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Treatment of tumor with an extract from the shell of an aquatic animal

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ABSTRACT A kind of marine animal with a unique evolution status, has many bioactive substances with special functions. In the present study, we investigated the antitumor effects of four different extracts derived from shells (aqueous and methanolic extracts of burned and normal shell powder). Aqueous and methanolic extracts of burned shell powder were shown to be cytotoxic and inhibit the in vitro proliferation of human laryngeal carcinoma tumor cell lines (Hep-2) and human Rhabdomyosarcoma cell line (RD) and one murine mammary adenocarcinoma tumor cell line (AMN3), in the dose and time-dependent manner. Clinical and laboratory results of burnt crust extracts showed a strong antitumor effect. Inhibited % in vitro proliferation of human laryngeal carcinoma tumor cell lines (Hep-2) was 66.1%, 76.45%, and 76% at 24hr,48 hr, and 72 hr respectively. While at human Rhabdomyosarcoma cell line (RD) Inhibited % in vitro proliferation cell lines were 67.4%, 50.6%, and 51.8% at 24 hr,48 hr, and 72 hr respectively, in murine mammary adenocarcinoma tumor cell line (AMN3), the main inhibition were 66.1 % at 250 µg/ml at 72 hr.

1. INTRODUCTION

The research started from the year 1978 until now (44 years) on a new treatment for cancer, as the research continued after several studies.

Because chemotherapy for cancer is a concern for many people today . Laboratory scientists, clinicians, cancer victims and news reports always get their hopes up when a new chemotherapeutic agent is discovered in the lab and shows some promising properties for clinical applications. (Curado, 2011).

The study of the antitumor activities of riverine and marine active substances is an important field in the exploitation of marine active substances and antitumor drugs (Tincu & Taylor, 2004).

In recent years, many active substances, including clotting factors, protease inhibitors, antibacterial substances, lectins, etc., have been found in blood cells and blood plasma of horseshoes. (tachypleysin, a cationic peptide) isolated from acidophilic extracts from the blood cell debris of the animal horseshoe (Tachypleus tridentatus) It can inhibit the growth of Gram-negative and Gram-positive bacteria at low concentrations.

Tachyglycin has anti-tumor effects, some scientists have investigated the biological effects of tachyglycin on human gastric cancer cell line and found that it can effectively change the morphological and malignant characteristics of it and has a certain differentiation-inducing effect on human gastric cancer (Markham, 1982).

Examined a chemically synthesized preparation of tachyplesin bound to an RGD sequence and found that synthetic RGD-tachyplesin could inhibit the proliferation of TSU prostate cancer cells, B16 melanoma cells as well as epithelial cells in a dose-dependent manner in vitro and reduce tumor growth in vivo. Tumor growth was inhibited by inducing apoptosis in cancer cells. It is believed that tachiplecin activates the classical complement pathway to kill cancer cells.

Marine animal scales contain many natural substances; (chitin) which is one of the most important substances that ranks second after cellulose as the most abundant organic compounds on earth chemically is polysaccharides, which are large molecules strung together like pearls. Unlike most polysaccharides, chitin has a strong positive charge that allows it to bind to negatively charged surfaces (Li et al., 2002).

a. Chitin and derrivatives

b. Chitosan

c. Chitin oligosaccharides

d. Chitosan oligosaccharides

It has many properties that make it attractive for a variety of health applications. For example, research indicates that it is antibacterial, antifungal, antiviral, non-toxic, and non-allergenic. Chitosan synthesized from the shell of marine animals has anticoagulant activities. Scientists have found that chitosan may enhance the antitumor activity of cancer chemotherapy drugs and prevent side effects caused by cancer chemotherapy drugs, such as myelotoxicity, gastrointestinal toxicity and organo-immunotoxicity caused by 5-fluorouracil (Chen et al., 2005).

In addition, culture-soluble, low-molecular-weight chitosan may act as an immunomodulator in the intestinal immune system of animals and enhance the cytotoxic activity of intestinal intraepithelial lymphocytes against tumor. Another medicinal agent found in the animal's shell is glucosamine, which is used for patients with osteoarthritis and provides pain relief and to help regenerate damaged tissues in the joints.

Based on clinical observations about some patients who were given promising results and an ancient manuscript that inspired our attention from Islamic folk medicine that talks about the use of animal shell ashes to treat cancer patients, this research aims to explore the antitumor activity of marine animal shell extracts (Curado, 2011).

The marine animal shell extract contains a set number of amino acids, which are available in specific proportions. This amino acid content is of particular significance because all amino acids play an essential role in the biochemical activity and the value of nutrients.

