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Role of Catalase Enzyme in the Existence of Allicin on Hep-G$_2$ Cell Line

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Abstract

In recent years, there has been significant attention on the use of chemical and bioactive compounds extracted from medicinal plant sources to prevent and treat various diseases. Among the active components of herbal medicines, curcumin, resveratrol, quercetin, emodin, silybin, and their derivatives have been particularly discussed in the treatment of chronic and common disorders such as cardiovascular, metabolic, neurogenic diseases, and cancer. It is estimated that about sixty percent of currently widely used anticancer drugs are derived from natural sources. These compounds also play an important role in cancer prevention, due to their high ability to scavenge free radicals, inhibit cell growth, and induce apoptosis. For centuries, garlic and its derived compounds have attracted researchers' attention due to their health-promoting effects. Various bioactive compounds in garlic are responsible for its health benefits, among which allicin is one of the key components. Allicin is responsible for the pungent odor of garlic and is one of the most important organosulfur compounds in garlic, making up about 70% of the thiosulfate compounds. Allicin is produced during the crushing of garlic through the enzymatic activity of alliinase on alliin and is effective against a wide range of microorganisms. The antimicrobial and antioxidant properties of allicin are attributed to its reaction with the thiol groups of various enzymes. Allicin prevents cancer onset and progression by blocking metastasis and inhibiting excessive cell proliferation. This study investigates the role of the enzyme catalase in the presence of allicin on the Hep-G2 cell line. Allicin, the active ingredient in garlic, was applied in its pure form to Hep-G2 cancer cells in a concentration range from 0 to 250 µg/mL for 24 hours. The MTT assay revealed that allicin had an inhibitory effect on the proliferation of these cells. A proposed mechanism for the cytotoxic effect of allicin is the impact of oxygen free radicals on the biochemical structure of these cells. The general reduction in catalase enzyme activity leads to an increase in these free radicals. Ultimately, by inducing oxidative stress and decreasing catalase activity, allicin causes cellular toxicity and exhibits anticancer properties on Hep-G2 cells.

Keywords: Allicin, Catalase enzyme, Cytotoxicity, Free radicals.

Introduction

Cancer is one of the most common and destructive diseases, affecting millions of people worldwide each year [1].

Surgery, chemotherapy, and radiotherapy are the common treatments for cancer, often supplemented by other complementary and alternative therapies. Although the goal of chemotherapy is to eliminate tumor cells, various types of normal cells are also affected, leading to many side effects (including hair loss, anemia, fatigue, nausea, vomiting, diarrhea, infection, bruising, or minor bleeding, and even cognitive issues). In recent years, due to the fear of the side effects of chemotherapy drugs, people have preferred the use of some natural herbal products for cancer treatment [2].

Herbal medicines have been used in traditional medicine to treat various diseases. Scientific studies on medicinal plants for treating many diseases, including cancer, have shown promising results, and they can also reduce the toxicity of other drugs due to their antioxidant properties. Carcinogenic factors include physical factors such as ultraviolet radiation, chemical factors like carcinogenic compounds, cigarette smoke, an imbalanced diet, occupational hazards, genetic factors, hormonal, metabolic, and biological factors, especially certain bacteria and viruses.

In traditional medicine, many natural substances have been recognized for treating various cancers. Some of the most well-known natural anti-cancer agents globally include the Chinese herb *Rhodiola rosea*, flavonoid compounds like quercetin, *Scutellaria baicalensis*, garlic, curcumin, etc. The anti-cancer mechanisms of many of these substances are related to their antioxidant properties and their ability to inhibit tumor cell growth. Many of these substances are used traditionally in various regions worldwide. Some plants play a vital role in preventing and treating cancer. Among the active ingredients of herbal medicines, compounds like curcumin, silymarin, resveratrol, quercetin, emodin, silibinin, and their derivatives have been extensively studied for the treatment of common chronic disorders such as cardiovascular diseases, metabolic conditions, neurodegenerative diseases, and cancer[3].

Silymarin, a natural product, is gaining attention as a new therapeutic option in complementary medicine. It has a broad spectrum of mechanisms, both in vitro and in vivo, such as antioxidant, anti-inflammatory, dose-dependent anti-apoptotic effects, and cellular transporter modulation. Hence, it can be considered a promising drug in complementary medicine [3].
Silibinin, another active compound derived from *Silybum marianum* (milk thistle), is traditionally used to treat a wide range of liver diseases [4].

