Garlic Induced Apoptosis, Cell Cycle Check Points and Inhibition of Cancer Cell Proliferation

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Abstract   Present review article describing effects of garlic components on induction of apoptosis, cell cycle check points and inhibition of cancer cell proliferation. Garlic derived organic compounds hold unique therapeutic potential and induces apoptotic signaling pathways; do cell cycle arrest, and show cytotoxic and anti-invasive activity to a variety of cancer cells. Regular dietary intake of garlic cut down neoplastic growth, cancer cell proliferation, and induces programmed cell death. Garlic components act as tumor suppressor agents and prevent aberrant cell expansion by slowing down the cell cycle, or by inducing apoptosis. These apoptosis inducers can act on various apoptosis-related proteins to promote apoptotic cell death. Garlic based nanoformulations can target oncogenic mutations which disrupt apoptosis. These can inhibit cellular changes leading to tumor initiation, progression of metastasis. Garlic derivatives can control DNA damage thereby slow down oncogenic changes and induce caspase activity which halts or stalls mutation in p53 gene and thereby stops growth of lung, colon, and breast cancer. More often, new combinations of garlic components may stop cancer progression as well as multistage carcinogenesis. No doubt garlic components can be used in designing best possible molecular targets of cancer cells and that could be recommended for clinical management of different types of cancer.

Keywords: garlic, apoptosis, antioxidants, caspase protein, histone deacetylation, cell cycle check points, tumor suppression

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1. Introduction

Allium sativum, commonly known as lahsun in Hindi (Garlic in English) belongs to family Alliaceae and plant order liliales [1] Plant has been used for centuries as a nutraceutical, prophylactic and therapeutic medicinal agent. Garlic is a perennial, erect, bulbous herb indigenous to Asia but it is commercially grown in many parts of the world. It is a main singular and combined foodstuff which is used in Traditional Medicine of India. *Allium sativum* L. is an important aromatic plant and has multiple uses [2]. It is a rich source of organo-sulfur compounds, which are thought to be responsible for its flavor and aroma, as well as its potential health benefits [3]. It is also used for both culinary and medicinal purposes by many cultures for centuries [4]. Garlic is a multi-component pioneer food contains good nutraceuticals which are highly protective to lung cancer and increase the life expectancy in cancer patients [4,5,6]. Dietary consumption of green garlic bulbs reduce all types of cancer risks [7]. It stops invasion and progression of cervical cancer [8]. Homemade garlic ailments and preparations are used as herbal drug for complementary therapy of cancer. Garlic oil inhibits proliferation of AsPC-1, PANC-1, and Mia PaCa-2 cells while garlic tea shows strong therapeutic potential against lung and colorectal cancer [9]. Both black and green garlic

extracts (BGE) check proliferation of lung [10] and colorectal cancer and inhibits other types of carcinoma [11]. It contains S-allylcysteine (SAC), one of the major water-soluble compounds significantly inhibit oncogenesis [12] while, S-Allylmercaptocysteine (CySSA) is known to exhibit anti-cancer effects [13]. Alliin, isolated from garlic (*Allium sativum*) prevents LPS-induced inflammation in 3T3-L1 adipocytes. Similarly, DATS prevents tumor progression and promotes apoptosis in ectopic glioblastoma xenografts in SCID mice via HDAC inhibition [14] (Table 1). Cyclic sulfoxides garlicnins B2, B3, B4, C2, and C3 isolated from *Allium sativum* are toxic to cancer cells [15]. Conjugates of daidzein-alliinase are used as a targeted pro-drug enzyme system against ovarian carcinoma [16].

Both allicin and allyl-mixed disulfides with proteins and small thiol molecules show good anticancer activity [3] and are considered as safe botanicals [17] (Table 1). These compounds induce of apoptosis in tumor cells and show multiple therapeutic potential [18] against different types of cancers [19]. Alliin, prevents LPS-induced inflammation in 3T3-L1 adipocytes) [20] while thiacremonone shows anticancer activity and does down regulation of peroxiredoxin [21]. Black garlic extract is strong inhibitor of lung cancer cell proliferation and radio sensitization [10] (Table 1). Thiacremonone (2, 4-dihydroxy-2, 5-dimethyl-thiophene-3-one is sulfur compound from garlic [21] shows inhibition of NF-kappaB and cancer cell growth

with IC(50) values about 100 microg/mL in colon cancer cells) [22]. Garlic, silver bullets are used for carcinoma surveillance in upper endoscopy for Barrett's esophagus [23] (Table 1).

