Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Medicine
Department of Pharmacology

Effect Of Enoxaparin And Heparin On Apoptotic ,Inflammatory, And Antioxidant Biomarker in SW480, And LNCaP Cell Line

A thesis Submitted to the Council of the College of Medicine, the University of Babylon, as a Partial Fulfillment of the Partial Requirements for the Degree of Master (M.Sc.) in Pharmacology /Toxicology.

By:

Noor Kareem Mezal Obead

BSc Pharm. (2013-2014)

Supervised by:

Assist.Prof.

Majid Kadhim Abbas

MSc.pharmacology

2022 A.D

Prof. Dr.

Kaiser N. Madlum

Ph.D. Biology

1443 A.H

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

"يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَ الَّذِينَ أُوتُوا الْعِلْمَ دَرَجَات "

صدق الله العليَ العظيم

(سورة المجادلة : الاية 11)

Certification

We certify that this thesis entitled (**Effect of enoxaparin and heparin on apoptotic, inflammatory,and antioxidant biomarker in SW480 and LNCaP cell line**) was prepared by (**Noor Kareem Mezal Obead**) 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.

Signature
Assist.Prof.
Majid Kadhim Abbas
Supervisor

Signature
Prof. Dr.
Kaiser N. Madlum
Supervisor

Date:

The Recommendation of the Head of Department

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

Signature
Assist. Prof. Dr.
Selman Mohammed Selman
The Head of Department of Pharmacology
College of Medicine
University of Babylon, Iraq
Date:

Examining Committee Certificate

We certify that we have read this thesis entitled "(**Effect of enoxaparin and heparin on apoptotic, inflammatory, and antioxidant biomarker in SW480 and LNCaP cell line)** " and as an examining committee examined the student "**Noor Kareem Mezal**" in its contents and that in our opinion it meets the standard of a thesis for the degree of Master, in Pharmacology and Toxicology.

Signature

Prof. Dr.

Bassim I.Mohamad

(Chairman)

Signature

Prof. Dr.

Aliaa Saad Karkush

(Member)

Signature

Lecturer.Dr.

Reyadh H.Hashem

(Member)

Signature

Assist.Prof.

Majid Kadhim Abbas

(Supervisor and Member)

Signature

Prof. Dr.

Kaiser N. Madlum

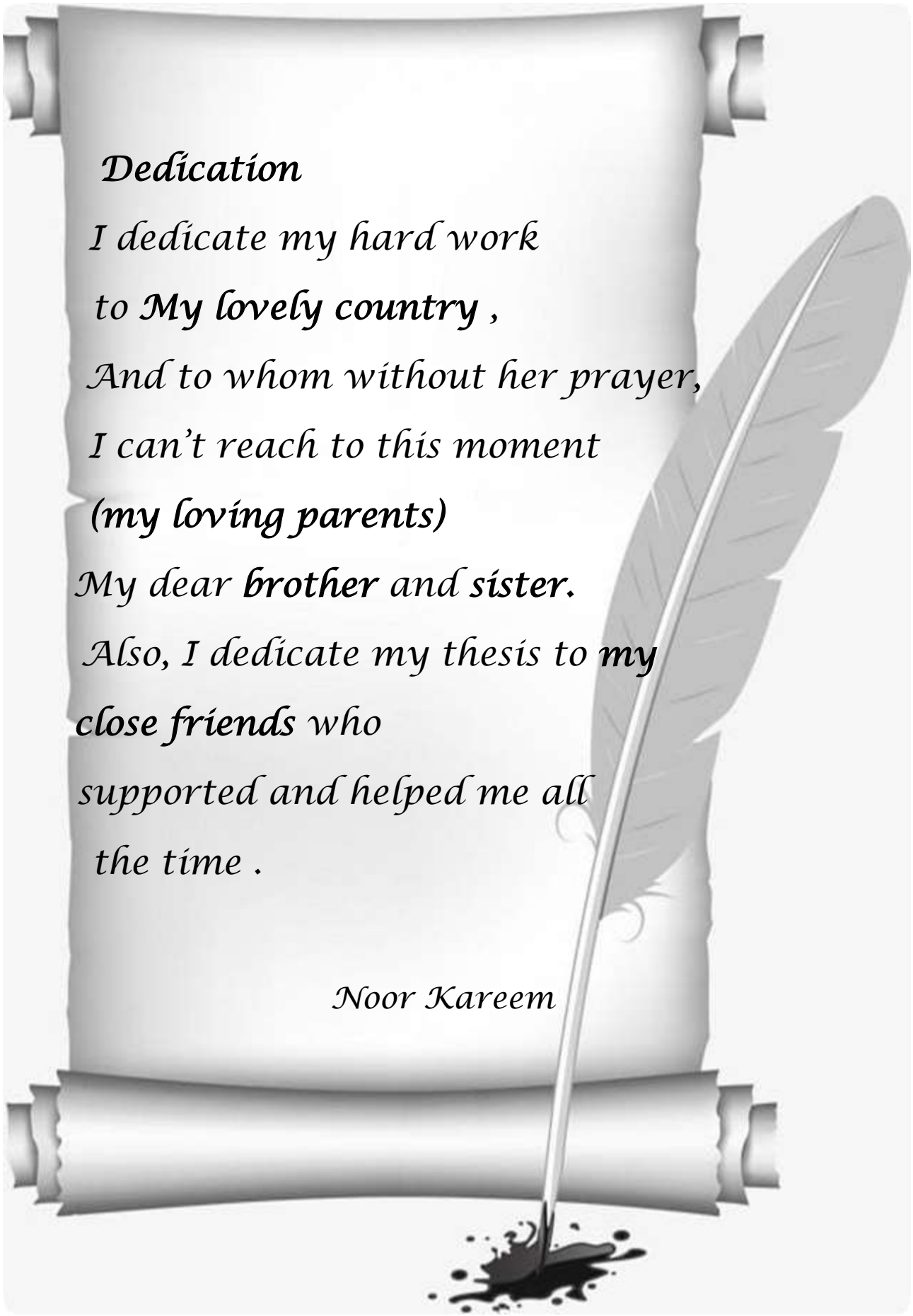
(Supervisor and Member)

Approved for the college council

Prof.

Dr. Muhanad Abbas Alshalah

(Dean of College Medicine)



Dedication

I dedicate my hard work

to ***My lovely country*** *,*

And to whom without her prayer,

I can't reach to this moment

(my loving parents)

My dear ***brother*** *and* ***sister.***

Also, I dedicate my thesis to ***my close friends*** *who*

supported and helped me all

the time .

Noor Kareem

Acknowledgments

I am very grateful to my God for helping me to perform and finish this work despite all difficulties …my faithful thanks and appreciation for my research supervisor's Assist. Prof. **Dr. Majid K.Abbas** and Prof. **Dr. Kaiser N. Madlumm** for their supervision, help, and continuous advice, I am very grateful for working under their guidance….

Special thanks go to the department of pharmacology for their support and cooperation…

My special thanks also go to **Dr.Angham Mohsen** for hers help and support.

Finally, All words cannot suffice in thanking my lovely family, my brother and sister, and my close friend, for their patience, support, and encouragement….

Summary

Cancer starts when cells in the body begin to grow out of control. Cells in nearly any part of the body can become cancer cells, and can then spread to other areas of the body. The most frequent type of cancer worldwide is colon cancer. Operation, radiation, targeted therapy, and immune therapy are all treatments for colon cancer. Prostate cancer is the most common cancer among men. In the present study, an effort has been made to assess the effect of heparin and enoxaparin on the viability of the normal Vero cell line, SW480 colon cancer cell line, and LNCaP prostate cancer cell line. The second objective was to evaluate the effect of heparin and enoxaparin on the expression of TNF-α and caspase3 in SW480 colon cancer and LNCaP prostate cancer cells. In addition, this study aimed to evaluate the antioxidant effect of heparin and enoxaparin on SW480 colon cancer and LNCaP prostate cancer cell lines.

The experimental work was performed in the post-graduate student's research laboratory at the College of Medicine/University of Babylon during the period from December 2021 to April 2022.

The first experiment was the cytotoxicity assay, Vero normal, prostate cancer (LNCaP), and colon cancer (SW480) Cell lines were seeded in 96 tissue culture plates. After 24 hours, cells were exposed to different concentrations of drugs (heparin or enoxaparin) at serial dilutions ranging from (1000,500,250,125,62 to31 µg\ml) four replicates were used for each concentration of both heparin and enoxaparin for each type of cells along with four replicates as a control group for each cell type. All plates were incubated for 24 hours a 37C°, then, the MTT cytotoxicity assay was performed.

The result showed that heparin causes a significant *(P≤0.001)* decrease in the viability of Vero cells at all concentrations. Heparin significantly *(P≤0.050)* reduces the viability of SW480 colon cancer cells at the concentrations of (1000 and 500µg/ml) while it has no significant effect on the viability of the LNCaP prostate cancer cell line. The result showed that enoxaparin significantly *(P≤0.050)* reduces the viability of SW480 colon cancer cell at all concentration but has no effect on the viability of Vero and LNCaP prostate cancer cell.

In the second experiment, LNCaP and SW480 Cell in 96 tissue culture plates were treated with different concentrations of the drugs (heparin or enoxaparin) at serial dilutions ranging from 1000 to 31 µg\ml (four replicates were used for each concentration) along with four replicates as a control group for each cell type. Then incubated for 24 hours, at the end of the exposure period, they were taken for immunoassay by ELISA method for the measurement of TNF-alpha and caspase3 concentrations.

The result showed that heparin has no significant effect on TNF-α level in SW480 colon cancer cells however causes a significant *(P≤0.050)* decrease in TNF-α level at the concentration (1000,250,125µg/ml) in the LNCaP prostate cancer cell line.

Enoxaparin significantly *(P≤0.050)* reduces TNF-α release at the concentration (1000,250,31µg/ml) in SW480colon cancer cells but has no significant effect on TNF-α release in the LNCaP prostate cancer cell line. Heparin does not affect caspase3 levels in SW480 colon cancer and LNCaP prostate cancer cell. Enoxaparin significantly *(P≤0.050)* increases caspase3 level in SW480 colon cancer cells while has no significant effect on caspase3 level in LNCaP prostate cancer cells

For measuring the total antioxidant activity, The CUPRAC Method was performed to detect the antioxidant effect of heparin and enoxaparin on SW480 colon cancer cells and LNCaP prostate cancer cell lines cultured in 96 well plates.

The result showed that heparin has no significant antioxidant effect in SW480 colon cancer cells but cause significant *(P≤0.050)* increase in antioxidant effect at the concentration (1000,250,31µg/ml) in the LNCaP prostate cancer cell line. The result showed that enoxaparin has no significant antioxidant effect in SW480 colon cancer cells but has significant *(P≤0.050)* increase in antioxidant effect at the concentration (250µg/ml) in LNCaP prostate cancer cells.

List of Contents

List of Tables

Figures

List of Abbreviations

Abbreviations	Meaning
AP-1	Activator protein-1
AR	Androgen receptor
AS	Active surveillance
BAX	Bcl-2-associated X protein
BC	Breast cancer
BCL-2	B-cell lymphoma 2
BPH	Benign prostatic hyperplasia
CC	Colon cancer
COPD	Chronic obstructive pulmonary disease
CRC	Colorectal cancer
CXCL1	C-X-C Motif Chemokine Ligand 1
CXCL12	C-X-C Motif Chemokine Ligand 12
CXCR4	C-X-C chemokine receptor type 4
DDW	Deionized distilled water
DMSO	Dimethyl sulfoxide

DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
EDTA	Trypsin- Ethyl ediaminetetraacetic acid
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
FAP	Familial adenomatous polyposis
FBS	Fetal bovine serum
FEV1	Forced expiratory volume in one second
GAG	Glycosaminoglycans
GC	Gastric cancer
GPx	Glutathione Peroxidase
GSH	Reduced glutathione
H2O2	Hydrogen peroxide
HCC	Hepatocellular carcinoma
HIT	Heparin-induced thrombocytopenia
HNPCC	hereditary non-polyposis colorectal cancer
HRP	Horseradish peroxidase
IBD	Inflammatory bowel disease
IL-10	Interlukin-10

IL-13	Interlukin-13
IL-18	Interlukin-18
IL-1α	Interlukin-1α
IL-1β	Interlukin-1β
IL-4	Interlukin-4
IL-5	Interlukin-5
IL-6	Interlukin-6
IL-8	Interlukin-8
ILs	Interlukins
JNK	c-Jun N-terminal kinase
LC	Lung cancer
LMWH	Low-molecular-weight heparin
LPS	Lipopolysaccharide
LUTS	Lower urinary tract symptoms
MAPK	The mitogen-activated protein kinases
MMP-2	Matrix Metallopeptidase 2
MMR	Measles,Mumps and Rubella
MUC5AC	Mucin 5AC
NCI	National Cancer Institute

NF-κB	Nuclear factor kappa-lightchain-enhancer of activated B cells
NK	Natural killer
OD	Optical density
OSCC	Oral squamous cell carcinoma
PBS	Phosphate buffer saline
PCa	Prostate cancer
PI3K	Phosphoinositide 3-kinase
PSA	Plasmatic levels of prostate-specific antigen
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RPMI-1640)	Roswell Park Memorial Institute
RT	Radiation therapy
SCC	Squamous cell carcinoma
SNPs	Single nucleotide polymorphisms
SOD	Superoxide dismutase
SPS	Serrated polyposis syndrome
TFPI	Tissue factor pathway inhibitor
TGF-β1	Transforming growth factor beta 1
Th	The helper T cells

TNF- R1	Tumor necrosis factor receptor 1
TNF- R2	Tumor necrosis factor receptor 2
TNF-α	Tumor necrosis factor-α
TRAF2	TNF receptor-associated factor 2
TS	Thymidylate synthase
UFH	Unfractionated heparin
WHO	World Health Organization

Chapter One

Introduction

and

Literature Review

1.1 Introduction

Cancers are defined by the National Cancer Institute (NCI) as a collection of diseases in which abnormal cells can divide and spread to nearby tissue. Cancer is one of the main causes of death worldwide. Despite the significant development of methods of cancer healing during the past decades, chemotherapy remains the main method of cancer treatment (Bukowski *et al* 2020).

Colorectal cancer (CRC) with 800 000 deaths per year globally is still one of the most common cancers in men and women This heavy public health burden is predicted to increase if no improvement in early detection and effective interventions for late-stage CRC are discovered (Lannagan *et al.*, 2021).

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 (Villers and Grosclaude, 2019).

Heparin, including unfractionated heparin (UFH), low-molecular-weight heparin (LMWH), and heparin derivatives, are commonly used in venous thromboembolism treatment and reportedly have beneficial effects on cancer survival. Heparin can affect the proliferation, adhesion, angiogenesis, migration, and invasion of cancer cells via multiple mechanisms (Ma *et al.*, 2020).

Enoxaparin mediated down-regulation of MAPK (mitogen-activated protein kinases) and PI3K (phosphoinosiride3-kinase), reduced MMP-2 (matrix metalloproteinzse 2) expression, and inhibited A549 cell migration. Additionally,

enoxaparin increased doxorubicin's efficacy by enhancing apoptosis, while no effect on cell-cycle progression was observed (Alturkistani *et al.*, 2019).

The cytotoxic effects of TNF-α-activated eosinophils on endothelial cells are also markedly inhibited by heparin (Page, 2013). Enoxaparin, the most widely used LMWH, is known to inhibit T cell-mediated release of multiple cytokines, including IL-4, IL-5, IL-13, and TNF-α, involved in various inflammatory disorders (Shastri *et al.*, 2015).

1.2 Aims of the study

1. To assess cytotoxic effect of heparin and enoxaparin on the viability of cancer and normal cell lines (SW480, LNCaP, and Vero cell lines).
2. To determine the anti-inflammatory and apoptotic effect of enoxaparin and heparin in the SW480 colon cancer cell line and LNCaP prostate cancer cell line.
3. To assess the antioxidant effect of heparin and enoxaparin on SW480 colon cancer cell and LNCaP prostate cancer cell line.

1.3 Cancer

Cancer remains the second most common reason for death in the United States and worldwide. According to an estimate by the American Cancer Society, about 1,806,950 new cancer cases and nearly 606,520 cancer-related deaths are projected for this year in the United States. Cancer is a complex disease driven by a variety of mechanisms. In many cases, its asymptomatic progression results in a late diagnosis and limiting therapeutic options (Sudan *et al.*, 2020).

The development of cancer involves a series of complicated events, often taking decades to occur. In most cases, the final result is altered genes that regulate differentiation and cell growth. In terms of gene alteration to form a cancer cell, a normal cell's genome acquires mutations in tumor suppressor genes, proto-oncogenes, and other genes involved in cell growth regulation (Tacar *et al.*, 2013).

Metastasis involves the spread of cancer cells from the primary tumor to surrounding tissues and distant organs and is the primary cause of cancer morbidity and mortality. To complete the metastatic cascade, cancer cells must detach from the primary tumor, intravasate into the circulatory and lymphatic systems, evade immune attack, extravasate at distant capillary beds, and invade and proliferate in distant organs (Seyfried and Huysentruyt, 2013).

1.4 Pathogenesis of cancers

It was estimated that there were about 18.1 million newly diagnosed cancer cases and about 9.6 million cancer-related deaths worldwide in 2018. Lung cancer (LC) has the highest incidence and mortality rate among human cancers. Female breast cancer (BC), prostate cancer (PCA), and colorectal cancer (CRC) are the

second, third, and fourth cancers with the highest incidence, respectively. CRC, gastric cancer (GC), and hepatocellular carcinoma (HCC) are the three cancers with the highest mortality rate beyond LC. The main risk factors that influence the incidence and mortality of cancers include rapid population growth and aging, socioeconomic development and patient's low screening compliance caused by lower education and income, and lack of health insurance and awareness So far, most cancers are not effectively diagnosed at the early stage (Chi *et al.*, 2019)

1.5 Colon cancer

The fourth deadliest cancer in the world is colon cancer (CC) and its incidence is increasing in many countries It represents approximately 10% of total cancer cases in both sexes. Many factors are strongly linked to CC, including genetics, environmental factors, physical activity, and age. Currently, surgery and chemotherapy are the two main treatment options for CC, according to tumor location and patient characteristics (Toolabi *et al.*, 2021).

CRC usually emerges from the glandular, epithelial cells of the large intestine. Cancer arises when certain cells of the epithelium acquire a series of genetic or epigenetic mutations that confer on them a selective advantage. With abnormally heightened replication and survival, these hyper-proliferative cells give rise to a benign adenoma, which may then evolve into carcinoma and metastasize over decades (Rawla *et al.*, 2019).

1.5.1 Epidemiology of colon cancer

More than 50% of all cases occur in more developed regions with wide geographic variation in incidence across the world. Incidence rates vary ten-fold in both sexes worldwide, the highest estimated rates are found in Oceania (age-

standardized rates of 44.8 and 32.2 per 100,000 in men and women, respectively), and the lowest in Western Africa (4.5 and 3.8 per 100,000). In 2012, 345,000 new cases and 152,000 deaths were reported in the European Union. In some regions with previously low incidence rates, e.g. Eastern Europe and East Asia, significantly increasing numbers of colorectal cancer cases have been noted and attributed to changes in risk factors and diet towards a lifestyle common to Western countries (Kolligs, 2016).

1.5.2 Diagnosis of colon cancer

Mostly, the diagnosis of CRC at first depends on an endoscopic biopsy or polypectomy. The key part of the microscopic assessment is to search for proof of invasion, when the biopsy is superficial or not well arranged, the diagnosis can be difficult. When the muscular mucosae can be recognized, it is critical to decide if it is present in neoplastic cells. Invasive CRC ordinarily invades through muscularis mucosae into the submucosa and can be seen near submucosal blood vessels. Desmoplasia is another important observation of invasion, which is a type of fibrous proliferation auxiliary to invasive tumor growth. Another feature, dirty necrosis, is necrotic debris in glandular lumina can be seen in invasive CRC and is routinely useful to indicate primary CRC when metastasis is encountered (Fleming *et al.*, 2012).

1.5.3 Stages Of Colon Cancer

1.5.3.1 Stage 0 Colon Cancer

When abnormal cells are found in the wall, or mucosa, of the colon, it is considered stage 0 colon cancer. This is also called carcinoma in situ.

1.5.3.2 Stage I Colon Cancer

Cancer which has invaded the mucosa and the submucosa is considered stage I colon cancer.

1.5.3.3 Stage II Colon Cancer

When cancer has spread past the colon wall but has not affected the lymph nodes, it is considered stage II colon cancer. This condition is subdivided into three categories, stage IIA cancer has spread to the serosa or outer colon wall, but not beyond that outer barrier, stage IIB cancer has spread past the serosa but has not affected nearby organs, stage IIC cancer has affected the serosa and the nearby organs.

1.5.3.4 Stage III Colon Cancer

Cancer that has spread past the lining of the colon and has affected the lymph nodes is considered stage III colon cancer. In this stage, even though the lymph nodes are affected, cancer has not yet affected other organs in the body. This stage is further divided into three categories: IIIA, IIIB, and IIIC, where the cancer is staged depending on a complex combination of which layers of the colon wall are affected and how many lymph nodes have been attacked.

