Garlic [*Allium sativum*]: A Review of its Potential Use as an Anti-Cancer Agent

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Abstract: Garlic [*Allium sativum*] is among the oldest of all cultivated plants. It has been used as a medicinal agent for thousands of years. It is a remarkable plant, which has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activity. In this review, we will discuss particularly the largely preclinical use of this agent in the treatment and prevention of cancer. A number of studies have demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a number of organosulfur compounds derived from garlic. The chemopreventive activity has been attributed to the presence of organosulfur compounds in garlic. How this is achieved is not fully understood, but several modes of action have been proposed. These include its effect on drug metabolizing enzymes, antioxidant properties and tumor growth inhibition. Most of these studies were carried out in the animal models. Also, recent research has focused on the antimutagenic activity of garlic. Recently, it has been observed that aged garlic extract, but not the fresh garlic extract, exhibited radical scavenging activity. The two major compounds in aged garlic, S-allylcysteine and S-allylmercapto-L-cysteine, had the highest radical scavenging activity. In addition, some organosulfur compounds derived from garlic, including S-allylcysteine, have been found to retard the growth of chemically induced and transplantable tumors in several animal models. Therefore, the consumption of garlic may provide some kind of protection from cancer development.

INTRODUCTION

Garlic (*Allium sativum*) is among the oldest of all cultivated plants. It has been used as a spice, food and folklore medicine for over 4000 years, and is the most widely researched medicinal plant [1, 2]. Codex Ebers, an Egyptian medical papyrus dating to about 1550 B.C., includes 22 therapeutic formulations that mention garlic as an effective remedy for a variety of ailments including heart problems, headache, bites, worms and tumors [3]. According to the Bible, the Jewish slaves in Egypt were fed garlic and other allium vegetables, apparently to give them strength and to increase their productivity [2]. In ancient Greece, garlic was consumed to treat intestinal and lung disorders [4]. As early as 1858, Louis Pasteur reported the antibacterial properties of garlic [3]. In India, garlic has been used for centuries as an antiseptic lotion for washing wounds and ulcers. During World War II, garlic was used to treat the wounds of soldiers [5]. Many workers have researched on garlic's insecticidal, antimicrobial, antiprotozoal and antitumor activities [6-8].

In traditional Chinese medicine, Islamic medicine, folklore medicine and the Ayurvedic system of medicine, several spices and herbs including garlic are described to possess medicinal properties (e.g. anti-thrombotic, hypolipidemic and anti-hypertensive) [9-16]. In the homeopathic system, garlic is also an effective remedy for many ailments. In China, garlic tea has long been recommended for fever, headache, cholera and dysentery. In rural Japan, miso-soup containing garlic is used as a remedy for the common cold with headache, fever and sore throat [17].

More recently, garlic has been reported to be effective in various ailments such as cardiovascular diseases because of its ability to lower serum cholesterol [18-23]. A component of garlic, S-methylcysteine sulfoxide (SMCS), has been shown to reduce both blood cholesterol and the severity of atherosclerosis [19-21]. Garlic has protective effects against stroke, coronary thrombosis [24, 25], atherosclerosis [26, 27], platelet aggregation [9, 28-31], as well as infections and vascular disorders [25]. However, we must note that there is considerable controversy concerning the cholesterol lowering effects of garlic and a number of studies have reported that some garlic preparations do not lower serum cholesterol [32-38].

The fibrinolytic activity of garlic in both man [39] and experimental animals [18] has been reported. Many claims of an antibiotic action [40], a hypoglycemic effect [41], antitumor [42], antioxidant [43] and antithrombotic properties have also been attributed to the garlic extracts [24, 25, 44].

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Thus, the chemoprotective action of garlic is well recognized, and it has been established that individuals who regularly consume large amounts of garlic (~20 grams or more per day) are less susceptible to cancer than those with a low intake, particularly in the case of gastric or intestinal cancers [45-50].

ORGANOSULFUR COMPOUNDS FROM GARLIC

Garlic and other members of the *Allium* family contain high levels of organosulfur compounds, to which many of the beneficial effects of garlic have been attributed [51, 52]. Garlic bulbs contain S-allyl cysteine sulfoxide (alliin) which is liberated when garlic is cut and the parenchyma is destroyed [25]. Alliin (an odorless compound) is acted upon by the enzyme allinase (alliin lyase) to produce diallylthiosulfinate (allicin). Allicin, which is responsible for the characteristic smell and taste of garlic, is the main component of freshly crushed garlic homogenates. Individuals who ingest raw garlic consume allicin. Although the details of allicin metabolism in humans is not well understood, it is know that allicin is converted into allyl mercaptan and diallyl disulfide (DADS) in the rat liver [53] and to allyl mercaptan in human blood [54]. Since these substances are also found in human breath after ingestion of raw garlic, it is believed that they are also formed *in vivo* [55-58]. When garlic is cooked under aqueous conditions, allicin decomposes, producing mostly DADS and diallyl trisulfide (DATS), plus smaller amounts of other allyl mono-, di- and polysulfides [59-61].

Garlic also contains S-propylcysteinesulfoxide (PCSO) and S-methylcysteine-sulfoxide (MCSO) [61-64]. PCSO can generate over 50 compounds depending on the temperature as well as the water content [65]. The action of allinase on the mixture of alliin, PCSO and MCSO can produce a number of other molecules including allyl methane thiosulfinate, methyl methanethiosulfinate and other mixed or symmetrical thiosulfinates (R—S(=O)—S—R'), where R and R' are methyl, propyl and allyl groups (**Fig. (1)**). GC/MS analysis of garlic extract has shown the presence of 3-vinyl-6H-1,2-dithiin and 3-vinyl-4H-1,2-dithiin. Methanolic extracts of garlic contain a number of non-polar compounds, including the optically active compounds E- and Z-4,5,9-trithiododeca-1,6,11-triene-9-oxide [25]. The E isomer is the major component, commonly called E-ajoene and has the structure shown in **Fig. (1)**.