It is estimated that about sixty percent of the current widely used anti-cancer drugs are derived from natural sources. These agents also play an important role in cancer prevention due to their ability to eliminate free radicals, inhibit cell growth, and induce apoptosis [5].

Literature Review

Allicin is one of the most important organosulfur compounds derived from garlic. It is the compound responsible for the characteristic odor and taste of garlic and is highly unstable, breaking down under heat and alkaline conditions. The aging process in garlic affects the amount of allicin, leading to its reduction. Preliminary studies by Semler et al. identified diallyl disulfide and diallyl trisulfide as the key compounds in garlic extracts responsible for its aroma [6].

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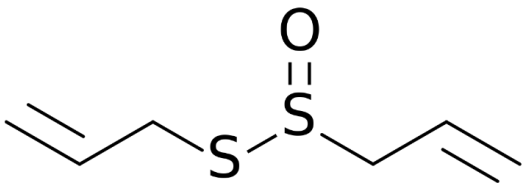

Figure 1-1: The most important bioactive compound in garlic, Allicin (Diallyl trisulfide) [6].

Allicin was the first discovered by Cavallito and colleagues in 1994, and the first published paper on allicin addressed its antimicrobial properties. Allicin is not inherently active in raw garlic; instead, it is activated through the repeated action of the enzyme alliinase, which occurs when garlic is crushed or chopped, converting its precursor, alliin, into allicin [6].

Alliin is the primary sulfur-containing compound in raw garlic and garlic powder, typically present at around 8 grams per kilogram in raw garlic cloves. Allicin (diallyl thiosulfinate) is a volatile compound responsible for garlic's pungent odor and is chemically an unstable, highly reactive molecule. Its antioxidant properties arise from the presence of SH (-) groups in garlic [7]. Under conditions such as concentration, pH, and heat, allicin frequently transforms into a fat-soluble oligosulfide (diallyl disulfide) [6].

Antioxidant Enzymes is a molecule that can prevent the oxidation of other molecules. During oxidation, electrons are transferred from one substance to another, and these reactions produce free radicals. These molecules terminate the chain of reactions by neutralizing free radicals and inhibit further oxidative reactions by oxidizing themselves. Antioxidants protect against cellular damage caused by molecules known as free radicals [8].

Catalase wast the first studied by Louis Tenard (the discoverer of hydrogen peroxide) in 1811, who hypothesized that hydrogen peroxide was broken down by an unknown substance. In 1900, Oscar Loew first coined the term "catalase" and found that the enzyme was present in many plants and animals. In 1937, James B. Sumner and Alexander D. Danes discovered catalase in bovine liver. In 1938, the molecular weight of catalase was determined. Catalase is an antioxidant enzyme found in most aerobic cells and plays a key role in detoxifying hydrogen peroxide (a reactive oxygen species). Hydrogen peroxide is a product of aerobic metabolism and is recognized as a byproduct of various oxidases and superoxide dismutases in eukaryotic cells. One of the reactive oxygen species is hydrogen peroxide, which forms as a result of oxidase activity or aerobic metabolism. Due to its toxic effects, the rapid removal of hydrogen peroxide is essential for all cells. One of the most effective ways to remove hydrogen peroxide is through the enzyme catalase, which is produced by all mammalian tissues [9].

Accumulation of hydrogen peroxide in cells leads to oxidation of DNA, proteins, and lipids, resulting in mutations and cell death. Catalase catalyzes the conversion of two molecules of H_2O_2 into molecular oxygen and two molecules of water. Catalase also exhibits peroxidation activity, where low-molecular-weight alcohols can act as electron donors. In humans, the highest levels of catalase are found in the liver, kidneys, and erythrocytes [9].

Research in **Catalase and Allicin** by Pedro Buc Calderon has shown that blocking catalase activity in cancer cells results in the accumulation of hydrogen peroxide, leading to the death of these cells. Flavonoids derived from medicinal plants, including garlic, have been shown to inhibit catalase, thereby eradicating cancers such as cervical, ovarian, and lung cancers. These compounds also reduce catalase mRNA levels in these cells [9].

