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EFFECT OF LYCOPENE FROM *SOLANUM LYCOERICUM* IN CELL LINE AND CELL DEATH

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EFFECT OF LYCOPENE FROM *SOLANUM LYCOERICUM* IN CELL LINE AND CELL DEATH

By Hadeel Khaleel Rahman RAHMAN

April 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

EFFECT OF LYCOPENE FROM *SOLANUM LYCOERICUM* IN CELL LINE AND CELL DEATH

Hadeel Khaleel Rahman RAHMAN

Master of Science in Biology

Advisor: Asst. Prof. Dr. Songül ŞAHİN

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April 2023

The aim of this study was to evaluate the effect of the lycopene extract from Solanum lycoericum (tomato) on the bacterial and proliferation of lymphocytes and three cancer cell lines (Human breast cancer AMN3, hepG2, and MEF. The first study affects the antimicrobial activity of lycopene extract. The second assay studies effect of lycopene extract on lymphocyte division in human blood. The third assay including the effect of lycopene extract Solanum lycoericum on the growth of tumor cell lines AMN3, HepG2, and MEF was done by using cytotoxic assay. The results show that the extraction lycopene extract revealed cytotoxicity on different cells line, and this effect depends on concentration and cell line types. In gene expression studies, the results showed the downregulation of the genes Hsp60, and Hsp70 in AMN3 and HepG2 cancer cell lines. While the gene expression of apoptosis genes, the Caspase 8 gene was high regulation for all used cancer cell lines. All results showed that the Lycopene extract of Solanum lycoericum (tomato) induced cell death by mitochondrial Intrinsic pathway after 24 hrs of exposure.

2023, 67 pages

Keywords: Cell line , Lycopene , Apoptosis

ÖZET

HÜCRE DİZİSİ VE HÜCRE ÖLÜMÜNDE *SOLANUM LYCOERICUM*'DAN LİKOPENİN ETKİSİ

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Nisan 2023

Bu çalışmanın amacı, Solanum lycoericum'dan (domates) elde edilen likopen ekstraktının, lenfositlerin ve üç kanser hücre hattının (İnsan meme kanseri AMN3, hepG2 ve MEF) bakteriyel ve proliferasyonu üzerindeki etkisini değerlendirmektir. İlk çalışma, likopen ekstraktının antimikrobiyal aktivitesini inceler. İkinci çalışma, likopen ekstraktının insan kanındaki lenfosit bölünmesi üzerindeki etkisini inceler. Likopen ekstraktı Solanum lycoericum'un AMN3, HepG2 ve MEF tümör hücre hatlarının büyümesi üzerindeki etkisini içeren üçüncü deney, sitotoksik analiz kullanılarak yapıldı. Sonuçlar, ekstraksiyon likopen ekstraktının farklı hücre dizilerinde sitotoksisite ortaya koyduğunu ve bu etkinin konsantrasyona ve hücre dizisi tiplerine bağlı olduğunu göstermektedir. Gen ekspresyonu çalışmalarında sonuçlar, AMN3 ve HepG2 kanser hücre hatlarında Hsp60 ve Hsp70 genlerinin aşağı regülasyonunu gösterdi. Apoptosis genlerinin gen ekspresyonu yapılırken, Caspase 8 geni kullanılan tüm kanser hücre hatları için yüksek regülasyon olmuştur. Tüm sonuçlar, Solanum lycoericum'un (domates) Likopen ekstraktının, 24 saat maruz kaldıktan sonra mitokondriyal içsel yolla hücre ölümüne neden olduğunu gösterdi.

2023, 67 sayfa

Anahtar Kelimeler: Hücre dizisi , Likopen , Apoptoz

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Hadeel Khaleel Rahman RAHMAN

Çankırı-2023

CONTENTS

LIST OF SYMBOLS

µg/mL	Microgram per milliliter
mg/mL	Milligram per milliliter
mL	Milliliter
µL	Microliter
µm	Micrometre

LIST OF ABBREVIATIONS

A. baumanni	*Acinetobacter baumanni*
A375	Human melanoma cell line
A549	Adenocarcinoma human alveolar basal epithelial cells
Abbreviation	Meaning
AMJ13	Invasive ductal carcinoma
AO\PI	*Acridine orange\propidium iodide stain*
ATP	Adenosine triphosphate
BAD	Bcl-2 associated agonist of cell death
BAK	Bcl-2 antagonist killer
BAX	Bcl-2 associated X-protein
Bcl-2	B-cell lymphoma 2
BID	*BH3 interacting domain death agonist*
C. albicans	*Candida albicans*
CBDs	Cembranoid-type diterpenes
cdk2	Cyclin-dependent kinase 2
cDNA	Complementary deoxyribonucleic acid
C-myc	Cellular-myelocytomatosis
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
E2F	Expression factor

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1. INTRODUCTION

Cancer is one of the major health problems in the world and is one of the important causes of an increase in deaths among children and adults. According to the WHO, cancer is the second most common cause of death in the world after cardiovascular diseases . as the number of people living with cancer reached 21.2 million people in 2021. Breast cancer is the most commonly diagnosed cancer and is followed by lung cancer, then colorectal cancer, prostate cancer, non-melanoma of skin cancer, and stomach cancer.Although there are many traditional approaches to treating cancer such as surgery, radiation, and pharmacological therapy (including chemotherapy, gene therapy, hormone therapy, and immunotherapy) they didn't achieve the required results and they have many side effects (Zhang *et al.* 2021).

All ancient communities have their own special and customized pharmacognosy and natural medicines specified for their common plants, some of them were difficult to be investigated at all. Investigations of traditional herbal medicines does not provide an over view about the development history of medicines only, it also provides a fascinating insight for our capability in developing a wide range of ivilized habits. The unique role of the natural product in the management of disorders and diseases has been a typical example by their presence almost all departments of disorders management Carotenoides are blue, brawn, and yellow pigmentes present in fruites and vegetables, which possess a wide range of propos biological functions, includ antioxidiant, anticarcinogen, and imuno-protective propierties.the extraction Lycopene, the most abundant *Solanum lycoericum* (tomato) carotenoid, has been the primary focus of both in Vitro and in Vivo study examining the relaitionship between increas intake of *Solanum lycoericum* (tomatoes) and reduced risk of kidney cancer. Numerous epidemiologic studies have shown that higher serum the extraction lycopene concentration is inversely related to prostate cancer risk. (Etminan *et al.* 2004).the extraction Lycopene, A major carotenoid component of *Solanum lycoericum* (tomato) , has a potential anti cancer activity in many types of cancer. Epidemiolog and clinical triales rare provided evidence for mechanismes of the compoundes action, and studies

on its effected on cancer of different cell origins are now being done. The aim of the present studies was to deteirmine the effect of the extraction lycopene on cell cycle and cell viability in three human cancer cell lines In on study was able to be found concerning effect of the extraction lycopene extract on mitotic index and ant microbal and study effect the lycopene may inhibit the growth cell line.and cell death.

1.1 Aims of the Study

- Extraction of active compound *lycopene* from *Solanum lycoericum*.

- Detecting the medical efficacy and the inhibitory effect of lycopene extract on some bacterial and yeast species.

- Detecting the medical efficacy and the inhibitory effect of lycopene extract on some bacterial and yeast species.

- Study extraction lycopene from *Solanum lycoericum* in Mitotic Index in lymphocytes of humans.

- Effect of extraction of lycopene from *Solanum lycoericum* plant on cancer and normal cell line.

- Effect of different concentrations of lycopene *Solanum lycoericum* extracts on gen expression in same gene of gene expression.

- The effect of lycopene from *Solanum lycoericum* extracts in cell death in same gene apoptosis.

2. LITERATURE REVIEW

2.1 Tumors

The tumors is a pathologic disturbances of cell growth, characterized by excessive and abnormal prolifeiration of cell. Tumors are an abnormal mass of tissue that may be fluid or sold -filled. When the growth of tumors cell is confined to the site of origin and are of normal physicality they are concluded as benign(normal) tumors (Sinha 2018).

Cell growth is a highly regulated process. Its control involves balancing positive regulation of anabolism processes with negative regulation of catabolism processes Many transcription factors play a key role in cellular differentiation and the delineation of cell phenotype. These cells are located in areas of the body that are exposed to damage by cell death and are then replaced by another cell by division (Iwata *et al.* 2020)

2.2 Types of Tumors

Can be identified by.

2.2.1 Benign tumors

Our bodies constantly produce new cells to replace old ones. Sometimes, DNA gets damaged in the process, so new cells develop abnormally, and they continue to multiply faster than the immune system can remedy, forming a tumor. A benign tumor is a mass of cells that can't either invade neighboring tissue or spread throughout the body (Metastasis). Benign tumors generally have a slower growth rate than malignant tumors, a benign tumor is not annoying if it is not pressing on nearby tissues, nerves, or blood vessels and causing damage the tumor is not removed completely may result in the compression of abdominal organs, tissues, blood vessels, and nerves, followed by continual tumor growth and possible fatality (Follain *et al.* 2020).

There are many types of benign tumors such as fibroids in the uterus, lipomas in the fatty tissue under the skin, or Hemangiomas in blood vessels and some of these types such as intestinal polyps are considered precancerous and should be removed to prevent them from becoming malignant (Wan *et al.* 2016).

2.2.2 Malignant tumors

Malignant means that the tumor is cancerous. This tumor is the result of changes in DNA, gene transcription, or translation, and this results in defective protein or proteins leading to the transformation of normal cell components into uncontrolled proliferation. Malignant tumors can invade nearby tissues. Some malignant cells can pass into the bloodstream or lymph nodes, and they can grow quickly and spread to other tissues of the body (metastasis) (Sinha 2018). These types of tumors are abnormal growth, and this is due to the possibility of possessing an altered number of chromosomes and the presence of abnormal changes within these chromosomes and their surfaces, and their loss of adhesion to neighboring cells (Vargas-Rondón *et al.* 2018).

The malignant cell is difficult to diagnose due to the occurrence of a variety of malignant tumors containing giant cells. Morphologically, the malignant cell is characterized by a large nucleus, with irregular size and shape, the nucleoli are eminent, the cytoplasm is rare and densely colored or pale, and undergoes changes, new structures appear or normal structures disappear The mitotic division is increased, and defects in the mitotic spindle appear. cancer cells can ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death or apoptosis (Fadaka *et al.* 2017). In cancer, most cells increased glycolysis and use this metabolic pathway for the generation of ATP even in the presence of O2. The possible causes of aerobic glycolysis in cancer have been explained by malfunction of mitochondria, adaptation to a hypoxic environment (Yang *et al.* 2020), oncogenic signals, altered metabolic enzymes, and physiological response to enhanced energy demand for membrane transporter activity required for cell division, growth, and migration. Malignant cells demand oxygen and nutrients to survive and proliferation

and therefore need to blood vessels to oncome the blood circulation system, initiation of tumor angiogenesis is required for tumor progression (Lugano *et al.* 2020).

Tumors can be vascularized either through co-option of the pre-existing vasculature the forming new blood vessels through a variety of molecular and cellular mechanisms It seems that tumors can control inhibitors and inducers of Angiogenesis by Altering Gene Expression (Ratushnyy *et al.* 2020).

2.3 Causes of Cancer Development

Cancer development is a multistep process in which cells acquire a certain number of progressive alterations. Many factors are responsible for the process of initiation and progression of cancer, including external (environmental) factors such as smoking, alcohol consumption, physical inactivity, radiation, chemicals, infection with microorganisms, overweight, and poor diet, and internal factors including inherited mutations, hormones, and immune changes. These factors can induce genetic changes, causing abnormal growth of cells that result in DNA damage or an alteration in the pathways that are responsible for protecting the normal cellular homeostasis and can cause cancer (Rea *et al.* 2018).

2.4 Carcinogenesis

Is a multistep process by which normal cells acquire genetic and epigenetic changes that result in cancer. Is the process whereby cells with a growth advantage over their neighbors are transformed by mutations in the genes that control cell division into cells that no longer respond to regulatory signals. This process can be divided into three distinct stages: initiation, promotion, and the progression (malignant conversion).

2.4.1 Initiation stage

This stage results from a spontaneous mutation in the genes, or mutations resulting from exposure to a carcinogenic agent and lead to dysregulation of biochemical pathways associated with proliferation, differentiation, and survival of cells. The exposed cell to one of the carcinogenic factors, will sufficient from a change in its genetic material, such as the breakdown of one strand of the double helix a, which affects the biological function of the cell and turn it into a mutant cell (Barnes *et al.* 2018).

The Human body naturally has special mechanisms for repairing that repair the genetic error and prevent it from changing into a mutation, but if this error remains uncorrected for a certain period (for five- or six-cell divisions), these cells may become precancerous, so the active immune system continues to destroy these cells, but if this does not happen, then these cells will enter the next stage (O'Grady *et al.* 2015).

2.4.2 Promotion stage

Is a selective clonal expansion of initiated cells into a detectable cell mass that is either benign or preneoplastic. In this stage, the mutated cells are exposed to external factors called promoters to induce a complete neoplastic by a process called. During this phase cancer cells may develop strategies allowing them to avoid recognition by immune cells (immunosurveillance escape mechanisms). The time that the normal cell needed to transform into a mutant cancerous cell and then develop into a noted tumor is called the latency period, and this period varies according to the type of cancer, it may last for a month or even a year (Iglesias *et al.* 2017).