1.5.3.5 Stage IV Colon Cancer

In this stage, cancer has spread to other organs in the body through the blood and lymph nodes (Li et al., 2016).

1.5.4 Symptoms of colon cancer

The definition of clinical variables of symptoms and hemoglobin measurements was as follows: Changes in bowel habits: any history of changes in the frequency of defecation, constipation, or diarrhea. Blood in stools: visible blood in the stool as well as melena. Abdominal pain: any history of bloating, dyspepsia, or other discomforts in the abdomen. Mucus in feces: mucus noted in feces. Tenesmus: continuous or unfounded need for defecation. General symptoms: fatigue, unintentional loss of weight, anorexia, fever of unknown origin, night sweats, unexplained tiredness. Acute symptoms: colonic obstruction, fresh bleeding, and/ or perforation which lead to immediate surgery or other procedures (Alexiusdottir et al., 2012).

1.5.5 Risk factors of colon cancer

The chance of developing CRC can be increased by environmental factors and/or genetic factors. The various risk factors for developing CRC include age above 50, low socioeconomic class, overweight and obesity, sedentary lifestyle, tobacco smoking, heavy alcohol intake, low-fiber, and high-fat diet, consumption of red meat, processed meat, and burnt or charred meat, diabetes mellitus and insulin resistance, acromegaly, renal transplantation with long-term immunosuppression, long-term androgen deprivation therapy, personal or family history of CRC or colorectal adenoma, long-standing inflammatory bowel disease (IBD), familial adenomatous polyposis (FAP), mutated MMR gene syndromes like hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome and Muir-Torre syndrome, hamartomatous polyposis syndromes like Peutz-Jeghers syndrome, Cowden syndrome and Juvenile polyposis syndrome, and non-inherited

polyposis syndromes like serrated polyposis syndrome (SPS) and Cronkhite-Canada syndrome (Ahmed, 2020).

1.5.6 Treatment of colon cancer

The treatment modalities for CC include surgery, cryosurgery, chemotherapy, radiation therapy, and targeted therapy. chemotherapy as the major therapeutic strategy utilizes different drugs or drug combinations to reduce cancer cell division. The conventional chemotherapy for CC involves delivering drugs to the non-target site and patients ultimately suffer from unwanted side effects such as neutropenia, anemia, hand-foot syndrome, diarrhea, gastrointestinal (GI) toxicity, mucositis, nausea, vomiting, fatigue, hematologic disorders, and liver toxicity (Banerjee *et al.*, 2017).

1.6 Prostate Cancer

Prostate cancer (PCa) is known to be the most commonly diagnosed malignancy in males as well as the second leading cause of cancer-related deaths that occur in males (Zaidi *et al.*, 2019). This is the presence of cancerous cells in the prostate gland. As in the rest of the organs, when cancer develops in the prostate the cancerous cells begin to multiply in an uncontrolled manner such that in time they can spread from the prostate to other parts of the body, especially to the bones and the lymph nodes near the gland (Azhar and Gill, 2015).

There are several types of PCa including acinar adenocarcinoma, transitional cell cancer, ductal adenocarcinoma, squamous cell cancer, and small cell PCa. Among them, acinar adenocarcinoma is the most common PCa (Sadri Nahand *et al.*, 2020).

When prostate cancer is suspected, tissue biopsy remains the standard of care for diagnosis. However, the identification and characterization of the disease have

become increasingly precise through improved risk stratification and advances in magnetic resonance and functional imaging, as well as from the emergence of biomarkers. Multiple management options now exist for men diagnosed with prostate cancer (Litwin and Tan, 2017).

1.6.1 Epidemiology of prostate cancer

Prostate cancer is one of the most frequently diagnosed cancers in men and is the fifth leading cause of death worldwide. In 2018, a total of 1,276,106 new cases of prostate cancer were diagnosed, and there were 358,989 related deaths. The prognosis of patients with early-stage prostate cancer is favorable compared with other malignancies; however, the high global incidence makes prostate cancer a critical health issue. Thus, understanding the global epidemiological trends for prostate cancer is a vital need (Villers and Grosclaude, 2019).

The burden of prostate cancer has a remarkably disproportionate distribution across racial groups. For example, in the USA, African Americans are twice as likely as individuals of European ancestry to develop or die from prostate cancer and have a more aggressive disease nature at diagnosis. In contrast, Asian American men have the lowest incidence and mortality rates of prostate cancer. (Hur and Giovannucci, 2020) The regions with the highest prostate cancer incidence are Australia/New Zealand (at 86.4/100,000) and Northern and Western Europe. The lowest rates were observed in South Central (5.0/100,000) and South-Eastern Asia (Barsouk *et al.*, 2020).

1.6.2 Diagnosis of prostate cancer

Many prostate cancers are detected based on 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, 2018)

1.6.3 Symptom of prostate cancer

Most prostate cancer diagnoses are made in symptomatic men. Prostate cancer should be suspected in men over 50 years old presenting with lower urinary tract symptoms (LUTS), visible haematuria, or erectile dysfunction. LUTS is also a common presenting symptom of benign conditions affecting the prostate, such as benign prostatic hyperplasia (BPH) and prostatitis, creating a diagnostic challenge. There is no strong evidence of the association between the severity of LUTS and the likelihood of prostate cancer or the stage at diagnosis. Digital rectal examination (DRE) is recommended in many countries alongside PSA to aid decision-making about referral for diagnostic testing (Merriel et al., 2018).

1.6.4 Risk factors of prostate cancer

Many studies have been carried out focusing on both inherited and acquired risk factors for the development of cancer of PCa. Family history and genetic alterations, such as SNPs and somatic mutations, were reported as congenital risk factors. Additionally, several environmental risk factors, such as diet, lifestyle, obesity, metabolic syndrome, sexual behaviors, and infections, have also been reported (Kimura and Egawa, 2018).

1.6.5 Treatment of prostate cancer

Men with clinically low-risk prostate cancer, and some selected patients with intermediate-risk disease, frequently opt for active surveillance (AS). Others in the

low-intermediate-risk category may choose minimally invasive ablative therapies, radiation therapy (RT), or radical prostatectomy with the intent of curing their disease. Men with localized or metastatic high-risk diseases will typically be treated with RT with or without hormonal therapy, while patients with recurrent or metastatic, castrate-sensitive, or resistant diseases can be offered chemotherapy (Evans, 2018).

1.7 Inflammation

Inflammation is a critical immune process that occurs in response to tissue damage caused by injury or infection. Inflammatory processes aim to clear pathogenic material and debris from damaged tissue areas and initiate wound healing. While this process is an essential defense mechanism to fight invading pathogens, persistent chronic inflammation can cause further tissue damage. This can result in the release of reactive oxygen and nitrogen species from cells, as well as increased genome instability leading to an increased risk of cancer. When the cells are damaged, or there is an infection, the cells release agents to activate inflammatory signaling pathways, release inflammatory mediators and cytokines, recruit inflammatory immune cells, and increase vascular permeability (Archer *et al.*, 2020).

Many cells of the innate immune system, such as macrophages, dendritic cells, and mast cells infiltrate inflamed tissues and have been implicated in the development and progression of cancer by contributing to the tumor microenvironment (Han *et al.*, 2020).

The cancer-inflammation connection has two pathways: an extrinsic pathway, where inflammatory conditions facilitate the development of cancer (by releasing chemicals, for example), and an intrinsic pathway, where genetic alterations

leading to cancer also stimulate the inflammatory process, thus contributing to the establishment of a microenvironment favorable to tumor development. For this reason, regardless of tumor origin, there are inflammatory cells in the vicinity of all tumors (Vendramini-Costa and Carvalho, 2012).

Inflammatory cytokines have a key role in cancer progression via many pathways, including a direct effect on tumor cells, interaction with the chemokine system, stimulation of the epithelial-to-mesenchymal transition, and augmentation of metastasis. Cytokines and immune mediators secreted in the tumor microenvironment affect both myeloid progenitors and mature myeloid cells by regulating the activity of several transcription factors, which, in turn, regulate the synthesis of their protein targets, thereby affecting myeloid cell functions (Diakos *et al.*, 2014).

1.7.1 Cytokine

Cytokines are small protein molecules (<60 kDa) that mediate messages among cells, especially immune cells. Cytokines are produced by many cell populations. The helper T cells (Th) and macrophages are the predominant ones. Cytokines are of different types: lymphokines (cytokines produced by lymphocytes), monokines (cytokines produced by monocytes), chemokines (cytokines with chemotactic activities), and interleukins (ILs) (cytokines that are made by one leukocyte but act on different leukocytes). These cytokines have a very high affinity for their receptors Therefore, they produce a biological effect at picomolar concentrations. Cytokines have a pleiotropic effect, that is, the same cytokine acts on different cell types. On the other hand, different cell types secrete the same cytokines. Similar functions are exhibited by different cytokines. Cytokines act synergistically or antagonistically (Gupta et al., 2020).

Cytokines play an essential role in maintaining physiological immune homeostasis and regulating pathophysiological processes, such as cancer and autoimmune diseases (Bonati and Tang, 2021).

Cytokines are key modulators of inflammation, participating in acute and chronic inflammation via a complex and sometimes seemingly contradictory network of interactions. A better understanding of how these pathways are regulated helps facilitate a more accurate identification of agents mediating inflammation and the treatment of inflammatory diseases (Turner *et al.*, 2014).

1.7.2 Pro-Inflammatory cytokine

It has been reported that pro-inflammatory cytokines such as TNF-α, IL-1α, IL-1β, and IL-6 produced during the inflammatory responses contribute to the pathogenesis of diseases such as degeneration of the intervertebral discs, epilepsy, osteoarthritis, initiation, and progression of cancer, depression, and up-regulate chemokine secretion from macrophages (Asanka Sanjeewa *et al.*, 2020).

1.7.3 Anti-Inflammatory cytokine

Anti-inflammatory cytokines are secreted by several immune cell subtypes including CD4+ Th2 cells, regulatory T cells, M2 macrophages, mast cells, and regulatory B-cells (Russell and Morgan, 2014).

Anti-inflammatory cytokines which include IL-4, IL-10, and IL-13, have the inverse effect, such as they reduce inflammatory reactions (Gupta *et al.*, 2020).

1.7.4 TNF-α

TNFα was identified in 1975 as a molecule capable of causing tumor necrosis at high concentrations. Many studies on TNFα showed that it is a pleiotropic pro-

inflammatory cytokine involved in a wide variety of cellular processes and has contradictory effects ranging from cell proliferation to cell death. First, it was described that TNFα was involved in immune system regulation and was mainly secreted by cells such as monocytes, macrophages, natural killer (NK) cells, T lymphocytes, mast cells, and neutrophils, but later several works showed that it is also produced by non-immune cells like endothelial cells, adipocytes, neurons, fibroblasts, and smooth muscle, among others (Mercogliano *et al.*, 2021).

TNF-α acts either by inducing apoptosis via its receptor TNF-α receptor type 1 (TNFR1) or activating pro-inflammatory survival genes (mainly via TNF-α receptor type 2, TNFR2 (Schröder *et al.*, 2020).

1.8 Apoptosis

Apoptosis is a mode of programmed cell death essential for maintaining tissue homeostasis by elimination of unwanted, superfluous, and damaged cells Apoptosis occurs discretely in individual cells of our body and is a highly regulated energy-dependent process (Sharma *et al.*, 2019).

Dysregulation of apoptosis is often associated with human diseases. Excessive apoptosis can cause degenerative disorders, whereas insufficient apoptosis can result in neoplastic diseases In this regard, cancer cells avoid apoptosis by disabling the cell death machinery through various mechanisms, thereby gaining an excessive survival advantage (Matsuura *et al.*, 2016).

Apoptosis is characterized by morphologic changes, such as chromatin condensation, nuclear fragmentation, and reduction of cell volume (known as pyknosis). as well as biochemical changes that include caspase activation, breakdown of DNA and protein, and membrane surface modifications that allow

the apoptotic cell to be recognized and engulfed by phagocytic cells (Koff *et al.*, 2015).

The mechanism of apoptosis mainly consists of two core pathways involved in inducing apoptosis; the extrinsic pathway and the intrinsic pathway Extrinsic pathway refers to the DR-mediated pathway, and the intrinsic pathway is mitochondrial-mediated. Both of these apoptotic pathways, extrinsic and intrinsic pathways might lead to the same terminal (execution pathway) (Rehmet and Gul-e-Saba, 2019).

1.8.1 Apoptosis In Cancer

Apoptotic cell death inhibits oncogenesis at multiple stages, ranging from transformation to metastasis. Consequently, for cancer to develop and progress, apoptosis must be inhibited. Cell death also plays a major role in cancer treatment, serving as the main effector function of many anti-cancer therapies (Lopez and Tait, 2015).

The loss of apoptotic control allows cancer cells to survive longer and gives more time for the accumulation of mutations which can increase invasiveness during tumor progression, stimulate angiogenesis, deregulate cell proliferation and interfere with differentiation There are many ways in which cancer cells evade apoptosis: caspase function can be inhibited or the trigger for apoptosis can be disabled The upregulation of antiapoptotic BCL-2 proteins and loss of BAX and/or BAK are the predominant methods of evasion. BCL-2 is not considered an oncogene, but mutations in it enhance tumor onset. The overexpression of BCL-2 protein is present in over half of all cancers, regardless of type This results in tumor cells that are resistant to any intrinsic apoptotic stimuli which include some anticancer drugs (Pfeffer and Singh, 2018) .

1.8.2 Extrinsic pathway

The extrinsic pathway is triggered by a family of death receptors located on the cell membrane, including TNF- R1, FAS (CD95), TRAIL-R1, and TRAIL-R2. In a simplified model, following ligand/receptor binding, the caspase protease, caspase-8, is activated through dimerization. Activated caspase-8 cleaves and activates the executioner caspase-3 and -7, where upon they leave hundreds of different substrates, including cytoskeletal proteins, nuclear structural proteins, and lipid metabolism (Cao and Tait, 2018).extrinsic and internsic pathway of apoptosis are demonstrated in figure 1.1.

1.8.3 The intrinsic mitochondrial apoptotic pathway

The events of the mitochondrial apoptotic pathway are controlled via the proteins of the Bcl-2 family which govern the permeability of the mitochondrial membrane. The P53 possesses a vital role in the regulation of the Bcl-2 family proteins (Nagoor Meeran *et al.*, 2019).

Upon exposure to stress or developmental cues, the pro-survival signal is overwhelmed, allowing BAK and BAX to become active, whereupon they oligomerize in the mitochondrial outer membrane and form pores, releasing mitochondrial constituents including cytochrome c. The latter triggers the apoptotic caspase cascade, which begins with the initiator caspase, Caspase-9, and culminates in the activation of the effectors, Caspase-3, and Caspase-7. These two enzymes cleave hundreds (potentially thousands) of intracellular substrates resulting in DNA damage, suppression of transcription and protein translation, and the disabling of many other essential cellular processes (McArthur *et al.*, 2017).

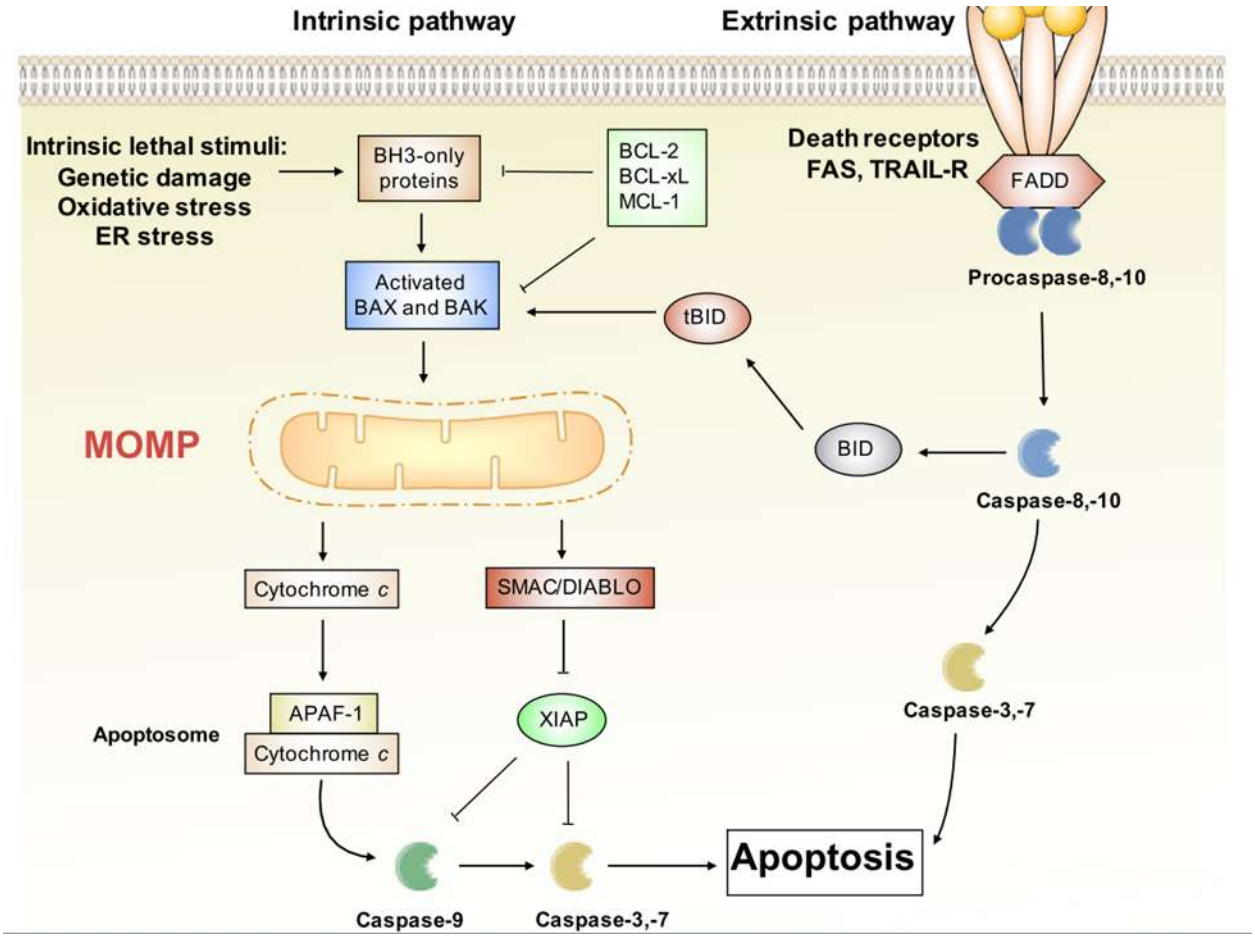


Figure1.1 Apoptotic signaling. intrinsic (mitochondrial) signaling pathway and the extrinsic pathway (Cao and Tait, 2018).

1.8.4 The intrinsic endoplasmic reticulum pathway

This intrinsic endoplasmic reticulum (ER) pathway is the third pathway and is less well known. It is believed to be caspase 12-dependent and mitochondria-independent. When the ER is injured by cellular stresses like hypoxia, free radicals, or glucose starvation, there is unfolding of proteins and reduced protein synthesis in the cell, and an adaptor protein known as TNF receptor-associated factor 2 (TRAF2) dissociates from procaspase-12, resulting in the activation of the latter (Wong, 2011).

1.8.5 Caspase

Caspases are a family of evolutionary conserved cysteine-dependent endoproteases that hydrolyze their substrates after specific aspartic acid residues. They consist of an amino-terminal domain of variable size sequentially followed by large and small catalytic subunits of respectively 20 kDa and 10 kDa that together form the protease domain. The process of proximity-induced autoactivation of the caspase zymogen into the active protease is driven by dimerization-induced conformational changes that lead to proteolytic excision of the flexible linker regions separating the prodomain and the large and small catalytic subunits. In contrast, executioner caspases lack an extended amino-terminal prodomain and require cleavage by initiator caspases for their activation (Van Opdenbosch and Lamkanfi, 2019).

Caspases are pivotal components of apoptosis. To date, two types of caspases have been defined, the initiator caspase and the effector/executioner caspase. caspase-8 and -9 are initiator caspases while caspase 3 is effector caspase. Furthermore, other caspases such as caspase-2, -10, and -11 belong to the initiator category while caspase-6 and -7 belong to the effector category (Xu *et al.*, 2019).

Caspases are a 15-member family of cysteine proteases playing essential roles in programmed cell death and inflammation. Among them, caspase-3 is a prototypical apoptotic executioner that, upon activation by initiator caspase-8 or caspase-9, cleaves many other functionally critical proteins within the cell, leading to apoptosis. Many anticancer therapies including cytotoxic drugs, radiotherapy, or immunotherapy can cause tumor cell death by activating caspase-3. As such, caspase-3 activation is used by numerous investigators as a surrogate marker for the efficacy of cancer treatment (Zhou *et al.*, 2018).

