CANCER AND NATURAL PRODUCTS

Editor
Mert Pekcan

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ANTI-CANCER POTENTIAL OF SHIKONIN

ÖĞÜNÇ MERAL[1]

Introduction

Cancer remains among the most serious challenges in global health, and conventional treatments such as chemotherapy and radiation are often plagued by problems of toxicity, drug resistance, and a lack of specificity. This has stimulated intensive research into new therapeutic agents derived from natural sources, which for centuries have served as a cornerstone in drug discovery. Among these promising natural compounds, shikonin is a naphthoquinone pigment isolated from plant roots belonging to the Boraginaceae family, such as Lithospermum erythrorhizon, Arnebia euchroma, and Onosma paniculatum, and has come to the forefront as a potent anti-cancer agent with a unique mechanism of action (Andújar et al., 2013).

Traditionally, shikonin and its derivatives have been used for thousands of years in traditional Chinese, Korean, and Japanese medicine, mainly for anti-inflammatory, antioxidant, and wound-healing purposes (Chen et al., 2002). However, modern

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pharmacological research has identified a much wider spectrum of bioactivity, with its anti-cancer potential being particularly noteworthy. Unlike many conventional chemotherapeutic agents that target a single pathway, shikonin exhibits a "multi-targeted" or "polypharmacology" profile, interfering with multiple hallmarks of cancer simultaneously (Hanahan & Weinberg, 2011). These include the induction of various modes of cell death, such as apoptosis, necroptosis, and autophagy, inhibition of metastasis and angiogenesis, and sensitization of resistant cancer cells to therapy.

Chemical Profile and Sources of Shikonin

The principal bioactive component of the "Zicao" root extract is shikonin, a compound with a molecular formula of $C_{16}H_{16}O_5$ and an IUPAC name of (R)-5,8-dihydroxy-2-[(1R)-1-hydroxy-4-methylpent-3-en-1-yl]-1,4-naphthoquinone. It mainly contains a naphthoquinone core, which is essential for the generation of its redox-cycling activity, while the presence of a side chain contributes to its lipophilicity and interaction with biological membranes (Kumar et al., 2020). This is responsible for its purple-red color. Shikonin consists of two enantiomeric forms, and the R-enantiomer has been identified as the natural and biologically active one. Its lipophilic nature allows the compound to easily penetrate cell membranes, which is one of the characteristic features of its potent intracellular activity.

The two most commercially important sources of shikonin are the roots of Lithospermum erythrorhizon Sieb. et Zucc. and Arnebia euchroma (Royle) Johnst. In these plants, shikonin biosynthesis flows through the phenylpropanoid pathway: condensation of p-hydroxybenzoic acid and geranyl pyrophosphate to yield the intermediate, which undergoes hydroxylation and cyclization to yield the final naphthoquinone structure (Yazaki, 2017). Because both L. erythrorhizon and A. euchroma have slow

growth and a very low yield, considerable efforts have been made on the technologies of plant cell culture as an ecological and controllable method of large-scale shikonin production (Fu et al., 2022).

Molecular Mechanisms of Anti-Cancer Action

Anti-cancer efficacy of shikonin does not arise from a single mechanism but through a collaborative action targeting the basic vulnerabilities of cancer cells. Cancer cells are defined by their capability to evade programmed cell death. Shikonin effectively counters this by triggering multiple death pathways.

Apoptosis

One of the major mechanisms of the cytotoxic action of shikonin is apoptosis, or programmed cell death type I. Shikonin exerts its inducing effect on apoptosis through both the intrinsic (mitochondrial) and extrinsic (death receptor) pathways. Shikonin promotes MOMP by modulating the Bcl-2 family proteins: it downregulates anti-apoptotic proteins (Bcl-2, Bcl-xL) while upregulating pro-apoptotic proteins (Bax, Bak). This triggers dissipation of mitochondrial membrane potential ($\Delta\Psi m$), release of cytochrome c into the cytosol, and activation of the caspase cascade, involving both caspase-9 and executor caspase-3, which in turn culminates in apoptosis (Wu et al., 2019). This process is often accompanied by ROS production, which can serve as one of the key mediators of the apoptosis-inducing action of shikonin. Shikonin may enhance the expression of death receptors, such as Fas and TRAIL receptors, on the cell surface. The ligation of these receptors initiates the formation of the death-inducing signaling complex (DISC) that causes activation of caspase-8, leading to further activation of caspase-3 (Gong & Li, 2011).

Necroptosis

One of the most exciting aspects of the actions of shikonin involves the induction of necroptosis, or regulated necrosis. This bears great importance in that most tumor cells develop resistance by evading apoptosis. Indeed, shikonin represents a well-documented inhibitor of the mixed lineage kinase domain-like protein, MLKL, which is an executor of necroptosis. By directly binding to MLKL, shikonin promotes oligomerization and translocation of the latter toward the plasma membrane, leading to its rupture and a lytic kind of death (Xie et al., 2020). Representing a mechanism effective in apoptosis-resistant cancer cells, this factor makes shikonin a promising agent in the treatment of resistant cancers.

Autophagy

The role of autophagy in the action of shikonin is complex and context-dependent. It may be an important prosurvival mechanism that degrades damaged organelles to supply energy and nutrients under stress. On the other hand, overactivation of this process may result in a cell death-inducing mechanism, generally referred to as autophagic cell death. Shikonin has been demonstrated to induce autophagy in many types of cancer cells by inhibiting the AKT/mTOR pathway, which negatively regulates autophagy (Wang et al., 2020). In some contexts, autophagy acts as a resistance mechanism. Thus, the combination of shikonin with autophagy inhibitors enhances its cytotoxic effects. In others, excessive autophagy contributes directly to cell death.

Inhibition of Metastasis and Angiogenesis

Metastasis is the major cause of cancer-related deaths. Shikonin has shown potent anti-metastatic action by targeting multiple steps of the invasion-metastasis cascade. Shikonin represses

EMT, which is an indispensable process for metastasis, through the downregulation of key transcription factors. This leads to increased expression of epithelial markers such as E-cadherin and reduces the expression of mesenchymal markers such as N-cadherin and Vimentin (Huang et al., 2021). Shikonin suppresses the activities and expressions of MMPs, especially MMP-2 and MMP-9, both of which are necessary for degrading the extracellular matrix to allow the invasion of cancer cells into other tissues (Sun et al., 2018). For tumor progression and spreading, the development of a new network of blood vessels is necessary. Shikonin strongly inhibits angiogenesis through the repression of vascular endothelial growth factor secretion from cancer cells and directly targeting vascular endothelial cells to inhibit tube formation (Yang et al., 2019). This anti-angiogenic action has been through the inhibition of the HIF-1α/VEGF signaling axis.

Targeting Cancer Cell Metabolism (The Warburg Effect)

Cancer cells prefer to use aerobic glycolysis; this phenomenon is known as the Warburg effect. Shikonin disrupts metabolic reprogramming by the direct inhibition of PKM2, a key glycolytic enzyme highly expressed in cancer cells (Wang et al., 2017). PKM2 inhibition by shikonin shunts glucose metabolites into anabolic pathways, leading to metabolic stress and ROS accumulation, contributing to cell death. This targeting of cancer-specific metabolism brings a high therapeutic index.

Modulation of Key Oncogenic Signaling Pathways

Shikonin thus exhibits its multi-targeted action by interfering with several important signaling pathways often altered in cancer. The PI3K/Akt/mTOR pathway is one of the key routes for cellular survival, growth, and proliferation. Indeed, shikonin has been able to inhibit the activation of PI3K, Akt, and mTOR consistently across a wide range of tumor types, thereby resulting in pro-apoptotic and

anti-growth effects (Cheng et al., 2021). NF-κB is a transcription factor and acts as a general regulator for inflammation, cell survival, and growth. The compound shikonin blocks NF-κB activation by impeding its nuclear translocation and subsequent transcription of prosurvival and metastatic genes (Andújar et al., 2013). STAT3 is one of the several transcription factors constitutively active in many cancers, driving oncogenesis. Shikonin thus inhibits the phosphorylation and activation of STAT3, further leading to downregulation by STAT3 target gene expression, such as that of Cyclin D1, Bcl2, and Survivin (Zhang et al., 2020).

