IJPSR (2022), Volume 13, Issue 3 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 June 2021; received in revised form, 27 July 2021; accepted, 28 July 2021; published 01 March 2022

ANTIOXIDANT EFFICACY OF GARLIC DERIVED ALLICIN ON CULTURED BREAST CANCER CELL LINES

Anubha Chaudhari [*1] and Rajendra Chauhan [2]

Department of Zoology [1], Government MLB Girls PG Autonomous College, Bhopal - 462026, Madhya Pradesh, India.
Department of Zoology [2], Government MVM College, Bhopal - 462026, Madhya Pradesh, India.

Keywords:

Enzymic antioxidants, Allicin, Cancer cell lines, Phytochemicals

Correspondence to Author:
Ms. Anubha Chaudhari

Department of Zoology, Goverment MLB Girls PG Autonomous College, Bhopal - 462026, Madhya Pradesh, India.

E-mail: anubha88chaudhari@gmail.com

ABSTRACT: The present study aims at analyzing the efficiency of allicin, agarlic derived phytochemical or neutraceutical in elevating the activities of various enzymic antioxidants on its treatment to cultured breast cancer cell lines. In the experimental work, cultured breast cancer cell lines were treated with different doses of allicin, and through standardized protocols, the cell lines were assayed for the activities of enzymic antioxidants such as Peroxidase, Superoxide dismutase, Catalase and Glutathione S Transferase as compared to untreated control. It was found that allicin treatment significantly increased the activities of the said enzymic antioxidants as compared to control. This study demonstrates the antioxidant potential of allicin in cancer cells, showing decreased antioxidant activity as a characteristic feature of cancer cells, leading to severe oxidation-induced damage. The study also proposes future prospects in the areas of cancer research and therapy to cobble for the designing of targeted plant-based therapies that are easily available and are cost effective in comparison to synthetic therapeutic chemicals and that too without side effects.

INTRODUCTION: The scientific revolution and urge for a more technologically advanced civilization has invited several new diseases and strengthened the severity of old and known diseases. The revolution has increased pollution, created physical and mental distress in humans, invited severe transformations in the microbial community, perished several life forms, and the last but not the least has made us forget the efficacy of the nature and natural products to heal and maintain a healthy standard of living. There has been extensive research regarding the curative and preventive potencies of various targeted molecules of dietary plant origin like Genistein, diazdin, curcumin, lycopene, emodin, allicin, sulphoraphain, vanillium *etc.* these have shown great actions to cure dreaded disease that either caused death or lead to several dreaded side effects when treated with synthetic drugs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(3).1322-31

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(3).1322-31

Cancer is a multifactorial disease characterized by changes in a group of normally dividing cells of the body that gain uncontrolled growth, causing a lump called a tumour. This is true of all cancers except

leukaemia (cancer of the blood). If untreated, tumour cells can grow and invade surrounding normal tissue or to distant parts of the body *via* the circulation and can cause tumourogenic growths in those parts [1]. Traditional cancer therapies under the allopathic system of medical practice rely on surgical ablation of the cancer tissue, subjecting the tissue to radiotherapy and treatment with chemotherapy. Other therapeutic strategies include immunotherapy, endocrine therapy, gene therapy, etc, depending upon the epidemiology of the disease and socioeconomic status of the patient and relatives [2].

Chemotherapeutic procedures rely on the administration of cytotoxic chemicals that block the cell cycle at various stages or impair the metabolism of cancer cells interfere with the fundamental processes of cancer cells. According to Collins and Workman 2006 [3], chemotherapy remains the main ammunition against cancer and is administered through different routes depending upon site, severity, and stage of cancer. In connection to these, Dougherty & Lister, 2010 [4], stated that Cancer treatments cause side effects and problems that occur when treatment affects healthy tissues or organs.

Owing to the severity of the disease, a large number of side effects of diagnostic and therapeutic procedures and cost of diagnosis and treatment, clinicians and researcher of today's era has turned their vision towards plant-based therapeutic strategies such as owing to their curable properties known through ages and traditions. *Allium* species of plants are now well known for their therapeutic potential against an innumerable number of diseases. *Allium*s pp., the most important representative genus of the Alliaceae family, comprises 700 species, widely distributed in the northern hemisphere [5].

The well-known garlic (*Allium* sativum) and onion (*Allium* cepa) and several other species are widely used as edible ingredients, spices, and medicinal applications. Among all known *Allium* species, the best known, Garlic (*Allium* sativum) is one of the most widely investigated plants over the past couple of decades for its use against a spectrum of diseases ranging from infectious and metabolic diseases to dreaded and complex ailments like cancer [6]. The use of garlic as an edible has been shown to be associated with human health and has been found to cobble the cure of many diseases since time immemorial. Dipado and Carruthers 1960 carried out experiments in which they implanted tumor explants, one allicin incubated and the other incubated to two groups of healthy mice respectively [7].

They found that allicin-treated tumors did not grow in mice it comparison to the allicin untreated tumor implanted mice. This study clearly showed the possibility of anticancer activities of Allicin. Allicin has been shown to target p21ras by causing its thioallylation, causing its activation resulting in enhanced phosphorylation of kinase involved in several signaling pathways [8]. p21ras is actively involved in the activation of lymphocytes [9,] which play an important role in immunosurveillance and against tumors. In connection to this allicin has also been found to trigger TNF-α secretion by macrophages [10], which is also a key cytokine in the regulation of immune responses [11]. Thus allicin can also be thought to alter macrophage activities.