2.4.3 Progression stage

Progression is the most relevant stage of cancers, it begins when malignant cells colonize adjacent sites, and it is characterized by increasing tumor heterogeneity, invasion, immune escape, and dissemination of cancer cells. In this stage, the cancer

cell becomes more virulent by having a property called metastasis, where the cancer cell separates from the primary tumor and breaks down the proteins that make up the extra-cellular matrix (ECM) that separates neighboring cells from each other, and by destroying of these proteins, the cancer cells it can penetrate the ECM and leave the tissue, (Kummerow *et al.* 2015) .

2.5 Relationship of Cancer with Genes

Cancer begins when genes in a cell become abnormal and the cell starts to grow and divide out of control**.** There are several genes involved in the induction and formation of cancer, the most important of these genes are tumor suppressor genes, oncogenes,

2.5.1 Tumor suppressor genes

Tumor suppressor genes (TSG) are normal genes that have a critical role in the genesis of neoplasia. The transcription and translation of these genes, resulting in the production of proteins that regulate the cell cycle, repair any DNA damage, and control apoptosis (When TSGs are mutated and lose their function, the cells lose control and this leads to neoplastic growth and transforms the normal cell into the malignant cell (Joyce and Kasi, 2018). The mutations inactivating tumor suppressor genes are of the recessive type. The most common tumor suppressor gene is P53, which is encoding for protein 53 and is located on chromosome 17p13 (Grenda *et al.* 2022).

2.5.2 Oncogenes

Oncogenes (OGs) are Genes whose expression causes cells to exhibit the properties of cancer cells. Most known oncogenes are derived from mutated versions of normal cellular genes (proto-oncogenes) that control cell proliferation, survival, and (Lyu *et al.* 2020). (OGs) are usually activated by gain-of-function mutations that stimulate cell growth and division (Chen *et al.* 2019).

2.5.3 DNA repair genes

DNA repair genes have a role in maintaining genomic integrity against both sources of DNA damage (exogenous and exogenous). Exogenous factors include ultraviolet radiation, ionizing radiation, or chemotherapeutic agents, and endogenous factors include mistakes in meiosis, reactive oxygen species, or replication errors.

Several mechanisms can activate the DNA repair genes to repair damaged DNA including direct repair, base excision repair (BER), nucleotide excision repair (NER), mismatch excision repair, and save our souls (SOS) (Alhmoud *et al.* 2020).

2.6 Gene Expression

Gene Expression is the process by which information from a gene is used to direct the synthesis of functional gene products with observable phenotype, these products are proteins (hormone, enzyme, or receptor). It is a tightly regulated process that allows the cells to respond to their environmental changes and is regulated at multiple levels Modulation of gene expression is a fundamental property of all living organisms allowing a cell's protein composition to be adjusted in response to a variety of environmental signals or during differentiation processes (Pascual-Ahuir *et al.* 2020).

2.7 Programmed Cell Death (Apoptosis)

Is a highly regulated mechanism that generally occurs during development and aging to keep cells in tissue. It can also act as a protective mechanism when the cells are damaged by toxic agents or by diseases (Kabel *et al.* 2016).

Programmed cell death is begun by the formation of apoptotic bodies that are rapidly engulfed and removed by phagocytes before the contents of the cell exit to the outside. In this way, cell death does not trigger an inflammatory reaction in the body (Battistelli and Falcieri 2020). This is fundamentally different in contrast with necrosis, which is a

form of traumatic cell death that results from acute cellular injury in which the cell suffers from swelling and then degrades, releasing its contents into the interstitial space causing damage to surrounding cells and thus leading to an inflammatory response (Gong *et al.* 2017).

2.7.1 Mitochondria and programmed cell death

Mitochondria are the powerhouses of the cell, and their main role is the production of adenosine triphosphate (ATP) and regulating cellular metabolism. They also act in calcium signaling, stress responses, reactive oxygen species signaling, steroid hormone and heme biosynthesis, cell division, cell differentiation, cell cycle, and cell death. Mitochondria have highly variable shapes, sizes, and positions (El-Hattab *et al.* 2018), and their diameter range from (0.75 to 3 m2. Mitochondria play an important role in the process of programmed cell death through the intrinsic pathway. This pathway is activated by changing the polarity of the mitochondrial membrane, leading to a change in membrane permeability, which is the start of programmed cell death. The initiation of the programmed cell death is under the control of proapoptotic proteins belonging to the B cell lymphoma (BCL) 2 family such as (BAD, and BID) and this lead to forming a membrane-spanning pore that allows the passing of apoptogenic factors (e.g cytochrome-C) to the cytoplasm (Picca *et al.* 2021).

2.7.2 Morphological changes in programmed cell death

Morphological changes of programmed cell death generally happen in the nucleus and cytoplasm. These changes are characterized by rounded cells and shrinkage, positional organelle loss, damaged cytoplasmic contents such as mitochondria, chromatin condensation, and DNA fragmentation. The blebbing of the plasma membrane is followed by these conditions which cause the nucleus to break, naturally associating with no inflammation, and Finally forming apoptotic bodies. After cell shrinking and losing connection with neighboring cells, the apoptotic bodies are engulfed by macrophages or r neighboring cells (Banerjee *et al.* 2016).

2.7.3 Biological changes in programmed cell death

Biological changes of programmed cell death can be observed through the activation of caspases, protein cleavage, and alteration of membrane permeability The overexpression of phosphatidylserine (PS) on the outer plasma membrane is considered a unique feature of programmed cell death The other feature of programmed cell death involved activating cysteine proteases enzymes called (Caspases) with specificity for aspartic acid residues in their substrates. Caspases of mammalian can divide into three functional groups (Zamaraev *et al.* 2017).

2.8 Heat Shock Proteins and Cancer

Heat shock proteins (Hsps) are a large family of molecular chaperones that are known for their roles in protein maturation, refolding, and degradation. These proteins are induced to repair damages occurring in other proteins, thereby preserving the integrity of the cell and giving stress tolerance against oxidative stress, hyper and hypothermia, hyper and hypoxia, anoxia, and a variety of other aggressive agents, (Moura *et al.* 2018). Hsps also play a key role in a variety of cancer-related activities such as cell proliferation, metastasis, and anti-cancer drug resistance. The interaction of the heat-shock factor (HSF1) with heat-shock elements (HSEs) with promoter regions of the heat-shock protein (HSP) gene leads to triggers the transcription of HSP genes (Zatsepina *et al.* 2021).

HSPs are classified into many types according to their molecular weights such as Hsp27, Hsp40, Hsp60, Hsp70, Hsp90, and Hsp100 . These proteins keep the cells viable under hypoxia, altered pH, oxidative stress, DNA damage response, and many other stress conditions). Hsp70 also has a role in promoting the growth of cancer cells by refolding denatured proteins and inhibiting protein aggregation. Overexpression of Hsp70 also leads to the development of cancers (Conway de Macario *et al*. 2020).

2.9 Treatment of Cancer

Since the discovery of cancer, the main goal of the research has been to discover new methods of good treatment. More than 60% of ongoing trials of high-quality medical treatment worldwide focus on cancer. The choice of treatment and its progress depends on the type of cancer, its locality, and stage of progression. In addition, the development in the field of cancer treatments, especially non-traditional methods, may open great prospects for a full recovery from this disease, including gene therapy and treatment with plant secondary metabolites (Theuretzbacher *et al.* 2020). Therapeutic modalities are divided into.

2.9.1 Surgical treatment

Surgery is one of the most effective treatments to remove tumors, especially small tumors, which can be easily eliminated and prevent their growth again. This process requires the removal of all cancer cells, and if not it will spread to other sites and tissues. chemotherapy or radiotherapy is often performed after surgery to kill potentially surviving cancer cells. Surgical treatment has a negative effect on the quality of life and a recovery period and sometimes there is difficulty in removing the tumor (Metcalfe *et al.* 2016).

2.9.2 Chemotherapy

Chemotherapy is one of the most clinically prevailing therapies and involves using chemotherapeutic agents that have played a key role in reducing mortality and morbidity rates and in increasing patients' quality of life. chemotherapy treatments cause DNA damage by forming acentric fragments of chromosomes resulting in the breaking of chromosomes (Alhmoud *et al.* 2020), or stopping the cell cycle in the metaphase of mitosis and target not only dividing cancer but also dividing normal cells.

2.9.3 Radiotherapy

Radiotherapy involves delivering a lethal dose of ionization radiation such as x-rays, gamma rays, and particulate radiation beams to a defined tumor volume. Radiation will damage the DNA, and cancer cells will die especially when they attempt to divide in mitosis with reducing the impact of damage on the surrounding healthy tissue. DNA damage includes abasic lesions, deoxyribose ring-opening, single-stranded and double-stranded breaks (Chen and Kuo 2017).

2.9.4 Immunotherapy

Immunotherapy is a type of treatment that enhances the ability of the body's immune system to attack cancer cells. Immunotherapies include various Strategies to activate immune cells against cancer including vaccination with tumor antigens or raising of antigen presentation to increase the ability of the patient's immune response against cancer cells (Poorebrahim *et al.* 2020). The immune response is either active immunity using vaccines prepared by using patient-derived tumor cells and this response is non-specific or Passive immunity by administration of agents such as Abs or cytokines that enhance anti-tumor response.

2.9.5 Gene therapy

Gene therapy is a therapeutic method used to correct or alleviate the symptoms of the disease by replacing a disfigured gene with a healthy one or inducing cell death. Gene therapy is the introduction of exogenous nucleic acids, such as gene segments, genes, oligonucleotides, siRNAs, or miRNAs into cells expression modulation of a target gene, or synthesis of an exogenous protein.Delivery of these exogenous nucleic acids to malignant cells allowed to avoid cancer by restoring the expression of TSGs or silencing oncogenes. Gene therapy can inhibit telomerase in tumor cells and lead to telomere shortening which leads to the aging of cancer cells and apoptosis (Quazi 2021).

2.10 *Solanum Lycoericum L.* Tomato

Can be identified by.

2.10.1 Classification of *Solanum lycoericum L.*

Table 2.1 Classification of (*solanum lycoericum L.* (Tomato) (Nunes *et al.* 2014)

Kingdom	**Plantae**
Phylum	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	*Solanum*
Species	*Solanum lycopericum*
Common name	Tomato

2.10.2 Description of *tomato*

The tomato is the edible berry of the plant *Solanum lycopersicum*, commonly known as the tomato plant. The speciees originated in western South America, Mexico, and Central America The Mexio Nahuatl word *tomatl* gave rise to the Spanish word *tomate*, from which the English word *tomato* derived. Its domestication and use as a cultivated food may have originated with the indigenous peoples of Mexico tomato, (*Solanum lycopersicum*), flowering plant of the nightshade family (Solanaceae), cultivated extensively for its edible fruits. Labell as a vegetables for nutroitional purposes, *Solanum lycopersicum* (tomatoes)are a good source of vitamin C and the phytochemical the lycopene. The fruits are commonly eaten raw in salads, served as a cooked vegetable, used as an ingredient of various prepared dishes, and pickled. Additionally, a larges percentage of the world the (tomato) crop is used for processing; products include canned *(Solanum lycopersicum*) tomatoes, tomato juice, ketchup, puree, paste, and "sun-dried" tomatoees or dehydrated pulp.

Physical description and cultivations (Tomato) plant are generally much branch, spreading 80–185 cm (25–75 inches) and somewhat trailing when fruiting, but a few forms are compact and upright. Leaves are more or less hairy, strongly odorous, pinnately compound, and up to 45 cm (20 inches) long. The four -petaled flowers are yellow, 2 cm (0.8 inch) across, pendant, and clustered. Fruites are berries that vary in diameters froms 1.8 to 9.5 cm (0.8 to 4 inches) or more. (Stacewicz-Sapuntzakis *et al.* 2005).

2.10.3 Chemical composition of *Solanum lycopericum* tomato

Solanum lycopericum (Tomato L.) is containing a diversity of biologically active secondary metabolites, and relative research showed that the *Solanum lycopericum* plant was rich in carotenoids especially lycopenen, they have 22mg per cup from lycopene and waterrmelon have 13 mg per cup (Shang *et al.* 2019). The high content of linoleic acid in tomato seed oil is very important for some chemical production. *Solanum lycopericum* is one of the richest sources of carotenoids such as β-carotene, which have the anticancer effect derived from its pro-oxidant activity, anti-oxidant action, regulation of cell differentiation, or cell cycle arrest. The study proved the *Solanum lycopericum* friut contain many chemical compounds such as terpene and phenylpropanoid volatiles, various classes of phenols, alkaloids, and other compounds, all of these compounds have antibacterial and ant tumor activities Figure 2.1.