Generation of Reactive Oxygen Species (ROS)

A common thread in the mechanism of shikonin is the induction of oxidative stress. As a naphthoquinone, shikonin can undergo redox cycling, producing large amounts of ROS, including superoxide anion and hydrogen peroxide. While low levels of ROS serve as signaling molecules, at high levels they cause oxidative damage to lipids, proteins, and DNA, leading to the activation of cell death pathways such as apoptosis and necroptosis (Wang & Yi, 2018). The anti-cancer activities of shikonin are often diminished by antioxidants, highlighting the key role of ROS in its cytotoxicity.

Anti-Cancer Effects Against Specific Cancer Types

The broad-spectrum activity of shikonin has been demonstrated in a wide array of human cancers, both *in vitro* and in preclinical *in vivo* models.

Hematological Cancers

Shikonin was strongly effective against leukemia and lymphoma. It showed the induction of apoptosis and necroptosis in AML cells and also evidenced a synergistic effect when used in combination with a standard chemotherapeutic agent, cytarabine

(Duan et al., 2014). It is also effective in cases of multiple myeloma and lymphomas, often targeting the NF-κB and STAT3 pathways.

Breast Cancer

Shikonin acts against breast cancer through cell proliferation inhibition, G2/M cell cycle arrest, and induction of apoptosis. More so, it has shown potent activity against TNBC, one of the subtypes that is very aggressive and resistant to treatment, through inhibition of the PKM2-mediated Warburg effect and EMT (Li et al., 2021).

Hepatocellular Carcinoma (HCC)

Shikonin exerts an inhibitory effect on both the growth and metastasis of liver cancer cells. It induced apoptosis via the ROS-JNK pathway and inhibited invasion by downregulating MMP-9 expression. Targeting the cancer stem-like cells in HCC further enhanced its therapeutic potential (Jiang et al., 2019).

Lung Cancer

In major types of lung cancers, including NSCLC and SCLC, shikonin has been shown to inhibit proliferation and sensitize cells to radiation and chemotherapy. Its anti-metastatic effects, largely through inhibition of the FAK/Src signaling pathway, are pronounced in lung cancer models (Wu et al., 2020).

Glioblastoma

GBM is a brain tumor for which there are few options for treatment. Shikonin easily crosses the blood-brain barrier and induces necroptosis in generally apoptosis-resistant GBM cells. Besides, it has been proved to inhibit angiogenesis and tumor growth in orthotopic mouse models of GBM (Fu et al., 2021).

Colorectal Cancer

Shikonin has been found to induce apoptosis and inhibit the Wnt/β-catenin signaling pathway, which plays an important role in the pathogenesis of colorectal cancer. Shikonin also altered gut microbiota and exerted anti-inflammatory effects via a colitis-associated tumor model, underlining its potential role in the prevention of cancer (Guo et al., 2021).

Shikonin in Overcoming Drug Resistance

The main obstacle in oncology is resistance to drugs. Several mechanisms make shikonin a promising strategy against such an obstacle. Many cancers acquire resistance by escaping from apoptosis; therefore, this constitutes a huge advantage for shikonin, considering it induces an alternative, regulated form of cell death-necroptosis (Xie et al., 2020). Shikonin has been shown to downregulate the expression of P-glycoprotein, one of the most important ABC transporters that extrude chemotherapeutic drugs outside cancer cells, and reverse MDR (Wu et al., 2019). A few subpopulations of tumor cells called CSCs are known to cause disease recurrence and drug resistance. Shikonin was found to target and ablate CSCs in various cancers by inhibiting their self-renewing capacity and some key signaling pathways (Jiang et al., 2019).

Synergistic Combinations with Conventional Therapies

In order to enhance efficacy and reduce side effects, shikonin is increasingly studied in combination with existing anti-cancer modalities. Shikonin synergizes with a variety of chemotherapeutics, including doxorubicin, cisplatin, and 5-fluorouracil. The synergy often arises from shikonin's ability to inhibit DNA repair mechanisms, induce ROS, and suppress anti-apoptotic signals, thereby sensitizing cancer cells to the cytotoxic drug (Cheng et al., 2021). Shikonin works as a radiosensitizer. It inhibits the repair of

radiation-induced DNA damage and increases radiation-induced apoptosis and necroptosis, thus making cancer cells more vulnerable to radiation treatment (Liu et al., 2020). Combinations of shikonin with targeted agents, such as receptor tyrosine kinase inhibitors, are being investigated. The multi-target nature of shikonin can help overcome resistance that often develops against single-target agents.

Pharmacokinetics, Toxicity, and Delivery Challenges

Despite its promising anti-cancer profile, the clinical translation of shikonin faces several difficulties. Shikonin has poor aqueous solubility and rapid metabolism and elimination, leading to low systemic bioavailability and a short half-life in vivo (Ma et al., 2022). After oral or intravenous administration, it is quickly converted to metabolites like acetylshikonin and isobutylshikonin, which may have different biological activities. While shikonin exhibits selective toxicity towards cancer cells in vitro, its in vivo toxicity is a concern. High doses have been associated with hepatotoxicity and nephrotoxicity in animal models (Lü et al., 2019). However, its therapeutic effect (the difference between an effective and a toxic dose) can be optimized through targeted delivery systems. To overcome the limitations of poor solubility, stability, and bioavailability, and to reduce off-target toxicity, advanced drug delivery systems are being developed for shikonin. Encapsulating shikonin in liposomes or biodegradable polymeric nanoparticles can improve its solubility, prolong its circulation time, and enhance its accumulation in tumor tissue via the Enhanced Permeability and Retention (EPR) effect (Zhao et al., 2021). Conjugating shikonin-loaded nanocarriers with ligands (e.g., folic acid, peptides) that recognize receptors overexpressed on cancer cells can further improve specificity and reduce side effects (Wang et al., 2023).

Conclusion

Shikonin is a fascinating natural compound with strong anti-cancer activity in both multiple ways and directions. This capability actually induces several modes of cell death, such as apoptosis and necroptosis, inhibits metastasis and angiogenesis, disrupts cancer metabolism, and modulates key oncogenic signaling pathways. To this date, targeting drug-resistant and cancer stem cells, two of the most demanding challenges in oncology today, is particularly valuable.

However, the road to the clinic is unforeseen upon overcoming its pharmacokinetic limitations and possible toxicity. The future of the shikonin-based therapy is focused on the rational design of novel analogs and complex systems of delivery targeted to tumors. Further interdisciplinary research will be very promising for shikonin in order for this traditional herbal treatment to turn into a modern, effective agent in cancer medicine and hopefully offer new hope for patients suffering from resistant and aggressive cancers.

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BIOLOGICAL AND CLINICAL IMPORTANCE OF ALPHA-LIPOIC ACID

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Introduction

Although lipoic acid synthesis is endogenous, lipoic acid, also referred to as alpha-lipoic acid or ALA/α-LA, was identified only in the 1930s. In 1937, it was identified as a component of potato extract that acted as a growth factor for Lactobacillus spp. (Byung 1994; Busby et al. 1999). Subsequently, Reed et al. succeeded in isolating 30 mg of lipoic acid from 100 kg of liver residue in the year 1951. The German clinicians initiated the clinical administration of racemic lipoic acid to patients who were admitted for treating liver cirrhosis, heavy metal toxicity, and mushroom poisoning, as well as for diabetic neuropathy, from the year 1966 onwards. These applications of lipoic acid in the clinics had been prompted by various observations of low lipoic acid levels in liver cirrhosis and diabetes mellitus and polyneuropathy patients. In the 1980s, alpha-

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lipoic acid was recognized as a powerful antioxidant (Kramer et al. 2002). Further studies elucidated the molecular structure of alpha-lipoic acid by identifying the core as 1,2-dithiolane-3-pantothenic acid (Morris et al. 1995).

Because of its structure, lipoic acid has also been referred to as "thioctic acid," "1,2-dithiolane-3-valeric acid," and "6,8-thioctic acid." Endogenously, lipoic acid is covalently bound to proteins as a prosthetic group in numerous multienzyme complexes such as the glycine cleavage system, pyruvate dehydrogenase complex, and α-ketoglutarate dehydrogenase complex. Mammalian tissues contain approximately 5-25 nmol/g lipoic acid, most of which is protein-bound (Kramer et al., 2002; Pfaffly, 2001).

