REPUBLIC OF TURKEY
HARRAN UNIVERSITY
HEALTH SCIENCES INSTITUTE
DEPARTMENT OF MEDICAL BIOCHEMISTRY

THE INVESTIGATION OF ANTICANCER EFFECT OF SCORPION CARAPACE

Shanaz Abdulkareem Mohammed MOHAMMED

MASTER THESIS

SUPERVISOR
Assoc. Prof. Dr. İSMAİL KOYUNCU

SANLIURFA

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Shanaz Abdulkareem MOHAMMED

2022

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INDEX OF ICONS AND ABBREVIATIONS

HCT-116	Colon Cancer Cell
MDA-MB-231	Breast Cancer Cell
DU-145	Prostate Cancer Cell
HELA	Cervical Cancer Cell
U2OS	Bone Cancer Cell
BEAS-2B	Normal Lung Cell
HEK-293	Normal Human Embryonic Kidney Cell
HUVEC	Human Umbilical Vein Endothelial Cell
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
ROS	Reactive Oxygen Types
DCFDA	Dichlorofluorescein diacetate
JS-1	1,1',3,3'-Tetra Methyl Benzimidazole Carcyanin Iodide
CRL-4010	Normal Breast Cell
IARC	International Agency for Research on Cancer
ER	Estrogen Receptor
CTX	chlorotoxin
NDBP	Disulfide Bond-Free Peptides
ICK	Inhibitory Cysteine Node
TsTx1	TityusToxin
IpTxa	Imperatoxin
ACE	Angiotensin Converting Enzyme
MMP-2	Metalloproteinase-2
DEEM	Diethyl Ethoxy methylene Manolete
HSPI	Heat Shock Protein
LAAOs	L-Amino acid Oxidase

ChTx	Charybdotoxin
IbTx	iberiotoxin
AgTx	Agitoxin
PLA_2	Phospholipase A2
µM	Micromolar
BMK-CBP	Serine Protease-Like Protein
AMP	Antimicrobial Peptide
DMSO	Dimethyl Sulfoxide
PBS	Phosphate Buffered Saline
EDTA	Ethylenediaminetetraacetic acid
RPMI	Roswell Park Memorial Institute Cell Media
DMEM	Dulbecco's Modified Eagle Medium Cell Media
FBS	Fetal Bovine Serum

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ABSTRACT

THE INVESTIGATION OF ANTICANCER EFFECT OF SCORPION CARAPACE

Shanaz Abdulkareem Mohammed MOHAMMED

Department of Medical Biochemistry, Master thesis

Scorpion is one of the arachnids which have several properties, there are many studies on the effectiveness of scorpion venom, despite the danger of poison to humans, studies found that there are many active peptides in scorpion venom, and they are a rich source of pharmacologically active compounds that have a role in helping to cure of infection, and cancer treatment ... etc. From there we developed the study and focused on other aspects of the treatment through Scorpion. The aim of this study is to determine the anti-cancer properties of the carapace of black scorpion (Androctonus crassicauda) that lives in Sanliurfa. This study was determined as the first study done on scorpion carapace to detect the anti-cancer activity .The scorpion's outer shell was collected and extractions of (hexane, dichloromethane, ethanol, methanol, and water) were extracted then the detection of the cytotoxic effect of scorpion shield was done on colon cancer (HCT-116), breast cancer (MDA-MB-231), prostate cancer (DU-145), lung cancer (A-549), bone cancer (U2OS), normal lung (BEAS-2B), kidney (HEK-293), normal prostate (PNT-1A), normal breast (CRL 4010), and endothelial cell (HUVEC) Obtained from ATCC by investigate them by MTT assay .The effect of the strongly cytotoxic fraction on cell apoptosis was investigated by Annexin-V and cell cycle flow cytometry. As a result of the study, it was determined that the hexane fraction showed the strongest cytotoxic effect on prostate cancer cells (DU-145), while its toxic effect on normal prostate cells (PNT-1A) was found

to be lower than on cancer cells. It was determined that the hexane fraction induced apoptosis on DU-145 and stopped the cell cycle in Sub G1 phase. According to the data obtained, it was concluded that the scorpion carapace has anticancer potential, therefore, further studies should be carried out to determine the components showing anticancer activity.

Key Words: Scorpion carapaces, anti-cancer activity, Apoptosis, Cell cycle, scorpion morphology, Annexin V.

1. INTRODUCTION

Despite recent breakthroughs in treatment options, cancer remains the main cause of death globally. Furthermore, the prevalence of many malignancies, including skin, prostate, breast, and kidney cancers, continues to rise. Cancer deaths are expected to grow further, reaching 11.5 million by 2030.

Conventional cancer chemotherapy, radiation, and surgical treatment largely focus on generating mass cell death without great specificity, resulting in substantial side effects and toxicity in numerous bodily tissues. As a result, there is an urgent need to develop novel tumor-targeting medicines that may efficiently and selectively destroy tumor cells while limiting harm to normal organs (214).

Cancer, is characterized by the spread of abnormal cells. Every sixth death globally is due to cancer, causing roughly 10 million fatalities in 2020, or nearly one in every six deaths.

Cancer may start anyplace in the body, which contains billions of cells. human cells proliferate to form new ones through a process known as cell division, this usually happens when the body needs new cells, however, in some cases, this process can break down and allow damaged or abnormal cells to multiply , these cells can then divide and form tumors, which are usually lumps of tissue, although they can be considered to be cancer, these tumors can also spread to other tissues, they can also become malignant, and spread to distant parts of the body which is called (Metastases).

Various types of drugs and procedures are available to treat cancer some of these are referred to as local treatments, which are usually used to treat a specific area of the body. Others are referred to as systemic treatments, which involve using drugs that can affect the entire body, most people are treated for cancer with various types of treatment, among these include surgery, chemotherapy, radiation treatment, stem cell therapy, and hormone therapy.

This disease led to more search for cancer treatment and new and more effective methods, so science resorted to natural products, including animal plants and their toxins, and they discovered through them that scorpion poison and some other animals are the most effective poisons in this field. However, where the scorpion's venom strength varies

according to the environment in which it lives, the effect occurs through those peptides found in scorpion venom that connect to the ion channels of the cell.

In 2004, a teenage girl had surgery to remove a brain tumor that took 14 hours, but surgeons were surprised that they left a thumb-sized tumor after they thought it was healthy tissue after seeing a patient with cancer, Jim Olson, an oncologist at the Fred Hutchinson Cancer Research Center, started searching for a molecule that could allow surgeons to visualize cancer using the naked eye, after searching the DNA database, scientists found only six weeks later chlorotoxin, it was extracted from the venom of a deathstalker scorpion, that researchers discovered in 1998 that binds to the surface of the cells to ion channels in the brain, this toxin is 500 times more accurate than an MRI, as it allows researchers to see cancerous tumors as small as 200 cells, other teams are working on ways to use Tozuleristide to differentiate cancerous tumors from other areas, such as the breast and spinal cord.

The field of cancer chemoprevention is a new medical discipline that's focused on developing natural agents that can prevent the development of cancer, among the medical benefits of scorpion venom is its use in medicine, as the substance "chlorotoxin" in the poison is used to treat brain and spinal cord cancer, and was used by researchers to eliminate malaria transmitted by mosquitoes, as well as to use it against bone diseases transmitted by rats, this made it a required material, which researchers are trying to obtain for use in medical research.

Research conducted at the University of Colima (southwest Mexico) has identified a carefully selected group of peptides (Centruroides tecomanus) that have anti-cancer properties, when the toxin attacks malignant cells, it promotes programmed cell death that leads to the death of cancer cells.

These researches and many other types of scorpion venom had a great impact on the world of cancer patients, as there are many peptides in scorpion venom that have a great effect on the malignant cell. from this perspective, we focused on other active substances in scorpions for the treatment of cancer, but this time from the scorpion carapace of Turkish scorpions in Sanliurfa, which makes this research one of the latest

researches conducted on the scorpion carapace, as we conducted on 10 types of different cancer cells.

As a result of our literature search; It has been determined that the majority of the studies conducted with scorpion are related to the anticancer activity of the scorpion venom. Therefore, in this study, the anticancer activity of the scorpion carapace was examined for the first time.

In this study, extracts of scorpion carapace in different solvents were obtained. The cytotoxic effects of these extracts on different cancer and normal cells were investigated. The mechanism of action of hexane extract, which has the highest cytotoxic effect, on prostate cancer was determined by annexin V and cell cycle analysis.

2. GENERAL INFORMATION

2.1. Cancer

Uncontrolled growth and the spreading of abnormal cells are the main causes of cancer, if the cancer cells are not controlled, they can eventually cause the death of the patient, various external factors such as exposure to radiation and chemicals may potentially lead to cancer formation.

Various factors can also increase a person's risk of getting cancer, some of these include dietary factors, environmental pollutants, and certain infections, these factors can then lead to the development of cancer, it is regarded as the main cause of mortality in humans.

Carcinogens are substances that can cause the development of cancer by damaging the DNA of the cells, some of these include exposure to radiation, chemicals found in car exhaust fumes, and asbestos (2).

The goal of cancer therapy is to kill the cancer cells without damaging the normal cells, understanding the mechanisms of apoptosis has allowed scientists to develop new drugs that can target cancer cells, however, resistance to these drugs can prevent them from working properly (3).

In terms of classification, cancer is categorized from I to IV and it depends on the stage it's in the more detailed information that comes with this system allows doctors to make more informed decisions when it comes to treating patients (4). For example, stages of melanoma cancer as shown in (Figure 2.1), Stage 0- This stage indicates the presence of abnormal cells in situ (inner layer) Stage 1- Notice the polyp or tumor is on the inner lining and has not invaded beneath the muscle layer, diagnoses and treatment at this stage have a good percentage of the recovery, it is possible to diagnose this stage as well through screening, the individual can be asymptomatic during this phase or have mild symptoms, if not investigated properly, which can be easily missed. Stage 2-The tumor penetrates through the strong muscle and affects other healthy tissue, Patients notice mild signs and symptoms but can overlook them if not investigated. Stage3- surrounds lymph nodes,

Lymph nodes are bean-shaped tissues that protect the human body from infection, now the cancer cells have attacked the immune system of the body. During this stage, the patient usually shows significant signs and symptoms that are easily diagnosed, investigated, and invasive treatment plan. Stage 4-The cancer cells now have spread to the secondary site (another organ) and then the cancer cells attack the lymph nodes in the third stage, so here they will be spread via blood or lymphatic channels, prognosis during this stage varies (5).

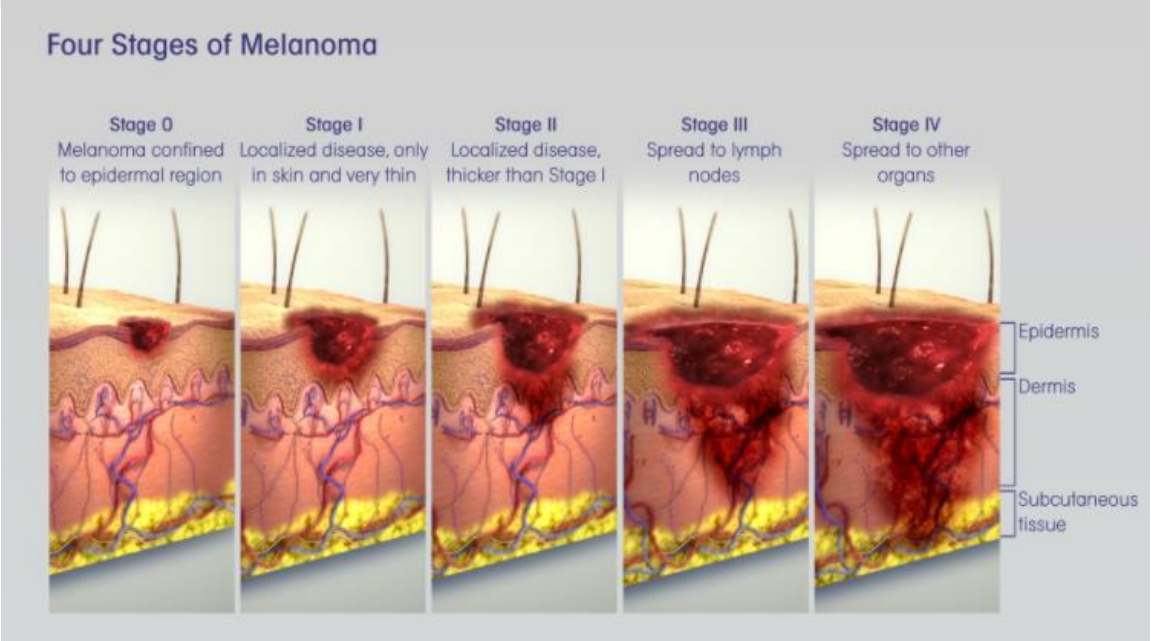


Figure 2.1. stages of melanoma cancer (6).

2.2. Cancer Epidemiology

During the 1980s, epidemiologists were able to identify many important causes of cancer, however, they did not identify the exact events that triggered human carcinogenesis, the advancements in molecular biology and cell biology over the last two decades have led in the development of innovative cancer-analysis methodologies, considerably broadening the scope and methodology of cancer research. Although some of the discoveries made during the 1980s were based on traditional methods, many of these have not been able to identify the exact causes of cancer, instead, they rely on the findings of modern molecular, viral, or genetic studies (7,8).

The environment and lifestyle factors that can contribute to the development of cancer are some of the factors that have been identified as the causes of the carcinogenic effects of tobacco (9). The incidence of lung cancer is highest among current smokers, who start to smoke when they are young and continue throughout their lives.

In Britain, after the First World War, the prevalence of male smoking increased significantly. By 1955, the threat of lung cancer among men under the age of 55 had become the highest in the world (10). Epidemiologists in the US and Britain noted that cigarette smoking was a contributing factor to the development of lung cancer. Over the years, the levels of tar in British cigarettes have decreased (11-14).

In China, the prevalence of male cancer deaths due to smoking in 1990 was 22%, whereas that in Britain fell from 44% in 1990 to about 36% by 2000 (15,16).

As people get older, their cancer risks may also be influenced by their diet. Aside from alcohol, they may also be exposed to other harmful substances (17). such as aflatoxin, which can cause nasopharyngeal cancer (18).

The IARC has released a status report that the global burden of cancer based on the 2018 GLOBOCAN estimates. It also highlights the variability in cancer incidence and mortality across 20 different geographical regions (19).

There were around 18.1 million new cancer diagnoses and approximately 9.6 million cancer deaths in 2018. Lung cancer is the most common cancer in both sexes (11.6 percent of all cases) and the main cause of cancer mortality (18.4 percent of all cancer

deaths), with female breast cancer coming in second (11 percent). as well as prostate cancer. Cancer (7.1%), colorectal cancer (6.1%), colorectal cancer (9.2%), stomach cancer (8.2%), and liver cancer (8.2%) are the leading causes of mortality (20In terms of incidence, lung cancer is the most prevalent cause of cancer-related death in males, followed by prostate and colorectal cancer, and liver and stomach cancer in terms of fatality. Breast cancer is the most frequent kind of cancer in women and the major cause of cancer death, followed by colorectal cancer, lung cancer, and cervical cancer; in terms of incidence and mortality, cervical cancer ranks fourth (21).

