Review Article

Fighting Cancer with Phytochemicals from *Allium* Vegetables

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ABSTRACT

Medicinal benefits of *Allium* are attributed to sulfur-containing compounds, collectively known as organosulfur compounds (OSCs), derived from garlic and other *Allium* vegetables. Most common OSCs are diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS) and ajoene. Epidemiological studies have suggested cancer risk reduction with increasing intake of *Allium* vegetables and this association has been documented for prostate, gastric, esophageal, breast and colorectal cancers. *In vitro* studies with different cancer cell lines, *in vivo* studies with chemically-induced carcinogenesis and tumor xenograft animal models proved that OSCs have chemopreventive as well as chemotherapeutic activity. DATS and other OSCs mediate their anticancer effects by altering the metabolism of carcinogens, by inhibiting cell cycle progression, inducing oxidative stress leading to DNA damage and consequently inducing apoptosis. Recent studies have found that these OSCs also possess anti-angiogenic activity. This review discusses the cancer chemopreventive and chemotherapeutic effects of DATS and other related OSCs from *Allium* vegetables in relevant models and its beneficial implications for humans.

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Keywords*: Allium*, Organosulfur compounds, cell cycle, apoptosis, chemoprevention, chemotherapeutic, anti-angiogenic.

INTRODUCTION

Allium vegetables have been used for centuries in traditional medicine from Asia. The genus *Allium* comprises of approximately five hundred species (Dorant et al., 1993). Scientific evidence exists to suggest that *Allium*-derived phytochemicals reduce the risk of diabetes by altering blood glucose levels (Mathew and Augusti, 1975), decreases the risk of cardiovascular disorders by reducing the cholesterol levels, triglycerides and low-density lipoproteins and increasing the level of high-density lipoproteins in blood (Rahman, 2001). *S*-Methylcysteine sulfoxide (SMCS), a component of garlic, reduces cholesterol level in the blood and also decreases the severity of atherosclerosis (Sainani et al., 1979), inhibits platelet aggregation (Thomson and Ali, 2003), increases fibrinolysis (Bordia et al., 1977), and reduces cancer risk by inhibiting the activation of carcinogens or increasing its detoxification (Milner, 2001). S-allyl mercaptocysteine (SAMC) is also reported to stimulate immune system by induction of T cell proliferation and activation of macrophages (Lau et al., 1991). Further studies suggest that SMCS can protect from radiation (Singh et al., 1995) and infections (Avato et al., 2000), and also possesses suppressive effect on the aging process (Nishiyama et al., 1997).

Population-based case-control and interventional studies have suggested a protective role for garlic and other *Allium* vegetables against cancer. Anticancer effects of organosulfur compounds have been documented in oral (Guercio et al., 2014), pharynx (Guercio et al., 2014), larynx (Guercio et al., 2014), breast (Hahm and Singh, 2014), esophageal (Wargovich et al., 1988), ovarian (Guercio et al., 2014), gastric (Guercio et al., 2014), colorectal (Galeone et al., 2006), prostate (Hsing et al., 2002) and renal cancers (Guercio et al., 2014).

We have discussed about beneficial effects of DATS and other related OSCs of *Allium* and their modes of action against cancer in this article. Epidemiological studies revealed the health benefits of *Allium* vegetables. Several population-based case control studies showed the inverse correlation between intake of *Allium* vegetables and cancer incidence. *In vitro* and *in vivo* studies have revealed that these OSCs inhibit cell proliferation and induce apoptosis in cancer cells by induction of cell cycle arrest, ROS production, histone acetylation, DNA damage and by modulating the mechanism of carcinogenesis. The anticancer effects of some important OSCs are discussed here in detail.

ORGANOSULFUR COMPOUNDS

Plants belonging to *Allium* family have sulfur containing organic compounds known as organosulfur compounds which are released from the vegetables upon their processing. The sulfur compounds are responsible for most of the health promoting effects of the *Allium* vegetables (Block, 1985). The primary sulfur compound in the vegetables of genus *Allium* is γ -glutamyl-*S*-alk(en)yl-L-cysteine which is hydrolyzed to *S*-alkyl(en)yl-L-cysteine sulfoxide that is also known as alliin (Block, 1985) (*Figure 1*). Alliinase is an enzyme which is released from vacuoles of *Allium* vegetables after chewing or cutting. Alliinase hydrolyzes alliin to allicin and other alkyl alkane thiosulfinates (Block, 1985). Allicin and alkyl alkane thiosulfinates are highly unstable compounds which further get decomposed to yield oil soluble compounds such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins and ajoene (Block, 1985). The γ-glutamyl-*S*-alk(en)yl-L-cysteine also yields water soluble OSCs like *S*-allyl cysteine (SAC) and *S*-allyl mercaptocysteine (SAMC) (Kodera et al., 2002). Depending upon the conditions like water content and temperature, up to fifty OSCs can be generated from *S*-allyl cysteine sulfoxides like SMCS and *S*-propyl cysteine sulfoxide (Kubec et al., 1999).

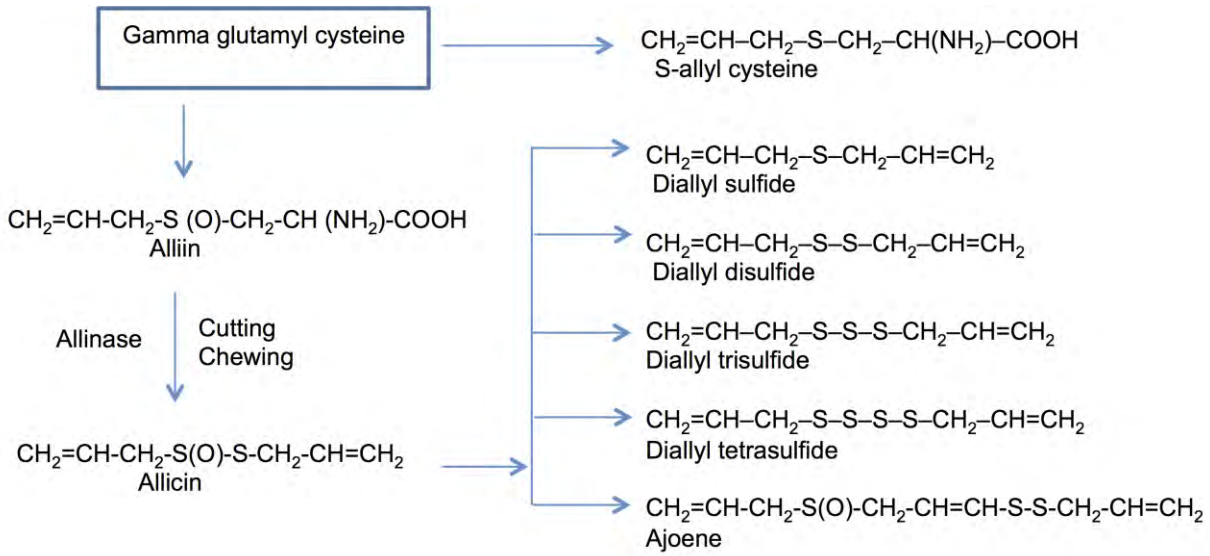

Figure 1. Metabolism of organosulfur compounds. Processing of *Allium* vegetables results in the formation of OSCs like DAS, DADS, DATS, Ajoene etc (Powolny and Singh, 2008).

DIVERSITY AND ABUNDANCE OF ORGANOSULFUR COMPOUNDS IN *ALLIUM* SPECIES

Many organosulfur compounds have been isolated from different *Allium* species listed in *Table 1*. DATS is the most predominant OSC extracted from garlic by steam distillation. Proportions of organosulfur compounds found in garlic and isolated by steam distillation with solvent extraction are reported as DATS (900-1100 µg/g), DADS (530-610 µg/g), AMT (250-270 µg/g), AMDS (100 µg/g), DAS (30-100 µg/g), DMTS (15-19 µg/g), AMS (3.8-4.6 µg/g), DMDS (2.4 – 2.5 µg/g) and PMS (0.7-0.8 µg/g) (Shukla and Kalra, 2007).