Although further studies regarding the activity of allicin on normal cells and the details of the mechanism of action of allicin on neoplasms and the most efficient routes of administration are required. Further studies would also decipher its molecular interactions within the living system so that its claimed applicability long-term and cost-effective benefits in assume terms of health and nutrition among consumers to create a cancer-free world. The present study investigates the antioxidant efficacy of allicin as an anticancer phytochemical on cultured breast cancer cell lines, which would add up to the process of designing plant-based cost-effective anticancer therapeutic agents without side effects.

Experiment design (Methodologies): The MCF-7 and T47D breast cancer cell lines were purchased from Sigma Aldrich and were maintained in DMEM (Dulbecco's Modified Eagle's medium) Sigma Aldrich kit according to the manufacturer's instructions. All the cell lines were maintained in the media supplemented with 10% f *et al.* calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C.

Experimental and control cells were suspended in 1X Assay Buffer and Homogenized on ice. They were then centrifuged at 15000 g for 10 min. Pellet was discarded, and supernatant containing the Cell lysate fraction was assayed undiluted. The assays and estimations were done on the said cultured cell lines by treatment with various concentrations of Allicin as compared to untreated control.

Analysis of Enzymic Antioxidants: The standard methodologies for analyses of enzymic antioxidants in untreated controls and allicin treated Cultured cell lines were taken up and are presented under the following subheadings marked A-D:

A Peroxidase Assay [12]: Cell Biolabs' Oxi Select™ hydrogen peroxide/peroxidase assay kit was used to assay the activity of Peroxidase.

B Catalase Activity Assay [12]: Cell Bio labs' Oxi Select™ catalase activity Assay was used for the direct measurement of catalase activity from cell lysate.

C Superoxide Dismutase Activity Assay [13]: Oxi Select™ superoxide dismutase activity assay kit was used to assay superoxide dismutase activity in which a xanthine/xanthine oxidase (XOD) system generates superoxide anions.

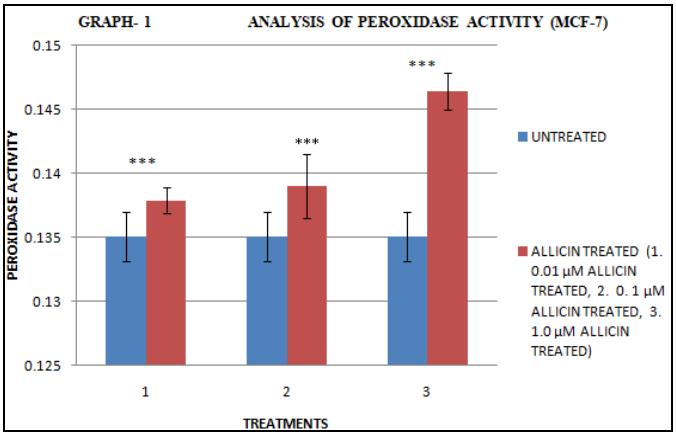
D GST Activity Assay [14]: Sigma GST Activity Assay Kit (Colorimetric) was used to measure the activity of Glutathione-S-transferase (GST).

Results of Analysis of Enzymic Antioxidants: The results of Enzymic antioxidant analysis are presented under the following subhad marked A-D:

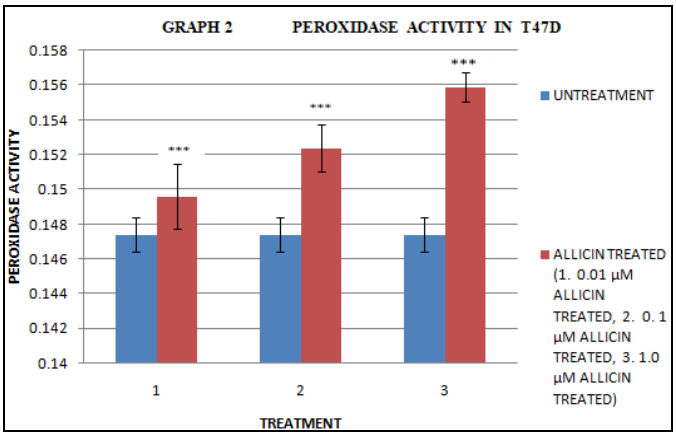
Analysis of Peroxidase activity: The results of the analyses of Peroxidase activity in the MCF-7 and T47D cell lines are presented in **Table 1.** followed by Graph 1 (for MCF-7) and Graph 2 (for T47D).

TABLE 1: PEROXIDASE ACTIVITY OF UNTREATED AND ALLICIN TREATED CELL LINES (MCF-7 AND T47D)

	UNTREATED (CONTROL)	0.01 µ M ALLICIN TREATED	0.1 µM ALLICIN TREATED	1.0 µ M ALLICIN TREATED
Per oxidase Activity In Mcf-7	0.13508 ± 0.001897	0.13788 ± 0.001004***	0.13904 ± 0.00249***	0.14640 ± 0.00144***
Per oxidase Activity In T 47 d	0.1474 ± 0.000975	0.1496 ± 0.001891***	0.1524 ± 0.001351***	0.1559 ± 0.000837***



GRAPH 1: ANALYSIS OF PEROXIDASE ACTIVITY (MCF-7)



