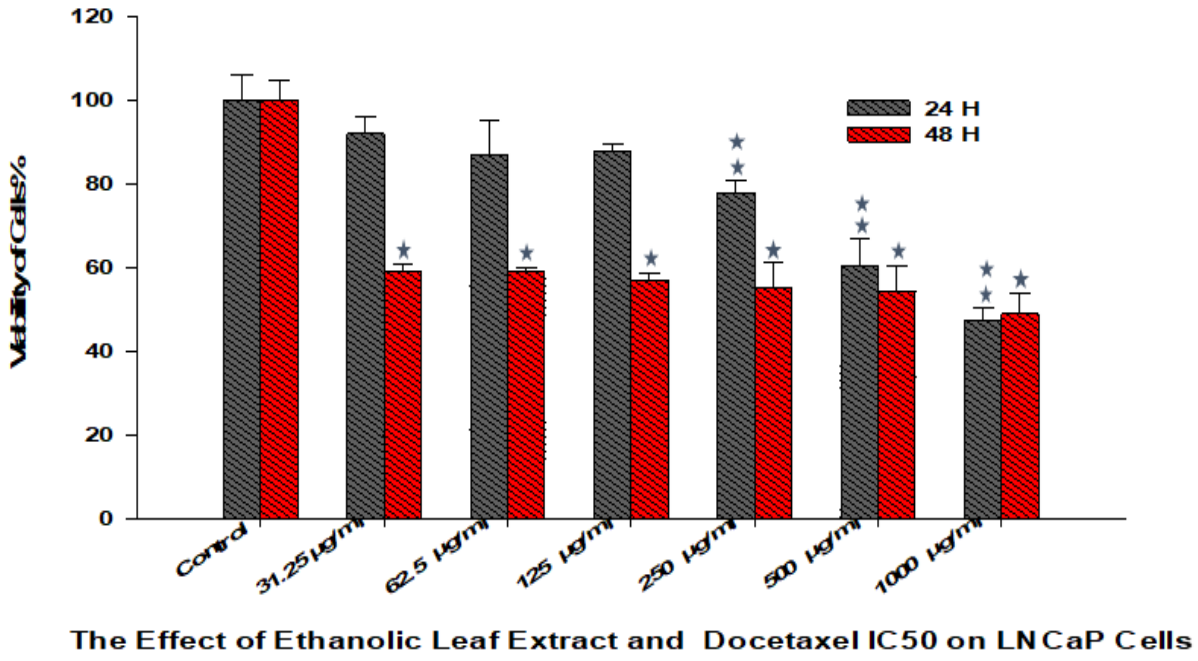


Figure (3-5)

3.6 Effect of ethanolic extract *Moringa oleifera* seeds on LNCaP cell line.

Results showed highly significant (P value ≤0.001) reduction in LNCaP cell viability at concentration ≥500 µg/ml after 24 hour of incubation and at concentration ≥250 µg/ml after 48hour incubation period as shown in figure (3-6).

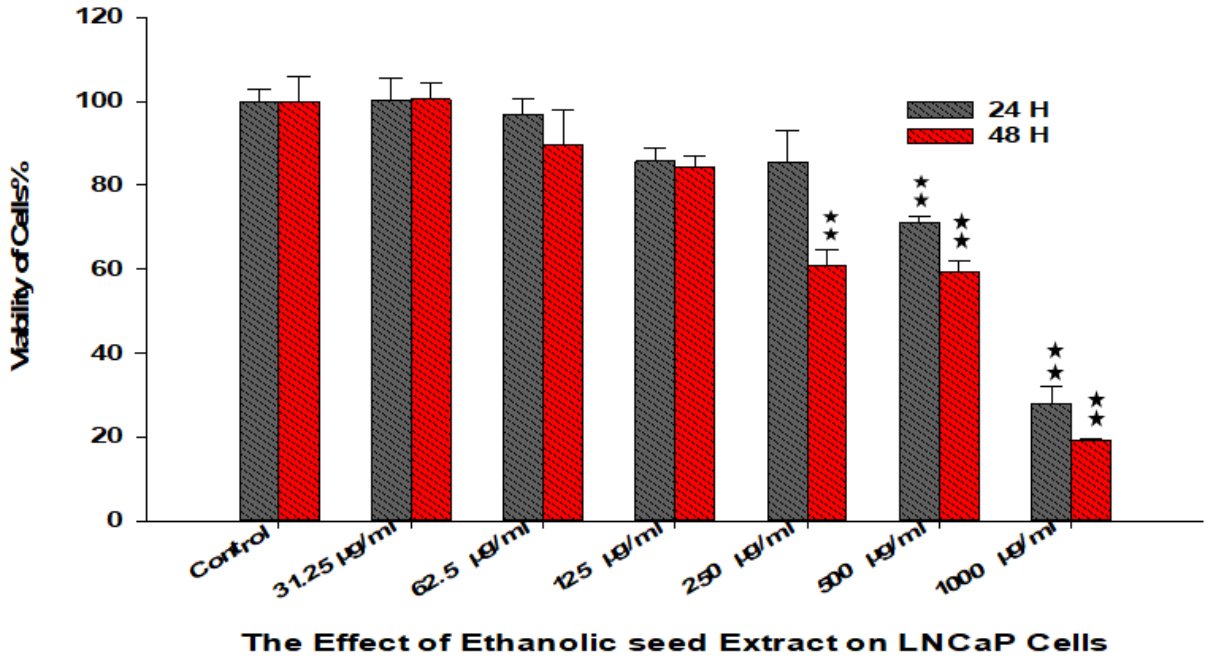
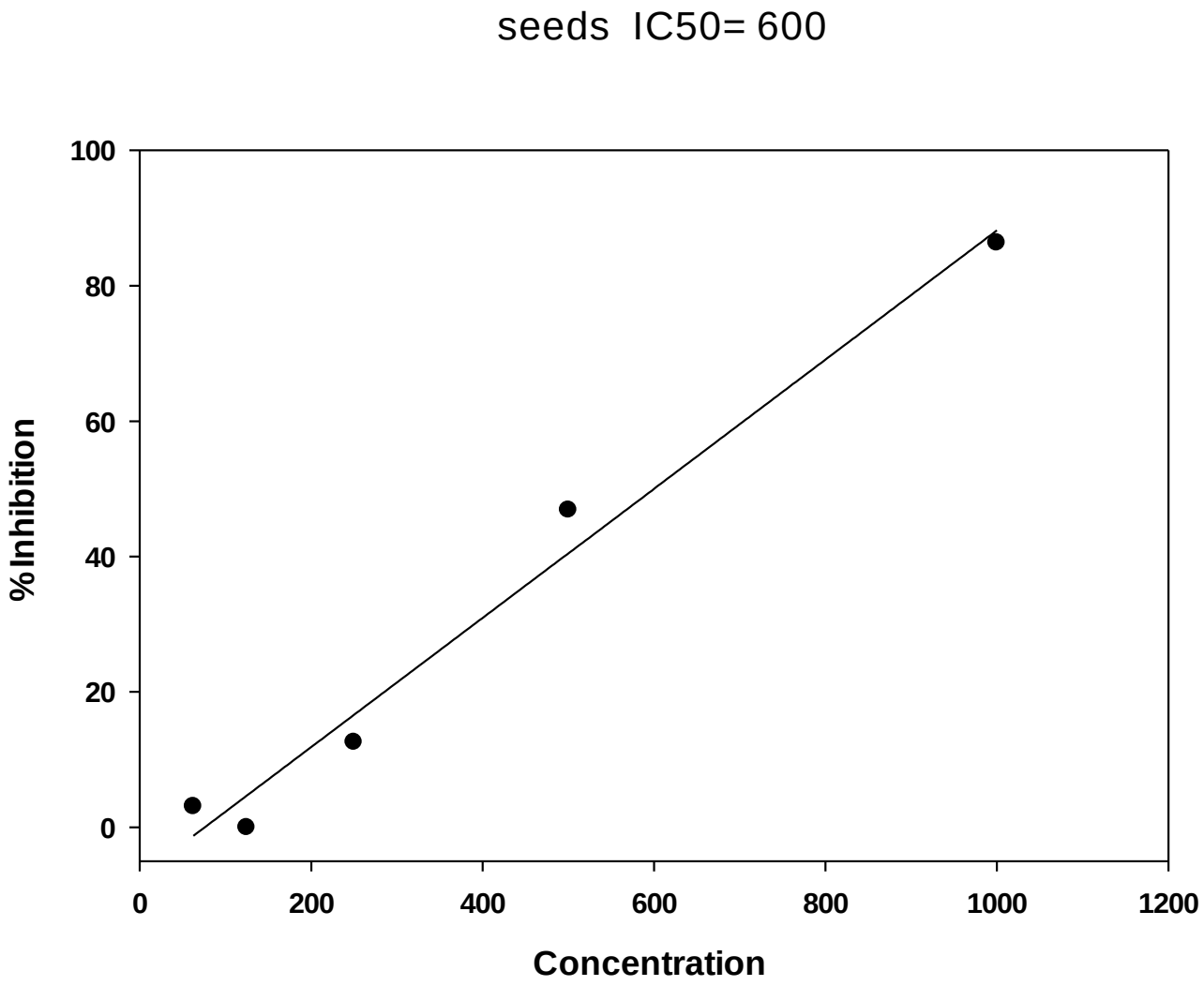