Structure of Lipoic Acid

Though LA or α-LA is endogenously synthesized, it was not discovered until the 1930s. In 1937, it was observed as a potato extract constituent needed as a growth factor by Lactobacillus spp. (Byung, 1994; Busby et al., 1999). Reed et al. then isolated 30 mg of lipoic acid from 100 kg of liver residue in 1951. German clinicians started administering racemic lipoic acid to those patients who were admitted for liver cirrhosis, heavy metal toxicity, mushroom poisoning, and diabetic neuropathy in 1966. Clinical application of lipoic acid was spurred by the low levels of this antioxidant observed in liver cirrhosis, diabetes mellitus, and polyneuropathy patients. In the 1980s, alpha-lipoic acid (ALA) was discovered to be a potent antioxidant (Kramer et al., 2002). The core of alpha-lipoic acid was further identified to be 1,2-dithiolane-3-pantothenic acid (Morris et al., 1995).

Due to its structure, lipoic acid has also been termed as "thioctic acid," "1,2-dithiolane-3-valeric acid," and "6,8-thioctic acid." Lipoic acid is endogenously bound covalently to proteins as a prosthetic group in a host of multienzyme complexes such as the

glycine cleavage system, pyruvate dehydrogenase complex, and α-ketoglutarate dehydrogenase complex. Mammalian tissues contain about 5-25 nmol/g lipoic acid, most of which is protein-bound (Kramer et al., 2002; Pfaffly, 2001).

Biosynthesis and Transport of Lipoic Acid

Lipoic acids are synthesized across a broad range of organisms — from bacteria to plants and animals — and in many eukaryotes and prokaryotes (Herbert & Guest, 1975; Busby et al., 1999). In animals and humans, the primary site of lipoic acid biosynthesis is the liver, although synthesis also occurs in other tissues (Biewenga & Haenen, 1997).

In eukaryotes, the biosynthetic pathway is located in mitochondria; in plants, a plastidial pathway exists in addition to mitochondrial synthesis (Yasuno & Wada, 2002). In mitochondria, lipoic acid is derived from octanoic acid (which provides the eight-carbon backbone) and sulfur donors that furnish the two thiol groups (Perham, 1991).

When administered orally or intravenously, lipoic acid is rapidly absorbed and transported into cells, where it can be enzymatically reduced to DHLA. Studies of hepatic uptake in perfused rat liver and isolated hepatocytes identified two transport mechanisms: a carrier-mediated uptake effective at concentrations below approximately 75 μM, and concentration-dependent passive diffusion at higher concentrations (Peinado et al., 1989).

Metabolism of Lipoic Acid

Over 93% of an orally administered dose of lipoic acid is absorbed from the intestine. Following absorption, ~20-30% transiently accumulates in the liver, with additional deposition in tissues such as heart, skeletal muscle, and brain. Following absorption, the 1,2-dithiolane ring is reduced to the active DHLA;

lipoic acid is also S-methylated and undergoes β-oxidation. In rats and humans, the principal urinary metabolite is 4,6-bismethylmercaptohexanoic acid. In rats, ~80% of orally administered radiolabelled lipoic acid is excreted in urine (Cremer et al., 2006; Shay et al., 2009).

In studies in which radiolabeled lipoic acid (0.5 mg/100 g body weight) was given intraperitoneally to rats, 56% of radioactivity was recovered in urine. Extraction and chromatographic analysis distinguished lipoic, bisnorlipoic and tetranorlipoic acids as metabolites, products of β-oxidation of the lipoic acid chain (Joseph & Donald, 1976). Human volunteer studies following a single 1 g dose of R-lipoic acid detected 3-ketolipoic acid and bisnorlipoic acid in plasma (Kramer, 2001).

Plasma half-life is relatively short, but extensive presystemic elimination occurs. Repeated oral dosing rapidly achieves peak plasma levels. In man, a single oral 600 mg dose produces plasma concentrations in the 10–24 μM range; maximal effects have been observed at plasma concentrations up to ~300 μM, and beneficial effects have been reported at ~10 μM (Kramer, 2001).

Functional Roles of Lipoic Acid in Mitochondrial Enzyme Complexes

Lipoic acid functions as a cofactor in the process of pyruvate's oxidative decarboxylation. The decarboxylation of pyruvate occurs after its interaction with thiamine pyrophosphate (TPP), producing a hydroxyethyl-TPP intermediate. This intermediate subsequently donates the hydroxyethyl group to the lipoyl disulfide moiety present in the E2 subunit (also known as lipoamid). The hydroxyethyl component undergoes redox reactions that transform it into an acetyl group, which is then donated to coenzyme A, resulting in the formation of acetyl-CoA. Subsequently, the lipoyl group becomes reduced to dihydrolipoic

acid (DHLA), followed by its reoxidation through the action of dihydrolipoamide dehydrogenase, with NAD+ serving as the final electron recipient. The process of attaching lipoic acid covalently to lysine residues on proteins requires ATP and is facilitated by lipoate-protein ligase enzymes, while its detachment is carried out by hydrolase enzymes. In this way, lipoic acid captures acyl groups and transports them among the various enzyme components within multienzyme complexes. Throughout these catalytic processes, the lipoic acid... system functions as a redox pair that facilitates electron transfer from the dehydrogenase substrate to NAD+ (Gözükara, 1989). Lipoic acid serves as a prosthetic group for no fewer than five mitochondrial proteins, which include: (i) the acyl-transferase component within the pyruvate complex, (ii) α-ketoglutarate dehydrogenase, (iii) branched-chain α-ketoacid dehydrogenase complexes, (iv) protein X found in the pyruvate dehydrogenase complex, and (v) the H protein that is part of the glycine cleavage system (Navari-Izzo et al., 2002).

Lipoic acid functions as a cofactor in the process by which pyruvate undergoes oxidative decarboxylation. When pyruvate reacts with thiamine pyrophosphate (TPP), it loses its carboxyl group and forms a hydroxyethyl-TPP intermediate. This intermediate then donates its hydroxyethyl group to the lipoyl disulfide moiety present on the E2 subunit (lipoamid). Through a series of oxidation-reduction reactions, this hydroxyethyl fragment is transformed into an acetyl group, which subsequently combines with coenzyme A to produce acetyl-CoA. The lipoyl group becomes reduced to dihydrolipoic acid (DHLA) and is subsequently reoxidized through the action of dihydrolipoamide dehydrogenase, which utilizes NAD+ as the final electron recipient. The covalent linkage of lipoic acid to lysine residues in proteins requires ATP and is facilitated by lipoate-protein ligase enzymes, while its detachment is accomplished by hydrolytic enzymes.

In this capacity, lipoic acid captures acyl groups and transports them among different enzyme components within multienzyme assemblies. Throughout these catalytic processes, the lipoic acid/DHLA pair functions as a redox system that facilitates electron transfer from the dehydrogenase substrate to NAD+ (Gözükara, 1989)

Lipoic acid serves as a prosthetic group for no fewer than five mitochondrial proteins: (i) the acyl-transferase subunit of the pyruvate dehydrogenase complex, (ii) the α-ketoglutarate dehydrogenase enzyme, (iii) the branched-chain α-ketoacid dehydrogenase enzyme complexes, (iv) protein X within the pyruvate dehydrogenase complex, and (v) the H protein involved in the glycine cleavage system (Navari-Izzo et al., 2002).

Research has shown that lipoic acid participates in the regeneration of vitamins C and E, both of which neutralize free radicals (Busse et al., 1992). Vitamin E acts as a primary membrane-bound antioxidant that halts chain reactions involving lipid radicals; during this process, vitamin E itself becomes a radical species that is subsequently reduced back to its tocopherol form by vitamin C. The oxidized form of vitamin C (semidehydroascorbate radical) is then restored by glutathione. Consequently, vitamins C and E, along with glutathione, work synergistically to minimize cellular damage and regulate free radical activity. The effectiveness of this antioxidant regeneration system is fundamentally dependent on adequate glutathione levels (Busse et al., 1992).

The R-enantiomer of lipoic acid aids in restoring vitamins C and E to their reduced states; DHLA demonstrates powerful antioxidant capabilities within mitochondria (Kramer et al., 2001).