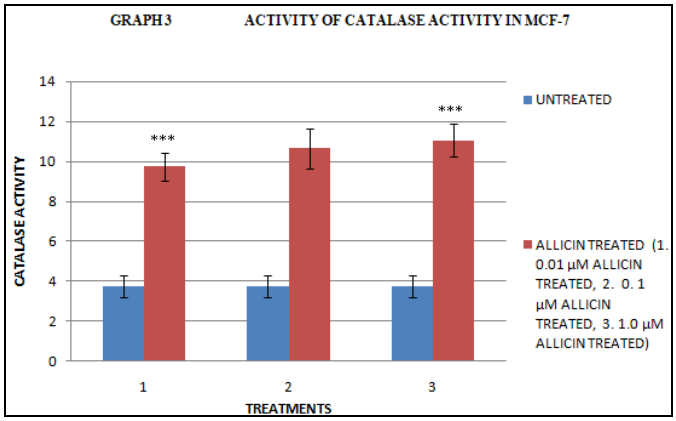
GRAPH 2: PEROXIDASE ACTIVITY IN T47D

The results **Table 1** and Graphs 1 & 2) of the analyses of Peroxidase activity in untreated and allicin treated cell lines show statistically significant increase in the activities of the said enzyme on treatment with increasing concentrations of allicin (*i.e* 0.01 µM, 0,1 µM and 1.0 µM). Numerical data are represented as Mean = SEM of five independent experiments (N=5). Statistical significance of the difference from untreated (control) values at $p < 0.01$ determined by unpaired t-test of two-sample unequal variance using Graph Pad software.

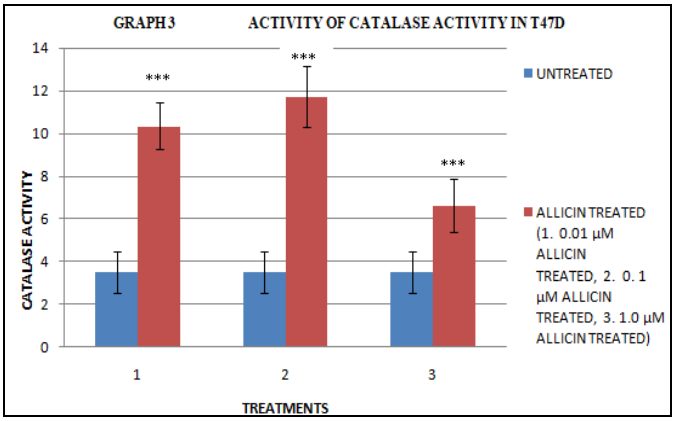
B Analysis of Catalase Activity: The results of the analyses of Catalase activity in the MCF-7 and T47D cell lines are presented in **Table 2**. followed by Graph 3 (for MCF-7) and Graph 4 (for T47D).

TABLE 2: CATALASE ACTIVITY OF UNTREATED AND ALLICIN TREATED CELL LINES (MCF-7 AND T47D)

	UNTREATED (CONTROL)	0.01 µM ALLICIN TREATED	0.1 µM ALLICIN TREATED	1.0 µ M ALLICIN TREATED
Catalase Activity In Mcf-7	3.7432 ± 0.53837	9.73 ± 0.688195***	10.6396 ± 0.993275***	11.038 ± 05.829727***
Catalase Activity In T 47 d	3.506 ± 0.964752	10.3688 ± 1.09216***	11.718 ± 1.427621***	6.6576 ± 1.240673***

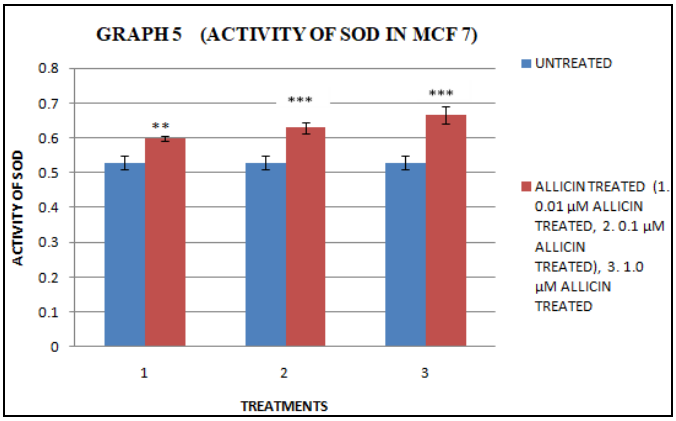


GRAPH 3: ACTIVITY OF CATALASE ACTIVITY IN MCF-7

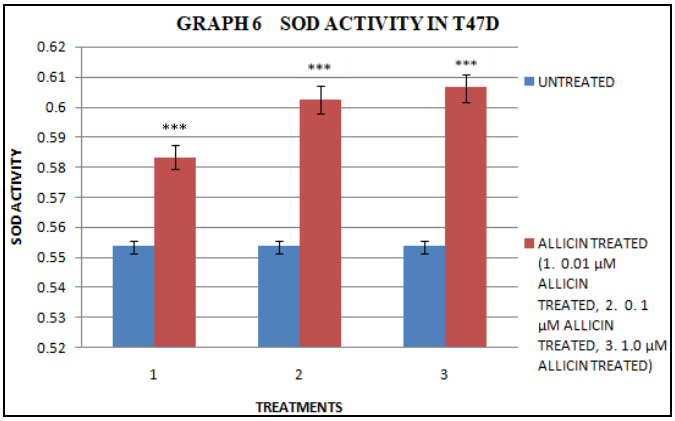


GRAPH 4: ACTIVITY OF CATALASE ACTIVITY IN T47D

The results **Table 2** and Graphs 3 & 4) of the analyses of Catalase activity in untreated and Allicin treated cell lines show statistically significant increase in the activity of the said enzyme on treatment with increasing concentrations of allicin (*i.e* 0.01 µM, 0,1 µM and 1.0 µM). However a greater activity of catalase was observed for treatment group 2 in T47D cell line. Numerical data are represented as Mean=SEM of five independent experiments (N=5). Statistical significance of the difference from untreated (control) values at $p < 0.01$ determined by unpaired T test of two sample unequal variance using Graph Pad software.