Other garlic preparations contain a number of compounds of interest. For example, the major water-soluble organosulfur compounds in aged garlic extracts are S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) [66]. Garlic oil is also enriched in the volatile components of garlic such as DADS and DATS [27].

GARLIC AND CANCER – EPIDEMIOLOGICAL STUDIES

The anticancer properties of garlic have been proposed since ancient times. The ancient Egyptians used garlic
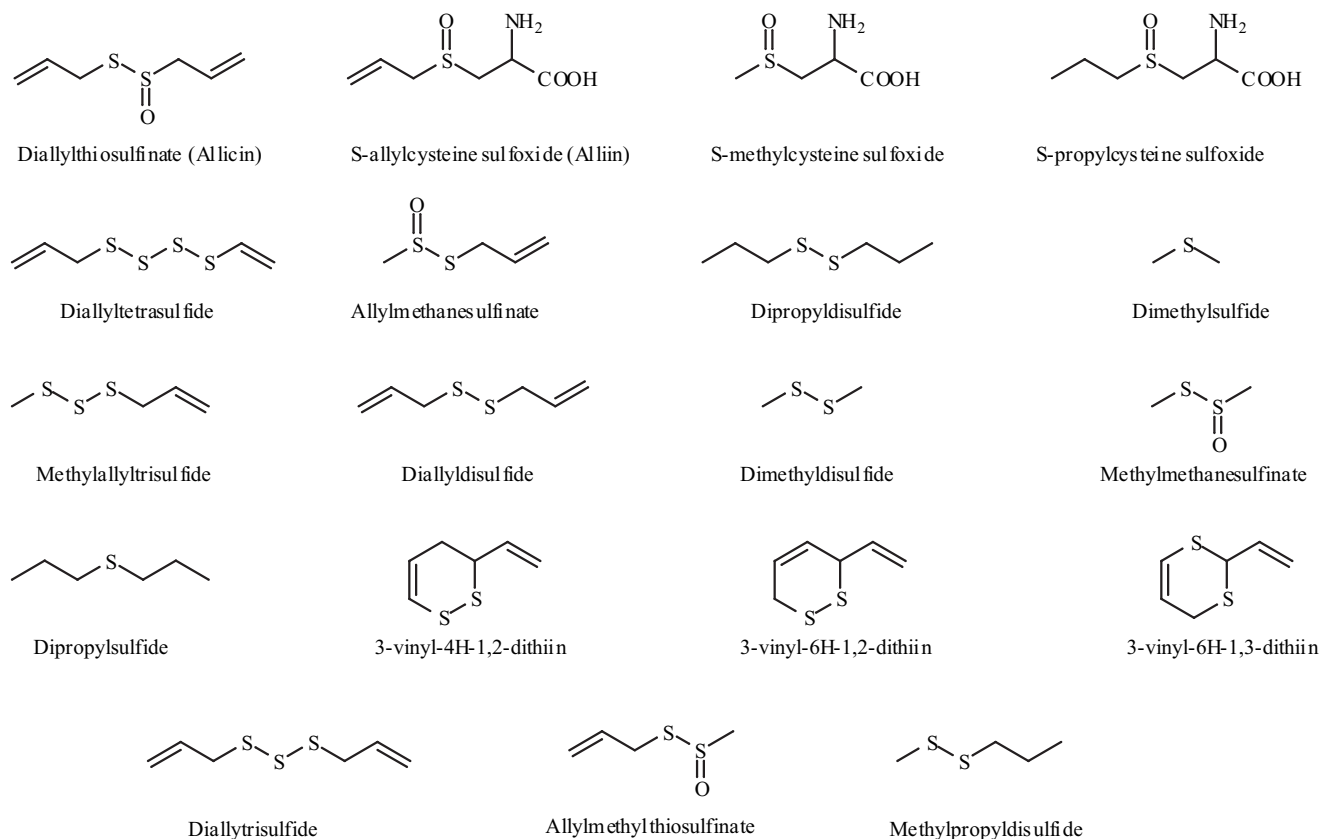

Diallylthiosulfinate (Allicin)   S-allylcysteine sulfoxide (Alliin)   S-methylcysteine sulfoxide   S-propylcysteine sulfoxide

Diallyltetrasulfide   Allylmethanesulfinate   Dipropyldisulfide   Dimethylsulfide

Methylallyltrisulfide   Diallyldisulfide   Dimethyldisulfide   Methylmethanesulfinate

Dipropylsulfide   3-vinyl-4H-1,2-dithiin   3-vinyl-6H-1,2-dithiin   3-vinyl-6H-1,3-dithiin

Diallyltrisulfide   Allylmethylthiosulfinate   Methylpropyldisulfide

Fig. (1). Sulfur compounds from garlic.

externally for the treatment of tumors [3], and Hippocrates and physicians in ancient India are reported to have used garlic externally for cancer treatment [10, 67].

In reviews of epidemiological studies conducted in the last 30 years, there is convincing evidence that the consumption of certain vegetables, such as garlic, reduces the risk of colorectal, stomach, lung and esophageal cancers [45-50]. In addition, there is probable evidence for cancers of the breast and bladder [68]. A variety of garlic preparations as well as garlic components have been studied for their effects on the prevention of cancer development in both humans and animals [69].

A number of epidemiological studies have been carried out in China to assess the effect of garlic consumption on stomach cancer. In one of the earliest studies, two large population groups in Shandong Province were compared for garlic intake and stomach cancer incidence. A group living in Cangshan county with the lowest stomach cancer death rate (3/100,000) in China were compared to a group of patients from Qixia county with a >3-fold higher death rate from stomach cancer (10/100,000) [70]. The cause for this difference was attributed to garlic consumption since the residents of Cangshan county usually consume about 20 g garlic per day, while the residents of Qixia county rarely eat garlic. It was postulated in this study that garlic reduced the concentration of nitrites in the stomach by inhibiting nitrate reduction by bacteria. Nitrites are precursors of nitrosoamines, which are potent carcinogens.