Induction of apoptosis depends on the redox-state of the cell, with anti-oxidants being able to prevent sulfide-induced apoptosis [24]. Both growth arrest and induction of apoptosis is associated with a considerable reduction of the level of cdc25C and p53 gene mutations and its expression in cells. Garlic derived polysulfides induce apoptosis [24] and can control cancer cell proliferation, angiogenesis and metastasis at physiological dose. Garlic components may participate in regulation of cellular differentiation and cell death much similar to oncogenes and tumor suppressor genes or induce their activity that play an important role in this process by regulating either cellular proliferation. There is a possibility that garlic may restore all three different categories of cancer associated genes. First category of proto-oncogenes and their oncogenic counterparts encodes proteins that induce cellular proliferation. Some of these proteins function as growth factors or growth factor receptors in normal cells, and its expression is carefully regulated. Garlic possesses apoptosis inducers which act on several apoptosis-related proteins to promote apoptotic cell death. Even its components also bind to most of the growth factor receptors and normalize any type of aberration related to gene function. A second category of genes called tumor suppressor genes or anti-oncogenes encodes proteins that inhibit excessive cell proliferation. Inactivation of these genes results in unregulated proliferation. Garlic antioxidants and enzymatic inhibitors check unregulated cellular proliferation by making interference in metabolic pathways which synthesize cancer growth promoting factors.

A defective or inefficient apoptosis is a characteristic feature of cancer cells that needs induction but garlic derived polysulfides lead to induction of apoptosis [24].

These may induce apoptosis through p53-dependent and independent pathways, which facilitate cytochrome c release from mitochondria. Hence, there should be more appropriate and authentic information is needed on target specificity of cancer agents which induce apoptosis. Though from biochemical researches cytotoxic potential of garlic components is established but target specificity explanation is still lacking. Therefore, all intriguing possibilities that are responsible for defects in apoptotic pathways which contribute to treatment failure can be resolved by making appropriate combinations of garlic components for cancer control. It also needs a thorough understanding about apoptotic signaling pathways to unravel novel drug targets and finding proper therapeutic solutions. Further, these should be compared for better cancer therapeutics with effect of certain oncogenes such as *E1A* and c-*myc*. Among all efficacies new induction of apoptotic signaling pathways in cancer cells be needed to resist apoptotic insults, with strong efforts to target the mitochondrion for restoring efficient cell death signaling in cancer cells. Hence, there is an immense need to make conceptual framework that can link cancer genetics with cancer therapy. Present review article describes use of garlic components for inducing apoptosis via small molecule drug treatments, its receptor ligation, and cytotoxic action. Further, nanoformulations which could target oncogenic mutations, disrupt apoptosis, reduce the chances of tumor initiation, progression and metastasis should prepare. These nanoformulations may induce apoptosis by making changes in mitochondrial membrane potential, phosphatidylserine membrane localization, DNA content, and antigen cleavage. Hence, best possible drug targets must explore to stop cancer progression and producing selective pressure to override apoptosis so then multistage carcinogenesis could be finished by opting new combinations of garlic components. Garlic based herbal products and targeted molecules can be recommended for clinical management of various cancer types.

