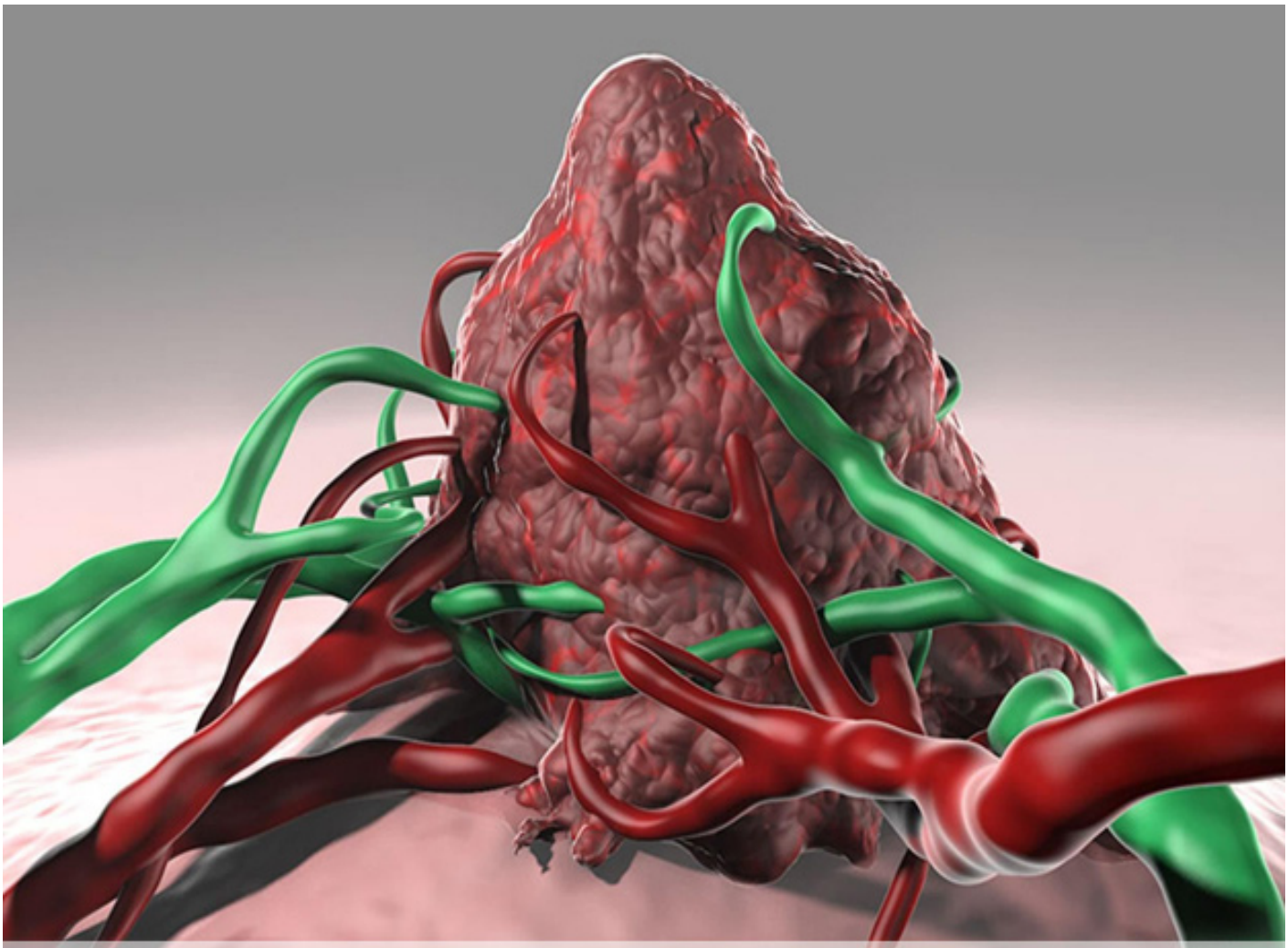

CANCER THERAPY

ISBN 978-81-936678-8-0

MEDDOCS
Open Access Publisher

Allium Species in the Fight Against Cancer

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Published Online: Apr 30, 2020

eBook: Cancer Therapy

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Introduction

Cancer is one of the most common causes of death in the developed world affecting people from any age, race or gender [1,2]. Breast cancer is the most common carcinoma observed in women followed by other forms of cancer like colorectal, lung, cervical and stomach cancers [3-5]. Chemotherapy and radiation therapy are currently the methods of choice along with new generation anti-tumoragents used alongside surgical procedures however current treatments end to have severe side effects that can reduce patients' quality of life. Immunotherapy, hormone therapy and targeted therapy methods are gaining momentum and success despite not being without side effects. Research and development of direct and complementary therapies for all cancers including breast cancer are essential and needed to help improve disease outcomes and patients' quality of life [1].

Plants have historically been used to treat various diseases including cancer where a number of cancer treatments originate from therapeutic plants [6]. World Health Organization (WHO) reports state that most of the world's population depends on traditional medications for treatment of diseases [5,6]. Plants and their constituents have been widely used for development of the new therapeutics for cancer with approximately 60% of current cancer drugs having been developed from plant products [7].

Genus *Allium* (including garlic) have been commonly used as therapeutic plants for treatment of hypertension and infectious diseases [8]. Specifically, constituents of *Allium sativum* is of interest for scientific research as it has anticoagulant, antihistaminic, antiparasitic, antifungal, antiprotozoal and antiviral properties [8,9].

Allium sativum (garlic) anticancer properties have been widely demonstrated where increased dietary consumption of garlic bulb was shown to decrease the risk of several cancer types like colon, pancreas, breast cancers [2] and increased intake of *Allium* class vegetables decrease stomach cancer [10]. The mechanisms associated with *Allium* species in controlling breast cancer include the suppression of DNA adduct formation, triggering drug metabolizing enzymes responsible for detoxification of carcinogen agents and induction of apoptosis [2].