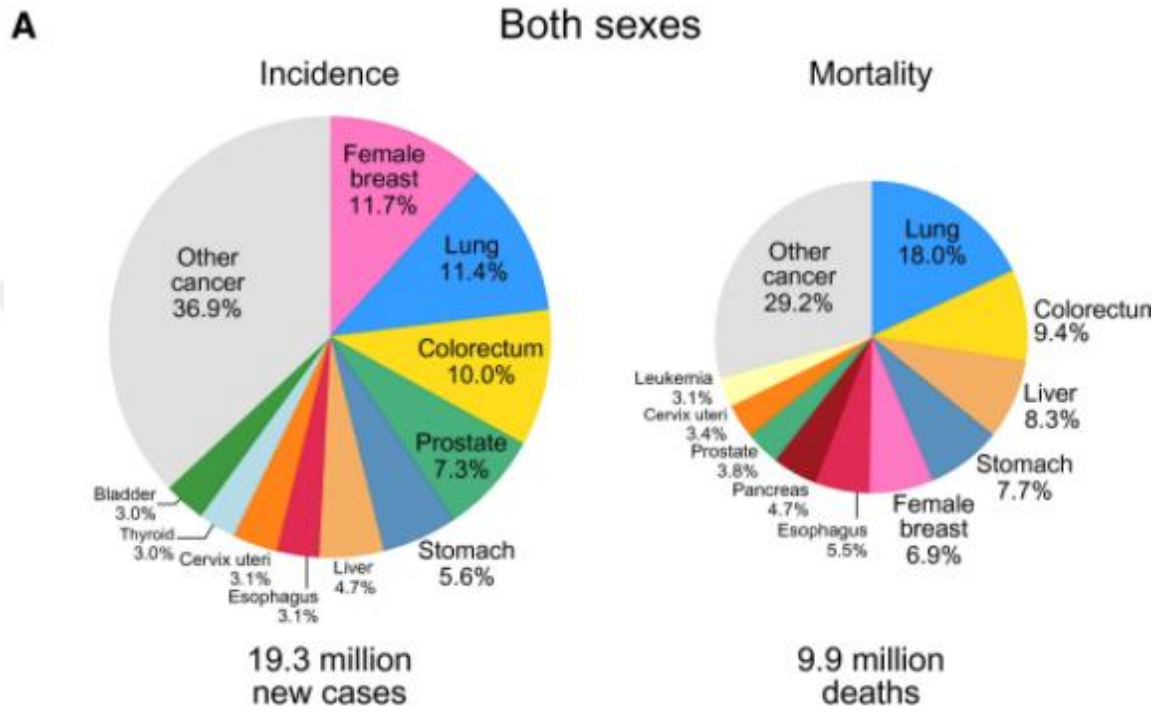
Following that, the International Agency for Research on Cancer updated the global cancer burden based on GLOBOCAN 2020 cancer incidence and death estimates. In 2020, it is anticipated that 19.3 million new cancer cases and around 10.0 million cancer deaths will occur. Female breast cancer has surpassed lung cancer as the most common malignancy. With 2.3 million new cases projected (11.7 percent), lung cancer (11.4 percent), colorectal (10.0 percent), prostate (7 percent), 3) and stomach cancer (5.6 percent) are the most often diagnosed malignancies.

Lung cancer continues to be the largest cause of cancer death, accounting for an estimated 1.8 million fatalities (18%), followed by colorectal (9.4 percent), liver (8.3 percent), stomach (7.7 percent), and breast (7.7 percent) among females. (6.9 percent).) In average, the incidence of both sexes was two to three times higher in transition countries, whereas the fatality rate varied twice for men and twice for women. Female death rates from breast and cervical cancer were substantially higher in transition nations than in non-transition countries (15.0 vs 12.8 per 100 000 and 5.2 vs 12.4 per 100 000, respectively) (22).

The worldwide burden of cancer was 28.4 million cases in 2040, a 47 percent rise from 2020, compared to countries in transition (from 32 percent to 56 percent) in transition nations (from 64 percent to 95 percent). is to be expected However, the increased risk factors connected with globalization and an expanding economy may worsen this.

Efforts to develop a sustainable infrastructure for cancer prevention and care in transition countries are crucial to the global fight against cancer (Fig. 2.2.), the top 10 malignancies in terms of estimated cases and deaths for men and women globally, together

and separately. The top ten cancer types account for more than 60% of newly diagnosed cancer cases and more than 70% of cancer deaths in both sexes.



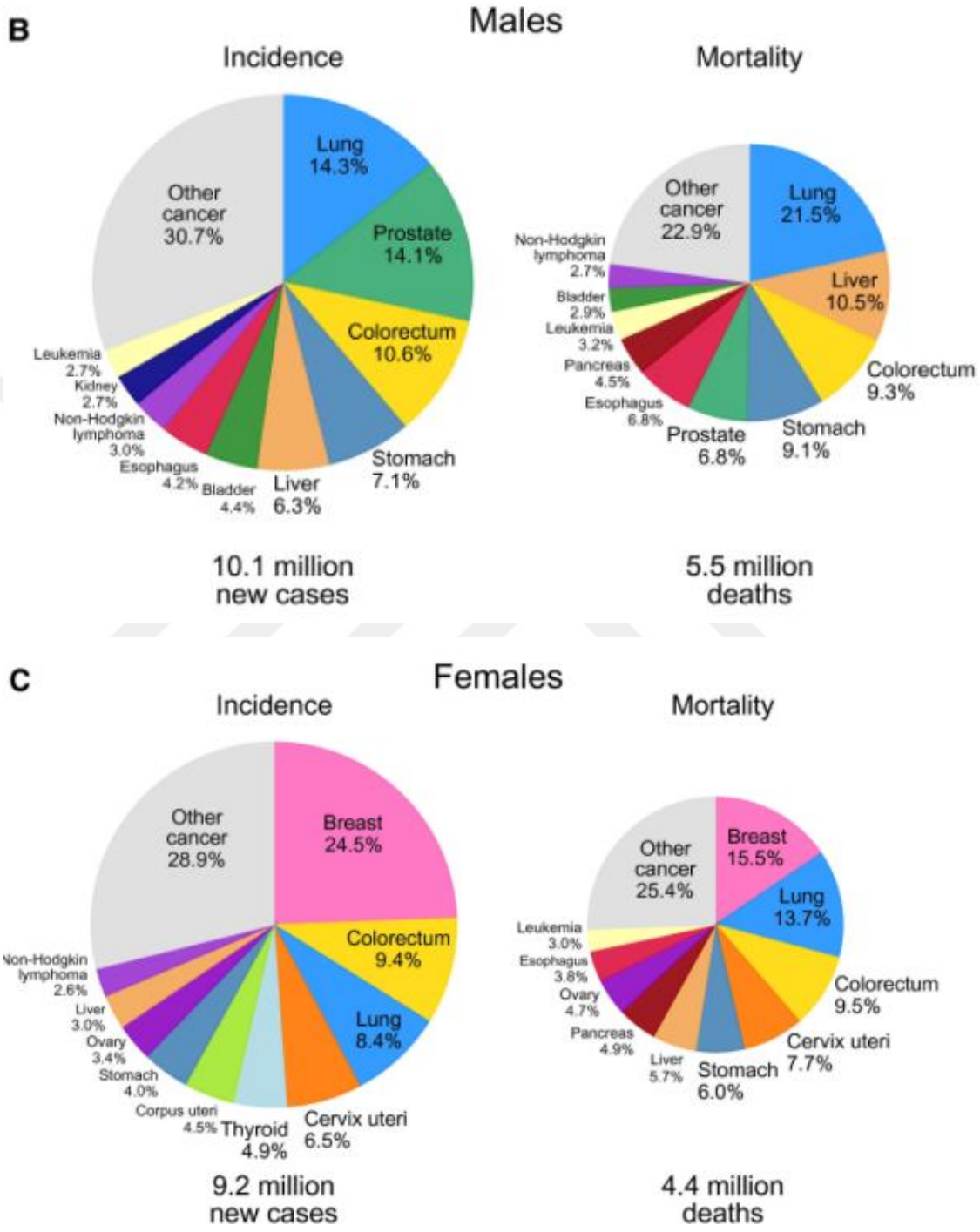


Figure 2.2. Cases and deaths from the top ten most common cancers in 2020. for (A) both sexes, (B) men, and (C) women. Nonmelanoma skin cancers (excluding basal cell carcinoma in terms of incidence) are classified as "other." GLOBOCAN 2020 is the source (22).

2.3. Cancer Treatment

There are several cancer therapy methods accessible, Surgery, radiation, chemotherapy, hormonal treatment, immunological therapy, and symptomatic and supportive therapy are among them, depending on the type of cancer, it can be treated with one or a combination of these treatments (23).

The goal of cancer treatment is to create a cure for patients and to allow them to live a normal life span However, this may or may not be possible depending on the type of cancer, if cancer doesn't respond to treatment, it can be treated by either slowing down the growth of cancer or shrinking it down (24).

The treatment of cancer may be used as:

- **Primary treatment:** One of the most frequent cancer treatments is referred to as primary treatment, this treatment seeks to either eliminate the entire malignancy from the body or destroy the cancer cells.
- **Adjuvant treatment:** An alternative treatment known as Adjuvant treatment is used to treat people with advanced cancer, it aims to kill the cancer cells that may have remained after the primary treatment.
- **Palliative treatment:** In palliative care, patients are allowed to relieve the side effects of their cancer treatment, some of the common treatment procedures that can be used to relieve these symptoms include surgery, chemotherapy, and radiation (24).

2.3.1. Surgery Treatment

Cancer surgery has been described in terms of thousands of years dating back as far as ancient Egypt (25). It's believed that the first documented example of an adequate surgical procedure for removing breast tumors was presented during the 1st century AD by the Greek physician Leonidas, this method involved carefully cutting into the healthy tissues surrounding a tumor followed by serial cautery until the tumor was fully removed (26,27).

The concept of cancer surgery emerged before the advent of antisepsis and anesthesia,150 years ago, only ill-advised or brave individuals had been able to undergo

surgery because the morbidity and mortality had a high curve, cancer surgery has been supported by continuous innovation and research in the field of medicine (28).

One of the most common and effective ways of treating various types of cancer is by having surgery, which is regarded as a less invasive and less damaging procedure compared to radiotherapy and chemotherapy, another reason why people choose to have surgery is that it allows the tumor to be removed without damaging the surrounding tissues, various types of surgical procedures can be performed depending on the patient's condition (29).

Preventive surgery is one of the most prevalent forms of cancer surgery that may be performed, this type of procedure is carried out to remove tissue that's likely to become cancer, for instance, a colposcopy is performed for the removal of atypical cervical lesions a biopsy is also important when it comes to identifying and treating cancer, aside from breast and colon cancer, other types of cancer such as testicular cancer can also be treated with surgery.

For patients with advanced breast cancer, surgical resection is often performed to remove cancer, it can also be done for other types of cancer, such as malignant bowel disease, in addition, reconstruction is also carried out to improve the cosmesis after a mastectomy (30).

2.3.2. Radiotherapy

Since Wilhelm Roentgen discovered the X-ray in 1895, which was quickly followed by the first therapeutic application of radiation in 1896, the discipline of radiotherapy has seen great scientific developments, these innovations have resulted in the creation of new approaches and techniques that have improved the quality of patient care and prevented cancer from spreading (31).

New knowledge of radiobiology has led to the development of models for integrating biological precision with physical targeting for customized cancer treatment (32). Currently, radiation is one of the major therapeutic methods utilized in more than half of all cancer patients, either alone or in combination with surgery and chemotherapy to treat a wide spectrum of malignancies (33).

Radiotherapy is based on the use of two kinds of radiation, namely Electromagnetic radiation and particles the first is caused by X-rays and gamma rays, whereas the second is caused by eis electrons, neutrons, and protons.

Depending on the type of cancer, radiation therapy can be delivered either internally or externally, the beam is supplied in the first modality from an external source of the body, while in the latter It is inserted into the damaged region and lesions that need

High-energy X-rays or particles are employed in radiation treatment to kill cancer cells (36). This method has been proven to be effective in killing the most cancerous cells while avoiding normal cells, the advancement of technology has allowed the delivery of precise and effective radiotherapy to tumors with minimal injury to normal tissues, understanding the mechanisms of radiation resistance and its effects on normal tissues has also improved the treatment of cancer (37).

Over the past few decades, technological advances have allowed significant advances in non-formal 3D radiotherapy, such as intensity-modulated radiotherapy (IMRT), stereotaxic (body-oriented) radiotherapy (SBRT), and improved imaging systems (i.e., targeted radiotherapy) Imagery, IGRT, has enabled accurate delivery of radiation doses matching exact runtime dimensions while minimizing radiation exposure to surrounding normal tissues (38). Ionizing radiation therapy has been regarded as a promising treatment for various types of cancer due to its ability to improve the survival rate of patients, this therapy is mainly based on the power of its cytocidal properties and the ability to selectively target the tumor (39).

2.3.3. Chemotherapy

During the 1900s, Paul Ehrlich, a German scientist, started looking into developing drugs to treat diseases, he was the first to introduce the concept of chemotherapy, which refers to the use of chemicals to treat a specific disease, he was also the first to show how animal models could screen various chemicals for their potential cancer-fighting properties.

In 1908, he used the rabbit model to develop arsenicals, which were then used to treat syphilis, he was also interested in developing drugs to treat cancer (40,41).

Chemotherapy is referred to as a type of drug that kills fast-growing organisms (Figure 2.3). Chemotherapy is known as a 'cytotoxic' medication Which means that it is toxic to the cells (47). The cancer cells multiply rapidly and in a large number of most cells of the body, so that why Chemotherapy is a common treatment for cancer.

There are several chemotherapy medicines available, Chemotherapy medications can be used alone or in combination to treat a variety of malignancies (42).

Chemotherapy is an effective treatment for many types of cancer, but it can have unforeseen effects, some chemotherapy side effects are modest and treatable, while others might have serious repercussions, using a variety of therapy to eradicate hidden cancer cells (43).

Following surgery, which often involves the removal of cancer cells, chemotherapy can be administered to eliminate any residual cancer cells in the body, this form of treatment is referred to as adjuvant therapy (44).