Materials and Methods

The Hep-G2 cell line was thawed and counted using a Neubauer chamber, then passed to ensure a sufficient number of cells. 100,000 cells were seeded in each milliliter of culture medium, using DMEM with 10% fetal bovine serum and 200 units of penicillin G. Three petri dishes were used for each concentration: 0, 50, 100, 150, 200, and 250 µg/mL, and incubated in a CO2 incubator at 37°C for 24 hours. After incubation, the contents of each petri dish were treated with lysis buffer, and the cells were lysed. The sample was centrifuged for 10 minutes, and the supernatant was collected for subsequent assays [10].

MTT Assay (Cell Viability)

The MTT assay is one of the most widely used methods to assess cell viability. The MTT protocol, introduced in 1983 by Mossman, is a colorimetric method for evaluating cell proliferation and survival. This assay is based on mitochondrial activity, which is stable in living cells, and the number of living cells correlates with mitochondrial activity. The tetrazolium dye in the MTT assay is reduced in metabolically active cells. Mitochondrial dehydrogenase enzymes in living cells break the tetrazolium ring, producing NADH and NADPH, which results in the formation of an insoluble purple formazan precipitate. This precipitate can be dissolved using isopropanol or dimethyl sulfoxide (DMSO). Dead cells do not have this ability, and thus do not produce a signal. The color formed is used as a marker of living cells, and its intensity is measured at a wavelength of 540 to 630 nm, which correlates directly with the number of living cells [10].

Catalase Assay

The catalase assay is based on the degradation of hydrogen peroxide, following the method of Aeby (1984). The reaction mixture consists of 100 mM phosphate buffer and 15 mM hydrogen peroxide. After adding the tissue extract, the change in absorbance was evaluated using a spectrophotometer at a wavelength of 240 nm [11].

Results

Cell Viability Percentage

The obtained data on cell viability percentage are shown in Figure 1. As shown in the graph, compared to the control (untreated), the cell viability percentage decreased in a concentration-dependent manner from 50 µM to the final treatment concentrations with the compound being investigated (allicin). A significant decrease in cell viability was first observed at a concentration of 100 µg/mL, followed by further reductions at higher concentrations. The highest reduction in cell viability (41.7%) was observed at a concentration of 250 µg/mL. In the control sample, the cell viability remained at 100%, indicating that the substance has cytotoxic effects on the tested cancer cells.