OSCs ANALOGS

Block et al was the first to synthesize the ajoene from allicin (Block et al, 1986). Later, different derivatives of ajoene were synthesized by R1 substitution, which are found to be more stable than the ajoene (Hunter et al., 2008). Further, Kaschula et al synthesized twelve ajoene derivatives out of which one of the derivatives with p-methoxybenzyl (PMB) end group showed better anticancer effects in cell viability assay of WHCO1 esophageal cancer cells than the E- and Z- ajoene of *Allium* vegetables in addition to that these analogs are found to have enhanced selectivity towards cancer cells. PMB end group ajoene derivative was found to be twelve times more potent than Z-ajoene with an IC-50 of 2.1 µM (Kaschula et al., 2011). DATS derivatives induced cell cycle arrest and apoptosis in PC-3 cells. Bis(2-methylallyl) trisulfide (2-M-DATS), dibutenyl trisulfide (DBTS) and dipentenyl trisulfide (DPTS) derivatives of DATS are found to be more cytotoxic to PC-3 cells than DATS (Chen et al., 2012). Recent study on macrophages using DATS analogs showed that 1,3-bis(2-methylallyl) trisulfane (DATS-2) induced the ROS which was stronger than its precursor DATS (HE, 2015).

Earlier studies showed that DATS mediates its anticancer effects on PC-3 cancer cells through macrophages by induction of cytotoxicity, phagocytosis, nitric oxide and TNF-α. DATS-2 and DATS-1 (1,3-di(but-3-enyl) trisulfane) are found to be more effective than DATS, DATS-3 (1,3-bis(3-methylbut-enyl) trisulfane and DATS-4 (1,3-di(pent-4-enyl) trisulfane) (Xiaoyan HE, 2013).These studies showed that it is possible to synthesize OSC analogs and screening of these analogs also revealed few of these synthesized derivatives have enhanced anticancer effects. Further studies are required in both *in vitro* and *in vivo* to investigate the parameters including their toxicity on normal cells, bioavailability, pharmacokinetics and stability.

EPIDEMIOLOGICAL EVIDENCE FOR ANTICANCER ACTIVITY OF *ALLIUM* VEGETABLES

Anticancer properties of *Allium* species are well known from the ancient times. Egyptians (Block, 1985), Hippocrates and Indian physicians (Moyers, 1996) used garlic externally for the treatment of tumors. Several epidemiological studies have shown an inverse correlation between the intake of *Allium* vegetables and risk of cancers and other chronic diseases. Consumption of *Allium* vegetables reduces risk of lung, esophageal (Gao et al., 1999), stomach (Dorant et al., 1996), pancreatic (Chan et al., 2005), prostate (Hsing et al., 2002), endometrial (Galeone et al., 2009), colorectal and breast cancers (Glade, 1999). For example one of the earliest epidemiological studies in China involving subjects from Cangshan and Qixia counties suggested stomach cancer risk reduction with garlic consumption (Mei et al., 1982). There was three-fold difference in death rate due to stomach cancer. This was attributed to the difference in garlic intake, Cangshan county group consumed about 20g garlic per day, whereas garlic consumption was rare in Qixia county group which comparatively showed three-fold higher death rate. From this study, it was suggested that garlic lowers the concentration of nitrites in the stomach, which are the precursors of potent carcinogenic nitrosamines, by inhibiting bacterial nitrate reduction (Mei et al., 1982). Another epidemiological study in China with 564 gastric cancer patients and 1131 controls whose age and sex were same and around 96% of them were born in Linqu county having high incidence rates for gastric cancer, suggested that consumption of *Allium* vegetables reduced the occurrence of gastric cancer (You et al., 1989) and *Helicobacter pylori* infection (You et al., 1998).

Table 1. Organosulfur compounds of *Allium* species

Oil soluble organosulfur compounds:

Chemical Structure	Compounds	Abbreviation
CH_2=CH–CH_2–S–CH_2–CH=CH_2	Diallyl sulfide	DAS
CH_2=CH–CH_2–S–S–CH_2–CH=CH_2	Diallyl disulfide	DADS
CH_2=CH–CH_2–S–S–S–CH_2–CH=CH_2	Diallyl trisulfide	DATS
CH_2=CH–CH_2–S–S–S–S–CH_2–CH=CH_2	Diallyl tetrasulfide	DTS
CH_2=CH-CH_2-S (O)-CH_2-CH (NH_2)-COOH	S-allylcysteine sulfoxide	Alliin
CH_2=CH-CH_2-S(O)-S-CH_2-CH=CH_2	Diallyl thiosulfinate	Allicin
CH_2=CH-CH_2-S(O)-CH_2-CH=CH-S-S-CH_2-CH=CH_2	Ally 3-allylsulfinyl-1 -propenyl disulfide	Ajoene
CH_3-S(O)-CH_2-CH(NH_2)-COOH	S-Methylcysteine sulfoxide	-
CH_3-CH_2-CH_2-S(O)-CH_2-CH(NH_2)-COOH	S-Propylcysteine sulfoxide	-
CH_3-CH=CH-S(O)-CH_2-CH(NH_2)-COOH	S-Propenylcysteine sulfoxide	-

CH_3-CH_2-CH=SO	Propanethial S-oxide	-
CH_2=CH–CH_2–S–CH_3	Allyl methyl sulfide	AMS
CH_2=CH-CH_2-S-S-CH_3	Allyl methyl disulfide	AMDS
CH_2=CH–CH_2–S–S–S–CH_3	Allyl methyl trisulfide	AMT
CH_3–S–S–S–CH_3	Dimethyl trisulfide	DMTS
CH_3–CH_2–CH_2–S–CH_3	Methyl propyl sulfide	MPS
CH_3-CH_2—CH_2-S-CH_3	Propylmethyl sulfide	PMS
CH_3-CH_2-CH_2-S-S-CH_3	Propylmethyl disulfide	PMDS
CH_3-CH_2-CH_2-S-S-S-CH_3	Propylmethyl trisulfide	PMTS
CH_3–CH_2–CH_2–S–CH_2–CH_2–CH_3	Dipropyl sulfide	DPS
CH_3–CH_2–CH_2–S–S–CH_2–CH_2–CH_3	Dipropyl disulfide	DPDS
CH_3–CH_2–CH_2–S–S–S–CH_2–CH_2–CH_3	Dipropyl trisulfide	DPTS
CH_3–CH_2–CH_2–S–S–S–S–CH_2–CH_2–CH_3	Dipropyl tetrasulfide	-

Water soluble organosulfur compounds:

Chemical Structure	**Compound Name**	**Abbreviation**
CH_2=CH–CH_2–S–CH_2–CH(NH_2)–COOH	S-allyl cysteine	SAC
CH_2=CH–CH_2–S–S–CH_2–CH(NH_2)–COOH	S-allyl mercaptocysteine	SAMC
CH_2=CH-CH_2-SH	Allyl mercaptan	AM

Further studies in Shanghai, China showed the inverse correlation between the risk of prostate cancer and consumption of *Allium* vegetables (Hsing et al., 2002). Meta-analysis of the epidemiological data on role of garlic intake and risk of head and neck, breast, lung, gastric, colon and prostate cancers concluded that consumption of raw and cooked garlic was associated with protective effect against gastric and colorectal cancer (Fleischauer et al., 2000). Another study in Switzerland by comparing 223 patients (119 colon and 104 rectal cancers) to 491 healthy controls found that garlic consumption was protective against colorectal cancer (Levi et al., 1999). This is further supported by studies showing garlic intake reduces the risk of developing the colorectal cancer (Le Marchand et al., 1997; Steinmetz et al., 1994).

Population-based diet study in England which included 18 food items including garlic found that higher intake of garlic was protective against prostate cancer (Key et al., 1997). Population-based case control studies in France and Switzerland showed that increased intake of *Allium* vegetables reduced the risk of breast cancer (Challier et al., 1998; Levi et al., 1993). Karinat, a supplement which consists of 150 mg of garlic powder was given to 33 subjects having benign breast cancer, and other 33 subjects were given placebo. Karinat reduced the benign breast cancer and showed beneficial effect in up to76% of subjects (Bespalov et al., 2004). Case control studies in India showed that onion consumption decreased the risk of lung cancer (Sankaranarayanan et al., 1994). Similar case control studies in China supported the notion that intake of onion was protective against brain cancer (Hu et al., 1999).