Figure 2.1 *Solanum lycopericum*

2.10.4 Medical importance of lycopene

Lycopene is a pigment belonging to the group of carotenoids and it is among the most carefully studied antioxidants found especially in fruit and vegetables. As a carotenoid, lycopene exerts beneficial effects on human health by protecting lipids, proteins, and DNA from damage by oxidation. Lycopene is a powerful oxygen inactivater in the singlet state Lycopene has anti proliferative effecte on kidny and Hert new tumor cell. In Hert new model , few expression of regulatory proteins cell cycle, such as cyclins D 1 and Ei and the cyclin-dependeent kinasees 3 and 5, as wells as suppression of insulin-like Growth factore (IGF-I) action have been correlat with lycopene's effected on proliferation Other studies reported that lycopene had limite effected on cell proliferation of canceroues and noncanceroues cell lines in an in vitro system with doses with in the physiological range, but only rare studi reporte no effected of lycopene on cell proliferation (Giovannucci,*et al.*1995).Deregulated cell srounded is one of the major sample of tumer cells. These celles may lose the ability to regulate the cell srounded and control their rate of proliferation. A rate-limiting stepe in the cell srounded that is often disturbtion in cancer is the progression of cells through the first gap (G1) phase .Recently, several reportes show that lycopene can induced cell cycle stop at the G1 phase reported that the growth of the human hepatoma cells line (Hep4B) was inhibited 25–50% by extraction lycopene at physiological concentrations as low as 0.5 μM. Lycopene was found to induce G0/G1 cell cycle arrest and S phase block. In a similaris study with the human kidny cancer cell lines HepG2 and CSMF7 (Hadley *et al.* 2002).

Although, the roled of extraction lycopene in prevention of prostate cancer cell has been studied more extensively, human studies with tomatoes and extraction lycopene on tumer affecting other organs are now being unidertaken. The majority of studies on cycle cell were carried out in kidny and Hert new cancer cell lines after 48 h of lycopene treatment. The aim of the present study was to determine the effected of extraxtion lycopene on cyle cell and cell prolifeiration in nine different human cancer cell lines at different time points (Hantz *et al.* 2005).

The extraction Lycopene is common found in *Solanum lycoericum*(tomato) products. Due to its lipoiphilic characters, the interactiones between extraction lycopene and fates enhance its bioavailability Therefore, cooiking processes using oiles in the preparation of *Solanum lycoericum* (tomato) sauces and pasted are very importants. In most foods sources,the extraction lycopene exists predominantly in the all-trans conformiation. In contrasts, the cis-isomers is thought to provide betters bioavailability and might be more easily absorbHowever, the extraction lycopene solubilization in warms water collapses of the cell walles; this weakenes the connection between the extraction lycopene and the tissue matrix. As a result, the extraction lycopene is more accessible and isomerization from the all-trans to the cis conformation is increased Previoues studies have suggested that the bioavailability of the extraction lycopene is more substantial in tomato paste than fresh *Solanum lycoericum* tomatoes (Renju *et al.* 2014).

3. MATERIALS AND METHODS

3.1 Materials

Can be identified by.

3.1.1 Equipment and apparatuses

The Equipment and Apparatuses used in this research are shown in the Table 3.1 below:

Table 3.1 Equipment and apparatuses

Equipment and Apparatuses	Manufacture company	Origin
ELISA	Quik Fit	Germany
Electrophoresis	Advance	Japan
Nucleic Acid Extractor (Anatolia gene works)	Anatolia gene works magnesia 16	Turkey
Micro Centrifuge	Hermal	Germany
Cooling Centrifuge	Hettich	Germany
Nano drop Spectrophotometer	Thermo Scientific	U.S.A
Conventional Polymerase Chain Reaction	Agilent MX3005P	Germany
Real-Time Polymerase Chain Reaction	Agilent MX3005P	Germany
Distiller	Kottermann	Germany
Incubator	Memert	Germany
CO_2 Incubator	Gallenkamp	England
Shaker Incubator	Selecta	Spain
Oven	Memaret	Germany
Laminar Flow Hood	K & K	Korea
Digital Microscope Camera	Lamin	U.S.A
Rotary Evaporator	Memmert	Germany
Micropipette	Volac	England
Deep Freezer	The Electron oporation	U.S.A
Inverted Microscope	Oltmpus	Japan
Vortex	Hermal	Germany
Florescent Microscope	Lumin	U.S.A
Magnetic Stirrer	K & K	Korea
PH-Meter	Radiometer	Denmark
Autoclave	Gallenkamp	U.K
Sensitive Balance	Sartorius	Germany
Filter unit 0.22 μm	Microlab Scientific	China

3.1.2 Biological and chemical materials

The biological and chemical materials used in this research are shown in the Table 3.2 below:

Table 3.2 Biological and chemical materials

Materials	Manufacture company	Origin
Agarose	Sigma	U.S.A
Culture Media (RPMI-1640)	Chemical Point	Germany
Phytohaemagglutinin (PHA)	Medicago	Sweden
Di-Sodium Hydrogen-O-Phosphate (Na_2HPO_4)	Quali Kems	India
Mono-potassium Phosphate (KH_2PO_4)	GCC	UK
Potassium chloride (KCl)	Alpha Chemika	India
Fetal Bovine Serum (FBS)	Avonchem	UK
Giemsa stain	Chemical Point	Germany
Ethanol	Sigma	U.S.A
Colchicine	Avonchem	UK
Trypsin\Versine	Sigma	U.S.A
Crystal violate	BDH	England
Trypan blue stain	BDH	England

3.1.3 Ready-made diagnostic kits

The ready-made diagnostic kits that used in this research are shown in the Table 3.3 below:

Table 3.3 Ready-made diagnostic kits

Diagnostic Kits	Manufacture company	Index No.
Acridine Orange \ Propidium Iodide staining (AO\PI) kit	US biological (U.S.A)	LGBD10012
ExCellentCT Lysis Kit	Abm (Canada)	G915
First Chain cDNA Synthesis Kit	TonkBio (U.S.A)	TB30001B
Kapa Express ladder	Kapa (U.S.A)	KK6304
Magnesia Genomic DNA Large Volume Kit	Anatolia (Turkey)	AE1051

3.1.4 Gene expression primers

The primers were used to investigate gene expression during this stuidy, where four genes closely related to induction programmed cell death were selected (caspase 8, Caspase9) in HepG2, AMN3, and MEF Cell lines. And three genes related to the phenomenon of resistance of cells to cellular stress conditions were selected, which are the genes encoding the heat shock proteins Hsp60, and Hsp70 in HePG2 and AMN3 cancer cell lines, In addition to the Glyceraldehyde-3-phosphate housekeeping initiator. The primers were designed through this study based on the information available on the Gene bank of Biotechnology Information (NCBI) website https://www.ncbi.nlm.nih.gov. The sequence of nitrogenous bases for the mRNA deposited at the NCBI website was used for each of the studied genes, and the sequence of the primers was determined using the ApE-plasmid program. The accuracy of the primers' work and their specificity towards the specific genes was confirmed by using the Primer-BLAST program as well as the in silico PCR tool program, in addition to having one peak in the Disassociation curve stage when conducting Real-Time Quantitative PCR tests. Table 3.4 shows the sequence of nitrogen bases in all the primers that were designed.

Table 3.4 Primers used in the study of gene expression

Human Primers	Forward\ Reverse	5´……...3´ sequence
HSP60	Forward	CCG TCC CAA GCA ATG GAT G
	Reverse	GAA GAT GAC AGG GGC CAG
HSP70	Forward	CCT CTC CCC ATC TTC AGA TCA
	Reverse	TCA AGT CAA GGT CAC AGT GAG
Caspase 8	Forward	CTC TTG AGC AGT GGC TGG TC
	Reverse	GCT GAT CTA TGA GCG ATA CT
Caspase 9	Forward	CAA GTG GAC CAG GAG GAA CC
	Reverse	TTC TCG ATT GGC AGG TCC AC

3.2 Lycopene Extract from *Solanum Lycoericum* (Tomato) Preparation

Solanum lycoericum **(**Tomato) -based food products from Iraq products were purchased in the local supermarket (Baghdad). Lycopene extracts The extraction method was

modified from our original method, which was developed and used for tomato cell exttraction Lycopene was extracted from tomato cells by placement of 5 g of cells and 5–15 mL of ethannol with 0.1% butylated hydiroxytoluene into a 40 mL centrifuge tube. Samples were thoroughly mixed on a vortex at level 8 homoggenized at level 10 for 0–120 s and then saponified by 0–2 mL of saturated KOH solution immersed in a 60 °C water bath. Subsequently, 5 mL of deionized water and 5–9 mL of hexane were added, and samples were mixed at vortex level 10 for 15–75 s and then centrifuged for 15 min at 5 °C The heexane phase was reemoved and retained. The process of hexane addition, mixing, and centrifugation was repeated three times. extracts were pooled and dried in a evaporator flushed with argon, and stored in a −20 °C freezer less than 24 h (Campbell *et al.* 2006).

3.3 Studying Effect of Lycopene Extract on Some Bacterial and Yeasts Species

Can be identified by.

3.3.1 Specimens of bacteria and yeasts

The isolates that were resistant to the following antibiotics:

- Antibacterial antibiotics include Vancomycin, Cephalosporine, Clindamycin, Erythromycin, and Fucidine.

- Antifungal antibiotics include Terbinafine, Fluconazole, Nystatine, Itraconazole.

3.3.2 Preparation of cultural media

The culture media used in this study was made according to the manufacturer's instructions, with the pH set to 7, then sterilized with an autoclave at 121°C 15 pound/inch2 for 15 min, and then incubated for 24 h at 37°C before being stored at 4°C until use (Saleem 2016).

3.3.3 Muller hinton agar

The medium was prepared according to the manufacturer's instructions (Hi-media), autoclave sterilized, cooled, placed into a disposable sterile petri dish, and stored at 4ºC until used (MacFaddin 2000).

3.3.4 Determination of the antibacterial and antifungal activity of plant extract

The agar-well diffusion method used to test the antibacterial activity is as follows:

- The surface of Muller Hinton Agar medium was inoculated with a sterilized swab of the bacterial and fungal culture containing (1.5X108) cells/mL in comparison with a Densichek solution and then the plates were left to dry at room temperature.

- Two holes with a diameter of 5 mm were made by using a sterilized Cork Borer in the middle of the cultured plates.

- After the sterilization of the extract by using a Millipore Filter unit with a diameter of 0.22 µm according to (Shneider and Ermel 1986), 0.1 mL (100 microliters) of the extract was added to each hole by using a micropipette and a control hole was made by adding sterile distilled water

- To allow the extract to diffuse through the medium, the plates were kept in the refrigerator at 4°C for a half-hour, as described by then the plates were incubated at 37ºC for 24 hours.

- The efficacy of the extract was determined by measuring the diameter of the inhibition zone around each hole.

3.4 Concentration Lycopene Used for Cytotoxicity Assay

A standard concentration of 100 mg/mL was prepared. Then the concentrations used for the cytotoxic assay were prepared from the standard concentration. Serum-free RPMI-1640 medium was used to make relative dilutions, and five concentrations of 25, 50, 100, 200, and 400 µg/mL were prepared for use in toxicity tests.

3.4.1 Solution used for cytotoxicity testing on lymphocytes

The solutions were prepared according to (Yaseen 1999) in the following Table 3.5:

Table 3.5 Solution used for cytotoxicity testing on lymphocytes

Solution	Preparation Method
Complete Growth Media	RPMI-1640 medium was used with the addition of calf fetal serum. The pH was adjusted to 7.2, then the medium was sterilized using 0.22 µm membrane filters. The medium was incubated at 37 °C for three days to ensure that it was free of contaminants, then poured into sterile tubes of 5 mL per tube, and kept at -20 °C.
Fetal Calf Serum	was prepared by the Iraqi Biotechnology Company
Phytohaemagglutinin (PHA)	The PHA was prepared simultaneously by dissolving 2.5 mg of PHA in 4 mL of sterile water, taking 0.1 mL of it and adding it to 0.6 mL of sterile water, then taking 0.1 mL of it to be added to each culture tube containing 5 mL of culture medium so that the final concentration reached 15 µg /mL and store at -20°C.
Colchicine	Dissolve 0.5 mg of colchicine powder in 10 mL of distilled water, taking care to prepare it immediately upon use and keep it away from light.
Fixative Solution	Prepared by simultaneous mixing of absolute methanol with glacial acetic acid in a ratio of 1:3 v/v and used cold.
Giemsa Stain	Dissolve 2 g of Giemsa stain powder in 100 mL of absolute methyl alcohol using a magnetic stirrer for 72 hours, then filter the dye using Whattman No.1 filter paper. Keep in an airtight, opaque vial. This solution is stored in stock, and when used, dilute immediately by mixing 1 mL of the dye with 4 mL of Sorensen buffer.

3.4.2 Blood sample collection

Human venous blood was drawn from apparently healthy persons aged 25-50 years by using a five mL syringe pre-washed with heparin to prevent blood clotting, and the simultaneously collected blood was used.

3.4.3 Slides preparation

The glass slides were cleaned by immersion in chromic acid for 72 hours and washed with hot and then cold water and kept in the refrigerator until use.

3.5 Study the Effect of Lycopene in Mitotic Index in Lymphocytes of Humans

The effect of the alkaloid extract at different concentrations on the mitotic index of lymphocytes was studied by using a short-term blood culture, based on the method of (Verma and Babu 1995)

3.5.1 Culture of blood with lycopene extract

The extract was added at concentrations of 25, 50, 100, 200, and 400 μg/mL to the prepared culture tubes containing the complete RPMI-1640 culture medium, and the final volume of the mixture should be 5 mL, and three replicates for each concentration. Then 0.5 mL of blood was added to each tube using a 5 mL syringe, then 0.1 mL of the lymphocyte-cleaving agent (PHA) was added and mixed with the medium gently, and then incubated at 37 °C on a tilted form for 24 hours, and the tubes should be mixed every 12 hours. Then a set of tubes was left without any extract added and this treatment was considered a control.