Figure (3-6)

3.7 Half maximal inhibitory concentration of *Moringa oleifera* seeds ethanolic extract.

Result of present study found that the concentration 600µg/ml of ethanolic seeds extract inhibit 50% of LNCaP cell as shown in figure (3-7).



Half maximal inhibitory concentration of *M. oleifera* seeds ethanolic extract

Figure (3-7)

3.8 Effect of the combination from different concentration of ethanolic *Moringa oleifera* seeds extract and IC50 concentration of docetaxel on LNCaP Cell Line.

Results showed significant reduction (P value ≤ 0.05) in LNCaP cell viability at concentration ≥62.5 µg/ml for incubation period 24 hour and also showed highly significant reduction (P value ≤0.001) in LNCaP cell viability after 48 hour of incubation or exposure to combination at concentration ≥32.25 µg/ml as shown in figure (3-8) .

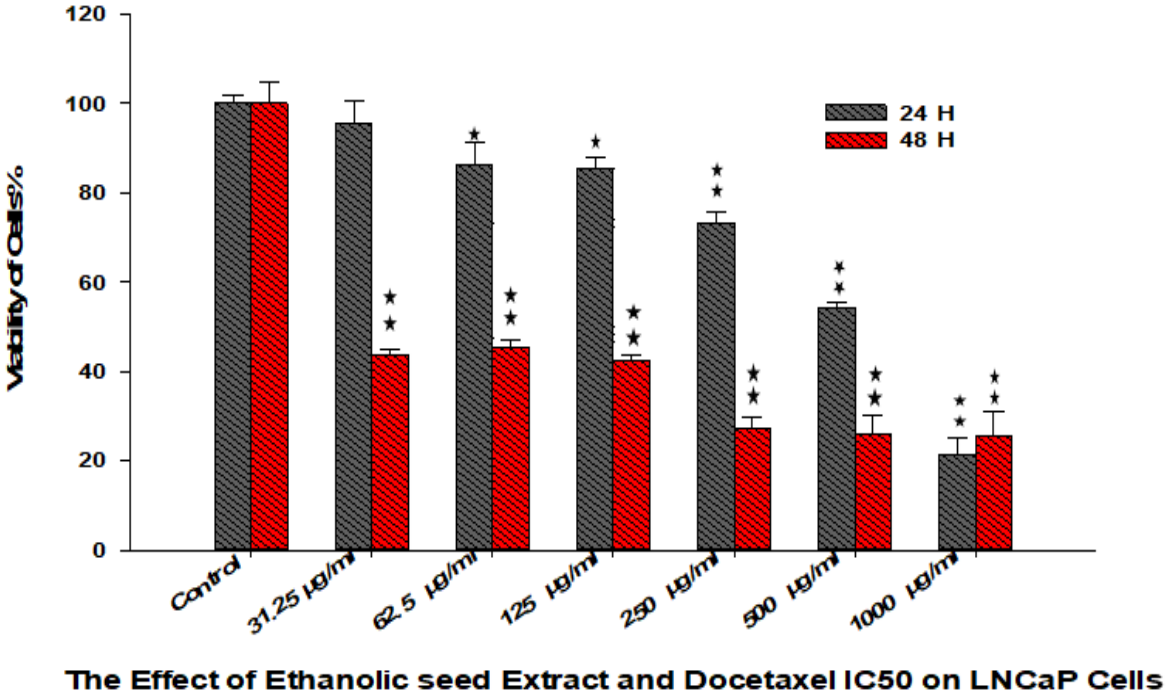


Figure (3-8)

3.9 Level of tumor necrosis factor related apoptosis inducing ligand (TRAIL) in docetaxel treated LNCaP cell line.

According to the result of the current study the level of TRAIL was significant increased (P ≤0.001) in a concentration dependent manner after the treatment of LNCaP cell line to different concentrations of docetaxel in comparison to the control group, this refers to apoptosis induction mechanism by extrinsic pathway done through using of docetaxel.

* The effect of docetaxel on LNCaP cells line through increasing the level of TRAIL where we measure the concentration of this parameter in pigo gram /ml (1 gm = 10, 00000000000 pigo) in different concentration of docetaxel.

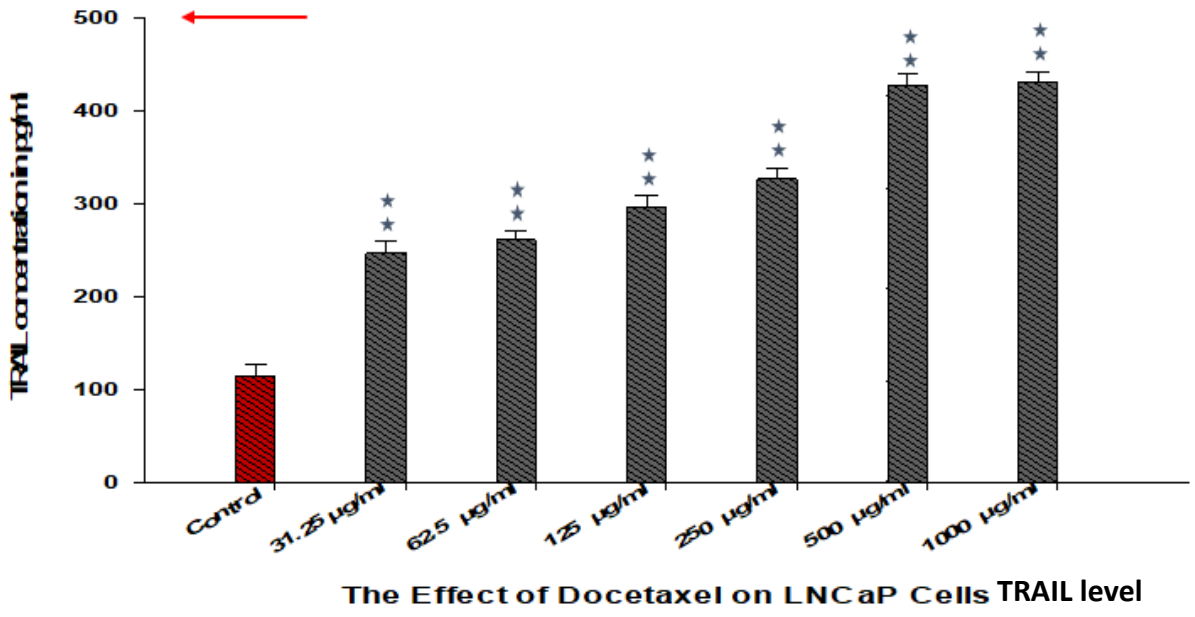


Figure (3-9)

3.10 Level of tumor necrosis factor related apoptosis induced ligand (TRAIL) in LNCaP cell line treated with ethanolic extract of *Moringa oleifera* seeds.