GRAPH 5: ACTIVITY OF SOD IN MCF 7



GRAPH 6: SOD ACTIVITY IN T47D

C Analysis of Superoxide Dismutase Activity: The results of the analyses of Superoxide Dismutase activity in the MCF-7 and T47D cell lines are presented as **Table 3** followed by Graph 5 (for MCF-7) and Graph 6 (for T47D).

TABLE 3: SUPEROXIDE DISMUTASE ACTIVITY OF UNTREATED AND ALLICIN TREATED CELL LINES (MCF-7 AND T47D)

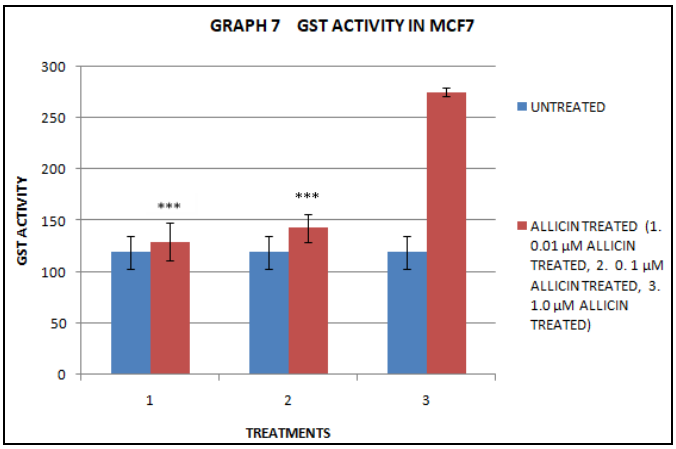
	UNTREATED (CONTROL)	0.01 µM ALLICIN TREATED	0.1 µM ALLICIN TREATED	1.0 µ M ALLICIN TREATED
Sod Activity In Mcf-7	0.5286 ± 0.020011	0.5974 ± 0.007259**	0.6296 ± 0.01627***	0.6666 ± 0.025965***
Sod Activity In Mcf-7 T 47 d	0.553 ± 0.020433	0.5832 ± 0.003995***	0.6024 ± 0.0045685***	0.6064 ± 0.0045873***

The results Table 3 and Graphs 5 & 6) of the analyses of Superoxide Dismutase activity in untreated and Allicin treated cell lines show statistically significant increase in the enzyme activity on treatment with increasing concentrations of Allicin (*i.e* 0.01 µM, 0,1 µM, and 1.0 µM). Numerical data are represented as Mean = SEM of five independent experiments (N=5). Statistical significance of the difference from untreated (control) values at $p < 0.01$ determined by unpaired t-test of two-sample unequal variance using Graph Pad software.

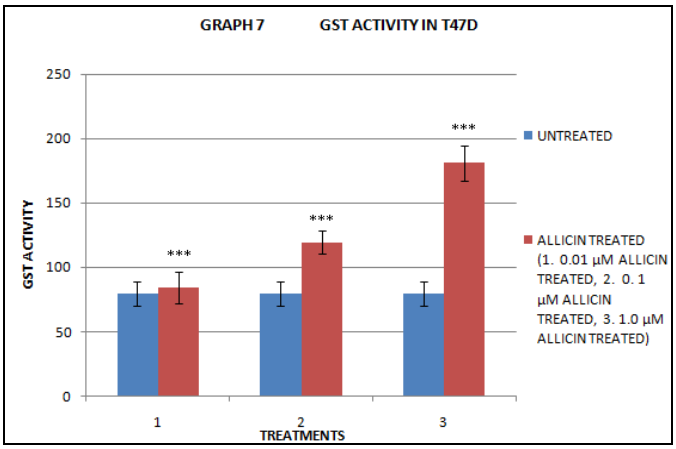
D Analysis of Glutathione-S- Transferase Activity: The results of the analyses of Glutathione S Transferase activity in the MCF-7 and T47D cell lines are presented as **Table 4**. followed by Graph 7 (for MCF-7) and Graph 8 (for T47D).

TABLE 2: GLUTATHIONE S TRANSFERASE ACTIVITY IN UNTREATED AND ALLICIN TREATED CELL LINES (MCF-7 AND T47D)

	UNTREATED (CONTROL)	0.01 µ M ALLICIN TREATED	0.1 µ M ALLICIN TREATED	1.0 µ M ALLICIN TREATED
GST_activity in MCF-7	119.28 ± 15.71652	129.6528 ± 18.510832***	142.9408±13.439233***	275.56 ± 4.162158**
GST activity in T47D	79.52 ± 9.001053	84.2912 ± 12.525978***	119.28 ± 9.021053***	180.8078 ± 13.574749***



GRAPH 7: GST ACTIVITY IN MCF7



GRAPH 8: GST ACTIVITY IN T47D

The results 4 and Graphs 7 & 8) of the analyses of Glutathione S Transferase activity in untreated and Allicin treated cell lines show a statistically significant increase in the activity of the said enzyme on treatment with increasing concentrations of Allicin (*i.e* 0.01 µM, 0.1 µM and 1.0 µM) in case of treatment group 3 in MCF-7 and treatment groups 2 and 3 in T47D cell lines. Numerical data are represented as Mean = SEM of five independent experiments (N=5). Statistical significance of the difference from untreated (control) values at $p < 0.01$ determined by unpaired t-test of two-sample unequal variance using Graph Pad software.