In vitro and *in vivo* studies revealed that garlic has reductive effect on tumour sizes by leading apoptotic cancer cell death. Active molecules in garlic are also found to function as antioxidant agentsin *in vitro* cell culture models [11].

Experiments on evaluation of bioactive molecule composition of *Allium sativum* has shown that the plant has high number of therapeutic molecules with anticancer effects such as allyl sulphide containing molecules. Different *Allium sativum* compounds have been shown to regulate molecular mechanism in cancer formation by regulating mutagenesis, affecting cell proliferation rates and their differentiation, DNA adduct formation, release of free radicals and angiogenesis in tumour [9].

Historical perspective on the medical and anticancer effects of *Allium sativum*

Anticancer effects of *Allium sativum* dates back to centuries ago. In Ancient Egypt garlic was used to heal tumour tissue formed outside of the body [12] and Hippocrates and ancient Indian physicians were also using garlic for external treatment of cancer tissues [13,14]. Aristophanes and Galenal mentioned in their writings that *Allium sativum* preparations and components have potency on treatment of uterine tumours [15].

Cultivation of *Allium sativum* dates back over 5000 years. It is believed that the native area of *Allium sativum* is West China, Kazakhstan and Kyrgyzstan as mentioned in records belonging to 3000 B.C in Mesopotamia. Ebers Papyrus and Egyptian art dating to 2700 B.C mention that garlic was already widely used in that region as well [12,16]. Hippocrates, father of medicine, Galen, father of galenic pharmacy, Pliny the Elder in Historia Naturalis (77 C.E), and Dioscorides in De Materia Medica used garlic for treatment of many conditions such as parasitic infections, respiratory system problems, digestion problems, and so on. *Allium sativum* was also mentioned in Chinese writings dating back to A.D. 510 [17], and took place in the Bible and the Koran [12]. Sumerians used *Allium sativum* L. for treatment purposes. Garlic that the Sumerians used was believed to have been brough from China and further spread to other countries like Korea or Japan afterwards [12]. In ancient China, garlic was such a popular remedy partly due to its heating effect and it was considered as in ying yang philosophy where "in every good there is a bad and in every bad there is a good in" [18].

In the Vedas, an ancient Indian book, garlic was recommended for treatment of common diseases like cough, skin conditions, rheumatism, weakened appetite and hemorrhoids. Ancient India's first pharmacists and physicians were widely prescribing garlic [12].

In addition to its ancient use in the treatment of various diseases, it was also used in the maintenance of mental health where it was further utilized in the treatment of disorders such as depression [18].

Usage of herbs and spices were common in ancient Egypt where mostly local herbs and medicinal plants from the Nile River area were preferred. Garlic was one of the most frequently used plants by Egyptians not only for treatment purposes but also to increase the strength and increase the capacity of work of their slaves [18]. In the Ebers Papyrus it was mentioned that garlic can heal 32 different illnesses [18]. Ancient Egyptians had great healing skills and preparations in remedies so they influenced all surrounding geographic regions such as the Israelis, Persians, Babylonians, Phoenicians and etc. Ancient Israelis described garlic as a body temperature increaser, appetitive, blood pressure increaser, antiparasitic remedy and etc. Advice on consumption of garlic is also mentioned in "The book of Judaism", "Talmud" [18].

In Ancient Greece, garlic was highly valued such that leaders fed soldiers garlic before battles. Greek Olympic athletes ate garlic before competitions to achieve good scores [19].

Various cultures historically used garlic and various forms of it to achieve good health where Tibetans still use ancient recipies for treatment of health problems like stomach ache. Slavic people used garlic in the 7th century to treat spider bites, against lice, anti-ulcer remedy, heal snake bites [20]. In the Middle Ages garlic was highly valued among Arabic people. In the 8th century, Byzantine Empire encouraged their people to increase the cultivation of garlic. In the 9th century garlic was suggested as a preventive for 'blood vessel aging'. Great Britain met with garlic in 1548. Lonicerus advised consumption of garlic as anti-helminthic remedy as well as treatment of skin diseases and dandruff. For centuries, people were encouraged to plant garlic, learned to prepare teas from garlic mixing it with honey to address various diseases related to gastrointestinal system, various fevers, diarrhoea or cold [18].

Among Russian people garlic is avalued herb with the nickname of "Russian penicillin". They used garlic for treatment of diseases affecting the pulmonary system. Even after discovery of penicillin Russian Red Army used garlic for treatment of war wounds as a natural remedy [18].

Epidemiological aspects to garlic consumption and cancer

There has been many epidemiological studies conducted inthe past three decades which revealed that dietary intake of garlic decrease the risk of several cancer types such as colorectal, oesophageal, stomach and lung etc [21-24]. Further studies have also demonstrated anti-cancer properties of garlic and its constituents both *in vitro* and *in vivo* [25]. These findings were supported with the study conducted in China by Mei and coworkers who investigated the correlation between stomach cancer and garlic consumption in two different areas. It was shown that in Cangshan area population consumes 20 grams of garlic whereas in Qixia area garlic consumption is rare and stomach cancer formation is three fold higher [26]. Another epidemiological study performed in China has shown that consumption of vegetables belonging to *Allium* family such as garlic, onion and scallions reduce the incidence of stomach cancer [27]. Many scientists have worked on the dietary intake of *Allium* species and its anticancer effects where Levi and co-workers; concluded that dietary garlic consumption play a protective role on colon cancer [28]. Similarly Fleischauer and co-workers have shown the correlation between garlic intake and protection against colon and stomach cancers [25].