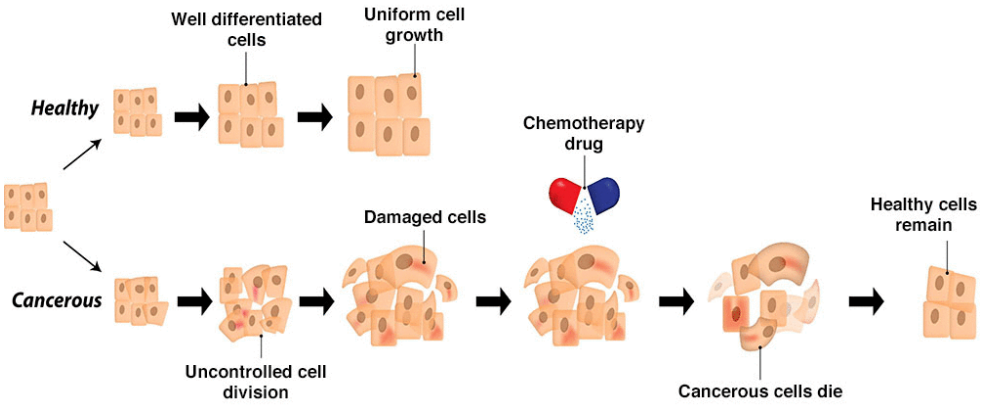


Figure 2.3. diagram showing accurately the progression of cancer and how it is halted using chemotherapy (47).

2.3.3.1. Chemotherapy for illnesses other than cancer

Other diseases that can benefit from chemotherapy include:

- **Bone marrow disorders:** A bone marrow transplant is a type of treatment that can be performed for patients with certain types of blood and bone marrow diseases also referred to as a stem cell transplant, Chemotherapy can also be administered to help the body prepare for transplantation.

- **Immune system dysfunction:** Lower chemotherapy dosages can aid in the reduction of an overactive immune system in people suffering from immune system illnesses such as rheumatoid arthritis and lupus (45,46).

2.3.3.2. Chemotherapy and cancer treatment

Depending on the type of cancer, chemotherapy can be given in various ways, it can be done through a vein, intravenously, or intravenously, if this is the case, then it will usually be given at a day-treatment center or hospital, after the treatment, patients can usually go home, depending on the type of chemotherapy you're receiving, these sessions may last for up to an hour or less or a few hours and depending on how you feel during and after treatment. If you need to give medicine for a short time, up to a few days it can be done through a small plastic tube that's inserted into a vein. It's usually done with a small needle. This method is similar to how a blood test is performed (47).

2.3.4. Overview of Natural Products in Cancer Treatment

Natural ingredients are now playing a significant part in cancer therapy these days, including the presence of anti-cancer agents in large numbers that are used in clinics, whether they are derived from nature or various natural products such as animals, plants, and microorganisms (also of marine origin) (48).

Natural products have recently generated interest in the realm of chemotherapy for cancer patients, due to the growing interest in natural products in the treatment of cancer, the field has been searching for new and effective ways to treat the disease, some

of the factors that have contributed to this interest include its non-toxic nature and its ability to work on multiple goals, the co-administration of natural products has also had a role with traditional chemical treatments in mitigating toxicity and reducing the use of sausages in traditional medicines (49).

Over the past half-century, there have been a lot of studies done on the potential of natural items to cure cancer, community efforts have resulted in the discovery of new therapeutic opportunities and the widespread use of natural products (50). Currently, more than 60% of anticancer medications are sourced from natural sources in some form or another (51).

Natural products can have various positive effects on human health, they can also help prevent cancer, for instance, certain compounds can suppress the growth of tumors, since cancer cells have developed multiple mechanisms to resist the induction of apoptosis ,by natural compounds, modulation of apoptotic signaling pathways is a major factor in antitumor activities (52).

For experts, marine organisms produce excellent natural chemicals that can be used as effective medicines, the increasing number of patents issued for these chemicals in the past few years has justified the importance of their use as therapeutic agents.

Despite critical supply challenges, active ingredients from the seas explored elements derived as sources of drugs and anticancer, antiviral, anti-inflammatory, and antimicrobial therapies for many other conditions (53).

Natural products have an incredibly wide variety of unique structures that can be used to create new compounds, over the past 30 years, various marine organisms, such as bacteria and algae, have started to produce new compounds, while terrestrial plants and soil microbes have typically been the main sources of natural products, marine organisms have started to produce new compounds (54,55).

2.3.4.1. Plant Product

Phytotherapy is believed to be the oldest form of therapy in the world, it involves the use of plants that have been traditionally used for healing, and According to estimates, two-thirds of the world's population uses therapeutic herbs derived from folk remedies. The cost of traditional medicines is lower than that of pharmaceutical companies, in most cases, folk medicine is the only source of health care.

Applicability to rural areas, folk medicine is a vital part of their health care, according to the World Health Organization, more than 21 thousand species of plants are used in herbal medicine (56).

The plant kingdom has always served long been known for its useful drugs, it has been used for treating various diseases, the search for anti-tumor agents from the plant kingdom has been inactive pursuit since the 1950s but the greatest impetus came after the discovery of vincristine and vinblastine from Catharanthus roseus around 1960, the folklore knowledge of its hypoglycemic activity stimulated the investigation of this plant which ultimately led to the discovery of the above active principles, later on, another semi-synthetic derivative namely vindesine has been developed.

Cancer research has led to a reassessment of many primitive plant cure and a massive search for anti-tumor principles have provided many active products belonging to the following classes of chemical compounds diterpenes, lignans, quassinoids, ansamacrolides, and alkaloids (57). Several compounds derived from plants are used successfully in the treatment of cancer (Table 2.1.) (58).

Table 2.1. anticancer agents derived from plant (58).

Compound	Cancer use	Status
Vinblastine	Breast, lymphoma, germ-cell, and renal cancer	Phase III/IV
Vincristine	Leukemia, lymphoma, breast, lung, pediatric solid cancers, and others	Phase III/IV
Paclitaxel	Ovary, breast, lung, bladder, and head and neck cancer	Phase III/IV
Docetaxel	Breast and lung cancer	Phase III
Topotecan	Ovarian, lung, and pediatric cancer	Phase II/III
Irinotecan	Colorectal and lung cancer	Phase II/III
Flavopiridol	Experimental	Phase I/II
Acronyciline	Experimental	Phase II/III
Thalicarpin	Experimental	Preclinical/phase I
Bruceton	Experimental	Preclinical/phase I

It has been known that some of the polyphenols found in green tea can have anti-cancer properties, one of these is Epigallocatechin-3-gallate in many studies, Epigallocatechin-3-gallate is known to have the ability to inhibit the various stages of carcinogenesis from initiation and consolidation to progression. It can also cause cell cycle disruption and apoptosis; it also shows the capacity to suppress cancer cell growth It has been demonstrated to be involved in the control of different signaling pathways involved in cancer cell growth and proliferation (59).

There are different types of marine plants that are important to ocean biomass, Bacteria, actinomycetes, cyanobacteria, fungus, microalgae, seaweeds, mangroves, and other halophytes are among them, together, these are known as more than 90% of the ocean's biomass (60). These organisms are highly productive and have a wide variety of biological activities, and are chemically unique they can also give important information for the development of novel cancer medicines (61).

Marine plants which mostly belong to polyphenols and sulfuric polysaccharides are rich in medicinally active chemicals, it has been proven that the chemicals showed some pharmacological properties, in particular, immunomodulatory, antioxidant, and antitumor properties, the phytochemicals may activate macrophages and induce cell-mediated apoptosis to prevent the damage caused by DNA oxidation, they can also prevent the formation of carcinogens (62). Despite the abundance of chemicals in marine life, the marine floras are still unexplored for the development of lead compounds for cancer treatment (63).

2.3.4.2. Animals' Product Overview

Zoo pharmacognosy is a multidisciplinary approach to the behavioral and physiological responses of animals, it involves the study of various aspects of their self-medication behavior recent research has revealed that several plants employed by animals for self-medication contain antitumoral secondary metabolites, these chemicals have the potential to be used therapeutically in humans (64).

Zootherapy is another related and sometimes confusing term it involves the use of animal parts and toxins to treat various human ailments, this is often referred to as a mixture of animal parts and by-products (65).

Animal-based remedies were used by ancient civilizations such as Egypt and Mesopotamia, according to historical writings, these medicinal methods were then passed down across many locations and exist in modern medical records.

Animal parts were used in approximately one-tenth of the treatments by Dioscorides' in Materia Medica, these medications were also included in the Islamic, Chinese, and Ayurvedic inventory, human illnesses can be healed via zootherapy, which involves the use of natural poisons and organs from animals, several research on the antitumoral by-products of terrestrial animals has been conducted (66,67).

One such research, for example, discovered that several meat-derived peptides (GFHI, DFHING, FHG, GLSDGEWQ) had a substantial effect on MCF-7 human breast

cancer cells, these peptides inhibited the viability of human gastric adenocarcinoma cell lines (AGS) (68).

Researchers (the Su group) isolated bioactive 3 peptides (ACPB-3) from goat spleen or anticancer goat liver, in vitro and in vivo anticancer activity of a human gastric cancer cell line (BGC-823) and gastric cancer stem cells were demonstrated (GCSEs). (69,70). For example, TCM uses ingredients extracted from 36 animal species, Such as rhinoceros, black bear, tiger, and sea horse - the most endangered animals. Indian Ayurvedic medicine recommends snake venom to treat arthritis, while in South America, Asia, and Africa the bite and crushed tusks of tarantulas are used in traditional medicine to treat many ailments, from cancerous tumors to dental pain and asthma (71).

2.3.4.3. Treatment Through Insects

An insect's defense against a pathogen is made up of a wide range of peptides and proteins, these are designed to protect against various infections, the use of these agents acts differently to target tumor cells or viruses by direct impact or by stimulating the insect's immune system.

It has been shown that some insect peptides can suppress protein expression, viral gene, and rybosilate DNA, and others can induce membrane lysis, cell cycle arrest, or induce apoptosis (72).

The anticancer factor Pierisin-1, which was derived from the Pupae pupae extract of butterfly, was discovered in 1998, Purification revealed that this is a 98 kDa protein (73). And also, Lectins, another type of protein, were found in the insect larvae of the species S. peregrine, it has been known that it can induce agglutination and a cytotoxic effect /antitumor (74).

In different cancer cell lines, the anticancer effectiveness of ethanolic extract of propolis (EEP) was examined, among these are MCF-7 human breast cancer, HT-29 human colon adenocarcinoma, Caco-2 human epithelial colorectal adenocarcinoma, and B16F1 murine melanoma, the cytotoxic and apoptotic effects of at various dosages were studied in four different cancer cell lines, the Trypan blue dye exclusion and MTT tests were used to

evaluate the impact of EEP on numerous cell lines, in all cell lines studied, EEP exhibited a mortality rate of greater than 50%. (i.e., IC50 value). The atomic force and light microscopes indicated different apoptotic features in all cell types after 24 hours of treatment with 250 g/mL EEP. Because of the early onset of this sickness, Indian stingless bee propolis is an effective anticancer treatment.

Furthermore, Indian stingless bee propolis' antioxidant capabilities have been shown to be useful in the therapy of certain cancer cells (75).So, it shows us that Many studies have shown that insects can enter into the treatment of cancer through the substances they secrete or by parts of their body also by eating themes.

A recent Italian study revealed that eating insects such as ants and cockroaches protect against cancer, this by thanks to a study by scientists from the University of Rome, in which they proved that insects have many benefits, especially ants when ingested, as they protect against cancer with antioxidants, and antioxidants reduce the development of free radicals, which protects against DNA damage.

Scientists studied the effectiveness of eating insects in fighting cancer after crushing various insects, including ants, cockroaches, and silkworms, and found a large number of antioxidant compounds in the insect powder such as vitamins A, C, E, and beta-carotene, caterpillars and African Silkworms had higher levels of antioxidants than olive oil, also, powdered crickets and silkworms have a higher which is 5 times higher antioxidant content than orange juice. Following these findings, one of the researchers responsible for the study said that about 25% of the world's population consumes insects, showing that the rest of the world should do so as well According to Mauro Serafini, an Italian professor, edible insects are nutritious and contain high levels of nutrients, unsaturated fatty acids, proteins, vitamins, minerals, and fiber (76). Also, the scientists discovered the venom of bees can use as a treatment to treat illnesses such as rheumatoid arthritis, and other neurological disorders (146-149).

2.3.4.4. Treatment Through Arachnids

Arachnids, which are also known as joint-legged invertebrates, are found mainly on land and in both freshwater and marine environments but most of them live on land.

There are over 100,000 species of spiders that are named, as well as various kinds of scorpions, mites, daddy-long-legs, and ticks, there may be up to 600,000 species in total, there are also unknown numbers of these creatures (77).

Most adult spiders have eight legs, which is more than the six legs of most insects However, spiders also have an extra pair of weapons designed to allow them to defend and feed themselves (78). Thus, we realized the difference between insects and arachnids the arachnids have four pairs of legs, eight totals, and insects have three pairs of legs, six totals (77). There areso many types of arachnids in general like the figure below (Figure 2.4.) but here I will only talk about two types of them the spiders and scorpions in anticancer activity.

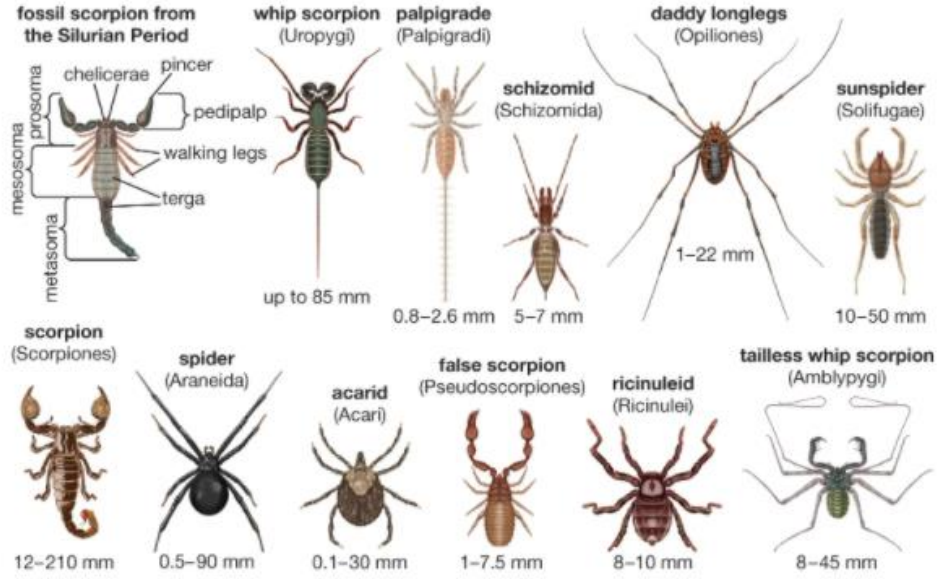


Figure 2.4. some types of arachnids (79).

a. Spiders

Spider venoms have complex mixtures of compounds, such as peptides, proteins, salts, and small organic molecules. However, the venoms of a few species are considered dangerous to humans.

Spider venoms are known to have high chemical diversity, which makes them an attractive target for chemical exploration, spider venoms can potentially be used to treat various diseases. However, their development is still in its early stages, understanding the mechanisms by which these toxins work is still needed to develop effective therapeutics (80).

Most spider venoms include over a thousand distinct peptides with weights ranging from 2 to 8 kDa. Including that the main component of most spider venoms is peptides (81). It is estimated that about 10 million or more biologically active peptides may have a high probability of being in the venom of different types of spiders (82).