1.9 Oxidative stress and antioxidant

Free radicals are highly reactive atoms or molecules with one or more unpaired electron(s) in their external shell and can be formed when oxygen interacts with certain molecules. These radicals can be produced in cells by losing or accepting a single electron, therefore, behaving as oxidants or reductants. The terms reactive oxygen species (ROS) and reactive nitrogen species (RNS) refer to reactive radicals and non-radical derivatives of oxygen and nitrogen, respectively (Liguori *et al.*, 2018).

Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed “oxidative stress.” Regulation of the reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function (Birben *et al.*, 2012).

Oxidative stress is tightly linked to the initiation and progression of cancer. Moderate amounts of ROS promote stress signaling and contribute to mutation, thus favoring cancer development (Pisoschi *et al.*, 2020).

Cells have several mechanisms to rework and eliminate ROS to avoid their harmful effects. The synergistic action of both antioxidant proteins and enzymes and exogenous antioxidants neutralize free radicals and modulate cells (Watson *et al.*, 2011).

Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate. Antioxidants can be synthesized in vivo (e.g., reduced glutathione (GSH), superoxide dismutase (SOD), etc.) or taken as dietary antioxidants (Kasote et al., 2015).

The human body put in place several strategies to counteract the effects of free radicals and oxidative stress, based on enzymatic (e.g., SOD, CAT, and GPx) and non-enzymatic (e.g., lipoic acid, glutathione, L-arginine, and coenzyme Q10) antioxidant molecules, all of them being endogenous antioxidants. Besides these, there are several exogenous antioxidant molecules of animal or vegetal origin, mainly introduced by diet or by nutritional supplementation (Pizzino *et al.*, 2017).

1.10 Heparin

Heparin is a naturally occurring, highly sulfated polysaccharide that plays a critical role in a range of different biological processes (Paluck *et al.*, 2016).chemical structure of heparin demonstrated in figure 1.2.

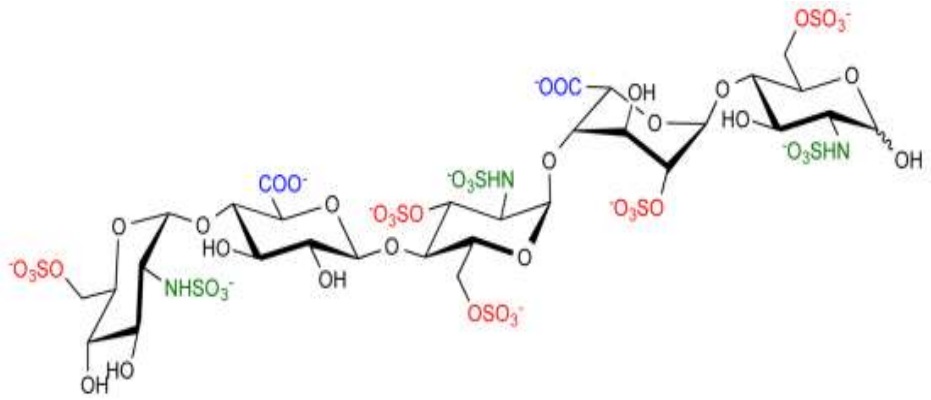


Figure1.2 The chemical structure of a heparin pentasaccharide shows various repeats containing the sulfate (red), sulfamate (green), and carboxylate (blue) groups (Paluck *et al.*, 2016).

Heparin is the oldest anticoagulant used in clinical medicine. Paradoxically, heparin was discovered by Mclean in 1916 in an attempt to isolate a thrombolytic agent. Heparin is a naturally occurring polysaccharide belonging to the family of glycosaminoglycans (GAG) ubiquitously present in mast cells. Unfractionated heparin (UFH) is the least processed form of the natural GAG produced via purification from animal tissue, most commonly the porcine intestine (Oduah *et al.*, 2016).

1.10.1 The anticoagulant mechanism of action

Heparin interacts with the coagulation system in multiple ways, but its interplay with AT for inhibiting the action of thrombin and factor (F) Xa, is unique. The active center serine of thrombin and other coagulation enzymes are inhibited by an arginine-reactive site on the AT molecule. Heparin exerts an anticoagulant effect by binding to the lysine site on AT, thereby inducing a non-reversible conformational change at the arginine-reactive site in such a manner that it inhibits thrombin by up to 1000 times (Li and Ma, 2017).

In addition, heparin can act through other serine protease inhibitors such as heparin cofactor II, protein C inhibitor, and tissue factor plasminogen inhibitor. The antithrombotic action of heparin in vivo, though dominated by anticoagulant mechanisms, is more complex, and interactions with other plasma proteins and cells play significant roles in the living vasculature (Gray et al., 2012).

1.10.2 Adverse effects of heparin

All the adverse impacts of heparins are linked to their wide range of biological activities, with bleeding becoming the most essential safety problem, results indicating the intensity of heparin as an anticoagulant (S. Alban, 2012).

Heparin-induced thrombocytopenia (HIT) is a well-known complication of heparin therapy. Two types of HIT have been described. HIT type I is due to a nonimmunogenic response to therapy characterized by a transitory, slight, and asymptomatic reduction in platelet count. HIT type II is an immune-mediated drug reaction, less frequent but more severe than HIT type I (Giossi et al., 2012).

Delayed skin lesions after subcutaneous heparin are the most common type of hypersensitivity reaction, followed by life-threatening heparin-induced thrombocytopenia (Gonzalez-Delgado and Fernandez, 2016).

Osteoporosis is the most frequent severe adverse effect of long-term UFH treatment, with a 2.2–5% incidence of heparin-induced osteoporotic fracture(S Alban, 2012).

1.11 Enoxaparin Sodium

The fractionated low molecular weight form of heparin (LMWH), developed in 1981 and approved for medical use in 1993, is known as enoxaparin and inhibits factor Xa in the coagulation process. Enoxaparin is among the safest and most effective essential medicines according to World Health Organization (WHO) reports (Shirazi *et al.*, 2021)

Low-molecular-weight heparins (LMWHs) are chemical or enzymatic depolymerization of unfractionated heparins (UFHs) with an average weight of 5,000 Da Unlike UFHs, short chains of LMWHs are less interacted with plasma proteins, directly inhibiting factor Xa (KUBAT *et al.*, 2021).

1.11.1 Mechanism of action of enoxaparin

Enoxaparin acts at the final common pathway of the coagulation cascade. It binds to antithrombin III, and the complex of enoxaparin anti-thrombin III inactivates clotting factors Xa, IIa, and IXa, which are responsible for the conversion of prothrombin to thrombin, which is responsible for the conversion of fibrinogen into fibrin and clot formation Enoxaparin has a higher ratio of anti-Xa to anti-IIa activity, which may be related with a lower trend of hemorrhagic effect (Febbraro *et al.*, 2021).

1.11.2 Uses of enoxaparin

Low molecular weight heparins (LMWH) are glycosaminoglycans obtained by fractionation of heparin. They are used in the prophylaxis of venous thromboembolism in surgical patients and the prophylaxis of venous thromboembolism in nonsurgical patients with an acute condition (such as acute heart failure, respiratory failure, severe infection, or rheumatic disease) and impaired mobility at increased risk for venous thromboembolism, in the treatment of deep vein thrombosis and pulmonary embolism, in the treatment of unstable angina and myocardial infarction without ST-segment elevation in combination with oral acetylsalicylic acid, and the treatment of acute myocardial infarction with ST-segment elevation (Vitiello and Ferrara, 2021).

Enoxaparin has the ability of anticoagulation because of its highly sulfated pentasaccharide part which binds to antithrombin and catalyzes the inactivation of factor Xa (Palassi et al., 2021).

1.11.3 Adverse effect

The most common adverse effect of enoxaparin is bleeding, as with all anticoagulants. ecchymosis, hematoma, thrombocytopenia, hematuria, and anemia may occur when this drug is administered (Cirne and Machado, 2020).

In particular, enoxaparin sodium is an effective and safe first-generation LMWH for the prevention and treatment of venous thromboembolism and the prevention of mechanical heart valve thrombosis, as well as in specific patient populations including pregnant and cancer patients Although LMWHs are indicated as safe drugs, LMWHs may lead to injection site problems such as irritation, pain, bruising, redness, and swelling. Delayed hypersensitivity reactions in the injection site are the most commonly reported reactions, although systemic reactions are rarely reported (KUBAT *et al.*, 2021).

1.12 Non-anticoagulant effects of heparin and enoxaparin

1.12.1 Anticancer effect

Along with its anticoagulant activity, the other activity that is gathering a lot of importance nowadays is its ability to arrest the progression of tumors especially some solid forms of tumors such as small cell lung carcinoma and pancreatic tumors (Lokwani *et al.*, 2014).

The mechanisms of the antineoplastic properties of heparin have been the subject of several studies. The antineoplastic activity appears to be unrelated to the anticoagulant properties. Proposed chemotherapeutic mechanisms include interference with cellular proliferation, the release of tissue factor pathway inhibitor (TFPI) from vascular endothelium, anti-inflammatory properties, and inhibition of heparanase activity resulting in decreased tumor invasion and

metastasis One of the most relevant mechanisms of heparin inhibition of the hematogenous spread of malignant cells appears to be via inhibition of P-selectin mediated platelet adhesion to tumor cells and L-selectin mediated leukocyte interaction with tumor cells (Oduah *et al.*, 2016).

LMWHs are commonly administered to cancer patients for treatment and prophylaxis of thromboembolic events. Importantly, several clinical trials have demonstrated that the administration of LMWHs improved the survival of cancer patients, as a result of preventing the metastasis of cancer cells. Given that the CXCL12 molecule contains heparin binding sites and heparin shifts CXCL12 monomer-dimer equilibrium to a dimerization state LMWH can diminish the interaction of CXCR4 and CXCL12, thus inhibiting cell proliferation, adhesion, migration, and invasion stimulated by CXCL1 (Ma et al., 2012).

1.12.2 Anti-Inflammatory effect

In a study involving 24 asthma patients, the effect of treatment with enoxaparin, an LMWH, was evaluated. The authors reported an increase in the forced expiratory volume in one second (FEV1), which is an assessment of airway obstruction or bronchoconstriction. They also reported a decrease in the percentage of eosinophils and lymphocytes upon bronchoalveolar lavage, which corresponds to a reduction in inflammation. A recent systematic review suggested the role of heparin was to reduce the histamine or leukotriene-induced bronchial hyper-reactivity, without inhibiting the bronchoconstriction response (Oduah *et al.*,2016).

Heparin and related molecules can bind electrostatically to the positively charged nuclear localization sequence of NF-κB and prevent it from translocating to the nucleus. Blocking of this transcriptional factor can potentially reduce inflammatory gene activation and regulate the gene expression and production of

proinflammatory cytokines, chemokines and adhesion molecules. Heparin and o-desulphated heparin have been reported to inhibit NF-κB activation in a tumour necrosis factor (TNF-α)-stimulated human endothelial cell line and in ischemic-reperfused rat myocardium (Young, 2007).

1.12.3 Antioxidant activity

Heparin down-regulates the iron homeostasis regulator hepcidin and reduces TGF-β2-mediated increase in ferritin and ROS It can also improve the imbalance between oxidation and antioxidation and protect hemodialysis patients from the adverse effects of oxidative stress the antioxidant mechanism of enoxaparin (EP) on endothelial cells stimulated by oxidative stress. They found that EP interferes with the hydrogen peroxide (H2O2)-induced P38 MAPK pathway in human endothelial cells (HECS). It also reduces acute pancreatitis (AP)-1 activation in H2O2-induced HECS by interfering JNK MAPK signal (Qiu *et al.*, 2021).

1.13 Comparison between UFH And LMWH

Unlike UFHs, short chains of LMWHs less interacted with plasma proteins, directly inhibiting factor Xa In addition, as LMWHs exert fewer effects on platelets, the incidence of thrombocytopenia with LMWHs is relatively low than UFHs Since LMWHs do not contain long polysaccharide chains which inhibit factor IIa, they do not alter the partial thromboplastin with a very high absorption with subcutaneous administration, thereby, leading to a bioavailability ratio of up to 90% As the behavior of LMWHs following absorption is more predictable than UFHs, monitoring is not necessary. Although LMWHs can be administered intravenously similar to UFHs, the high bioavailability ratio makes them suitable for subcutaneous administration, as well (KUBAT *et al.*, 2021).

1.14 Cell culture

Cell culture refers to laboratory methods that enable the growth of eukaryotic or prokaryotic cells in physiological conditions. Its origin can be found in the early 20th century when it was introduced to study tissue growth and maturation, virus biology and vaccine development, the role of genes in disease and health, and the use of large-scale hybrid cell lines to generate biopharmaceuticals. The experimental applications of cultured cells are as diverse as the cell types that can be grown in vitro (Segeritz and Vallier, 2017).

1.14.1 Primary cultures

Cells that are cultured directly from tissue are known as primary cells. A primary culture may be produced either by allowing cells to migrate out from the tissue after sterile dissection, which is adhering to a substrate or by disaggregating the tissue mechanically or enzymatically to produce a suspension of cells. These cells are, therefore, more representative of the cell types in the tissue from which they were isolated, although many cells are unable to attach and survive in vitro. Most primary cultures are usually heterogeneous, have a low growth fraction, and have a limited lifespan, except some derived from tumors. Cells that have attached are trypsinized and reseeded in a fresh flask and become a secondary culture (Walker, 2009).

1.14.2 Advantages and disadvantages of cell culture

The main advantage is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells. Cell cultures have a high control of the physicochemical environment (i.e., pH, temperature, osmotic pressure, oxygen, and carbon dioxide tension) which can be controlled very accurately, and the control of physiological conditions, which can be constantly examined.

The disadvantages of cell culture are: highly skilled personnel, techniques must be performed using strict asepsis techniques because animal cells grow slower than many of the common contaminants (e.g., bacteria, viruses, and fungi). Additionally, animal cells may not survive when isolated and therefore are not capable of independent sustainable existence without providing a complex environment. One of the main limitations of cell culture is the expense and effort that has to be applied to obtain a relatively low amount of cells (Levy *et al.*,2013).

1.14.3 Cell line

The term cell line refers to the propagation of culture after the first subculture. In other words, once the primary culture is sub-cultured, it becomes a cell line. A given cell line contains several cell lineages of either similar or distinct phenotypes. It is possible to select a particular cell lineage by cloning or physical cell separation or some other selection method. Such a cell line derived by selection or cloning is referred to as cell strain. Cell strains do not have infinite life, as they die after some divisions (Chaudhary and Singh, 2019).

The choice of a cell line for cell culture depends heavily on the functional prop parties and specific readouts required of the cell model. The selected cell lines will also need to align with the available equipment and requirements of their specific hazard group. Cells cultured in the lab can be classified into three different types: primary cells, transformed cells, and self-renewing cells. Primary cells, such as fibroblasts obtained from skin biopsies and hepatocytes isolated from liver explants, are directly isolated from human tissue (Segeritz and Vallier, 2017).

Immortal cell lines are often used in research in place of primary cells. They offer several advantages, such as they are cost-effective, easy to use, providing an unlimited supply of material, and bypassing ethical concerns associated with the

use of animal and human tissue. Cell lines also provide a pure population of cells, which is valuable since it provides a consistent sample and reproducible results (Kaur and Dufour, 2012).

Cell lines can be classified into two types, Finite and Continuous, depending upon the life span of the culture. Finite cell lines are characterized by a limited life span, have a limited number of cell generations (usually 20-80 population doublings), exhibit the property of contact inhibition, density limitation and anchorage dependence, slow growth rate, and doubling time around 24-96 hours. Continuous cell lines are those that are transformed under laboratory conditions or in vitro culture conditions. These lines show the property of ploidy (aneuploidy or heteroploidy), absence of contact inhibition, absence of anchorage dependence, rapid growth rate, and doubling time is 12-24 hours. They grow in monolayer or suspension form (Singh and Goswami, 2011).

1.14.4 Cell Viability

Cell viability is defined as the number of healthy cells in a sample. the proliferation of cells is a vital indicator for understanding the mechanisms inaction of certain genes, proteins, and pathways involved in cell survival or death after exposure to toxic agents. Generally, methods used to determine viability are also common for the detection of cell proliferation. Cell cytotoxicity and proliferation assays are generally used for drug screening to detect whether the test molecules have effects on cell proliferation or display direct cytotoxic effects. Regardless of the type of cell-based assay being used, it is important to know how many viable cells are remaining at the end of the experiment (Adan *et al.*, 2016).

Indirect techniques that assess cell viability by monitoring cell membrane integrity after drug exposure, i.e., dye exclusion and preferential dye uptake, also destroy or

interfere with the cell's functioning and hence are terminal assays (Rampersad, 2012).

1.15 Cell models

1.15.1 Vero cell line

Derived from the kidney of an African green monkey (Cercopithecus aethiops) in the 1960s, Vero cells are a common mammalian continuous cell line used in research. This anchorage-dependent cell line has been used extensively in virology studies, but has also been used in many other applications, including the propagation and study of intracellular bacteria (e.g., Rickettsia spp.) and parasites (e.g., Neospora), and assessment of the effects of chemicals, toxins, and other substances on mammalian cells at the molecular level. In addition, Vero cells have been licensed in the United States for the production of both live (rotavirus, smallpox) and inactivated (poliovirus) viral vaccines, and throughout the world (Ammerman *et al.*,2008).

1.15.2 SW480 Colon cancer cell line

The colonic cancer SW480 cell line originates from the primary tumor of adenocarcinoma of the colon in a 50-year-old male . The SW480 cell line showed small groupings of irregular-shaped cells (80% of fully adhered cells were irregular in shape, with only 20% representing a more spindle-like morphology), (Shanehbandi *et al.*, 2019).

1.15.3 LNCaP prostate cancer cell line

LNCaP cell lines which are exhibiting epithelial morphology that was isolated from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old, White, male (blood type B+) with a confirmed diagnosis of metastatic prostate carcinoma (Castanares *et al.*, 2016).

Chapter Two

Materials and Methods

2. Materials and Methods

The experimental work was performed in the Postgraduate lab /Department of Pharmacology at the College of the Medicine /University of Babylon from (December 2021 - April 2022).

2.1 Materials

2.1.1 Chemicals

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

Table (2. 1) chemicals used in the study

Chemical	Company	Country
Alcohol spray (ethanol 70%)	Aljoud	Iraq
Dimethyl sulfoxide (DMSO)	Roth	Germany
Fetal bovine serum (FBS)	Gibco	UK
Gentamycin (80 mg vial)	The Arab pharm.	Jordan
MTT(3-(4,5-Dimethylthiazole-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) dye powder	Roth	Germany
Phosphate buffer saline tablet	Gibco	UK
Roswell Park Memorial Institute-1640 (RPMI-1640) powder medium	Gibco	UK

Sodium bicarbonate powder	Ludeco	Belgium
Trypsin- Ethyl ediaminetetraacetic acid (EDTA) powder	US biological	USA

2.1.2 Instruments and Tools:

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

Table 2.2 List of Instruments and Tools Used in the Study

Instrument or tool	**Company**	**Country**
Autoclave	Jeiotech	Korea
Automatic micropipettes (different sizes)	DRAGON MED	Germany
Cell culture flask (25ml)	SPL	Korea
Cell culture plate (96-wells)	SPL	Korea
Distiller	ROWA	Germany
Double distillation water stills	GFL	Germany
Electric oven	Memmert	Germany
ELISA Reader	Memmert	Germany
Incubator	Memmert	Germany
Water bath	Memmert	Germany
Inverted microscope	T.C Meiji techno	Japan
Laminar airflow cabinet	Labtech	Korea
Liquid nitrogen container GT38	Air Liquide	France

Magnetic stirrer	Labinco	Netherland
Microcentrifuge	Memmert	Germany
Millipore filter (0.45, 0.22µm)	Biofil	Australia
pH Meter	WTW	Germany
Refrigerator	Arcelik	Turkey
Sensitive Balance	Labtech	Korea
Sterile freezing vial (1.5 ml)	Biofilm	Australia
Whatman filter paper	Merck	Germany
Vortex	Kottermann	Germany

2.1.3 Drugs:

The drugs used in the study were listed in **(Table 2.3)** with their suppliers.