Chemopreventive properties of *Allium sativum*

The well known anticancer properties of garlic are now being detailed as recent studies demonstrated the chemopreventive activity of *Allium sativum* preparations using fresh garlic extracts, garlic oil, aged garlic extract etc. Balasenthil *et al* [29]. have demonstrated the inhibitory effect of *Allium sativum* L. on 7,12dimethylben[a]anthracene- induced buccal pouch cancer formation in hamsters [29]. Garlic exerts its chemopreventive activity on buccal pouch cancer formation by regulating peroxidation of lipid and increasing the amounts of glutathione, glutathione peroxidase and glutathione S-transferase [30]. Another study conducted by Wargovich and co-workers have shown

that Diallyl sulphide (DAS), one of the main components of garlic, reduce the incidence of 1,2-dimethylhydrazine induced colorectal adenocarcinoma in C57BL/6J mice [31]. Wargovich and co-workers, have also shown that Diallyl Sulphide (DAS), one of the most widely studied thioether compounds in *Allium sativum,* inhibits N-nitrosomethylbenzylamine (NMBA)-induced oesophageal cancer in rats. Besides, it was also demonstrated that tumour formation in rats is diminished in NMBA treated mice [32]. Sparnins *et al.*[33], studied the effects eight organosulfur compounds obtained from *Allium sativum* on Benzo[a] Pyrene (BP) induced forestomach and lung neoplasias in A/J mice. Allyl Methyl Trisulfide (AMT), Allyl Methyl Disulphide (AMD) Diallyl Sulphide (DAS), Diallyltrisulfide (DAT) and four other saturated compounds with allyl groups were tested and it was shown that these allyl compounds have inhibitory effect on BP induced neoplasias in mice [33].

Samaranayke *et al*. [34], studied the anticarcinogenic activity of garlic on rat liver induced by Diethylnitrosamine (DEN) and it was shown that treatment with *Allium sativum* significantly decreased the carcinogenic activity [34]. Chemopreventive activity of organosulfur compounds obtained from *Allium sativum* studied in diethylnitrosamine induced hepatocarcinogenesis in male F344 rats and it was revealed that S-Methlycystein (SMC) and cysteine reduced the number tumours and Glutathione S-Transferase Placental form (GST-P) positive hepatocellular loci area.

These findings all together demonstrate the chemopreventive activity of *Allium sativum* soluble organosulfur compounds on hepatocarcinogenesis and colon carcinogenesis [35].

Impact of *Allium sativum* on drug metabolizing enzymes

Allium sativum contains many bioactive molecules that prevent cancer formation by inducing detoxification through activation of the enzymes at phase I and II. Munday et al, [36] showed that diallyldisulphides activate phase II detoxification enzymes such as Quinone Reductase (QR) as well as Glutathione Transferase (GT) in tissues of Gastrointestinal System (GIT) such as forestomach, glandular stomach, duodenum, jejunum, ileum, caecum, colon, liver, kidney, spleen, heart, lungs and bladder. All these findings together suggest that such enzyme induction contributed to the defending activity of *Allium sativum* against gastrointestinal system cancers [36-42].

Raqmesh Dalvi, used garlic oil to study biotransformation of enzymes at Phase I and II in healthy rats and interestingly it was shown that rats given 500mg/kg of garlic oil had decreased quantity of liver cytochrome P450, aminopyrine N-demethylase enzyme in liver microsomes as well as aniline hydroxylase [43]. Findings of Dalvi indicate that oil extracted from *Allium sativum* inhibiting methabolism of drugs in rats may cause inhibition of cancers induced by drugs by activating drug metabolizing enzymes.

Siess *et al*. [44], studied alkyl sulphide activity and its impact on drug metabolizing enzymes found in rat liver and the results have shown that administration of allyl disulphides induced phase I and II enzymes with similar pattern to phenobarbital [44].

Another significant enzyme responsible from detoxification is Glutathione S-Transferase (GSTs) which is involved in detoxification of carcinogen chemicals with electrophilic properties. GSTs and their relationship with the garlic derived Organosulfur Compounds (OSCs) were thoroughly studied. Sparnins *et al.* [33], studied the activity of Allyl Methyl Trisulfide (AMTS) on glutathione S-transferase on several mice organs such as liver, small intestines, forestomach and lungs and it was shown that administration of AMTS increased levels and activity. GSTs in all investigated tissues as well as Benzo[a]Pyrenes (BP) induced forestomach tumours are inhibited [33]. All these findings indicate that organosulphur compounds found in garlic has protective effect against cancers by inducing glutathione transferase enzyme.

Singh and Shukla, published "Differential Induction of NAD(P)H: Quinone Oxidoreductase by Anticarcinogenic Organosulfides from Garlic" which elucidated the effects of organosulfur compounds: Diallylsulphide (DAS), Diallyltrisulfide (DATS), Diallyl Disulphide (DADS) Dipropylsulphide And Dipropyldisulphide (DPS and DPDS) obtained from garlic on NAD(P)H: Quinone oxidoreductase expression. NAD(P)H: Quinone oxidoreductase is an enzyme responsible from elimination of activated quinone metabolites of Benzo[a]Pyrenes (BP). Experimental results revealed that treatment with Diallyl Disulphide (DADS), Diallyltrisulfide (DATS) inhibited benzo[a]pyrenes induced forestomach tumour formation by increasing NAD(P)H:quinone oxidoreductase significantly. Moreover it was also shown that Diallyldisulphide (DADS) and Diallyltrisulfide (DATS) had more robust inductor effect on NAD(P)H: quinone oxidoreductase than Diallyl Sulphide (DAS) in benzo[a]pyrenes induced forestomach tumour formation. Interestingly Dipropyl Sulphide and Disulphide (DPS and DPDS) obtained from garlic have shown no inhibitory effect on benzo[a]pyrenes induced tumour formation and no effect was observed at NAD(P)H: quinone oxidoreductase activity of forestomach [45]. In addition diallyl sulphide (DAS) was demonstrated to be the most potent NAD(P)H: quinone oxidoreductase activity inducer in lungs [45]. All these results indicate that the treatment of different cancers with DAS significantly postpone tumor formation as well as decrease the number of tumors per animal.