Table 1. Multiple uses of various plant parts of Garlic (*Allium sativum*) for treatment of different diseases

Medicinal	Preparation/ Ailment	Treatment
Leaves	Hot concoction	Common cold,
Leaves	Tea	Reduce serum total cholesterol and triglyceride levels
Bulbs green	Crushed paste	Reduce platelet aggregation, hyperlipidemia
Leaves	Oil	Blood-thinning
Bulb	Sticky juice	Adhesive in mending glass and porcelain
Bulb	Solvent extract (w/v)	Nematicide and insecticide for cabbage root fly and red mite in poultry,
Folk medicine	Crushed bulbs and dry stem	Relieving pain, defense against malaria, flu, cold and , sneezing deterring animals such as birds, insects, and worms from eating the plant.
Cherokee	Hot syrup	Expectorant for coughs and croup
Bulb	Luke warm paste	Antiseptic to prevent gangrene
Garlic + cinnamon	Bulb and bark	Fish and meat preservative, and antimicrobial
Spiritual and religious	Total plant	Both good and evil
Europe	Bulbs	White magic
Central European	Total garlic plant could be worn, hung in windows, or rubbed on chimneys and keyholes	Powerful ward against demons, werewolves, and vampires
Muslims	Green and raw garlic	Good for prayer
Hinduism	Green and raw garlic	Garlic is thought to stimulate and warm the body and to increase one's desires
Jain	Green and raw garlic	Religion avoids eating garlic and onion on a daily basis.
Buddhist traditions	Green and raw garlic	Increase drives to the detriment of meditation practice

2. Anticancer Activity

2.1. Induction of Caspase Activity

CASP3 protein is an important member of the cysteine-aspartic acid protease (caspase) family [25]. Caspases exist as inactive proenzymes in the cells which undergo proteolytic processing at conserved aspartic residues to produce two subunits, large and small, that dimerize to form the active enzyme. This protein cleaves and activates caspases 6 and 7; and the protein itself is processed and activated by caspases 8, 9, and 10. It is the predominant caspase involved in the cleavage of amyloid-beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease. After alternative splicing this gene results in two transcript variants that encode the same protein [26] Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways [27]. Garlic induces caspase activity which halts or stalls mutation in p53 gene and thereby stops growth of lung, colon, and breast cancer. It supports non-apoptotic roles of many effectors to the apoptotic signaling pathways. Similarly, diallyl trisulfide-induce apoptosis of bladder cancer cells which is caspase-dependent and regulated by PI3K/Akt and JNK pathways [28]. DADS also induce phosphatidylserine translocation from the inner to the outer leaflet of the plasma membrane and activate caspase-3 activity. Like caspase 3, caspase 2, plays important role in cell cycle regulation, DNA repair, and tumor suppression [29]. Garlic components act upon two pathways of target cell apopotosis stimulated by CTLs and through Fas pathway in which ligation of trimeric Fas units by CTL borne Fas ligands leads to the association of death domains of Fas with FADD. These components regulate a series of reactions that could lead to apoptosis of target cell. Garlic components may induce CTL functions and activating caspase cascade that results in the apoptotic death of the cell death by invoking mitochondrially mediated death pathways.

Normally in healthy cell three important genes reside i.e. proto-oncogenes, oncogenes, and a third category of cancer associated genes which regulate programmed cell death. These genes encode proteins that either block or induce apoptosis; bcl-2 is an anti-apoptosis gene. DATS-induce apoptosis and does down-regulation of Bcl-2, Akt and cyclin D1 protein levels, and up-regulation of Bax, Fas, p53 and cyclin B protein levels in Capan-2 cells [30] (Table 1). More specifically carcinogens and viruses might alter the regulated function of these genes and converting them into potent cancer causing genes. In such condition garlic derived components mainly sulfur compounds, saponins and vitamins can evoke tumor suppressor gene and stop conversion of proto-oncogenes in to oncogenes. All this is possible due to genetic rearrangements of proto-oncogenes and mutations. Obviously, conversion of proto-oncogenes into oncogenes involves mutation that may result in production of qualitatively different gene products that may harm normal physiological metabolism of a normal cell. Further, viral integration into the host genome serves to convert a proto-oncogene into a transforming oncogene. Retroviruses have shown to integrate within the c-myc proto-oncogene. Such transformed or metastatic cells can be depleted by programmed cell death only thorough apoptosis induction pathways. That is possible by garlic component dosage as DADS modulates the cellular levels of Bax, Bcl-2, Bcl-xL, and Bcl-w in a dose-dependent manner, and induce apoptosis in the MCF-7 breast-cancer cell line through interfering with cell-cycle growth phases in a way that increases the sub-G(0) population and substantially halts DNA synthesis [28].