Macrothele venom can kill human hepatocellular carcinoma cells (BEL-7402), it also inhibits DNA synthesis and proliferation of these cells, it also plays a function in cell cycle arrest during the G0/G1 phase, and C-mycin BEL-7402 downregulated cells and induced cell apoptosis, after treatment with Macrothele spider venom, it killed programmed cells at a very high rate, and treatment with this venom led to remarkable morphological changes, inhibition of proliferation, and the effects of this toxin were observed on human cervical cancer cells (HeLa), caspase-3 reulation, and inhibition of G0/G1 cell cycle arrest (83).

Lycosin-I is a cationic peptide that can be used to inhibit the growth of cancer cells which is isolated from Lycosa singoriensis (Figure 2.5). It can also be used to treat other diseases, the lycosin-I peptide can be used to block the growth of cancer cells by activating various signaling pathways in vitro as well as in vivo. These include the triggering of apoptosis, and the inhibition of proliferation, Lycosin-I treatment with (40 μM) give a resulted in the death of over 90% of human tumor cells in the following cell lines: HCT-116 colon adenocarcinoma, fibrosarcoma (H1080), cervical cancer (HeLa), prostate cancer (DU145), The (HepG2) which is Hepatocellular carcinoma, and (H1299, A549) which is lung adenocarcinoma and other types of cancer, when utilized at the same dose,

Lycosine-1 is less dangerous to non-cancerous cells, Lycosin-I stimulated the death process mediated by mitochondria, sensitizing cells to the effects of apoptosis, and increased p27, preventing cell growth. (Figure 2.6) (84). IToproduce effects, it is necelycosin-I must bind rate the cell membrane (85).

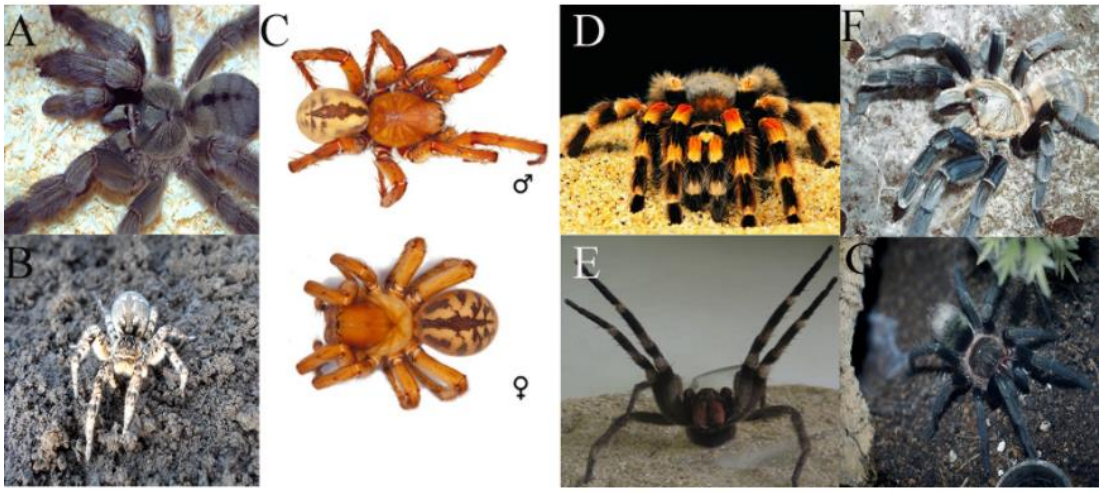


Figure 2.5. images of spiders. (A) Psalmopoeus cambridgeiMicha L Rieser, a molecular biologist, provided this picture. (B) Avereanu provided this photo of Lycosa singoriensis. (C) Lachesana tarabaevi was also provided by Alexey et al (86). (D) Ornithoctonus huwena; (E) Peigneur et al provided this photo of the Phoneutria nigriventer (87). (F) OrnithoctonusHainana; (G)Vanessa S. provided this photo of the Thrixopelma pruriens.

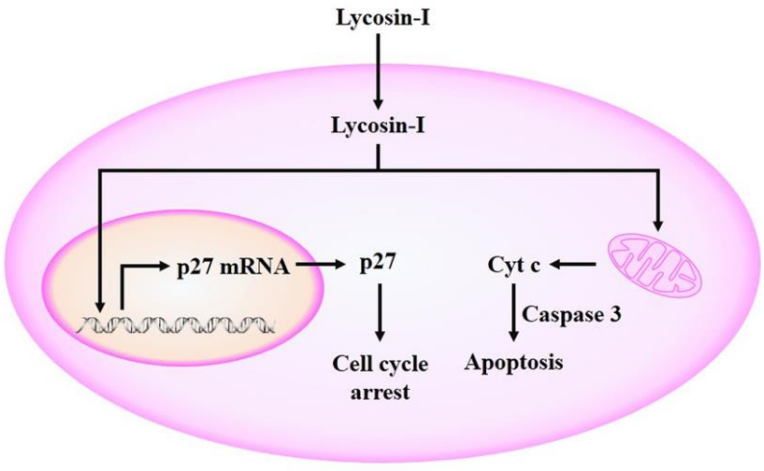


Figure 2.6. Lycosin-anti-tumor I's mechanism, Lycosin-I defies the biological need to limit cell cycle progression by regulating p27 mRNA and protein expression. Lycosin-I can attach to the itochondria membrane and activate the mitochondrial-mediated death pathway, promoting cancer cell apoptosis. Cyt c stands for cytochrome c.

Polyamine toxins (PATs) are formed when polyamines (PAs) combine with lipophilic acids derived from wasp or spider venom (Figure 2.7). PATs are being studied largely for their ability to block the ionotropic glutamate receptor (iGluR). They are also useful in developing drug targets for psychiatric and neurological disorders (88-91). Recent studies have shown that the presence of PATs has remarkable activity with antiproliferative properties.

In MCF-7 breast cancer cells, for example, PA366 is more active than PA389. which appears to depend on the lipophilic head group structure (PATs PA366 and PA389 from spider venom). In the head group, both PATs have the spermine (Spm, a 3-4-3 PA) chain and either a 4-hydroxyphenyl (PA366) or a 1H-indol-3-yl subgroup (PA389). These PATs' prospective targets were selected based on their distinct functions in breast cancer and tumor progression (92).

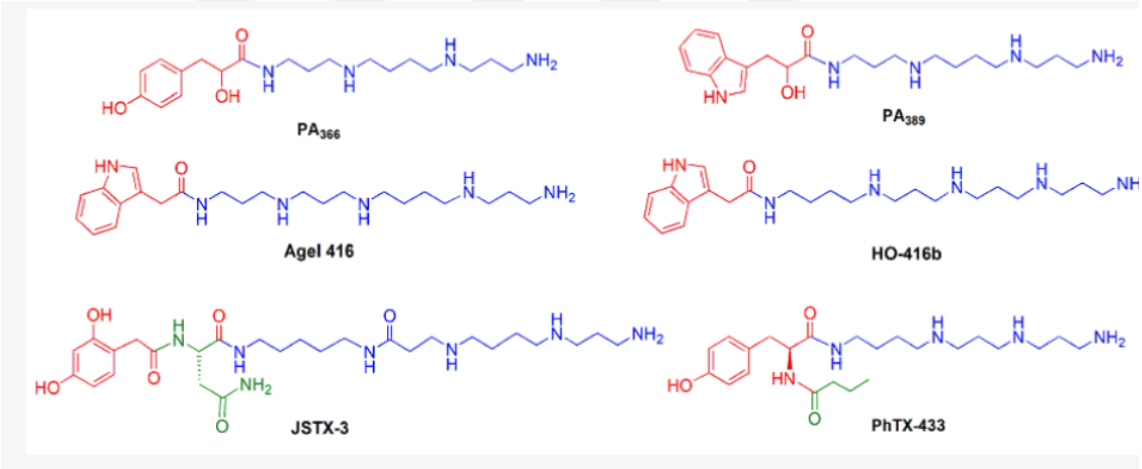


Figure 2.7. the organic PATS Spider and wasp venom structures are connected to the current investigation

Spider is one of the examples of cancer treatment with it is venom, many types of research show this technic but the more important animal in arachnids in our research is a scorpion, which we study the carapaces (outer shell) of scorpion farther than the venom that makes this researcher unique.

2.4. Scorpion

Scorpions are arthropods that measure about 13 to 220mm long, it is easily recognized due to its various structures (93). Although all scorpions are poisonous, only around fifty species are dangerous to humans. (94, 95).

Scorpions can be very dangerous, especially for children and the elderly who may have cardiovascular or respiratory conditions (96-99). These animals are considered medically important because they defend themselves by biting humans, most of the time, they sting to protect themselves (100,101).

Certain species in the Buthidae family have medicinal relevance, Buthus, Parabuthus, Mesobuthus, Tityus, Leiurus, Androctonus, and Centruroides are a few examples (102,103). The most significant species among them are: (T. bahiensis), (T. serrulatus), (T. trivittatus), and (T. stigma,rus) in South America (104,105), and (C. notorious), (C. suffusess), (C. limpid), and (C. sculpturatus) in Mexico (106,107). and the following species are found in the Middle East and North Africa:(L.quinquestriatus), (A.crassicauda),(A.mauretanicus) ,(A.australis),(A.amoreuxi), and (B.occitanus) (108). In South African nations, the following species are found: (P.granulatus) and (P.transvaalicus) (109).In India, as well as M. tamulus and Palamneus Swammerdam (93).

A type of arachnid known as a Scorpion or Scorpionida is a long-tailed arachnid about 2500 species, that has a curved tail that's tipped with a venomous barb, it also has two grasping pincers at the front, there are over 2,500 recognized scorpion species, with 22 confirmed living families, although they are commonly found in deserts, they can also live in other habitats.

Scorpions are among the largest terrestrial arthropods, measuring about 6 cm in length on average about (2.5 inches) (110). Scorpions have few sexual characteristics; however, males have longer tail then females and are frequently slenderer.

The black emperor scorpion (Pandinus imperator) is a Guinean African species that is 18 cm (7 inches) in length and weighs 60 kilograms, is a behemoth among scorpions (more than 2 ounces). South Africa's rock scorpion, which is also known as a Hadogenes troglodytes, is the world's longest known scorpion.

Females can reach a length of over 20 centimeters (8.3 inches), the Caribbean Microtityus fundraise, the tiniest scorpion, measures 12 mm long (0.5 inches), a handful of the ancestors of current scorpions were enormous, two species (Gigantoscorpio will and Brontoscorpio anglicus) contain fossils that range in size from 35 cm (14 inches), while an unexplained species is believed to have been 90 cm in length (35.5 inches), the majority of desert and other arid-zone species are yellowish or light brown in damp or alpine settings; however, the hue is brown or black (110).

2.4.1. Scorpion Morphology

A scorpion's body is covered by an exoskeleton, which is a skeleton on the outside of the body, it's made up of chitin, a tough, flexible material composed of organic compounds (111).

The prosoma (cephalothorax) and opisthosoma (abdomen) are the scorpion's two main body parts (112). The anterior (front) part of the prosoma is covered dorsally (on top) by a hard-sclerotized plate known as the carapace, the 8 eyes (which range from 0 to 10 depending on the family, genus, and species) are distributed over the carapace in three groups: a median pair and two lateral groups of three (113).

The mesosoma and metasoma of the pre-abdomen, as well as a tail-like posterior part the majority of animals have no outward differences between sexes, the male metasoma is sometimes longer than the female metasoma, the mouthparts (carapace, eyes, and chelicerae), pedipalps, and four pairs of walking legs comprise the cephalothorax.

Scorpions have two eyes on top of their cephalothorax and two to five pairs of eyes in the front corners of their cephalothorax, chelicerae can be seen on the front and beneath the carapace, they have three segments and are pincer-like with sharp teeth, the scorpion's brain is placed in the cephalothorax in the back, immediately below the esophagus.

The pedipalp is divided into segments and utilized as a clawed limb for immobilizing prey, defense, and sensory purposes. Pedipalp segments include the coxa, trochanter, femur, patella, tibia, and tarsus on the pedipalp segments and other body components, Scorpions feature keels or carinae, which are darker or granular elevated

linear ridges. These are useful taxonomic characteristics. Proprioceptors, sensory setae, and bristles cover the legs. Some species' legs may have spines and spurs.

Pre-abdomen or Mesosoma or pre-abdomen makes up a sizable section of the opisthosoma. It is made up of the opisthosoma's seven anterior somites (segments), each of which has a tergite sclerotized plate covering it dorsally, Somites 3–7 is highly structured, with sternites (matching plates) located in the center. Somite 1 has gonopore-covering genital opercula on its ventral side.

Sternite 2 produces the basal plate, which contains pectins that act as sensory organs, following that, three to six somites have spiracles. They serve as access points for the scorpion's respiratory organs, also known as the novel's lungs. The spiracle openings of a certain species may be circular, elliptical, slits, or oval. Scorpio's seventh and last body, is devoid of appendages and exterior characteristics. The mesosome contains a dorsal blood artery or the heart, which is the center of the scorpion's open circulatory system.

The female gonads are made up of three or four tubes connected by two to four parallel transverse anastomoses, these tubes are the sites of oocyte formation as well as embryonic development, they are linked by two oviducts, which connect to a single atrium and go to the genital orifice. Males have two gonads, which are two ladder-like cylindrical tubes containing cysts that produce spermatozoa. Both tubes on each side of the mesosoma are connected by a sperm duct, they are connected to glandular symmetrical structures, which are paraxial organs that terminate at the genital opening, the spermatophore is made up of chihat that join together to create these hidden structures.

Certain animals' tails feature light sensors, a vesicle holding a symmetrical pair of venom glands makes up the telson. The hypodermic aculeus, a curled stinger with sensitive hairs, holds it externally To transfer its secretion to the bottom of the aculus, each venom gland contains a duct from the gland follicle to the direct sub-linear position of the aculeus, where there is a hole for each of the paired ducts to expand its secretion. The zinc-containing metal proteins of the stinger harden the blade (Figure 2.8) (114).

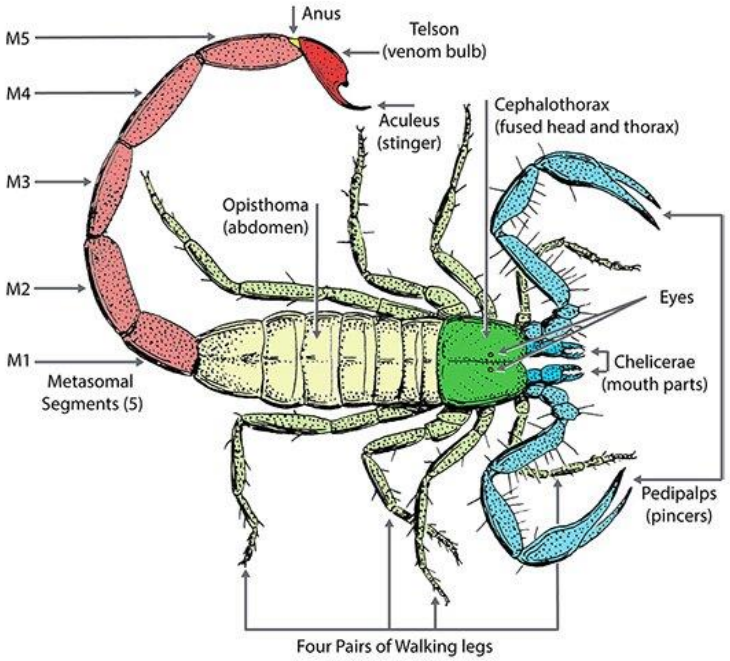


Figure 2.8. scorpion morphology (115).