Table 2. 3 List of Drugs Used in the Study

Enoxaparin sodium 4000 IU	Sanofi	Germany
Heparin sodium 5000 IU/ml	Duopharma	Malaysia

2.1.4 Assay kit

The assay kits used in this study include:

Table 2.4 list of ELISA assay kits used in the present study

ELISA kit caspase3	BT LAB	Korea

ELISA kit TNF-alpha	BT LAB	Korea

ELISA kit contents include the following:

Table 2.5 List of contents of the ELISA assay kit

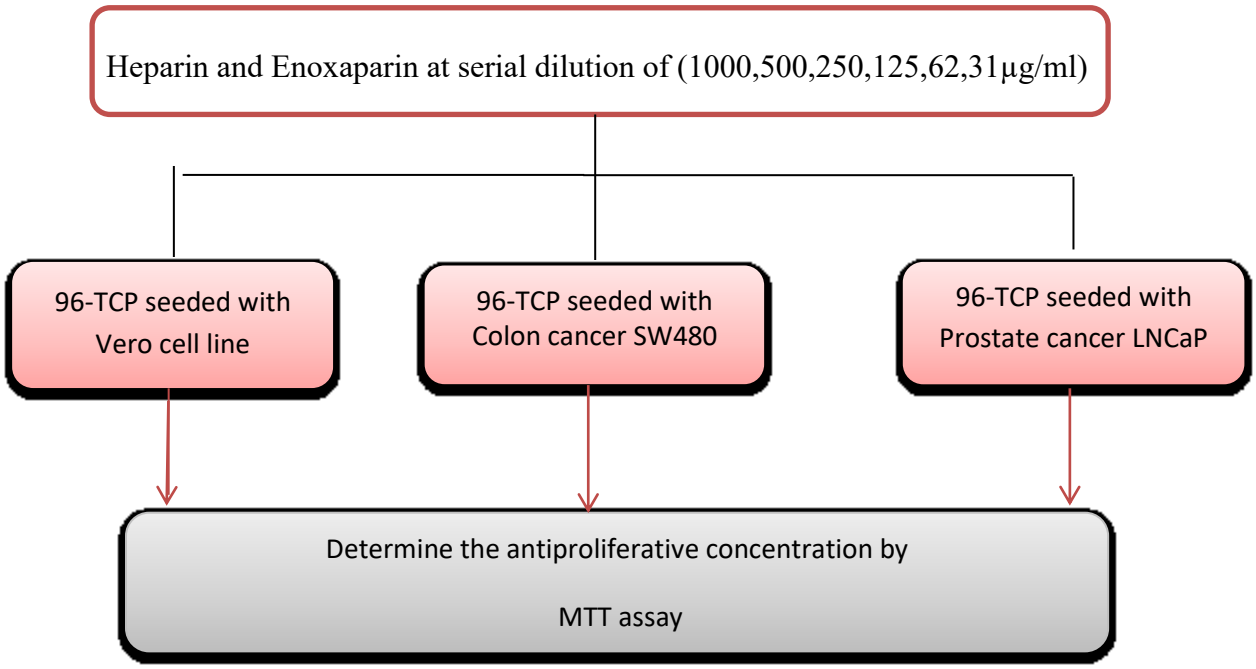
Micro ELISA Plate (Dismountable)	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips
Reference Standard	96T: 2 vials 48T: 1 vial
Concentrated Biotinylated Detection Ab (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL
Concentrated HRP Conjugate (100×)	96T: 1 vial, 120 µL 48T: 1 vial, 60 µL
Reference Standard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

2.1.5 Cell Lines

Frozen vials of human prostate cancer LNCaP, Colon cancer SW480, and Vero cell lines were obtained from the Tissue Culture Laboratory in the College of Medicine / University of Babylon.

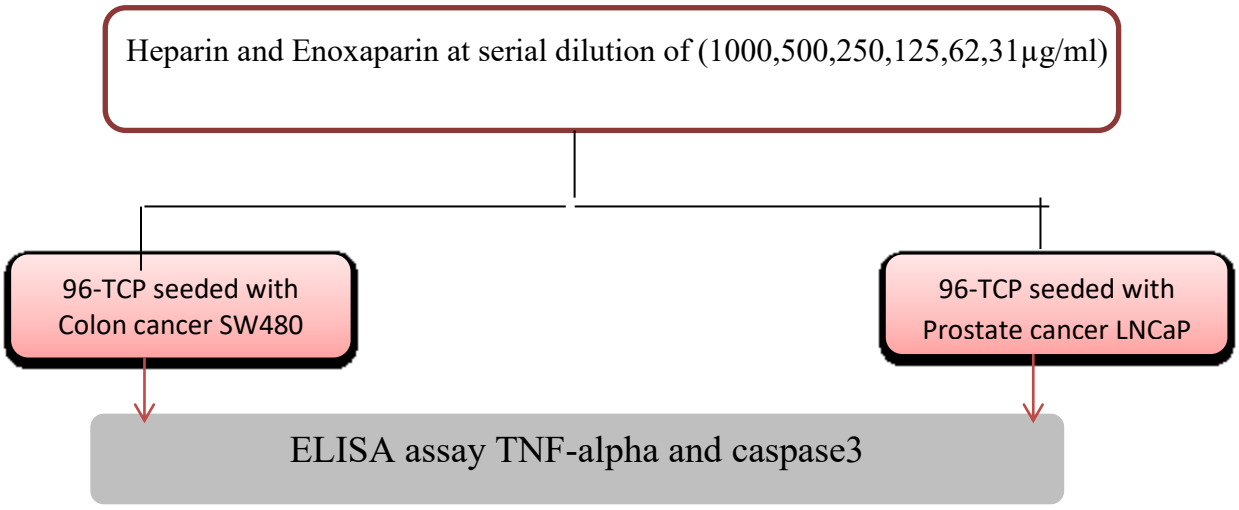
Experiments protocol

Experiment No.1
Cytotoxicity assay



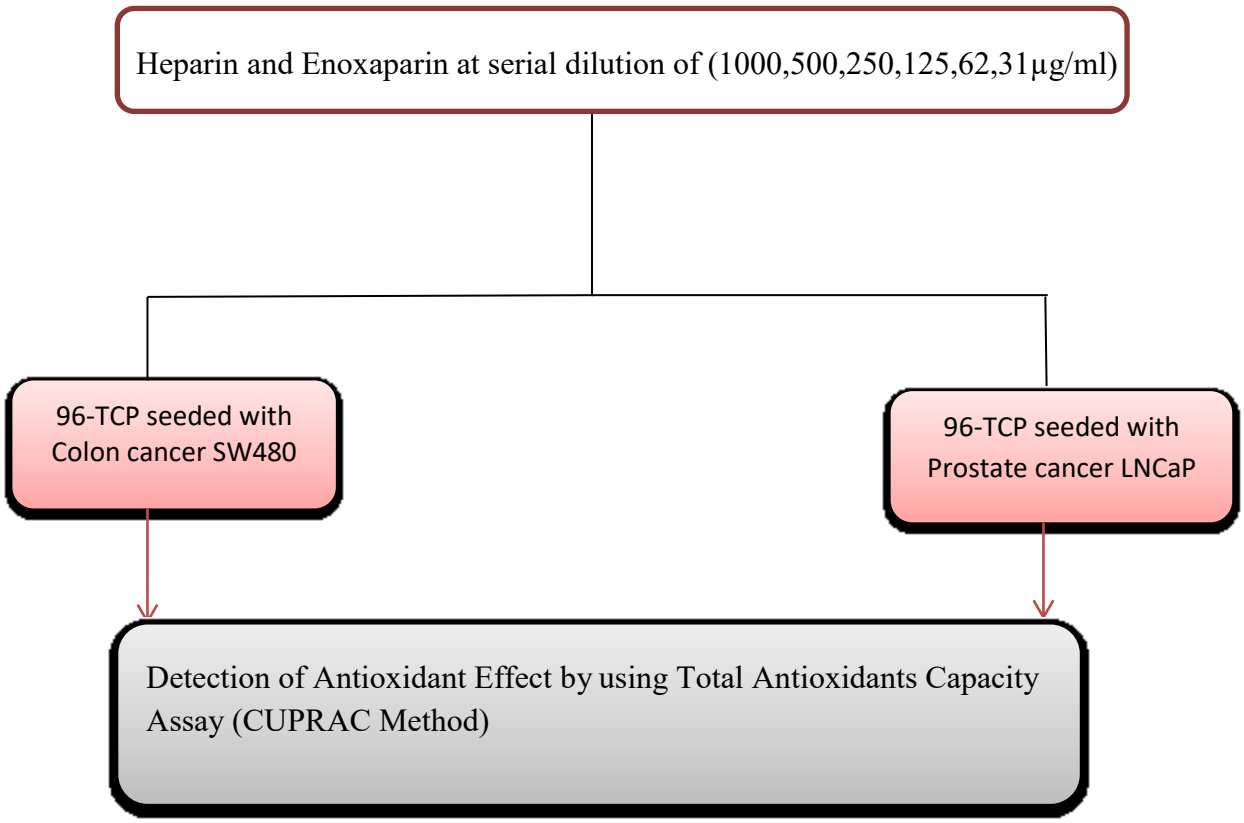
Experiment No.2

Detection of apoptosis and inflammatory markers



Experiment No.3

Detection antioxidant effect



2.2 Methods

2.2.1 Preparation of Reagents and Solutions

2.2.1.1 Phosphate Buffer Saline (PBS):

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

2.2.1.2 Trypsin-(EDTA) Solution:

As indicated by US Biological headings, a weight of a 10.1 gm of trypsin-EDTA powder and dissolving in 0.9 Liter of double distilled water (DDW) with continuous mixing at room temperature. 7.2 of PH value should be reached and complete the volume to 1 Liter by DDW, the solution 56 was sterilized using Millipore filters of 0.45 and 0.22 μm respectively, after that, the solution was kept at (- 20C˚) temperature.

2.2.1.3 Gentamycin Stock Solution:

Gentamycin vial of 40 mg/ml solution was considered as a stock solution and stored at 4˚C for use. the working concentration of gentamicin in the medium is 50 μg/ml, so 1.25 ml of gentamycin solution was added to 1L of RPMI-1640 medium (Khokhlova et al., 2019).

2.2.1.4 MTT Solution:

MTT powder (0.5 gm) was dissolved in PBS (100 ml) to achieve 5 mg/ml concentration. A 0.2 μm millipore filter was utilized to sterilize the MTT solution and stored in a sterile and light-protected bottle. The solution was stored at 4°C of

temperature for multiple uses or at -20°C of temperature for long storage (Meerloo, 2011).

2.2.2 Preparation of Tissue Culture Medium:

2.2.2.1 Preparation of Serum-Free Medium:

Liquid Roswell Park Memorial Institute (RPMI-1640) medium: Liquid RPMI-1640 medium was prepared according to the Gibco product manual from RPMI-1640 medium powder as the following: 10.43 gm of RPMI-1640 medium powder was dissolved in 0.9 L of DDW in a volumetric flask. Other constituents added include: 2 gm sodium bicarbonate powder as needed and 80 mg of gentamycin were added with continuous stirring. The solution was completed to 1 liter by DDW with adjusting the PH at 7.4. Use 0.4 and 0.2 μm Millipore filters respectively to sterilize the solution under the airflow cabinet. For examination of any contamination, the prepared medium was incubated at 37 ºC overnight with continuous following up, if there is no contamination, the medium could be used or stored at 4ºC temperature until use (Phelan and May 2016).

2.2.2.2 Preparation of Serum-Medium:

Medium with serum was prepared as described in the preparation of serum-free medium in (2.2.2.1) by adding 10 percent of fetal bovine serum.

2.2.2.3 Preparation of Freezing Medium

The freezing medium was prepared from the following compositions: 6 ml serum-free medium, 3 ml FBS, and 1 ml DMSO. The solution was stored at (- 20) C˚ temperature between uses (Meleady and O'Connor, 2006).

2.2.3 Preparation of Cell Line

2.2.3.1 Preparation of Cell Line (Vero, LNCaP, and SW480).

The frozen cell line vial was removed from the liquid nitrogen container with caution and directly placed into a beaker containing pre-warmed (37°C) sterile DDW. The vial was removed from the water before the ice floccule dissolved completely, then it was wiped with 70% ethanol. Without delay, the cell suspension content of the vial was pipetted under a laminar flow cabinet into a 15 ml sterile plastic centrifuge tube containing 10 ml of pre-warmed serum-free medium. Centrifugation was done at 1000 rpm for 5 minutes and the supernatant was aspirated and decanted. The cells pellet was re-suspended into a 5ml warm (37C˚) serum medium and transferred into a 25 ml size cell culture flask, incubated at 37C˚ and the serum medium was replaced on the next day (Phelan and May 2017).

2.2.3.2 Sub-Culturing of Vero, LNCaP, and SW480 Cell Lines.

1-The cells are checked and examined using the inverted microscope with phase contrast capabilities to ensure that the cells are healthy and sub-confluent and free of contamination.

2-The laminar flow is sanitized by wiping off the surface of the working area with 70% ethanol.

3-The growth medium is removed from the flask using a pipette and wash the monolayer with a sufficient volume of PBS to ensure the removal of all media from the flask.

4-An appropriate volume of the trypsin/EDTA solution is added to the flask and incubated at 37 c° to allow the cells to detach from the inside surface of the flask (within 2-10 min).

5-The cells were examined using an inverted microscope to ensure that all the cells are detached and in suspension. Gently tap the flask with the palm of the hand a couple of times to release any remaining detached cells.

6-The trypsin is inactivated by adding equal volume of serum-containing media to the flask.

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

8-The cell line was incubated at 37C°for 24 hr. (Meleady and O'Connor, 2006).

2.2.3.3 Harvesting of Vero, LNCaP, and SW480 Cell Lines.

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).

2.2.3.4 Freezing of Vero, LNCaP, and SW480 Cell Lines.

The cell lines source were kept frozen at (-196) ºC in a nitrogen tank according to the following protocol:

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

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

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

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

5. The vial was kept for 10 minutes at room temperature and transferred to – 80C° deep freezer for 24 h and then stored for a long time in the liquid nitrogen tank after one minute (Yang *et al.*, 2019).

2.2.4 Preparation of The Drugs

2.2.4.1 Preparation of Heparin

Heparin vial (5000 IU/ml) equal to (50mg/ml) solution was considered as stock solution from this stock a serial dilution was made.

2.2.4.2 Preparation of Enoxaparin

Enoxaparin syring (4000IU/0.4ml) equal to (100mg/ml) solution was considered as stock solution from this stock a serial dilution was made.

2.2.5 MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)Assay:

2.5.1 MTT Principle:

The general purpose of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) 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 that for most viable cells mitochondrial activity is constant and thereby an increase or decrease in the number of viable cells is linearly related to mitochondrial activity. The mitochondrial activity of the cells is reflected by the conversion of the tetrazolium salt MTT into formazan crystals, which can be solubilized for homogenous measurement. Thus, any increase or decrease in viable cell number can be detected by measuring formazan concentration reflected in optical density (OD) using a plate reader at 540 and 720 nm. For drug sensitivity measurements the OD values of wells with cells incubated with drugs are compared to the OD of wells with cells not exposed to drugs (Meerloo, 2011).

2.5.2 Procedure (Meerloo et al; 2011):

1- At the end of the drug exposure period, the medium was removed from the wells, and then the cells were washed with PBS. A blank control was carried out to assess unspecific formazan conversion.
2- A volume of 1.2 ml of MTT solution (5 mg/ ml) was added to 10.8 ml medium to obtain final concentration of 0.5 mg/ml. Then, 200 µl of the resulting solution was added to each well.
3- The plate was incubated for 3 hours at 37°C until intracellular purple formazan crystals were visible under the inverted microscope.
4- The supernatant was removed and 100 µl DMSO was added to each well to dissolve the resultant formazan crystals.
5- The plate was incubated at room temperature for 30 minutes until the cells have lysed and purple crystals have dissolved.
6- Absorbance was measured by a microplate reader 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 a percentage of cell viability or proliferation. Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation.

The percent of cell viability or percent 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

2.2.6 The Experiments

2.2.6.1 Study The Cytotoxic Effect of Heparin and Enoxaparin on (Vero, LNCaP, And SW480) Cell Lines.

Pilot study was done inorder to choose the appropriate concentration. Cell lines including Vero, prostate cancer (LNCaP), and colon cancer (SW480) cell lines were seeded and labeled in 96 tissue culture plates. Each cell type exposed to different concentrations of drugs (heparin or enoxaparin) at serial dilutions (1000,500,250,125,62,31µg\ml). Four biological replicates were used for each concentration of both heparin and enoxaparin for each type of cells) along with four replicates as a control group for each cell type. Then the plate was covered with a self-plastic lid and incubated for 24 hours, at the end of the exposure period, the cell line growth was assessed by cytotoxicity assay (MTT assay).

2.2.6.2 Detection of Apoptosis and Inflammatory markers in LNCaP Prostate Cancer And SW480 Colon Cancer Cell Line.

Prostate cancer (LNCaP) and colon cancer (SW480) cells lines were seeded in 96 tissue culture plates. all cells were treated with different concentrations of drugs (heparin or enoxaparin) at serial dilutions ranging from 1000 to 31.25 µg\ml (three biological replicates were used for each concentration of both heparin and enoxaparin for each type of cells) along with three biological replicates as a control group for each cell type. Then the plate was covered with a self-plastic lid and incubated for 24 hours, at the end of the exposure period, the cells supernatants ~~lines~~ were taken for immunoassay by ELISA method using TNF-alpha, IL10, and caspase3 according to the protocol mentioned below

Test Principle:

This ELISA kit uses the sandwich-ELISA principle in which the plate is precoated with an antibody specific to the human cytokine of interest. Samples or standards are added to the plate and combined with the antibody. The biotinylated detection antibody and avidin-HRP conjugate are added to the plate changing the color to blue. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density is measured at a wavelength of 450 nm. The concentration of cytokine of interest is measured by comparing the OD of the sample to the standard curve (Paulie and Perlmann, 2016).

Assay procedure: all reagents and samples are brought to room temperature before use. Centrifuge the sample again after thawing before the assay. All the reagents should be mixed thoroughly by gently swirling before pipetting, foaming should be avoided. It is recommended that all samples and standards be assayed in duplicate.

1. Addition of Sample: 100μL of Standard, Blank, or Sample is added per well. The blank well is added with Reference Standard & Sample diluent. Solutions are added to the bottom of the micro-ELISA plate well, avoiding inside wall touching and foaming as possible. Mix it gently. Cover the plate with the sealer we provided. Incubate for 90 minutes at 37℃.

2. Biotinylated Detection Ab: then the liquid of each well is removed, without washing. Addition of 100μL of Biotinylated Detection Ab working solution to each well. Then the plate is covered with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.

3. Wash: each well is aspirated and washed three times, then the wash process is done by filling each well with Wash Buffer (approximately 350μL) (a squirt bottle, multi-channel pipette, manifold dispenser, or automated washer are needed).

Complete removal of the liquid at each step is essential. After the last wash, the remained wash Buffer is removed by aspirating or decanting. Then the plate is inverted and placed against thick, clean absorbent paper.

4. HRP Conjugate: 100μL of HRP Conjugate working solution is added to each well, then incubate for 30 minutes at 37°C.

Wash: the wash process is repeated five times as conducted in the third step.

5. Substrate: 90μL of Substrate Solution is added to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When an apparent gradient appeared in standard wells, the user should terminate the reaction.

Stop: 50μLof Stop Solution is added to each well. Then, the color turns yellow immediately. The order to add the stop solution should be the same as the substrate solution.

6. OD Measurement: the optical density (OD value) of each well is determined at once, using a microplate reader set to 450 nm. The user should open the microplate reader in advance, preheat the instrument, and set the testing parameters.

After the experiment, all the unused reagents are placed back into the refrigerator according to the specified storage temperature respectively until their expiry.

2.2.4.3 Detection of Antioxidant Effect of Heparin and Enoxaparin on LNCaP Prostate Cancer and SW480 Colon Cancer Cell Lines using Total Antioxidants Capacity Assay (CUPRAC Method)

A- Principle:

The CUPRAC method is comprised of mixing the antioxidant solution (directly or after acid hydrolysis) with a copper(II) chloride solution, a neocuproine (2,9-dimethyl-1,10-phenanthroline) alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and subsequently measuring the developed absorbance at 450 nm after 30 min (normal measurement). Since the color development is fast for compounds like ascorbic acid, gallic acid, and quercetin but slow for naringin and naringenin, the latter compounds were assayed after incubation at 50 0 C on a water bath for 20 min (after Cu(II)- Nc reagent addition) so as to enable complete oxidation (incubated measurement). The flavonoid glycosides were hydrolyzed to their corresponding aglycones by refluxing in 1.2 M HCl-containing 50% MeOH to exert maximal reducing power towards Cu(II)-Nc (hydrolyzed measurement). Thus the total CUPRAC antioxidant capacity of a mixture containing various antioxidants should be finally measured after a suitable combination of hydrolysis and incubation procedures so as to obtain maximum absorbance at 450 nm **(**Apak et al., 2007**).**

Total antioxidants + Cu^{+2} ⟶ Cu^{+}

Cu^{+} + 2,9-dimethyl-1,10-phenanthroline ⟶ complex (λ_{max} at 450 nm)

B- Reagents preparation

1. Copper(II) chloride solution at a concentration of 10^{-2}M was prepared from $CuCl_2 \cdot 2. H_2O$ weighing 0.4262 g, dissolving in H_2O and diluting to 250 ml with water.
2. Ammonium acetate (NH_4Ac) buffer pH = 7.0 was prepared by dissolving 19.27 g of NH_4Ac in water and completing the volume to 250 ml.
3. Neocuproine (Nc){2,9-dimethyl-1,10-phenanthroline} solution at a concentration of 7.5 * 10^{-3}M was prepared by dissolving 0.039 g Nc in 96% EtOH, the volume was completed to 25 ml with ethanol.
4. The standard solutions of sample antioxidants were prepared at 1.0 * 10^{-3}M Torolox.