2. LITERATURE REVIEW

A tumor is a solid mass of tissue that forms when abnormal cells group together. Tumors can affect bones, skin, tissue, organs and glands. Many tumors are not cancer (they're benign). But they still may need treatment. Cancerous, or malignant, tumors can be life-threatening and require cancer treatment (Chen et al., 2001).

A tumor is an abnormal growth of cells that serves no purpose. A benign tumor is not a malignant tumor, which is cancer. It does not invade nearby tissue or spread to other parts of the body the way cancer can. In most cases, the outlook with benign tumors is very good. But benign tumors can be serious if they press on vital structures such as blood vessels or nerves. Therefore, sometimes they require treatment and other times they do not (Curado, 2011). Cancer is a major health problem and one of the main causes of mortality worldwide (Rani et al., 2019).

A tumor is a mass or group of abnormal cells that form in the body. If you have a tumor, it isn't necessarily cancer. Many tumors are benign (not cancerous).Tumors can form throughout the body. They can affect bone, skin, tissues, glands and organs. Neoplasm is another word for tumor.

A tumor may be:

a. **Cancerous:** Malignant or cancerous tumors can spread into nearby tissue, glands and other parts of the body. The new tumors are metastases (mets). Cancerous tumors can come back after treatment (cancer recurrence). These tumors can be life-threatening.

b. **Noncancerous:** Benign tumors are not cancerous and are rarely life-threatening. They're localized, which means they don't typically affect nearby tissue or spread to other parts of the body. Many noncancerous tumors don't need treatment. But some noncancerous tumors press on other body parts and do need medical care.

c. **Precancerous:** These noncancerous tumors can become cancerous if not treated (Kateb et al., 2011).

2.1 What Causes A Tumor?

Your body is constantly making new cells to replace old or damaged ones that die off. Sometimes, the cells don't die off as expected. Or, new cells grow and multiply faster than they should. The cells start to pile up, forming a tumor.

2.2 What are the Risk Factors for Tumors

Tumors affect people of all ages, including children. Factors that increase the chances of developing a tumor include:

a. Gene mutations (changes), such as mutated BRCA (breast cancer) genes.

b. Inherited conditions, such as Lynch syndrome and neurofibromatosis (NFS).

c. Family history of certain types of cancer like breast cancer or prostate cancer.

d. Smoking, including exposure to secondhand smoke

e. Exposure to toxins like benzene or asbestos

f. Previous radiation exposure.

g. Viruses like HPV

h. Having Obesity

2.3 Previous Studies

A great number of studies and researches have been carried out over the course of many years and continue to be carried out to this very day regarding fresh discoveries of cancer and how to treat it. When it comes to finding treatments for cancer, researchers have used a wide variety of research methodologies and types. Where there is research on the use of active materials for this animal based on modern techniques in nanotechnology, where this animal was converted to the nano state by first converting it into powder and then converting the powder into nanoparticles in a modern way, which is the method of moving balls and then being treated with deep freezing, as well as where there is research on the use of active materials for this animal based on modern techniques in nanotechnology, where there is research on the use of active materials for this animal based on modern techniques There is empirical evidence. It comprises an extract of active chemicals that are effective in treating specific cancer kinds (Shigenaga et al., 2016).

In its identification of therapy and resistance to malignant diseases, the most recent research followed a novel approach that did not imitate the methodologies of earlier studies. Mice were implanted with human mammary adenocarcinoma cell line (AMN3), human rhabdomyosarcoma (RD) cell line, human laryngeal carcinoma (Hep-2) cell line, and human rhabdomyosarcoma (Hep-2) cell line.

Where the clinical and laboratory results obtained showed a rapid therapeutic effect and a lack of negative side effects caused by chemically manufactured drugs, because the majority of the drugs for treating cancer diseases, if not all of them, are in relatively high concentrations and have to be taken at certain and spaced out intervals (weekly, semi-monthly, or monthly) and cause symptoms. Whereas, the results obtained showed that there was no such thing as a negative side effect caused by chemically manufactured drugs. Constipation is just one of several negative side effects that patients may have, including severe pain, loss of hair, loss of weight, general weakness, neuropathies, and skin ulcers. Patients may also experience generalized weakness and neuropathies (Chen et al., 2005).

Because the effective and bioactive compounds that were extracted from this marine animal are natural and have relatively low concentrations, and because the concentration of the drug substance present within the body is within its carrying capacity and does not cause damage to healthy cells, it is safe to use on a daily basis and the effect of the drug substance is continuous and cumulative. Herein lies the wisdom of God Almighty. In addition, there are no negative consequences associated with its use (Curado, 2011).