According to the result of the current study the level of TRAIL was highly significant increased (P ≤0.001) in a concentration dependent manner after the treatment of LNCaP cell line to different concentrations of ethanolic seeds extract in comparison to the control group, this refers to apoptosis induction mechanism by extrinsic pathway done through using of ethanolic seeds extract as shown in figure (3-10).

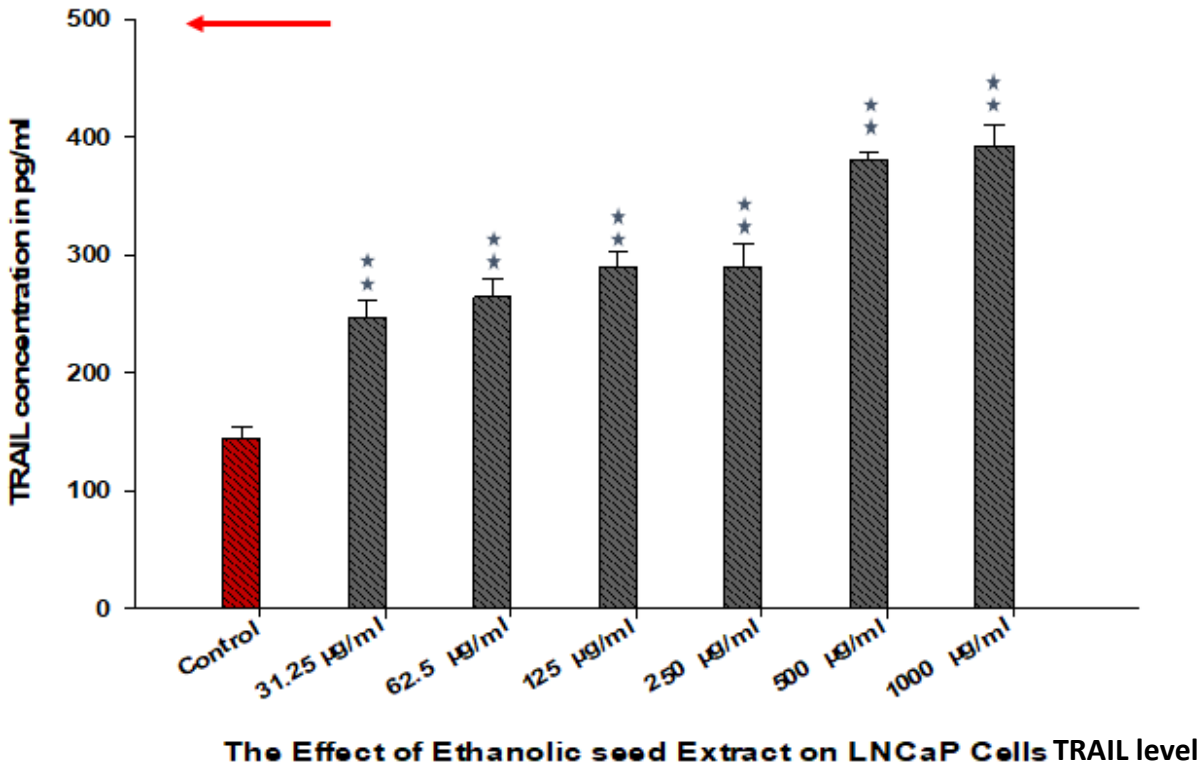


Figure (3-10)

3.11 Level of tumor necrosis factor related apoptosis induced ligand (TRAIL) in LNCaP cell line treated with combination of different concertation from ethanolic extract of *Moringa oleifera* seeds plus IC50 of docetaxel.

Result showed that the level of TRAIL was significant increase ($P \leq 0.001$) after the treatment of LNCaP cell line with a combination containing different concentration of ethanolic extract from *Moringa oleifera* Seeds plus IC50 of docetaxel and this increase was concentration dependent starting at concentration (31.25µg/ml) as shown in figure (3-11).

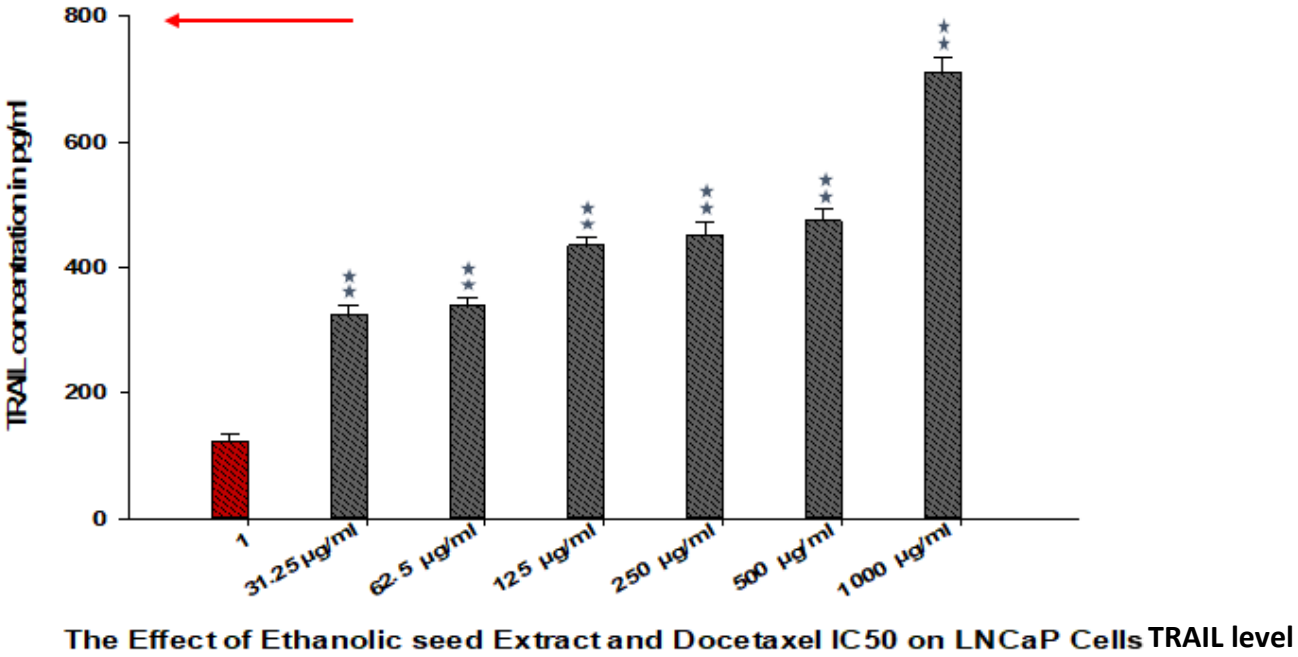


Figure (3-11)

3.12 Level of tumor necrosis factor related apoptosis induced ligand (TRAIL) in LNCaP cell line treated with ethanolic extract of *Moringa oleifera* leaves.