Another study in China involved comparing 564 patients with stomach cancer to 1131 controls in an area of China where cancer rates are high. It was found that consumption of all *Allium* vegetables, including garlic, onions and scallions, reduced the occurrence of stomach cancer [40, 71]. This conclusion is supported by the results of You and co-workers who reported that garlic consumption reduced the

incidence of *Helicobacter pylori* infection and precancerous gastric lesions in individuals of Cangshan county of Shandong province, an area of China at relative low risk of gastric cancer [72].

Takezaki and co-workers have also studied the lifestyles of residents in high and low risk areas of Jiangsu province, China, to elucidate the relationship between gastric cancer incidence and diet [73]. In the study, they reported that frequent consumption of allium vegetables (particularly garlic) might be a factor in low mortality from gastric cancer. In an analogous study, Gao and co-workers studied the protective effect of allium vegetables on both esophageal cancer (EC) and stomach cancer (SC) in a high-epidemic area of Jiangsu province, China [74]. In this study, dietary habits were studied in relation to cancer incidence in Yangzhong city, one of the areas of highest incidence for both SC and EC. They reported that frequent intake of allium vegetables (including garlic) was inversely associated with the risk of both esophageal and stomach cancer.

Fleischauer and his co-workers recently conducted a meta-analysis of the epidemiological literature on the association between garlic consumption and risk of stomach, colon, head and neck, lung, breast and prostate cancers [75]. In their analysis, they assessed consumption of raw garlic, cooked garlic or both (RC garlic). They concluded that a high intake of RC garlic might be associated with a protective effect against stomach and colorectal cancers.

Similarly, Levi and co-workers also assessed the relationship between diet and risk of colorectal cancer [76]. Their case-control study conducted in Vaud, Switzerland included 223 patients with incident colon (119) or rectal (104) cancers. Controls (491) included age-matched patients with non-neoplastic diseases unrelated to obesity or chronic conditions inducing long-term modification of diet. In their analysis they found consumption of garlic to be protective

Table 1.   Examples of Experimental Inhibition of Chemical Carcinogenesis by Garlic

Chemicals	Carcinogen	Organ/Species	Reference
Fresh garlic extract	DMBA	Buccal pouch/hamster	80
Garlic oil	DMBA/PMA	Skin/mouse	81
Diallyl sulfide (DAS)	DMH NMBA BP DMBA	Colon/rat Esophagus/rat Forestomach, lung Skin/mouse	82 83 84-86 87
Allyl methyl sulfide	BP	Forestomach/mouse	87
Allyl methyl trisulfide	BP	Forestomach/mouse	84
Diallyl trisulfide (DATS)	BP	Forestomach/mouse	84
Methanol extract of garlic	AFB1	Liver/rat	88
Fresh garlic powder	DEN	Liver/rat	89
S-allyl cysteine (SAC)	DMBA	Buccal pouch/hamster	90
S-methyl cysteine (SMC)	DEN	Liver/rat	91

DMBA, 7,12-dimethylbenz(a)anthracene; PMA, phorbol-myristate-acetate; DMH, 1,2-dimethylhydrazine; NMBA, *N*-nitrosomethylbenzylamine; BP, benzo[a]pyrene; AFB1, aflatoxin B1; DEN, diethylnitrosamine

Table 2.   Antimutagenic Activity of Some Garlic Constituents

Chemicals	Mutagen	Organism/Organ	Reference
Methanolic garlic extract	AFB1	Salmonella TA98	88
Aqueous garlic extract	AFB1	Salmonella TA100	88
Aqueous garlic extract	4-NQO	E. coli	92
Aqueous garlic extract	Gamma rays Hydrogen peroxide Cumene hydroperoxide t-butyl hydroperoxide	Salmonella TA102	93
Diallyl sulfide	PhIP	Rat colon	94
Garlic powder	PhIP	Rat colon	94

AFB1, aflatoxin B1; 4-NQO, 4-nitroquinoline -1-oxide; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

against colorectal cancer. This study is in agreement with the results of Marchand *et al*. who also observed that the intake of garlic was inversely associated with the risk of developing colorectal cancer [77].

A number of other studies have focused on the effects of diet and incidence of other types of cancers. Key and co-workers reported that consumption of garlic decreased the risk of developing prostate cancer [79]. In a French study, the role of diet on breast cancer risk was assessed. It was shown that breast cancer risk decreases with the consumption of garlic and onions (p value for trend $< 10^{-6}$) [79].

A recent review of the epidemiologic literature by Fleischauer and Arab surveyed the effects of garlic consumption on cancers of the stomach, colon, head and neck, lung, breast and prostate [69]. From this survey, they concluded that the available evidence suggests a preventative effect of garlic consumption in stomach and colorectal cancer, while the data concerning other types of cancer was inconclusive.

GARLIC, CANCER AND CHEMOPREVENTION

Although the anticancer properties of garlic have been recognized for centuries, most recent studies involving garlic have focused on several aspects including chemoprevention. Studies on the chemopreventive activity of garlic have assessed several different garlic preparations including fresh garlic extract, aged garlic, garlic oil and several organosulfur compounds derived from garlic. Some of these studies are summarized in Table 1.

In addition to studying the anticarcinogenic activity of garlic components, a number of researchers have recently focused on the antimutagenic activity of garlic. A number of these studies are summarized in Table 2.

From these studies, it is clear that the chemopreventive activity of garlic is related to the organosulfur compounds (OSCs) derived from it. Thus, in the last five years, research has focused on elucidation of the mechanism of action of OSCs, both *in vivo* and in culture. Although how garlic

achieves chemoprevention is not fully understood, several modes of action have been proposed on the basis of these studies.

Effects on Drug Metabolizing Enzymes

There is evidence that at least part of the chemopreventive action of garlic in carcinogenesis is due to the induction of phase II detoxification enzymes including glutathione transferases, quinone reductase, epoxide hydrolase and glucuronosyl-transferase, that inactivate toxic substances and facilitate their excretion [95]. These enzymes are highly inducible in animals and humans and a strong inverse relationship exists between tissue levels of phase II enzymes and susceptibility to chemical carcinogenesis [96-103].