2.2. Induction of Apoptosis-related Proteins

Apoptosis is followed by two types of death pathways, namely, the extrinsic pathway and the mitochondria-mediated pathway. Both are linked and molecules used in one pathway can influence the other [31]. Apoptosis imparts cell shrinkage, blebbing of plasma membrane, affect maintenance of organelle integrity, condensation and fragmentation of DNA, followed by ordered removal of phagocytes [32]. Apoptosis is a suicidal self managed operation that kills cancer cells and maintain minimal damage to surrounding tissues. Apoptosis is regulated by the Bcl-2 family of proteins i.e. Bcl-2, Bcl-XL, and Mcl-1 which are anti-apoptotic proteins that possess all the four domains (BH1-4). These proteins regulate apoptotic events and share homology in any of the four common Bcl-2 homology (BH) domains [33]. The second category of Bcl-2 family of proteins contains BH domains 1, 2 and 3. These proteins would include Bax, Ki-67 and Bak. Bax is a pro-apoptotic protein that resides in the cytosol under physiological conditions. The third group of Bcl-2 family of proteins is the BH3-only proteins. The BH3-only family members include Bim, Bad, Bmf, Noxa and Puma. They act by neutralizing the anti-apoptotic proteins [33]. Apoptosis is induced in experimental systems through a variety of methods but two known methods i.e. biological induction and chemical induction are well used. In biological induction activation of Fas or TNF receptors by their respective ligands, or by cross-linking with an agonist antibody, induces apoptosis of Fas- or TNF receptor-bearing cells. In chemical apoptosis inducers act on several apoptosis-related proteins to promote apoptotic cell death [30].

Few important derivatives from garlic such as allicin, ajoene, DAS, DADS, DATS, and SAMC, have been found to induce apoptosis and cell cycle arrest in various cancer cell lines grown in culture. There are many more possibilities that different natural products may choose different pathway to maintain apoptosis in cancer cells. Garlic oil induce programmed cell death, cell cycle arrest, and show pro-apoptosis effects in AsPC-1 cells [34] and human pancreatic carcinoma cells [34]. Depending on the agent selected and the concentrations used, apoptotic events can be detected between 8–72 h post-treatment. S-allylcysteine is a potential therapeutic compound isolated from garlic derivative that suppresses proliferation and induces apoptosis in human ovarian cancer cells *in vitro* [35]. S-allylcysteine is also used to enhance apoptosis in oral cancer (Table 1) and human breast cancer cells through ROS-mediated activation of JNK and AP-1 [36]. Allicin inhibits cell growth and induces apoptosis in U87MG human glioblastoma cells through an ERK-dependent pathway [37]. It induces p53-mediated autophagy in Hep G2 human liver cancer cells [4,38] and

induces apoptosis in EL-4 cells *in vitro* by activation of expression of caspase-3 and -12 and up-regulation of the ratio of Bax/Bcl-2 [39]. It also induces apoptosis in gastric cancer cells through activation of both extrinsic and intrinsic pathways [40]. Allicin purified from fresh garlic cloves induces apoptosis in colon cancer cells via Nrf2 [41]. while diallylpolysulfides induce growth arrest and apoptosis in cancer cells (Busch et al., 2010) [24]. Allicin induce autophagic cell death in human HCC Hep G2 (p53(wild type) cells, it may also induce apoptotic cell death through caspase-dependent and caspase-independent pathways by overproduction of reactive oxygen species (ROS) in human HCC Hep 3B (p53(mutation)) cells. Moreover, in cell death mechanism p53 knocked down Hep G2, and silenced the p53 gene using siRNA-mediated silencing. However, allicin treatment induces apoptotic cell death in p53 knocked down Hep G2 cells similar to that of Hep 3B cells [4]. Allyl sulfides inhibit cell growth of skin cancer cells through induction of DNA damage mediated G2/M arrest and apoptosis [39].