3.5.2 Cells harvest

0.1 mL of colchicine was added at a concentration of 10μg/mL to each control tube before 150 minutes of the end of the original culture time. While no colchicine was added to the treated tubes, and then all tubes were returned to the incubator. Then all tubes were placed in the centrifuge for 10 minutes at a speed of 1500 rpm, the filtrate was discarded and the precipitate was shaken well with the remnants of the culture medium. Added 5-10 mL of 0.075 M hypotonic solution warmed at 37 °C to each tube gradually and gently with shaking. The tubes were incubated in a water bath at 37°C for

30 minutes. Then the tubes are centrifuged at 1500 rpm for 10 min, and the filtrate was discarded.

3.5.3 Fixation

The precipitate was shaken well and a few drops of cold Fixative were added on the tube wall with continuous shaking and the volume was completed to 5 mL. The samples were mixed by the Vortex and then the tubes were placed at 4°C for 30 minutes.

3.5.4 Washing

The tubes were placed in a centrifuge for 10 min. at a speed of 1500 rpm, the filtrate was discarded and the precipitated cells were left. The fixation process was repeated until the clear color of the suspension appeared. The precipitate was suspended by 1 mL of the fixative and kept at -20°C.

3.5.5 Dropping

Clean, cool, wet, and fat-free glass slides were prepared, the cells were mixed well and dropped on the cold slides by a Pasteur pipette from a distance of 0.5-1m and allowed to dry.

3.5.6 Staining and microscope examination

Slides were stained with prepared Giemsa stain diluted with warm Sorensen buffer solution prepared at a ratio of 4:1 for 2-3 minutes, then washed with Sorensen buffer, allowed to dry, and then examined by light microscopy to calculate the Mitotic Index (MI) according to (King *et al.* 1982) method. As the cells in the metaphase were divided by the total number of cells examined (1000), as in the following Equation (3.1):

$$Mitotic\ Index\ (MI) = \frac{The\ number\ of\ dividing\ cells}{The\ total\ number\ of\ cells\ is\ 1000} \times 100 \quad (3.1)$$

3.6 Solutions Used for Tissue Culture

The solutions were prepared according to the method (Giuliano 2007) as shown in the following Table 3.6:

Table 3.6 Preparation solutions used for tissue culture

Solution	Preparation Method
Sodium Bicarbonate	The solution was prepared by dissolving 4.4 g of sodium bicarbonate monohydrogen in 100 mL of distilled water, then sterilizing the solution with an oxidizer at a temperature of 121 °C for 15 minutes, then keeping at 4 °C until use.
Culture Media RPMI-1640	The medium was prepared by mixing 10.4 g of RPMI-1640 with 0.5 mg/L of Penicillin and Streptomycin with 100 mL of fetal calf serum. The components of the medium were mixed and 4.4% of the sodium bicarbonate solution was added and completed the volume to 900 mL by distilled water. Then the pH was adjusted to7.2, then complete the volume to 1 liter with distilled water. The medium was sterilized with a 0.22 μm filter unit and incubated at 37 °C for 72 hours to ensure that it was free of contaminants.
Trypsin–Versin solution	This solution was prepared by dissolving 1 gm of trypsin/versin powder in 100 mL of distilled water, adding sodium hydroxide to it to adjust the pH to 7, then sterilizing it with a 0.22 μm filter unit and refrigerating until use.
Crystal Violet Stain	Dissolve 5 g of stain in 200 mL of absolute methanol and filter with Whattman No. 1, then add to the filtrate 50 mL of 37% formaldehyde AND complete the volume with distilled water to 1 liter.
Trypan blue stain	Dissolve 1 gm. of stain in 100 mL of PBS, then filter using Whattman Paper No. 1 and store at 4°C until use.

3.7 Cancer Cell Lines

All cancer cell lines were prepared by the Iraqi Center for Cancer and Medical Genetics Research from Experimental Therapy Department/Cell Bank Unit.

3.7.1 Human hepatic cancer cell line (HepG2)

Received this line at pass No. 64, sample was taken from cervical cancer cells of a 31-years- old of African American woman. This line was grown in RPMI-1640 medium equipped with 10% calf fetal serum. The cells were treated with trypsin/viresine solution when the complete subculture layer was formed.

3.7.2 AMN3 cell line

Received this line at pass No. 51, the sample was taken from the breast cancer of a 69-year-old woman. This line was grown in RPMI-1640 medium equipped with 10% calf fetal serum. The cells were treated with enzyme trypsin/viresine solution when the complete subculture layer was formed.

3.7.3 MEF cell line

Received this line at pass No. 53, the sample was taken from a pelvic RMS of a 7-years-old female. This line was grown in RPMI-1640 medium equipped with 10% calf fetal serum. The cells were treated with trypsin/viresine solution when the complete subculture layer was formed.

3.8 Growth of AMN3 and HepG2 Cancer Cell Lines, and MEF Normal Cell Line

The method of (Freshney 2021) was used to grow cancerous line cells as follows:

1- Cells of each of the lines were placed in a culture container with a diameter of 25 cm^2 containing RBMI-1640 culture medium and 10% calf B serum.

2- The containers containing the cell suspension and culture medium were incubated in a 5% CO2 incubator at 37°C for 24 hours.

3- After a day of incubation, and when it was confirmed that there was growth in the cell culture and that it was free of contamination, secondary cultures were conducted for it.

4- The cells were examined using an inverted microscope to ensure their viability, freedom from contamination, and their growth to the required number of approximately 500 to 800 thousand cells/mL.

5- The cells were transferred to the growth booth and the used culture medium was disposed of.

6- The cells were washed with PBS solution and then discarded, and the process was repeated twice for 10 minutes each time.

7- A sufficient amount of trypsin/veresin enzyme was added to the cells and incubated for 30-60 seconds at 37°C and monitored until they changed from a monolayer of cells to single cells, then the enzyme was stopped

8- The cells were collected in centrifugal tubes and placed in a centrifuge at 2000 rpm/min for 10 minutes at room temperature, to precipitate the cells and get rid of the trypsin and the used culture medium.

9- Examine the number of cells by taking a specific volume of the cell suspension and adding to it the same volume of Trypan Blue stain to determine the number of cells and their vitality by using a Hemacytometer slide, according to the Equation (3.2):

$$C = N \times 10^4 \times \frac{F}{mL} \qquad (3.2)$$

Since:- C = number of cells in one mL of solution

N = number of cells in the slide F = dilution factor

10^4 = Slide Dimensions

10- The percentage of cell vitality in the sample was also calculated by using a Hemacytometer slide also according to the Equation (3.3):

$$Live\ cell\ viability\ = \frac{live\ cells}{dead\ cells} \times 100 \quad (3.3)$$

11- The cell suspension was distributed in new containers and then incubated in a 5% CO2 incubator at 37°C for 24 hours.

3.9 Cytotoxic of Lycopene Extracted from *Solanum lycoericum* on Cancer Cell Lines

Five concentrations of Lycopene extract 25, 50, 100, 200, and 400 µg/mL were used and sterilized by using a filter unit with a diameter of 0.22 µm under sterile conditions. All prepared concentrations were used immediately after completing the preparation process.

1- Prepare the cell suspension by treating the contents of a 25 cm² tissue culture container with trypsin/versine solution after emptying the old culture medium and gently moving the bottle, then incubated in the incubator at a temperature of 37 °C for 10 minutes, then 20 mL of the culture medium containing serum was added to it. The cell suspensions were mixed well and 0.2 mL was transferred to each hole of the flat-bottomed plate for tissue culture by using an automatic fine pipette.

2- The plate was left in the incubator at a temperature of 37 °C for 24 hours until the cells adhere to the hole, after which the old culture medium was disposed of in the holes and 0.2 mL of the previously prepared concentrations of the extract was added with three replicates for each concentration, in addition to that three replicates were made For control (cell suspension only) plates were incubated at 37°C.

3- After 24 hours of exposure time, remove the plate from the incubator and add crystal violet stain solution to the all holes that containing the cells at a rate of 100 µL

4- The plate was returned to the incubator for 20 minutes, after which it was taken out, and its contents were removed and the cells were washed with water until the excess stain is removed, as the living cells take the stain while the dead ones do not.

5- The results were read by using the ELISA with a wavelength of 492 nm.

The inhibiting ratio was calculated according to the Equation (3.4):

$$Percentage\ of\ cell\ inhibition\ = \frac{absorbance\ reading\ of\ control\ cells - absorbance\ reading\ of\ treated\ cells\ for\ each\ concentration}{absorbance\ reading\ of\ control\ cells} \times 100 \quad (3.4)$$

6- Determination of IC50 for each cancer line after an exposure period of 24 hours (Freshney, 2021)

3.10 Real-Time Polymerase Chain Reaction

Gene expression measurements were carried out according to the following stages

3.10.1 Cell seeding stage

Two culture bottles with a size of 25 cm² were used to implant the cancer cells for each cancer cell line AMN3, HepG2, and MEF at a rate of one million cells per container. The cells were incubated for 24 hours at 37°C until the adhesion and proliferation of cells in each container and the formation of a monolayer.

3.10.2 Exposure stage

After monolayer formation, cells were exposed to nicotine alkaloid extract at IC50 concentration for each cell line for 24 hours, and one culture bottle for each cell line was left untreated and returned as a positive control.

3.10.3 Harvesting cells stage

The cells were harvested by using a sterile scraper without removing the culture medium, collected in sterile test tubes, and transferred to a centrifuge for cell sedimentation, then the culture medium was discarded, the precipitate was washed with PBS solution, then the cells were suspended in 50 µL of cooled PBS solution and kept at -80 °C.

3.10.4 RNA extraction from cancer cell lines (AMN3 ,HepG2, and MEF)

Total RNA was extracted from untreated cells (control) and treated with lycopene using Excellent CT Lysis kit This kite contains a solution to analyze cells, remove DNA, and proteins. The resulting RNA can be used directly for the manufacture of complementary DNA and in Polymerase chain reaction experiments.

Components of the RNA extraction diagnostic kit Table 3.7.

Table 3.7 Components of the diagnostic kit for RNA extraction

Components	Volume
Decomposition Solution	1.25*2mL
Stop Solution	300 µL
Protease Enzyme	50 µL
Protease Inhibitor	50 µL

Procedure:

1- 1µL of Protease enzyme was added to every 10-100000 cells to get rid of proteins and mixed with 50 µL of the decomposition solution which is acts on the cell wall and DNA and incubated for 10 minutes at 37 °C.

2. After the incubation period was over, 1µL of protease inhibitor solution was added to the mixture to stop the action of the protease enzyme.

3. The decomposition was then completed by adding 5 µL of stop solution and the mixture was incubated at room temperature for 2 minutes and transferred to a deep freezer.

3.11 Reverse Transcription (Convert RNA to Complementary DNA (cDNA))

Reverse transcription (cDNA synthesis) was performed by using PCR technology by using the First Chain cDNA Synthesis Kit from TonkBio.

3.11.1 Components of the diagnostic kit for reverse transcription

Diagnostic tools for reverse transcription are listed in Table 3.8.

Table 3.8 Components of the diagnostic kit for Reverse transcription

Components	Volume
TonkBio™ M-MLV Solution	120 µL
OligodT Primer	120 µL
Reaction Buffer Solution * 5	500 µL
dNTP Mixture (10 µM)	240 µL
RNase Inhibitor Solution	60 µL
RNase-Free Water	1*2 mL

Principle:

This kit is used to effectively synthesize cDNA from the first strand of RNA. This kit uses M-MLV reverse transcriptase with lower RNase H activity, which avoids RNA-DNA hybrid from fragmentation in the progression of cDNA synthesis.

Procedure: Mix the ingredients in a centrifuge and put them on ice.

1- The following ingredients are added in a sterile Nuclease-Free Tube on Ice:

Components of this step, as shown in Table 3.9.

Table 3.9 Ingredient of this step

ingredients	volume
RNA / mRNA	1 µL
OligodT Primer	1 µL
RNase-Free Water	10.5 mL

2- Incubated at 65°C for 5 minutes, then cooled on ice.

3- After that, the following ingredient are added:

Added ingredients, Table 3.10.

Table 3.10 Ingredient added in this step

ingredients	Volume
Reaction Buffer Solution * 5	4 µL
RNase Inhibitor Solution	0.5 µL
dNTP Mixture (10 µM)	2 µL
TonkBio™ M-MLV Solution	1µL

4- Then, it was incubated at 42°C for an hour.

5- Finish the reaction by heating to a temperature of 70°C for 5 minutes.

3.12 Measuring the Concentration and Purity of cDNA

Complementary DNA concentration and purity were measured by using a Nano drop spectrophotometer, which can detect the concentration of cDNA in ng/microliter units and its purity by measuring the optical density ratio (OD) at a wavelength of 260/280 nm according to the absorption wavelength of DNA and protein. The acceptable purity of cDNA samples is 1.8 (Sambrook and Russel 2001). The concentrations of cDNA in the samples used were equalized according to the sample with the lowest concentration using the following Equation (3.5):

$$V_n = \frac{C_\circ X V_\circ}{C_n} \quad (3.5)$$

Since:

V_n : The volume to be taken from the current sample and supplemented to

100 µL by adding sterile water.

$V_\circ$: Standard volume (100 µL).

$C_\circ$: Low sample concentration.

C_n : cDNA concentration in the current sample.