In 1992, Dalvi studied the effects of garlic oil (diallyl sulfide) on hepatic Phase I and Phase II biotransformation enzymes in normal rats [104]. He found that rats treated with a single dose of garlic oil (500 mg/kg i.p.) showed a significant depression of hepatic cytochrome P-450, aminopyrine N-demethylase and aniline hydroxylase, while microsomal protein content, cytochrome b_5, NADPH-cytochrome c reductase, benzphetamine N-demethylase and cytosolic glutathione S-transferase remained unaffected after 24 hours following treatment. In contrast, daily administration of garlic oil (50 mg/kg i.p. for 5 days) produced a significant increase in hepatic cytochrome P-450, aminopyrine N-demethylase and benzphetamine N-demethylase activities only.

Siess and co-workers also studied the effects of alkyl sulfides on hepatic drug metabolizing enzymes in normal rats [105]. These workers investigated the effects of a number of alkyl sulfides found in garlic on the modulation of drug-metabolizing enzymes in rat liver. They reported that disulfides with two propyl groups or two allyl groups (but not two methyl groups) provoked a pleiotropic response on drug metabolizing enzymes, with the induction of both phase I and phase II enzymes. The pattern of induction of drug-metabolizing enzymes was similar to that elicited by the enzyme inducer, phenobarbital.

Manson and co-workers also studied the effect of oral administration of garlic oil to rats on a number of drug metabolizing enzymes in liver tissues [106]. They reported that garlic oil induced phase II enzymes such as GSTs and the conjugating enzyme, gamma-glutamyltranspeptidase.

Glutathione-S-transferases (GSTs) are important detoxifying enzymes that remove harmful electrophiles by conjugating them with glutathione [107]. GSTs are a well-known class of detoxifying enzymes in Phase II metabolism of drugs and can therefore play a detoxifying role in metabolism of carcinogens that may be electrophilic in nature. Any substance that increases the levels and/or activity of GSTs has the potential to be chemopreventive. The effect of garlic-derived OSCs on GSTs has been investigated by a number of laboratories.

Sparnins and co-workers studied the effect of oral administration of allyl methyl trisulfide (AMTS) on GST activity in the liver, forestomach, small intestine and lung of mice [84, 87]. They observed that 96 h after oral administration of AMTS, GST activity was increased in all tissues and, in addition, benzo[a]pyrene induction of forestomach tumors was suppressed [87]. Similarly, three other garlic-derived compounds (allyl methyl disulfide, diallyl trisulfide, diallyl disulfide) stimulated GST activity in these organs [84]. In contrast, saturated compounds (propyl derivatives) did not affect GST activity in these organs in mice [84]. These results suggest that allyl groups are important for this stimulation of GST activity.

Similarly, Sumiyoshi and Wargovich reported that the oral administration of diallyl sulfide (DAS, 400 mg/kg) stimulated mouse hepatic GST activity after a time lag of about 18 hours [108]. In this same paper, they also reported elevated colonic GST activity by as much as 149 % and 170 %, 24 and 48 hours, respectively, after DAS administration. In both the liver and colon, the increased GST activity was DAS dose- dependent.

As mentioned above, garlic-derived compounds have the ability to suppress the induction of tumors by benzo[a]pyrene (BP) [87]. Therefore, the study of the effect of garlic-derived compounds on the metabolism of BP may provide an explanation for this effect.

In a recent study, Singh and co-workers investigated the effect of diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dipropyl sulfide (DPS) and dipropyl disulfide (DPDS) on the expression of NAD(P)H:quinone oxidoreductase (NQO), an enzyme implicated in the detoxification of activated quinone metabolites of BP [109]. They observed that treatment of mice with DADS and DATS, which are potent inhibitors of BP-induced forestomach tumorigenesis, resulted in a statistically significant increase (2.4 and 1.5 fold, respectively) in forestomach NQO activity. In addition, DADS and DATS were much more potent inducers of forestomach NQO activity than DAS, which is a weaker inhibitor of BP-induced tumorigenesis than the former compounds. Propyl group containing OSCs (DPS and DPDS), which do not inhibit BP-induced tumorigenesis, did not affect forestomach NQO activity. In addition, there was a good correlation between the effects of these OSCs

against BP-induced pulmonary tumorigenesis and their effects on NQO expression in the lung. DAS was found to be the most potent inducer of pulmonary NQO activity (3.2 fold increase over control levels).

In a similar study, Munday and Munday investigated the effect of DADS on tissue activities of quinone reductase (QR) and glutathione S-transferase in the gastrointestinal tract of rat [110]. In this study, increased activities of QR and GST were recorded in the forestomach, glandular stomach, duodenum, jejunum, ileum, cecum, colon, liver, kidneys, spleen, heart, lungs and urinary bladder of rats given DADS over a wide range of doses. Large variations in response were recorded among the different organs, with forestomach, duodenum and jejunum being the most sensitive to enzyme induction by DADS. In these organs, significant increases in QR activity were observed at a dose of only 0.3 mg/kg/day, a dose that could be achieved by human consumption.

Chung and co-workers have recently reported that DAS and DADS can decrease arylamine N-acetyltransferase (NAT) activity in the strains of *Helicobacter pylori* from peptic ulcer patients [111]. NAT is thought to be involved in the detoxification of endogenous amines including carcinogens. In this study, *p*-aminobenzoic acid (a non-carcinogen) and 2-aminofluorene (a carcinogen) were used as substrates for NAT. DAS and DADS decreased the apparent K_m and V_{max} values of NAT enzyme in both *H. pylori* cytosol and suspensions suggesting noncompetitive inhibition. It was also observed that DAS and DADS elicited dose-dependent bactericide effects on *H. pylori* cultures. Garlic components may be useful in limiting the proliferation of this organism in the digestive system of *H. pylori,* which has been shown to have a possible causative role in gastric cancer [112]. This observation is in agreement with the results of You and co-workers who reported that *H. pylori* is a risk factor for the development and progression of precancerous gastric lesions and that consumption of garlic may be protective in this respect [72].