Rex Munday and Christine M. Munday, studied interactions of Diallyl Disulphide (DADS) on Glutathione S-Transferase (GSTs) and Quinone Reductase (QR) activity on gastrointestinal system of rat model. The data obtained from *in vivo* studies revealed that rats given Diallyl Disulphide (DADS) had increased Quinone Reductase (QR) and glutathione s-transferase (GSTs) activity in gastrointestinal system organs such as forestomach, as well as parts of intestine such as duodenum, colon, jejunum, ileum, cecum etc. in rats [36].

In another study conducted by Shukla and co-workers, examined the possible effects of Diallyl Sulphide (DAS) on γ-Glutamyltranspeptidase (GGT) on mice skin carcinoma induced by chemical application. For this study, polycyclic hydrocarbons were used to induce cancer in the mice skin and it was demonstrated that diallyl sulphide inhibited carcinogenic activity significantly. Besides of these, Diallyl Sulphide (DAS) pre-treated mice were shown to have inhibited γ-glutamyltranspeptidase activity induced by 12-O-tetradecanoyl phorbol-13-acetate. As a result diallyl sulphide was shown to be a good inhibitor for chemical induced carcinogenesis [46].

Interaction of phase I enzymes and organosulfur compounds are another area of interest. In 2001 [47], studied the Diallyl Sulphide (DAS) and its effects on cytochrome P450 specifically focused on cytochrome P450 2E1 which is a known phase I metabolizing enzyme. The experimental results have shown that cytochrome P450 2E1 (CYP2E1) transformed Diallyl Sulphide (DAS) to Diallylsulfoxide (DASO) and Diallylsulfone ($DASO_2$). All

these molecules were shown to deactivate cytochrome P450 2E1 also decrease bioactivation of Carbontetrachloride (CCl_4), N-nitrosodimethylamine [47]. Those studies have revealed that different forms *Allium sativum* exerts inhibitory role on carcinogenesis as well as decrease the formation of cancer causing molecules. It was also shown that bioactive molecules such as Diallyl Disulphide (DADS) and Diallyltrisulfide (DATS), Dipropyl Sulphide and Disulphide (DPS and DPDS) have inhibitory effect on Phase I and II enzymes. Overall it was shown that; DAS, DASO and $DASO_2$ have inhibitory properties on formation of chemical carcinogenesis as well as mutagenesis.

Antioxidant activity of *Allium sativum*

Antioxidant effects of *Allium sativum* is another area of interest for research. Many studies point out that formation of tumour tissue may be triggered by free oxygen radicals [48,49]. Imai and co-workers used organosulfur compounds of *Allium sativum* to determine its antioxidant effects. In the study, antioxidant effects of fresh garlic extracts and Aged Garlic Extracts (AGE) were compared and it was demonstrated that aged garlic extract has significant antioxidant activity. The most robust radical scavenging activity was observed on S-Allylcysteine (SAC) and S-allylmercapto-L-cysteine supporting the data obtained from previous research done by Naito *et al*. [50] that S-Allylcysteine (SAC) exhibit antioxigenic activity.

Effects of *Allium sativum* on lipid peroxidation and levels of antioxidants were studied by Balasenthil *et al*. [29], in male Syrian hamsters where buccal cancer formation was induced by Dimethylbenz(a)Anthracene (DMBA). The experimental results showed that administration of *Allium sativum* extract diminished lipid peroxidation of buccal pouch tumour tissue. Besides the lipid peroxidation, elevated Glutathione (GSH) and Glutathione S-Transferase and Peroxidase (GST and GPx) were observed along with the decreased incidence of neoplasms in hamsters treated with garlic extracts [29].

Ajoene (4, 5, 9-trithidodeca-1, 6, ll-triene 9-oxide), one of the major components of garlic, was shown to act as an apoptosis inducer for HL-60 human promyelocytic leukaemia cells. Experiments revealed that ajoene treated human leukaemia cells HL-60 have shown increased intracellular peroxide production, where peripheral mononuclear blood cells came from healthy donor cells exhibit no sign of apoptosis. Moreover treatment with ajoene leads to activation in the translocation of transcription factor kB which plays an important role in inflammation, cell proliferation and survival [51,52].

Allium sativum inhibits tumour formation

Many studies were conducted to determine the effects of garlic and its bioactive compounds on tumour cells and their growth on laboratory animals. The history of *Allium sativum* utilization on test animals goes back to 1950s. Two scientists, Weisberger and Pensky studied the interaction of sarcoma tumour cells with diethyl thiosulfinate. Their experimental results revealed that pretreatment inhibited formation of sarcoma in CFW Swiss mice [53]. Same scientists worked on the tumour formation of lymphosarcoma cell (Murphy-Sturn) in Wistar rat model where tumourigenesis was inhibited by pre-administration of diethyl thiosulphinate [53,54].

Belman conducted experiments on mice where tumour growth was promoted by phorbol-myristate-acetate and these tumours were treated with garlic and onion oil. Experimental results revealed that both garlic and onion oil decreased the number of tumours in mice [55].

Effects of Aged Garlic Extract (AGE) was also another area of interest of scientists where Riggs et al., [56] studied oral ingestion and subcutaneous administration of aged garlic extract on mouse murine transitional cell carcinoma model. Outcomes of the experiment showed that immunization of mice with AGE reduced the incidence of tumour growth compared to the control group which only received saline solution as a control. Moreover 50mg per 100 ml administration of AGE significantly decreased the volume of tumours and 500mg per 100 ml administration of AGE did not only decrease tumour volume but also significantly increased the survival rate of the tested mice [56].