In normal cells growth occurs at regular interval through well controlled phases of mitotic cell division. Apoptosis is highly important event because it controls cell number and support healthy cells to live and operate to kill old cells or defective cells. Diallyl tetrasulfide induce mitotic arrest to apoptosis [42]. It also induces apoptosis in human primary colorectal cancer cells) [43] and AGS gastric cancer cell line [44]. Diallyl trisulfide sensitizes human melanoma cells to TRAIL-induced cell death by promoting endoplasmic reticulum-mediated apoptosis) [45]. It induces apoptosis and inhibits proliferation of A549 cells *in vitro* and *in vivo* [46]. Similarly, di-allyl disulfide plays important role of in NF-κB mediated transient G2-M phase arrest and apoptosis in human leukemic cell-lines [47]. Allyl sulfides inhibit cell growth of skin cancer cells through induction of DNA damage mediated G2/M arrest and apoptosis) [38] (Wang et al., 2010). Similarly, diallyl trisulfide [30] and S-alkenylmercaptocysteine (CySSR) with sodium selenite induce apoptosis in pancreatic cells [13]. DATS-induced apoptosis is related with induction of pro-apoptotic Bax protein and p53 protein expression that was found upregulated and translocation to nucleus in MCF-7 cells [48]. DATS inhibits phosphatidylinositol 3'-kinase/Akt activation that, in turn, results in modulation of Bcl-2 family proteins, leading to enhanced apoptosis of T24 cells) [39]. This is the possible mechanism by which allyl sulfides suppress neoplastic cell proliferation[49]. S-benzyl-cysteine mediate cell cycle arrest and apoptosis involving activation of mitochondrial-dependent caspase cascade through the p53 pathway in human gastric cancer SGC-7901 cells [50] (Table 1). Thiacremonone inhibits lung cancer cell growth in a concentration dependent manner through induction of apoptotic cell death accompanied by induction of cleaved caspase-3, -8, -9, Bax, p21 and p53, but decrease of xIAP, cIAP and Bcl2 expression and inhibition of glutathione peroxidase activity of of PRDX6 through interaction) [21].

2.3. Histone Deacetylation Inhibition

Histone deacetylase inhibitors (HDIs) interfere with the function of histone deacetylase and are as possible treatments for cancers [51,52]. Sulfur compounds from garlic showed modulation of acetylation [53]. These are cytostatic agents that inhibit the proliferation of tumor cells in culture in vitro while *in vivo* induce cell cycle arrest, cell differentiation and/or apoptosis. Histone deacetylase inhibitors exert their anti-tumor effects via the induction of expression changes of oncogenes or tumour suppressor genes, through modulating the acetylation/deactylation of histones and/or non-histone proteins such as transcription factors [55]. Histone deacetylation inhibitors (HDACi) also suppress cancer growth and induce apoptosis in cancer cells. Similarly, DADS shows similar properties as HDACi in MCF-7 cells as it lowers the removal of an acetyl group from an acetylated substrate and induces histone-4 (H4) hyper-acetylation that may be responsible for the induction of apoptosis in breast cancer cells [56]. In addition, di-allyl disulfide causes inhibition of histone deacetylation may also an important check point [56]. Histone deacetylase inhibition induces the accumulation of hyperacetylated nucleosome core histones in most regions of chromatin but affects the expression of only a small subset of genes, leading to transcriptional activation of some genes, but repression of an equal or larger number of other genes. Garlic compounds inhibit histone deacetylase activity and induce histone hyperacetylation *in vitro* as well as *in vivo* [56]. Thus both histone acetylation and deacetylation play important roles in the modulation of chromatin topology and the regulation of gene transcription [56] and are responsible for the induction of apoptosis in breast cancer cells [56]. DADS is able to increase PGC1α expression in a ROS-dependent manner and to induce mitochondrial biogenesis at early stage of treatment that precede cell cycle arrest and apoptosis outcome [57]. DADS also elicits the increase of PGC1α within nuclear compartment, the decrease of PGC1α non-active acetylated form and does induction of nuclear-encoded mitochondrial genes such as TFAM and TFBM1) [57]. There are clear evidences about involvement of histone acetylation in modulation of gene expression by diallyl disulfide and allyl mercaptan [57].