These observations agree with those of Chung who studied the effects of DAS and DADS on arylamine N-acetyltransferase (NAT) activity in human bladder tumor cells [113]. He observed that NAT activity as well as cell viability in human bladder tumor cells was inhibited by DAS and DADS in a dose-dependent manner. The data also indicated that DAS and DADS decrease the apparent values of K_m and V_{max} of NAT in human tumor cells for two substrates, 2-aminofluorene and *p*-aminobenzoic acid.

In a study published in 1999, Shukla *et al*. studied the inhibition of carcinogen-induced activity of γ-glutamyl transpeptidase (GGT) in mouse skin by DAS [114]. They reported that GGT activity induced by carcinogenic polycyclic hydrocarbons was significantly inhibited by DAS in mouse skin. Pretreatment with DAS also inhibited GGT levels induced by the mitogen, 12-O-tetradecanoyl phorbol-13-acetate. Therefore, DAS appeared to be a strong modifier of chemically induced carcinogenesis.

Although the reports described thus far have focused on the effects of garlic and its organosulfur compounds on phase II enzymes, Yang and co-workers have studied the

effects of DAS and related compounds on cytochrome P450 activity, specifically cytochrome P450 2E1 (CYP2E1), a phase I drug metabolizing enzyme [115]. In these studies, it was observed that DAS is converted to diallylsulfoxide (DASO) and diallylsulfone (DASO$_2$) by CYP2E1. All three compounds were shown to be competitive inhibitors of CYP2E1 and possess the ability to reduce bioactivation of carbon tetrachloride, N-nitrosodimethylamine and acetoaminophen by CYP2E1 to toxic products. In addition, these compounds reduced the incidence of chemically induced tumors in the animal models.

In contrast to the results described above, Khanum and co-workers observed the variable results of feeding fresh garlic and garlic oil on detoxifying enzymes and micronuclei formation in rats treated with a carcinogen, azoxymethane (AOM) [116]. The results showed that feeding fresh garlic and garlic oil-supplemented diets tended to reduce hepatic lipid peroxidation, though not to any significant levels. Glutathione content was also not altered. The catalase activity in the liver of rats fed a fresh garlic-supplemented diet was reduced compared to that of the control diet; however, the activity was not affected by AOM treatment. Ingestion of garlic caused a 40 % increase in hepatic glutathione peroxidase activity, whereas carcinogen treatment reduced it. The activity of hepatic glutathione-S-transferase was unaffected by the feeding regimen, while it was lowered in the garlic oil diet group treated with AOM. The gamma-glutamyl transpeptidase activity was elevated more than 7 fold in the kidney of rats treated with AOM, while it was reduced almost to half when the AOM-treated rats were fed fresh garlic and garlic oil. Micronuclei formation was increased fourfold in rats exposed to AOM, whereas the increase was reduced to half when AOM-injected groups had either fresh garlic or garlic oil in their diet. From these studies, it was concluded that long-term feeding of garlic (fresh or oil) reduced the toxic effect of AOM in rats.

Since many garlic preparations exist in the marketplace, Song and Milner have studied the effect of heating on the ability of garlic to reduce the *in vivo* bioactivation of DMBA in Sprague-Dawley rats [117]. They observed that heating uncrushed garlic in either a microwave or convection oven decreased the protective activity of the garlic upon crushing. Preincubation of crushed garlic prior to microwave heating significantly restored its anticarcinogenic activity. The activity of crushed garlic was attributed to the formation of allyl sulfur compounds by alliinase.

It is clear from the studies reviewed here that garlic in several forms can change carcinogen metabolism and reduce formation of carcinogenic products. These studies have also shown that a number of garlic components including DAS, DADS, DATS, DPS and DPDS have a variety of effects on Phase I and Phase II enzymes.

Antioxidant Effects of Garlic

Tumor promotion may involve oxygen radicals [118-120]. Various anti-oxidants, free-radical scavengers, stimulators of superoxide dismutase and/or glutathione peroxidase, and inhibitors of cyclooxygenase (COX) and/or lipoxygenase (LOX) were found to affect oxidative stress and the formation of molecular species involved in tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). Perchellet and co-workers demonstrated that garlic oil stimulates glutathione peroxidase activity and inhibits the decrease in intracellular ratio of reduced to oxidized glutathione produced by TPA in epidermal cells [121]. Garlic oil was also found to inhibit LOX, a required enzyme in TPA-stimulated metabolism of arachidonic acid (AA) [122, 123].

Imai and co-workers studied the antioxidant properties of three garlic preparations and organosulfur compounds in garlic [66]. They observed that aged garlic extract, but not the fresh garlic extract, exhibited radical scavenging activity. Among the organosulfur compounds tested, the two major compounds in aged garlic extract, SAC and S-allylmercapto-L-cysteine, had the highest radical scavenging activity. Similarly, Naito *et al.* reported about the anti-oxygenic activity of SAC [124]. These reports suggest that oral administration of SAC and its analogs could be effective for radical scavenging, as these compounds play an important role in the antioxidant activity of aged garlic extract.

In the last few years, Balasenthil and co-workers have investigated the effect of garlic on lipid peroxidation and antioxidant levels during DMBA-induced hamster buccal pouch carcinogenesis in male Syrian hamsters. They observed that administration of an aqueous extract of garlic (250 mg/kg) to hamsters being treated with DMBA diminished lipid peroxidation in oral tumor tissue [125]. This decrease in lipid peroxidation was accompanied by a significant increase in the levels of glutathione (GSH), glutathione peroxidase (GPx) and glutathione S-transferase (GST). It should be noted that the incidence of neoplasms was also significantly reduced in the garlic-treated hamsters.