Diallyl Sulphide (DAS) treatment of mice on skin cancer model has shown that diallyl sulphide postpones tumour formation and decreases the average number of tumours per mouse. On the other hand, the experimental group that received diallyl sulphide at the initial stages of the experiment stayed tumour free until the experiments were terminated; overall suggesting that diallyl sulphide treatment exhibits a significant inhibitory effect on mouse skin carcinogenesis [45]. Organosulfur Compounds (OSC) and their cancer preventive activities were studied by Fukushima and co-workers to determine liver carcinogens and liver promote 6-8-week-old male Lewis rats. In these experiments, chemopreventive activities of organosulfur compounds were studied at the post initiation phase of carcinogenesis. Development of cancer in rats was determined by evaluation and quantification of Glutathione-S-Transferase Placental type (GST-P) positive foci. Interestingly it was observed that oil soluble organosulfur compounds, methyl propyl disulphide as well as propyl disulphide, had inhibitory effect on Glutathione-S-Transferase Placental type (GST-P) positive foci [35,57]. Furthermore organosulfur compounds that are soluble in water such as S-Methlycystein (SMC) along with cysteine also were found to have inhibitory effects on formation of Glutathione-S-Transferase Placental type (GST-P) positive foci. Thus experimental outcomes strongly suggest that fresh *Allium sativum* and organosulfur compounds derived from *Allium sativum* exhibit chemopreventive role on hepatocarcinoma [34,35,57].

The effects of Diallyl Disulphide (DADS) and Diallyltrisulfide (DATS) were investigated by Sakamoto *et al*. [58], on human lung cancer cell line A549. Experimental data concluded that both Diallyldisulfide (DADS) and Diallyltrisulfide (DATS) decreased the growth of A549 cells compared to the non-cancerous lung cells, where effects of diallyltrisulfide were found to be more robust compared to diallyl disulphide treatment. The mechanism of action of diallyltrisulfide was explained by its ability to increase levels of calcium in the cell triggering apoptosis [58].

Further, the effects of garlic constituents along with the S-cysteinyl compounds were studied on human prostrate carcinoma (LNCaP) cells. Experiments revealed that treatment with S-mercaptocysteine lead to a significant reduction of human prostate carcinoma (LNCaP) cells whereas less antiproliferative activity was observed on treatments with S-allyl cysteine. Utilization of S-cysteinyl analogues in experiments revealed that specifically disulphide or diallylcontaining molecules caused the most reduction in cell proliferation of prostate carcinoma cells. Although when these experiments were extended to investigate the effects of S-Allylmercaptocysteine (SAMC) on human prostrate carcinoma (LNCaP) cells, experiments showed the inhibitory effects of this molecule on the known markers of LNCaP cells, prostate specific antigen, leading increased catabolism of testosterone where it it well known that androgens increase the

risk of prostate cancer [59,60].

Effects *Allium sativum* on apoptosis and cell cycle

Many experiments have repetitively revealed that garlic and its constituents have significant antiproliferative activities on cancer cells. Pinto and Rivlin conducted a study, "Antiproliferative Effects of *Allium* Derivatives from Garlic" [19,61]. Knowles and Milner studied both the effects of allyl sulphides on cell growth as well as their inhibitory effects on neoplastic cell proliferation [62,63]. These studies revealed that several bioactive molecules obtained from *Allium sativum* are involved in induction of apoptosis, alterations in cell cycle and modified signal transduction in different cell types. Also these scientists showed that *Allium sativum* derivatives altered the regulation of nuclear factors associated with inflammation as well as the immune system. Allicin was previously shown to inhibit the cell cycle of leukaemia cells at S/G_2M phase where this effect was not observed on non-neoplastic cells [64].

Hong *et al* [65]. studied effects of *Allium sativum* extract, Diallyl Sulphide (DAS) and Diallyl Disulphide (DADS) on Non-Small Cell Lung Cancer (NSCLC) cells that are p53-wild type H460 and p53-null type H1299 [65]. It was demonstrated that Diallyl Disulphide (DADS) had more potent apoptotic activity on H460 non-small cell lung cancer cells by triggering DNA damage via p53 activation. On the other hand treatment with garlic extract and diallyl disulphide caused increased levels of Bax and decreased levels of Bcl-2 consistent with the well known mechanism where Bax is increased and Bcl-2 is decreased in p53 mediated apoptotic cell death mechanism. Altogether these data suggest that garlic extract, Diallyl Sulphide (DAS) and Diallyl Disulphide (DADS) promote apoptotic mechanisms by regulation and increasing levels of p53 protein in Non-Small Cell Lung Cancer (NSCLC) [66,67].

Effects of Diallyl Disulphides (DADS) on breast cancer cell lines were studied by Nakagawa and co-workers [68]. The team investigated the effects of synthetic diallyl disulphide on estrogenpositive breast cancer cell lines MCF-7 and KPL-1 as well as estrogen negative breast cancer cell lines MDA-MB-231 and MKL-F. MTT assay data suggested that treatment with diallyl disulphide with all four breast cancer cell lines caused antiproliferative activity at 72h incubations. This data was also supported by investigation of apoptotic mechanisms where it was shown that Bax protein is up regulated; Bcl-x was down regulated and caspase-3 levels were elevated in the cells compared to the control experiments. Moreover, *in vivo* studies on KPL-1 breast cancer cells transplanted in female nude mice treated with diallyl disulphide were shown to have decreased primary tumour weight compared to the untreated mice. PCNA-labelling technique was used to determine cell proliferation in mouse experiments where it was demonstrated that control group had 59.6% tumour proliferation and 1 and 2 mg diallyl disulphide treated nude mice had these percentages at 44.6 and 44.5 % respectively. All these data suggest that diallyl disulphide found in the constituent of *Allium sativum* oil extract at around 25.23% is a potent therapeutic agent for both estrogen positive and negative breast cancers [68].