Critical characteristics that define an antioxidant's effectiveness include its capacity to chelate metal ions, scavenge radical species, interact synergistically with other antioxidants,

influence gene expression patterns, achieve adequate bioavailability, and reach sufficient intracellular levels. In contrast to most antioxidants, both the oxidized form (ALA) and the reduced form (DHLA) of lipoic acid possess antioxidant properties; they can directly neutralize reactive oxygen species and demonstrate antioxidant activity against various radical species (Pfaffly, 2001).

Characteristics that establish lipoic acid as an optimal antioxidant include:

• Antioxidant activity in both its reduced and oxidized states;

• Efficient absorption kinetics;

• Solubility in both lipid and aqueous environments;

• Capacity to chelate metal ions. (Güvenç, 2008)

Methods for the Determination of Lipoic Acids

Methods used to detect α-lipoic acid and DHLA include:

- microbiological assays,
- colorimetric assays,
- gas chromatography (GC),
- gas chromatography–mass spectrometry (GC-MS),
- high-performance liquid chromatography (HPLC),
- high-performance liquid chromatography with electrochemical detection (HPLC-EC),
- capillary electrophoresis,
- enzyme immunoassays.

HPLC-EC is one of the few methods that can independently quantify free α-lipoic acid and DHLA; many other methods measure only total lipoic acid (Pfaffly, 2001).

Biological Effects of Lipoic Acids

Lipoic acid was first used clinically to treat Amanita mushroom poisoning and subsequently reported to be useful for neuropathic complaints (Gomes & Negrato, 2014). Subsequent studies report that lipoic acid reduces the oxidized forms of other antioxidants, acts as a metal-binding agent, modulates NF-κB and insulin signaling pathways, inhibits atheroma formation, improves endothelial dysfunction and reduces exercise-induced oxidative stress (Moini et al., 2002; Packer et al., 1995).

Antioxidant Effects

Thiol compounds are biologically active antioxidants. Antioxidant action of lipoic acid is due to the reactivity of its dithiolane ring. This activity is manifested in radical scavenging, metal chelation, the repair of oxidative damage, and the regeneration of other antioxidants. (Biewenga et al., 1997)

Both LA and DHLA were reported to scavenge reactive oxygen and nitrogen species like hydrogen peroxide, hypochlorous acid, hydroxyl radical, and peroxynitrite (Moini et al., 2002; Packer et al., 2001; Scott et al., 1994). They also regenerate antioxidants like ascorbate, vitamin E, and ubiquinol, hence supporting cellular antioxidant capacity (Packer et al., 1995). Lipoic acid can cross the blood–brain barrier (Shay et al., 2009).

Lipoic acid detoxifies by chelating pro-oxidant metals; LA preferentially chelates Mn^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}, while DHLA chelates Hg^{2+} and Fe^{3+} (Frizzell & Baynes, 2013). Such metals as iron, copper, mercury, and cadmium catalyze hydroperoxide decomposition and generation of ROS; chelation can, therefore, contribute to antioxidant effects (Muller & Menzel, 1990).

In models of vitamin C and E deficiency, lipoic acid prevented deficiency symptoms by reducing oxidized antioxidants

back to active form. For instance, vitamin E radical, that is, tocopheroxyl, may be reduced by DHLA back to tocopherol (Wolinsky, 2004).

Some prooxidant effects of lipoic acid have been reported: in one study, lipoic acid increased protein carbonyl and nitrotyrosine levels in aged rat brain while decreasing non-protein thiol, total thiol and lipid hydroperoxide levels, indicating potential prooxidant actions under some conditions (Kayali et al., 2006).

Anti-carcinogenic effects

Karaca and Bayşu Sözbilir (2007) demonstrated in a rat model of diethylnitrosamine (DEN)-induced hepatocarcinogenesis that oral α-lipoic acid exerted protective effects compared with controls: ALA treatment increased GSH levels and body weight while reducing MDA, AST, ALT, GGT, ALP and ADA. Both 7-day and 14-day protective regimens were tested, with 14-day treatment being more effective.

Lipid peroxidation increases during hepatocarcinogenesis after DEN exposure; α-lipoic acid, via antioxidant mechanisms, may slow cancer progression and inhibit cells with oncogenic programs (Shih, 1983; Arivazhagan et al., 1999).

In combination with doxorubicin, lipoic acid reduced the cardiotoxicity of the chemotherapeutic agent and improved survival in murine leukemia models. Low concentrations of lipoic acid (≈1 μM) may act as growth promoters, whereas high concentrations (≈100 μM) exhibit antiproliferative effects; timing and dosing are therefore critical (Dovinova et al., 1999; Berkson et al., 2006).

Malarkodi et al. (2003) demonstrated that lipoic acid reduced adriamycin-induced nephrotoxicity in rats, consistent with reduced lipid peroxidation and improved antioxidant status. Similar protective effects were observed against adriamycin-induced cardiac

lipid peroxidation and renal lipid peroxidation in follow-up studies (Novotny et al., 2008).

Clinical case reports have also described intravenous lipoic acid combined with low-dose naltrexone (and lifestyle modification) in advanced pancreatic cancer with prolonged survival in individual patients (Berkson et al., 2006). Crucially, while these findings suggest a promising clinical avenue for novel combinations, it is essential to note that case reports represent anecdotal evidence. They serve as valuable indicators for directing future, large-scale randomized controlled trials necessary to definitively establish the mechanism and clinical benefit of this synergistic regimen.

Diabetes Mellitus

Alpha-lipoic acid enhances insulin signaling in skeletal muscle and adipocytes by activating tyrosine and serine/threonine kinases, increasing glucose uptake (Haugaard & Haugaard, 1970; Yaworsky et al., 2000). In stereoisomer studies, acute supplementation with the R-enantiomer significantly increased insulin-dependent glucose transport (~64%), whereas the S form showed no effect (Streeper et al., 1997). Chronic R-form supplementation reduced plasma insulin and free fatty acids and increased insulin-stimulated glycogen synthesis and glucose oxidation. Chronic S-form supplementation decreased GLUT4 levels, whereas R-form did not (Streeper et al., 1997).

Clinical studies indicate beneficial effects of ALA in both type 1 and type 2 diabetes. In lean and obese diabetic subjects, 1200 mg/day for 4 weeks decreased plasma pyruvate and lactate and improved insulin sensitivity (Konrad et al., 1999). In type 2 diabetics, a 4-week ALA regimen increased insulin-mediated glucose disposal from 25% (control) to 62% (intervention); maximal benefit was observed at 600 mg/day, with no additional benefit at 1200–1800 mg/day (Jacob et al., 1999).

Hyperglycemia increases flux through the polyol pathway, glycation end-products and ROS, contributing to diabetic complications; the antioxidant and insulin-sensitizing properties of lipoic acid may therefore help prevent diabetic complications (Borcea et al., 1999; Nourooz-Zadeh et al., 1997; Oberley, 1988).

Memory and Cognitive Function

In aged mouse models, long-term lipoic acid supplementation improved cognitive function and reduced β-amyloid accumulation and oxidative damage (Farr et al., 2003). Chronic supplementation increased total superoxide dismutase activity and overall antioxidant capacity while reducing malondialdehyde, and attenuating neurodegeneration in the hippocampus, thereby improving cognitive performance (Cui et al., 2006).

In transgenic mouse models of Alzheimer's disease, lipoic acid supplementation improved learning and memory (Quinn et al., 2007). A human pilot study administering 600 mg/day for one year to 9 Alzheimer's patients reported preservation of cognitive function (Hager et al., 2001).

Pre-irradiation lipoic acid prevents radiation-induced motor and memory deficits in mice, protecting cerebellar proteins and lipids from oxidative damage (Manda et al., 2007). In intracerebroventricular streptozotocin models, lipoic acid mitigated cognitive deficits and normalized brain glucose and energy metabolism (Sharma & Gupta, 2003).

Cardiovascular System and Diseases

Reactive oxygen species and mitogen-activated protein kinases play central roles in myocardial reperfusion injury. ALA has anti-apoptotic and cardioprotective effects; pre-reperfusion

supplementation reduced reperfusion injury in humans and animal models (Oh et al., 2009).