According to the result of the current study the level of TRAIL was significant increased (P ≤0.001) in a concentration dependent manner after the treatment of LNCaP cell line to different concentrations of ethanolic leaves extract in comparison to the control group, this refer to apoptosis induction mechanism by extrinsic pathway done through using of ethanolic leaves extract starting at concentration (31.25µg/ml) as shown in figure (3-12)

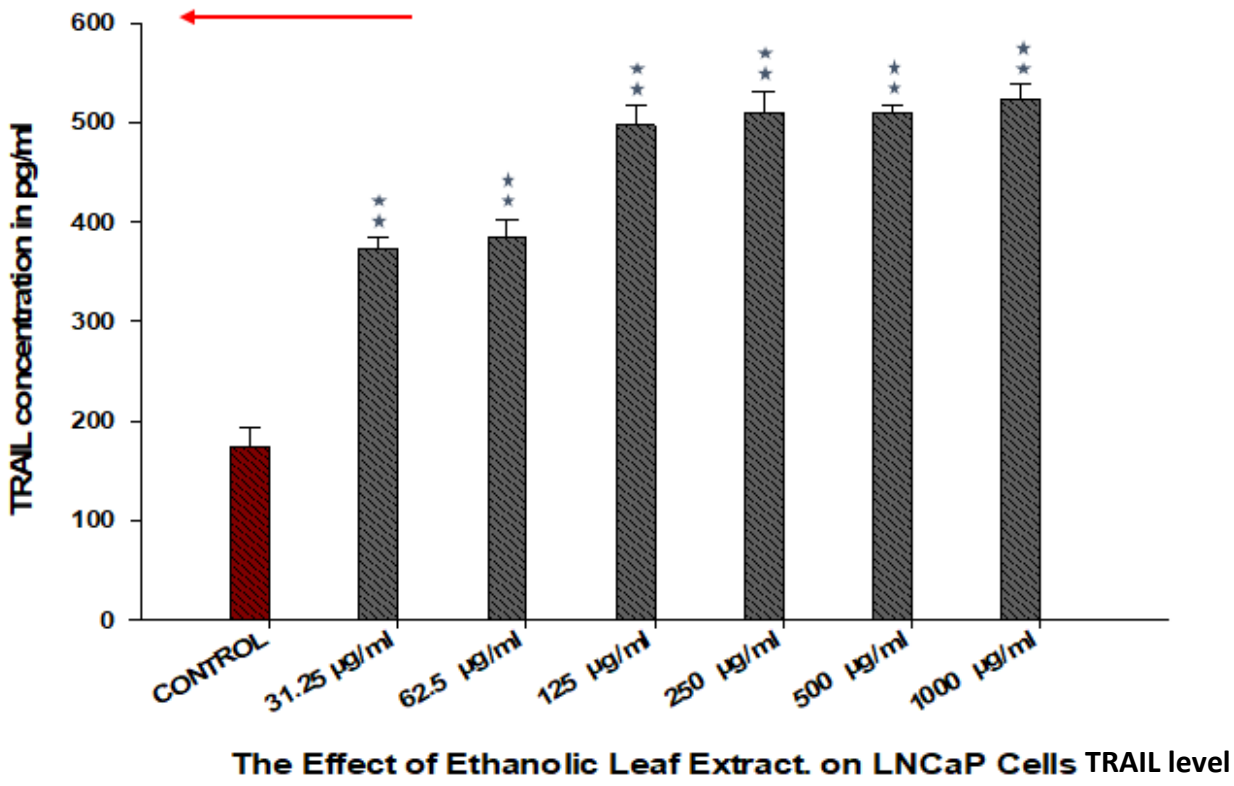


Figure (3-12)

3.13 Level of tumor necrosis factor related apoptosis induced ligand (TRAIL) in LNCaP cell line treated with combination of different concertation from ethanolic extract of *Moringa oleifera* leaves plus IC50 of docetaxel.

Result showed that the level of TRAIL was highly significant increase ($P \leq 0.001$) after the treatment of LNCaP cell line with a combination containing different concentration of ethanolic extract from *Moringa oleifera* leaves plus IC50 of docetaxel and this increase was concentration dependent starting at concentration (31.25µg/ml) of ethanolic leaves extract as shown in figure(3-13) .

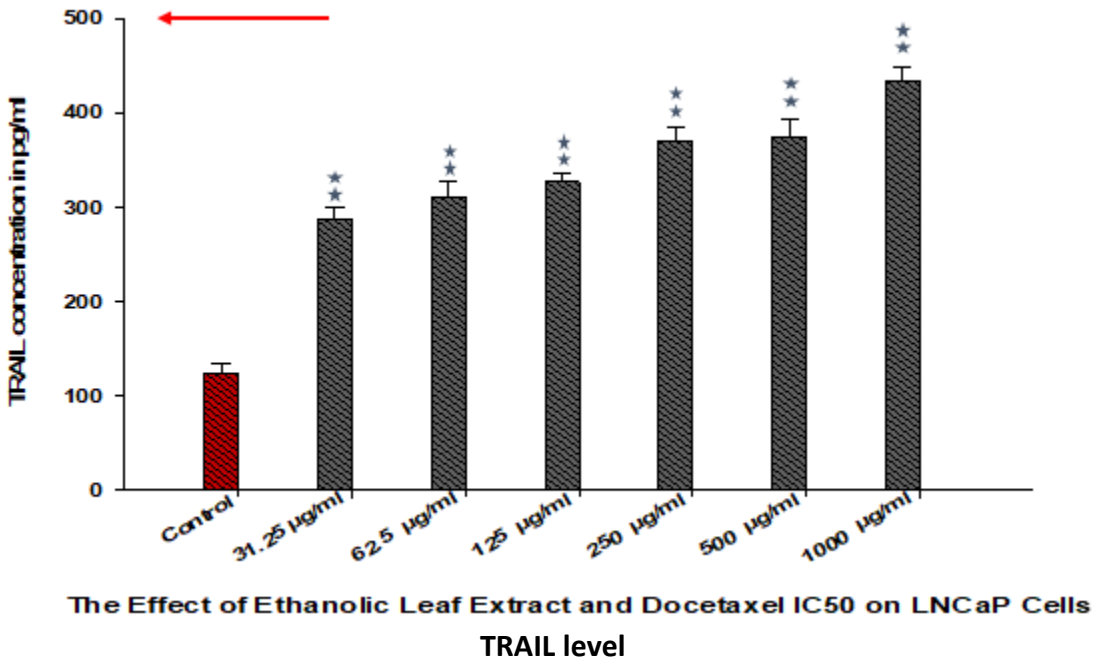
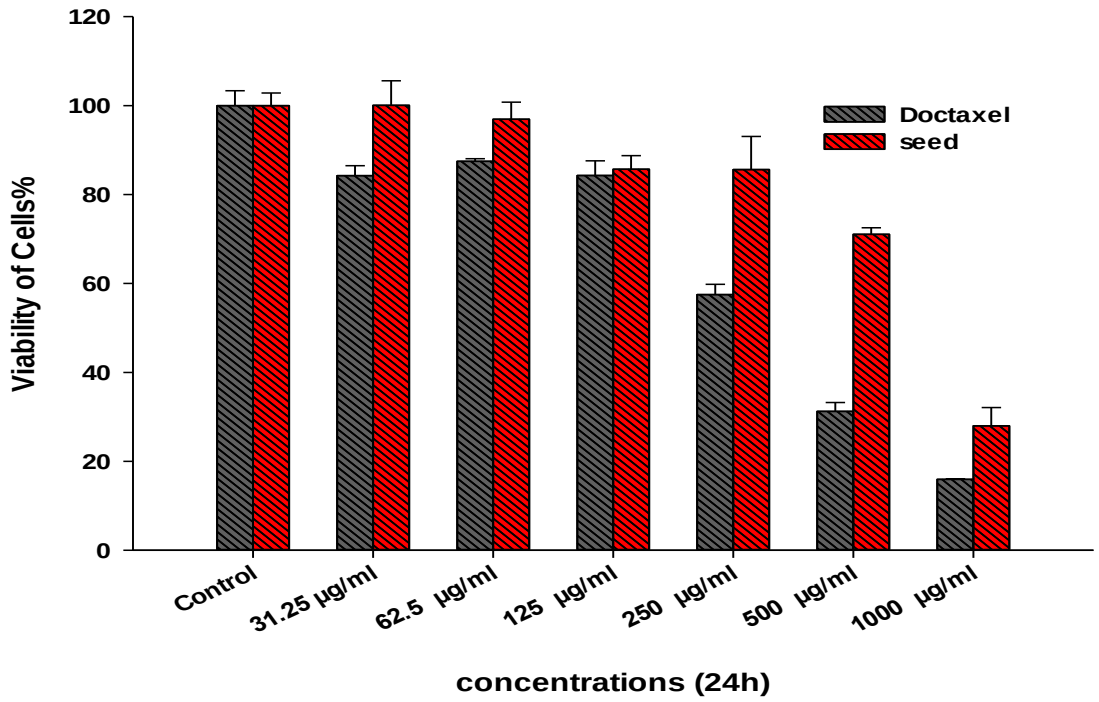


Figure (3-13)

3.14 Effect of different concentrations of ethanolic extract of *Moringa oleifera* seeds and docetaxel on the viability of LNCaP cell line after 24 hours of incubation.