Antiproliferative activity of S-Allylmercaptocysteine(SAMC) and S-Allylcysteine (SAC) on SW-480 and HT-29 human colon cancer cell lines were studied by Shirin and co-workers [69]. Researchers also investigated the effects of these two compounds on colon cancer cell cycle. Sulindac Sulphide (SS) was used as a positive control since chemopreventive effects of this compound was previously shown [70]. Experiments revealed that SAMC inhibited the growth of both SW-480 and HT-29 colon cancer cell lines at similar doses with SS, where no effect was observed on SAC treated cells. SAMC was shown to induce apoptosis by increasing caspase-3 activity. Moreover SS inhibited the cell cycle from G1 to S phase where SAMC inhibited the cell cycle progression from G2 to M phase. All these findings together suggest the anticancer effects of SAMC on colon cancer cell lines *via* a cell cycle blocking molecular mechanism differing from that of the sulindac sulphide [69]. Similar inhibitory effect from G2 to M phase of cancer cell line was observed on allicin treated HCT-15 leukaemia cells. Knowles and Milner's experiments showed that allicin treatment of HCT-15 cells caused inhibition on cell cycle from G2 to M phase along with the inhibition in p34cdc2 kinase activity which explains the antiproliferative property associated with allicin treatment [71].

Farhadi *et al*. [72], studied the effects of fresh *Allium sativum* juices on KB cell line, the oral squamous cell carcinoma. Researchers conducted MTT assays to determine antiproliferative activity of fresh garlic juice on KB cells. Moreover, in order to determine apoptosis induction in KB cells; Terminal deoxynucleotidyltransferase-mediated dUTP Nick End Labelling (TUNEL) and deoxyribonucleic acid fragmentation test were performed. MTT assay results showed significant cytotoxic activity associated with fresh garlic juice extracts on KB cell line as well as increased apoptosis related with increased Bax:Bcl-2 and caspase-3 activity. Their results suggested that treatment of oral squamous cell carcinoma with fresh garlic juice promoted apoptosis by inducing Bax:Bcl-2 activity [72].

All these results collectively reveal that bioactive molecules found in *Allium sativum* such as S-allylmercaptocysteine, S-allylcysteine, diallyl disulphides, diallyl sulphides induce apoptosis *via* several pathways.

Immunomodulatory effects of *Allium sativum*

Previous findings have shown that *Allium sativum* immune boosting activity by supporting the host immune system around the tumor microenvironment against the immunosuppressive effect of developing tumor [73]. Aged Garlic Extract preparations (AGE) was shown to stimulate the proliferation of lymphocytes, inducing phagocytotic activity of macrophages, promoting infiltration of lymphocytes and macrophages into tumour sites, moreover promoting release of many cytokines IFNγ, interleukin 2, interleukin 12 and tumour necrosis factor α [74-76]. This type of induction of immune system is known as Th-1-type immune response which often observed at the pro-inflammatory reactions responsible for killing intracellular parasites as well as for propagating autoimmune responses [74-77]. A 2006 study performed by Ishikawa and co-workers have shown that aged garlic extract has increasing effect on natural killer cell number along with their activation [78] moreover Kuttan mentioned that aged garlic extract increases the number of White Blood Cells (WBC) [79]. Bhattacharyya *et al*. [81], showed that *Allium sativum* promote interferon α expression [80]. Moreover antioxidant activity of *Allium sativum* leads to inhibition of effects of oxidized low density lipoprotein and decrease of peroxides as well as inhibition of Nuclear Factor κB (NFκB) suggesting that *Allium sativum* extract has ability to minimize intracellular oxidative stress of endothelial cells caused by oxidized low density lipoprotein [81].

Histone modifying effect of *Allium sativum* OSCs

Organosulfur compounds of *Allium sativum* were previously shown to modify acetylation of histone proteins in several mechanisms. Druesne and co-workers used diallyl disulphides on human colon cancer cell line Caco-2 cells which revelaed that OSC has the ability to increase histone acetylation as well as induce p21(waf1/cip1) expression [82]. Same year, Druesne and coworkers, published another article suggesting that repetitive diallyl disulphide treatments on HT-29 cells cause lengthened histone H3 K14 hyper acetylation [83]. Additional experiments on histone acetylation showed that diallyldisulphides have the ability to inhibit histone deacetylases in mouse erythroleukaemia DS19 and human leukaemia cells K562 in histone H4 and histone H3 [84]. Increased acetylation of the histone proteins are strictly associated with inhibition of growth of cancer cell lines, Caco-2 and T47D after S-allylmercaptocysteine treatment and DS19 cells after allicin, S-allylmercaptocysteine and S-allyl cysteine treatment [85].

Inhibition of angiogenesis by *Allium sativum*

Alliin treatment of human endothelial cells induced by fibroblast growth factor-2 was previously shown to inhibit tube formation and angiogenesis. This anti-angiogenic effect of alliin was shown to be controlled by elevated p53 and cellular nitric oxide levels [86]. Moreover diallyltrisulfide treatment caused inhibition on human umbilical vein endothelial cell migration and capillary like tube formation which was highly correlated with inhibition of vascular endothelial growth factor secretion, Akt inactivation and VEGF receptor-2 protein down regulation (Xiao *et al*., 2006). Ajoene was administered inside the peritoneum of C57BL/6 mice along with B16/BL6 melanoma cells caused strong inhibition of lung metastasis and metastasis of B16/BL6 melanoma cells [87]. These result altogether indicate the anti-angiogenic activity of bioactive molecules of Allium via VEGF receptor-2 and Akt related pathway.

Other *Allium* species with anticancer activities

Cancer formation can be classified under sections such as initiation stage of cancer, promotion stage and progression such that chemopreventive agents might take action at any of these stages [88]. Besides the traditional therapy procedures, development and discovery of complementary therapeutics is under focus of researchers [89]. Plant originated phytochemicals with known antioxidative and anti-inflammatory effects are also known to have high potency as chemopreventive agents on cancer at any stages stated above [90,91].