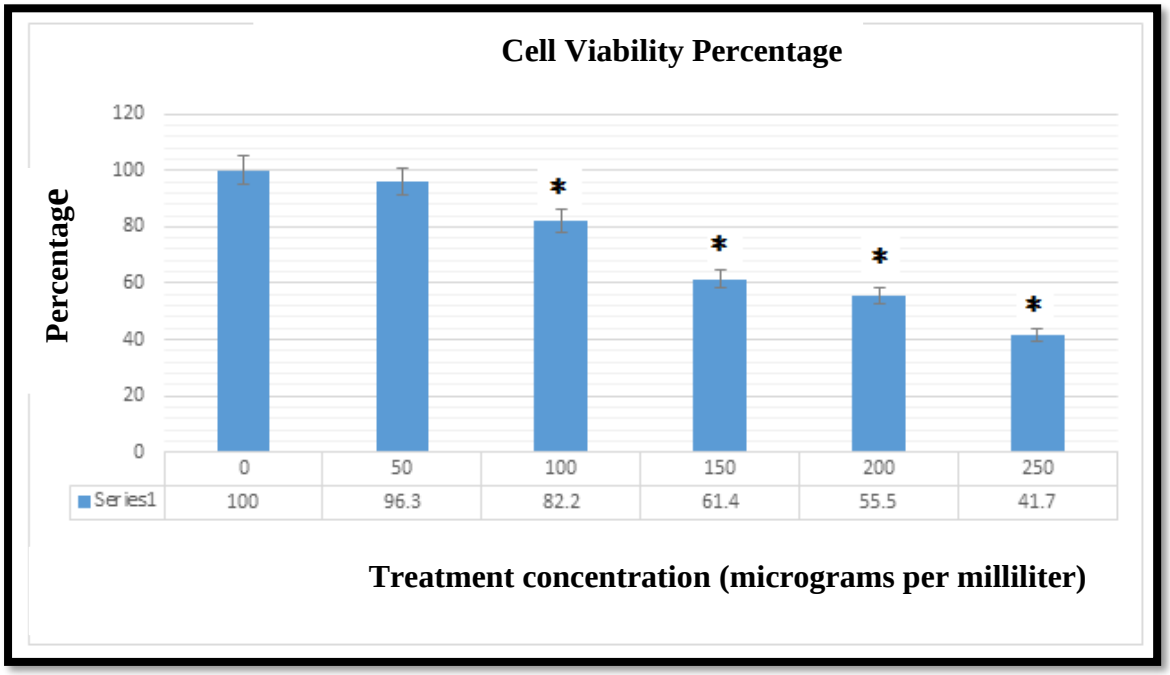

Figure 3-1 Changes in the cell viability percentage of Hep-G2 cells treated with different concentrations of allicin. Significant difference compared to the control.

Catalase (CAT) Activity

The results obtained from the catalase enzyme activity are shown in Chart (2). As observed in the chart, the data indicate that, compared to the control, the treatment with allicin at concentrations of 50 and 100 µg/mL led to a relative decrease in enzyme activity, though the difference was not significant. However, at concentrations starting from 150 µg/mL, there was a notable concentration-dependent decrease in activity, which was significant compared to the control ($p<0.05$). Furthermore, at the final treatment concentration of 250 µg/mL, the enzyme activity decreased by approximately 60% compared to the control, representing the greatest reduction. At 150 µg/mL, the decrease was about 68% of the control.

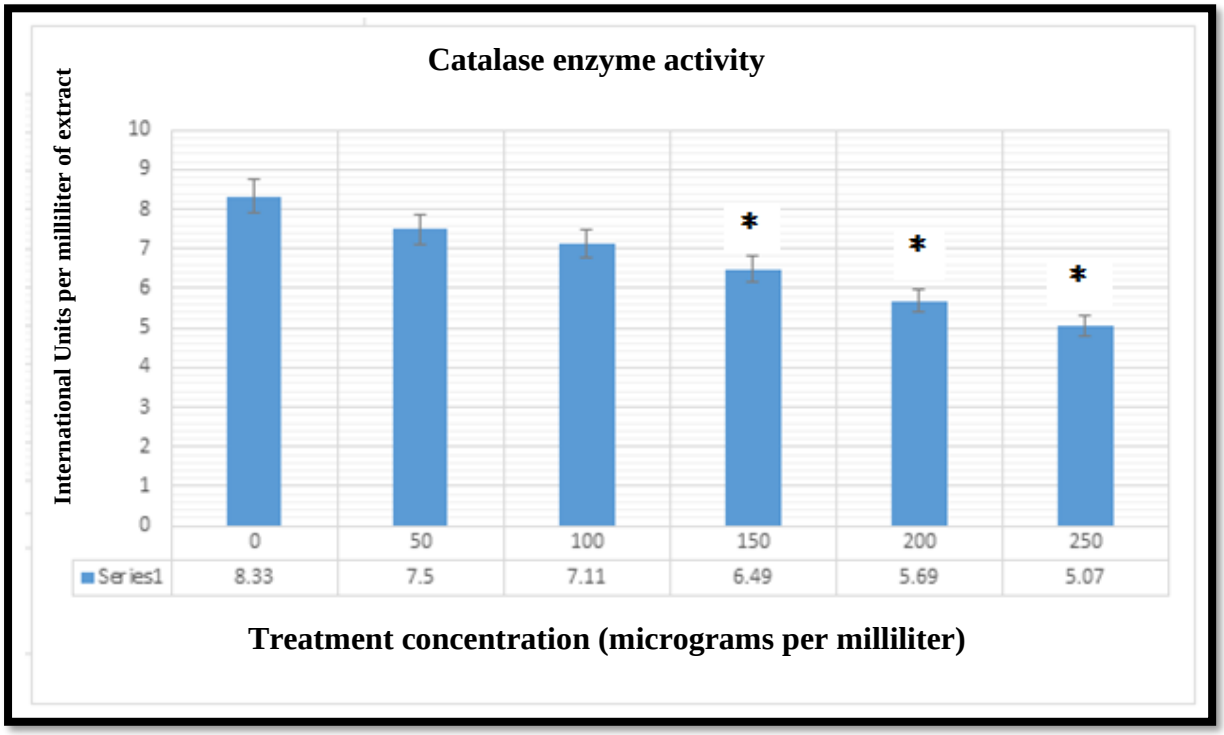

Chart 3-2 Changes in catalase enzyme activity in the cell extract under the effect of different concentrations of allicin.