MOLECULAR MECHANISMS OF CANCER CHEMOPREVENTION BY DATS AND OTHER OSCs

Induction of apoptosis in cancer cells

Apoptosis is a highly regulated process which is usually regulated by two different pathways (Green and Beere, 2000). One is intrinsic pathway mediated by mitochondria and other one is extrinsic pathway executed by death receptors and death ligands. Both of these pathways result in activation of caspases; albeit different isoforms (Ferri and Kroemer, 2001). Caspases play crucial roles in programmed cell death (Budihardjo et al., 1999). In intrinsic pathway, translocation of proapoptotic molecules like Bax, Bim and Bid to mitochondrial membrane and release of cytochrome c, second mitochondrial activator of caspases (SMAC) from mitochondria and formation of apoptosome complex by cytochrome c, oligomerization of apoptotic protease activating factor -1 (Apaf-1) in presence of dATP activates initiator caspase 9 which further activates effector or executioner caspases 3/7 and finally leads to disruption of nuclear membrane and DNA fragmentation (Kroemer and Reed, 2000); (Vucic et al., 2011). In extrinsic pathway, death receptors get activated by death ligands, which leads to the activation of caspases by the recruited adaptor proteins (Enari et al., 1998). Studies have shown that DATS induces apoptosis in cancer cells, which is usually deregulated *(Figure 2)*.

Apoptosis induction is one of the mechanisms by which DATS and other OSCs controls the cancer growth. Earlier studies have shown that DATS induces apoptosis in human lung cancer A549 cells (Sakamoto et al., 1997). Milner and colleagues also showed DNA fragmentation and other characteristic features of apoptosis induction in human colon cancer cells that were treated with another organosulfur compound DADS. Bcl-2 family proteins play critical role in DATS-mediated apoptosis. DATS induced the phosphorylation of Bcl-2 protein, *via* JNK activation and to certain extent by ERK1/2 activation in prostate cancer cells. Overexpression of Bcl-2 conferred resistance to DATS-induced apoptosis in PC-3 cancer cells (Xiao et al., 2004).

DADS-induced apoptosis in the breast cancer cell lines was associated with overexpression of Bax and decreased levels of Bcl-xL (Nakagawa et al., 2001). Treatment with DAS and DADS increased the ratio of Bax/Bcl-2 in lung cancer (Hong et al., 2000) and neuroblastoma cell lines (Karmakar et al., 2007). DADS-treated MDA-MB-231 breast cancer cells exhibited up-regulation of Bax and a decrease in Bcl-xL protein level. The intracellular ratio of Bcl-2/Bax was decreased when non-small cell lung cancer cell lines were treated with DAS and DADS (Hong et al., 2000). Exposure of HL-60 cells to ajoene resulted in caspase mediated cleavage of Bcl-2 protein leading to apoptotic cell death (Li et al., 2002).

In a comparative study using prostate cancer DU145 and PC-3 cells, DATS appeared to be a much more potent inducer of apoptosis than DAS and DADS (Xiao et al., 2004). The role of p53 in apoptosis induction by OSCs has also been studied as this tumor suppressor can repress the expression of Bcl-2 and increase the expression of Bax. DAS and garlic extract were found to be ineffective, but DADS increased the levels of p53 protein in H460 cells (Hong et al., 2000). Using lung cancer cells with wild-type and null/mutant p53, all these preparations influenced the levels of Bax and Bcl-2 in favor of apoptosis suggesting that OSCs have the ability to induce apoptosis through p53-dependent and p53-independent signaling pathways (Hong et al., 2000). Bcl-2 is an anti-apoptotic protein whose activity is regulated by the post-translational modification like proteolytic cleavage and phosphorylation (Chadebech et al., 1999). Allitridi (a synthetic DATS) induced apoptosis in BGC823 human gastric cancer cells by increasing the expression of caspase-3 and decreasing the expression of Bcl-2 (Lan and Lu, 2004). DATS treatment led to apoptosis induction in prostate cancer cells was accompanied by a decrease in phosphorylation of Akt and Bad, which further promoted the mitochondrial translocation of Bad and also activated caspases (Xiao and Singh, 2006). DATS covalently modified the cysteine residues of β-tubulin (Cys-12β and Cys-354β) to form S-allylmercaptocysteine (Hosono et al., 2005). However, this modification was only shown *in vitro*. DATS altered the expression levels of 41 proteins in BGC823 human gastric cell line, out of which 19 proteins were associated with apoptosis (Li et al., 2006). Another proteomic study showed that DATS changed the expression of 27 proteins in Saos-2 human osteosarcoma cells, out of which 13 proteins were related to apoptosis and cell cycle regulation (Zhang et al., 2009). In a comparative study of OSCs, cell death inducing effect of DATS was found to be relatively higher in Caco-2 and HT-29 human colon cancer cells as compared to DAS, DADS and allicin (Jakubikova and Sedlak, 2006).

Hyperphosphorylation of Bcl-2 by JNK1/2 and ERK1/2 was shown in DATS-treated prostate cancer cells (Xiao et al., 2004). Significant protection from the DATS-induced apoptosis was seen when Bax and Bak proteins were depleted in the prostate cancer cells (Kim et al., 2007). DATS increased phosphorylation of

check point kinase 1 (Chk1) independent of p53 in LNCaP and HCT-116 cells (Xiao et al., 2009). DATS down-regulated Akt activation and expression of Bcl-2, and increased the expression of Bax in T24 human bladder cancer cells (Wang et al., 2010b). DATS induced hypophosphorylation of Akt *via* increased phosphorylation of p66shc adaptor protein was reported in PC-3 prostate cancer cells (Borkowska et al., 2012). OSCs induced ROS production which was coupled with inhibition of cell proliferation by arresting cells in G2/M phase and apoptosis induction in A375 and BCC cells (Wang et al., 2010a). Mechanism of DATS-induced apoptosis in prostate cancer cells are summarized in Figure 2.

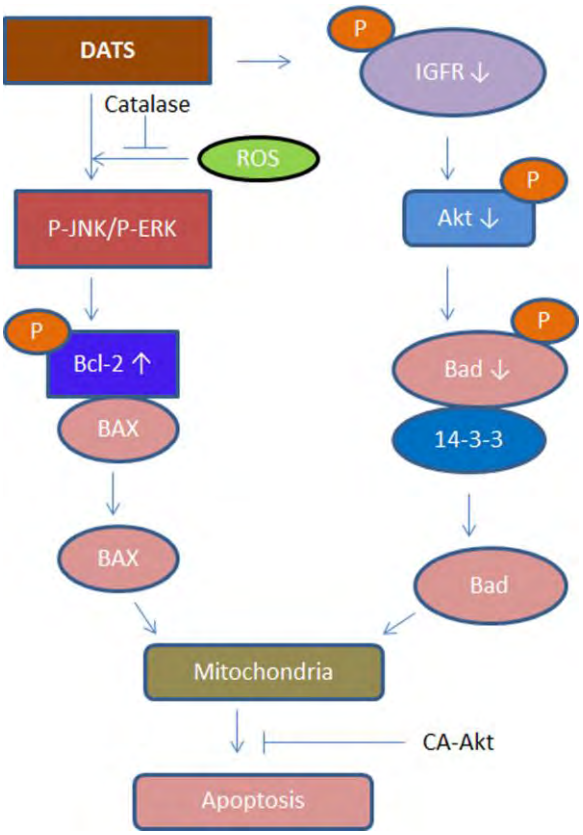

Figure 2. Molecular mechanisms of DATS-induced apoptosis in human cancer cells. DATS-induced apoptosis was accompanied by the activation of JNK (to certain extent ERK1/2) dependent phosphorylation of Bcl-2 leading to its reduced interaction with Bax and increase in mitochondria-mediated activation of caspase pathway and apoptosis. DATS also leads to the inactivation of Akt which results in the reduced phosphorylation and translocation of Bad into mitochondria. Effects of DATS were attenuated by ectopic expression of constitutively active Akt (CA-Akt) or by knock-down of Bak and Bax expression.