In endotoxin-induced models, ALA suppressed endothelial fractalkine expression and reduced endothelin-1-positive cell infiltration and lipopolysaccharide-induced myocardial dysfunction (Sung et al., 2005; Goraca et al., 2009). ALA preserves mitochondrial enzyme activities in diabetic cardiomyopathy and delays deterioration of cardiac function, attenuating structural changes and serving a protective role (Artwohl et al., 2007).

In obese rats, ALA activated AMP-activated protein kinase (AMPK) in endothelial cells, preventing endothelial dysfunction; in experimentally induced hypercholesterolemic rabbits, ALA reduced aortic intimal lesion formation (Lee et al., 2005; Amom et al., 2008). In older humans, ALA restored endothelial glutathione levels and improved vascular endothelial function (Smith et al., 2008).

Respiratory System and Diseases

ALA protects against endotoxin-induced oxidative stress in the lung: in LPS-treated rats ALA reduced lipid peroxidation, decreased H_2O_2, and increased free sulfhydryl groups in bronchoalveolar lavage fluid (Goraca & Skibska, 2008). In experimental asthma models, ALA reduced airway inflammation, plasma extravasation and eosinophilic infiltration, and decreased VEGF expression and vascular permeability (Lee et al., 2006).

Gastrointestinal System and Diseases

In acute pancreatitis models, ALA reduced serum amylase and lipase and decreased pancreatic weight-to-body weight ratio (Park et al., 2005). In patients undergoing hepatic resection, ALA lowered serum AST and ALT levels (Dünschede et al., 2006). ALA showed therapeutic potential in experimental gastric ulcer models by suppressing neutrophil accumulation, preserving endogenous

glutathione, inhibiting ROS generation and apoptosis (Karakoyun et al., 2009).

Urinary System and Diseases

ALA has been proposed for the treatment of ischemia- and repeated-stimulus-induced bladder contractile dysfunction (Lin et al., 2008). In mesangial proliferative glomerulonephritis, ALA halved ROS increases (Budisavljevic et al., 2003).

Muscular System and Diseases

Muscles in Duchenne muscular dystrophy (DMD) are exposed to increased oxidative stress and calcium dysregulation leading to necrosis and apoptosis. In mdx mouse models, ALA reduced plasma creatine kinase, decreased lipid peroxidation products and affected antioxidant enzyme activities in diaphragm muscle (Hnia et al., 2007). However, ALA supplementation did not consistently improve skeletal muscle contractile properties or fatigue resistance (Coombes et al., 2001).

Retinal Protection

Alpha-lipoic acid (ALA) has demonstrated substantial protective efficacy against both age-related mitochondrial dysfunction and oxidative stress within retinal pigment epithelial (RPE) cells. Intriguingly, comparative studies have been conducted between ALA and its neutral amide derivative, lipoamide (also known as LA amide). These investigations suggest that lipoamide may function as a more potent antioxidant in shielding RPE cells from oxidative damage than ALA itself (Volobоueva et al., 2005; Li et al., 2008). This enhanced efficacy is often attributed to lipoamide's improved lipophilicity, which may facilitate more efficient cellular uptake and accumulation within the retina.

Nervous System and Diseases

Combined supplementation of ALA and N-acetylcysteine protected Alzheimer's disease patient fibroblasts from oxidative and apoptotic determinants, suggesting potential for combined therapies (Moreira et al., 2007). In multiple sclerosis patients, ALA reduced relapse frequency and symptom severity; dose-dependent reductions in serum inflammatory markers were observed. Animal models of MS also showed anti-inflammatory effects (Kidd, 2005).

Cataract

Cataract formation is associated with reduced antioxidant activity in the lens. Glutathione, a primary lens antioxidant, is regenerated by ALA (Karaca & Bayşu Sözbilir, 2007). Aldose reductase is important in diabetic cataractogenesis; ALA inhibits aldose reductase activity in the diabetic rat lens. Experimental studies showed that ALA reduced lens opacification, increased lens GSH, ascorbic acid, α-tocopherol, and enhanced GPx, catalase and ascorbate reductase activities (Ou et al., 1996; Maitra et al., 1995; Bilska & Wlodek, 2005).

Glaucoma

In glaucoma patients, sulfhydryl groups reflecting glutathione levels are substantially decreased in the aqueous humor, particularly in advanced stages; erythrocyte glutathione is also reduced and MDA levels in the anterior chamber are elevated. ALA supplementation raised low glutathione levels and improved biochemical and visual parameters in open-angle glaucoma patients (Filina et al., 1995; Head, 2001).

Ischemia and Reperfusion Injury

Ischemia followed by reperfusion triggers a substantial surge in reactive oxygen species (ROS) generation, a phenomenon known

to be a major contributor to extensive tissue damage observed in conditions such as stroke, cardiac arrest, hemorrhage, and traumatic brain injury.

In experimental models of these injuries, the administration of alpha-lipoic acid (ALA), whether preceding or immediately following the ischemic event, successfully mitigated reperfusion-induced ROS levels. This intervention consequently resulted in reduced infarct sizes and improved survival rates. Furthermore, the reduced form, dihydrolipoic acid (DHLA), also demonstrated comparable therapeutic efficacy (Bilska & Wlodek, 2005).

Regarding renal injury, studies employing kidney ischemia-reperfusion models showed that ALA treatment—delivered both pre-ischemically and immediately post-ischemically—served to preserve renal hemodynamics, maintain the kidney's urinary concentrating ability, and facilitate the normalization of sodium excretion (Bae et al., 2008).

AIDS (Acquired Immunodeficiency Syndrome)

ALA has been administered to AIDS patients; it reduced NF-κB activity, a transcription factor implicated in inflammatory gene expression and HIV replication. While DHLA increased NF-κB DNA-binding activity, ALA decreased it. ALA treatment in AIDS patients increased glutathione, vitamin C and helper T cell counts and reduced oxidative stress markers, suggesting potential adjunctive benefits (Packer et al., 1995).

Exercise and Sport-related Conditions

Exercise increases oxidative stress and glutathione oxidation is a key indicator. Animal studies emphasize the necessity of adequate tissue glutathione to withstand exercise-induced oxidative stress (Sen & Packer, 2000). Excessive exercise-related declines in cardiac glutathione-S-transferase activity were prevented by ALA;

ALA reduced oxidative lipid damage in muscle and ameliorated differences in glutathione antioxidant systems between trained and untrained individuals (Khanna et al., 1999; Zembron-Lacny et al., 2009).

Other Indications

In ataxia-telangiectasia, ALA reduced chronically activated damage-responsive proteins associated with ROS (Gatei et al., 2001). An antioxidant mixture including ALA protected photoreceptor cones after rod cell loss in a retinitis pigmentosa model (Komeima et al., 2006). In metabolic syndrome, ALA reduced proinflammatory markers and improved endothelial function (Sola et al., 2005). There are case reports linking ALA with insulin autoimmune syndrome; caution is therefore warranted (Takeuchi et al., 2007; Ishida et al., 2007). In transient focal ischemia models, ALA decreased infarct volumes (Clark et al., 2001). Antioxidant diets containing ALA attenuated radiation-induced bone marrow suppression and increased survival in irradiated rats (Wambi et al., 2008). ALA may partially reverse age-related mitochondrial decline and oxidative stress (Evans & Goldfine, 2000).

Dosage

Reported values pertaining to the lethal dose 50 (LD50) demonstrate considerable variability: The intravenous LD50 in Rattus norvegicus is documented to be approximately 400-500 mg/kg, a range that is similarly reflected in the oral LD50 in canines, which is also approximately 400-500 mg/kg. It is noteworthy that, in the canine population, the lowest observed adverse effect level (LOAEL) was determined to be 121 mg/kg/day, as evidenced specifically by the elevation of hepatic enzymes. An overdose of this substance poses a risk of presenting as allergic cutaneous reactions and hypoglycemia. Therefore, due to the potential for substantial pharmacological interactions, meticulous caution is imperative when

administering this compound to patients who are concurrently receiving insulin, oral hypoglycemic agents, or treatments involving thyroid hormones (Fragakis, 2007).