- Significant difference compared to the control.

Discussion

An emerging strategy for cancer treatment and prevention involves the use of bioactive compounds from medicinal plants. Allicin is one such bioactive compound that has not been extensively studied on Hep-G2 cells until now.

In our initial experiment, we tested the effect of allicin on cell viability. The results of the cell viability test showed that allicin reduced cell viability in a concentration-dependent manner.

Epidemiological studies and experimental investigations have shown that both synthetic and natural compounds from medicinal plants can act as anti-cancer agents in the inhibition of breast cancer. Allicin can cross the phospholipid bilayer and inhibit the growth of cancer cell lines from breast, endometrial, and colon cancers. The anti-proliferative effects of allicin have been attributed to its ability to halt the cell cycle and reduce intracellular glutathione levels, which surpass its antioxidant activity. Additionally, allicin likely induces apoptosis by altering the expression of proteins involved in apoptosis regulation in tumor cells. These findings are consistent with the data obtained from evaluating the cytotoxicity of allicin in this thesis [12].

Another researcher, Mr. Chin and colleagues, demonstrated that allicin extracted from garlic induces apoptosis through oxidative stress in colon cancer cells. Their research revealed that at a concentration of 50 µg/mL, 50% inhibition of cell proliferation occurred [13].

Oxidative stress is generated by reactive oxygen species (ROS), and this concept has been discussed by John D. Hayes and colleagues in 2020. They showed that cancer cells produce such radicals to destroy normal (non-cancerous) cells. However, these cells themselves can also undergo

apoptosis due to the increased levels of free radicals, and this mechanism forms the basis of cancer cell treatment, particularly with respect to oxygen free radicals, which is emphasized in our study [14].

Research by Hayes and colleagues in 2020 also confirmed that ROS resulting from oxidative stress plays a crucial role in the initiation, progression, and tumor formation of cancer. Alterations in the structure of active genes and the antioxidant system under the influence of these radicals have been identified as significant mechanisms in cancer development [14]. Investigations by Asad Zaman Khan and colleagues in 2010 led to a review of multiple studies on the role of antioxidant enzymes in cancer [15]. Studies by Elchuri and colleagues in 2005 indicated that all types of superoxide dismutase activity show a reduction in liver cancer, and this decrease leads to a reduced antioxidant capacity, playing a critical role in cancer progression [16].

In a 2022 study by Anubha, it was shown that allicin, the most potent compound in garlic, plays a significant role in demonstrating a range of therapeutic actions in genetically-originated diseases like cancer. Furthermore, another part of the research focused on catalase enzyme activity in cell lines treated with different concentrations of allicin compared to the control over a given period, indicating that allicin's effect on catalase activity is positively correlated with its anti-cancer properties. This was accompanied by a reduction in enzyme activity [17].

Research by Christoph and colleagues in 2018 revealed that after cell culture and treatment with 0.5 mM allicin, chromatin remodeling is a key regulatory process that reduces catalase expression in breast cancer cells during the acquisition of resistance to oxidative stress. This reduction corresponds to the concentration-dependent decrease in catalase activity observed in our study [18].

Conclusion

Allicin, the main bioactive compound of garlic, was tested in its pure form on Hep-G2 cancer cells in the concentration range of 0 to 250 µg/mL for 24 hours. The MTT assay showed that allicin had an inhibitory effect on the proliferation of these cells. A proposed mechanism for the cytotoxic effect of allicin likely involves the influence of reactive oxygen species (ROS) on the biochemical structure of these cells. The general reduction in catalase enzyme activity leads to an increase in ROS levels. The elevated ROS levels result in oxidative damage to lipids and proteins, ultimately leading to cellular toxicity and anti-cancer effects in Hep-G2 cells.

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