2.4.1.1. Anatomy of Androctonus crassicauda Scorpion

Habitus (measurement unit : cm) :

a. Dorsal view

b. Ventral view

Chela : a. Dorsal

b. Ventral

c.Dorso- external side and moveable finger

Pectines

Spiracles

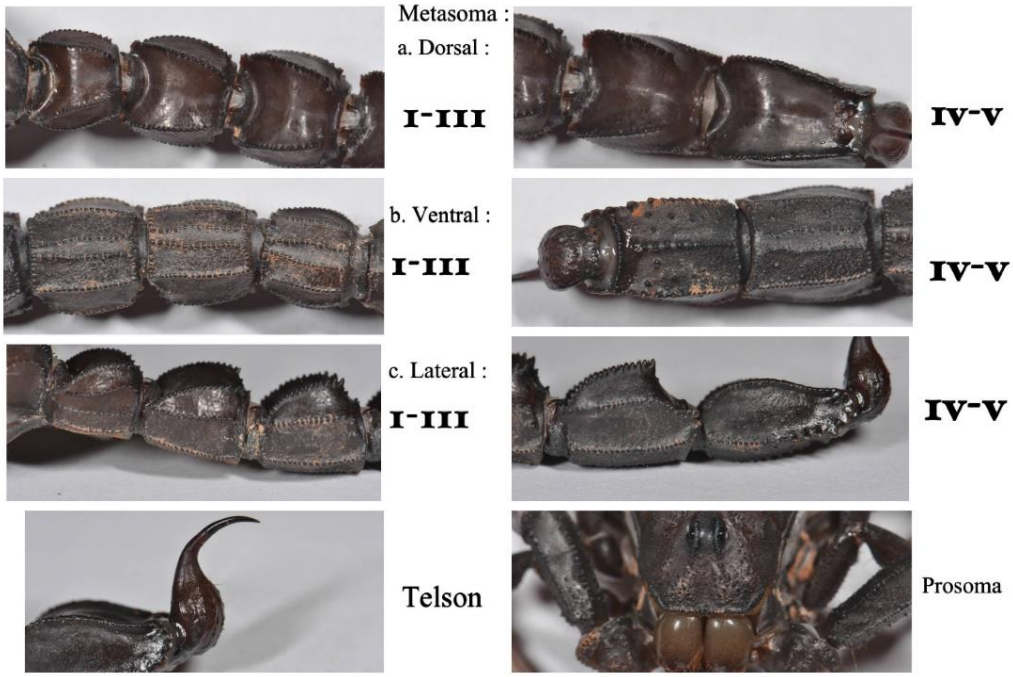


Figure 2.9. anatomy of Androctonus crassicauda scorpion (183).

2.4.2. The Biological Properties of Scorpion

Living fossils, such as scorpions have changed their shape very little over the past 420 million years can be defined as Scorpions having the lowest metabolic rate of all arthropods, for this reason, it spends most of its time in ambush for its passing prey.

Scorpions change their coats 6-9 times in 1-3 years before they become adults, the first step in control is sanitation, scattered in areas near residential boards, rocks, piles of wood and debris must be removed (116).

Pincers are employed to imprison and kill animals as well as to prevent predation., one of the scorpion's distinguishing features is its segmented tail, which is carried in a distinctive forward curve over the back and always ends with a stinger (117). In a defensive strike, Leiurus quinquestriatus may whip its tail at speeds of up to 128 cm/s (50 in/s) (124). The poisonous sting is utilized offensively as well as defensively.

Scorpions mostly eat insects such as grasshoppers, crickets, termites, beetles, and wasps. Spiders, solifugids, woodlice, and even small vertebrates like lizards, snakes, and

mammals are among the other prey. Earthworms and mollusks may be preyed upon by species with enormous claws (125). The majority of desert scorpions acquire their hydration from the food they consume, although some may take water from the damp soil water is used by scorpions that live in dense foliage and at moderate temperatures, using water on plants and in ponds (123).

Desert scorpions have a number of water-saving adaptations, Non-soluble substances such as xanthine, guanine, and uric acid are excreted without the use of water, Guanine is the most important component, as it optimizes nitrogen excretion, Scorpio skin is protected from UV rays by the sebaceous and wax glands, it can withstand high osmotic pressure which allows it to survive even when it is dry (122).

All of the continents except Antarctica have populations of these animals, and their diversity varies depending on their geographical location, in subtropical climates, the variety of scorpions is highest however, as you get closer to the equator and poles, the variety declines, that is why Scorpions prefer to live in temperatures of around 11 to 40 degrees Celsius, but they can also survive extremely cold temperatures, such as below zero degrees Celsius (121).

The majority of scorpion species are nocturnal or twilight, hiding throughout the day in burrows, crevices, and bark (119). Many species excavate a bunker several millimeters long beneath stones, others, such as spiders, reptiles, and tiny mammals, may exploit tunnels produced by other creatures, other animals dig tunnels that vary in nitric, Hadrurus species excavate tunnels up to 2 meters (6 feet 7 inches) deep. Mouthparts, claws, and legs are used to dig, individuals may assemble in the same shelter in a few species, notably the Buthidae family; shell scorpions can gather up to 30 individuals, female and juvenile families are occasionally seen together in some species (120).

After mating, the couple makes contact with their pedipalps and dances the promenade à deux (French for "a walk for two"). The male and female walk back and forth while facing each other in this dance while the male seeks for an appropriate location to place his spermatophore, in addition to kissing, the wooing ritual may include other activities, other behaviors involve touching and lifting each other's mouthparts, such as the Arbre Droit, which involves the partners rubbing their tails together, the male also

performs sexual stinging by squeezing the female's chelae, the dance can last for several hours (126,127).

Some scorpion species' gestation periods can extend up to a year (128). Every identified species gives live birth, and the mother tends for her offspring until their exoskeletons solidify, carrying them on her back (118).

Scorpions flash a spectacular blue-green when exposed to particular wavelength ranges of UV irradiation, such as those produced by a black lamp, due to fluorescent compounds in the cuticle such as beta-carboline, because this chemical substance is found in the scorpion's backbone, as a result, nighttime field surveys of these creatures have long used a hand-held UV light as a regular instrument, Figure 2.4.2 The scorpion's capacity to perceive light may be aided by this fluorescence (129).

Figure 2.10. the biological properties of scorpion (129).
a. The bright blue of the black emperor scorpion (Pandinus imperator).
b. The mother is a vibrant turquoise, while the infant is a dreary grey.

2.4.3. Toxin and Venom

Toxins are biomolecules generated by living organisms that already have different biological impacts on metabolic pathways of the exposed organism or are simply toxic substances originating from biological systems. Toxins can be utilized as both a protection mechanism and a hunting method (130). As mentioned earlier, all toxins originate from biological sources.

Toxins have evolved in a large number of organisms belonging to all spheres of life, a wide variety of species produce toxins, from primitive bacteria to higher eukaryotic organisms.

Toxins can be divided into groups according to different parameters and properties, are classified into two types based on their structure: non-peptide toxins and peptide toxins, Non-peptide toxins are mostly found in microbial organisms, plants, and fungi, the vast majority of the complex variety of animal venoms, usually peptide toxins, are orally active and act as a poison when ingested, this complex mixture is injected into prey or threat by subcutaneous, intradermally, or intravenous routes through a bite, sting, or any special apparatus venomous creatures, on the other hand, developed with a venom gland and a system that allows them to inject their toxins classified as venomous.

All poisonous creatures' organisms are classified as Animalia. so, they are often referred to as venomous animals, toxins from venomous animals are valuable natural resources for screening for unknown bioactive characteristics (130).

2.4.4. The Use of Animal Venom as Medicine and Anti-cancer Treatment

Toxins have developed among plants, animals, and microorganisms as a means of defense and prey capture (131). In nature, the majority of these poisons are classified as peptides, many of these peptides have been proven to be highly selective and relatively safe potent medicines (132). Non-peptide toxins are normally orally active, but peptide toxins are frequently found in specific organ-associated animal venoms designed to deliver them intramuscularly, subcutaneously, or intravenously (131). They are also

biochemical arsenals made up of combinations of bioactive substances known as toxins, such as salts, small molecules, proteins, and peptides (133).

Toxins have therefore been a substantial supply of molecular tools for dissecting physiological processes (134). as well as lead molecules for the creation of medications for a variety of illnesses, including chronic pain, diabetes, cancer, stroke, and autoimmune disease (135-139). Currently, seven venom-derived medications are on the market, including a cone snail toxin derivative for therapy.

One of the most promising advances in cancer research is the extraction of cancer-specific and anti-proliferative medicines from animal venom, because of its high specificity and selectivity for proteins and protein subtypes, venom is a valuable source of possible cancer medicines, for millennia, for example, Chinese traditional medicine and Indian Ayurvedic medicine have extensively documented the use of snake venom to treat arthritis and dried toad skin secretions to relieve pain (140).

Peptides' pharmacological properties, such as specificity, selectivity, stability, and smaller size (about 10–80 residues), make them a promising competitor and probable spearhead in the future battle against cancer, in comparison to synthesized peptides, venom peptides are disulfide-rich entities with strong target binding affinity (141,142).

The introduction of captopril, an antihypertensive medication, marked a milestone in the application of venom-peptide (143). Captopril, a dipeptide Ala-Pro analog derived from the snake Bothrops jararaca, binds well to the active site of the Angiotensin-converting enzyme (ACE). ACE is an enzyme that converts angiotensin I into the active vasoconstrictor angiotensin II, which controls blood volume, in the renin-angiotensin system. Captopril, an ACE inhibitor, is used to treat high blood pressure, a tri-peptide Phe-Ala-Pro analog enalapril was created in the same vein as captopril (143).

Captopril was first approved for usage in 1981, Cancer cells, unlike normal cells, may bypass the cell cycle checkpoint, which maintains intracellular balance in vivo (144). The ability to sustain proliferative signals, escape growth inhibitors, trigger invasion and metastasis, allow replication immortality, produce angiogenesis, and resist cell death, besides, two hallmarks of cancer are the regulation of cellular energetics and the avoidance of immunological destruction (145).

Also, we have other animals' Venom that can treat certain diseases like cancer and the importance of them is the scorpion which our study gets care of scorpion, here an example, then we will talk about the carapaces of the scorpion which an interesting study of our research.

The venom of the Indian black scorpion has been discovered to break DNA and inhibit the growth of human leukemic cells (150). Furthermore, Gonearrestide, a new peptide derived from scorpion venom, inhibited the development of primary colon cancer cells and solid tumors (151).

Commercialized medications based on bradykinin peptides obtained from the venom of the snake Bothrops jararaca, such as Captopril® and Enalpril®, are two effective hypertension treatments (152,153).

Ziconotide is another FDA-approved analgesic drug produced from -conotoxin, which was discovered in the venom of the sea snail Conus magus (154). We conclude that the complex variety of proteins in animal venoms has the potential to be a significant source of therapeutic medicines after assessing their pharmacological potential.

2.4.4.1. Scorpion Venom in Anticancer Activities

Scorpion venom is a complex collection of chemicals that perform critical roles in defensive systems, scorpion venoms have lower levels of enzyme activity in general, Phospholipases, hyaluronidases, mucopolysaccharides, low molecular weight molecules, mucus, inorganic salts, and other substances are among them. (155). Scorpion venom can connect to certain types of cells, such as cancer cells (156).

From roughly 1500 scorpion species, around 250 physiologically active proteinous compounds with anticancer potential have been found (157). Long-chain toxins are the most researched venoms because they typically activate sodium channels (158). whereas short-chain toxins activate potassium or chloride channels, which is necessary for predator defense (158).

The scorpion venom peptide arsenal is divided into two categories: disulfide-bridged peptides (DBPs), which often target membrane-bound ion channels, and non-disulfide-bridged peptides (NDBPs), which are a smaller group with multifunctional

capabilities (159-163). The majority of scorpion DBPs have three to four disulfide bridges and are divided into four types based on the type of membrane channels they interact with, the DBPs family of scorpion peptides targets membrane-bound ion channels like Na+, K+, Ca2+, and Cl (164). In many mammalian organisms, these channels play an essential role in regulating normal cellular physiology, interfering with their function by interacting with scorpion venom peptides can cause major alterations in their normal function, resulting in the numerous symptoms produced in mammals after scorpion envenomation (165).

The NDBPs are a separate class of peptides found in scorpion venom that has recently gained attention; many peptides from this class within the previous decade have been discovered and functionally defined, there has been a lot of interest in these peptides because of their diverse biological functions and multifunctional actions.

NDBPs, among other biological functions, exhibit antibacterial, as well as anticancer, hemolytic, anti-inflammatory, immune-modulatory, and bradykinin-potentiating properties, in contrast to DBPs, which have conserved structure-function connections, scorpion NDBPs are structurally varied and exhibit action against a wide range of biological targets (166,167).

The venom of Buthus martensitic Karsch suppressed human Inhibiting cell cycle progression and triggering apoptosis in breast cancer cells, and decreasing cyclin D1 protein levels, the author's Li et al (168). gonearrestide possesses significant anticancer action and has a significant impact in a variety of human malignancies, they observed that gonearrestide restricts growth via inducing cell cycle arrest in the G1 phase of primary colon cancer cells and solid tumors by inhibiting cyclin-dependent kinases 4 and increasing the production of cyclin D3, p27, and p21.

The scorpion venom Mesobuthus martensi Karsch (BmK) is likely the first scorpion species discovered to exhibit anticancer characteristics among all scorpions investigated for cancer research (170).

Bengali, a protein of 72 kDa derived from the Indian black scorpion Heterometric bengalensis kKoch, in human leukemic cells, has been demonstrated to trigger apoptosis, while not affecting normal human lymphocytes, reduced expression of heat shock proteins

(HSP) 70 and 90, as well as activation of caspase-3,9, have been associated with damaged nuclei and DNA fragmentation (171). Tityus disagreement yielded two novel apoptogenic peptides, Neopladine 1 and Neopladine 2, with molecular weights of around 30 kDa. Venom from scorpions (171-175).

2.4.5. The History of Scorpions in Turkey

In terms of climate, Turkey is a good place for scorpions to live, there are 2500 species of scorpions in the world today, divided into 21 families and 195 genera, with the development in recent years in Turkey, some scholars predict that the number of scorpion species will climb to 50.

Although Leiurus abdullahbayrami is the most poisonous scorpion species known in Turkey, scorpion antivenom is made from Androctonus crassicauda. According to the investigations, the antivenom from A.crassicauda has been manufactured constantly in Turkey since 1942 and is more impacted than other antivenoms.