Interactions with Other Nutrients

Synergistic benefits resulting from the co-administration of alpha-lipoic acid (ALA) with other established antioxidants have been well-documented in pre-clinical models. Specifically, research in embolic stroke models has demonstrated that combining ALA with vitamin E significantly ameliorates neurological deficits and reduces reactive gliosis (Gonzalez-Perez et al., 2002). Furthermore, the combined therapeutic effect of ALA and acetyl-L-carnitine proved superior to monotherapy in preventing age-related decline in mitochondrial function and mitigating subsequent cognitive impairments (Hagen et al., 2002; Liu et al., 2002a,b). Lastly, the co-administration of ALA and coenzyme Q has been shown to successfully enhance the antioxidant capacity of cellular membranes (Kozlov et al., 1999).

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ORGANOSULFUR COMPOUNDS OF ALLIUM SATİVUM L. AND ITS ROLE IN CANCER PREVENTION

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Introduction

Medicinal plants are recognized as excellent sources of herbal medicines due to the minimal adverse effects they produce (Surh, 2003). Garlic has been reported in many cultures and in folklore as a therapeutic agent with disease-preventive properties (Charu et al., 2014). Garlic contains a wide range of bioactive compounds, including organosulfur compounds, saponins, phenolic

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compounds, and polysaccharides (Bradley et al., 2016; Diretto et al., 2017; Wang et al., 2018; Szychowski et al., 2018b). Numerous studies have demonstrated that garlic and garlic preparations exert various health-promoting effects, such as the reduction of blood lipid levels and blood pressure, as well as the inhibition of microbial growth (Myneni et al., 2016).

A substantial number of preclinical studies have been conducted to investigate the cancer-preventive and therapeutic effects of garlic and its derivatives. These studies have focused on mechanisms including the modulation of xenobiotic metabolism, activation of metabolizing enzymes for carcinogen detoxification, inhibition of reactive oxygen species generation, enhancement of DNA damage repair, regulation of the cell cycle, induction of apoptosis, inhibition of angiogenesis, and histone modification (Ross, 2025).

In recent years, another garlic preparation known as black garlic has been introduced to the market (Y.-M. Lee et al., 2009; M. H. Kim et al., 2011). Black garlic has been consumed for centuries in countries such as South Korea, Japan, and Thailand. It is produced by fermenting white garlic (Allium sativum L.) under conditions of high temperature and high humidity for a specified period (Yuan et al., 2016). Both in vitro and in vivo studies have reported that black garlic exhibits stronger antioxidant activity than raw garlic (Seo et al., 2009; Lee et al., 2009). Numerous studies have also demonstrated that black garlic extracts possess various biological activities, including antioxidant, antiallergic, antidiabetic, anti-inflammatory, and anticarcinogenic effects (Kim et al., 2014; Park et al., 2014a; Yoo et al., 2014b; Ha et al., 2015; Jeong et al., 2016).

In contemporary medical practice, complementary and alternative medicine approaches are increasingly being integrated alongside modern therapeutic methods. In particular, plant-derived

drugs exhibit diverse mechanisms of action against cancer. Medicinal plants contain numerous antioxidants capable of scavenging free radicals, such as carotenoids, vitamin E and vitamin C, as well as quinones, polyphenols, amines, alkaloids, and flavonoids with high antioxidant activity (Cai et al., 2004; Kadifkova Panovska et al., 2005).

***Allium sativum* L.**

Garlic (Allium sativum L.) belongs to the class Monocotyledones, the superorder Liliiflorae, the order Asparagales, the family Alliaceae, and the genus Allium (Friesen et al., 2006). Raw garlic bulbs consist of approximately 65% water, 28% carbohydrates, 2% protein, 1.2% amino acids, 1.5% dietary fiber, as well as fatty acids, phenolic compounds, trace elements, and more than 33 sulfur-containing compounds (approximately 2.3%) (Butt et al., 2009). Ozalp Unal and Sel (2024) reported that black garlic exhibits lower moisture content, crude protein, total fat, total ash, and pH values compared with white garlic. The moisture content, crude protein, total fat, total ash, and pH values of white garlic were determined to be 69.3%, 11.2%, 1.94%, 2.5%, and 6.93, respectively.

Garlic (Allium sativum) is not only important for human health but has also been one of the most significant dietary sources used as a traditional medicinal agent worldwide (Szychowski et al., 2018a; Liyanagamage et al., 2020).

Among garlic-derived products, black garlic is recognized as one of the most well-known functional foods available on the market. Owing to its high nutritional value, black garlic has gained increasing popularity in Asian countries such as Japan, Singapore, and China in recent years (Liu et al., 2015). Various bioactivities of black garlic, including anticancer, anti-obesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects, have been reported in the literature (Kimura et al., 2017).

For centuries, people in Asian countries such as Thailand, South Korea, and Japan have produced and consumed black garlic as a traditional food. Black garlic is produced by fermenting whole fresh garlic bulbs under conditions of high humidity and temperature. This process leads to the transformation of garlic into a black-colored product through a series of non-enzymatic browning reactions, including the Maillard reaction, phenolic oxidation, and caramelization. During fermentation, not only do the physicochemical properties of garlic change, but the concentration of bioactive compounds is also enhanced (Kimura et al., 2017).

Natural Products in Cancer

In recent years, a growing body of evidence has demonstrated that various bioactive compounds derived from dietary sources confer health benefits beyond basic nutrition and play a significant role in the prevention of chronic diseases (Ross, 2025; Suman and Shukla, 2016). In cancer inhibition, natural compounds do not directly cause DNA damage in the same manner as conventional chemotherapeutic drugs. Moreover, the incidence of DNA mutations induced by natural compounds in viable cells is extremely low. In the fight against cancer, natural compounds exert their effects by:

- Enhancing the immune system,
- Inhibiting angiogenesis, thereby preventing the spread of cancer cells and the growth of new blood vessels that supply tumors,
- Creating an unfavorable environment for cancer development (by increasing oxygen levels in the body, enhancing metabolism, body temperature, and alkalinity, and reducing glucose levels),

- Promoting detoxification and preventing the accumulation of excessive toxic substances in the body,
- Supporting all target organs, particularly those affected by cancer,
- Combating free radicals that trigger carcinogenesis (Lam, 2003).

Currently, more than 60% of available anticancer drugs are derived from natural sources. Natural sources such as plant-based foods and their extracts contain various micronutrients (minerals and vitamins), dietary fibers, and bioactive compounds known as phytochemicals. In particular, plant-based dietary patterns have been associated with a reduced incidence of chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Doré and Blottière, 2015; Mozaffarian, 2016).

Garlic has long been used as a medicinal food. Recent studies have confirmed the diverse biological activities of garlic, including cancer prevention, antithrombotic effects, and cardioprotection (Isensee et al., 2010). The health benefits of garlic are largely attributed to its bioactive constituents, particularly organosulfur compounds such as diallyl trisulfide, S-allylcysteine, vinyldithiins, allyl propyl disulfide, ajoene, and allicin (Bhandari, 2012). In addition to these compounds, garlic is also characterized by phenolic constituents that possess pharmacological properties (Beato et al., 2011; Matysiak et al., 2015).

Organosulfur Compounds in Garlic

Garlic owes its characteristic flavor, odor, and a substantial proportion of its biological activity to the organosulfur compounds it contains (Block, 1992). In Allium species, the major organosulfur compounds are classified into two groups: water-soluble and oil-soluble organosulfur compounds. Lipophilic

organosulfur compounds have been reported to be more potent than their water-soluble counterparts (Bianchini and Vainio, 2001). Diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and other allyl polysulfides collectively constitute the allyl sulfide group in garlic and account for approximately 94% of garlic oil (Fisher et al., 2007).

Whole garlic bulbs contain two types of non-volatile organosulfur molecules: γ-glutamyl-L-cysteine peptides [γ-glutamyl-S-allyl-L-cysteine] and L-cysteine sulfoxides [containing S-allyl-L-cysteine sulfoxide] (Cetinkaya and Suntar, 2021). Approximately 80% of the cysteine sulfoxides present in garlic consist of S-allyl-L-cysteine sulfoxide, also known as alliin. When raw garlic cloves are crushed, the enzyme alliinase is released. Alliin is an amino acid that is converted by alliinase into 2-propenesulfenic acid, accompanied by the release of pyruvic acid and ammonia. 2-Propenesulfenic acid is unstable and highly reactive at room temperature. Two molecules of 2-propenesulfenic acid spontaneously react to form allicin, with the release of water (Ross, 2025).