3.13 Primers Preparation

Integrated primers were obtained as a dried product in different concentrations. According to the manufacturer's instructions (Integrated DNA Technologies, USA), the materials are dissolved in sterile, nuclease-free distilled water to give a final concentration of 100 picomoles which was used as a stock solution, and 10 µL of this

solution was added to 90 µL of distilled water to get 10 picomoles of the final concentration of the working solution that used in the real-time polymerase chain reaction (RT-PCR) technique.

3.14 Measuring the Gene Expression of Programmed Cell Death Genes and Genes Encoding Heat Shock Proteins

The PCR reaction was carried out by using a Kapa syber green master mix kit from (KAPA, USA). The reaction was carried out in a volume of 20 µL by adding the reaction components shown in bellow Table 3.11:

Table 3.11 Components of r real-time polymerase chain reaction

Components	Con.	Volume
KAPA SYBR® FAST qPCR Master Mix Mixture (2X)	1x	10 µL
Forward Primer	10	1µL
Reverse Primer	10	1µL
cDNA Template	-	6µL
Nuclease-Free Water to 20 µL	-	2µL

By using the thermal cycle program described in the following Table 3.12:

Table 3.12 Thermal cycle program of RT-PCR

Step	Temperature	Period	Cycle
Initial denaturation (DNA polymerase activation)	95 ℃	3 second	Hold
Denaturation	95 ℃	1-3 sec.	40
Annealing	62 ℃	20 sec.	40
Elongation	72 ℃	30 sec.	-
Extention	72 ℃	10 sec.	-

3.14.1 Calculate of gene expression in RT-PCR reaction

The gene (GAPDH) was used as a positive control to calculate the value of the change in the threshold cycle ΔCT. The GAPDH gene is one of the most common genes used in

gene expression data collection. The reason for using the GAPDH gene as a positive control in molecular studies is that its expression remains constant in the cells or tissues to be detected (Rebouças *et al.* 2013). The percentage of gene expression for the studied genes in the DNA genetic material was calculated according to the method, according to the following equations:

ΔCT: Where ΔCT was calculated between the GAPDH gene (References Gene) and the studied genes (Target Genes) according to the following Equation (3.6):

$$\Delta CT = Ct_{TG} - CT_{RG} \quad (3.6)$$

Since:

CT_{TG} : threshold cycle of studied genes.

CT_{RG} : threshold cycle of GAPDH gene.

ΔΔCT: The value of ΔCT was calculated for cells treated with lycopene extract (Target Sample) and untreated cells (control Sample) for all studied genes and these values were used to calculate ΔΔCT according to the following Equation (3.7):

$$\Delta\Delta CT = \Delta CT_{TS} - \Delta CT_{RS} \quad (3.7)$$

Since:

ΔCT_{TS}: the value of ΔCT for cells treated with nicotine alkaloid extract.

ΔCT_{RS}: the value of ΔCT for cells untreated with nicotine alkaloid extract.

$2^{-\Delta\Delta CT}$: The value of $2^{-\Delta\Delta CT}$ represents the number of times of change in gene expression in the studied genes.

3.15 Detection of Morphological Changes

Principle: This stain is used to dye DNA and detect changes that occur in cells due to programmed cell death. (AO) stain is considered a positive ion with the ability to stain the nuclei of living and dead cells, as the nuclei of living cells appear in a bright green, spherical shape, and a healthy structure when examined under a fluorescent microscope by using a wavelength filter (blue), while PI stain enters the nuclei of dead cells in which a breakdown occurs in their membranes and which appear bright red color when examined under a fluorescence microscope by using a wavelength filter (green). This method was performed to assess morphological changes (imaging nuclear changes and the formation of apoptotic bodies) and to identify living cells and dead cells in which programmed cell death has occurred (Alrawi 2017).

The solution used:

- Acridine Orange stain solution at a concentration of 5 mg/mL.
- Propidium Iodide Stock stain Solution at a concentration of 3 mg/mL.
- PBS (pH 7.2).

Preparation of working solution: Add 100 µL of AO and 100 µL of PI to 1000 µL of PBS, mix well, and keep at room temperature for two weeks.

Procedure:

1- Grown the cancer cells in a 24-hole tissue culture plate, 2 mL of cell suspension is added to each hole and incubated for 24 hours at 37°C.

2- When the cells adhere to the plate, the cells are treated with alkaloid extract at IC50 concentration and incubated for 24 hours.

3- After the incubation period is over, the culture medium is discarded and 500 µL of AO/PI stain mixture is added to each hole and then incubated at 37°C for 20-30 minutes in the dark.

4- After that, the cells are washed with PBS solution, and the washing process is repeated more than once until the excess stain is removed.

5- Finally, 500 µL of PBS is added to each hole, and then the cells are visualized with an inverted fluorescence light microscope by using blue and green filters.

3.16 Statistical Analysis

The results were statistically analyzed using the Graphpad Prism Version 6 statistical analysis system according to the ANOVA analysis of the variance test, and the arithmetic means were compared with the Tukey polynomial test (arithmetic mean ± standard deviation M±SD) with a significant difference in the probability level of > 0.05 (Wang *et al.* 2010).

4. RESULTS AND DISCUSSION

4.1 Preparation of Lycopene Extract for Solanum Lycoericum

A 100 mL was obtained from 5 gm. of dry ground of Solanum lycoericum fruit and the extract was brown.by crushing tomatoes to produce crude tomato juice that separated into serum and pulp the pulp is subsequently extracted using ethyl acetate as asolvent the final extraxte consists of tomato oil which lycopene together with number of other consititument that occur naturally . Then used to prepare the concentration that used in this study which are 25, 50, 100, 200, 400, µg/mL.. There are synergistic effects of chemical compounds in all parts of the plant that are responsible for their biological properties such as anti-inflammatory, antimicrobial and anticancer (Garcia-Oliveira *et al.* 2021).

4.2 Study the Effect of Lycopene Some Bacteria and Yeast Species

The antimicrobial activity of lycopene extract was investigated against Streptococcus. mutans, *S.vestiularis, Klebsilla Protues, C. albicans, and C. neoformans* by using the agar wells diffusion technique. The higher inhibition zone was observed in *Strreptococc mutans* and *C. albicans*, were (24,2 mm and 16.7 mm) respectively, followed by *S.vestibularis* (13.9), *klebsilla* (14.2), *Rhodotorula* (12.2), and *Protues* (12.7), as shown in Table 4.1, Table 4.2, Figure 4.1 and Figure 4.2.

Table 4.1 Effect lycopene extracted on some bacterial species

Type of Bacteria	**Inhibition zone (mm)**
	Mean ± SD
Streptococcus . mutans	24.2 ± 2.0
S. vestibularis	13.9 ± 1.3
Klebsiella .spp	14.2 ± 1.0
Protues	12.7 ± 2.1

*Significant difference among more than two independent means at the level (P≤0.05)

Table 4.2 Effect lycopene extracted. on some yeasts species

Type of Yeasts	Inhibition zone (mm)
	Mean ± SD
Candida albicans	16.7 ± 2.0
Rhodotorula	12.2 ± 2.1

*Significant difference among more than two independent means at the level (P≤0.05)

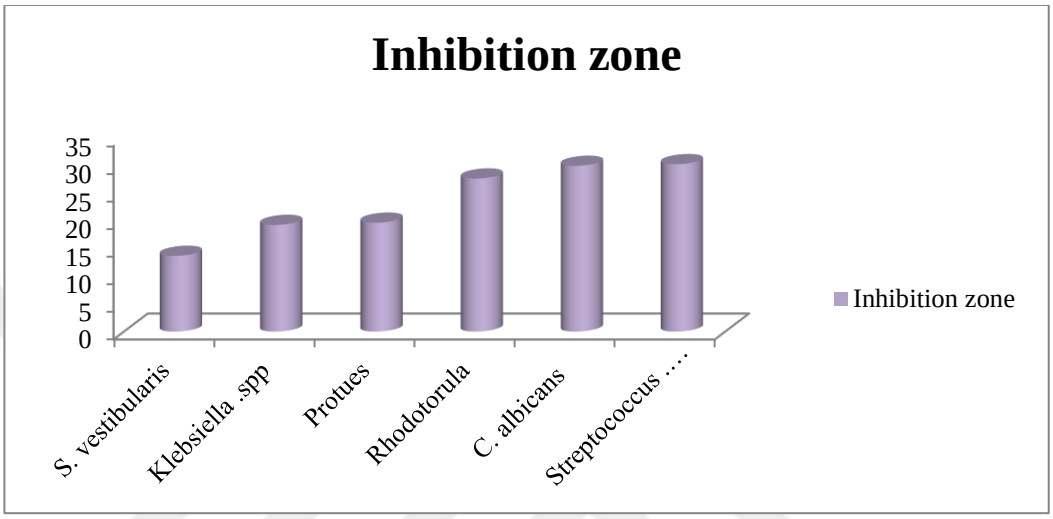

Figure 4.1 Inhibition zone of bacterial and yeasts species

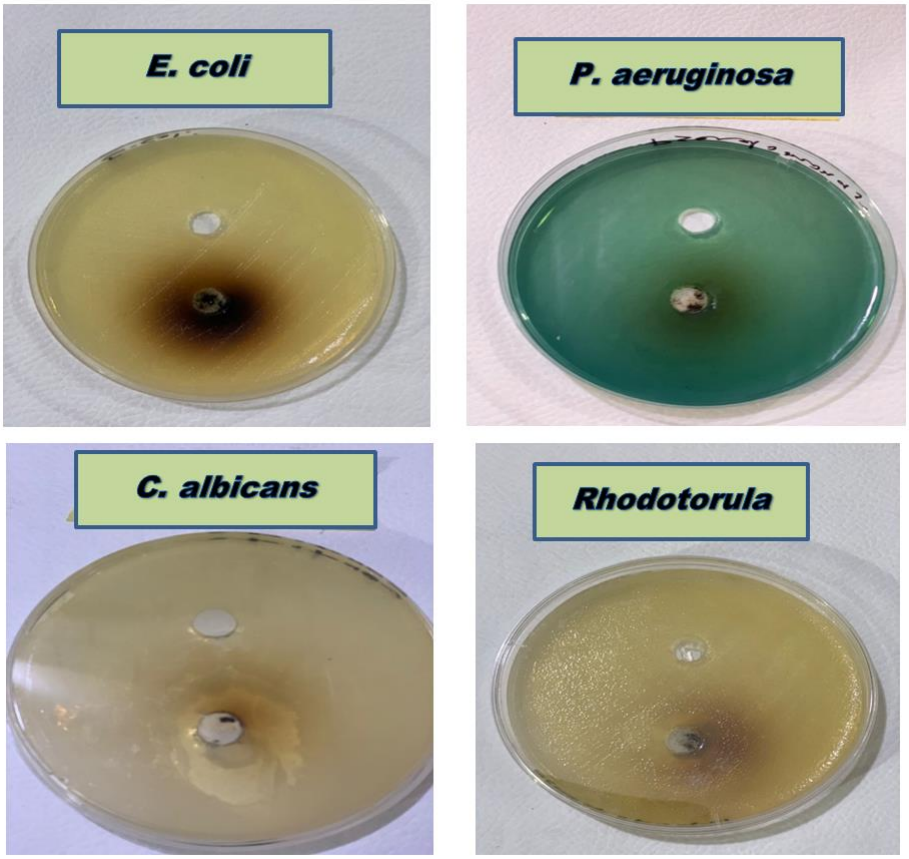

Figure 4.2 Inhibition zone of the effect of lycopene extract on some bacterial and yeast species for 24 hours at 37ºC

The plant extracts are considered a natural source of antimicrobial agents, regarded as nutritionally safe and easily degradable, and the antimicrobial activity exhibited by plant extracts against bacterial infection has been demonstrated by several researchers (Mostafa *et al.* 2018). The study done by (Al-Lahham *et al.* 2020) showed that the acetone and hexane extracts of *tomato L.* had a good antimicrobial effect against *Staph. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans.* Also, the ether and ethanolic extracts of seed and leaves of *lycopene L.* had antimicrobial activities on *Staphylococcus* species, and the methanol extract has an antifungal effect on *C. albicans* (Giovannucci,*et al.* 1995).

4.3 Effect of lycopene in Mitotic Index in Human Lymphocyte

Human lymphocytes were treated with five concentrations of lycopene *Solanum lycoericum* extract for 24 hours at 37°C. This test included treatment of lymphocytes with extract in the presence of PHA.

4.3.1 Effect of lycopene as a stimulant for lymphocyte division

The lycopene extract of *Solanum lycoericum L.* was used by adding it instead of the PHA by using five concentrations ranging between 25, 50, 100, and 200 and 400 µg/mL. The results showed that was no stimulation for lymphocyte division for all concentrations used.

4.3.2 Studying the anti-division effect of lycopene on a lymphocyte

To test the efficacy of tycopene in stopping lymphocyte division, the treatment led to an increase in the rate of lymphocyte division as the concentration increased, as shown in Table 4.3. The percentage of suspended cells stopped in the metaphase was 1.55, 1.61, 2.32, 4.02, 4.11 at concentrations 25, 50, 100,200 and 400 µg/mL respectively, while the percentage of stopped cells in the control was 4.32. There was no significant difference between the two concentrations 200 and 400 µg/mL respectively and control, and the difference was significant between the percentages of suspended cells at other concentrations. When cells were treated with a concentration of 50 µg/mL, the percentage of suspended cells in the metaphase phase was 17.68%, and this percentage increased to 46.39% at the concentration was 100 µg/mL and the increase continued until the percentage reached 97.32% at the concentration of 400 µg/mL as shown in Figure 4.3, Figure 4.4 and Figure 4.5.