Further study of the effects of garlic in this animal model of carcinogenesis demonstrated the protective effects of garlic [126]. It was observed that enhanced lipid peroxidation in the circulation of tumor-bearing animals was accompanied by a significant decrease in levels of ascorbic acid, vitamin E, GSH, GPx, superoxide dismutase (SOD) and catalase. Administration of garlic extract significantly decreased lipid peroxidation with simultaneous enhancement of the circulating levels of antioxidants suggesting that garlic exerts its protective effects by decreasing circulatory lipid peroxides and enhancing antioxidant levels.

In an extension of the above study, Balasenthil and Nagini studied the effects of S-allylcysteine (SAC), a water-soluble constituent of garlic, on lipid peroxidation in 7,12-dimethylbenz[a]anthracene-induced hamster buccal pouch carcinogenesis [90]. They observed that administration of SAC significantly suppressed DMBA-induced oral carcinogenesis. However, in contrast to their previous studies, they observed increases in both lipid peroxidation and the levels of antioxidants (GSH) and detoxifying enzymes (GPx, GST) in SAC-treated animals. The authors speculated that modulation of lipid peroxidation, enhancement of GSA and GPx together with carcinogen-detoxifying enzymes such as GST are major mechanisms by which SAC exerts its chemopreventive effect.

Dirsch and co-workers have investigated the ability of ajoene, a major component of crushed garlic [127], to induce apoptosis in the human promyelocytic leukemia cell line HL-60 [128]. They observed that ajoene induces apoptosis in human leukemic cells, but not in peripheral mononuclear blood cells of healthy donors. The effect was both dose- and time-dependent. Ajoene increased the production of intracellular peroxide in a dose- and time-dependent manner, which could partially be blocked by preincubation of the human leukemic cells with the antioxidant N-acetylcysteine. N-acetylcysteine also blocked ajoene-induced apoptosis suggesting that peroxide production is instrumental in apoptosis of these cells. Moreover, ajoene was demonstrated to activate nuclear translocation of transcription factor kB, an effect that was abrogated in N-acetylcysteine-loaded cells. These results suggested that ajoene may induce apoptosis in human leukemic cells via the stimulation of peroxide production and activation of nuclear factor kB.

In a recent study, Dirsch and Vollmar have further investigated the ability of ajoene to affect the inducible isoform of cyclooxygenase, COX-2, in lipopolysaccharide (LPS)-activated RAW 264.7 macrophages as an *in vitro* model [129]. The study was undertaken in the light of reports that aberrant or excessive expression of COX-2 has been implicated in carcinogenesis [130, 131]. Ajoene was found to inhibit COX-2 enzyme activity by a mechanism similar to the non-steroidal anti-inflammatory drug, indomethacin.

These studies demonstrate that garlic preparations exhibit radical scavenging activity and decrease lipid peroxidation. It is difficult to speculate on a common mechanism for these effects. However, the presence of sulfur-containing compounds in all garlic preparations suggests that sulfur may be the key to these biological effects.

Tumor Growth Inhibition

Several early studies focused on the preincubation of tumor cells with garlic or its components, and the analysis of the resulting ability of the tumor cells to produce tumors in test animals. In the late 1950s, Weisberger and Pensky reported that preincubation of sarcoma 180 tumor cells with diethyl thiosulfinate (prepared by incubating S-ethyl L-cysteine sulfoxide with alliinase from fresh garlic) completely inhibited the ability of these cells to produce tumors in mice [132]. In the same study, tumorigenesis by Murphy-Sturm lymphosarcoma cells in rats was significantly inhibited by preincubation with diethyl thiosulfinate. In both cases, treatment of animals with the thiosulfinate at the same time as tumor cell inoculation also significantly inhibited tumor development [132, 133].

Belman studied the effect of both onion and garlic oil on tumor promotion by phorbol-myristate-acetate in mice [134]. He observed that onion oil both decreased the number of tumors and delayed the rate of tumor development with the highest dose of onion oil (10 mg) causing a ~40 % decrease in tumors over a 164-day period. Garlic oil was found to be a less potent inhibitor of tumor promotion than onion oil in this study.

Riggs and co-workers investigated the effect of subcutaneous and oral administration of aged garlic extract (AGE) on the development of murine transitional cell carcinoma (bladder cancer) in mice [135]. They observed that a series of 5 weekly immunizations with AGE (cumulative dose = 13 mg) significantly reduced tumor incidence, tumor growth, and increased survival when compared to animals that received the saline control. Mice that received 50 mg AGE per 100 ml drinking water orally had significant reductions in tumor volume ($P<0.05$), and mice that received 500 mg oral AGE per 100g drinking water had significant reductions in both tumor volume and mortality ($P<0.05$).

Investigation of DAS in a mouse skin model of carcinogenesis has indicated that DAS treatment can effectively delay the onset of tumorigenesis and reduce both the cumulative number of tumors and the average number of tumors per mouse [136]. In groups in which DAS was applied prior to initiation or promotion, a significant population of the mice remained tumor-free until the termination of the experiment. These results suggested that DAS can effectively inhibit chemically induced mouse skin carcinogenesis.

Cancer prevention by organosulfur compounds (OSC) from garlic has been studied in a medium-term bioassay system (Ito assay) to detect liver carcinogens and promoters in rats [137, 138]. In these studies, attention was focused on OSC chemoprevention in the post-initiation phase of cancer development. In addition, the modifying effects of SMC and cysteine on the initiation stage of rat hepatocarcinogenesis were investigated. Cancer development was quantified by the number of glutathione-S-transferase placental type (GST-P) positive foci. It was observed that oil-soluble OSCs such as methyl propyl disulfide and propylene disulfide demonstrated inhibitory effects on the development of GST-P positive foci. Moreover, water-soluble OCSs such as SMC and cysteine similarly decreased GST-P positive focus formation. In contrast, OSCs such as diallyl sulfide, diallyl trisulfide and allyl methyl trisulfide enhanced the formation of such altered hepatocellular foci. When given during the initiation stage, both SMC and cysteine significantly inhibited foci formation. Using the same assay system, Samaranayake *et al*. reported that rats administered a therapeutic dose of garlic (20mg/kg body weight) daily exhibited fewer hepatic GST-P positive foci in response to administration of diethylnitrosoamine than rats that received distilled water [139]. Thus, the results indicate that fresh garlic and some garlic-derived OSCs exert chemopreventive effects on chemical carcinogenesis.