C-Reagents mixtures

Reagents	Test	STD	Blank
Copper(II) chloride solution	1ml	1ml	1ml
Sample	50 µl	------	-------
Working standard solution	-------	50 µl	-------
D.W	-------	-------	50 µl
Neocuproine (Nc) solution	1ml	1ml	1ml
Ammonium acetate (NH_4Ac) buffer	1ml	1ml	1ml
Test tubes were mixed by vortex and incubated for 30 minutes at 37°C, after that the absorbance was read on a spectrophotometer at 450 nm.			

D-Calculation:

$$\text{Total antioxidants levels} = \frac{\text{A.test}}{\text{A.STD}} * Conc.of \quad STD \ \text{(mmol/l)}$$

2.2.7 Statistical Analysis

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

Chapter Three

Results

3. Results

3.1 Effect of heparin and enoxaparin on cell viability

3.1.1 Effect of Heparin on the viability of Vero normal, SW480 colon cancer and LNCaP prostate cancer cell line.

The results showed that heparin at the concentration (1000-31µg/ml) causes a significant *(P≤0.001)* decrease in the viability of the Vero cell line in comparison with the control group after incubation for 24hr as shown in (figure 3.1).

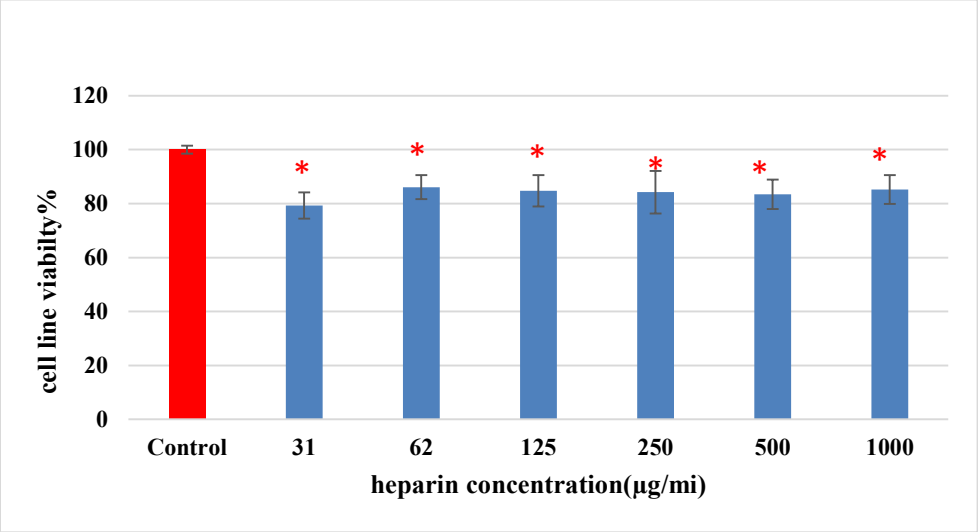


Figure 3.1: Effect of heparin on the viability of Vero cell line. * :significant

Results shown in figure 3.2 revealed that heparin at a concentration (1000,500 µg/ml) causes significant *(p≤0.050)* decrease in the viability of SW480 colon cancer cells while the concentrations (250, 125,62,31 µg/ml) cause no significant effect compared to the control group.

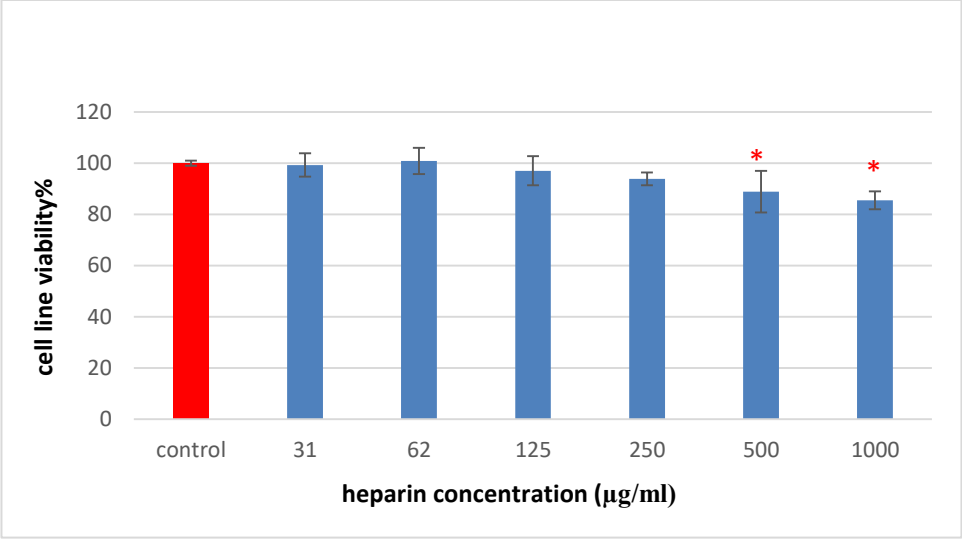


Figure 3.2: Effect of heparin on the viability of SW480 colon cancer cell line.

The results showed no significant difference at all concentration in the viability of the LNCaP prostate cancer cell line when compared with a control group (figure 3.3).

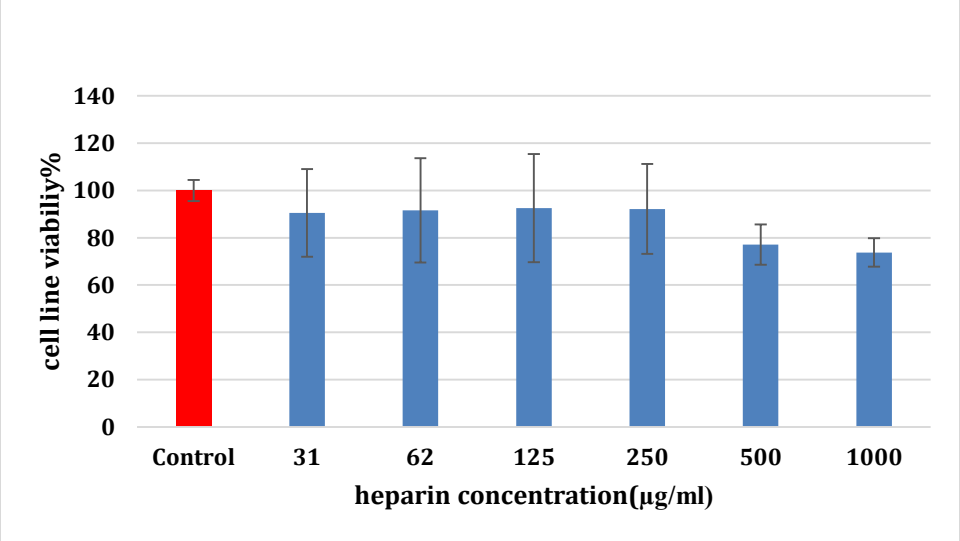


Figure 3.3: Effect of heparin on the viability of LNCaP prostate cancer cell line.

3.1.2 Effect of Enoxaparin on the viability of Vero normal, SW480 colon cancer, and LNCaP prostate cancer cell line.

Results shown in figure 3.4 revealed that enoxaparin has no significant effect on the viability of Vero cell when compared with the control group.

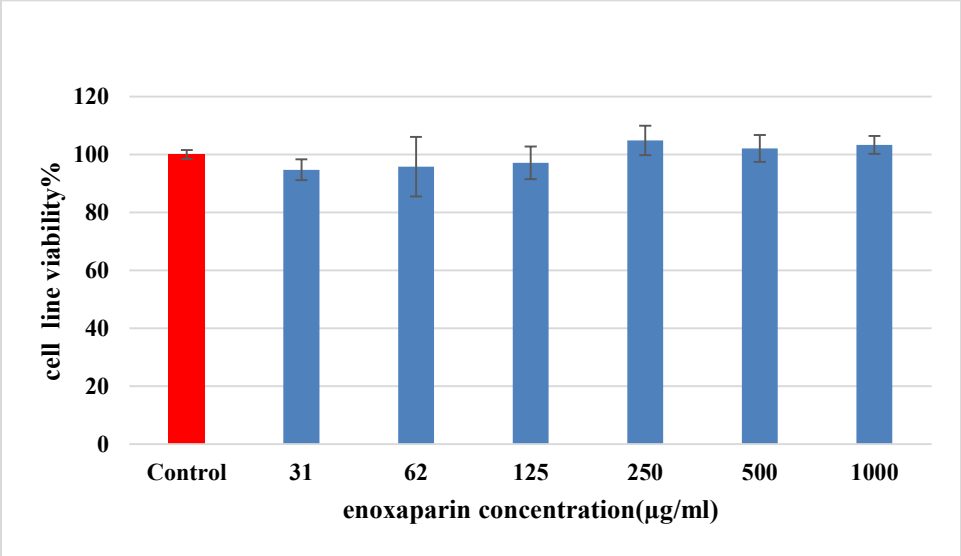


Figure 3.4: Effect of enoxaparin on the viability of Vero cell line.

For SW480 colon cancer cells, The result showed in figure 3.5 that enoxaparin at all concentrations causes a significant *(P≤0.050)* decrease in viability of colon cancer cells when compared with the control group.

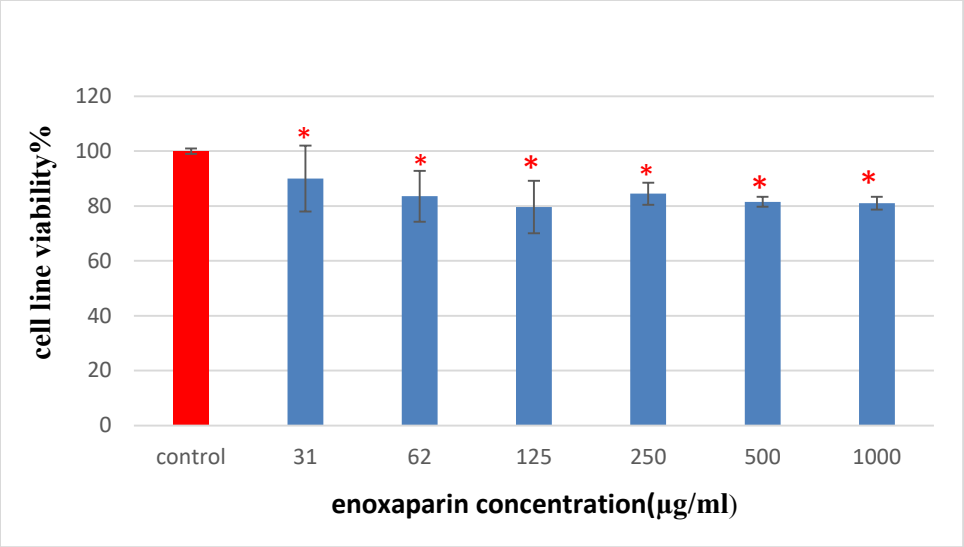


Figure 3.5: Effect of enoxaparin on the viability of SW480 colon cancer cell line.

The result is shown in figure 3.6 that enoxaparin has no significant effect on the viability of LNCaP prostate cancer cells at all concentrations when compared with the control group.

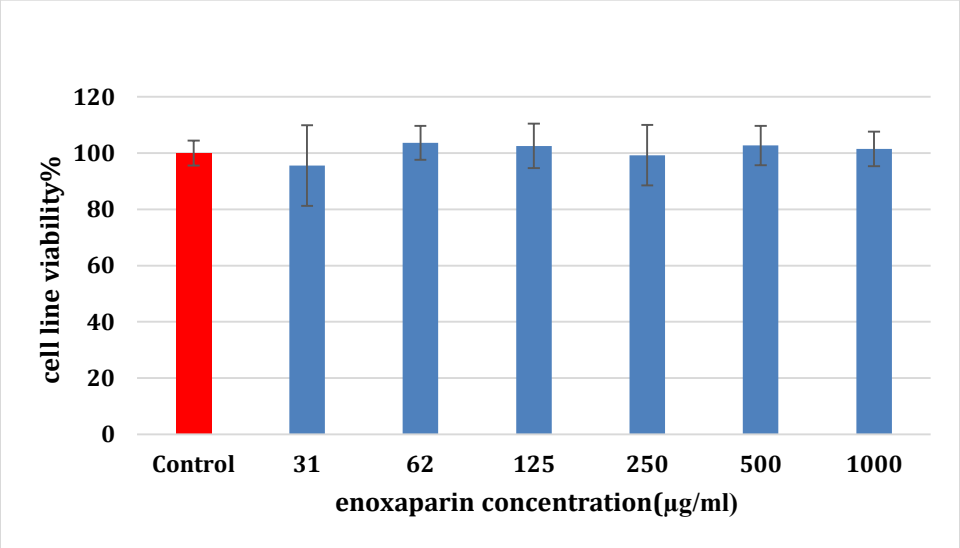


Figure 3.6: Effect of enoxaparin on the viability of LNCaP prostate cancer cell line.

3.2 Detection apoptotic and inflammatory effect of Heparin and Enoxaparin

3.2.1 Effect of Heparin on TNF-α release in SW480 colon cancer and LNCaP prostate cancer cell line.

For SW480 colon cancer cells, the result showed that heparin has no significant effect on TNF-α level at all concentrations when compared with the control group (figure 3.7).

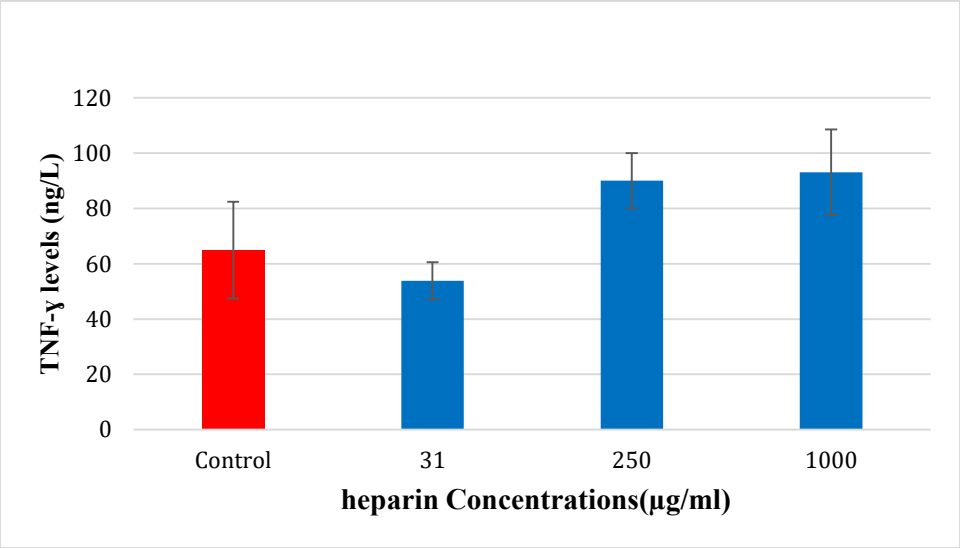


Figure 3.7: Effect of heparin on TNF-α release in SW480 colon cancer cell line.

The results showed that heparin at the concentration (1000,250,125 µg/ml) causes a significant ($P \leq 0.050$) decrease in TNF-α level in the LNCaP prostate cancer cell line (figure 3.8).

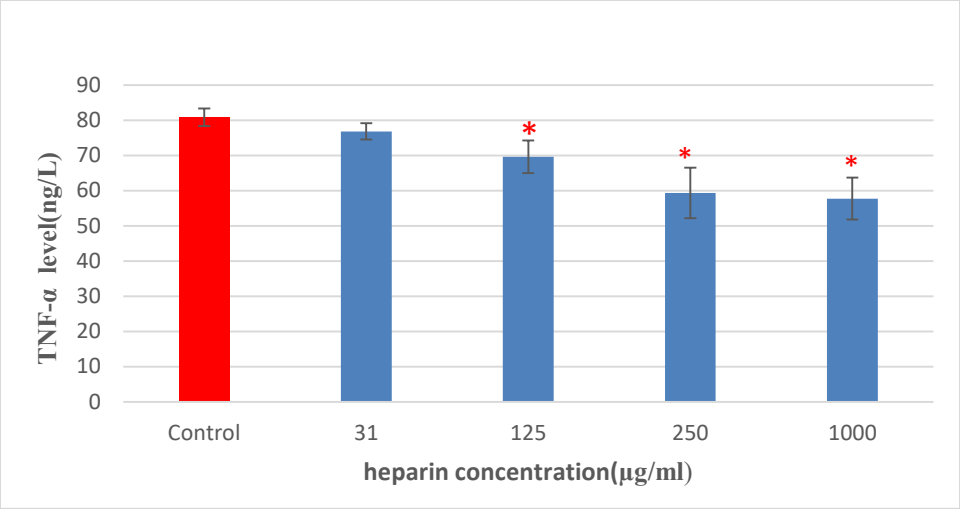


Figure 3.8: Effect of heparin on TNF-α in LNCaP prostate cancer cell line.

3.2.2 Effect of Enoxaparin on TNF-α release in SW480 colon cancer and LNCaP prostate cancer cell line.

The results showed that enoxaparin at the concentration (1000,250,31 µg/ml) causes a significant (*P≤0.050*) decrease in TNF-α level in the SW480 colon cancer cell line (figure 3.9).

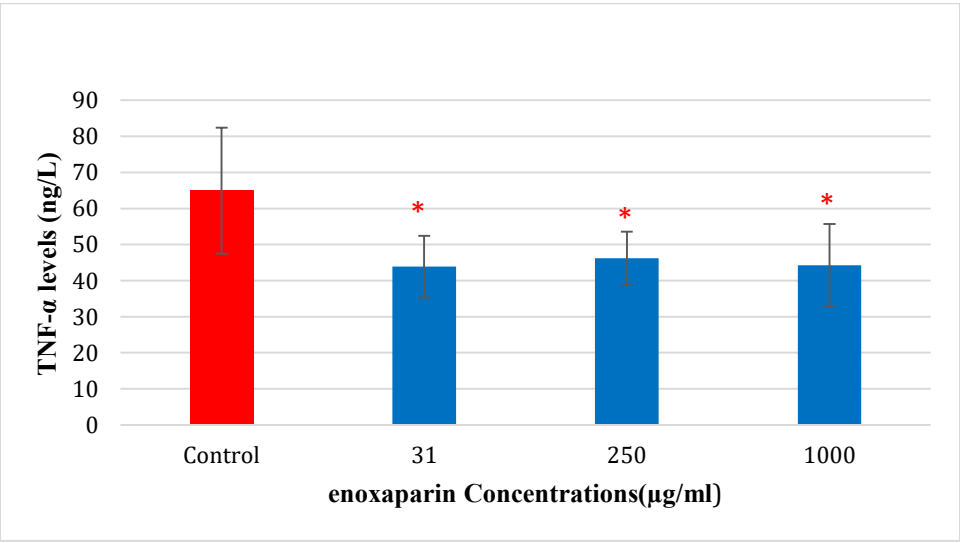


Figure 3.9 Effect of enoxaparin on TNF-α level in SW480 colon cancer cell line.

For the LNCaP prostate cancer cell line, the result showed no significant difference at all concentrations when compared with the control group (figure 13.10).

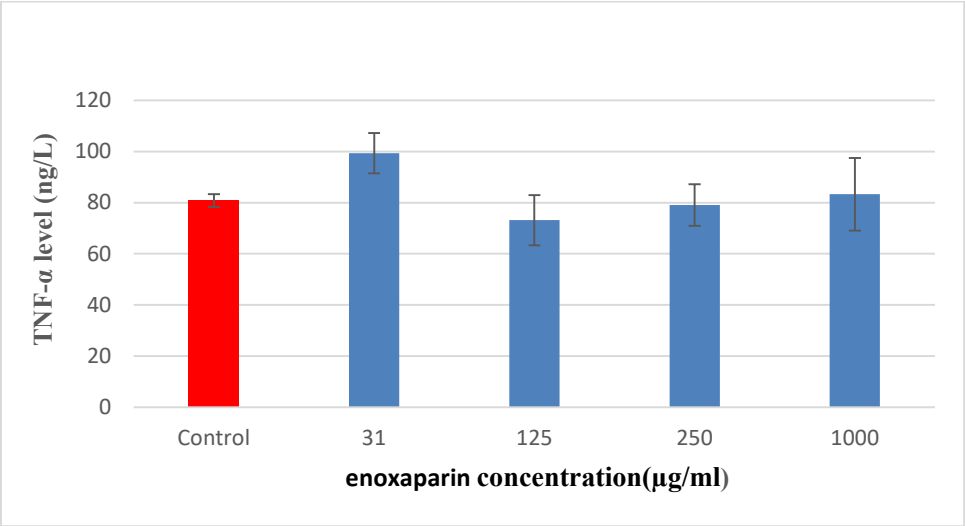


Figure 3.10: Effect of enoxaparin on TNF-α level on LNCaP prostate cancer cell line.