Table 4.3 Effect of lycopene extract in mitotic index on human lymphocyte in 24 hours of exposure at 37℃ and compared with colchicine

Con. µg/mL	Cell ratio in metaphase inhibition ratio ± Standard deviation	A percentage from control %
Control(Colchicine)	4.32 a ± 0.01	-
25	1.55 d ± 0.14	17.68
50	1.61 c ± 0.11	46.39
100	2.32 b ± 0.07	65.48
200	4.02 a ± 0.14	90.67
400	4.11 a ± 0.24	97.32

*The different letters in the same column indicate that there are statistical differences at the level of (0.05≥P)

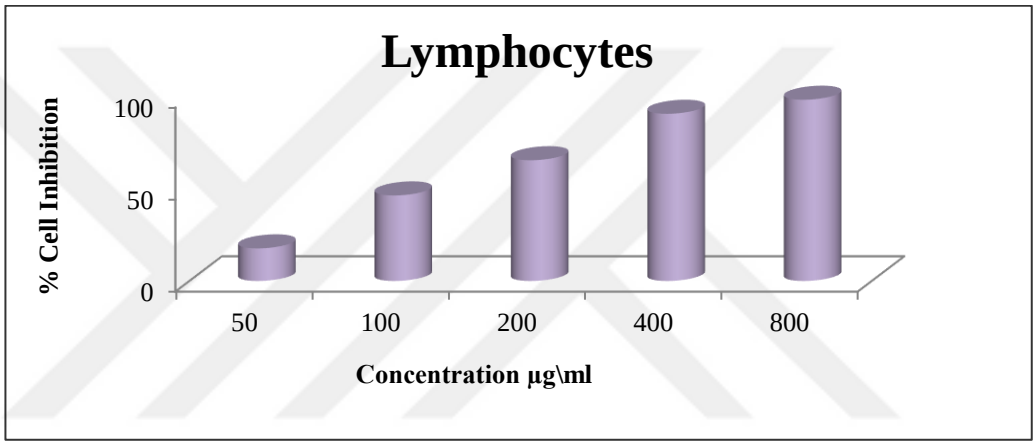

Figure 4.3 Effect of lycopene extract in mitotic index on human lymphocyte in 24 hours of exposure at 37℃

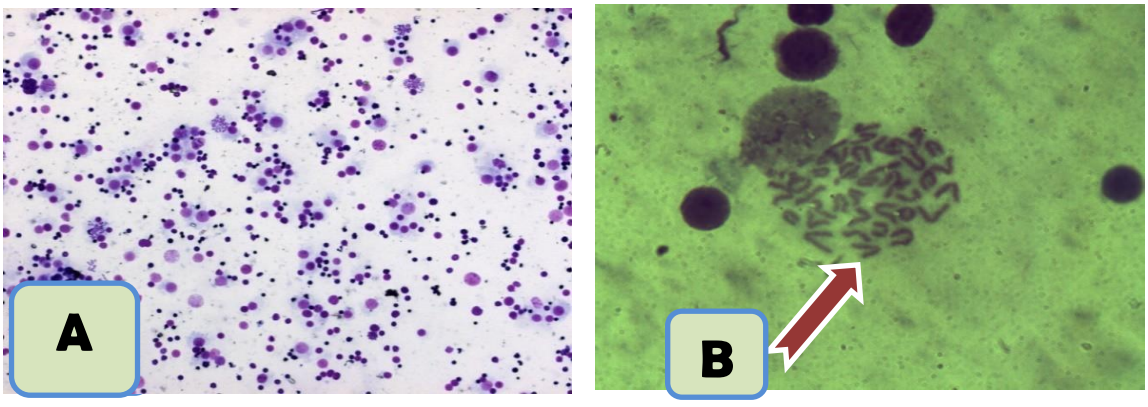

Figure 4.4 Comparison between lymphocytes that were treated and untreated with lycopene extract at a concentration of 400 µg/mL for 24 hours of exposure at 37° c (x20) by using crystal violate stain. (A) representing control lymphocytes. (B) representing treated lymphocytes with lycopene at a concentration of 400 µg/mL and showing

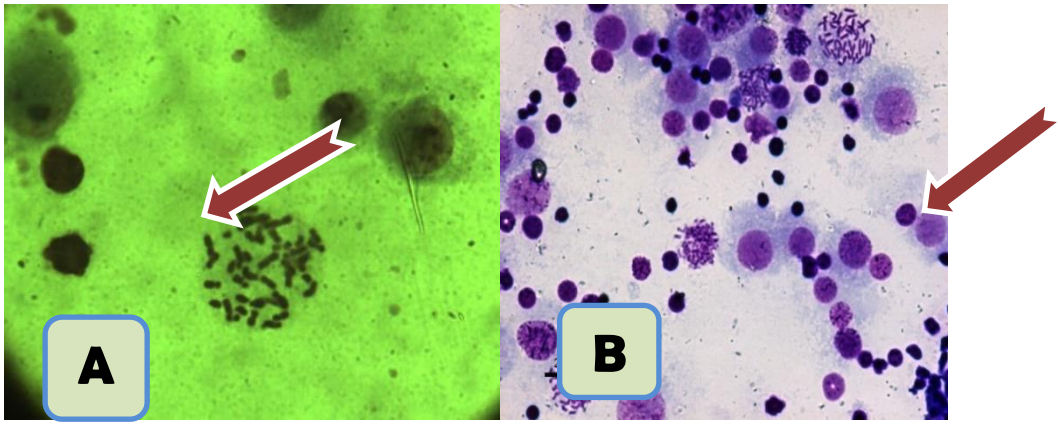

Figure 4.5 Comparison between lymphocytes that were treated and untreated with lycopene extract at a concentration of 400 µg/mL for 24 hours exposure at 37° c (x100) by using crystal violate stain. (A) representing control lymphocytes. (B) treated lymphocytes with lycopene at a concentration of 400 µg/mL

From these results, we can be inferred that the lycopene extract of *Solanum lycoericum* can stop the dividing of lymphocytes in metaphase. (Ahmed *et al.* 2019) also indicated that the methanolic extract of an alkaloid of *Equisetum arvense* led to a decrease in lymphocyte division with all concentrations that were used and this decrease was significant when compared with control, which was 4.20%. Ethyl alcohol extract of the *Nerium Oleander* leaves has an inhibitory effect on the human lymphocyte when treated with different concentrations of the extract for 48 hours (Khaleel *et al.* 2019). The aqueous extract of *Tribulus terrestris* fruit decreases the cell division of human lymphocyte at metaphase, at a rate of 1.8% in compared with control which was 3.33% (Qari and El-Assouli 2019).

4.4 Effect of Lycopene from *Solanum lycoericum* on Cancer and Normal Cell Lines

To test the effect of the lycopene extract's ability in the growth of cancerous tumors, the test was conducted on two cancer lines (AMN3, HepG2) and one normal cell line (MEF). Cancer cell lines were treated with five concentrations and three replicates for each concentration of the plant extract for 24 hours at a temperature of 37 by using different concentrations that are 25, 50, 100, 200, and 400 µg/mL, and the cytotoxicity

test was adopted to determine the effect of the concentrations of the extract on the growth of the cells, in a term of the percentage of the rate of inhibition of growth.

Table 4.4 showed that the lycopene had an inhibitory effect on the growth of cancer cells of the AMN3 line, starting with a concentration of 25µg/mL, as the percentage of inhibition was 30.08%, and this percentage increased to 56.46%, 79.42 %, 93.33 %, and 93.68 % for the concentrations 50, 100, 200, and 400 µg/mL, respectively. A significant difference was observed between concentrations as shown in Figure 4.6 and Figure 4.7. Table 4.5 showed that the lycopene an inhibitory effect on the HepG2 cancer cell line that started with a concentration of 25 µg/mL, as the percentage of inhibition reached 24.88% and increased to 39.10%, 51.15%, 88.90%, 90.50% for the concentrations 50, 100, 200, and 400 µg/mL, respectively. No significant difference was observed between concentrations 200 and 400µg/mL as shown in Figure 4.8 and Figure 4.9. Table 4.6 showed that the lycopene extract had a little inhibiting effect on the normal cell line (MEF). At concentrations 25, 50, and 100 µg/mL, the percentage of inhibition was 0% and increased to 25.11% and 35.90% for concentrations 200 and 400 µg/mL, respectively. No significant difference was observed between the concentrations 25, 50, and 100 µg/mL as shown in Figure 4.10 and Figure 4.11. According to the statistical results, it was found that there was a significant difference when comparing the effect of the lycopene extract on cancerous lines after using the IC_{50} concentration, which is for the AMN3 line (255), for the HepG2 line (80), and the normal line MEF (250) as shown in the Table 4.7.

Table 4.4 Inhibition percentage in the AMN3 cancer cell line by the effect of different concentrations of lycopene for 24 hours of exposure at 37° C

Con. µg /mL	inhibition ratio ± Standard deviation
25	30.08 d ± 2.3
50	56.46 c ± 3.1
100	79.42 b ± 4.0
200	93.33 a ± 7.3
400	93.68 a ± 8.4

*The different letters in the same column indicate that there are statistical differences at the level of (0.05≥P)

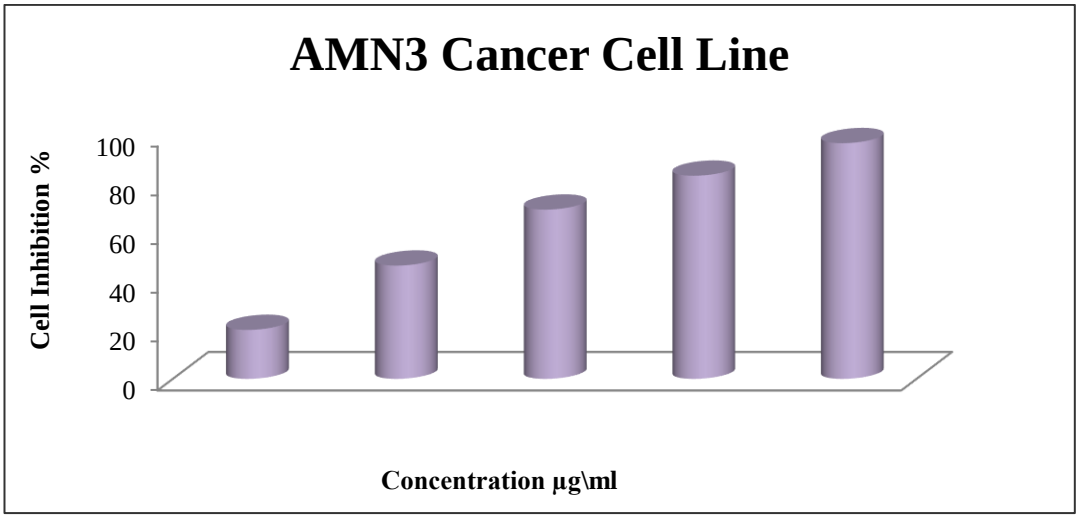

Figure 4.6 Inhibition percentage in the AMN3 cancer cell line by the effect of different concentrations of lycopene for a 24 hours of exposure at 37° C

Table 4.5 Inhibition percentage in the HepG2 cancer cell line by the effect of different concentrations of lycopene for a period of exposure for 24 hours at 37° C

Con. µg /mL	inhibition ratio ± Standard deviation
25	24.88 d ± 2.6
50	39.100 c ± 7.1
100	51.15 b ± 8.3
200	88.92 a ± 8.6
400	90.50 a ± 7.1

*The different letters in the same column indicate that there are statistical differences at the level of (0.05≥P)

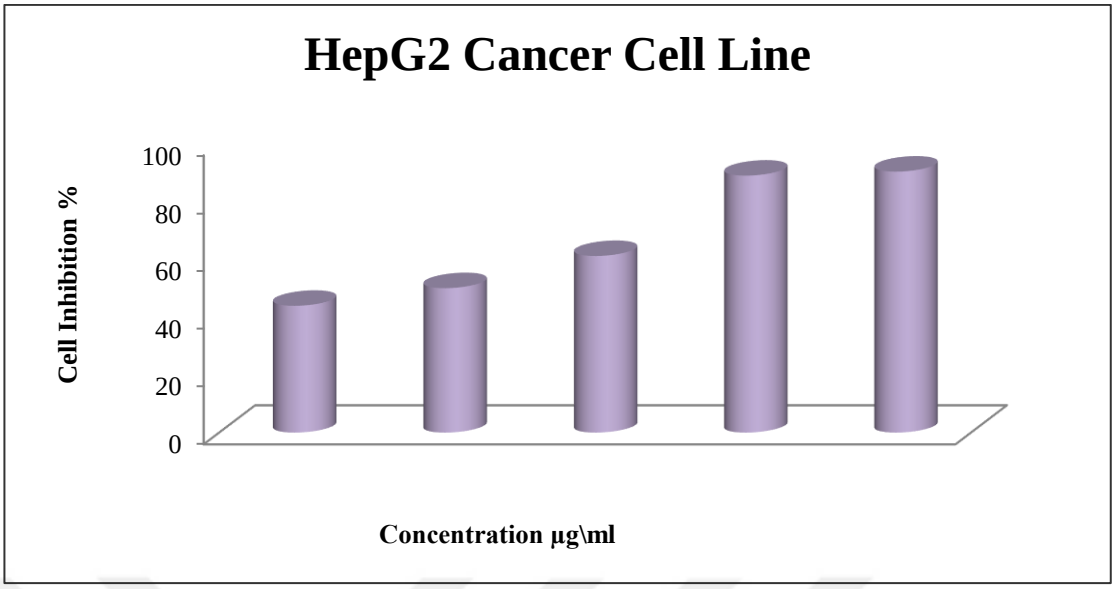

Figure 4.7 Inhibition percentage in the HepG2 cancer cell line by the effect of different concentrations of lycopene for a 24 hours of exposure at 37° C