Acrassicauda, one of the five most deadly scorpions in the world, is roughly 90 to 100 mm long, dark brown or black in color, with hefty claws and a curled tail. In Turkey, it is one of the most essential medical terminologies, A. crassicauda was discovered in greater numbers in Turkey's Southeastern Anatolia area (176).

Scorpion stings can cause serious envenomation, which is major public health concern in Turkey, morbidity and death from scorpion stings have previously been observed in several locations in Turkey (177). According to the most recent statistics, Turkey has twenty-eight scorpion species classified into four groups and thirteen genera (178, 179). These are the Buthidae, Scorpionidae, Iuridae, and Euscorpiidae families. Androctonus, Buthacus, Compsobuthus, Hottentotta, Leiurus, Mesobuthus, and Orthochirus are the seven genera that comprise the Buthidae family (180).

The venom of B. macro centers was also examined, and the 65 amino acid mammal-specific Na+ channel -toxin Bu1 was discovered. By screening the cDNA library from venom gland-produced mRNAs, three new -KTx type K+ channel inhibitors, MegTx1, MegTx2, and MegTx3, were generated from the venom of the scorpion M.

gibbosus. These poisons were determined to be members of the -KTx families -KTx3.x, -KTx9.x, and -KTx16. x. (181). In addition, the venom protein profile of several M. gibbosus individuals from the Aegean population was determined (182,183).

2.4.6. Carapaces of Different Animals Tested in Anticancer Treatment and Their Biological Functions

Exploration of the sea and its species is still in its infancy, even though the seas contain far more variety the search for new biological activity has just recently begun, chitin and chitosan are proteinaceous substances found in crustaceans and other marine sources (184).

Chitin and Chitosan were extracted from the carapace of a farmed Penaeus monodon species in this study (185). Chitin is a non-toxic, biodegradable polymer with a high molecular weight that is a nitrogen-containing modified polysaccharide, chitosan is deacetylated chitin, a non-toxic and biodegradable hydrophilic biopolymer found in nature. The current work aims to extract Chitin and Chitosan from farmed Penaeus monodon species eco-waste and investigate its antioxidant and in-vitro anticancer potential, chitin and chitosan are commercially available nutritional supplements (186).

Chitin and chitosan were extracted from the carapaces of grown Penaeus monodon species using chemicals such as 4% HCl for decalcification, 3% NaOH, 0.5 percent KMnO4, and 0.5 percent oxalic acid for decoloration NaOH at 40% concentration. The antioxidant activity was measured by DPPH scavenging, Nitric Oxide Scavenging activity, and Reducing Power activity, and Chitosan demonstrated higher DPPH inhibitory activity, better Nitrogen Scavenging activity than Chitin and Chitin, and similar Reducing activity MTT Assay and DAPI were used to investigate in-vitro anticancer effectiveness against MDA cancer cell lines (human breast cancer cell lines). Chitin is more cytotoxic than Chitosan to MDA cancer cell lines. According to the DAPI test, Chitin has higher apoptotic activity (187).

The research was published in the Egyptian Academic Journal of Biological Sciences with the title (Antioxidant and Anticancer Activities of Some Maggots Methanol Extracts) (2019). The study shows that whole-body extracts of maggots can serve as

antioxidants and anticancer agents. Surprisingly, these extracts, particularly C. albiceps extract, were discovered to exhibit therapeutic potential. For long years, insects have provided several natural items, including (silk, honey, beeswax, propolis and royal jelly) (220).

The extracts examined in this study demonstrated that C. albiceps extract had a more effective scavenging activity, although M. Domestica had a poorer scavenging ability than L. sericata The results of this study also revealed that Caco-2 cells exposed to different concentrations of tested extracts reduced the normal morphology of cells and cell adhesion capacity, with a preference for C. albiceps crude maggots extract, which decreased cell viability in a concentration-dependent manner, indicating the same principles involved in other anticancer agents. These findings back up recent research that found M. domestica maggots might be used clinically to treat stomach cancer and that alloferon, a peptide derived from Calliphora vicina, showed cytotoxic effects on cancer cells at a dosage of 25g.

The association between the order of potency in terms of antioxidant effects and the order of efficacy against Caco-2 cells, however, remains uncertain (221).

3. MATERIAL and METHOD

a. Martials and devices used in this research:

1. TissueLyser LT, a tiny bead mill that delivers quick, efficient disruption of up to 12 samples at the same time, is employed.

2. BD FACSVia™ Flow Cytometry System device.

3. (freeze-drying apparatus) Laboratory Vacuum freeze-drying technology, also known as sublimation drying, is a process of pre-freezing and sublimating water-containing materials under a vacuum. The original biological and chemical properties of the items are virtually the same after freeze-drying, making long-term preservation easier.

4. The Cancer Research Laboratory's CO2 incubator, laminar flow cabinet, inverted microscope, centrifuges, and refrigerators will be used in cell culture feeding, growth, cytotoxic and apoptotic testing, incubations, and fixation research.

5. Colorimetric MTT, protein quantification, and fluorometric intracellular radical and ELISA measurements will be performed using a multi-plate reader.

6. Fluorescent Microscope (Olympus CKX-51): For fluorescent staining research.

7. Flow cytometry (BD FACS-VIA): The Annexin-V test, as well as JC-1 and cell cycle analyses, will be used to detect apoptotic and necrotic cells.

8. Deep Freezer (-86oC) (Thermo Forma -86 Freezer): This freezer will be used to back up cell cultures and keep protein isolations.

9. [Core] Autoclave: Plastic and thermolabile material sterilization

b. Materials to be Provided:

Consumables (cell culture media, pipette set, cell line and apoptosis kit, and so on) for the analytical procedures used in the examination of the anticancer properties of the scorpion's outer shell are required within the scope of this project.

3.2. Preparation of Scorpion Outer Shell (Carapace) Extracts

In our study, scorpions that died in the scorpion venom center of Harran University were used. After the dead scorpions were thoroughly dried, the carapace parts

were separated with the help of forceps and powdered. The obtained samples were sterilized under UV-C light.

A group of (Androctonus crassicauda) scorpions were collected after we took from it the outer shell that includes (the shield and tail, without the needles), after collecting the scorpion's scales under suitable conditions and sterilizing them under ultraviolet light. Then draying process will be done, the scorpion shells are crushed well until the powdery state is formed.

After obtaining the powder, extracts of hexane, dichloromethane, methanol, Ethanol and water were synthesized, respectively. And then the cytotoxicity of the extracted extracts will be determined in identifying healthy and cancerous cell lines.

Figure 3.1. The outer shell of (Androctonus crassicauda) scorpions, and the process of making them a powder for using.

3.1.1. Preparation of Extractions

The method we used to extract the active substances from the scorpion's carapaces is: We weighed 0.250 g of scorpion powder in an Eppendorf tube (2 ml measurement) and used a pipette to take about 1000 µl (1 ml) of hexane and placed it in the, in the latter step all Eppendorf tubs placed in the device (TissuLayser LT) for 15 to 20 minutes and 50 1/sec twice.

The second step is to leave the tubes overnight in the refrigerator for further homogeneity. On the second day, return the tubs to the (tissue Layser LT) for the third time and then start using the centrifuge for approximately 15 min and 15,000 rpm.

Next, we took all the solvents that we got which in this way, we were able to take the active substances inside the solvent of hexane, dichloromethane, methanol, ethanol, and water.

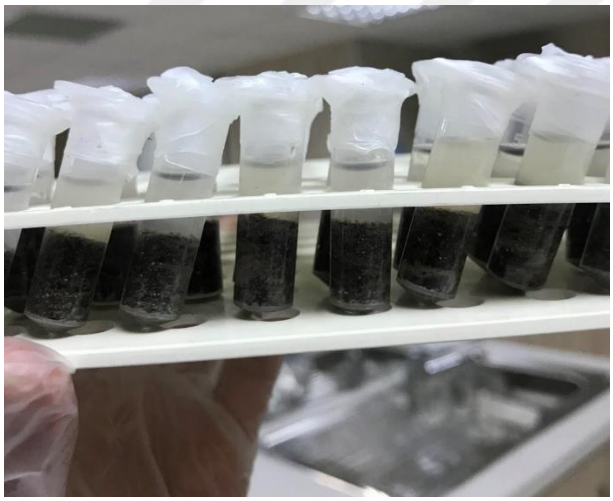


Figure 3.2. the centrifuge tubs which had a scorpion carapace powder with hexane covered by parafilm.

Figure 3.3. the extraction after using the (TissueLyser LT) device.

3.1.2. Filter And Drying

After completing these steps, we were able to filter all of our obtained extractions to remove any problematic particles to be used in the cell culture method, Then using a (a freeze dryer) device , the device began to convert all the solvent into pure powder without pollution, and useing (dimethyl sulfoxide) DMSO as solvent .

Figure.3.4. the (Freeze Dryer) device used by our study**.**

3.2. Investigation of Cytotoxic Efficacy of Scorpion Shell on Cancer and Normal Cells

3.2.1. Cells to Be Used in The Study

We will employ colon cancer (HCT-116), breast cancer (MDA-MB-231), prostate cancer (DU-145), lung cancer (A-549), bone cancer (U2OS), normal lung (BEAS-2B), kidney (HEK-293), normal prostate (PNT-1A), normal breast (CRL 4010), and endothelial cells (HUVEC) obtained from ATCC in the investigation. Cell lines will be put in the incubator at 37°C in a 5% CO2 environment. in DMEM-F12, RPMI-1640 broth with 10% FBS and 1% glutamine and antibiotic. Cells will be removed with a mixture of 0.25% trypsin, and 0.03% EDTA as recommended by the ATCC.

3.2.2. Care of Cells

To ensure cell growth and nutrition, DMEM F-12 medium containing a 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin will be used. While growing surface-dependent cells, the media will be drawn routinely every 2 days with the help of a sterile glass Pasteur pipette until the cells completely cover the surface, and then the environment of the cells will be changed by adding fresh sterile nutrient medium on top of the cells.

3.2.3. Cell Passage and Freezing Process

When the grown cells proliferate enough to cover the entire surface, the nutrient medium on the cells is removed with sterile Pasteur pipettes. Then, the surface of the cell culture is treated with commercially available sterile 1X PBS that does not contain Ca^{+2} and Mg^{+2} to increase the trypsin-EDTA treatment activity, and the cells are washed once and the PBS is removed from the medium ,by adding the Trypsin-EDTA to the cell culture, and the cells should separate from one another and from the plate surface. It is then centrifuged at 1200 rpm at +4°C.

Cell freezing solution prepared to contain 95% medium and 5% DMSO was added to the collapsed cells, In this solution, the cells are first stored in a deep freezer at -200 ^{0}C

or in a deep freezer at -860 ^{0}C for a short time, while they are stored in liquid nitrogen for a long time.

3.2.4. Cell Count

To obtain the desired number of cells from cells to new cultures, Thoma slide cell counts are performed. While 10µl of the current cell suspension is collected for counting, 90 µl of trypan blue is taken and the mixture is made up to 100 µl. A coverslip is placed on the Thoma slide and the homogenized cell dye mixture is slowly transferred from the edge between the Thoma slide and the coverslip with the help of a pipette. The cells in the large 9 squares on the Thoma slide are counted, to calculate the cells in millimeters (number of cells/ml), use the formula below.

Number of cells/ml = ((Number of cells counted X Dilution rate (10)) X 104) / 8.

3.3. Cell Culture Studies

3.3.1. Determination of Cytotoxic Effect by MTT Method

The cytotoxic effects of drugs will be studied using an MTT cell proliferation reagent. Cells will be opened from the stock and planted in 25 cm3 flasks, and when 80-90% full, they will be removed by trypsinization and inoculated into sterile 96-well plates as 1x104 cells/well. After 24 hours, the medium is withdrawn and scorpion carapace is injected into the cells at doses of 0, 2.5, 5, 10, 25, 50, 100, and 200 µg/ml, with the cytotoxic dose being attempted to be determined. The medications are given to each well in 200 µl increments and then incubated in a 37°C oven with a 5% CO_2 environment for 24 hours.

In this way, the cytotoxic effect of the outer shell of the scorpion is examined depending on the dose and time. After the incubation period, the medium in the wells is withdrawn, 90 µl of MTT solution is added onto 200 µl of the medium of the serum-free and incubated for 4 hours on average.

Following incubation, the plates' medium is removed, the formazan crystals are dissolved in 100 µl of DMSO, and absorbance values at 470 and 690 nm wavelengths are measured and recorded using a spectrophotometer. The cytotoxic dose (IC50) of the outer shell of the scorpion on the cells and the incubation time will be determined by the MTT method.

3.3.2. Morphological Examination of The Apoptotic Activity of The Scorpion Outer Shell by Fluorescent Microscope

The Acridine Orange / Ethidium Bromide method will be used for the morphological determination of the apoptotic effect of the scorpion's outer shell on the cells. Cell coverslips treated with the cytotoxic dose of carapace to be determined are taken and washed 2 times with PBS for 2 minutes. Then, it is left to stain the cells with acridine orange/Ethidium bromide working solution for 15 minutes. At the end of the staining, after the coverslips are washed with PBS, the dye is removed by keeping them upright on the blotter and examined under a fluorescence microscope.

3.3.3. Investigation of Apoptotic and Necrotic Activity on Cancer Type by Flow Cytometric Analysis (the Apoptotic Effect of Substances) With Annexin-v

The cytotoxic effect of the outer shell of the scorpion on cells, whether by activating the apoptotic pathway or by necrosis in the cells, will be analyzed by flow cytometry (BD FacsCanto) after the Annexin V Apoptosis Detection Kit FITC kit protocol is applied. FITC-conjugated Annexin-V lectin used in Annexin-V apoptosis assay; apoptotic It attaches to the phosphatidylserine phospholipid present on the cell membrane's outer surface. FITC causes fluorescent (FL1 detector; excitation= 488nm, emission=535nm) radiation of Annexin-V bound cells. This fluorescent glow in cells can be detected by the FL1 detector in flow cytometry.

Cells are classified according to the intensity of the radiation and placed on a diagram. Dead (necrotic) cells are detected with fluorescent PI (FL2 detector, excitation=

488nm, emission=562-588nm) dye that can bind to nucleic acids. PI passes through the damaged cell membrane of necrotic cells and stains their DNA. The fluorescent glow in cells whose DNA is stained is detected by the FL2 detector in flow cytometry. Cells are classified according to the intensity of the radiation and placed on a diagram. In the study, the kit protocol will be applied as follows after the lower and upper doses of the determined IC50 dose are applied to the cells to be seeded in 6 well plates in the incubation periods to be determined.

1. After the cells are trypsinized and removed, washed with PBS, centrifuged for about 5 minutes at 1200 rpm, and washed with 1X binding buffer.
2. The pellet will be suspended at 5 x 106 /ml cells with 1X binding buffer.
3. After transferring 100 µL of the cell suspension to a fresh Eppendorf tube, 5 µL of fluorochrome-conjugated Annexin V will be added.
4. It was placed in the incubator for 15 minutes at room temperature. It was then rinsed with 200 µL of 1X binding solution. The pellet will then be suspended in 200 µL of 1X Binding Solution and 5 µL of Propidium Iodide Staining solution before being examined in flow cytometry.