Result showed that there was insignificant difference (P≥0.05)between ethanolic extract of *Moringa oleifera* seeds and docetaxel for all concentrations taken in study .

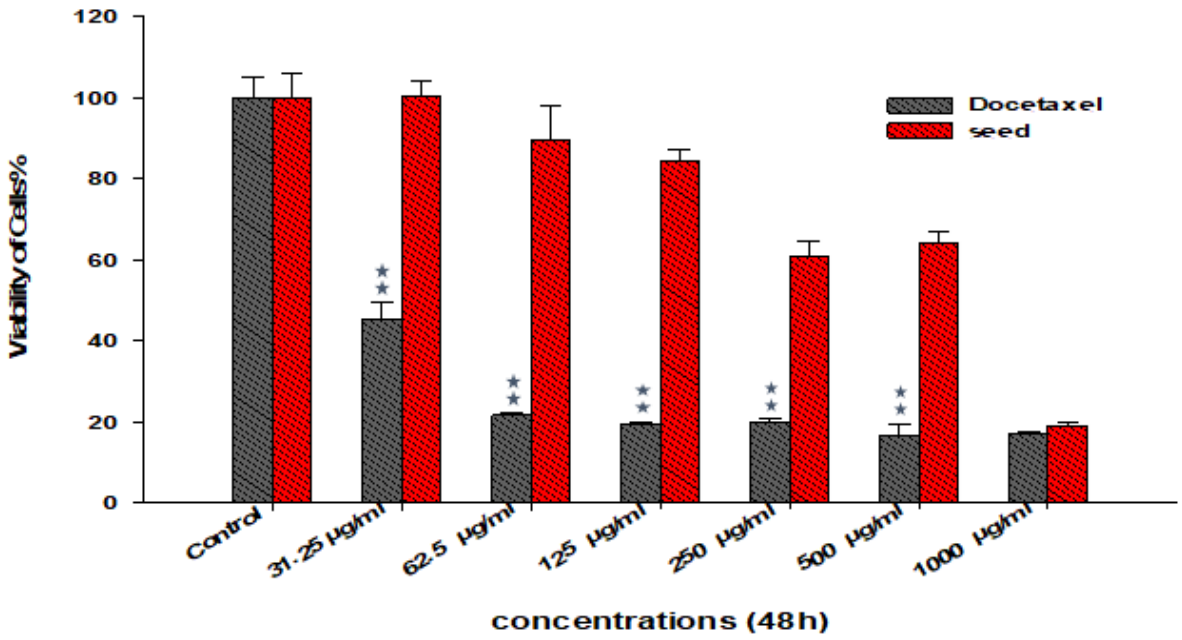


Effect of ethanolic extract of *Moringa oleifera* seeds and docetaxel on the viability of LNCaP cells for 24h

Figure (3-14)

3.15 Effect of different concentrations of ethanolic extract of *Moringa oleifera* seeds and docetaxel on the viability of LNCaP cell line after 48 hours of incubation.

Results showed there were highly significant difference (P value ≤0.001) in reduction cells viability of LNCaP cell line after exposure for 48 hours at different concentrations (31.25 µg/ml, 62.5 µg/l, 125µg/ml, 250 µg/ml, 500µg/ml) and there was nonsignificant difference (P≥0.05) at concentration 1000µg/ml as show in figure (3-15).

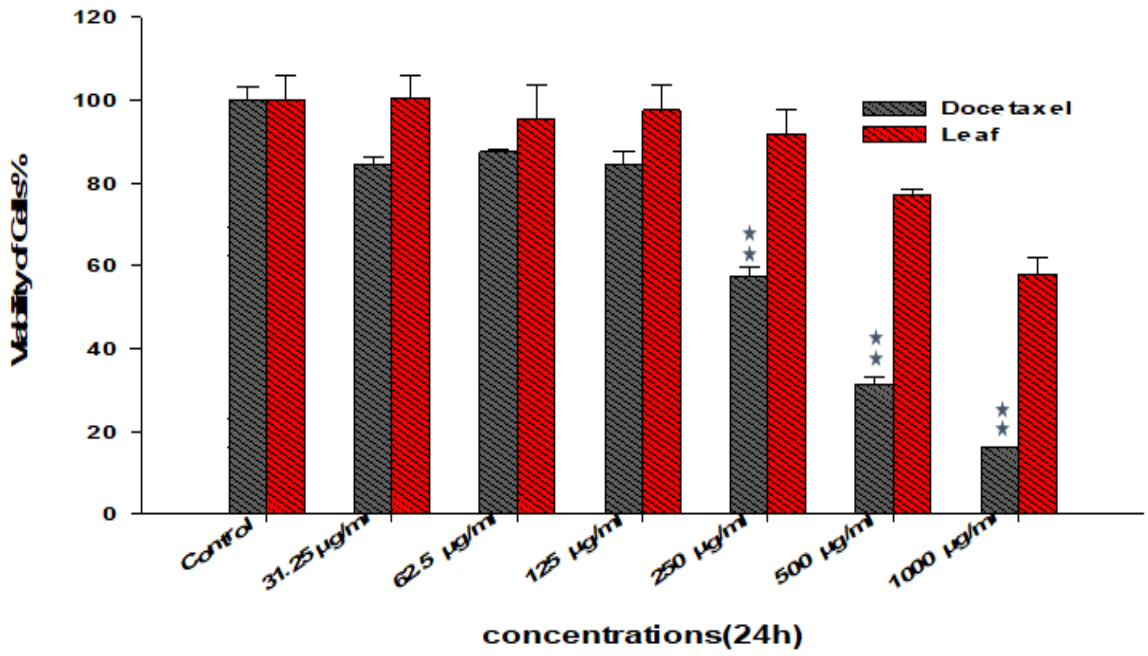


Effect of ethanolic extract of *Moringa oleifera* seeds and docetaxel on the viability of LNCaP cells for 48h

Figure (3-15)

3.16 Effect of different concentrations of ethanolic extract of *Moringa oleifera* leaves and docetaxel on the viability of LNCaP cell line after 24 hours of incubation.

Result showed that there was a highly significant difference ($P \leq 0.001$) between the effect of docetaxel and ethanolic extract of *Moringa oleifera* leaves at only high concentrations (250, 500, 1000) of both agents after 24 hours of incubation as shown in figure (3-16).