DISCUSSIONS: In the past decades, there has been an increased interest in using herbal medicines in the management, prevention, and therapy for a wide range of human diseases, including cancer. The herbal formulations from Ayurveda, traditional Chinese medicines, Japanese (Kampo), and others are composed of a mixture of different herbs, which synergistically work to produce maximum treatment efficacy with minimum or no side effects [15]. Extensive research in the very arena has revealed that these formulations can down-regulate cancer cells progression and stimulate the immune system to rise against cancer [16].

Garlic and its components & products have also been found to have anticancer activities through diverse mechanisms. Several studies on experimentally induced cancers in animal models and their amelioration using crude garlic extract, garlic powder, or isolated garlic phytochemicals such as allicin and other compounds have shown that garlic and its chemical derivatives have immense potential to fight cancers [17]. *Allium* species are supposed to be one of the world's oldest

cultivated vegetables of the world. It is presumed that our ancestors consumed wild *Allium* species long before actual farming methods was invented. *Allium* plants are small in size and leave no archaeological evidence; their exact origin still remains a mystery. *Allium* species are characterized by their rich content of thiosulfinates and other organosulfur compounds. The thiosulfinates are formed by the action of the enzyme alliinase from their respective S-alk(en)yl cysteine sulfoxides, which are mainly responsible for flavor and smell of garlic and onion.

The eye irritating compounds that induce tears are also a result of these sulphur rich compounds hence called Lachrymatory factors. Moreover, depending upon *Allium* species and differing conditions, thiosulfinates can decompose to form additional sulfur constituents, including diallyl, methyl allyl and diethyl mono-, di-, tri-, tetra-, penta-and hexasulfides, vinyldithiins, etc. This piece of work examines the biological activities of *Allium* thiosulfinate, allicin, as anticancer neutraceuticals. AS already described, Allicin (diallyl thiosulfinate or diallyl sulphide) is a biologically active compound found in crushed garlic due to stress-induced activation of an enzyme called allicinase that converts its precursor, alliin (s-allyl cysteine sulphoxide) into allicin [18]. Studies throughout the globe on the efficiency of allicin during the last two decades have shown its overwhelming potential to cure many chronic degenerative diseases that had threatened mankind for a long time.

The work emphasizes the utility of this multifaceted Nutraceutical allicin on cancer. Furthermore, allicin has shown a tremendous potential to modulate the immune system that constantly invigilates the body tissues for tumorogenic activities and help to clear off transformed cells were ever encountered. In connection to these, research has also revealed the immense antioxidant potential of allicin which impart prevention and protection against free radical-induced damages. The terms “free radicals” and “active oxygen” have attracted the attention of large numbers of scientific workers and clinicians. Human beings face constant attacks by exogenous factors such as ultraviolet rays and tobacco smoke, which cause oxidative stress. Such stress also arises from drugs (including anticancer drugs) that are used in common medical practice. In addition to those exogenous sources, endogenous sources of oxidative stress include those which are derived from metabolic activities of mitochondria or microsomes, peroxisomes during the electron transfer, and those from the enzymes used by macrophages and neutrophils during the immune response. Reactive radicals oxygen may be involved in carcinogenesis through two possible mechanisms that induce mutations and the effects of signal transduction and transcription factors. The mechanism of damage induction depends on factors such as the type of active oxygen species involved and the intensity of stress [19].

Active oxygen species are supposed to act directly or indirectly via effects on gene expression and cell signaling, and communications. AS examples, Glutathione and Thioredoxin work in the mechanisms of redox regulation [20]. Many studies on oxidative stress and carcinogenesis have been carried out in animal experiments and clinical practice. It was reported that lower activity of SOD is at a higher risk of developing breast cancer [21]. In general, cancer therapy with drugs and radiation creates a state of oxidative stress in the body, and active oxygen triggers apoptosis of exposed cells *via* p53 and cytochrome release from mitochondria. Anticancer drugs such as anthracyclines, bleoymcin, mitomycin C and cisplatin have active oxygen mechanisms of activity [22]. Research has shown that excessive ROS accumulation leads to cellular injuries, such as damage to DNA, proteins, and lipid membranes [23]. Peroxides, such as hydrogen peroxide (H_2O_2), are an example of the most well-documented ROS produced under oxidative stress conditions [24].

Hydrogen peroxide is a toxic product of normal aerobic metabolism and poisonous to eukaryotic cells. High doses can initiate oxidation of DNA, lipids, and proteins, leading to deleterious effects and cell death. The cellular damage caused by peroxides has been correlated with the development of many pathological conditions and cancers. It is now clear that injury to cells by such stresses is too significant to be ignored. Under normal physiological conditions, the cellular generation of ROS is counteracted by the action of antioxidant enzymes and other redox molecules. However, excessive ROS accumulation will lead to cellular

injuries, such as damage to DNA, protein, and lipid membrane. Because of their potentially harmful effects, excessive ROS must be immediately eliminated from the cells by a variety of antioxidant defense mechanisms. Superoxide dismutase (SOD catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen & is one of the most important antioxidative enzymes. SOD enzymes are classified into three groups: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular Ec-SOD [25]. Moreover, catalase is an antioxidant enzyme omnipresent in mammalian and non-mammalian cells that destroys hydrogen peroxide by dismutation. Eukaryotic catalases are heme-containing enzymes found in the liver, kidney, and erythrocytes in high concentrations, while the lowest concentrations occur in the connective tissues.