3.2.3 Effect of Heparin on caspase 3 release in SW480 colon cancer and LNCaP prostate cancer cell line.

For SW480 colon cancer cells, the result showed that heparin has no significant effect on caspase3 levels at all concentrations when compared with the control group (figure 3.11).

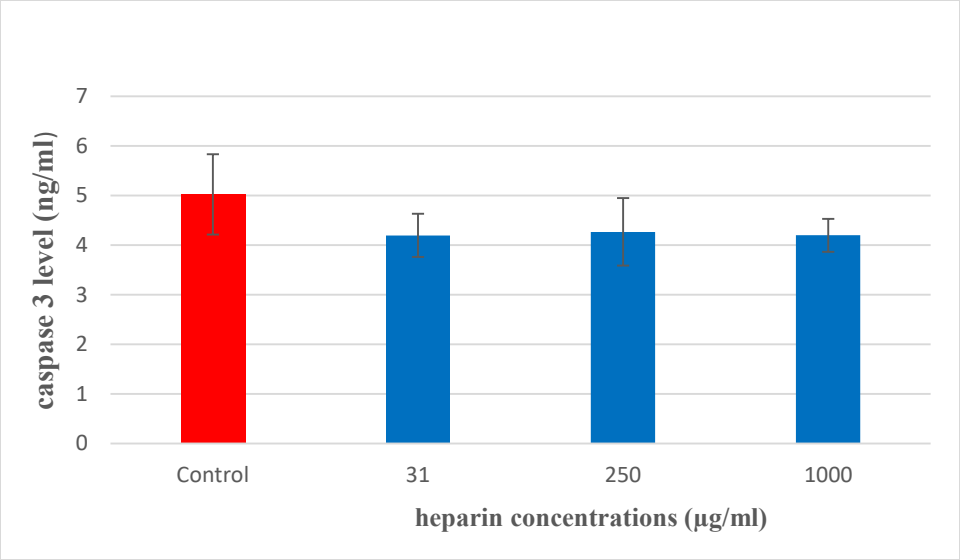


Figure 3.11: Effect of heparin on caspase 3 in SW480 colon cancer cell line.

For LNCaP prostate cancer cells, the result showed no statistically significant difference at all concentrations when compared with control group(figure 3.12).

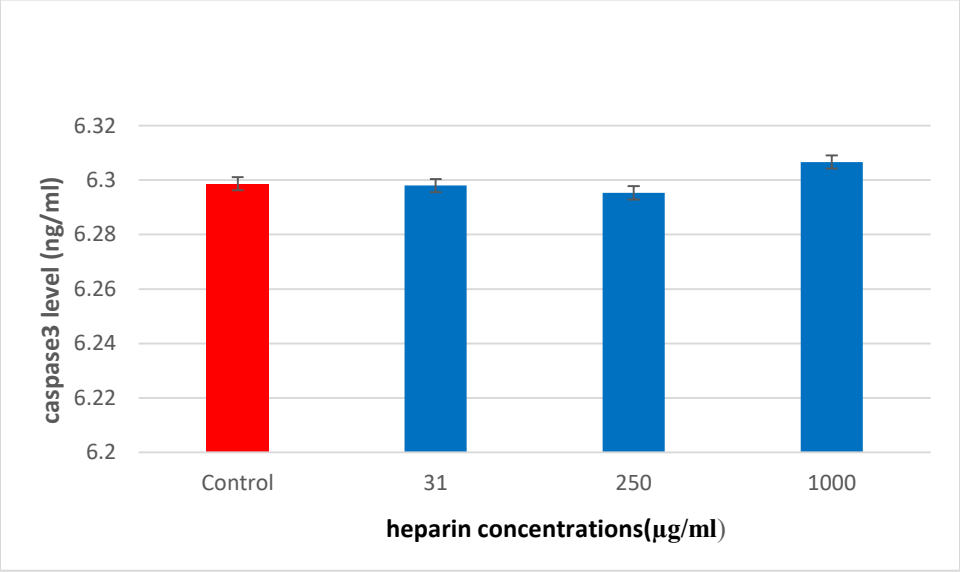


Figure 3.12: Effect of heparin on caspase 3 in LNCaP prostate cancer cell line.

3.2.4 Effect of Enoxaparin on caspase 3 release in SW480 colon cancer and LNCaP prostate cancer cell line.

The result is shown in (figure 3.13) that enoxaparin at the concentration (1000,250,31µg/ml) causes a significant *(P≤0.050)* increase in caspase3 level in SW480 colon cancer cells when compared with the control group.

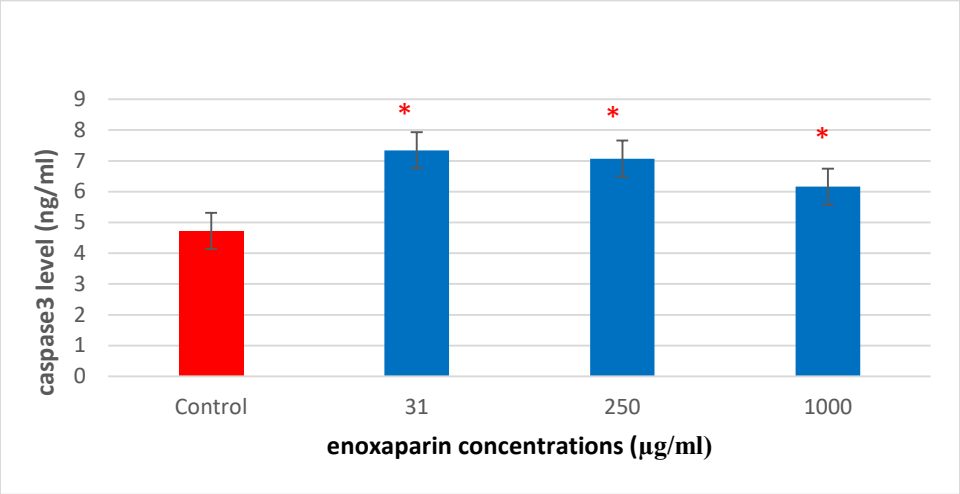


Figure 3.13: Effect of enoxaparin on caspase3 in SW480 colon cancer cell line.

For LNCaP prostate cancer cells, the result showed no statistically significant difference at all concentrations when compared with control group (figure 3.14).

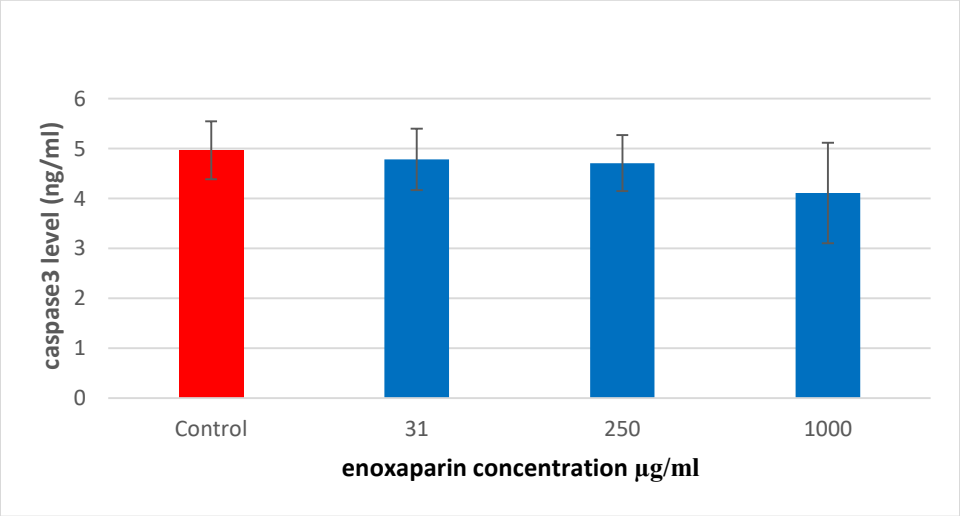


Figure 3.14: Effect of enoxaparin on caspase3 in LNCaP prostate cancer cell line.

3.3 Evaluation of the Total Antioxidant Capacity

3.3.1 Evaluation the Total Antioxidant Capacity of Heparin on SW480 colon cancer cell line and LNCaP prostate cancer cell line.

For the SW480 colon cancer cell line, the result showed there is not a statistically significant difference (figure 3.15).

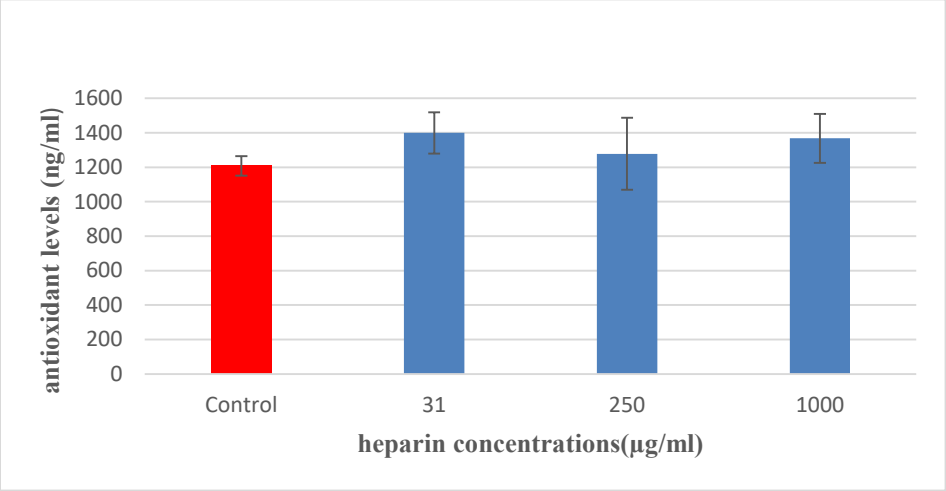


Figure 3.15: Antioxidant effect of heparin on SW480 colon cancer cell line.

For the LNCaP cell line, the result showed that heparin caused significant *(P≤0.050)* increase in total antioxidant at the concentration (31,250,1000µg/ml) when compared with the control group (figure 3.16).

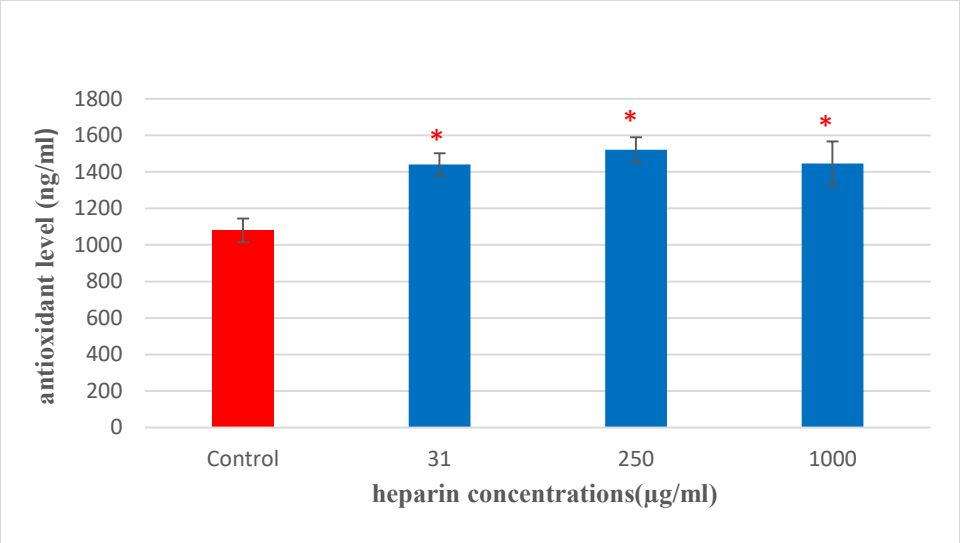


Figure 3.16: Antioxidant effect of heparin on LNCaP prostate cancer cell line.

3.3.2 Evaluation the Total Antioxidant Capacity of Enoxaparin on SW480 colon cancer, and LNCaP prostate cancer cell line.

For the SW480 colon cancer cell line, the result showed not a statistically significant difference at all concentrations when compared with the control group (figure3.17) .

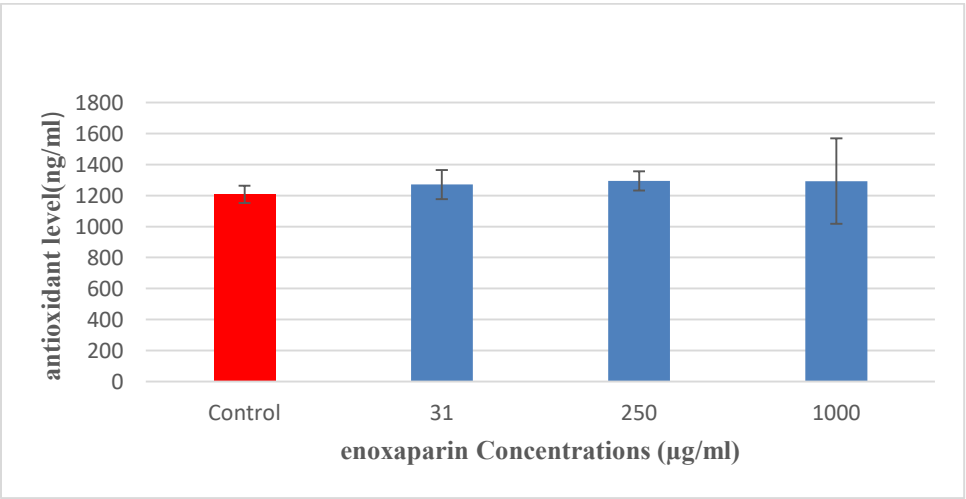


Figure 3.17: Antioxidant effect of enoxaparin on SW480 colon cancer cell line.

For the LNCaP prostate cancer cell, the result showed in figure3.18 that enoxaparin at the concentration (250µg/ml) cause a significant ($P \leq 0.050$) increase in total antioxidant when compared with the control group.

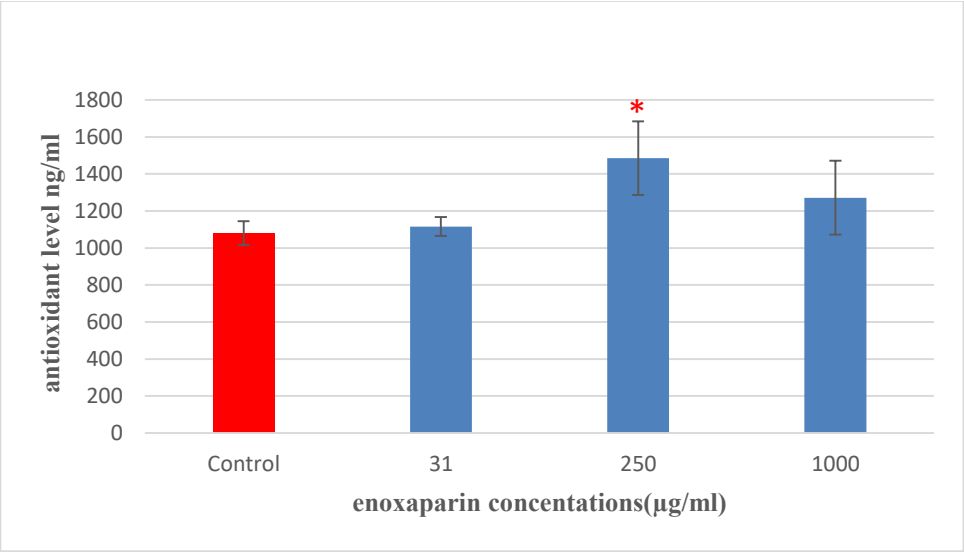


Figure 3.18:antioxidant effect of enoxaparin on LNCaP prostate cancer cell line.

Chapter Four

Discussion

4. Discussion

4.1 Cytotoxicity Evaluation

4.1.1 Effect of Heparin on the viability of Vero normal, SW480 colon cancer and LNCaP prostate cancer cell line.

For the Vero cell, The result of the current study revealed that heparin at all concentrations causes decrease in the viability of the Vero cell line (figure 3.1).

Various studies were made to determine the effects of heparin on different cell types like endothelial cells, smooth muscle cells, fibroblasts, and blood cells. Its effects on reproduction inhibition and cell cycle are still being investigated reported the growth inhibition effects of long-term heparin treatment in bovine pulmonary artery smooth muscle cells. They also concluded that besides growth inhibition, heparin may also have apoptotic and necrotic effects on normal pulmonary artery smooth muscle cells (Gurbuz *et al.*, 2013).

Regarding cancer cells, Results shown that heparin at the concentrations (500,1000µg/ml) causes a significant decrease in the viability of SW480 colon cancer cells (figure3.2).

Heparin preparations are mixtures of heterogeneous polysaccharides containing a large variety of biological activities; this makes the identification of the role of heparin during cancer progression rather complicated. There is accumulating evidence that heparin, apart from its anticoagulant activity, can block P- and L-selectin, affect the activity of growth factors and cytokines, release tissue factor pathway inhibitor (TFPI), inhibit heparanase and angiogenesis, alter interactions with integrins, and modulate protease activity and thereby the composition of extracellular matrix. While any of these activities may affect cancer progression

the experimental evidence from some studies strongly indicates that heparin affects early events in the metastatic cascade. as shown (Borsig, 2010).

Various types of heparin and its derivatives can inhibit tumor cell proliferation, migration, and invasion, and enhance the chemosensitivity of tumor cells. Heparin derivatives modulate hematogenous metastasis by inhibiting the interaction between platelets and tumor cells and control lymphatic metastasis by inhibiting lymphangiogenesis. Furthermore, heparin derivatives bind to a range of heparin-binding proteins to block signal pathways including TGF-β1, integrins, CXCL12-CXCR4 axis, and VEGFC/VEGFR-3 axis (Ma *et al.*, 2020).

LNCaP is a cell line derived from a metastatic lymph node lesion of human prostate cancer which is androgen receptor (AR) positive, and exhibits androgen-sensitive growth (Castanares *et al.*, 2016)

The result of the current study revealed that heparin has no effect on the viability of LNCaP prostate cancer cells when compared with the control group (figure3.3).

Heparin and enoxaparin turned out to have no influence on the viability of tumor cell lines. However, in contrast to these findings, the viability of the human choriocarcinoma cell line JEG-3 was described to be stimulated. whereas the viability of three of four SCC (squamous cell carcinoma) cell lines after treatment with unfractionated heparin for 12, 24, and 48 h was reduced Such discrepant results underline that tumor cells may largely differ from each other and thus also in their reactions to any compounds (Bittkau *et al.*, 2019).

4.1.2 Effect of Enoxaparin on the viability of Vero normal , SW480 colon cancer and LNCaP prostate cancer cell line.

For the Vero cell, The result of the current study revealed that enoxaparin has no significant effect on the viability of the vero cells (figure 3.4). This result agree with previous study by Bittkau on normal cell lines. They found that enoxaparin, showed no effect on the proliferation of human pulmonary epithelial cells A-549 and primary human osteoblasts. This is comprehensible, as heparin is an endogenous compound of the human body (Bittkau *et al.*, 2019).

For the SW480 colon cancer cell line, The result of the current study revealed that enoxaparin at all concentration causes significant decrease in the viability of SW480 colon cancer cells (figure 3.5).

Patients with advanced tumors often have hypercoagulable blood, requiring anticoagulant therapy. Clinical studies have found that tumor patients receiving anticoagulant therapy with heparin or LMWH have an extended survival period. The initial explanation is that heparin can inhibit tumor angiogenesis. Growing evidence exists that drugs such as LMWH may inhibit cancer growth and metastasis through various mechanisms. Moreover, tumor growth and metastasis depend on angiogenesis. Thus, blocking angiogenesis is a very promising strategy for treating tumors (Qiu *et al.*, 2021).

The hepatic growth of colon carcinoma metastases was strongly inhibited by enoxaparin compared to the (Ctrl) group ($p=0.001$). This effect was associated with an inhibition of heparanase mRNA expression and protein production both in vivo and in vitro (Djaafar *et al.*, 2016)

The combination of cisplatin and enoxaparin sodium showed a synergic effect in reducing cell viability and migration capacity and increased the apoptosis of H357

human OSCC cells. The present results suggest enoxaparin sodium could be beneficial in chemotherapy for OSCC patients (Camacho-Alonso *et al.*, 2020).

For the LNCaP prostate cancer cell line, The result of the current study revealed that enoxaparin does not affect the viability of LNCaP prostate cancer cells when compared with the control group (figure 3.6).

Heparin and enoxaparin turned out to not influence the viability of tumor cell lines. However, in contrast to these findings, the viability of the human choriocarcinoma cell line JEG-3 was described to be stimulated. whereas the viability of three of four SCC (squamous cell carcinoma) cell lines after treatment with unfractionated heparin for 12, 24, and 48 h was reduced Such discrepant results underline that tumor cells may largely differ from each other and thus also in their reactions to any compounds (Bittkau *et al.*, 2019).