3. METHODS

The cochlear tissue samples from all of the animals were obtained from southern Iraq and were then transported to the Experimental Treatment Department of the Iraqi Center for Cancer and Medical Genetic Research in Baghdad.

After removing the material from the shell, it is allowed to dry at room temperature and is then fastened. The powder that has been dried is separated into two separate samples for each of the samples:

a. The first sample is taken without any sort of intermediary.

b. The second sample is baked in the oven at a temperature of 200 degrees Celsius for two hours, which results in the formation of a burnt crust that may then be removed from the oven.

c. After that, the combination should be filtered, and finally, the findings should be examined.

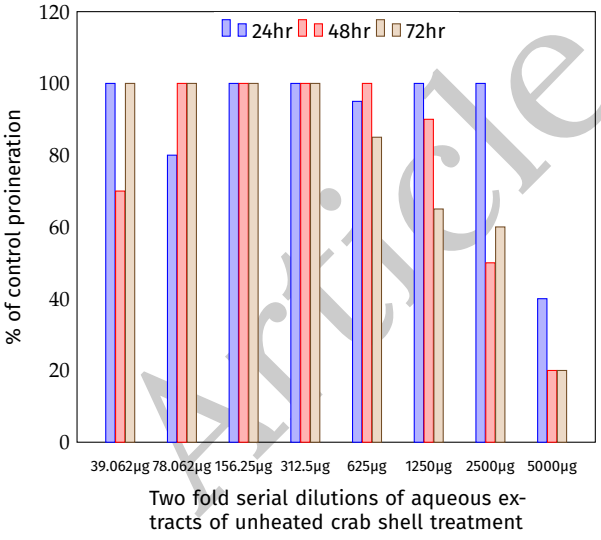

Figure 1. Shows the proliferation kinetics of 24-h, 48-h and 72-h, aqueous extract from unburned shell treatment on a Hep-2 cell line.

4. RESULTS & DISCUSSION

4.1 Results

4.1.1 Human rhabdomyosarcoma (RD) cell line

Figure 5 depicts the kinetics of cell proliferation after 72 hours after exposure to a water and ethanolic extract of burnt and unburned animal skin, showing a significant decrease in the rate of cell proliferation. When RD cells were

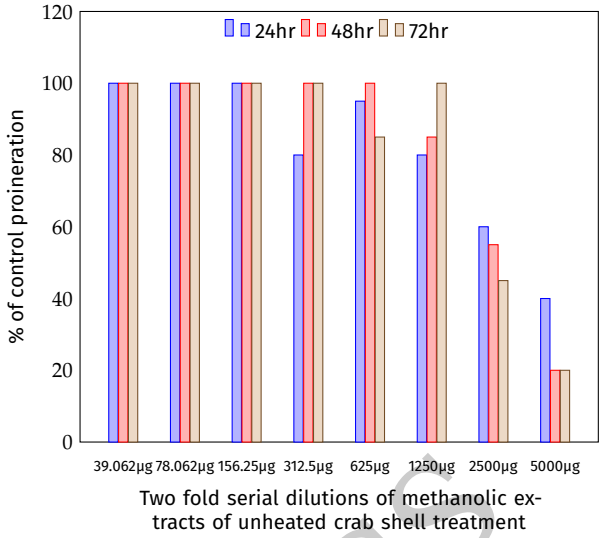

Figure 2. Shows the proliferation kinetics of 24 h, 48 h, and 72 h, methanolic extract from unburned shell treatment on a Hep-2 cell line.

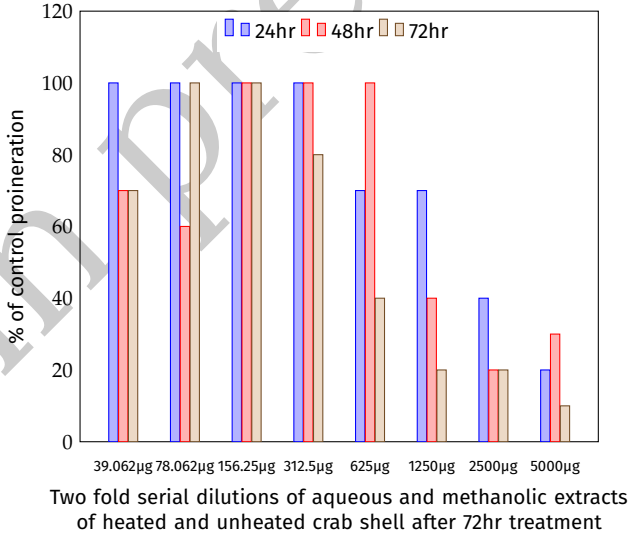

Figure 3. Shows the proliferation kinetics of 24-h, 48-h and 72-h, aqueous extract from burnt shell treatment on a Hep-2 cell line. Growth inhibition (LC50) was increased to 66.1%, 76.4%, and 76% at 24 hours, 48 hours, and 72 hours, respectively