Park and co-workers, studied antitumoral and cytotoxicity activity of *Allium tuberosum* L. Their interest of research was *in vitro* cytotoxic effects of thiosulfinates such as S-methyl 2-propene-1-thiosulfinate and S-methyl methanthiosulfinate obtained from column chromatography on MCF-7 breast cancer cell lines as well as the effects of thiosulfinates on a mouse model with Sacorma-180 tumour cells. Experiments revealed that thiosulfinates exhibited significant cytotoxicity against human breast cancer cell line MCF-7. Furthermore MCF-7 cells also showed signs of apoptosis. Moreover administration of thiosulfinates to mice model increased the lifespan of mice with Sarcoma-180. All together these experimental data suggest that *Allium tuberosum* L.have cytotoxic and antitumoural activities both *in vitro* and *in vivo* models [92].

Baba and co-workers investigated bioactive compounds such as saponins, laxogenin, laxogenin 3-O-alpha-arabinopyranosyl (1-->6)-beta-glucopyranoside, xiebai-saponin I, aglycone, isoliquiritigenin-4-O-glucoside etc. from *Allium chinense* G. Don. Researchers studied 32Pi incorporation of phospholipids in HeLa cells stimulated by 12-O-Tetradecanoylphorbol-13-Acetate (TPA). Laxogenin molecule was further studied on stage two lung cancer and was shown to have antitumor stimulating activity [93]. Furthermore in 2015 Yu *et al.* [94], used ethanolic extraction method to collect saponins of *Allium chinense* to evaluate and examine their anticancer activity on melanoma cells B16 and breast cancer cell line 4T1. Their experiments revealed that *Allium chinense* saponin treated cells had significant change in their morphologies moreover saponins from this plant induced the death of B16 cell and 4T1 cells. Number of apoptotic cells was also found to be increasing in a dose dependent manner when saponin concentrations were increased. In addition, proliferation of cancer cell lines B16 and 4T1 were inhibited in a dose dependent manner. The group also designed an *in vivo* study using C57 BL/6 mice to determine the antitumor activities of saponins obtained from *Allium chinense* where an inhibitory effect was observed on melanoma growth by saponins [94].

Ghodrati and co-workers studied the *in vitro* effects of chloroformic extract of *Allium hirtifolium* (Persian shallot) and allicin obtained from the same plant on the proliferation of cervical cancer cell line HeLa, human breast cancer cell line MCF-7 and C3H/An, and mouse connective tissue fibroblasts L929. Their experiments revealed that chloroformic extracts of the *Allium hirtifolium* exhibited dose and time dependent antiproliferative activity on HeLA and MCF-7 cells. Allicin was determined by Thin Layer Chromatography (TLC) and molecular analyses were performed by using (HPLC). MTT assay was used to determine antiproliferative activity of allicin on designated time periods 24, 48 and 72h where a significant effect was observed on MCF-7 and HeLa cells compared to mouse fibroblast L929. High number of apoptotic HeLa and MCF-7 cells were determined by DNA fragmentation analysis after the chloroformic *Allium hirtifolium e*xtract treatment, where L929 cells remained unaffected [95] suggesting that *Allium hirtifolium* may be a promising candidate for tumor suppression studies.

In 2011, Mohammadi-Motlagh and co-workers studied impact of *Allium ascalonicum* on cancer together with plants' anti-inflammatory properties. Anti-inflammatory and anticancer effects of *Allium ascalonicum* was studied using trypan blue exclusion assay, LDH cytotoxicity assay, and proliferation potency of cells were evaluated by using coulter counter after treatment of human acute T-cell leukemia Jurkat cells, mouse fibrosarcoma cells Wehi164, human erythroleukemia cells K562 , and Human Umbilical Vein Endothelial Cells (HUVEC). Viability data revealed that aqueous *Allium ascalonicum* bulb extract exhibited significant cytotoxic activity on cancer cell lines where human umbilical endothelial cells were not affected on the same concentrations applied where IC_{50} value for K562 was 100 ± 8 µg/ml, for Jurkat cell line IC_{50} was 100 ± 10 µg/ml, for cell line Wehi164 IC_{50} was 400 ± 6 µg/ml and for HUVEC IC50 was 1600 ± 8 µg/ml. Furthermore significant antiproliferative effect was observed on cancer cell lines where again HUVEC cells were found to be the least affected cell line from different incubations of *Allium ascalonicum* extract incubations. Acetic acid-induced vascular permeability model on mice was used to determine anti-inflammatory potency of the *Allium ascalonicum* aqueous extract which revealed a significant *in vivo* anti-inflammatory effect [96]. All these finding from experimental results suggest that *Allium ascalonicum* be a candidate as a therapeutic and preventive agent for cancer.

Anticancer effects of aqueous extract of *Allium ursinum* on gastric cancer cell line AGS was studied by Xu and coworkers [97]. They used flow cytometry technique to determine whether AGC cells *Allium ursinum* extract treated and non-treated cells leading to apoptosis and cell cycle arrest or not. Proteins obtained from flow cytometry evaluated by Western blotting technique for determination of cell cycle proteins as well as colorimetric assay was used to determine caspase activity. Aqueous extracts of *Allium ursinum* demonstrated G2/M phase arrest in gastric cancer cell line AGS together with induction in apoptosis of cells. Data obtained from Western blot analysis pointed out that cyclin B, a protein involved in mitosis, is inhibited. Interestingly, it was shown that G_1 phase-related proteins remain same after treatment with *Allium ursinum* [97] suggesting that the extract is involved in interfering with the cell cycle at the M-stage.

Timite *et al*. [98], investigated structure of glycosides from *Allium schoenoprasum* and its cytotoxic effects on human colon cancer cell lines HCT 116 and HT-29. Researchers used methanolic extracts obtained from crude *Allium schoenoprasum*to shown *in vitro* effects of the extract. Many bioactive molecules and trace elements obtained from the plant leaves exhibited antioxidant and anticancer activity. MTT assay applied on HCT116 and HT-29 cells showed promising cytotoxic effect on colon cancer cell lines [98].