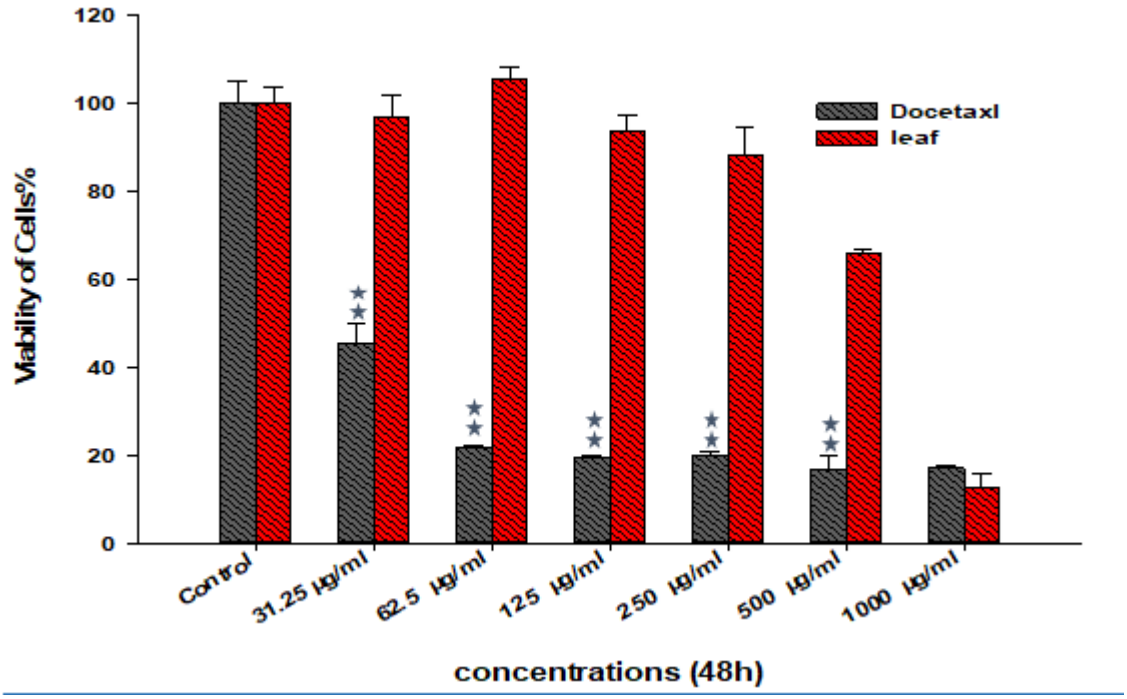


Effect of ethanolic extract of *Moringa oleifera* leaves and docetaxel on the viability of LNCaP cells for 24h

Figure (3-16)

3.17 Effect of different concentrations of ethanolic extract of *Moringa oleifera* leaves and docetaxel on the viability of LNCaP cell line after 48 hours of incubation.

Results of this comparison showed there were highly significant (P ≤0.001) difference in reduction cells viability of LNCaP cell line after exposure to test agents for 48 hour at different concentrations (31.25 µg/ml , 62.5 µg/l , 125µg/ml , 250 µg/ml , 500µg/ml) and will become nonsignificant at concentration 1000µg/ml where the P value ≤0.988) as shown in figure(3-17) .



Effect of ethanolic extract of *Moringa oleifera* leaves and docetaxel on the viability of LNCaP cells for 48h

Figure (3-17)

CHAPTER FOUR DISCUSSION

4.1 The antiproliferative effect for ethanolic extract of *Moringa oleifera* seeds and leaves against LNCaP cell line and IC50 measurement.

Natural materials, or plant-derived bioactive compounds, have already received increased attention in cancer chemotherapy because they are thought to be more biologically responsive, co-evolved with their target sites, and less toxic to normal cells (Cragg and Newman, 2005; Chaudhary *et al*., 2019).

Furthermore, there is a plethora of evidence that supports the anticancer properties of natural product-derived drugs as an alternative mode of cell death promotion. Based on these tidbits of information, many researchers are now focusing their efforts on the plants' potential to deliver natural products that can benefit the pharmaceutical industry (Iqbal *et al*., 2017; Kumar *et al*., 2018a). *Moringa oleifera* is a plant that is commonly found throughout the world and has numerous medicinal properties, including anti-inflammatory, antifungal, antibacterial, and anticancer properties (Karim *et al*., 2016; Elsayed *et al.*, 2016; Kou *et al.*, 2018).

In the current study, ethanolic seed extract of ***Moringa oleifera*** highly significant ($P \leq 0.001$) reduces the cell viability of the LNCaP cell line in a dose and time-dependent manner. This outcome corresponds to Another study published in 2015 confirmed the antiproliferative effect of ***Moringa oleifera*** seed extract, demonstrating that increasing the concentration of seed oil significantly reduced the viability of various cell lines after 24 hours of incubation, depending on the concentration used(Elsayed *et al*., 2015).The current study also found that ethanolic leaves extract of *Moringa oleifera* significantly reduced LNCaP cell viability ($P \leq 0.01$), and that this reduction was dose and time dependent. This

finding is consistent with the findings of many other studies, including (Wang *et al*., 2020) study, which discovered that the anti-proliferative effects of *Moringa oleifera* extracts were time- and concentration-dependent after incubation for various periods (12-24–48 hours). In addition to another study published in 2011, (Sreelatha *et al*., 2011) conducted a semi-similar study to the present study by using the MTT reduction assay to determine the effect of ***Moringa oleifera*** leaves extracts on the extent of survival of KB tumor cells. Moringa oleifera leaves extract inhibited KB cell proliferation in a dose-dependent manner. Another study discovered that increasing the concentration of ***Moringa oleifera*** leaves extract (seeds or leaves) resulted in a significant dose-dependent increase in its anticancer effect. a 2013 study (Berkovich *et al*., 2013a). Based on the all above findings and research, it is possible that the ethanolic extract of *Moringa oleifera* leaves and seeds has antiproliferative properties.

Docetaxel in the present study showed highly significant ($P \leq 0.001$) reduction in cell viability of LNCaP in dose and time dependent manner. Which agreed with study done in 2010 referred to ability of docetaxel to inhibit growth of LNCaP cell (Hwang *et al*., 2010).

The results of the current study's half inhibitory concentration finding for docetaxel, ethanolic extract of ***Moringa oleifera*** *seeds and leaves are (423 g/ml,600 g/ml,700 g/ml) respectively, demonstrating the superiority of seeds over leaves in antiproliferative effect and correlating with other studies. Moringa oleifera* leaves extract had a wide spectrum of antiproliferative effect in different cancer cells as shown in study of (del Mar Zayas-Viera Pablo Vivas-Mejia & Reyes, 2016) where find the antiproliferative effect of ***Moringa oleifera*** and half inhibitory concentration differ according to cell line as showed in previous studies where The *Moringa oleifera* ethanolic extract was tested in ovarian, prostate and

breast cancer cell lines. Seventy-two hours post-treatment, the cell viability was measured by a colorimetric analysis with the AlamarBlue dye. The concentration inhibiting 50% of cell growth (IC50) was calculated. The IC50 of *Moringa oleifera* extract in the cisplatin-resistant ovarian cancer cells, A2780CP20, was 270 µg/ml. The IC50 for the prostate cancer cells, PC3, was 170 µg/ml. this difference in Moringa oleifera efficacy and IC50 with different cell type is corresponding with current study , where according to the value of IC50 recorded by the current study the ethanolic extract of *Moringa oleifera* seeds was more potent as antiproliferative agent against LNCaP cell line in comparison to the ethanolic extract of *Moringa oleifera* leaves (IC50 was 600µg/ml versus 700µg/ml) despite that the flavonoid content, tannins and phenolic compounds are higher in leaves than seeds of *Moringa oleifera* to which antiproliferative effect is attributed this may be related to type of cell line or to presence of additional contents in seed do this effect on LNCaP cell line which used in the current study . Our findings are consistent with those of (Elsayed *et al*., 2015), who found that increasing the concentration of seed oil significantly reduced the viability of different cell lines after 24 hours of incubation, with variations between cell lines when treated with different concentrations of seed oil. In this case, HeLa cells had the greatest decrease in cell viability, followed by HepG2, MCF-7, L929, and CACO-2, which agreement with current study where the IC50 for seeds is lower than that of leaves.

4.2 Effect of ethanolic extract of *Moringa oleifera* (leaves and seeds) on level of TRAIL in LNCaP cell line.

Apoptosis is a major process for cell death that plays an important role in many diverse processes ranging from development to stress responses (Gechev *et al*., 2006) . The inactivation of apoptosis is a central cause to many cancers. Therefore, induction of apoptosis seems to be an effective strategy against tumor progression (Shiraishi *et al*., 2005) Screening of plants and plant derived products as potential inducers of apoptosis have become the major target in anticancer drug research. In the present study the TRAIL was used as indicator to the induction of apoptosis that occurred after the treatment of LNCaP cell line with ethanolic extract of ***Moringa oleifera*** (seeds or leaves) and docetaxel. The highly significant concentration dependent increase in the level of TRAIL after the treatment of LNCaP cell line with each ethanolic extract of ***Moringa oleifera*** (seeds and leaves) and docetaxel reported by the current study indicate the ability of this extract to induce apoptosis in the LNCaP cell line. There are several preclinical research works had confirmed the ability of seed, leaves and bark of ***Moringa oleifera*** to induce apoptosis in many cancer cells leading to their ultimate death (Adebayo *et al*.,
2017).Another study discovered that an ethanolic extract of M. oleifera seeds mildl y induced apoptosis in HCT8 cell lines (Al-Asmari A, 2015). The findings of present study are consistent
also with those of Khan *et al*. (2020), in which we demonstrated that ***Moringa oleif era*** extract inhibits growth and induces apoptosis via the Notch signaling pathway. Also, according to additional research, the anti-proliferative activity of Moringa oleifera's ability to scavenge ROS in cancer cells results in cell apoptosis(chhikara *et al*.,2021).