Table 4.6 Inhibition percentage in the MEF normal cell line by the effect of different concentrations of lycopene for 24 hours of exposure at 37° C

Con. µg /mL	inhibition ratio ± Standard deviation
25	0 c
50	0 c
100	0 c
200	25.11 b ± 2.7
400	35.90 a ± 2.8

*The different letters in the same column indicate that there are statistical differences at the level of (0.05≥P)

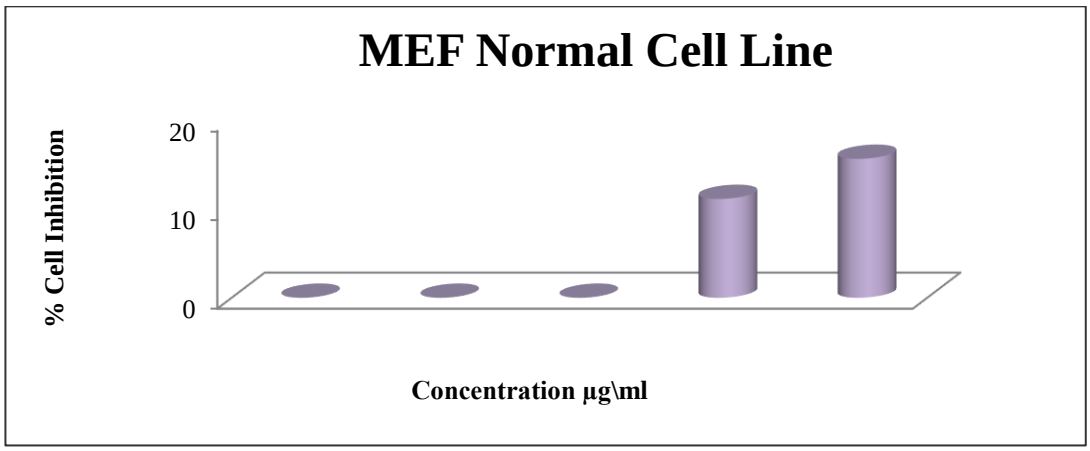

Figure 4.8 Inhibition percentage in the MEF normal cell line by the effect of different concentrations of lycopene for 24 hours of exposure at 37° C

Table 4.7 IC_{50} concentration values in the cancer cell lines AMN3, HepG2, and the normal cell line MEF

Cell Line	IC_{50} con. µg/mL
AMN3	255
HepG2	80
MEF	250

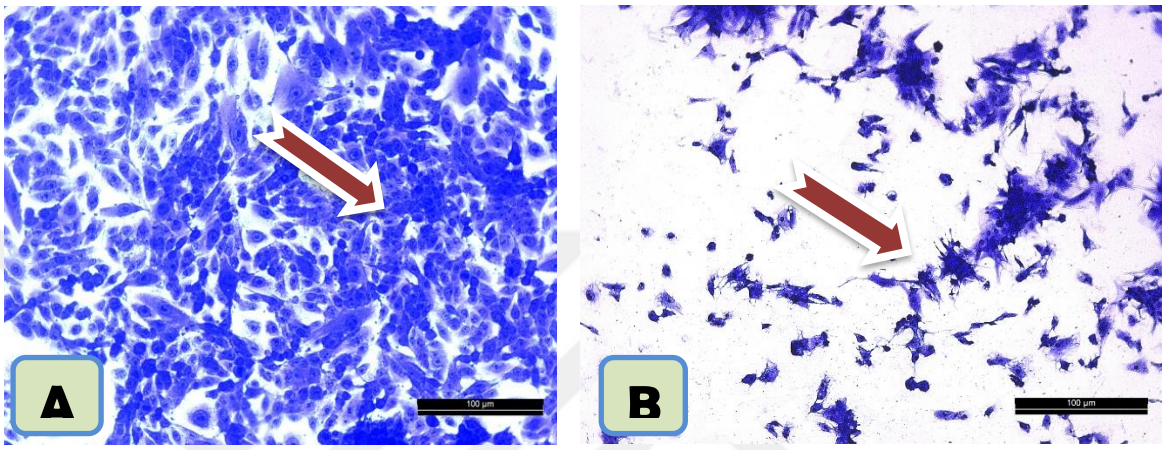

Figure 4.9 Comparison between cells of AMN3 line that treated and untreated with lycopene extract at a concentration of 400 µg/mL for 24 hours exposure at 37° C (x100) by using crystal violate stain. (A)AMN3 cancer cell line representing control and showing dense cells. (B) AMN3 cell line treated with lycopene extract at a concentration of 400 µg/mL and showing dead cells and voids between cells

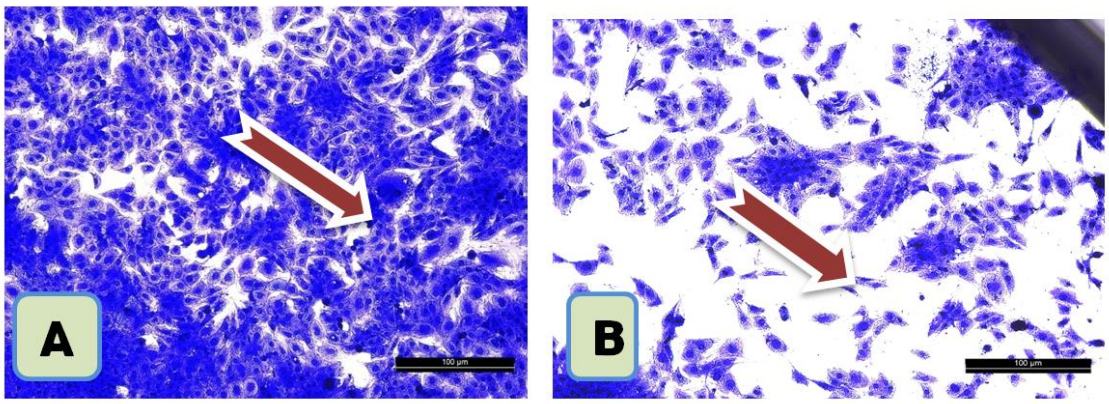

Figure 4.10 Comparison between cells of HepG2 line that treated and untreated with lycopene at a concentration of 400 µg/mL for 24 hours exposure at 37° C (x100) by using Crystal Violate stain.(A) HepG2 cancer cell line representing control and showing dense cells.(B) HepG2 cell line treated with extract at a concentration of 400 µg/mL and showing dead cells and voids

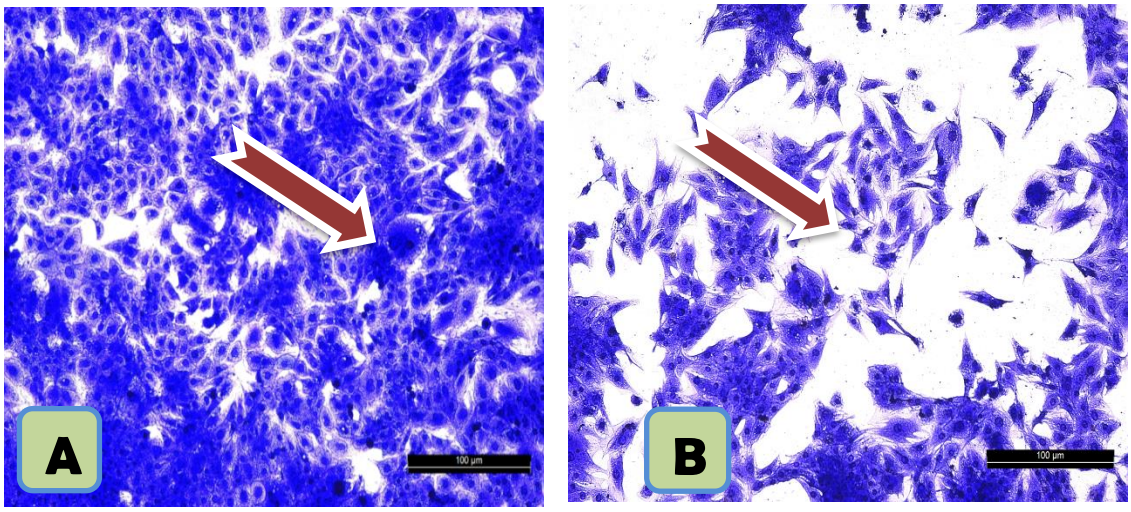

Figure 4.11 Comparison between cells of MEF line that treated and untreated with lycopene extract at a concentration of 400 µg/mL for 24 hours exposure at 37° C (x100) by using Crystal Violate stain.(A) MEF cell line representing control and showing dense cells.(B) MEF cell line treated with extract at a concentration of 400 µg/mL and showing dead cells and voids between cells

The results of this study reinforced the findings of many researchers about plant extracts having anti-cancer activity. This effectiveness depends on the type and concentration of extract, in addition to the sensitivity of cancer cells. The results showed the toxic effects of the lycopene extract of *Solanum lycoericum L.* on the growth of cancer cell lines(AMN3 and HepG2) for all the concentrations that used as shown in Table 4-4, Table 45. The results also showed an effect of the lycopene extract on the normal cell line (MEF) at higher concentrations, but at lower rates than in the cancer cell lines, as shown in the Table 4.6.

The inhibitory effect of the extract on cancer cells is that it contains many chemical compounds, especially lycopene , which is effective in inhibiting or stopping the growth of cancer cells. The study of (Al-Lahham *et al.* 2020) showed that the aqueous and methanol extracts of *Solanum lycoericum L.* roots have an anti-proliferative effect on HeLa cervical adenocarcinoma. The aqueous acetone extract 70% of tomato L. stems has a toxic impact on various human cancer cell lines such as A549, PC3, and MCF7, according to the study of. Also (Thawabteh *et al.* 2019) It was discovered that the alkaloid extract of *Coptis Chinensis Franch* inhibits the multiplication of breast and

liver cancer cells by halting the cell cycle and producing stress in the endoplasmic reticulum, resulting in cancer cell metastasis inhibition. Another study looked at the effects extract on the development of lung cancer (A549), breast cancer (MCF-7), hepatocellular carcinoma (HepG-2), and normal human fetal lung fibroblast (WI-38) cell lines by using ether, chloroform, and aqueous extracts of *Cynanchum acutum L*. seeds (Youssef *et al.* 2019).

From the above, it is clear that the lycopene extract of *Solanum lycoericum L*. has cytotoxicity against cancer cells. The use of cytotoxicity tests for drugs is an important part of detecting new drugs. It is a complex process that affects multiple metabolic pathways after cells are exposed to a toxic substance and, as a result, causes cells to die either by programmed cell death, which is characterized by morphological changes such as cell shrinkage, apoptotic bodies formation, (Kokhdan *et al.* 2018), or by loss integrity of cell membrane, changes in Nucleolar shape, and loss of mitochondrial membrane function and release of Cytochrome-C from mitochondria (Xiang *et al.* 2019) .

4.5 Extraction of RNA and its Conversion to Complementary DNA from Cell Lines

Total RNA was extracted from cells of the AMN3 cancer cell line that were treated and untreated with lycopene extract at IC_{50} (255 μg/mL) for 24 hours, as well as from cells of the HepG2 cancer cell line that were treated and untreated with lycopene extract at IC_{50} (80 μg/mL) for 24 hours. Also from cells of MEF normal cell line that treated and untreated with lycopene at IC_{50} (250 μg/mL). The concentration of RNA from each cancer cell line that transferred to cDNA was determined by using a Spectrophotometer Nano Drop, and it varied from 1289 to 1311 ng/microliter, with purity between 1.78 and 1.81.

4.6 Effect of Lycopene Extract on Gene Expression of Heat Shock Proteins Genes (Hsp60 and Hsp70) in Cancer Cell Lines

The level of gene expression was determined by using RT-PCR and Syber green dye as a fluorescent dye, which can distinguish any DNA, including complementary DNA. The treatment with lycopene extract at IC_{50} (255, 80) for 24 hours leads to inhibiting the gene expression of heat shock proteins encoding genes in cancer cell lines. Table 4.8 showed that the gene expression of the genes encoding heat shock proteins Hsp60, and Hsp70 in the AMN3cancer cell line was (0.43, and 0.16) respectively, in treated cells compared with the control group, and in the HepG2 cancer line it was (0.11, and 0.77) respectively, in treated cells compared with the control group as shown in Figure 4.12.