4.2 Detection of apoptosis and inflammatory effect of Heparin and Enoxaparin

4.2.1 Effect of Heparin on TNF-α levels in SW480 colon cancer and LNCaP prostate cancer cell lines.

The result showed that heparin has no significant effect on TNF-α release by SW480 colon cancer cells as illustrated previously in (figure 3.7). While For LNCaP prostate cancer cell line, heparin causes a significant decrease in TNF-α level at the concentration (1000,250,125µg/ml) when compared with control group.

Among many inflammatory cytokines, tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β play a major role in activating neutrophils and inducing T cell proliferation and activation, leading to the occurrence of inflammatory reaction.

Heparin also reduces the protein levels of IL-1 β, TNF-α, IL-6, IL-8, and IL-18 induced by lipopolysaccharides (LPS), which may be related to heparin interfering with mitogen-activated protein kinase (MAPK), nuclear factor kappa-lightchain-enhancer of activated B cells (NF-κB), and c-Jun signaling pathways (Qiu *et al.*, 2021).

Heparin is known to inhibit the degranulation of isolated human mast cells in response to a variety of stimuli, and hence the release of histamine. Cytotoxic effects upon endothelial cells of TNF-a activated eosinophils are markedly inhibited by heparin (Lever and Page, 2012).

The mechanisms behind the anti-inflammatory effect of heparin have yet to be completely elucidated. Proposed mechanisms include binding of inflammatory cytokines and acute phase reactions by heparin, inhibition of adhesion molecules involved in the inflammation response, and most importantly P-selectin and L-selectin. inhibition of NF-κB translocation from the cytoplasm to the nucleus (Oduah et al., 2016).

4.2.2 Effect of Enoxaparin on TNF-α level in SW480 colon cancer and LNCaP prostate cancer cell line.

The results showed that enoxaparin at the concentrations all caused a significant decrease in TNF-α level in the SW480 colon cancer cell line (figure 3.9). Enoxaparin, the most widely used LMWH, is known to inhibit T cell mediated release of multiple cytokines, such as IL-4, IL-5, IL-13 and TNF-α, involved in various inflammatory disorders including asthma (Shastri *et al.*, 2015).

Enoxaparin Reduced levels of cytokines were detected in patients treated with enoxaparin In clinical trials, enoxaparin, and other LMWHs, have also been

reported to be effective in diseases with complex pathology, including diabetic foot ulcers and lichen planus (Yan *et al.*, 2017).

For the LNCaP prostate cancer cell line, the result showed no significant difference when compared with the control group (figure 13.10). This agrees with (Sun *et al.*, 2009).

3.2.3 Effect of Heparin on caspase 3 level in SW480 colon cancer and LNCaP prostate cancer cell line.

For SW480 colon cancer cells, the result showed that heparin has no significant effect on caspase3 levels as shown in (figure 3.11). This result agree with previous findings of Uzun . they reported that heparin has no significant anti-proliferative and apoptotic effects on colon cancer cells in vitro (Uzun *et al.*, 2009).

For LNCaP prostate cancer cells, the result showed no statistically significant difference (figure 3.12). heparin induces apoptosis in CNE2 cells, which may be probably regulated by increased expression of c-myc and the rates of bax/ bcl-2. These findings suggest that heparin may function as an inducer of apoptosis in carcinoma cells (Li *et al.*, 2001).

3.2.4 Effect of Enoxaparin on caspase 3 level in SW480 colon cancer and LNCaP prostate cancer cell line.

The result is shown in (figure 3.13) that enoxaparin at the concentration at all causes a significant increase in caspase3 level in SW480 colon cancer cells when compared with the control group.

For LNCaP prostate cancer cells, the result showed no statistically significant difference (figure 3.14).

The combination of cisplatin and enoxaparin sodium showed a synergic effect in reducing cell viability and migration capacity and increased the apoptosis of H357 human OSCC cells. The present results suggest enoxaparin sodium could be beneficial in chemotherapy for OSCC patients (Camacho-Alonso *et al.*, 2020).

4.3 Evaluation of the Total Antioxidant Capacity

4.3.1 Evaluation of the Total Antioxidant Capacity of Heparin on SW480 colon cancer and LNCaP prostate cancer cell line.

If we compare between the results in figures 3.15 and 3.16, it appear that heparin has no significant antioxidant effect on SW480. While for LNCaP prostate cancer cell line, heparin caused a highly significant increase in total antioxidant at the concentration (31,250,1000µg/ml)

Cancer cells exhibit aberrant redox homeostasis, but while ROS are pro-tumorigenic, high ROS levels are cytotoxic Specifically, hyperproliferation of tumor cells is accompanied by high ROS production, but they are adapted to thrive under conditions where this oxidative burden pushes redox balance away from a reduced state; tumor cells achieve this by increasing their antioxidant status to optimize ROS-driven proliferation, while at the same time avoiding ROS thresholds that would trigger senescence, apoptosis, or ferroptosis (Hayes *et al.*, 2020)

Oxidative stress activates the epidermal growth factor receptor (EGFR) through a ligand-independent reverse activation method. EGFR activates downstream signaling pathways and mucin 5AC (MUC5AC) expression, leading to excessive mucus secretion, increasing morbidity and mortality from chronic obstructive pulmonary disease. Studies have shown that heparin can reduce the production of dual oxidase 1 and ROS, block phorbol 12-myristate 13- acetate-induced EGFR

activation, and inhibit overexpression of MUC5AC. Heparin downregulates the iron homeostasis regulator hepcidin and reduces TGF-β2-mediated increase in ferritin and ROS. It can also improve the imbalance between oxidation and antioxidation and protect hemodialysis patients from the adverse effects of oxidative stress (Qiu *et al.*, 2021)

4.3.2 Evaluation of the Total Antioxidant Capacity of Enoxaparin on SW480 colon cancer and LNCaP prostate cancer cell line.

For SW480 colon cancer cells, The result showed in (figure 3.17) no significant difference when compared with the control group.

For the LNCaP prostate cancer cell, the result showed in (figure3.18) that enoxaparin at the concentration (250µg/ml) cause a significant ($P<0.050$) increase in total antioxidant when compared with the control group.

The antioxidant mechanism of enoxaparin (EP) on endothelial cells stimulated by oxidative stress. They found that EP interferes with the hydrogen peroxide (H2O2)-induced P38 MAPK pathway in human endothelial cells (HECS). It also reduces acute pancreatitis (AP)-1 activation in H2O2-induced HECS by interfering JNK MAPK signal (Qiu *et al.*, 2021).

Higher doses or longer treatment duration may reveal a more pronounced antioxidant potential for ENX that may perhaps extend to upregulating other antioxidant molecules (e.g., GSH, CAT, SOD, and/or GSH-P); however, more studies are warranted to confirm this speculation (Shaker *et al.*, 2018).

Conclusions

and

Recommendations

Conclusions

1-From our study found heparin cytotoxic to vero normal cell line and has antiprolifrative effect on SW480 colon cancer cell line at higher concentrations.

2-Enoxaparin has antiprolifrative ,anti-inflammatory, apoptotic effect on SW480 colon cancer cell line.

3-Heparin and enoxaparin increase the total antioxidant capacity in the LNCaP prostate cancer cell line .

Recommendations

1-Study the molecular assessment of enoxaparin on (MAPK, MMP-2, PI3K) in cancer cell line.

2-Study the possible interaction of heparin and enoxaparin with anticancer drug in vivo and in vitro.

References

References

- Abdulkhaleq, L. A., M. A. Assi, Rasedee Abdullah., M. Zamri-Saad, Y. H. Taufiq-Yap, and M. N. M. Hezmee. (2018) 'The crucial roles of inflammatory mediators in inflammation: A review', Veterinary World, pp. 627–635. doi: 10.14202/vetworld.2018.627-635.
- Adan, A., Kiraz, Y. and Baran, Y. (2016) 'Cell Proliferation and Cytotoxicity Assays', Current Pharmaceutical Biotechnology, pp. 1213–1221. doi: 10.2174/1389201017666160808160513.
- Ahmed, M. (2020) 'Colon Cancer: A Clinician's Perspective in 2019', Gastroenterology Research, pp. 1–10. doi: 10.14740/gr1239.
- Alexiusdottir, K. K., Pall Helgi Möller, Petur Snaebjornsson, Larus Jonasson, Elinborg j. Olafsdottir, Einar Stefan Björnsson, et al. (2012) 'Association of symptoms of colon cancer patients with tumor location and TNM tumor stage', Scandinavian Journal of Gastroenterology, pp. 795–801. doi: 10.3109/00365521.2012.672589.
- Alban, S. (2012) 'Adverse effects of Heparin', Handbook of Experimental Pharmacology, pp. 211–263. doi: 10.1007/978-3-642-23056-1_10.
- Ali, M. S., Al-Rubaei, S. H., and Ahmed, N. S. (2020) 'Tumor necrosis factor alpha as a potential marker for prostate cancer', Journal of Biotechnology Research Center, pp. 5–9. doi: 10.24126/jobrc.2020.14.1.582.
- Alturkistani, A., Nisanne Ghonem, Verna-Ann Power-Charnitsky, Alejandro Pino-Figueroa, mattia m. Migliore. (2019) 'Inhibition of PAR-1 receptor signaling by enoxaparin reduces cell proliferation and migration in A549 cells', Anticancer Research, pp. 5297–5310. doi: 10.21873/anticanres.13723.

- Ammerman, N. C., Beier-Sexton, M., and Azad, A. F. (2008) 'Growth and maintenance of vero cell lines', Current Protocols in Microbiology, pp. 1–7. doi: 10.1002/9780471729259.mca04es11.
- Apak, R., Kubilay Güçlü, Birsen Demirata , K. Işıl Berker , Saliha Esin Çelik, Burcu Bektaşoğlu, et al. (2007) 'Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay', Molecules, pp. 1496–1547. doi: 10.3390/12071496.
- Archer, M., Dogra, N. and Kyprianou, N. (2020) 'Inflammation as a driver of prostate cancer metastasis and therapeutic resistance', Cancers, pp. 1–24. doi: 10.3390/cancers12102984.
- Asanka Sanjeewa, K. K., D. P. Nagahawatta, Hye-Won Yang ,Jae Young Oh, Thilina U. Jayawardena, You-Jin Jeon, et al. (2020) 'Octominin inhibits LPS-induced chemokine and pro-inflammatory cytokine secretion from raw 264.7 macrophages via blocking TLRS/nf-κb signal transduction', Biomolecules, pp. 1–16. doi: 10.3390/biom10040511.
- Azhar, R. A., René Sotelo, Juan Arriaga and Gill, I. S. (2015) Prostate Cancer A Patient ' s Guide.
- B. Vendramini-Costa, D. and E. Carvalho, J. (2012) 'Molecular Link Mechanisms between Inflammation and Cancer', Current Pharmaceutical Design, pp. 3831–3852. doi: 10.2174/138161212802083707.
- Banerjee, A. ,Surajit Pathak, Vimala Devi S, Dharani Vasan, Ramachandran Murugesan, Rama S. Verma . (2017) 'Strategies for targeted drug delivery in treatment of colon cancer: current trends and future perspectives', Drug Discovery Today, pp. 1224–1232. doi: 10.1016/j.drudis.2017.05.006.
- Barsouk, Adam, Sandeep Anand Padala, Anusha Vakiti, Azeem Mohammed, Kalyan Saginala, Krishna Chaitanya Thandra 5, et al. (2020)

'Epidemiology, Staging and Management of Prostate Cancer', Medical Sciences, p. 28. doi: 10.3390/medsci8030028.

- Birben, E., Umit Murat Sahiner MD, Cansin Sackesen MD, Serpil Erzurum MD and Omer Kalayci, MD. (2012) 'Oxidative stress and antioxidant defense', World Allergy Organization Journal ,pp.9–19
- Bittkau, K. S., Philipp Dörschmann, Martina Blümel, Deniz Tasdemir, Johann Roider, Alexa Klettner, et al. (2019) 'Comparison of the Effects of Fucoidans on the Cell Viability of Tumor and Non-Tumor Cell Lines . doi: 10.3390/md17080441.
- Bonati, L. and Tang, L. (2021) 'Cytokine engineering for targeted cancer immunotherapy', Current Opinion in Chemical Biology, pp. 43–52. doi: 10.1016/j.cbpa.2021.01.007.
- Borsig, L. (2010) Heparin as an inhibitor of cancer progression, Progress in Molecular Biology and Translational Science. Elsevier Inc. doi: 10.1016/S1877-1173(10)93014-7.
- Bukowski, K., Kciuk, M. and Kontek, R. (2020) 'Mechanisms of multidrug resistance in cancer chemotherapy', International Journal of Molecular Sciences, doi: 10.3390/ijms21093233.
- Camacho-Alonso, F., Gómez-Albentosa, T., R. E. Oñate-Sa´nchez, M. R. Tudela-Mulero, M.Sanchez-Siles, Francisco J. Go´mez-Garcıa, et al. (2020) 'In Vitro Study of Synergic Effect of Cisplatin and Low Molecular Weight Heparin on Oral Squamous Cell Carcinoma', Frontiers in Oncology.
- Cao, K., and Tait, S. W. G. (2018) 'Apoptosis and Cancer: Force Awakens, Phantom Menace, or Both?', International Review of Cell and Molecular Biology, pp. 135–152. doi: 10.1016/bs.ircmb.2017.12.003.

- Castanares, M. A., Ben T. Copeland, Wasim H. Chowdhury, Minzhi M. Liu, Ronald Rodriguez ,Martin G. Pomper, et al. (2016) 'Characterization of a novel metastatic prostate cancer cell line of LNCaP origin', Prostate, pp. 215–225. doi: 10.1002/pros.23115.
- Chaudhary, M. V. and Singh, P. (2017) 'CELL LINE : A REVIEW', (May).
- Chi, Y., Di Wang, Junpei Wang, Weidong Yu, and Jichun Yang . (2019) 'Long Non-Coding RNA in the Pathogenesis of Cancers', Cells, pp. 1–44. doi: 10.3390/cells8091015.
- Cirne, C. A. and Machado, T. A. C. (2020) 'Utilization profile of enoxaparin in hospitalized patients with chronic kidney disease in southern brazil', Brazilian Journal of Pharmaceutical Sciences. doi: 10.1590/s2175-97902019000318296.
- Descotes, J. L. (2018) 'Diagnosis of prostate cancer', Asian Journal of Urology. doi: 10.1016/j.ajur.2018.11.007.
- Diakos, C. I., Kellie A Charles, Donald C McMillan, Stephen J Clarke. (2014) 'Cancer-related inflammation and treatment effectiveness', The Lancet Oncology, pp. 493–503. doi: 10.1016/S1470-2045(14)70263-3.
- Djaafar, S., Isabelle Dunand-Sautier, Carmen Gonelle-Gispert, Stephanie Lacotte Ariane DE Agostini, Majno Petro, et al. (2016) 'Enoxaparin attenuates mouse colon cancer liver metastases by inhibiting heparanase and interferon-inducible chemokines', Anticancer Research, pp. 4019–4032.
- Evans, A. J. (2018) 'Treatment effects in prostate cancer', Modern Pathology, pp. 110–121. doi: 10.1038/modpathol.2017.158.
- Febbraro, S., Javier Leal Martínez-Bujanda, Concepción Nieto Magro, and Paolo Bettica . (2021) 'Bioavailability study of Enoxaparin Sodium Chemi (80 mg/0.8 mL) and Clexane (80 mg/0.8 mL) subcutaneous injection in

healthy adults ', Int. Journal of Clinical Pharmacology and Therapeutics, pp. 734–744. doi: 10.5414/cp204022.

- Fleming, M., Sreelakshmi Ravula , Sergei F. Tatishchev , Hanlin L. Wang.(2012) 'Colorectal carcinoma : Pathologic aspects', pp. 153–173. doi: 10.3978/j.issn.2078-6891.2012.030.
- Giossi, A. ,Elisabetta Del Zotto, Irene Volonghi, Paolo Costa, Rita Bertuetti, Paolo Remida, et al. (2012) 'Thromboembolic complications of heparin-induced thrombocytopenia', Blood Coagulation and Fibrinolysis, pp. 559–562. doi: 10.1097/MBC.0b013e3283502989.
- Gonzalez-Delgado, P. and Fernandez, J. (2016) 'Hypersensitivity reactions to heparins', Current Opinion in Allergy and Clinical Immunology, pp. 315–322. doi: 10.1097/ACI.0000000000000281.
- Gray, E., Hogwood, J. and Mulloy, B. (2012) 'The anticoagulant and antithrombotic mechanisms of heparin', Handbook of Experimental Pharmacology, pp. 43–61. doi: 10.1007/978-3-642-23056-1_3.
- Gupta, K. K., Khan, M. A. and Singh, S. K. (2020) 'Constitutive Inflammatory Cytokine Storm: A Major Threat to Human Health', Journal of Interferon and Cytokine Research, pp. 19–23. doi: 10.1089/jir.2019.0085.
- Gurbuz, H. A., A. Baris Durukan, Handan Sevim, Ekin Ergin, Aylin Gurpinar, and Cem Yorgancioglu. (2013) 'Heparin toxicity in cell culture: A critical link in translation of basic science to clinical practice', Blood Coagulation and Fibrinolysis, pp. 742–745.
- Han, I. H., Song, H. O. and Ryu, J. S. (2020) 'IL-6 produced by prostate epithelial cells stimulated with Trichomonas vaginalis promotes proliferation of prostate cancer cells by inducing M2 polarization of THP-1-derived

macrophages', PLoS Neglected Tropical Diseases, pp. 1–23. doi: 10.1371/journal.pntd.0008126.

- Hayes, J. D., Dinkova-Kostova, A. T. and Tew, K. D. (2020) 'Oxidative Stress in Cancer', Cancer Cell, pp. 167–197.
- Hur, J. and Giovannucci, E. (2020) 'Racial differences in prostate cancer: does timing of puberty play a role?', British Journal of Cancer, pp. 349–354. doi: 10.1038/s41416-020-0897-4.
- Kasote, D. M., Surendra S. Katyare, Mahabaleshwar V. Hegde, Hanhong Bae . (2015) 'Significance of antioxidant potential of plants and its relevance to therapeutic applications', International Journal of Biological Sciences, pp. 982–991. doi: 10.7150/ijbs.12096.
- Kaur, G. and Dufour, J. M. (2012) 'Cell lines', Spermatogenesis, pp. 1–5. doi: 10.4161/spmg.19885.
- Kimura, T. and Egawa, S. (2018) 'Epidemiology of prostate cancer in Asian countries', International Journal of Urology, pp. 524–531.
- Koff, J. L., Ramachandiran, S. and * L. B.-M. (2015) 'A Time to Kill: Targeting Apoptosis in Cancer'. doi: 10.3390/ijms16022942.
- Kolligs, F. T. (2016) 'Diagnostics and epidemiology of colorectal cancer', Visceral Medicine, pp. 158–164. doi: 10.1159/000446488.
- KUBAT, E., Özer Aylin Gürpınar, Gökşen Ertuğrul, Hakan Işık, Duru Karasoy, Mehmet Ali Onur. (2021) 'Enoksaparin sodyum subkutan fibroblast için tam olarak güvenli midir?: Bir hücre kültürü çalışması', Acta Medica Alanya, pp. 18–23. doi: 10.30565/medalanya.822679.
- Lannagan, T. R., Rene Jackstadt, Simon J Leedham, and Owen J Sansom . (2021) 'Advances in colon cancer research: in vitro and animal models',

Current Opinion in Genetics and Development, pp. 50–56. doi: 10.1016/j.gde.2020.12.003.

- Lever, R. and Page, C. P. (2012) 'Non-anticoagulant effects of heparin: An overview', Handbook of Experimental Pharmacology, pp. 281–305. doi: 10.1007/978-3-642-23056-1_12.
- Levy, A. R., Adriana Rojas-Villarraga, Juan-Manuel Anaya, Yehuda Shoenfeld, Ricard Cervera. (2013) AUTOIMMUNITY From Bench to Bedside.
- Li, J., Cheng-Hao Y, MM, Ye-Ting Hu, MM, Jin-Song Li, PhD, Ying Yuan, MD, Su-Zhan Zhang, et al. (2016) 'TNM Staging of Colorectal Cancer Should be Reconsidered According to Weighting of the T Stage', Medicine (United States), pp. 1–8. doi: 10.1097/MD.0000000000002711
- Li, H. L., Ye, Kai He, Zhang, Hai Wei, Luo, Ying Ru, Ren, Xian D , Xiong, Ai Hua et al. (2001) 'Effect of heparin on apoptosis in human nasopharyngeal carcinoma CNE2 cells', Cell Research, pp. 311–315. doi: 10.1038/sj.cr.7290101.
- Li, X. and Ma, X. (2017) 'The role of heparin in sepsis: much more than just an anticoagulant', British Journal of Haematology, pp. 389–398. doi: 10.1111/bjh.14885.
- Liguori, I., Gennaro Russo, Francesco Curcio, Giulia Bulli, Luisa Aran, David Della-Morte, et al. (2018) 'Oxidative stress, aging, and diseases', Clinical Interventions in Aging, pp. 757–772. doi: 10.2147/CIA.S158513.
- Litwin, M. S. and Tan, H. J. (2017) 'The diagnosis and treatment of prostate cancer: A review', JAMA - Journal of the American Medical Association, pp. 2532–2542. doi: 10.1001/jama.2017.7248.