4.3 Effect *Moringa oleifera* ethanolic extract (Leaves, Seeds) and docetaxel on the viability of LNCaP cell line After 24 and48 hours incubation period.

Results of current study it shown highly significant ($P \leq 0.001$) difference in cell viability reduction effect where the docetaxel is superior than ethanolic extract of ***Moringa oleifera*** leaves extract at treatment period 24 hour and got rapprochement in effect after increasing period of treatment to 48 hour and increased the extract concentration to 1000µg/ml as shown in figures (3-16) and (3-17). from upper mentioned finding of present study, the period of treatment and concentration are important to get antiproliferative effect from ethanolic extract of *Moringa oleifera* leaves near to effect getting from docetaxel.

Regarding ***Moringa oleifera*** ethanolic seeds extract the finding in present study referred to rapprochement in reduction cell viability effect between ethanolic extract of ***Moringa oleifera*** seeds and docetaxel at 24hour treatment period at all concentration taken in this study and loss of this rapprochement by the noted increase in effect of docetaxel when increase period of treatment to 48 hour in all concentrations taken except at 1000µg/ml as showed in figures (3-14) and (3-15).

unfortunately, until now, there are few or no studies make comparison in antiproliferative effect (reduction of cell viability) of ***Moringa oleifera*** and chemotherapy but there are few studies refer to antiproliferative effect of ***Moringa oleifera*** extract in compared with another plant like (Supaporn Pamok, 2012) which get the following conclusion the ***Moringa oleifera*** extracts had better antiproliferative activity on all types of cells than the *Pseuderanthemum palatiferum* extracts. Moreover, the aqueous and ethanol extracts of ***Moringa oleifera*** had better antiproliferative effect on all types of tested colon cancer cell lines than other plant extracted with the same solvent for example, ***Moringa***

oleifera *hortensis* which has higher IC50 than that of ***Moringa oleifera***. Antiproliferative activity measured by MTT dye is used to determine the cell viability in assays of cell proliferation and cytotoxicity (shoemaker *et al*., 2004). So, in present study our comparison between ***Moringa oleifera*** ethanolic extract and docetaxel was consider from few studies done in this concept and it gives hope to the possibility of using *Moringa oleifera* as an alternative to chemotherapy one day after conducting clinical studies on it. Also shown from current study the ethanolic seeds extract of *Moringa oleifera* is superior than Leaves extract in comparison with docetaxel and this is corresponded with new study done recently refer to results showed that the ethanolic extract from ***Moringa oleifera*** seeds exhibited the highest antiproliferative activity against CRC cell lines compared to the rest of the ***Moringa oleifera*** extracts (Fuel *et al*., 2021).

4.4 Effect of the combination from different concentration of ethanolic *Moringa oleifera* seeds or leaves extract and IC50 concentration of docetaxel on LNCaP cell line.

The use of plant extracts, either alone or in association with other therapeutic agents, have high potential in the field of cancer therapy due to their safety, efficacy, reduced toxicity, and low propensity for the development of resistance (Fuel *et al*., 2021). Many efforts have been made to study the synergistic effects of combined therapy between conventional and traditional herbal medicines. Regarding the synergistic effect of ethanolic extract of *M. oleifera* seeds with docetaxel IC50, the current study found that the reduction in viability of the LNCaP cell line was significant ($P \leq 0.05$) at a concentration of 62.5 µg/ml after 24 hours of incubation as compared to control, and that this reduction effect in cell viability will be highly significant ($P \leq 0.001$) at a concentration of 31.25µg/ml.

The ethanolic extract of ***Moringa oleifera*** leaves caused a highly significant ($P \leq 0.001$) reduction in cell viability of the LNCaP cell line starting at 250µ g/ml in a 24-hour exposure period and a significant ($P \leq 0.01$) reduction in cell viability starting at 31.25µg/ml in a 48-hour exposure period in this study. This result of current study refers to the presence of an enhancement in the anti-proliferative effect of docetaxel when combined with an ethanolic extract of *Moring oleifera* (seeds and leaves). This finding is consistent with a recent study by (Sahruddin *et al*., 2021), which found that combining gemcitabine with M oleifera extract improved the chemotherapeutic effect of gemcitabine on pancreatic cancer. The current study's findings are also consistent with the findings of(Brown *et al*., 2020), who found that an ethanolic extract of ***Moringa oleifera*** synergized the antiproliferative effect of vesicular stomatitis virus, which has been used as oncolytic viral therapy against cervical cancer cells. In addition, another study

found that ***Moringa oleifera*** acted synergistically with doxorubicin to induce cytotoxicity and increased apoptosis in human HeLa cervical cancer cells. Finaly

(Berkovich *et al*., 2013b) which revealed to a dose-dependent significant increase in anticancer effect of ***Moringa oleifera*** leaves extract. which inhibits the growth of pancreatic cancer cells, the cells NF-κB signaling pathway, and increases the efficacy of chemotherapy in human pancreatic cancer cells with increase plant extract concentration.

Conclusions

1. Ethanolic extracts of *Moringa oleifera* seeds and leaves have antiproliferative effects against LNCaP (androgen-sensitive human prostate adenocarcinoma cells) cell line.
2. The antiproliferative effect of *Moringa oleifera* seeds ethanolic extract against LNCaP cell line is more than that of leaves extract.
3. Ethanolic extracts of *Moringa oleifera* seeds and leaves have apoptotic effects (increase TRAIL level) against LNCaP cell line.
4. The antiproliferative effects of seeds and leaves ethanolic extracts of *Moringa oleifera* against LNCaP cell line are less than that of Docetaxel.

Recommendations

1. Investigate the effect of *Moringa oleifera* seeds and leaves extracts on other type of cancer cell lines.
2. Further researches are required to use *Moringa oleifera* seeds and leaves extracts with other anticancer agents.
3. Experimental studies are required to assess the antiproliferative effect of *Moringa oleifera* seeds and leaves extracts in animal models-induced cancer.
4. Clinical trials to estimate the antiproliferative effect of *Moringa oleifera* seeds and leaves extracts on different types of cancer are recommended.

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الخلاصة :

السرطان مرض غير معدي لكن اغلب الاحصائيات تشير لكونه السبب الرئيسي للوفاة في جميع أنحاء العالم. يعد سرطان البروستات ثاني أكثر أنواع السرطانات شيوعًا بين الرجال وخامس سبب رئيسي للوفاة في جميع أنحاء العالم. تعود الاختلافات في معدلات الإصابة الى الاختلاف في الطرق المختبرية المستخدمة للكشف عن المرض بين دولة واخرى. ترتبط معدلات الإصابة بسرطان البروستات ومعدلات الوفيات ارتباطًا وثيقًا بالعمر، حيث لوحظت أعلى نسبة إصابة تكون في الرجال المسنين فوق سن 65. بينما تنمو بعض أنواع سرطان البروستات ببطء وقد تحتاج الى علاج بسيط أو لا تتطلب علاجًا، بالرغم من ذلك فإن البعض الآخر يكون عدوانيًا وينتشر بسرعة.