The effects of naturally occurring garlic derivatives and synthetic S-cysteinyl compounds that resemble aged garlic constituents, on the proliferation of human prostate carcinoma (LNCaP) cells have been investigated [140]. The results showed that S-allyl mercaptocysteine significantly decreased LNCaP cell growth while S-allyl cysteine had a lesser effect. Studies using synthetic S-cysteinyl analogs showed that growth inhibition was most effective with compounds containing a disulfide or an active diallyl moiety. In an extension of this study, these researchers have shown that SAMC exhibits differential effects on the

recognized biomarkers for LNCaP cells similar to those produced by androgen deprivation and strongly suggests that this effect may be mediated, in part, by diminishing the trophic effects of testosterone, likely converting it to metabolites less reactive toward androgen receptors [141].

These observations were confirmed and extended by the work of Sakamoto *et al.* who studied the effects of allyl sulfides on the *in vitro* proliferation of human A549 lung tumor cells [142]. They reported that both DATS and DADS reduced the growth of A549 cells but were less effective toward non-neoplastic lung cells. DATS was more potent that DADS in this respect. DATS (10 μM) was found to increase the intracellular levels of Ca^{2+} and induce apoptosis as indicated by increased DNA fragmentation.

Effects of Garlic and its Compounds on the Cell Cycle and Induction of Apoptosis

There is increasing evidence that garlic and compounds isolated from garlic have significant antiproliferative effects on human cancer cells. Much of this work has recently been reviewed by Pinto and Rivlin [143] and Knowles and Milner [144, 145]. The effects shown by garlic derivatives include induction of apoptosis, regulation of cell cycle progression and modification of pathways of signal transduction. Additionally, they reported that garlic derivatives appear to regulate nuclear factors associated with immune function and inflammation. Some of the pertinent reports will be summarized here.

In 1997, Zheng and co-workers reported that the inhibitory effects of allicin on proliferation of leukemia cells were associated with the cell cycle blockage at the S/G_2M boundary phase and induction of apoptosis [146]. This effect was exhibited on neoplastic (leukemia) cells, but not non-neoplastic cells.

In the last few years, a number of reports have appeared concerning the antiproliferative effects of several compounds derived from garlic. Hong and co-workers studied the effects of DAS, DADS and garlic extract on p53-wild type H460 and p53-null type H1299 non-small cell lung cancer (NSCLC) cells [147]. They reported that DAS or DADS treatment, but not garlic treatment, of both cell types resulted in the highest number of cells in an apoptotic state. DADS was found to be more effective in inducing apoptosis in NSCLC cells. In H460 cells, the level of p53 protein, which is involved in the activation of apoptosis by DNA damage, was increased following DADS treatment. DAS and garlic treatment of H460 cells induced a rise in the level of Bax (a cell death agonist) and a fall in the levels of Bcl-2 (a cell death antagonist). It is well known that p53 activates the transcription of Bax and represses the expression of Bcl-2 [148, 149]. Thus, this study demonstrated that DAS, DADS and garlic extract are effective in reduction of an anti-proliferative gene in NSCLC and suggested that modulation of apoptosis-associated cellular proteins by DAS, DADS and garlic extract may be the mechanism for induction of apoptosis.

Similarly, Nakagawa *et al.* investigated the growth inhibitory effects of DADS on human breast cancer cell lines

and in an *in vivo* assay [150]. In both estrogen receptor-positive and –negative cells, DADS caused growth inhibition that was due to apoptosis as seen by the appearance of a sub G_1 fraction. The apoptosis cascade was comprised of up-regulation of Bax protein (142 %), down-regulation of Bcl-X_L protein (38 %) and activation of caspase-3 (438 %) compared to controls. In estrogen receptor-negative cells, DADS antagonized the effect of linoleic acid, a potent breast cancer cell stimulator and synergized the effect of eicosapentaenoic acid, a potent cancer cell suppressor. In an *in vivo* assay, DADS treatment caused growth retardation of tumors and reduction of tumor weight compared to DADS-untreated mice. Thus, this study suggested that DADS is a promising anticancer agent for both hormone-dependent and –independent breast cancers, and may also synergize with polyunsaturated fatty acids, known as modulators of breast cancer growth.

Shirin and co-workers have also studied S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) for their effects on cell proliferation in human colon cancer cell lines [151]. They found that SAMC but not SAC inhibited growth of colon cancer cell lines. SAMC also induced apoptosis and this was associated with increases in caspase-3- like activity. These effects of SAMC were accompanied by induction of jun kinase activity and a marked increase in endogenous levels of reduced glutathione. SAMC inhibited progression of cells at G_2-M in the cell cycle.

As mentioned previously, blockage at G_2-M has also been observed with leukemia cells treated with allicin [146]. Knowles and Milner have recently reported that the G_2-M phase arrest induced by DADS coincides with a suppression of p34^{cdc2} kinase activity [152]. The p34^{cdc2} kinase complex governs the progression of cells from the G_2 to the M phase of the cell cycle [153]. The formation of p34^{cdc2} kinase complex is controlled by association of the p34^{cdc2} catalytic unit with the cyclin B_1 regulatory unit [154]. Knowles and Milner have also recently shown that 12 hours of exposure of cultured HCT-15 colon cancer cells to 50 μmole/L DADS causes a twofold increase in cyclin B_1 protein expression; thus, suggesting that other factors involved with the p34^{cdc2} kinase must account for the ability of DADS to inhibit its activity [155].