- Lokwani, R., Nina Suhaity Binti Azm, Mashitah Binti Mohd Yusoff and Solachuddin Jauhari Arief Ichwan . (2014) 'Beyond anticoagulant: Heparin as a potential anti-cancer agent', Journal of Biochemistry, Microbiology and Biotechnology , pp. 76–82. doi: 10.54987/jobimb.v2i2.160.
- Loo, S. W. and Pui, T. S. (2020) 'Cytokine and cancer biomarkers detection: The dawn of electrochemical paper-based biosensor', Sensors (Switzerland), doi: 10.3390/s20071854.
- Lopez, J. and Tait, S. W. G. (2015) 'Mitochondrial apoptosis: Killing cancer using the enemy within', British Journal of Cancer, pp. 957–962. doi: 10.1038/bjc.2015.85.
- Ma, L., Haiquan Qiao, Changjun He, Qian Yang, Chun Hei Antonio Cheung, Jagat R. Kanwar, et al. (2012) 'Modulating the interaction of CXCR4 and CXCL12 by low-molecular-weight heparin inhibits hepatic metastasis of colon cancer', Investigational New Drug, pp. 508–517. doi: 10.1007/s10637-010-9578-0.
- Ma, S. N., Zhi-Xiang Mao, Yang Wu, Ming-Xing Liang, Dan-Dan Wang, Xiu Chen , et al. (2020) 'The anti-cancer properties of heparin and its derivatives: a review and prospect', Cell Adhesion and Migration, pp. 118–128. doi: 10.1080/19336918.2020.1767489.
- Matsuura, K., K. Canfield, W. Feng and M. Kurokawa . (2016) 'Metabolic Regulation of Apoptosis in Cancer', International Review of Cell and Molecular Biology, pp. 43–87. doi: 10.1016/bs.ircmb.2016.06.006.
- McArthur, K., Chappaz, S. and Kile, B. T. (2017) 'Apoptosis in megakaryocytes and platelets: life and death of a lineage'. doi: 10.1182/blood-2017-11-742684.

- Meerloo, V. kaspres and cloos (2011) ‘Cancer Cell Culture’, Cancer Cell Culture. doi: 10.1385/1592594069.
- Merriel, S. W. D., Funston, G. and Hamilton, W. (2018) ‘Prostate Cancer in Primary Care’, Advances in Therapy, 35(9), pp. 1285–1294. doi: 10.1007/s12325-018-0766-1.
- Meleady, P. and O’Connor, R. (2006) ‘General procedures for cell culture’, Cell Biology, Four-Volume Set, pp. 13–20. doi: 10.1016/B978-012164730-8/50003-4.
- Mercogliano, M. F., Sofía Brun, Florencia Mauro, Patricia Virginia Elizalde and Roxana Schillac . (2021) ‘Harnessing tumor necrosis factor alpha to achieve effective cancer immunotherapy’, Cancers, pp. 1–33. doi: 10.3390/cancers13030564.
- Nagoor Meeran, M. F., Farah Laham, Sheikh Azimullah, Saeed Tariq, Shreesh Ojha . (2019) ‘α-Bisabolol abrogates isoproterenol-induced myocardial infarction by inhibiting mitochondrial dysfunction and intrinsic pathway of apoptosis in rats’, Molecular and Cellular Biochemistry, pp. 89–102. doi: 10.1007/s11010-018-3434-5.
- Oduah, E. I., Linhardt, R. J. and Sharfstein, S. T. (2016) ‘Heparin: Past, present, and future’, Pharmaceuticals, pp. 1–12.
- Van Opdenbosch, N. and Lamkanfi, M. (2019) ‘Caspases in Cell Death, Inflammation, and Disease’, Immunity, pp. 1352–1364.
- Palassi, S., Hadi Valizadeh, Saeideh Allahyari, and Parvin Zakeri-Milani . (2021) ‘Preparation and in vitro characterization of enoxaparin nano-liposomes through different methods’, Advanced Pharmaceutical Bulletin, pp. 295–300. doi: 10.34172/apb.2021.043.

- Page, C. (2013) ‘Heparin and Related Drugs: Beyond Anticoagulant Activity’, ISRN Pharmacology, pp. 1–13. doi: 10.1155/2013/910743.
- Paluck, S. J., Nguyen, T. H. and Maynard, H. D. (2016) ‘Heparin-Mimicking Polymers: Synthesis and Biological Applications’, Biomacromolecules, pp. 3417–3440. doi: 10.1021/acs.biomac.6b01147.
- Pfeffer, C. M. and Singh, A. T. K. (2018) ‘Apoptosis: A Target for Anticancer Therapy Claire’. doi: 10.3390/ijms19020448.
- Phelan, K. and May, K. M. (2017) ‘Mammalian cell tissue culture’, Current Protocols in Human Genetics, 2017(July), p. A.3G.1-A.3G.22. doi: 10.1002/cphg.41.
- Pisoschi, A. M. A. Pop, F. Iordache, L. Stanca, G. Predoi, A.I. Serban . (2020) ‘Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status’, European Journal of Medicinal Chemistry, p. 112891. /doi.org/10.1016/j.ejmech.2020.112891.
- Pizzino, G., Natasha Irrera, Mariapaola Cucinotta, Giovanni Pallio, Federica Mannino, Vincenzo Arcoraci, et al. (2017) ‘Oxidative Stress: Harms and Benefits for Human Health’, Oxidative Medicine and Cellular Longevity.
- Qiu, M., Shengjie Huang , Chuanhong Luo, Zhenfeng Wu, Binzhu Liang, Haozhou Huang, et al. (2021) ‘Pharmacological and clinical application of heparin progress: An essential drug for modern medicine’, Biomedicine and Pharmacotherapy, p. 111561. doi: 10.1016/j.biopha.2021.111561.
- Rampersad, S. N. (2012) ‘Multiple applications of alamar blue as an indicator of metabolic function and cellular health in cell viability bioassays’, Sensors (Switzerland), pp. 12347–12360.

- Rawla, P., Sunkara, T. and Barsouk, A. (2019) 'Epidemiology of colorectal cancer: Incidence, mortality, survival, and risk factors', Przeglad Gastroenterologiczny, pp. 89–103. doi: 10.5114/pg.2018.81072.
- Rehmet, J. and Gul-e-Saba, C. (2019) 'Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics', Advanced Pharmaceutical Bulletin, pp. 205–218.
- Russell, M. A. and Morgan, N. G. (2014) 'The impact of anti-inflammatory cytokines on the pancreatic β-cell', Islets, pp. 37–41. doi: 10.4161/19382014.2014.950547.
- Sadri Nahand, J., Maryam Esghaeia, Seyed Hamidreza Monavari, Mohsen Moghoofei, Seyed Jalal Kiani, Shayan Mostafaei, et al. (2020) 'The assessment of a possible link between HPV-mediated inflammation, apoptosis, and angiogenesis in Prostate cancer', International Immunopharmacology, p. 106913. doi: 10.1016/j.intimp.2020.106913.
- Schröder, S. K., Anastasia Asimakopoulou, Stefan Tillmann, Steffen Koschmieder, Ralf Weiskirchen, et al. (2020) 'TNF-α controls Lipocalin-2 expression in PC-3 prostate cancer cells', Cytokine, 135(May).
- Segeritz, C. P. and Vallier, L. (2017) Cell Culture: Growing Cells as Model Systems In Vitro, Basic Science Methods for Clinical Researchers. Elsevier Inc. doi: 10.1016/B978-0-12-803077-6.00009-6.
- Seyfried, T. N. and Huysentruyt, L. C. (2013) 'On the origin of cancer metastasis', Critical Reviews in Oncogenesis, pp. 43–73.
- Shaker, R. A., Samer Hassan Abboud, Hayder Chasib Assad and Najah Had. (2018) 'Enoxaparin attenuates doxorubicin induced cardiotoxicity in rats via interfering with oxidative stress, inflammation and apoptosis', BMC Pharmacology and Toxicology. doi: 10.1186/s40360-017-0184-z.

- Shanehbandi, D. and Habib Zarredar2 (2019) ‘Anticancer Impacts of Terminalia catappa Extract on SW480 Colorectal Neoplasm Cell Line Dariush’. doi: doi.org/10.1007/s12029-019-00349-z.
- Sharma, A., Boise, L. H. and Shanmugam, M. (2019) ‘Cancer metabolism and the evasion of apoptotic cell death’, Cancers.
- Shastri, M. D., Niall Stewart, James Horne, Syed Tabish R. Zaid, Sukhwinder Singh Soha, Gregory M. Peterson, et al. (2015) ‘Non-anticoagulant fractions of enoxaparin suppress inflammatory cytokine release from peripheral blood mononuclear cells of allergic asthmatic individuals’, PLoS ONE. doi: 10.1371/journal.pone.0128803.
- Shirazi, M., Zahra Naeiji, Fatemeh Rahimi Sharbaf, Fateme Golshahi, Marzieh Fathi, Farzaneh Nazari, et al. (2021) ‘Therapeutic role of enoxaparin in intra-uterine growth restriction: A randomized clinical trial’, Journal of Gynecology Obstetrics and Human Reproduction, p. 102070.
- Singh, J. and Goswami, A. (2011) ‘Applications of cell lines as bioreactors and in vitro models’, pp. 4–6.
- Strober, W. (2015) ‘Trypan Blue Exclusion Test of Cell Viability’, Current Protocols in Immunology, p. A3.B.1-A3.B.3.
- Sudan, S. K., Sachin Kumar Deshmukh, Teja Poosarla, Nicolette Paolaungthong Holliday, Donna Lynn Dyess, Ajay Pratap Singh, et al. (2020) ‘Resistin: An inflammatory cytokine with multi-faceted roles in cancer’, Biochimica et Biophysica Acta - Reviews on Cancer, doi: 10.1016/j.bbcan.2020.188419.
- Sun, Z., Zong-lan Hu, Xiao-hong Ning, Jian-feng Zhou, Ya-juan Shao, Jin-hong Duan, Xian-da Yang, et al. (2009) ‘[Inhibitory effect of low molecular weight heparin on the secretion of vascular endothelial growth factor by

tumor cells in vitro].', Zhonghua zhong liu za zhi [Chinese journal of oncology], pp. 826–830.

- Tacar, O., Sriamornsak, P. and Dass, C. R. (2013) 'Doxorubicin : an update on anticancer molecular action ', pp. 157–170. doi: 10.1111/j.2042-7158.2012.01567.x.
- Toolabi, N., Fattane Sam Dalir, Amir Mokhlesi, Mahmood Talkhab . (2021) 'Identification of key regulators associated with colon cancer prognosis and pathogenesis', Journal of Cell Communication and Signaling, pp. 115–127. doi: 10.1007/s12079-021-00612-8.
- Turner, M. D., Belinda Nedjai, Tara Hurst, Daniel J. Pennington . (2014) 'Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease', Biochimica et Biophysica Acta - Molecular Cell Research, pp. 2563–2582.
- Uzun, Y., Akdogan E, Ozdemir F, Ovali E . (2009) 'The effects of heparin on DLD-1 colon cancer cell line', Bratislava Medical Journal, pp. 3–6.
- Vitiello, A. and Ferrara, F. (2021) 'Low Molecular Weight Heparin, Anti-inflammatory/Immunoregulatory and Antiviral Effects, a Short Update', Cardiovascular Drugs and Therapy. doi: 10.1007/s10557-021-07251-6.
- Viazzi, S. et al. (2015) 'Real-time characterisation of the harvesting process for adherent mesenchymal stem cell cultures based on on-line imaging and model-based monitoring', Biosystems Engineering, pp. 104–113. doi: 10.1016/j.biosystemseng.2015.06.006.
- Villers, A. and Grosclaude, P. (2019) 'Epidemiology of Prostate Cancer', Medecine Nucleaire, pp. 2–4. doi: 10.1016/j.mednuc.2007.11.003.
- Walker, J. M. (2009) 'p53 protocols - IN M OLECULAR B IOLOGY TM Series Editor', Life Sciences, p. 588.

- Watson, A. S., Mortensen, M. and Simon, A. K. (2011) 'Autophagy in the pathogenesis of myelodysplastic syndrome and acute myeloid leukemia', Cell Cycle, pp. 1719–1725. doi: 10.4161/cc.10.11.15673.
- Wong, R. S. Y. (2011) 'Apoptosis in cancer: From pathogenesis to treatment', Journal of Experimental and Clinical Cancer Research, pp. 1–14. doi: 10.1186/1756-9966-30-87.
- Xu, X., Lai, Y. and Hua, Z. C. (2019) 'Apoptosis and apoptotic body: Disease message and therapeutic target potentials', Bioscience Reports, pp. 1–17. doi: 10.1042/BSR20180992.
- Yan, Y., Yang Ji, Nan Su, Xiang Mei, Yi Wang, Shanshan Du, et al. (2017) 'Non-anticoagulant effects of low molecular weight heparins in inflammatory disorders: A review', Carbohydrate Polymers, pp. 71–81. doi: 10.1016/j.carbpol.2016.12.037.
- Yang, T., Ji Peng, Zhiquan Shu, Praveen K. Sekar, Songjing Li, and Dayong Gao . (2019) 'Determination of the membrane transport properties of jurkat cells with a microfluidic device', Micromachines, pp. 1–12. doi: 10.3390/mi10120832
- Young, E. (2007) 'The anti-inflammatory effects of heparin and related compounds', Thrombosis Research, pp. 743–752. Watson, A. S., Mortensen, M. and Simon, A. K. (2011) 'Autophagy in the pathogenesis of myelodysplastic syndrome and acute myeloid leukemia', Cell Cycle, pp. 1719–1725. doi: 10.4161/cc.10.11.15673.
- Zaidi, S., Jason Gandhi, Gunjan Joshi, Noel L. Smith, Sardar Ali Khan. (2019) 'The anticancer potential of metformin on prostate cancer', Prostate Cancer and Prostatic Diseases, pp. 351–361.

- Zhou, M., Xinjian Liu,Zonghai Li,Qian Huang,Fang Li,Chuan-Yuan Li et al. (2018) ‘Caspase-3 regulates the migration, invasion and metastasis of colon cancer cells’, International Journal of Cancer, pp. 921–930. doi: 10.1002/ijc.31374.

الخلاصة

يبدأ السرطان عندما تبدأ خلايا الجسم في النمو خارج نطاق السيطرة.و يمكن أن تتحول الخلايا الموجودة في أي جزء من الجسم تقريبًا إلى خلايا سرطانية ، كما يمكن أن تنتشر بعد ذلك إلى مناطق أخرى من الجسم. سرطان القولون هو أكثر أنواع السرطانات شيوعًا على مستوى العالم. وتشمل علاجات سرطان القولون الجراحة والعلاج الكيميائي والعلاج الموجي والعلاج المناعي. اما سرطان البروستات فيعد أكثر أنواع السرطانات شيوعًا بين الرجال ، بعد سرطان الجلد.

في هذه الدراسة ، تم إجراء محاولة لتقييم:

1. تأثير الهيبارين والإينوكسابارين على حيوية الخلايا الطبيعية ، وخط خلايا سرطان القولون SW480وخط خلايا سرطان البروستات. LNCaP
2. الهدف الثاني تقييم تأثير الهيبارين والإينوكسابارين على TNF-α و caspase3 في خط خلايا سرطان القولونSW480 وخلايا سرطان البروستات LNCaP
3. الغرض الثالث تقييم التاثير المضاد للاكسده للهيبارين والإينوكسابارين في خط خلايا سرطان القولون SW480 وخط خلايا سرطان البروستاتLNCaP

تم تنفيذ العمل التجريبي في مختبر الدراسات العليا/فرع الادوية / كلية الطب / جامعة بابل خلال الفترة من كانون الاول2021 الى نيسان 2022

التجربة الأولى هي اختبار السمية : خطوط الخلايا بما في ذلك خط الخلايا الطبيعية ال Vero وخلايا سرطان البروستات (LNCaP) و خلايا سرطان القولون (SW480) .تم زرع خطوط الخلايا في 96 لوحة زراعة الأنسجة.بعد 24 ساعة تم تعريض جميع الخلايا لتراكيز مختلفة من الأدوية (الهيبارين و الإينوكسابارين بتخفيفات متسلسلة تتراوح من 62،125،250،500،1000 إلى 31 ميكروغرام / مل)ولقد تم استخدام أربع مكررات لكل تركيز من الهيبارين و الإينوكسابارين لكل نوع من الخلايا مع اربع مكررات كمجموعة تحكم لكل نوع من الخلايا ، تمت حضانة كل الصفائح لمدة 24 ساعة عند 37 درجة مئوية ، وثم تم إجراء اختبار MTT لقياس السمية الخلوية.

أظهرت النتائج أن الهيبارين يسبب انخفاضًا معنويًا ($P \leq 0.050$) في قابلية بقاء خلايا الVero في جميع التراكيز. بينما يسبب الهيبارين انخفاض معنوي($P \leq 0.050$) في حيوية خلية سرطان القولون SW480 بتركيز (1000,500 ميكروغرام / مل) مع عدم وجود تأثير معنوي على حيوية خط خلايا سرطان البروستاتLNCaP . وكذلك أظهرت النتائج أن الإينوكسابارين يسبب انخفاضًا معنويًا ($P \leq 0.050$) في حيوية خلايا سرطان القولون sw480 بتركيز (500،1000 ميكروغرام / مل) وليس له أي تأثير على حيوية خلايا سرطان البروستاتا Vero و LNCaP.

تضمنت التجربة الثانية: سلالات الخلايا بما في ذلك سرطان البروستات (LNCaP) وسرطان القولون (SW480) حيث تم زرع خطوط الخلايا في 96 لوحة زراعة الأنسجة. وتم تعريض جميع الخلايا الى

تراكيز مختلفة من الأدوية (الهيبارين و الإينوكسابارين) بتخفيفات متسلسلة تتراوح من 1000 إلى 31 ميكروغرام / مل (تم استخدام ثلاثة مكررات لكل تركيز من كل من الهيبارين والإينوكسابارين لكل نوع من الخلايا) جنبًا إلى جنب مع ثلاثة مجموعة تحكم لكل نوع خلية. ثم تم تغطية اللوحة بغطاء بلاستيكي ذاتي وحضنتها لمدة 24 ساعة ، في نهاية فترة التعرض ، تم أخذ خطوط الخلايا للمقايسة بطريقة ELISA Tالقياس NF-alphaو Caspase3

بينت النتائج أن الهيبارين ليس له تأثير معنوي على TNF-α في خلايا سرطان القولون SW480 ويسبب انخفاضًا معنويًا (*P ≤0.050*) في TNF-α عند التركيز (125،250،1000 ميكروغرام / مل) في خط خلايا سرطان البروستاتLNCaP

يسبب enoxaparin انخفاضًا كبيرًا (*P ≤0.050*) في TNF-α عند التركيز (31،250،1000 ميكروغرام / مل) في الخلايا السرطانية SW480colon وليس له تأثير كبير على TNF-α في خط خلايا سرطان البروستات LNCaP اما الهيبارين فليس له تأثير على caspase3 في سرطان القولون SW480 وخلايا سرطان البروستات LNCaP يسبب Enoxaparin زيادة كبيرة

(*P ≤0.050*) في caspase3 في خلايا سرطان القولون SW480 وليس له تأثير كبير على caspase3 في خلايا سرطان البروستاتLNCaP

تضمنت التجربة الثالثة: إجراء طريقة CUPRAC للكشف عن التأثير المضاد للأكسدة للهيبارين والإينوكسابارين على خلايا سرطان القولون SW480 وخط خلايا سرطان البروستاتLNCaP

أظهرت النتائج أن الهيبارين ليس له تأثير مضاد للأكسدة معنوي في خلايا سرطان القولون SW480 ويسبب زيادة معنوية *(P ≤0.050)* في تأثير مضادات الأكسدة عند التركيز (31،250،1000 ميكروغرام / مل) في خط خلايا سرطان البروستات LNCaP .أظهرت النتائج أن الإينوكسابارين ليس له تأثير مضاد للأكسدة معنوي في خلايا سرطان القولون SW480 ويسبب زيادة معنوية *(P ≤0.050)* في تأثير مضادات الأكسدة عند التركيز (250 ميكروغرام / مل) في خلايا سرطان البروستات LNCaP .

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامـــعة بابـل

كليـة الطب

تأثير الاينوكسابارين والهيبارين على موت الخلايا المبرمج ، وعلامات الالتهاب ومضادات الأكسدة في خط الخلايا SW480 وLNCaP

رســالـــــة

مقدمـــة إلـــى مجلس كليـــة الطـــب / جامعـــة بابـــل

كجزء من متطلبـات نيـل درجـة الماجستيـر في الأدوية /السموم

من قبل

نور كريم مزعل عبيد

بكالوريوس صيدلة

2014-2013

اشراف

أ.م. ماجد كاظم عباس
ماجستير ادوية

أ.د قيصر مظلوم
دكتوراه علوم حياة

1444هجري

2022ميلاد