Allicin is a volatile compound present in garlic. Allicin (diallyl thiosulfinate) is the most well-known bioactive component in freshly crushed garlic extract (Bruck et al., 2005). It is responsible for the characteristic aroma and flavor of garlic (Amagase, 2006; Corzo-Martínez et al., 2007). Allicin rapidly reacts with free thiol groups and readily penetrates biological membranes. However, the mechanisms underlying the effects of allicin on various cellular systems are not fully elucidated. Its biological activities are thought to be associated with thiol (–SH) modification and antioxidant properties (Miron et al., 2002). Allicin has been reported to induce activities affecting vital cellular pathways, including mitochondrial membrane potential, intracellular redox regulation, and cell division, in human colon carcinoma cell lines (Xiao et al., 2005). Furthermore,

allicin induces G2/M cell cycle arrest, disrupts mitochondrial membrane potential, and modulates intracellular glutathione levels (Jakubíková and Sedlák, 2006). Allicin decomposes to form a series of oil-soluble organosulfur compounds, including diallyl trisulfide (DATS), diallyl disulfide (DADS), diallyl sulfide (DAS), and allyl methyl sulfide (AMS) (Sato et al., 2020; Shang et al., 2019). DADS, which accounts for approximately 66% of all organosulfur compounds in garlic, exhibits health-promoting effects such as antimicrobial, antioxidant, antidiabetic, and anticancer activities (De Greef et al., 2021). In various cancer models, the anticancer efficacy of DADS has been demonstrated through multiple signaling pathways that regulate cell proliferation, apoptosis, and metastasis (Hong et al., 2000; Druesne et al., 2004; Yuan et al., 2004).

DADS has been shown to reduce migration and invasion of human colon cancer cells and MDA-MB-231 breast cancer cells by inhibiting matrix metalloproteinases (MMPs) and tumor necrosis factor-α (TNF-α) via the NF-κB, PI3K/Akt, and MAPK/ERK signaling pathways (Lai et al., 2013; Yin et al., 2018). Apoptosis induction through p53-mediated pathways has been observed in DADS-treated breast and colorectal cancer cells (Hong et al., 2000; Siddhartha et al., 2018; Kim et al., 2019). Additionally, several studies have demonstrated that DADS induces autophagy in RAW264.7, leukemia, and osteosarcoma cells by inhibiting phosphorylation of the PI3K/Akt/mTOR signaling pathway (Suangtamai and Tanyong, 2016; Yue et al., 2019; Choromanska et al., 2020). However, the clinical application of DADS is limited due to its poor solubility in aqueous environments (Alam et al., 2013; Siddhartha et al., 2018).

Allicin also gives rise to the production of sulfur-containing compound, thiacremonone (Kim et al., 2012). When fermented with organic solvents, allicin produces ajoene (Viswanathan et al., 2014).

In the body, allicin interacts with L-cysteine to form S-allylmercaptocysteine (SAMC) (Sigounas et al., 1997).

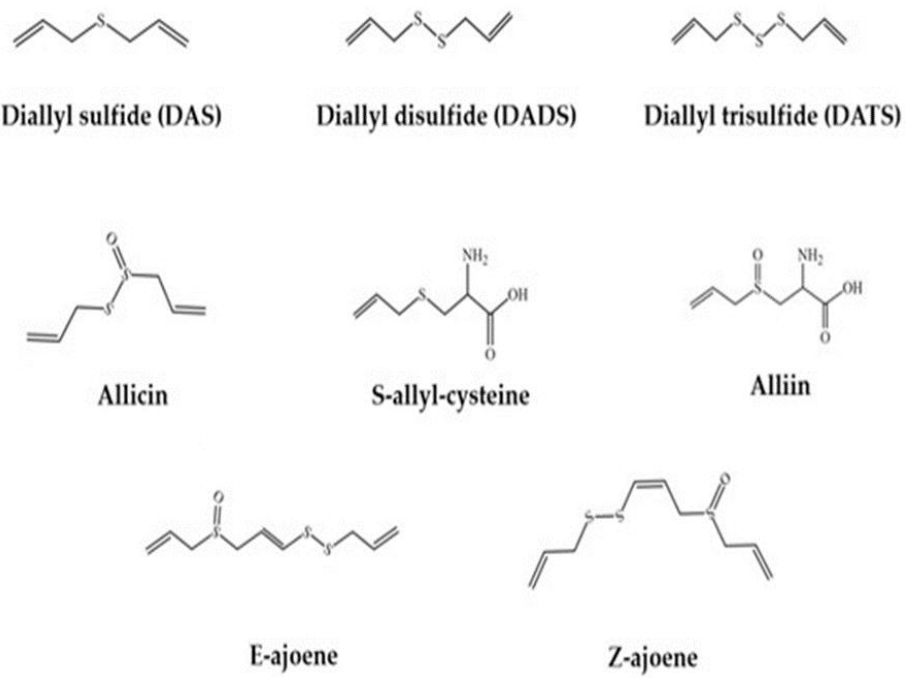
N-trans-feruloyloctopamine (FO), a hydroxycinnamic acid derivative, is isolated from garlic husks. Garlic also contains natural organoselenium compounds, including Se-methyl-L-selenocysteine (MSeC), a derivative of S-methyl cysteine. γ-Glutamyl-L-cysteine is a peptide containing the water-soluble dipeptide γ-glutamyl-S-allyl-L-cysteine. S-allyl-L-cysteine (SAC) and SAMC are water-soluble organosulfur compounds produced from γ-glutamyl-S-allyl-L-cysteine following prolonged aqueous fermentation of crushed garlic. S-benzyl-L-cysteine (SBC) is a water-soluble structural analog of SAC and can be isolated from aged garlic extract. S-propargyl-L-cysteine (SPRC) is a derivative of SAC and functions as a hydrogen sulfide (H_2S) donor derived from garlic extract (Rose et al., 2019). SAC contributes to the health benefits of garlic by conferring antidiabetic, antioxidant, and anti-inflammatory activities (Saravanan and Ponmurugan, 2013; Colín-González et al., 2015).

Following oral intake of garlic, SAC can be detected in plasma, liver, and kidney tissues (Nagae et al., 1994). Due to its stability in the bloodstream, SAC is considered the most reliable biomarker of garlic consumption (Steiner and Lin, 1998). In contrast, allicin, sulfides, ajoene, vinyldithiins, and other oil-soluble organosulfur compounds cannot be detected in blood or urine. (Lawson and Hughes, 1992).

Alliin, an unstable compound with antioxidant activity, is converted into SAC, a stable compound, during the aging process of fresh garlic (Corzo-Martínez et al., 2007; Lee et al., 2009). Aged garlic has been reported to exhibit higher antioxidant capacity than fresh garlic (Jang et al., 2018). Lee et al. (2009) reported that the reduction in free radical levels in aged black garlic was significantly greater than that observed in fresh garlic. Özalp Ünal and Sel (2024)

found that the total phenolic and flavonoid content and the DPPH activity of aqueous black garlic extract was significantly higher than that of white garlic extract.

Figure 1. Chemical structures of the main organosulfur compounds in garlic.



Kaynak: Shang et al.,2019

Organosulfur Compounds and Cancer

Garlic is known to possess anticancer properties due to its content of various sulfur-containing and organic compounds. These compounds exert their effects through multiple mechanisms, including cell cycle arrest, inhibition of signaling pathways, induction of apoptosis, autophagy, and antioxidant activity. Through these mechanisms, they interact with different stages of cancer cell development, proliferation, growth, invasion, migration, and

metastasis. Garlic contains both water-soluble and lipid-soluble sulfur compounds that exhibit anticancer properties by reducing oxidative stress, suppressing carcinogen metabolism, and enhancing immune function (Ross, 2025).

The anticancer properties of various sulfur-containing compounds present in garlic have been extensively investigated. According to previous studies, allicin is the primary active sulfur compound and serves as a precursor to several lipid-soluble allyl sulfur compounds with anticancer activity (Bat-Chen et al., 2010; F. Chen et al., 2016; Dhanarasu, 2017; Zou et al., 2016). One of the bioactive components of garlic, diallyl disulfide (DADS), has been reported to induce apoptosis in the T24 human bladder cancer cell line (Shukla and Taneja, 2002). DADS has also been shown to suppress 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors in rats (Song and Milner, 1999).