3.3.4. Investigation of scorpion outer shell on the cell cycle by flow cytometry

One of the most significant properties of chemotherapeutic medicines is their ability to limit the spread of malignant cells by interrupting the cancer cell cycle at various stages. The investigation in our study will look at the influence of the scorpion's outer shell on the cell cycle.

Effect Investigation of scorpion outer shell on the cell cycle by flow cytometry. It is a method that contains propidium Iodide dye and is used to distinguish cells according to the amount of DNA they contain at the stage of the cell cycle. In this method, cells are seeded into 6 well plates, approximately 1X105 cells. The cell cycle kit protocol will be applied to the cells after the determined dose is applied to the cells for the specified time. 500 µl of trypsin is applied to the cells inoculated on a 6-well plate, and 1 ml of media is added when the cells are observed to be removed off the surface.

Cells are deposited in Eppendorf tubes and centrifuged for 5 minutes at 5000 rpm to remove the supernatant and get pellets. The 1X buffer solution from the kit is then added to the cell suspension. and the cells are rinsed into the cells by centrifuging for 5 minutes at 1500 rpm.

250 µl of solution A (trypsin buffer) from the kit is gently mixed into the cell suspension. It is incubated in the dark for 10 minutes. After incubation, 200 µl of solution B (trypsin inhibitor and RNase buffer) is added to the cell suspension and incubated in the dark for 10 minutes. Following incubation, 200 µl of solution C is added to the cell suspension, and the tubes are mixed and incubated for another 10 minutes in the dark. Following that, cell suspensions are inspected using a flow cytometer, and each sample is evaluated using the kaluza analysis tool.

4. RESULTS

4.1. Cytotoxic Efficacy of Scorpion carapace on Cancer and Normal Cells

After examining the cytotoxic effects of scorpion carapace on various cancer and normal cells by MTT method, IC50 values were determined and all results are shown in Table 4.1. The change in cell viability was examined in a dose-dependent manner. The cytotoxic effects of scorpion carapace were investigated 24 hours after the application of 0, 2.5, 5, 10, 25, 50, 100, and 200 μg/ml doses to the cells. The viability of the cells in the control group without substance added (0μM) was accepted as 100% for all time periods, and the viability values of the other samples were calculated proportionally from the absorbance values.

When the IC50 values of scorpion carapace were examined, it was determined that the strongest cytotoxic effect was observed on prostate cancer cell DU-145 in hexane and water, show in table 4.1

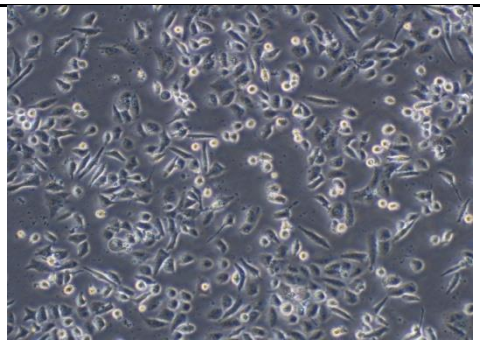
While scorpion carapace showed a strong cytotoxic effect on prostate cancer cells (DU-145), it was observed that its cytotoxic effect on normal prostate tissue cells (PNT1-A.) was lower than on cancer cells. For this reason, it has been determined that scorpion carapace has a selective effect on prostate cancer, and this cancer cell line will go under the other steps.

Table 4.1. IC50 values of scorpion carapace on normal and cancerous cells.

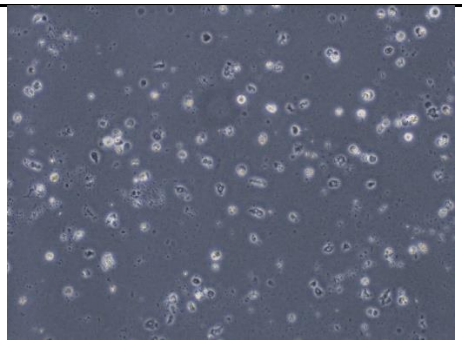
CELLS	HEXAN	DICHLOROMETHANE	METHANOL	DH2O	ETHANOL
CANCER CELLS					
MDA-MB-231	5.87	38.48	40.28	66.18	37.51
A549	7.95	60.17	51.01	61.31	42.18
HCT-116	14.19	171.37	68.36	328.03	44.68
DU-145	3,95	42.37	44.42	113.21	35.46
U2OS	13.22	54.09	47.34	213.62	36.90
NORMAL CELLS					
HEK-293	3.76	39.20	55.47	347.70	39.64
PNT1-A	14.63	51.74	37.11	62.99	32.51
BEAS-2B	6.29	50.01	37.55	74.14	34.38
CRL-4010	3.74	26.86	37.77	75.75	33.04
HUVEC	3.73	39.66	34.48	132.49	14.83

4.2. Effects of Hexane Extract on cell morphology of DU-145 cell

The effects of hexane extract on DU-145 cell morphology were investigated. The results are given in figure 4.1. When the images were examined, it was observed that 5µg/ml hexane extract led the cells to apoptosis. In the morphological images, it was determined that the number of cells decreased depending on the dose, and the membrane structures of the cells changed, giving a typical apoptotic cell appearance (Figure 4.2).



KONTROL DU-145



DU-145 Hexane Extraction 5 µg/ml

Figure 4.1. Morphological representation of hexane extract on DU-145 cell line

4.2.1. Investigation of the effects of Hexane Extract on the cell DNA of DU-145 cell by fluorescent staining method

The Acridine Orange / Ethidium Bromide method used for the morphological determination of the apoptotic effect of the scorpion's outer shell on DU-145 cell line. Cell coverslips treated with the cytotoxic dose of carapace to be determined are taken and washed 2 times with PBS for 2 minutes. Then, it is left to stain the cells with acridine orange/Ethidium bromide working solution for 15 minutes. This staining method was used to observe the apoptotic and necrotic effects of hexane extract on DU-145 cells. Viable cells were observed as homogeneous green stained chromatin, necrotic cells as homogeneously orange stained chromatin, early apoptotic cells as condensed and fragmented green stained chromatin, and late apoptotic cells as condensed and fragmented orange stained chromatin (Figure 4.2).

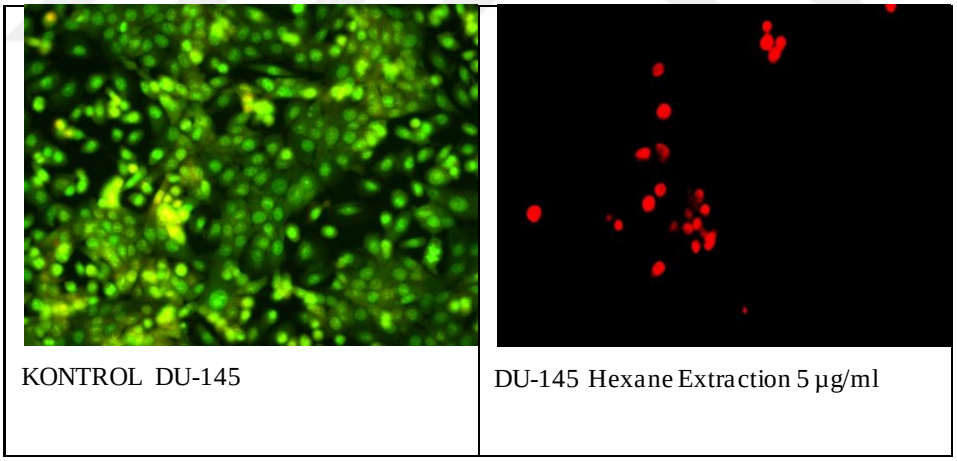


Figure 4.2. Images of hexane extract on acridine orange-ethidium bromide staining on DU-145 cell line

4.3. Investigation of the effects of hexane extract on cell apoptosis of DU-145 cell by Flow Cytometric Annexin-V Apoptosis/Necrosis Analysis

Apoptosis is another process that causes cell death. We found a proportion of sub-G1 population resulting from DNA fragmentation in flow cytometry examination of the cell cycle (Fig. 4.6), indicating the possibility of apoptosis induction as an additional mechanism of hexan mediated anti-proliferative impact. The Annexin V-propidium iodide (PI) test was used to confirm the participation of apoptosis in DU-145 cell death (Fig. 4.4). Early apoptosis is distinguished by the translocation of phosphatidylserine to the plasma membrane's external side, as determined by Annexin V staining. Flow cytometry analyses of Annexin V and propidium iodide staining demonstrated that the same chemical had varying efficacy for inducing apoptosis in the DU-145 cell line. in late apoptotic cells (Annexin V+, PI+) were significantly increased by hexan extract in DU-145 cell line .

The flow cytometric annexin -V technique was utilized to test if the cytotoxic impact of scorpion carapace on prostate cancer cells is apoptotic.

It was observed that 5 µg/ml dose of hexane extract on DU-145 cells caused 44% of cell death. It was observed that 38.6% of these deaths were caused by late apoptosis and 5.4% by necrotic origin. (Figure 4.3).and (4.4)

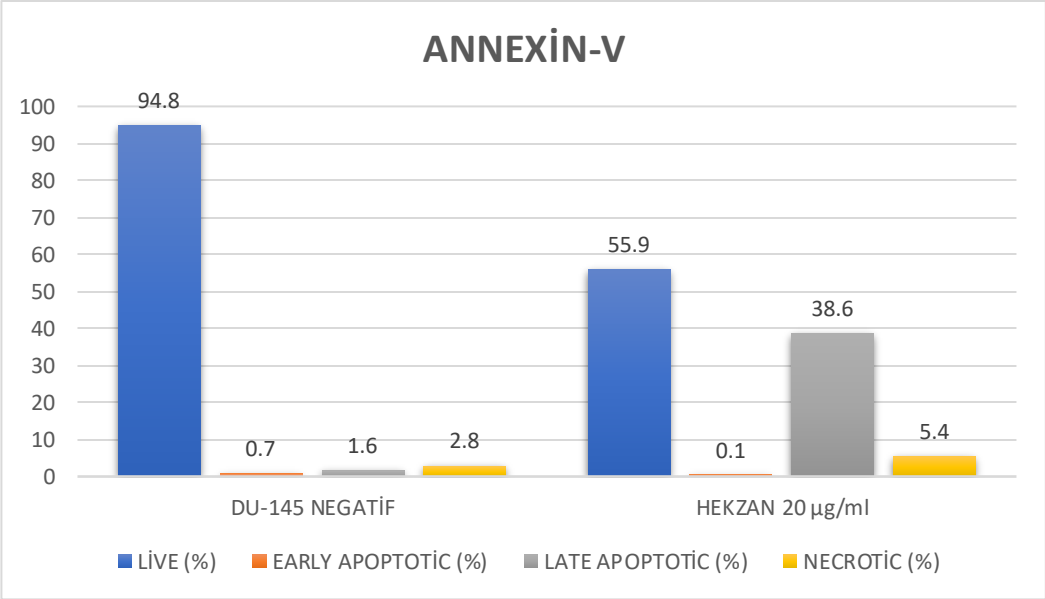
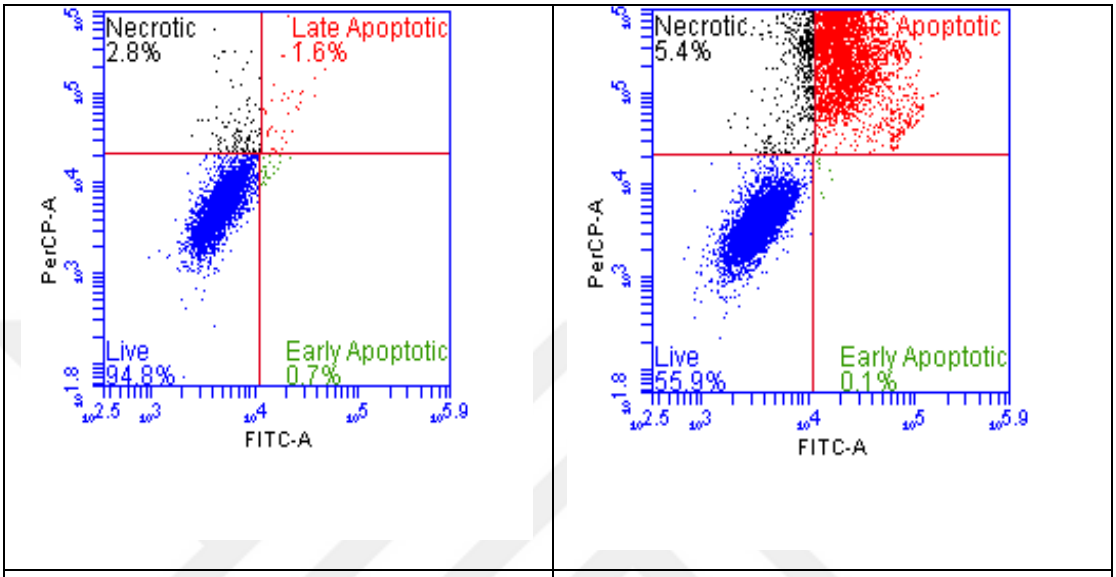


Figure 4.3. Annexin-V Apoptosis/Necrosis analysis of hexane extract on DU-145 cell line



A01 Du-145 negative Gate :cells	B02 Du-145 5 µg -Hexane Gate :cells

Figure 4.4. Investigation of the effect of scorpion carapace on DU-145 cell apoptosis with Annexin-V

4.3.1. Investigation of the effects of hexane extract on cell apoptosis of DU-145 cell by Flow Cytometric Cell Cycle Analysis

The analysis of the effects of Hexan extract from scorpion carapace on the cell-cycle phases and apoptosis of DU-145 cells was performed. Cell proliferation is dependent on the cell-cycle progression, in which cells pass through the G0/G1 phase to the S phase and the G2/M phase. the cells treated with hexane, and there was a consequent increase in the number of cells in the G0/G1 phase, it has also been shown that induced cell cycle arrest in the G0/G1 phase is happening in a concentration-dependent manner, The effect of hexane on the cell cycle was shown to be time-dependent

We found that the 5 µg/ml dose of hexane extract on DU-145 cells kept the cells in G0/G1 phase at a rate of 79.7%. (Figure 4.6) and (4.7).