2.4. Cell Cycle Checks Points

Cell cycle checkpoints are control mechanisms in eukaryotic cells which ensure proper division of the cell. Each checkpoint serves as a potential halting point along the cell cycle, during which the conditions of the cell are assessed, with progression through the various phases of the cell cycle occurring when favorable conditions are met. There are three known checkpoints: the G1 checkpoint, also known as the restriction or start checkpoint; the G2/M checkpoint; and the metaphase checkpoint, also known as the spindle checkpoint. The cell cycle checkpoints play an important role in the control system by sensing defects that occur during essential processes such as DNA replication or chromosome segregation, and inducing a cell cycle arrest in response until the defects are repaired [58]. Cell cycle in eukaryotes is a complex process, which is maintained by a network of regulatory proteins, or cell cycle control system. This system monitors and dictates the progression of the cell through the cell cycle [59]. Cell cycle checkpoints are maintained through the regulation of the activities of a family of protein kinases known as the cyclin-dependent kinases (CDKs), which bind to different

classes of regulator proteins known as cyclins, with specific cyclin-CDK complexes being formed and activated at different phases of the cell cycle. Those complexes, in turn, activate different downstream targets to promote or prevent cell cycle progression [60].

In normal cells, the cell cycle is tightly regulated to ensure faithful DNA replication and chromosomal segregation prior to cell division. Following DNA damage, the cell cycle can be transiently arrested to allow for DNA repair or activation of pathways leading to cell death (apoptosis). But in normal cells DNA damage is self repaired and cell tries to maintain cell cycle normal. There are certain cell cycle check points such as late G2 check point controls progression of cell cycle from G2 to M phase. Here, active Cdk1 (cdc2) for formation of complex, cyclin B1 is required. In next step for regulation of the cdc-B1 complex inhibitory phosphatases are needed at a pair of amino acids in the roofs of the active site by Wee1 (Figure 1 and Figure 2). After dephosporylation of these sites by the phosphatase Cdc25C increases Cdk activity. At this stage DNA damage activates Chk1 which inactivates Cdc25C through phosphorylation of cdc25C. This results in the phosphorylation and activity of cdc2-B1 and G2-M arrest (Figure 1 and Figure 2). Thus, G1/S is an important checkpoint that controls progression