Diallyl sulfide (DAS) and diallyl trisulfide (DATS) have been observed to protect mice against DMBA-, phorbol ester-, and benzo[a]pyrene-induced skin tumorigenesis (Arora et al., 2006). DATS inhibited the growth of PC-3 human prostate cancer xenografts in mice (Xiao and Singh, 2006). Additionally, DATS has been shown to reduce mitotic activity in tumor tissues, decrease histone deacetylase activity, increase acetylation of histones H4 and H3, inhibit cell cycle progression, and downregulate pro-tumorigenic markers such as Bcl-2, survivin, mTOR, EGFR, c-Myc, and VEGF (Zhang et al., 1989). In another study, DATS inhibited migration and invasion of SGC-901 gastric tumor cells by regulating the protein expression of MMP-7901 and E-cadherin in BALB/c nude mice (Jiang et al., 2017).

In addition to lipid-soluble organosulfur compounds, the water-soluble compound S-allylcysteine (SAC) inhibited tumor growth and malignant progression of highly metastatic non-small

cell lung carcinoma in mice (Tang et al., 2010). SAC has been shown to induce G1/S phase cell cycle arrest in A2780 human epithelial ovarian cancer cells (Xu et al., 2014). A structural analog of SAC, S-propargyl-L-cysteine (SPRC), reduced proliferation and induced G2/M phase cell cycle arrest in human pancreatic ductal adenocarcinoma cells (Wang et al., 2015).

S-allylmercaptocysteine (SAMC), derived from garlic, suppresses proliferation of hepatocellular carcinoma cells and adversely affects cell cycle progression by decreasing the proportion of cells in the S phase while increasing the proportion in the G0/G1 phase (Xiao et al., 2018).

Allicin has also been found to inhibit proliferation of gastric adenocarcinoma cells by inducing S-phase cell cycle arrest without affecting normal intestinal cells (INT-407) (Mansingh et al., 2018).

Diallyl trisulfide (DATS) has been reported to induce apoptosis in gastric cancer cells by downregulating Bcl-2 expression (Jiang et al., 2017). S-allylcysteine (SAC) induces apoptosis in ovarian cancer cells by decreasing the expression of procaspase-3, poly (ADP-ribose) polymerase-1 (PARP-1), and Bcl-2, while increasing the expression of active caspase-3 and Bax proteins (Xu et al., 2014).

S-allylmercaptocysteine (SAMC) induced apoptosis in colorectal carcinoma cells through activation of the Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (p38 MAPK) signaling pathways (Zhang et al., 2014).

Alliin induced apoptosis in gastric adenocarcinoma cells by generating reactive oxygen species (ROS). In addition, alliin was shown to reduce mitochondrial membrane potential through downregulation of the Bax/Bcl-2 protein ratio and upregulation of cytochrome c (Mansingh et al., 2018).

Overall, organosulfur compounds present in raw garlic exhibit higher bioavailability compared to those in cooked garlic (Torres-Palazzolo et al., 2018). Garlic, which has a richer phytochemical composition than many vegetables, contains more than 20 phenolic compounds (Ross, 2025). The major phenolic constituents include β-resorcylic acid, pyrogallol, gallic acid, rutin, protocatechuic acid, and quercetin (Nagella et al., 2014).

Numerous studies have demonstrated that garlic and its bioactive compounds possess anti-inflammatory properties (Ozalp-Unal and Sel, 2024a; Park et al., 2014b). Administration of aged garlic extract has been shown to reduce the levels of tumor necrosis factor-α (TNF-α) and interleukin-1 receptor–associated kinase 4 (IRAK4), while increasing the activity of adenosine monophosphate–activated protein kinase (AMPK) in the liver (Morihara et al., 2017).

Both in vitro and in vivo studies have indicated that garlic suppresses inflammation by inhibiting inflammatory mediators such as nitric oxide (NO), TNF-α, and interleukin-1 (IL-1). Due to its low or negligible toxicity, garlic holds considerable potential for the treatment of inflammatory diseases such as arthritis in humans (Shang et al., 2019).

In fermented garlic, numerous sulfur-containing compounds that contribute to health benefits are formed. Black garlic is rich in antioxidant compounds, including polyphenols, flavonoids (Ozalp-Unal and Sel, 2024), tetrahydro-β-carboline derivatives, and organosulfur compounds such as S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) (Bae et al., 2014).

The organoleptic properties of black garlic are improved as unstable and pungent compounds are converted into stable and odorless compounds such as S-allyl-L-cysteine (SAC), or decomposed into organosulfur compounds including diallyl sulfide

(DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins, and ajoene (Amagase et al., 2001; Corzo-Martínez et al., 2007), resulting in a characteristic sweet-sour taste (Montaño et al., 2004; Corzo-Martínez et al., 2007).

Alliin and its derivatives have been identified as the main precursors of the sulfur-containing compounds present in high concentrations in black garlic (Yu et al., 2014). Through the action of alliinase, alliin and its derivatives are converted into allicin, which is rapidly transformed into various sulfides, including diallyl disulfide, diallyl trisulfide, and diallyl sulfide. These compounds are responsible for the strong pungent odor of fresh garlic (Block et al., 1984).

The presence of allyl methyl trisulfide allows black garlic to retain part of the characteristic flavor of fresh garlic. Heterocyclic compounds, including thiophenes, alkyl pyrazines, furanones, and furans, are mainly produced through Maillard reactions during the aging process of black garlic (Schwab, 2013).

Black garlic contains abundant antioxidant compounds, including polyphenols, alkaloids, flavonoids, S-allylcysteine, and antioxidant intermediates derived from the Maillard reaction (Choi et al., 2014; Kimura et al., 2017).

SAC and SAMC are the principal unique water-soluble organosulfur compounds in black garlic. Diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and diallyl tetrasulfide are lipid-soluble compounds. These organosulfur compounds derived from allicin contribute significantly to the antioxidant activity of black garlic (Amagase et al., 2001; Borek, 2001). Notably, the concentration of S-allylcysteine, one of the most important bioactive organosulfur compounds in garlic, increases by approximately 4.3–6.3-fold in black garlic as a result of the heating and aging process (Bae et al., 2014).

Garlic exhibits a wide range of health benefits, including anti-inflammatory, anti-angiogenic, antidiabetic, anti-arthritic, antihyperglycemic, anticoagulant, antispasmodic, antihistaminic, antibacterial, antiviral, antifungal, and antiparasitic activities. In addition, garlic has been shown to reduce oxidative stress, lower hyperlipidemia and hypertension, stimulate prostaglandin synthesis, enhance immune function, suppress osteoarthritis and osteoporosis, and protect cardiac and hepatic functions. Epidemiological studies indicate that garlic consumption is associated with a reduced risk of cancer, particularly gastrointestinal malignancies. Owing to its significant effects on cancer, garlic has become a major focus of chemopreventive and chemotherapeutic research, with most of these effects attributed to its organosulfur compounds (Ross, 2025).

Chronic diseases such as cardiovascular disorders, cancer, and diabetes pose significant threats to human health and economic development. Garlic is regarded as one of the most effective dietary components for the prevention of chronic diseases and has been used therapeutically for approximately 5,000 years. High garlic consumption has been shown to be associated with a reduced risk of various cancers. Garlic oils exhibit strong antibacterial activity and potent antioxidant properties. Garlic consumption provides notable cardioprotective effects due to its antioxidant and anti-inflammatory properties, as well as its ability to lower cholesterol levels and reduce the risk of cardiovascular disease. When appropriately consumed, garlic is well known to confer potential cardiovascular benefits, including lowering blood pressure, improving elevated serum cholesterol levels, reducing platelet aggregation, and protecting vascular endothelial cells from low-density lipoprotein (LDL)-induced damage (Steiner and Lin, 1998).

In conclusion, despite technological and pharmaceutical advances, cancer remains a global health challenge. Natural products and phytotherapy have long been employed as complementary

approaches in cancer treatment. Moreover, the adverse side effects associated with chemotherapy are well documented, highlighting the importance of identifying novel natural agents capable of mitigating these effects. Natural products also have the potential to enhance the efficacy of conventional anticancer drugs without exerting toxic effects. However, further clinical studies are required to determine appropriate therapeutic doses for cancer prevention and treatment.

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