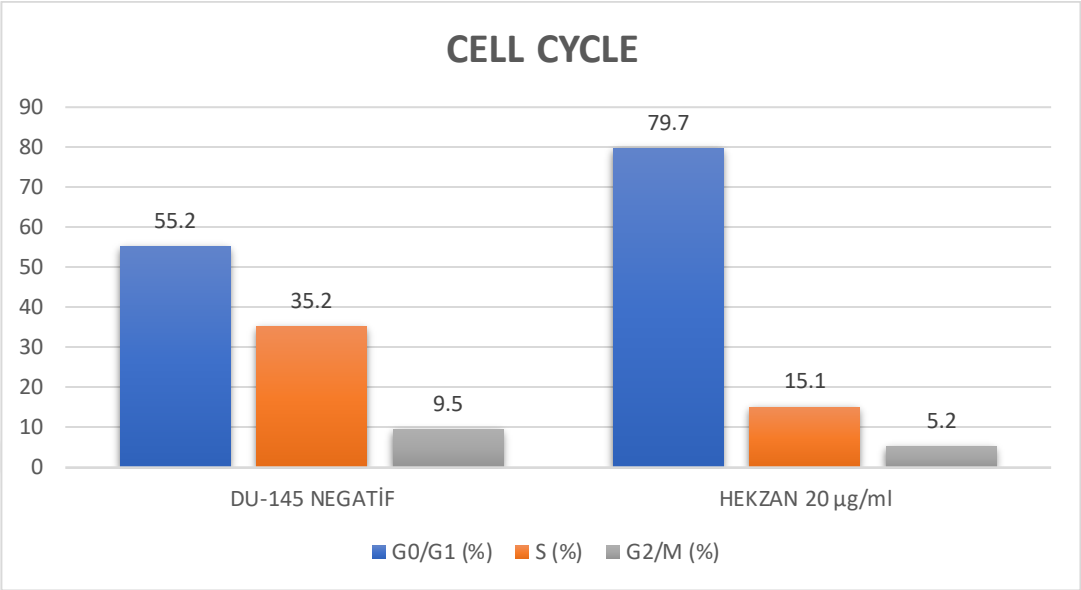
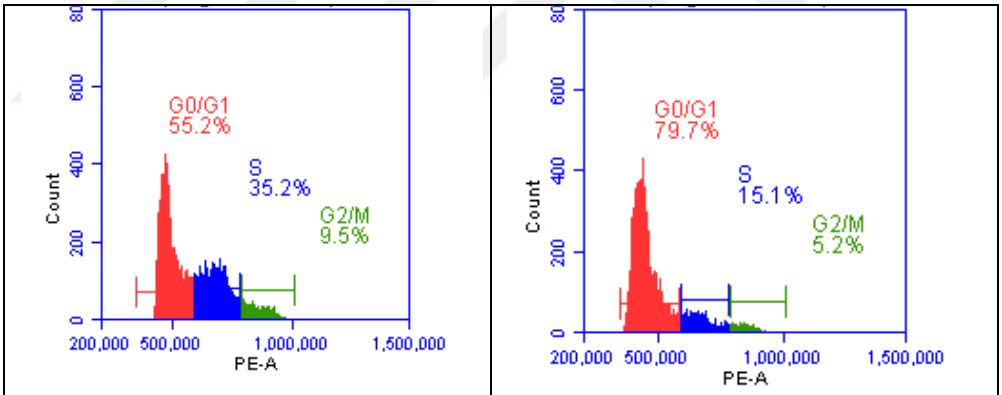


Figure 4.5. Cell Cycle analysis of hexane extract on Du-145 cell line



A01 Du-145 negative Gate : (Singlets in Cells)		A02 Du-145 5 µg-Hexane Gate : (Singlets in Cells)		B02 Du-145 5 ug-H Gate :cells	

Figure 4.6. Effects of scorpion carapace on DU-145 cells in cell cycle.

5. DISCUSSION AND CONCLUSION

Cancer is distinguished by the spread of aberrant cells It is the world's second biggest cause of death, after only cardiovascular disease, cancer is responsible for one out of every six deaths worldwide, causing about 10 million deaths in 2020, or nearly one out of every six deaths (188).

Cancer is becoming more prevalent in developing countries, in particular, emerging countries account for 55% of new cases, a ratio that might climb to 70% by 2050, in 2010 the cost of cancer globally was approximately US$1.16 trillion, which is equivalent to more than 2% of global GDP, this is because of the enormous suffering and economic cost that cancer causes, nonetheless, this large sum represents a lower bound that ignores the huge long-term consequences to families and careers (196).

In cancer treatment, many therapeutic procedures such as surgery, radiation, chemotherapy, and immunotherapy are employed, these treatments can hurt normal cells, causing a variety of harmful side effects including neurotoxicity, hepatotoxicity, and nephrotoxicity (189).

Because of significant side effects, therapeutic deficiencies, and problems with selectivity in the effects of drugs used in cancer chemotherapy, a treatment method that could be an ultimate solution has not been fully developed, so research in this area is ongoing and increasing, even today, as a result, research into effective and selective chemotherapy approaches is critical for cancer treatment, as a result, experts are searching for new alternative treatment options to replace chemotherapy in cancer treatment, science turned to chemotherapy in natural ways, so they discovered many animals and plants product that have anti-tumors.

Traditional anticancer medicines, such as chemotherapy and radiation, as well as they are inadequate specificity, produce substantial side effects and toxicities, because peptides preferentially target cancer cells while being less harmful to normal tissues, they are a better alternative for cancer prevention and therapy. Recent research has focused on anticancer peptides obtained from natural animal sources such as terrestrial mammals, marine animals, amphibians, and animal venoms. (195).

For ages, nature has provided life-changing and life-saving remedies. Nature's contributions to medicine include aspirin, penicillin, and morphine. These findings accelerated the area of natural product medication research, which mostly concentrated on herbs. Insects, on the other hand, have more than doubled the number of species, and entomotherapy with medicinal plants has been practiced for a very long time and is a significant alternative to modern medicine in many areas of the world. Natural products isolated from ants, bees, wasps, beetles, cockroaches, termites, flies, real bugs, moths, and other insects were examined. These natural compounds derived from insects have antibacterial, antifungal, antiviral, anticancer, antioxidant, anti-inflammatory, and immunomodulatory properties (215).

Natural products have played an essential role as recognized cancer chemotherapeutic agents for over 40 years, whether in their unmodified (naturally occurring) or synthetically modified forms (190).

Regarding marine plants, several compounds originating from marine sources have proved to be effective in addressing this illness by either reducing malignant cell growth or acting as apoptosis enhancers (191). Also, Plants, fungi, algae, and microbes produce carotenoids, which can help prevent diseases including cancer, cardiovascular disease, and a variety of other acute/chronic ailments (191).

Aside from maritime plants and plants, consider insects. In folk medicine and traditional Chinese medicine, extracts of insect corpses have been widely used to treat throat and ear infections, TB, influenza, cancer, and a variety of other illnesses and maladies (192). In China, insects from 77 different species, 14 different families, and 8 different orders have traditionally been utilized to treat oncology and cancer (193,194).

Immunomodulatory, antimicrobial, antihypertensive, antithrombotic, anticancer, antioxidative, and cholesterol-lowering properties have been reported in an increasing number of peptides derived from natural animal sources (197). This research, on the other hand, is focused on bioactive peptides derived from animals that may specifically target cancer cells and so function as anticancer medicines that are less hazardous to normal organs (198,199).

In this work, we collected and powdered the outer shell of the scorpion species of (Androctonus crassicauda) which lives in (Sanliurfa) , and then divided the outer shell into distinct extracts. Although there are no other studies on scorpion carapaces for determining the anticancer activity therefore our study is one of the first studies on the outer shell of scorpion.

In this study, the anticancer properties of scorpion carapace were investigated for the first time. As a result of our study, it was observed that the hexane fraction of scorpion carapace had a cytotoxic effect on different cancer cells. compare to other fractions which were (dichloromethane , methanol ,ethanol ,and water) hexane show the high cytotoxicity to the cell It was determined that the strongest cytotoxic effect was on prostate cancer DU-145 which the IC50 was 3,95 compare to other cancer cell lines, and it was determine that hexane extract less harmful to the normal prostate cell PNT-A1 by determining the IC50.

Apoptosis, also known as programmed cell death, is a natural process produced by numerous cell defects that regulates proper cell quantity by removing dangerous cells. When a cell undergoes apoptosis, it undergoes morphological and biochemical changes such as membrane enlargement, chromosomal condensation with lamin-B1 dissociation, cytochrome c release in mitochondria, and DNA fragmentation (216,217). The Annexin V/PI staining findings revealed that the scorpion carapace hexane extract triggered apoptosis in prostate cells. It was observed that 5 µg/ml dose of hexane extract on DU-145 cells caused 44% of cell death. It was observed that 38.6% of these deaths were caused by late apoptosis and 5.4% by necrotic origin

Cell cycle arrest is another major mechanism that has been linked to the anticancer effects of numerous well-known medicines (218). Some anticancer medications prevent cells from moving from one phase of the cell cycle to the next by targeting particular proteins that cause cancer cells to concentrate at a given stage. Cell cycle arrest inhibits the cancer cell from developing into a tumor and spreading to other regions of the body (219). Consistent with this, we discovered that scorpion carapace hexane extract causes G0/G1 cell cycle arrest in prostate cancer cells. Cell cycle arrest at the G1/S and/or G2/M borders is one of the biological responses to DNA damage that prevents cells from

entering key S and M phases with damaged DNA. This cell cycle stop permits cells to repair damaged DNA before DNA synthesis or mitosis begins.

we were determined that the hexane fraction induced apoptosis in cells by stopping the cell cycle in the G0-G1 phase. We found that the 5 µg/ml dose of hexane extract on DU-145 cells kept the cells in G0/G1 phase at a rate of 79.7%.

(Antitumor Activity in Scorpion and Frog Organ Lysates) (2020). Animals were collected, and their organ lysates and sera were prepared and tested against Michigan Cancer Foundation-7 breast cancer (MCF-7), prostate cancer (PC3), Henrietta Lacks cervical cancer (HeLa), and normal human keratinocyte cells. The scorpion's exoskeleton, appendages, and hepatopancreas were dissected, while the frog's liver, lungs, heart, oviduct, gastrointestinal tract, gall bladder, kidneys, eggs, and sera were collected and organ lysates/sera were prepared. Growth inhibition and cytotoxicity tests were conducted out.

Scorpion appendages, exoskeleton lysates, and hepatopancreas all inhibited growth and were cytotoxic. Furthermore, growth inhibition and cytotoxicity were seen in frog lungs, liver, gastrointestinal system, heart, oviduct, kidneys, eggs, and sera (222).

The lysates of the scorpion's upper body, hepatopancreas, exoskeleton, and appendages inhibited the proliferation of HeLa cancer cells by 56.2, 100, 82.1, and 41%, with P values of 0.155, 0.056, 0.095, and 0.45, respectively. Scorpion lysates also suppressed the development of MCF-7 cells by 73.4, 100, 92.6, and 65.5 percent, with P values of 0.061, 0.0023, 0.11, and 0.069, respectively .The lysates suppressed PC3 development by 43.3, 100, 100, and 83.1 percent, with P values of 0.79, 0.025, 0.013, and 0.075, respectively (223).

Animal poisons and toxins have recently been studied for their anticancer potential (200). Poisons are well-known for their damaging effects and the significant health problems they cause in individuals (201). Despite their deleterious repercussions, these toxins provide a plentiful supply of pharmacologically active molecules (202). in our study we work with a scorpion, so Scorpion venom has promising medicinal applications and is the most costly and lethal venom with the potential to be a therapeutic candidate. Several scorpion venom peptides have shown potential in treating a variety of disorders.

Because of their structural and functional specificity, scorpion peptides have been frequently used in the development of specialty therapeutics, notably for cardiovascular and immunological diseases (200). Scorpion venom contains a variety of bioactive compounds, including as mucopolysaccharides, free amino acids, phospholipases, hyaluronidases, amines, and nucleotides (203).

Several recent investigations have found that scorpion venom includes biologically active chemicals that can also be used to treat cancer (204,205).

Scorpion venom is thought to be a natural cancer therapy. The venom of the uncommon blue scorpion, Rhopalurus junceus, discovered in Cuba, was used to create Vidatox 30-CH, a potential novel cancer treatment, in 2011. This medication is made up of five low molecular weight peptides extracted from blue scorpion venom in a 33% alcohol solution. More than 10,000 cancer patients have been tested with the medication Vidatox. The study found that the medicine improved cancer patients' quality of life by decreasing tumor development (205-207).

Furthermore, a recent study employing (Androctonus crassicauda) species scorpion venom shown that the venom has dose- and time-dependent cytotoxic effects on breast and colorectal cancer cell lines and induces apoptosis in cancer cells by producing reactive oxygen species (206-208).

Several studies found that venoms taken from diverse scorpion species suppressed cancer cell proliferation in vitro, were efficient in lowering tumor development and decreased metastasis in various tumor models with no non-specific adverse effects. Furthermore, such inhibitors have been proven to decrease tumor development, particularly when used in conjunction with conventional chemotherapy or radiation, humans, on the other hand, have benefited from scorpions.

In China, fried scorpions are popular in cuisine, Scorpion or snake wine can boost the immune system. It can also help decrease the risk of getting sick (209). For thousands of years, scorpions and their venom are utilized in traditional Chinese, Indian, and African medicine. In China, for example, scorpion dried complete bodies have been commonly utilized as an ant epilepsy and analgesic drug since the Song Dynasty (A.D. 960-1279) (210,211).

The black widow spider has deadly components not just in its venom glands, but also in other regions of its body, such as its legs and abdomen, in its eggs, and even in newborn spiderlings. It is both theoretical and practical to investigate their potential impacts on cancer cells. In this work, they looked at how newborn black widow spiderling extract affected human HeLa cells in vitro.

The whole extract suppressed HeLa cell growth in a dose-dependent manner when administered at varied doses, with an IC50 value of 158 g/ml. Flow cytometry revealed that treatment of HeLa cells with spiderling whole extract triggered apoptosis in a dose-dependent manner and resulted in cell cycle arrest in S phase. The whole extract, at various doses, caused apoptosis and elevated caspase 3 activity in a dose-dependent manner. A portion of the HeLa cells treated with the whole extract looked to be morphologically altered, with membrane blebbing, nuclear disintegration, and chromatin condensation.

Further separation and activity screening revealed that the entire extract's cytotoxic and apoptotic activities were mostly related to its high-molecular-mass proteins, one of which was isolated and described for its anti-tumor properties on HeLa cells.

Buthus martensii Karsch (BmK), often known as the Chinese red scorpion, is a Buthidea family member that ranges from northwest China to Mongolia and Korea. The BmK scorpion has been used medicinally since the Song Dynasty in China (A.D. 960-1279). According to research, BmK was likely the first to be identified as having anticancer properties (212).

Zhang Futong created a solution from the dried whole body of the BmK scorpion (Quan Xie in traditional Chinese medicine) and administered it subcutaneously to mice with reticulum cell sarcoma and MA-737 mammary cancer every other day for five days in (1987) (213). On the eighth day after delivery, the inhibitory rate of development in reticulum cell sarcoma was 55.5 percent and 30.4 percent in mammary cancer, respectively. After BmK venom therapy, the DNA content of tumor tissues decreased. This seminal discovery paved the way for later research into the anticancer properties of BmK scorpion venom.

6. CONCLUSION

In this study, we showed that the scorpion carapace hexane fraction is a new and promising anticancer therapeutic agent candidate. We determined that the scorpion carapace extract has the potential for therapeutic application in cancer and leads to apoptosis by stopping the cell turnover of prostate cells.

7. REFRENCE

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