تلعب الطبيعة دورًا هاما في تطوير العديد من المركبات التي يمكن أن تساعد في علاج الأمراض المختلفة مثل السرطان الذي له اهتمام خاص بالطب. هدفت هذه الدراسة في المقام الأول إلى تقييم تأثير المستخلصات الإيثانولية لأوراق وبذور *Moringa oleifera* و docetaxel (علاج كيميائي) على نمو خط الخلايا LNCaP كل مادة على حدة ومجتمعة ، وثانياً لتقدير تأثير هذه العوامل على مستوى تحفيز عامل TRAIL. ،والذي يلعب دورا مهم في تنظيم عملية موت الخلايا المبرمج (apoptosis)في خط خلية LNCaP.

تم إجراء هذا البحث في مختبر بحثي لطلاب الدراسات العليا في كلية الطب بجامعة بابل من كانون الثاني 2021 إلى تشرين الأول 2021.

لأداء الهدف الأول ، تم زرع خلايا LNCaP في لوح مختبري خاص يتكون من 96 لوح صغير خاص في زراعة الأنسجة و حيث تم معالجتها بتراكيز مختلفة (1000 ، 500 ، 250 ، 125 ، 62.5 ، 31.25 ميكروغرام / مل) من كل مستخلص إيثانولي (بذور وأوراق)) تم وضع *Moringa oleifera* docetaxel حيث تم وضع الالواح في حاضنة خاصة لمدة 24 ساعة ، وتم عمل نفس الإجراءات ولكن بدلاً من ذلك كانت فترة الحضانة 48 ساعة. من أجل تقييم تأثير مستخلصات *Moringa oleifera* على النشاط المضاد للسرطان docetaxel ، تم قياس التركيز اللازم لقتل 50 بالمائة من الخلايا (IC50) لاحقًا ، ثم تم إجراء نفس الإجراء ولكن تمت معالجة خط الخلايا LNCaP بمزيج من تراكيز مختلفة لكل منهما المستخلص الإيثانولي (البذور والأوراق) مع docetaxel IC50 والمحتضنة أيضًا لفترات مختلفة (24 و 48 ساعة). بعد ذلك تم إجراء فحص السمية الخلوية MTT لقياس عدد الخلايا الحية باستخدام جهاز مختبري خاص.

لقياس مستوى TRAIL في خلايا LNCaP بعد 24 ساعة من العلاج بالمستخلص الإيثانولي من *Moringa oleifera* (الأوراق والبذور) ، docetaxel ومزيج من المستخلص النباتي الإيثانول بالإضافة إلى docetaxel IC50. تم استخدام TRAIL Kit خاص من نوع (Sandwich- ELISA) . تحتوي هذه المجموعة على لوحة micro-ELISA مطلية مسبقًا بجسم مضاد خاص بـ Human TRAIL. يتم خلط خلايا LNCaP المعالجة مع الجسم المضاد المحدد في لوحة Micro-ELISA. بعد ذلك، تم استخدام الجسم المضاد للكشف عن البيروكسيديز الخاص بـ Human TRAIL.

يتم حفظ كل لوح مع متشابه له من Avidin-Horseradish Peroxidase (HRP) . ثم يتم حساب تركيز TRAIL في العينة باستخدام معادلة خاصة بالكثافة البصرية (Optical density).

أظهرت نتائج هذه الدراسة انخفاضًا معنويًا ($P\leq 0.001$) في عدد الخلايا الحية في خط الخلايا LNCaP بعد العلاج بالمستخلص الإيثانولي لبذور *Moringa oleifera* في كلتا فترتي الحضانة وبطريقة تعتمد على الوقت والتركيز حيث كان التركيز المطلوب لقتل 50% من عدد الخلايا هو IC50 الخاص به 600 ميكروغرام / مل.

في حين أن المستخلص الإيثانولي لأوراق *Moringa oleifera* قلل من قابلية بقاء خط خلية LNCaP بشكل كبير ($P\leq 0.01$) وعالي بشكل ملحوظ ($P\leq 0.001$) بعد 24 و 48 ساعة من الحضانة على التوالي بطريقة تعتمد على التركيز والوقت ، وكان (IC50) الخاص به هو 700 ميكروجرام / مل.

قلل Docetaxel من عدد الخلايا الحية في خط خلايا LNCaP بشكل كبير ($P\leq 0.001$) بطريقة تعتمد على التركيز والوقت بعد كلتا فترتي الحضانة ، وكان IC50 الخاص به 423 ميكروغرام / مل.

أظهرت النتائج أنه بعد مرور 24 ساعة من الحضانة ، أدى الجمع بين تراكيز مختلفة من مستخلص بذور *Moringa oleifera* الإيثانولي بالإضافة إلى docetaxel IC50 إلى انخفاض معنوي (P 0.05) في عدد الخلايا الحية في خط الخلايا LNCaP. في حين أن الجمع بين تراكيز مختلفة من مستخلص أوراق *Moringa oleifera* الإيثانولي بالإضافة إلى docetaxel IC50 يؤدي إلى انخفاض معنوي ($P\leq$ 0.001) في عدد خلايا الحية في خط الخلايا LNCaP ، بينما بعد 48 ساعة من الحضانة ، يتم الجمع بين تراكيز مختلفة من مستخلص بذور *Moringa oleifera* الإيثانولي بالإضافة إلى docetaxel IC50 ينتج عنه دلالة عالية ($P\leq 0.001$) ، ومع ذلك فإن الجمع بين تراكيز مختلفة من مستخلص أوراق *Moringa oleifera* الإيثانولي بالإضافة إلى docetaxel IC50 يؤدي إلى انخفاض معنوي ($P\leq 0.01$) في عدد الخلايا الحية في خط الخلايا LNCaP.

فيما يتعلق بتأثير فترة الحضانة ، أظهرت النتائج أنه بعد 24 ساعة من تعرض الخلايا للمستخلص الايثانولي للنبات وكذلك docetaxel لم يكن هناك فرق معنوي ($p \geq 0.05$) بين تأثير المستخلص الإيثانولي لبذور *Moringa oleifera* والأوراق و docetaxel على حيوية خط الخلايا LNCaP بعد 24 ساعة الحضانة ، بينما كان هناك فرق معنوي ($P \leq 0.001$) في تقليل قابلية بقاء خط خلية LNCaP بعد 48 ساعة من التعرض لكل من العوامل المختبرة في هذه الدراسة ولجميع التراكيز باستثناء 1000 ميكروغرام / مل حيث كان الفرق ضئيلاً ($P \leq 0.05$).

فيما يتعلق بمستوى نتائج TRAIL أظهرت زيادة معنوية عالية($P< 0.001$) وهذه الزيادة تعتمد على التركيز في مستوى TRAIL بعد 24 ساعة من معالجة خط خلية LNCaP مع المستخلصات الإيثانولية من *Moringa oleifera* (البذور والأوراق) و docetaxel كل مادة على حدة وفي تجربة أخرى تم جمع المستخلص الايثانولي للنبات (البذور او الأوراق) مع مادة docetaxel ، لوحظ كل من المستخلصات الإيثانولية للبذور والأوراق من *Moringa oleifera* تتسبب في زيادة مستوى TRAIL مما يثبت ان لها تأثيرات على عملية الموت المنظم التي تحدث في الخلايا (Apoptosis) ضد خط خلايا LNCaP ، على الرغم من أن مستخلص البذور له تأثير أكثر من مستخلص الأوراق لكن التأثيرات على عملية الموت المنظم التي تحدث في الخلايا (Apoptosis) لبذور *Moringa oleifera* والمستخلصات الإيثانولية للأوراق ضد خط خلايا LNCaP أقل من تأثيرات docetaxel.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل – كلية الطب

فرع الادوية

دراسة خارج الجسم لتأثير مستخلص المورنكا اوليفيرا و علاج الدوسيتاكسل على تكاثر خلايا LNCaP وعلى عامل نخر الورم المرتبط بتحفيز الاستماتة (TRAIL)

رسالة

مقدمة في مجلس كلية الطب / جامعة بابل

كجزء من متطلبات نيل درجة الماجستير في الادوية والسموم

من قبل

ايمن احمد جواد محمد

بكالوريوس صيدلية (2010 – 2011)

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