treated with the aqueous extract of unburned animal cortex, there was no effect at any dosage, however treatment with the 67.4% ethanolic extract inhibited cell growth at a concentration of 5000 g/mL after 72 hours. On the other hand, treatment with aqueous extracts of burnt bark proved to be more efficient and resulted in a growth inhibition of 50.6% (LC 50) after 72 hours of exposure to 625 g/mL. The unburned extracts and the aqueous extracts of the burnt animal bark were efficient at a concentration of 156.25 g/mL in 72 hours by inducing 51.8% growth inhibition (LC 30). However, the ethanolic extract of the burnt animal bark was more effective than both of these.

4.1.2 Mice Mammary Tumor Cell Line

It used to study the effect of extracts on the mammary gland, tumors, water and ethanolic extracts of unburned animal cortex had no effect on all concentrations used, and the aqueous extract of burnt animal cortex also had no

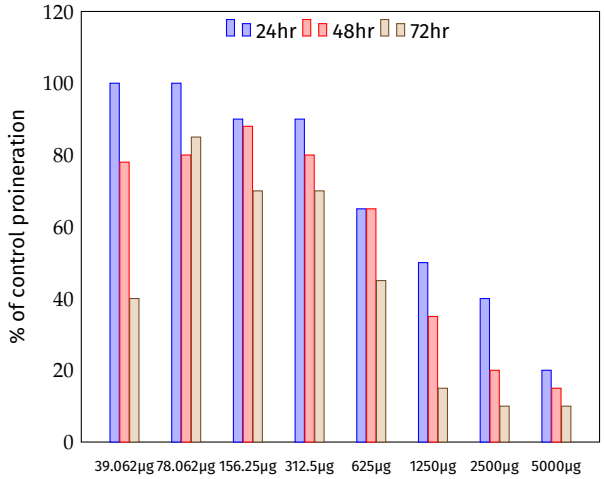

Figure 4. shows the diffusion kinetics of 24 h, 48 h, and 72 h, methanolic extract from burnt cortex treatment on a cell line. Hep-2 growth inhibition (LC50) increased to 42%, 45.3%, and 50.8% at 24 hours, 48 hours, and 72 hours, respectively.

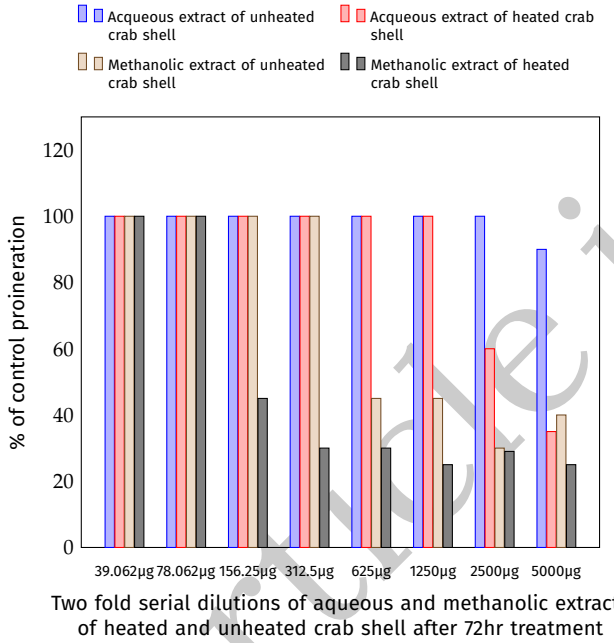

Figure 5. Shows the proliferation kinetics of 72 h, aqueous and methanolic extracts from burnt and unburned shell treatment on an RD cancer cell line. The ethanolic extract showed 67.4% growth inhibition at a concentration of 5000 µg/mL at 72 hours.

growth inhibitory effect on AMN3 cells, while ethanolic extracts were effective at 2500 µg / ml (G.I=66.1%) (LC 50) within 72 hours. Figure 6 shows the kinetics of reproduction from a 72 h recap of treated burnt and unburned animal cochleas.