In 2013, a study by Rezgui *et al*. [99] showed antitumor effects of *Allium flavum* on human colorectal cancer cell line SW480. Cytotoxicity effect of the molecules obtained from the extraction demonstrated moderate effect on colorectal cancer cell line compared to the doxorubicin which is used as positive control experiments [99].

Kim and co-workers studied both *in vitro* and *in vivo* anticancer effects of methanolic extracts of *Allium victorialis* var. *platyphyllum.* Researchers used human promyelocytic leukemia cells HL-60 and HL-29 treated with *Allium victorialis* var. *platyphyllum* and measured superoxide anion production induced by 12-O-Tetradecanoylphorbol 13-acetate (TPA). Their experiments revealed that methanolic extracts prepared by using rhizomes and stems of *Allium victorialis* var. *Platyphyllum* exhibited inhibitory effect of TPA induced superoxide anion formation in HL-60 cells in dose dependent manner. Ear oedema in mice model induced by TPA was also reduced by treatments with rhizome and stem extracts. MTT assay data showed that treatments of stem and rhizome exhibited antiproliferative activity in dose dependent manner, induction of apoptosis observed and HL-29 cells were undergone inter-nucleosomal deoxyribonucleic acid fragmentation. Overall research group showed that *Allium* species that they were using exhibited significant antioxidant, antiproliferative, chemopreventive and apoptosis promoting activities [100].

Khazaei *et al*. [101], studied *in vitro* antiproliferative and apoptosis promoting activities of methanolic extract of *Allium atroviolaceum* bulb on breast cancer cell lines MCF-7 and MDA-MB-231, human cervical cancer cell line HeLa and liver cancer cell line Hep-G2 and mouse embryo fibroblast 3T3. Bulb extracts demonstrated significant antiproliferative activity on MDA-MB-231, MCF-7, HepG2 and HeLa cell lines in concentration and time dependent manner. Flow cytometry technique was used to determine the effects of the bulb extract on cell cycle where treated MCF-7 cells exhibited ell cycle arrest at S and G2/M phase related with cyclin-dependent kinase 1 down regulation. Moreover treated MDA-MB-231 cells exhibited S phase cell cycle arrest associated to cyclin-dependent kinase 1 (*Cdk1*) and p53 protein. On the other HeLa cells demonstrated arrest in sub-G0 cell cycle accompanied with down regulation of *Cdk1* and HepG2 cells exhibited cell cycle arrest at G0/G1, S, G2/M phases which was related with p53 activity. Effects of methanolic extract of *Allium atroviolaceum* bulb on apoptosis was determined by using morphological investigations, caspase-3, caspase-8 and caspase-9 activity assays and *Bcl-2* gene expression. In MCF-7 cells *Bcl-2* gene was down regulated where caspase-3 and caspase-8 activity was determined to be significantly increased compared to control experiments. HeLa cells exhibited increase caspase-3 and caspase-9 activity with down regulation of *Bcl-2* gene expression. Interestingly HepG2 cells exhibited apoptosis mechanism activation which is independent to caspase activity. Research group also studied synergistic effect of tamoxifen and *Allium atroviolaceum* extract to point out that these extracts may be useful therapeutics as in combinations with conventional drugs [101].

Another study published in 2017 by Bhandari *et al*. [102], showed anticancer effects of *Allium wallichii*. Researchers used ethanolic extract of the plant against PC3 prostate cancer cell line, MCF-7 breast cancer cell line and HeLa cervical cancer cell line compared to the doxorubicin as a positive control showed moderate inhibitory effect on selected cell lines. Moreover extracts were used on Burkitt's lymphoma cell line showed promising anticancer activity suggesting that crude extract itself has promising anticancer impact such that further studies must be performed to determine underlying molecular mechanisms [102].

Ghasemi *et al*. [103], isolated tricin from *Allium atroviolaceum* to show a synergistic effect of the molecule when combined with docetaxel on prostate cancer cell line PC3. MTT assay analysis was used to determine PC3 cell proliferation where experimental data revealed that cells treated with tricin docetaxel exhibits significant antiproliferative activity. Moreover researchers found that MiR-21 protein, normally overexpressed in PC-3 cells was reduced significantly compared to the control experiments [103].

Isbilen *et al*. [104], studied anticancer effects of *Allium autumnale* .H. Davis, an endemic *Allium* species to island Cyprus [104,105]. The team investigated antiproliferative and cytotoxic effects of *Allium autumnale* P. H. Davis bulb and stem extracts on weakly metastatic breast cancer cell line MCF-7 and strongly metastatic breast cancer cell line MDA-MB-231. Their findings revealed that *Allium autumnale* P.H. Davis bulb and stem extract has significant antiproliferative and cytotoxic effect on both cancer cell lines.

Li and co-workers studied direct antitumoral activities of raw garlic extract on highly metastatic, aggressive sarcoma 180- and EL4-induced lethal ascite which can not be treated with the conventional treatment approaches. The aim of the research was to compare oral administrations of raw extract versus direct intraperitoneal injections. Their experimental results revealed daily injections of raw garlic extract for 21 days completely diminished the tumors in mice athough oral administrations did not show any significant therapeutic activity on the sarcoma 180- and EL4-induced lethal ascites. There results alltogether indicate that direct injections of raw garlic extract has high potency on treatment of cancers compared to the consumption of cooked garlic in diet suggesting a novel anticancer effect of raw garlic extract on mouse models.

Conclusion

Allium sativum and other *Allium* species are an area of research interest because of their wide range of health benefits. Chemical composition of the *Allium sativum* and organosulfur compounds in the bulb of the plant revealed to have anticancer effects both *in vitro* and *in vivo*. The anticancer effects of *Allium sativum* include inhibition of tumor formation, antioxidant activity, induction of apoptosis, stimulation of drug metabolizing enzymes etc. Overall several studies performed to determine the anticancer effects of *Allium* species revealed that active biological molecules obtained from these species could serve as highly promising anticancer agents, either as mono- or combination-therapies, depending on their mechanisms of action at cellular level on different cancer cell types.

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