The role of ROS in anticancer effects of OSCs

ROS plays crucial role in cell survival. Z-ajoene treatment decreased Bcl-2 protein level and survival in HL-60 cells (Li et al., 2002). Generally Bcl-2 acts upstream of the caspases but, it also has a role in the downstream as a substrate of caspase-3 enzyme creating a feedback loop, thus helps in the amplification of apoptotic signal by activation of mitochondrial pathway. Involvement of ROS was suggested, as the Bcl-2 cleavage was inhibited by the antioxidant *N*-acetyl cysteine (NAC) which also inhibited the nuclear translocation of NF-kB (Li et al., 2002). Similarly, DADS treatment led to apoptosis induction in HL-60 cells, which was associated with generation of hydrogen peroxide (Kwon et al., 2002). DADS rapidly increased the production of hydrogen peroxide and triggered the mitochondrial intrinsic pathway in SH-SY5Y neuroblastoma cells (Filomeni et al., 2003).

DATS treatment activated the c-jun N-terminal kinase pathway concomitant with ROS production in MCF-7 cells as well as its xenograft in Balb/c mice (Na et al., 2012). Ajoene treatment induced ROS production in human leukemic cells (Dirsch et al., 1998). DATS and DADS treatment induced apoptosis in human lung adenocarcinoma cells through the activation of c-jun N-terminal kinase and induction of ROS (Wu et al., 2009). The JNK activation was attenuated by overexpression of zinc or copper superoxide dismutase or by treatment with 5,5'dimethyl-1-pyrroline N-oxide (Filomeni et al., 2003). Generation of ROS and activation of p38 MAPK and JNK1 have been shown in human glioblastoma cells after treatment with OSCs (Das et al., 2007). In prostate cancer cells, DATS-induced apoptosis was shown to be mediated by JNK activation and hyperphosphorylation of Bcl-2 protein (Xiao et al., 2004). It was also shown that overexpression of catalase conferred significant protection against DATS-induced JNK1/2 activation, but not ERK1/2, suggesting that ROS/hydrogen peroxide act as secondary messenger in transmitting the DATS induced apoptotic signals in human prostate cancer cells (Xiao et al., 2004). DATS induced ROS production was attenuated in presence of superoxide dismutase enzyme (Chandra-Kuntal et al., 2013). DATS is also shown to inhibit STAT3 activation and x-linked inhibitor of apoptosis protein levels in prostate cancer (Chandra-Kuntal and Singh, 2010; Kim et al., 2011). Recently, Wallace et al showed that DATS increased the acetylation of histone proteins, decreased the HDAC activity and many pro-tumor markers, and upregulated the proapoptotic proteins in U87MG xenograft (Wallace et al., 2013). These studies show that OSCs trigger apoptosis through induction of ROS.

DATS-induced apoptosis involves calcium

DATS and other OSCs influence calcium homeostasis. Higher levels of calcium leads to the activation of the endonucleases which are calcium-dependent mediators of apoptosis (Rizzuto and Pozzan, 2003). DADS increased calcium level in HCT15 cells and this effect was correlated with cell cycle arrest and apoptosis. DADS and DATS have shown similar effects in lung cancer cells (Sakamoto et al., 1997). Increased calcium levels upon treatment with OSCs is due to a decrease in the activity of membrane bound calcium dependent ATPase which helps in the active pumping of calcium (Sundaram and Milner, 1996a). Increase in intracellular calcium levels have been observed in human glioblastoma cells after the treatment with OSCs (Das et al., 2007). DADS has also induced intracellular calcium level in N18 retina ganglion cells (Lin et al., 2006).

Inhibition of angiogenesis and metastasis

Tumor growth beyond 1-2 mm diameter requires neovascularization (Folkman, 2003). Studies have shown that garlic extracts inhibit blood vessel formation in tumors. Garlic extract inhibited proliferation, capillary tube formation and invasiveness of endothelial cells, and increased their adhesion to the collagen and fibronectin (Matsuura et al., 2006). Alliin inhibited vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF-2) induced tube formation by endothelial cells involving an increase in cellular nitric oxide level and p53 protein expression (Mousa and Mousa, 2005). DATS reduced the capillary tube formation in human umbilical vein endothelial cells (HUVEC), which was accompanied by suppression of VEGF secretion and down-regulation of VEGFR2 (Xiao et al., 2006b). DADS and DAS also inhibited endothelial cell growth, migration and reduced matrix metalloproteinases, MMP-2 and MMP-9 (Meyer et al., 2004). DATS treatment down-regulated the expression and also suppressed the activity of MMP-2 and MMP-9 by inhibition of ERK/MAPK signaling pathway leading to inhibition of invasion and migration of triple negative breast cancer (TNBC) cell lines (Liu et al., 2015).Treatment of B16F-10 melanoma tumor in C57BL/6 mice with DAS decreased the levels of proangiogenic factors including VEGF, interleukin-1β (IL-1β), IL-6, GM-CSF, and TNF-alpha and increased the levels of anti-angiogenic factors and tissue inhibitor of matrix metalloproteinase (TIMP-1) (Thejass and Kuttan, 2007). Treatment of garlic extract attenuated the metastatic spread of rat sarcoma cells (Hu et al., 2002). Intraperitoneal administration of ajoene to B16/BL6 melanoma cells injected in C57BL/6 mice showed inhibition of pulmonary metastasis (Taylor et al., 2006). SAMC injection to CB-17 SCID/SCID bearing PC-3 human prostate cancer cells showed inhibition of adrenal gland and lung metastasis (Howard et al., 2007). In another study, DATS-mediated inhibition of angiogenesis was associated with suppression of Notch-1 expression and induction of tumor suppressive microRNAs (Li et al., 2013). Collectively these studies show that OSCs inhibit angiogenesis, invasion and migration by down-regulation of proangiogenic factors like VEGF, FGF-2, IL-1β and MMPs.

Inhibition of carcinogen-induced DNA adduct formation

The role of chemical carcinogen-induced DNA damage in cancer development is well understood. For example, polycyclic aromatic hydrocarbons induce formation of DNA adducts by alkylation, arylation, etc. (Hemminki, 1993). The OSCs have the ability to inhibit formation of DNA adducts induced by chemical carcinogens. Milner and colleagues studied effect of garlic from various sources in different forms (raw dietary garlic powder, commercial high sulfur garlic, water and ethanol extracts of garlic and SAC) on DNA adduct formation in presence of carcinogen 7,12,dimethylbenz(a)anthracene in mammary cells (Amagase and Milner, 1993). Garlic powder (1-4%, w/w) also reduced DMBA-DNA adduct formation which in turn delayed the mammary tumor formation and tumor incidence in rats (Liu et al., 1992). In another study, it also decreased the formation of 7-N-methyldeoxyguanosine and 6-O-methylguanosine in mammary epithelium and presence of N-nitroso compounds in rats (Lin et al., 1994). Garlic powder, DADS and SAC dietary supplementation inhibited N-methyl-N-nitrosourea induced mammary tumorigenesis in rats (Amagase et al., 1996). Treatment of human peripheral blood lymphocytes with different concentrations of SAC and water extract of raw garlic decreased the benzo(a)pyrene-DNA adduct formation (Hageman et al., 1997). Studies proved that OSCs like SAC and DADS and garlic powder significantly inhibited the formation of DNA adducts with DMBA in mammary epithelial cells of female rats and also enhanced the effect of selenite in the inhibition of DNA adducts formation (Amagase et al., 1996).

Heating of garlic reduced its ability to inhibit DMBA-DNA adduct formation, which was attributable to inactivation of alliinase required for biochemical genesis of OSCs (Song and Milner, 1999). Treatment of human leukemia cells with DAS and DADS was associated with inhibition of N-acetyl transferase

activity and levels of 2-aminofluorene-DNA adduct (Lin et al., 2002). DAS interfered with metabolism of diethylstilbesterol (DES) leading to inhibition of DES-DNA adducts in liver of male Sprague-Dawley rats. DAS prevented the formation of DES-induced DNA adducts in a dose dependent manner (Green et al., 2003). Further, DAS inhibited DES-induced breast cancer by reducing the level of its DNA adducts in mitochondria and nucleus (Green et al., 2005). Boiled garlic powder inhibited the formation of O-6-methylguanine-DNA adducts in colorectum in presence of 1,2-dimethyl hydrazine in F344 rats (Chihara et al., 2010). From these findings it was revealed that OSCs prevent DNA damage by altering the metabolism of carcinogens.