Effects on Immunocompetence

Studies on the effects of garlic to enhance immunocompetence in cancer cells have focused mainly on bladder cancer, since superficial bladder tumors have been shown to be sensitive to several biological response modulators and especially to immunomodulators. In 1986, Lau *et al.* compared aged garlic extract (AGE) therapy with effective immunotherapies for bladder cancer such as BCG (Bacillus Calmette-Guérin) [156]. They reported that AGE was effective in inhibiting tumor growth in the transplantable murine bladder tumor model MBT2. For the last few years, Lamm and Riggs have also been studying the effects of garlic in the treatment of murine transitional cell carcinoma [157, 158]. Using the MBT2 model, they have observed that AGE is as effective a therapy as BCG when administered subcutaneously. It was also observed that oral

AGE added to drinking water at doses of 5, 50, and 500 mg/100 ml inhibited the growth of transitional cell carcinoma in a dose-dependent manner. In a similar study, Zlotta and Schulman also observed that mice receiving oral garlic at 50 or 500 mg had significant reductions in tumor volumes and mortality was also reduced at the higher dose level [159]. These observations are also supported by the report of Kyo and co-workers who studied the effects of AGE on immune functions in a variety of models [160].

The effects of AGE on murine transitional cell carcinoma are remarkably similar to those of BCG [158]. Both inhibit tumor growth, and microscopic examination of the site of tumor transplantation reveals infiltration by macrophages and lymphocytes. In animal models, both AGE and BCG induce hypertrophy of the reticuloendothelial system measured by splenic hypertrophy. AGE, like BCG, increases natural killer (NK) cell activity. In addition, animal studies have shown that AGE induces the release of cytokines such as IL-2, TNF-α and INF-γ [161]. In this study, it was also reported that AGE enhanced phagocytosis, an early immunostimulatory action, and killer cell activity and immunoproliferation of lymphocytes in response to mitogen stimulation. These effects suggest that AGE, like BCG, stimulates a Th1 cellular immune response that is characteristic of effective antitumor immunotherapies.

Efforts at determining the component of AGE responsible for these immunostimulatory effects have not been conclusive and thus suggest that multiple ingredients in AGE may be immunologically active [158]. Protein factor 4 (F4) from garlic has been shown to enhance the cytotoxicity of human peripheral blood lymphocytes against NK-sensitive and NK-resistant cells [162]. These effects were markedly augmented by the addition of low doses of IL-2 suggesting that the activity of F4 is mediated by IL-2.

However, the F4 fraction of garlic may not be the only garlic component involved in the immunostimulatory effects. Sundaram and Milner have reported that DADS was as effective as 5-fluorouracil (a potent anti-cancer drug) in inhibiting growth of tumors in transplanted human colon carcinoma cells [163]. Concurrent administration of DADS and 5-fluorouracil significantly reduced the depression of leukocyte counts and splenic weight associated with administration of 5-fluorouracil. In another study, Geng *et al.* studied the effects of SAC on human T cells [164]. SAC was found to inhibit activation of the nuclear protein of the Rel oncogene family (nuclear factor-κB). This protein, which is induced by TNF-α or H_2O_2, regulates immune function, inflammation and cellular growth. Thus, these studies suggest that low molecular weight compounds as well as protein found in garlic have antitumor and immune effects [158].

SUMMARY

Garlic (*Allium sativum*) is a member of the Lily family. Today, garlic is still employed worldwide as a natural remedy for the treatment of a variety of diseases including cancer. As detailed in this review, many researchers have reported pharmacological evidence supporting the use of garlic as an anti-carcinogenic agent.

A number of sulfur compounds that have been extracted and identified from the garlic, in particular diallylsulfide, diallyldisulfide, diallyltrisulfide, S-allylcysteine and S-allylmercapto-L-cysteine have been shown to possess anti-carcinogenic activity. As summarized in this review, this anti-carcinogenic activity involves many aspects including effects on drug metabolizing enzymes, antioxidant and free radical scavenging activity, inhibition of tumor initiation and promotion as well as effects on the cell cycle and induction of apoptosis in cancer cells. It is clear from this review of the recent literature that considerable progress has been made in recent years on the mechanisms by which garlic and the organosulfur compounds isolated from garlic suppress cancer initiation and development. It is also increasingly clear that garlic is most likely a powerful anticancer agent and inclusion of garlic in the diet should be considered to be mandatory to maintain good health.

LIST OF ABBREVIATIONS

AGE	=	Aged garlic extract
AMTS	=	Allyl methyl trisulfide
AOM	=	Azoxymethane
BCG	=	Bacillus Calmette-Guérin
BP	=	Benzo[a]pyrene
COX	=	Cyclooxygenase
DADS	=	Diallyl disulfide
DASO	=	Diallylsulfoxide
DASO$_2$	=	Diallylsulfone
DATS	=	Diallyl trisulfide
DMBA	=	Dimethylbenz(a)anthracene
DPS	=	Dipropyl sulfide
DPDS	=	Dipropyl disulfide
EC	=	Esophageal cancer
GGT	=	γ-Glutamyl transpeptidase
GPx	=	Glutathione peroxidase
GSH	=	Glutathione
GST	=	Glutathione-S-transferase
GST-P	=	Glutathione-S-transferase placental type
LOX	=	Lipoxygenase

LPS	=	Lipopolysaccharide
MCSO	=	S-Methylcysteinesulfoxide
NAT	=	N-Acetyltransferase
NK	=	Natural killer
NQO	=	Oxidoreductase
NSCLC	=	Non-small cell lung cancer
OSCs	=	Organosulfur compounds
PCSO	=	S-Propylcysteinesulfoxide
QR	=	Quinone reductase
RC	=	Raw garlic
SAC	=	S-Allylcysteine
SAMC	=	S-Allylmercaptocysteine
SC	=	Stomach cancer
SMCS	=	S-Methylcysteine sulfoxide
SOD	=	Superoxide dismutase
TPA	=	12-O-Tetradecanoylphorbol-13-acetate

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