The enzyme is concentrated in the peroxisome sub-cellular organelles [26]. In addition to enzymatic antioxidant, Glutathione S-transferase (GST) is a family of enzymes that plays an important role in the detoxification of xenobiotics. GST catalyzes the attachment of the thiol of glutathione to electrophiles. Glutathione is used to scavenge potentially toxic compounds, including those produced as a result of oxidative stress, and is part of the defense mechanism neutralizing the mutagenic, carcinogenic and toxic effects of such compounds.

The GSTs constitute catalyse the conjugation of electrophilic substances with the ubiquitous nucleophile GSH and the non-selenium-dependent reduction of organic hydroperoxides [27]. Several evidences have implicated the role of GST enzymes in the resistance of cancer cells to alkylating agents and anthracyclines [28]. It is well known that some genetic variations present in the antioxidant enzymes bring about an alteration in the activity or roles of the enzymes, which may result in the modulated ability to scavenge ROS. These modulations explain some relationships between specific gene variants and breast cancer risk suggesting the protective role of variants linked to the increased antioxidant protection. Thomson M & Ali M, 2003, have shown that consumption of garlic caused a 40% increase in peroxidase activity compared to treatment with carcinogens in experimental subjects [29].

In connection to the above findings, the results **Table 1** and Graphs 1 & 2 of our study show that Peroxidase activity in allicin treated cell lines show a statistically significant increase in treatment with increasing concentrations of allicin (*i.e.* 0.01 µM, 0,1 µM, and 1.0 µM) in comparison to untreated control which is in well correlation with the data reported by Skrzydlewska 2001 [30]. Nature has provided the animal and human body with a complex antioxidant defense mechanism that includes the antioxidant enzyme of which Catalase (CAT) is a major player to provide protection against oxidative damage include by reactive oxygen species. The Enzyme block the initiation of free radical chain reactions [31].

When free radicals are excessively produced or when the cellular antioxidant defense mechanism has failed, chain reactions can result in the interaction of proteins, lipids, and nucleic acids with such oxidants causing cellular dysfunctions that may lead to ailments and even death. Enzyme Catalase plays a role in the follow-up of breast cancer disease. Catalase, along with several other such enzymes such as SOD, peroxide, GST, etc., nullify the deleterious action of Reactive Oxygen Metabolites (ROMs) such as singlet oxygen, superoxide anions, hydroxyl radical, hydrogen peroxide [32]. AS compared to the above studies, the findings of our study on the effect of allicin treatment on cultured breast cancer cell lines indicate that catalase activity significantly increased **Table 2** and Graphs 3 & 4) with increasing concentrations of allicin, *i.e.*, 0.01 µM, 0.1 µM, 1.0 µM in comparison to the untreated control. However, greater activity of catalase was observed for treatment Group 2 in T47D cell lines.

The results of our study well correlate with the fact that as strong antioxidant properties imparts radioprotective effects by free radical scavenging abilities and increase the efficiency of scavenging systems in the cells [33]. Allicin has been shown to have pronounced antitumor activity hepatocellular cancer cells through ROS-mediated mitochondrial pathways [34]. It was reported by scientists that the antioxidant defense system changed in various human tumors. It was reported that a reverse relationship was found between antioxidant enzyme activities and lipid peroxidation in patients with tumor of various types [35].

Alteration in the tissue and serum level activities of superoxide dismutase and other free radicals Scavengers have been studied in various pathological conditions about tumours and cancers [36]. In connection to the above studies Iwase *et al.*, 1997 [37], however, reported that obtained Data remains unclear as the activity of superoxide dismutase very greatly according to the alterations of conditions in each of the different study groups. Studies have shown that there is a general increase in total activity of superoxide dismutase in the plasma and issue tissue samples of various subjects of malignant breast tumours. Furthermore significant increase in tissue activity of superoxide dismutase was recorded in malignant tumours as compared to benign tumors [38].

In general, an increase in the superoxide dismutase activity might reflect our response to oxidative stress and thus may predict a state of increased stress of relative oxygen species in the process of carcinogenesis. In our study, the total activity of superoxide dismutase in breast cancer tissues was found to be higher than that of the control group, although the differences were statistically insignificant on treatment with allicin. Our results are in accordance to the Results of a study in Japan, which showed a positive correlation between the activity of SOD in serum of cancer subjects and cancer mortality [39]. Tsubura A *et al.*, 2011, [40] showed that garlic-derived Sell for compounds shows antioxidant activity by elevating superoxide dismutase activity in breast cancer and has a potential therapeutic implication by acting as an anti-proliferative agent.