Histone modification

OSCs are shown to inhibit cell proliferation by modifying the acetylation of histone proteins thus affecting the expression of growth responsive genes (Myzak and Dashwood, 2006). DADS increased acetylation of histone H3 and H4 in DS19 mouse erythroleukemia and K562 human leukemia cells (Lea et al., 1999). DADS and its metabolite Allyl mercaptan inhibited the activity of HDAC in human breast cancer and rat hepatoma cells (Lea et al., 1999). DADS treatment inhibited proliferation of HT-29 and Caco-2 human colon cancer cells by inducing G2/M phase cell cycle arrest in association with increased acetylation of histone H3 and H4 (Druesne et al., 2004). Treatment with OSCs (allicin, SAMC and SAC) to DS19 mouse erythroleukemia cells and SAMC to Caco-2 human colon cancer cells and T47D human breast cancer cells induced growth inhibition which was correlated with increased acetylation of histone proteins (Lea et al., 2002). Allyl mercaptan strongly inhibited the HDAC activity as compared to other OSCs like SAMC, SAC, and DADS (Nian et al., 2008). Allyl mercaptan inhibition of HDAC resulted in increased binding of SP3 transcription factor on promoter region of *p21/WAF1* gene, which enhanced its expression (Nian et al., 2009). These findings suggest that histone acetylation is one of the mechanisms through which these OSCs could exert their anticancer effects.

Inhibition of cell cycle progression

Cell cycle progression consists of growth stimulus, DNA replication, segregation of the chromosomes, and division of eukaryotic cell. Cell cycle is a highly regulated process that involves many proteins including cyclin-dependent kinases (Cdk) and cyclins (Murray, 2004). Cell cycle check points are meant to regulate critical steps in cell cycle progression including completion of phase specific events, maintenance of genome stability and initiation of apoptosis program in extreme damaged states (Murray, 2004). During cellular stress, cell cycle checkpoints could be activated at G1/S phase to prevent the replication of the damaged DNA or at the G2/M phase to prevent segregation of damaged chromosomes to maintain genetic integrity (Hartwell and Weinert, 1989). Entry into M phase requires activation of Cdk1/cyclin B kinase complex by Cdk1 dephosphorylation at Thr14/Tyr15 by Cdc25 family of phosphatases. This dephosphorylated Cdk1 then binds to cyclin B1 to form an active Cdk1/cyclin B1 complex, which enters the nucleus to promote cell cycle progression (Toyoshima et al., 1998).

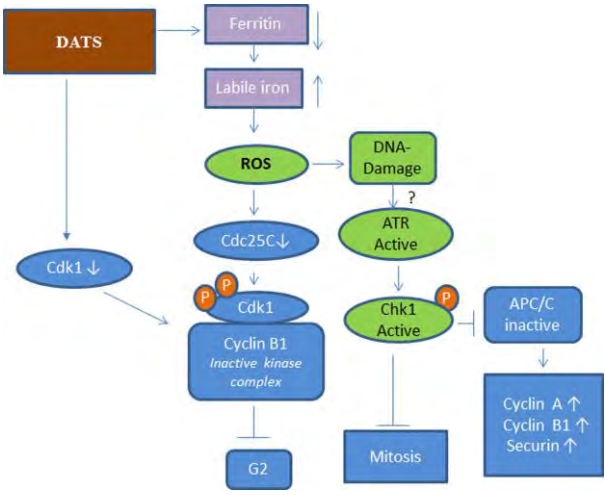

Figure 3. Molecular mechanisms of DATS-induced cell cycle arrest in human prostate cancer cells. DATS treatment to human prostate cancer cells induced the degradation of ferritin which increases the level of chelatable iron thus leading to the generation of ROS which results in the down-regulation of Cdc25C. Effect of DATS can be reversed in presence of antioxidants like NAC, superoxide dismutase and EUK134. DATS also down-regulates Cdk1 protein along with the accumulation of inactive Tyr15-phosphorylated Cdk1/cyclin B1 kinase complex. DATS-induced mitotic arrest was characterized by the accumulation of cyclin B1 and securin due to the inactivation of anaphase promoting complex/cyclosome by checkpoint kinase.

Many studies have demonstrated that the garlic derived OSCs can induce G2/M phase cell cycle arrest *(Figure 3)*. DATS treatment resulted in growth inhibition of A549 human lung cancer cells (Sakamoto et al., 1997). DADS caused dose- and time-dependent accumulation of HCT-15 human colon cancer cells in G2/M phase at the expense of G1 and S phases (Knowles and Milner, 1998). This G2/M arrest was accompanied by reduced kinase activity of the Cdk1/Cyclin B1 complex. DADS treatment increased intracellular calcium levels which resulted in dissociation of the Cdk1/cyclin B1 complex (Sundaram and Milner, 1996a). DADS treatment induced the inactivating phosphorylation of Cdk1 kinase in HL-60 human promyelocytic leukemic cells (Yuan et al., 2004). DADS-induced G2/M cell cycle arrest in PC-3 human prostate cancer cells was associated with a reduction in levels of Cdk1 (Arunkumar et al., 2006). Similar results were seen in HT-29 human colon cancer cells (Robert et al., 2001) and MGC80 human gastric cancer cells (Yuan et al., 2004).