4.2 Discussion

In our research, we compared the extracts obtained from burnt animal shells to those obtained from unburned animal shells. The findings indicated that the extracts obtained from burnt animal shells are more potent and have a greater cytotoxic effect than the extracts obtained from

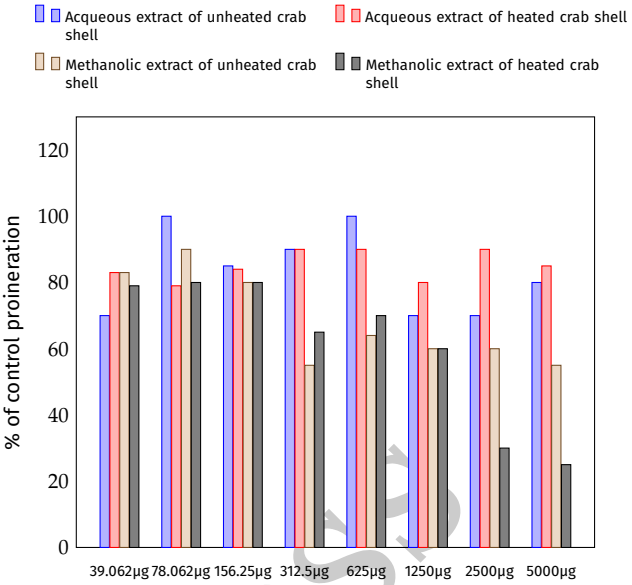

Figure 6. Shows the diffusion kinetics of 72 h, aqueous and methanolic extracts from burnt and unburnt animal cortex treatment on an AMN3 cell line. The ethanolic extracts were effective at 2500 µg/mL (G.I=66.1%) (LC 50) within 72 hours.

unburned animal shells, particularly the methanolic extract of burnt animal shells.

The presence of chitin and its derivatives, which we have identified as an anticancer component, may be responsible for the small antitumor impact that is exhibited by non-burnt cortical extracts. This finding was made possible by the fact that the cortex was not burned. Previous research has indicated the existence of animal anticancer chemicals such as tachipelicin, which can be found in the leukocytes of horseshoes.

A member of our team participated in a study in the past that indicated the existence of Ge, Se, Zn, Cr, Mg, and Cu in the ashes of the animal shell. This study also mentioned the presence of chitin, which was cited in some research as an anti-tumor chemical. Chitin is found in animal shells.

As a result, we postulated or hypothesized that during the heating in the oven for two hours at a temperature of 200 degrees Fahrenheit, these minerals, along with any additional amino acids or vitamins, and any sugars that were present in the animal's cortex, would combine to generate a new molecule that requires further examination.

Furthermore, a recent report showed that low-molecular-weight chitosan nanoparticles containing galactosylate could carry a positively charged anticancer, doxorubicin (adreomycin) (DOX) to target hepatocytes, and they tested it on a hepatocellular carcinoma cell line in vitro.

In conclusion, we provide evidence that extracts from burnt animal cochleae have a cytotoxic and growth-inhibitory effect on cancer cells and the effect was dose- and time-dependent and may indicate potential anti-tumor activity that should be tested further.

4.2.1 Applications

The Ministry of Industry/The General Company for the Pharmaceutical Industry in medical supplies companies and is used in:

a. It is used to treat throat cancer
b. It is used to treat blood cancer.
c. It is used to treat breast cancer.
d. It is used to treat prostate cancer.
e. It is used to treat bladder cancer.
f. It is used to treat other cancerous diseases that have been treated surgically, chemically, radiologically and immunologically.

4.2.2 Characteristics

The most important characteristic of this research is that:

a. The first clinical study to treat cancer.
b. A crustacean aquatic animal, which is widely available in rivers and seas, was used.
c. The extracted and used materials and compounds are natural and safe, and there are no side effects.
d. The animal can be obtained easily.
e. It is cheap and can be prepared for use as a treatment easily.
f. It can be used on the general public, especially those desperate for cancer.
g. More than 50 patients who used it were reviewed and analyzes were done and the results were positive.

4.2.3 Allegations

a. Tumor treatment with crustacean extract.
b. Referring to Claim No. 1 The shell material was removed, dried at room temperature, stabilized, and then ground. The dried powder was divided into two samples: the first sample was extracted directly and the second sample was burned in an oven at a temperature of 200◦C for two hours.
c. Referring to Claim No. 1, methanol (75%) and water (25%) were used to complete the extraction process for a period of 7 hours for the purpose of extracting biologically active materials with high efficiency.
d. Referring to the protection member No. 1, the highest inhibition rate is 80%.
e. With reference to Claim No. 1, it is utilized in the treatment of :
    1. It is utilized in the treatment of throat cancer.
    2. It is employed in the treatment of cancer of the blood.
    3. It is a component of a treatment for breast cancer.
    4. It is used as a treatment for cancer of the bladder.

It is used to treat other cancerous diseases that have been treated surgically, chemically, radiologically and immunologically.

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