Table 4.8 Change in the gene expression of heat shock proteins genes (Hsp60, Hsp70) in cancer cell lines

Gene type	AMN3 Gene Expression	HepG2 Gene Expression
Hsp60	0.43	0.11
Hsp70	0.16	0.77

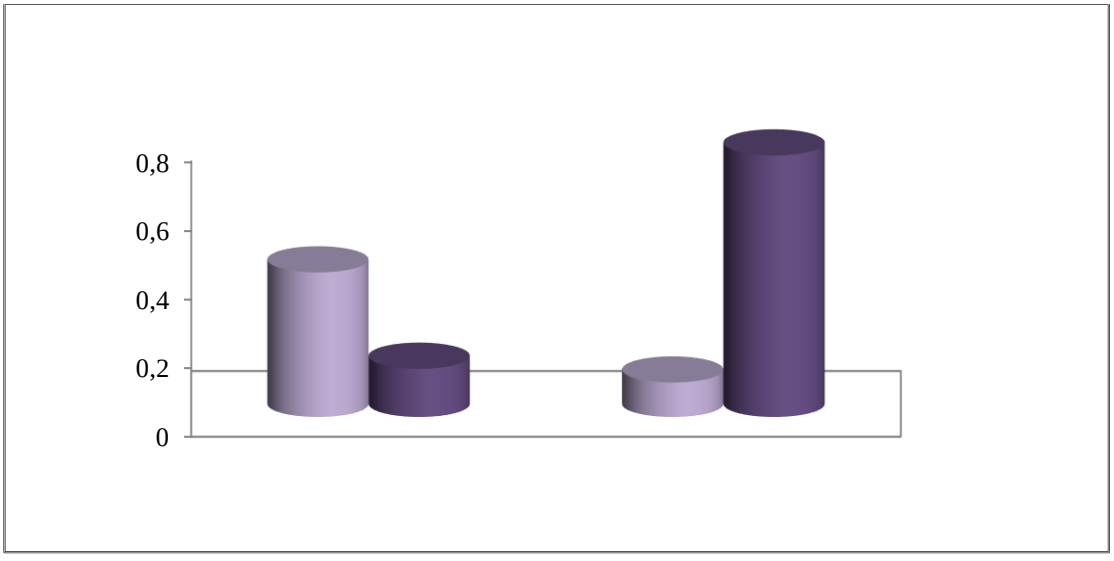

Figure 4.12 Change in the gene expression of heat shock proteins genes (Hsp60, Hsp70) in cancer cell lines

Heat shock proteins have a wide range of functions in the suppression of programmed cell death. Hsp60 overexpression has been indicated as a marker in some malignancies, including breast, lung, ovarian, and others. Because HSP60 acts as an anti-apoptotic factor, it has been identified as a possible target for anticancer treatment (Pirali *et al.* 2020). Hsp70 inhibition stops progressing and proliferating lung cancer cell lines through its cell cycle. Overexpression of Hsp70-1in many types of cancers promotes cancer progression through a variety of pathways, such as angiogenesis, reprogramming of metabolic processes to compensate for the hypoxia, and then metastasis (Rashid *et al.* 2021).

4.7 Effect of lycopene on Gene Expression of Programmed Cell Death Genes (caspase 8 and caspase 9) in Normal and Cancer Cell Lines

In cancer (AMN3, HepG2), and normal (MEF) cell lines, treatment with lycopene extract at IC50 (255,80, 250), respectively, for 24 hours increased gene expression of the Hsp60 encoding gene. In AMN3, HepG2, and MEF cell lines, the percentage change in the level of expression of the Caspase8 encoding gene was 0.41, 0.54, and 0.43, respectively, in cells treated with the percentage change in the level of expression of the Caspase9 1.90,2.70,3.44 respectively, as shown in Table 4.9 and Figure 4.13.

Table 4.9 Change in the gene expression of programmed cell death genes (caspase 8 and Caspase 9) in cancer cell lines

Gene Type	AMN3 Gene Expression	HepG2 Gene Expression	MEF Gene Expression
Caspase 8	0.41	0.54	0.43
Caspase 9	1.90	2.70	3.44

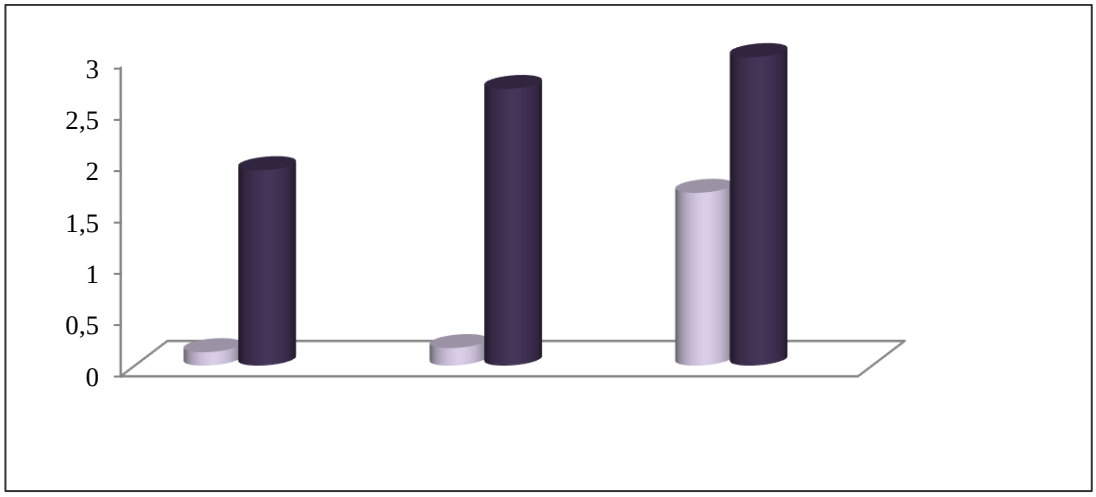

Figure 4.13 Change in the gene expression of programmed cell death genes (caspase 8, and caspase 9) in cancer cell lines

The study showed that the lycopene extract of *Solanum lycoericum L.* has an effect in to programmed cell death at IC_{50} after 24 of exposure through the intrinsic pathway by activating caspase8 and caspase 9 gen. Apoptosis is an evolutionarily conserved cell death mechanism that is involved in proper eukaryotic development and the maintenance of organismal homeostasis. The BCL-2 protein family, which includes both pro-apoptotic and pro-survival members, regulates this process. Many studies show that plant extracts can induce cancer cells to programmed cell death. The study indicated by (Pathiranage *et al.* 2020) showed that the organic solvent extracts of the polyherbal mixture containing seeds of *Nigella sativa*, roots of *Hemidesmus indicus*, and rhizomes of *Smilax glabra* have a significant up-regulation of the Bax gene in the NCI-H292 cancer cell line. The methanolic and acetone extract of the fruit of *Vatica diospyroides Symington* showed an effective impact in increasing the expression of caspase 8 protein in the Hela cervical cancer cell line. This leads to the release of cytochrome-C from mitochondria and binding with Apaf-1 and then with caspase9 to form a complex called apoptosome and this leads to apoptosis (Chothiphirat *et al.* 2019). Also, the water extract of the *Hibiscus sabdariffa* leaf belonging to the Malvaceae family can induce apoptosis in the prostate (LNCaP cells) cancer cell line by increasing the expression of caspase 8 and cytochrome-C of cytoplasm and then activating caspase9. (Al-Shammari *et al.* 2021) also showed that the alkaloid extracted from *Cyperus rotundus L*. by using methanol 80% can affect the gene expression of P53

in human digestive system cancer cell lines (SKGT-4 and HRT), where the treatment of cells with lycopene for 24 hours of incubation leads to upregulation of the P53, but downregulation of it when incubated for 48 hours. Vinca alkaloids, such as vinblastine, derived from leaves of *Catharanthus roseus*, can interact with tubulin proteins and inhibit them from polymerizing into microtubules (Garcia-Oliveira *et al.* 2021).

4.8 Exposure Extraction Lycopene and Detection of Morphological Changes in Cell Lines by using Immunofluorescence

When cancer and normal cells were exposed to lycopene extract at IC_{50} for 24 hours, their impact on AMN3, HepG2, and MEf cell lines was seen by inducing them to die when inspected by fluorescence microscopy as shown in Figure 4.14, Figure 4.15 and Figure 4.16. Through direct observation or through using fluorescence microscopy, which exposes normal cells and cells that have died due to programmed cell death. This approach was used to analyze morphological alterations (such as nuclear modifications and the creation of apoptotic bodies) and to distinguish between live and dead cells that had undergone programmed cell death. Due to the penetration of (AO) stain into the cell membrane and DNA bundles, all nuclei in living cells in the control group (untreated cells) looked green in color, with a normal spherical shape and regular chromatin. But the nuclei of cells that had undergone programmed cell death appeared red with fragmented nuclei and compacted chromatin.

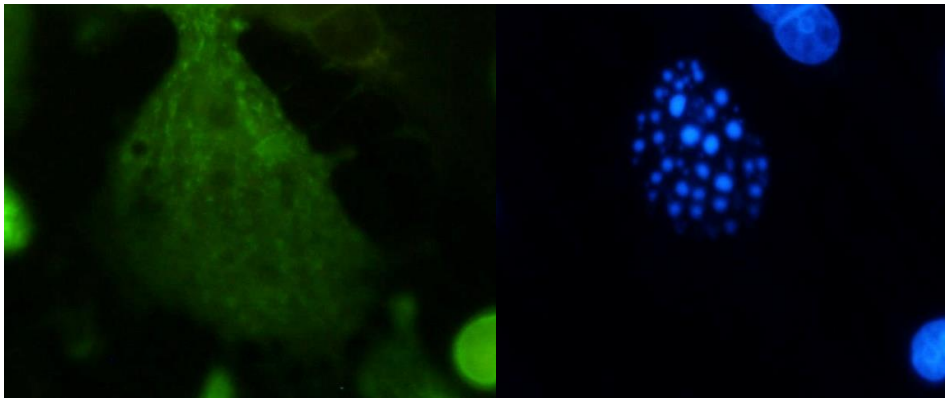

Figure 4.14 Detection of morphological changes of programmer cell death by immunofluorescence stain in MCF7 cancer cell (100X)

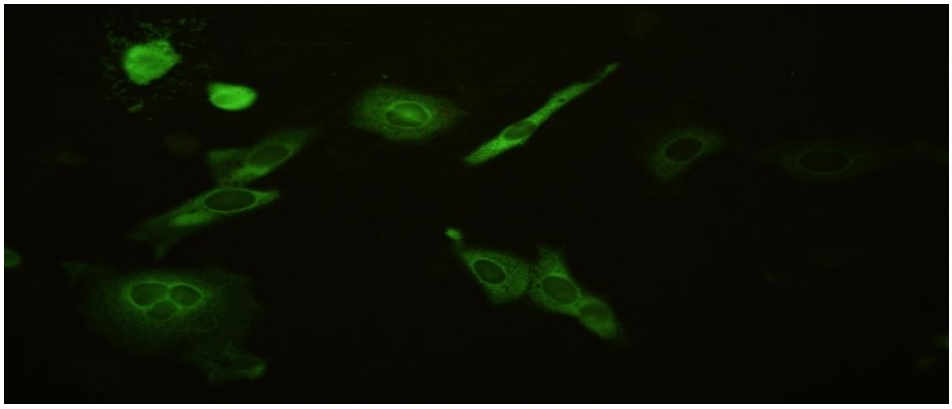

Figure 4.15 Detection of morphological changes of programmer cell death by using immunofluorescence HepG2 cancer cell line (100X)

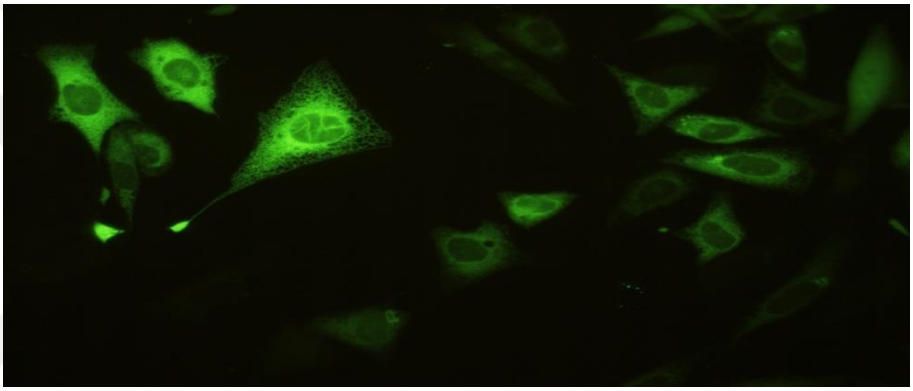

Figure 4.16 Detection of morphological changes of programmer cell death by using immunofluorescence stain in mef cancer cell line (100X)

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

It could be concluded from the present study that:

- The extract of lycopene from Solanum lycoericum fruit. contained a high percentage from him.

- The lycopene extract from Solanum lycoericum has an anti- division effect on lymphocytes.

- The lycopene extract has an inhibitory effect on the growth of (AMN3 and HepG2) cancer cell lines.

- The lycopene extract has an inhibitory effect on the expression of heat shock proteins encoding genes (Hsp60 and Hsp70) in cancer cell lines and increased the expression of programmed cell death encoding genes (Caspase-8, Caspase 9) in cancer (AMN3 and HepG2) and normal (MEF) cell lines.

- The lycopene extract can cause the death of cancer cells by inducing of programmed cell death process through many biological activities.

5.2 Recommendation

- Testing the antioxidant activity of the lycopene extract.

- Studying the effect of lycopene extract on the gene expression in some bacterial and yeast species.

- Studying the effect of lycopene extract on other cancer cell lines, especially lung cancer.

- Studying the effect of lycopene extract on the expression of other heat shock proteins encoding genes.

- Studying the effect of lycopene extract on the expression of other programmed cell death encoding genes.

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