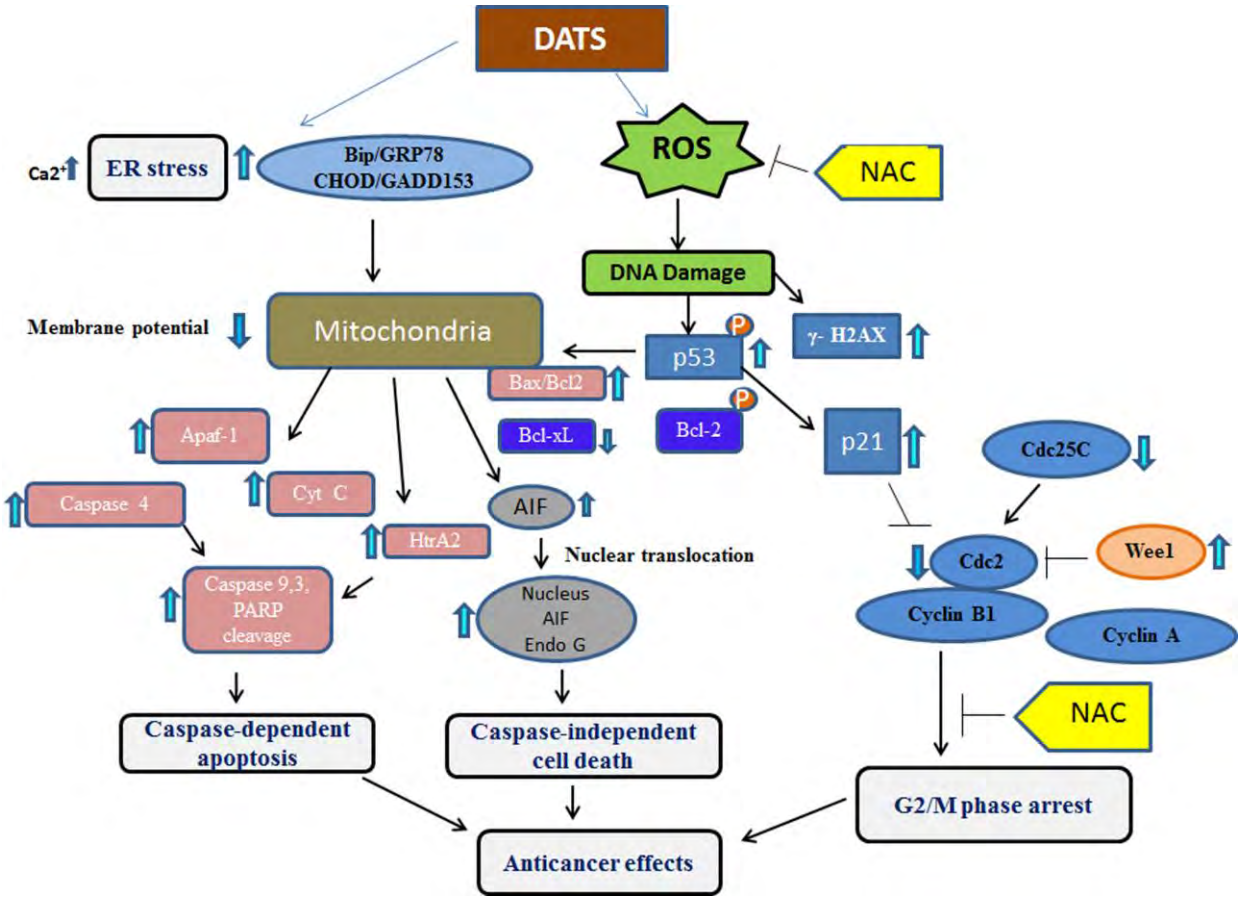

Figure 4. Mechanisms of DATS-induced cell cycle arrest and apoptosis in skin cancer cells. DATS induced G2/M cell cycle arrest and apoptosis in skin cancer cells by altering the levels of G2/M regulatory proteins of cell cycle and apoptotic proteins. DATS induced ROS production which in turn induces DNA damage resulting in phosphorylation of H2AX known as γ-H2AX predominantly by ATM. It further results in the activation of p53, p21 and regulatory proteins of G2/M phase Cdc2, cyclin B1, Cdc25C. DATS treatment induces the endoplasmic reticulum stress related molecules like CHOP/GADD153 and Bip/GRP78 which activates caspase-mediated apoptosis. DATS increased levels of calcium, down-regulated the expression levels of Bcl-xL, increased the hyperphosphorylation of Bcl2, ratio of Bax/Bcl2 ratio inducing apoptosis. DATS decreased the mitochondrial membrane potential and triggered the release of mitochondrial molecules like AIF (apoptotic inducing factor), Cyt C (Cytochrome C), Endo G (endonuclease) and HtrA2 mitochondrial serine protease. Release of Cyt C further activates the apoptotic molecules like Apaf1 (apoptotic protease activating factor 1), caspases -9, caspase-3 and cleavage of PARP (poly (ADP-ribose) polymerase) inducing caspase dependent apoptosis. DATS also induces caspase independent apoptosis by AIF (apoptotic inducing factor) and Endonuclease G. NAC (N-acetyl cysteine) blocks DATS-induced ROS production, cell cycle arrest and apoptosis in skin cancer cells.

DATS-induced G2/M arrest in PC-3 and DU145 cancer cells was correlated with increased phosphorylation of Cdk1(Tyr15), reduced Cdk1/cyclin B1 kinase activity, and increased inhibitory phosphorylation of Cdc25C(Ser216) and its reduced level (Herman-Antosiewicz and Singh, 2005). These effects were not seen in PrEC normal prostate epithelial cells suggesting its selectivity for cancer cells. DATS-induced cell cycle arrest in DU145 cells was accompanied by altered nuclear localization of Cdk1 and cyclin B1 (Xiao et al., 2009).

A role for ROS was also implicated in DATS-induced cell cycle effects (Herman-Antosiewicz and Singh, 2005). DATS-induced ROS generation was due to JNK-mediated degradation of ferritin which is an iron storage protein, thus increasing the level of free labile iron (Antosiewicz et al., 2006). DATS treatment caused activation of DNA damage checkpoint kinases Chk1 and Chk2 in PC-3 and DU145 cells (Herman-Antosiewicz and Singh, 2005). Activation of Chk1 was accompanied by accumulation of anaphase promoting complex/cyclosome (APC/C) and increased phosphorylation of securin, Cdc20 and Cdh1 (Antosiewicz et al., 2006). Furthermore, DATS treatment resulted in prometaphase arrest in PC-3 cells *via* ATR/Chk1 signaling (Herman-Antosiewicz et al., 2007b). It has been reported that OSCs affect the microtubule network. For example, SAMC treatment induced depolymerization of microtubular network and disruption of cytoskeleton network in interphase cells of SW480 human colon cancer cells and NIH3T3 mouse fibroblasts (Xiao et al., 2003).

DATS treatment induced mitotic arrest in human colon cancer cells and also inhibited the formation of spindle and disrupted microtubular network but this was not seen with DAS and DADS treatments (Hosono et al., 2005; Hosono et al., 2008). It was shown that ajoene-induced G2/M arrest was accompanied by disruption of cytoskeleton network in HL60 human promyelocytic leukemia cells (Kwon et al., 2002). However, G2/M phase cell cycle arrest by OSCs is not a universal phenomenon. For example, DADS-induced S phase arrest in CNE2 human nasopharyngeal carcinoma cells (Zhang et al., 2006). Cell cycle arrest in both G0/G1 and G2/M phase was reported in allicin-exposed human mammary cancer cells (Hirsch et al., 2000). Structure-activity relationship studies have revealed a critical role for the oligosulfide chain length in G2/M arrest by OSCs with allyl groups. DATS was more effective in inducing G2/M phase cell cycle arrest than DADS or DAS in J5 human liver tumor cells (Wu et al., 2004). Allitridi, a synthetic DATS, induced G1 phase arrest in BGC823 human gastric cancer cells (Lan and Lu, 2003). These findings showed that garlic derived DATS and other OSCs have the potential to inhibit cell proliferation of cancer cells by inducing cell cycle arrest. Mechanisms of DATS-induced apoptosis and cell cycle arrest in prostate and skin cancer cells have been summarized in *Figure 2-4* and *Table 2*.

Table 2. Chemopreventive and chemotherapeutic effects of organosulfur compounds (OSCs)

OSCs	Models	Effects	References
DAS	Mice	Chemopreventive	(Hong et al., 1992; Wargovich, 1987)
		Chemotherapeutic	(Hu et al., 2012)
	Rats	Chemopreventive	(Wargovich et al., 1992)
	Human cancer cells	Chemotherapeutic	(Chiu et al., 2013)
DADS	Mice	Chemotherapeutic	(Liao et al., 2007; Zhao et al., 2006)
	Rats	Chemopreventive	(Arunkumar et al., 2006)
	Human cancer cells	Antimetastatic	(Park et al., 2011)
DATS	Mice	Chemotherapeutic	(Li et al., 2012) (Wu et al., 2011)
		Anti-metastatic	(Shankar et al., 2008)
	Human cancer cells	Chemotherapeutic	(Li et al., 2012) (Chandra-Kuntal and Singh, 2010)
AGE	Mice	Chemotherapeutic	(Ebrahimpour et al., 2013)
	Rat cancer cells	Anti-metastatic	(Hu et al., 2002)
	Human cancer cells	Chemotherapeutic	(Dong et al., 2014)
GE	Mice	Chemopreventive	(Shukla and Taneja, 2002)
	Human cancer cells	Chemotherapeutic	(Yedjou and Tchounwou, 2012) (Su et al., 2006)
GO	Rats	Chemopreventive	(Zhang et al., 2012)
	Human cancer cells	Chemotherapeutic	(Lan et al., 2013)

Allicin	Mice	Chemopreventive	(Chu et al., 2012)
	Human cancer cells	Chemotherapeutic	(Chu et al., 2012)
Ajoene	Mice	Anti-metastatic	(Taylor et al., 2006)
		Chemopreventive	(Nishikawa et al., 2002)
		Chemotherapeutic	(Li et al., 2002)
	Human cancer cells	Chemotherapeutic	(Tilli et al., 2003)
SAC	Mice	Anti-metastatic	(Pai et al., 2012)
		Chemotherapeutic	(Tang et al., 2010)
	Human cancer cells	Chemotherapeutic	(Xu et al., 2014)
		Anti-metastatic	(Ng et al., 2012)