Concerning the above studies, the results of our study show **Table 3** and Graph 5 & 6 a statistically significant increase in superoxide dismutase activity in Allicin treated cell lines in comparison to untreated cells with increasing concentration of Allicin (*i.e.* 0.01 uM, 0.1 uM, and 1.0 uM as compared to the untreated control. The antioxidant properties of *allium* vegetables and active ingredients of garlic, such as allicin, might result from the contributory activities of sulphur-containing compounds at different steps of neutralizing reactive oxygen species that result in tumour progression and carcinogenesis. Glutathione S-transferase (GST) is an enzyme belonging to the proteins involved in phase-II detoxification of endogenous compounds and xenobiotics. Regulation and functional activity of GSTs have influences on cell growth in various ways, oxidative stress, disease progression, and prevention. Glutathione S-transferases (GSTs) are involved in detoxification by bringing about the removal of harmful electrophiles by conjugating them with glutathione [41]. GSTs are well-known enzymes that participate in Phase-II metabolism of drugs and also take part in detoxification during the metabolism of carcinogens that may be electrophilic.

Moreover, any substance that increases the levels and/or activity of GSTs is said to be chemopreventive. Our study in context to the analyses of glutathione S-transferase activity in untreated and allicin treated cell lines show a statistically significant increase in the activity of the said enzyme on treatment with increasing concentrations of allicin (*i.e* 0.01 µM, 0,1 µM and 1.0 µM) in case of treatment group 3 in MCF7 and treatment groups 2 and 3 in T47D cell lines. The results of our study are well in accordance to the studies shown by various workers throughout the globe pertaining to the use of plant-derived antioxidant compounds to neutralize the increased free radical status of cancerous tissue in various model systems.

CONCLUSION: The present study aimed at analyzing the effectiveness of garlic-derived phytochemical, ‘allicin’ on cultured human breast cancer cell lines to analyze the alterations in the activity of enzymic antioxidants such as Peroxidase, Catalase, Superoxide dismutase, and GST in such cells.

It was found that allicin treatment significantly increased the activities of the enzymes mentioned above in cultured cancer cells as compared to untreated control. The findings of this study throw a glimpse of light towards the development of plant-derived or herbal drugs to fight the dreaded disease as such drugs are cost-effective and free from side effects.

More studies and research are required to get an in-depth and comprehensive understanding of more effects of allicin so that an accurate strategy for cancer therapy can be designed.

ACKNOWLEDGEMENT: The authors would like to thank Deshpandey Laboratories, Bhopal for providing technical support and assistance for the successful completion of the said work.

CONFLICTS OF INTEREST: The authors don't have any conflict of interest regarding the work and its publication.

REFERENCES:

1. Sener G, Sehirli O, Ipçi Y, Ercan F, Sirvanci S, Gedik N and Yeğen BC: "Aqueous garlic extract alleviates isehaemia-reperfusion-induced oxidative hepatic injury in rats. J of Pharmacy and Pharmacology 2005; 57(1):145-50.
2. Gunn WG: Ackerman and del regato's; cancer: diagnosis, treatment and prognosis. JAMA 1985; 254(9): 1227.
3. Collins I and Workman P: New approaches to molecular cancer therapeutics. Nature Chemical Biology 2006; 2(12).
4. Dougherty and Lister: The royal marsden hospital manual of clinical nursing procedures. 7th Ed Wiley Black 2010.
5. Tsiaganis MC, Laskari K and Melisari E: Fatty acid composition of *Allium* species lipids. J Food Comp Anal 2006; 19(6-7): 620-27.
6. Abdullah TH, Kandil O, Elkadi A and Carter J: Garlic revisited: therapeutic for the major diseases of our times. J Natl Med Assoc 1988; 80: 439-45.
7. Dipaolo Ja and Carruthers C: The effect of allicin from garlic on tumor growth. Cancer Res 1960; 20: 431-4.
8. Patya M: Allicin stimulates lymphocytes and elicits an anti-tumor effect: A possible role of p21ras. Int Immunol 2004; 16: 275-81.
9. Tocque B, Delumeau I, Parker F, Maurier F, Multon MC and Schweighoffer F: Ras-GTPase activating protein (GAP): A putative effector for Ras. Cell Signal 1997, 9: 153-58.
10. Haase H, Hieke N, Plum LM, Gruhlke MCH, Slusarenko AJ and Rink L: Impact of allicin on macrophages activity. Food Chem 2012; 134: 141-48.
11. Ellerin T, Rubin RH and Weinblatt ME: Infection and anti tumor necrosis factor alpha therapy. Arthritis Rheum 2003; 48: 3013-22.
12. Pütter J and Becker R: In methods of enzymatic analysis bergmeyer. IJPSR 1983; 3: 286-93.
13. Kakkar PS, Das B and Viswanathan PN: A modified spectrophoto-metric assay of superoxide dismutase. Ind J Biochem Biophys 1984; 21: 130-32.
14. Habig WH, Pabst MJ and Jacoby WB: Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 249: 7130-39.
15. Dildar Ahmed, Saman Zara and Hira Baig: *In-vitro* analysis of antioxidant activities of oxalis corniculata linn. fractions in various solvents; Afr J Tradit Complement Altern Med 2013; 10(1):158-65.
16. T Liu, Y Song, H Chen, S Pan and X Sun: "Matrine inhibits proliferation and induces apoptosis of pancreatic cancer cells *in-vitro* and *in-vivo*. Biological and Pharmaceutical Bulletin 2010; 33(10): 1740-45.
17. Galeone C, Pelucchi C, Levi F, Negri E, Franceschi S, Talamini R, Giacosa A and La Vecchia C: Onion and garlic use and human cancer. Am J Clin Nutr 2006; 84(5): 1027-32.
18. Block E: Garlic and other alliums-the lore and the science. RSC publishing Cambridge UK 2010.
19. Mates JM, Perez-Gomez C and De Castro IN: Antioxidant enzymes and human diseases. Clin Biochem 1999; 32: 595-03.
20. Arrigo AP: Gene expression and the thiol redox state. Free Radic Biol Med 1999; 27: 936-44.
21. Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, Graham S, Laughlin R, Nemoto T and Shields PG: Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. Cancer Res 1999; 59: 602-06.
22. Weijl NI, Cleton FJ and Osanto S: Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat Rev 1997; 23: 209-40.
23. DN Douglas, Christopher Hao Pu, Jamie T Lewis, Rakesh Bhat, Anwar Anwar-Mohamed, Michael Logan, Garry Lund, William R Addison, Richard Lehner and Norman M Kneteman: Oxidative stress attenuates lipid synthesis and increases mitochondrial fatty acid oxidation in hepatoma cells infected with hepatitis c virus. J Biol Chem 2016; 291(4): 1974-90.
24. Zhang K, Wang G, Zou Z, Jia X, Wang S, Lin P, Chen Y, Zhang Z and Wang Y: Cloning, characterization and TBT exposure response of CuZn superoxide dismutase from Haliotis diversicolor supertexta. Mol Biol Rep 2009; 36: 583-94.
25. Zelko IN, Mariani TJ and Folz RJ: Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution, and expression. Free Radical Biol Med 2002; 33: 337-49.
26. Flavio Torres, Alejandro González-Candia, aCmilo Montt, Germán Ebensperger, Magdalena Chubretovic, María Serón-Ferré, Roberto V Reyes, Aníbal J Llanos and Emilio A Herrera: Melatonin reduces oxidative stress and improves vascular function in pulmonary hypertensive newborn sheep. J Pineal Res 2015; 58(3): 362-73.
27. Hayes JD and Pulford DJ: The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Bioch Mol Biol 1995; 30(6): 445-600.
28. Tew KD: Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 1994; 54: 4313-20.
29. Thomson M and Ali M: Garlic *Allium satium*: a review of its potential use as an anti-cancer agent. Cancer Drug Targets 2003; 3: 67-81.
30. Skrzydlewska E, Stankiewicz A, Sulkowska M, Sulkowski S and Kasacka I: Antioxidant status and lipid peroxidation in colorectal cancer. J Toxicol Environ Health A 2001; 64: 213-22.
31. Rao DN, Desai PB and Ganesh B: Epidemiological observation on cancer of the esophagus- a review of Indian studies. Ind J Cancer 1976; 33: 55-75.
32. Negahdar M, Djalali M, Abtahi H, Sadeghi MR, Aghvami T, Javadi E and Layegh H: Blood super oxide dismutase and catalase activities in women affected with breast cancer. Iranian J Pub Health 2005; 34(3): 39-43.
33. Wei Z and Lau BHS: Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. Nutr Res 1998; 18: 61-70.
34. Zou X, Liang J, Sun J, Hu X, Lei L, Wu D-H and Liu L: Allicin sensitizes hepatocellular cancer cells to anti-tumor activity of 5-fluorouracil through ROS-mediated mitochondrial pathway. J Pharma Sci 2016; 131: 233-40.
35. Alagol H, Erdem E, Sancak B, Turkmen G, Camlibel M and Bugdayci G: Nitric oxide biosynthesis and malondialdehyde levels in advanced breast cancer. Aust N ZJ Surg 1999; 69: 647-50.

36. Nakamura S, Hara K, Suchi T, Ito M, Ikeda H, Nagahama M, A Nakayama, A Nakagawa, H Kaneshima and J Asai: Interdigitating cell sarcoma: a morphologic, immunohistologic and enzyme-histochemical study. Cancer 1988; 61: 562-8.
37. K Iwase, K Kato, S Ohtani, T Tsujimura, T Hanai, A Inagaki, S Jimbo, N Kobayashi, H Yamamoto, A Nagasaka and K Miura: The relation between superoxide dismutase in cancer tissue and clinico-pathological features in breast cancer. Breast Cancer Tokyo Japan 1997; 4(3): 155-60.
38. Hathama Razooki Hasan, Thikra Hasan Mathkor and Mohammed Hasan Al-Habal: Superoxide dismutase is enzymes activities in plasma &tissues of Iraqi patients with breast cancer. A Pacific J of Cancer Prev2012; 13.
39. Pham TM, Fujino Y, Nakachi K, Suzuki K, Ito Y, Watanabe Y, Inaba Y, Tajima K, Tamakoshi A and Yoshimura T: JACC study group relationship between serum levels of superoxide dismutase activity and subsequent risk of cancer mortality: findings from a nested case-control study within the japan collaborative cohort study. Asian Pac J Cancer Prev 2009; 10: 69-73.
40. Tsubura A, Lai YC, Kuwata M, Uehara N and Yoshizawa K: Anticancer effects of garlic and garlic-derived compounds for breast cancer control. Anti-Cancer Agents in Medicinal Chemistry 2011; 11: 249-53.
41. Jakoby WB: The glutathione-s-transferase: a group of multifunctional detoxfication proteins. Adv Enzymol 1978; 46; 383-14.

How to cite this article:
Chaudhari A and Chauhan R: Antioxidant efficacy of garlic derived allicin on cultured breast cancer cell lines. Int J Pharm Sci & Res 2022; 13(3): 1322-31. doi: 10.13040/IJPSR.0975-8232.13(3).1322-31.