BIOAVAILABILITY AND METABOLISM OF OSCs

Oral administration of 27 mg of vinyldithiins, one of the constituents of garlic, to rats led to detection of dithiins in serum, kidney, fat tissue and liver in 24 h (Egen-Schwind et al., 1992a). Administration of 8 mg/Kg 35S-labeled alliin, allicin and vinyldithiine in the form of oil macerate in rats shown that 35S-allicin is absorbed and eliminated faster than 35S-allicin and 35S-vinyldithiine (Lachmann et al., 1994). In case of 35S-labeled DADS, the uptake by mouse liver was found to be maximum at 90 min, 70% of radioactivity was seen in liver cytosol out of which 80% was metabolized to sulfate (Pushpendran et al., 1980). Intake of 38g garlic resulted in detection of OSCs including DAS, DADS, DATS, dimethyl sulfide, allyl methyl sulfide, ally methyl disulfide and acetone in human breath (Taucher et al., 1996). Pharmacokinetic studies of SAC shown its half-life was comparatively lesser in rats and mice than in dogs (Nagae et al., 1994). Freeman et al carried out stability studies with allicin in blood, simulated gastric and intestinal fluids. Allicin remained stable in gastric and intestinal fluids suggesting that pH doesn't have any role in metabolism of allicin. Allicin levels were undetectable in heparinized blood after five minutes of incubation. Similarly in blood cell fraction, allicin was not detected but in plasma fraction its level decreased gradually (Freeman and Kodera, 1995). It is reported that 1 g of garlic contains 2.5 mg of allicin and 60 µg of SAC (Lawson and Gardner, 2005). One gram of freshly blended garlic contains up to 1.1 mg of DATS and up to 0.61 mg of DADS (Shukla and Kalra, 2007). SAC is rapidly absorbed in kidney, liver and plasma of dogs and mice (Nagae et al., 1994). Allicin was metabolized into DADS and AM in rat liver (Egen-Schwind et al., 1992b). Sheen et al shown that AM is the metabolite of DADS and found AM along with AMS in the extracellular fluid when they perfused rat liver with DADS, the peak levels of AM was found to be 46.2 µg/mL which was much higher than that of 0.93 µg/mL for AMS at their peak times 60 and 90 minutes, respectively (Sheen et al., 1999). When DADS was administered orally to rats at 200 mg/Kg of body weight dose, highest concentration was seen within 24 h in stomach and is converted into AM, AMS, Allyl methyl sulphoxide (AMSO) and Allyl methyl sulphone ($AMSO_2$) (Germain et al., 2002). When 10 mg/Kg of body weight dose of DATS was administered intravenously, it resulted in 31 µM of DATS in blood (Sun et al., 2006). Different drug delivery systems have been used to improve the stability and bioavailability of OSCs. The bioavailability of DATS and allitridin were improved when it was delivered in oil free microemulsions (Li et al., 2011) and pegylated liposomes (Sun et al., 2006). DATS nanoparticles were also used to study its bioavailability in rats and rabbits (Xu et al., 2009). Further studies should be done to find out the effective metabolite of OSCs in chemoprevention and chemotherapy since most of these OSCs get readily hydrolyzed. Bioavailability and pharmacokinetics of OSCs is one of the major issues that is affecting its clinical applications. It is yet to be determined how much dietary intake of garlic is good enough to achieve biologically active concentrations of DATS, DADS, DAS and other OSCs in humans.

IN VIVO EVIDENCE OF ANTICANCER EFFECTS OF DATS AND OTHER OSCs

Several studies have shown the *in vivo* anti-cancer effects of DATS and other OSCs *(Table 3).* Perchellet et al was the first to show the anticancer effects of garlic oil in a chemically-induced skin tumorigenesis model (Perchellet et al., 1990). Ajoene treatment inhibited skin carcinogenesis in mice (Nishikawa et al., 2002). Oral gavage of DAS inhibited the dimethyl hydrazine-induced colon cancer (Wargovich, 1987). DAS also inhibited the N-nitrosomethylbenzylamine-induced esophageal tumor formation in rats when administered before the initiation phase but it was not effective when given in the promotion phase (Wargovich et al., 1988).

Application of DAS on skin during the early stages of skin cancer decreased the number of tumors per mouse induced by polycyclic aromatic hydrocarbons (Singh and Shukla, 1998). Similarly DADS

inhibited the neoplasia of forestomach and lung induced by *N*-nitrosodiethylamine in mice. DADS, AMDS, and AM reduced the tumor formation in the lung and forestomach (Wattenberg et al., 1989). Anticancer properties of naturally occurring OSCs have been observed in carcinogen-induced esophageal cancer in rats (Wargovich et al., 1988). SAC inhibited the incidence and frequency of colon tumor induction by 1,2-dimethylhydrazine (Sumiyoshi and Wargovich, 1990). Chemoprevention of cancer by other OSCs were observed for pulmonary carcinogenesis and forestomach cancer in mice induced by benzo(a)pyrene (Sparnins et al., 1988). DADS treatment prevented the growth of human colon cancer cells implanted in athymic mice (Sundaram and Milner, 1996b), and also inhibited the growth of human breast cancer xenografts, which was correlated with decreased cell proliferation (Nakagawa et al., 2001). Oral gavage of DATS inhibited the growth of benzo(a)pyrene-induced forestomach cancer in mice (Hu et al., 1997). Growth of PC-3 xenografts in nude mice was inhibited by DATS treatment (Xiao et al., 2006a). DATS administration inhibited the growth of CT-26 murine colon cancer allograft implanted in BALB/c athymic mice, as well as the incidence and growth of phorbol ester-induced skin papilloma in female ICR mice (Wu et al., 2011), (Shrotriya et al., 2010).

SAC prevented the growth of CWR22R human androgen-independent prostate cancer cells implanted in athymic mice (Chu et al., 2007). It was shown that DATS polybutylcyanoacrylate nanoparticles inhibited the growth of implanted HepG2 liver hepatocellular carcinoma cells in athymic mice (Zhang et al., 2007). Overall, these studies provide the evidence for pre-clinical cancer chemopreventive efficacy of OSCs in various cancer models.

In vivo toxicity of OSCs

Most of the studies with OSCs have shown the anticancer effects without any harmful side effects even at higher doses in *in vivo* but few reports have revealed that these OSCs are also toxic. Nakagawa et al reported the toxic effects of raw garlic juice. Oral administration of raw garlic juice in Wistar rats led to retarded growth, decrease in body weight, and various histological and morphological changes were noticed in liver, adrenal glands, spleen, and decrease in red blood cell count. Also few rats died due to severe stomach injury administered with 5 ml/Kg of raw garlic juice but none of these toxic effects were noticed in rats administered with extracted aged garlic juice (Nakagawa et al., 1980). Another study in rats with garlic extract and garlic oil revealed its toxic effects. Intragastric administration of 2 ml/100g of garlic extract led to histological changes in liver, inhibited alkaline phosphatase, increased the levels of D-aspartate aminotransferase and urea. Similarly 10 mg/100g of garlic oil was found be harmful when administrated after 24h fasting led to death due to severe congestion of all organs (Joseph et al., 1989). Fehri et al too reported administration of 300 and 600mg/Kg garlic extract daily for 21 days showed toxic effects (Fehri et al., 1991). Iciek et al reported in a review that 62 mg/Kg dose of DATS in swiss mice was found to be lethal (Iciek et al., 2009). Administration of 30 ml/Kg dose of garlic extract by P.O., (per oral) and S.C. (subcutaneous) in Wistar rats and ddY mice was not lethal but few rats were reported to be died within one day of administration of garlic extract in I.P. group (intraperitoneal) (Nakagawa et al., 1984) . Contrary to this, chronic toxicity tests for 6 months revealed oral administration of garlic extract five times/ week even at 2000 mg/Kg body weight found to be not harmful (Alnaqeeb et al., 1996; Sumiyoshi et al., 1984) (Thomson et al., 1998). Taken together these studies suggest extracts from *Allium* vegetables may be toxic at certain concentrations further toxicity studies have to be done to find out effective and safe doses of OSCs.

Table 3. Inhibition of chemically-induced carcinogenesis by organosulfur compounds (OSCs)

OSCs	Carcinogens	Organs	References
DAS	DMH	Colon	(Wargovich, 1987)
	NMBA	Esophagus	(Wargovich et al., 1988)
	BP	Forestomach & Lung	(Singh and Shukla, 1998)
	DMBA	Skin	(Nigam and Shukla, 2007)
	VC, NDMA	Skin	(Surh et al., 1995)
	PhIP	MCF-10A/breast	(Wilson et al., 2007)
	DES	Breast	(Green et al., 2007)
	AA	Forestomach	(Hadjiolov et al., 1993)

	NMBA	Esophagus	(Wargovich et al., 1992)
	NNK	Lung	(Hong et al., 1992)
	DMH	Colon	(Wargovich, 1987)
DADS	B(a)P	MCF-10A/breast	(Nkrumah-Elie et al., 2012b)
	MNU	Prostate	(Arunkumar et al., 2006)
	PhIP	Mammary	(Mori et al., 1999)
	DMBA	Skin	(Dwivedi et al., 1992)
	DEN	Colon & renal	(Takahashi et al., 1992)
DATS	B(a)P	MCF-10A cells	(Nkrumah-Elie et al., 2012a)
	DMBA + TPA	Skin	(Shrotriya et al., 2010)
	B(a)P	Forestomach	(Sparnins et al., 1988)
AGE	DMH	Colon	(Matsuura et al., 2006)
	DMBA	Buccal pouch	(Balasenthil et al., 1999)
GE	DMBA	Skin	(Rao et al., 1990)
GO	NDEA	Liver	(Zhang et al., 2012)
	DMBA	Skin	(Perchellet et al., 1990)
Ajoene	DMBA+TPA	Skin	(Nishikawa et al., 2002)
SAC	DMBA	Buccal pouch	(Balasenthil et al., 2001)

Chemopreventive effects of OSCs *(Table 4)* led to some intervention trials in humans. A double blind placebo controlled intervention was done with higher doses of DATS and selenium in China (Li et al., 2004). Selection of subjects included family history of tumor, medical history of stomach disorder and smoking or alcohol consumption. Intervention trial included 2526 subjects in the intervention arm and 2507 controls. The subjects of intervention group were given orally 200 mg of DATS every day and 100 µg of selenium every other day for a period of one month (1989-1991), while the control group was subjected to two placebo capsules. Decrease in the incidence of cancer was seen after the five years follow-up. Intervention group subjects showed 47.3% lower incidence of gastric cancer and 22% lower incidence of all cancers (Li et al., 2004). This study indicated that large doses of DATS were tolerated without any side effects. Relative risk for all cancers was 0.67 (95% confidence limit CL = 0.43 – 1.03) and relative risk for gastric cancer was 0.48 (95% confidence limit CL = 0.21 – 1.06). Follow up studies with the same intervention group (1989-1991) was done up to 2001 to assess the long term effects of DATS, revealed a decrease in incidence risk of gastric and digestive system cancers, and mortality rate of all cancer (Zheng et al., 2005). It was also reported that aged garlic extract suppressed the colorectal cancer (Tanaka et al., 2006). Higher and lower doses of aged garlic extracts were given to the subjects having colorectal polyps for a period of one year. After one year, it was found that higher dose (2.4 mL/day) of aged garlic extract reduced the size and number of colon adenomas (Tanaka et al., 2006). Another study showed that ajoene administration reduced skin cancer by inducing apoptosis of basal cell carcinoma (Tilli et al., 2003). This study was rather small and included 21 subjects with basal carcinoma. Topical application of ajoene on tumors for a period of six months resulted in significant reduction in the tumor size in 17 out of 21 subjects. Further immunohistochemical analyses showed that ajoene application reduced the expression of Bcl-2 and induced apoptosis in basal carcinoma (Tilli et al., 2003). Meta-analysis studies conducted on 8,621 cases and 14,889 controls to study the effect of garlic intake and risk of gastric cancer, revealed garlic intake reduced the risk of gastric cancer (Kodali and Eslick, 2015). Similarly, Zhou et al reported that consumption of garlic lowered the risk of gastric cancer by meta-analysis of 543,220 subjects (Zhou et al., 2011). Meta-analysis of case control studies conducted in Italy between 1997-2007 revealed that high intake of *Allium* lowers the risk of gastric cancer (Turati et al., 2015).

Table 4. Mode of action of organosulfur compounds (OSCs) on different cancer cell lines

OSCs	Cell lines	Effects	References
DAS	Ca Ski human cervical	G0/G1 arrest & apoptosis	(Chiu et al., 2013)

	Human thyroid	G2/M arrest & apoptosis	(Shin et al., 2010)
	Colo 205 human colon	Antimetastatic	(Lai et al., 2013)
	Colo 320 DM human colon	G2/M arrest & Apoptosis	(Sriram et al., 2008)
DADS	BGC823 human gastric	G2/M arrest	(Bo et al., 2014)
	MGC-803 human gastric	Apoptosis	(Tang et al., 2013)
	U937 human leukemia	G2/M arrest & Apoptosis	(Dasgupta and Bandyopadhyay, 2013)
	MCF-7 human breast	Apoptosis	(Altonsy et al., 2012)
	HL-60 human leukemia	G2/M arrest	(Yi et al., 2012)
	B16F-10 melanoma	Apoptosis	(Pratheeshkumar et al., 2010)
DATS	SKOV-3 human ovarian	Apoptosis	(Wan et al., 2013)
	T24 human bladder	Apoptosis	(Shin et al., 2014)
	5637 human bladder	Antimetastatic	(Shin et al., 2013)
	PC-3 human prostate	Cytotoxic	(Borkowska et al., 2013)
	MCF-7, MDA-MB-231 human breast	Apoptosis & antimetastatic	(Chandra-Kuntal et al., 2013)
	H460 human lung	Apoptosis	(Xiao et al., 2009a)
	BCC human basal	Apoptosis	(Wang et al., 2012)
	PC-3	Apoptosis	(Kim et al., 2011)
	DU145 human prostate	G2/M arrest	(Chandra-Kuntal and Singh, 2010)
	LNCaP, HCT-116	G2/M arrest & Apoptosis	(Xiao et al., 2009b)
Ajoene	HL-60 human leukemia	Apoptosis & down-regulation of telomerase activity	(Ye et al., 2005)
	B16/BL6 melanoma	Antimetastatic	(Taylor et al., 2006)
	HL-60	G2/M arrest & inhibits 20S proteasome	(Xu et al., 2004)
	B16F10 melanoma	Apoptosis & Anti- adhesion	(Ledezma et al., 2004)
SAMC	SGC 7901 human Gastric	Apoptosis	(Yan et al., 2013)
	SW480, SW620, Caco-2 colorectal	Apoptosis & antimetastatic	(Liang et al., 2011)
	PC-3	Antimetastatic	(Howard et al., 2007)
	SW480, NIH3T3	Apoptosis & microtubule depolymerization	(Xiao et al., 2003)
	DS19 mouse Erythroleukemia	Induces histone acetylation & growth inhibition	(Lea et al., 2002)
	HEL, OCIM-1 MCF-7, CRL-1740	Inhibits cell proliferation	(Sigounas et al., 1997)

FUTURE DIRECTIONS

The studies conducted so far suggest the strong cancer chemopreventive potential of *Allium* vegetables. This is mostly attributed to DATS and other OSCs, which inhibit the growth of cancer cells by inducing cell cycle arrest, induction of apoptosis, suppression of aberrant signaling, modification of histones, inhibition of angiogenesis and metastasis, and detoxification of carcinogen. DATS oxidatively modifies the cysteine residues of beta tubulin, Keap1 and thioredoxin. However, still a complete understanding is lacking of how these OSCs can target a given aberrant molecule in cancer cells. Further studies are needed to explore the direct and selective molecular targets in cells and associated mechanisms of their alterations, transient or permanent, of OSCs. Still, very little is known about the actual effective metabolites of OSCs, since many of these OSCs are unstable and get metabolized. Future research should focus on following issues like bioavailability, pharmacokinetics, toxicity and clinical trials. Efforts are being done to increase the bioavailability of OSCs and standardize the doses for its translational potential because bioavailability and its plasma levels are the one of key issues affecting its clinical trials. Further, the combination study of DATS

and selenium suggests that more such studies are needed to find an effective combination for a given cancer type. Now time has come for the large multi-centric and multi-ethnic cancer chemoprevention trials with selected *Allium* vegetables or supplements in prevention set-up and selected OSCs such as DATS for their anti-tumor effects in therapeutic set-up. Furthermore, we believe that *Allium* vegetables could be an essential component in the prevention and management of cancer.

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