of cells through the restriction point (R) into the DNA synthesis S-phase (Figure 1 & Figure 2). During G1 stage, the tumor suppressor Rb binds and inhibits transcription factor E2F. More specifically, diallyl trisulfide-induced G2/M phase cell cycle arrest in DU145 cells is associated with delayed nuclear translocation of cyclin-dependent kinase 1 [61,47] while synthetic polysulfane derivatives induce cell cycle arrest and apoptotic cell death in human hematopoietic cancer cells [62] (Table 1). DAS also induce G0/G1 cell cycle arrest [63] and apoptosis in HeLa cells through caspase- and mitochondria and p53 pathways. It significantly inhibits the growth and induces apoptosis of human cervical cancer HeLa cells *in vitro* *[64]*. DAS-induced G0/G1 phase arrest is mediated through the increased expression of p21, p27, and p53 with a simultaneous decrease in CDK2, CDK6, and CHK2 expression. DAS exposed Ca Ski cells undergo apoptosis, with significant morphological changes and DNA condensation. It leads to alter the ratio of Bax/Bcl-2, and induce mitochondrial dysfunction, that results in release of cytochrome c for causing apoptosis in Ca Ski cells. Due to these properties DAS is considered as potential chemotherapeutic agent for the treatment of cervical cancer [65]. S-Allylmercaptocysteine does cell cycle arrest at the G2/M and sub-G1 interphases [13].
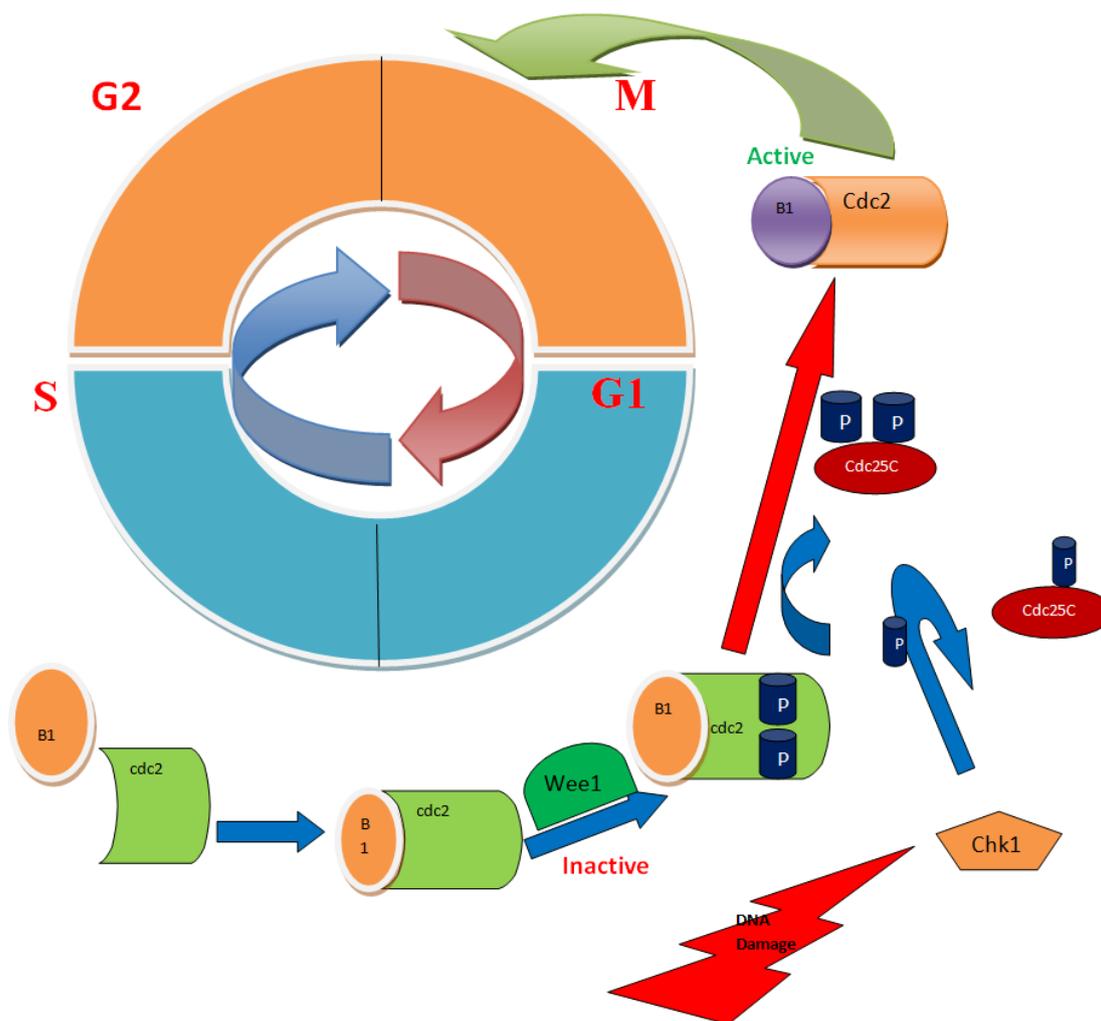
Figure 1. showing late G2 check point controlling progression from G2 to M phase. Active Cdk1(cdc2) complexed to cyclin B1 is required for progression from G2 to M phase. Regulation of the cdc-B1 complex involves inhibitory phosphatases at a pair of amino acids in the roofs of the active site by Wee1. Dephosporylation of these sites by the phosphatase Cdc25C increases Cdk activity. DNA damage activate Chk1 which inactivates Cdc25C through phosphorylation of cdc25C, resulting in the phosphorylation and inactivity of cdc2-B1 and G2-M arrest

Figure 2. showing important cell cycle check points to stop cancer progression in stressed and cancer prone cells

2.5. Activation of phosphorylation of Rb by Cyclin-bound Cyclin Dependent Kinases

Diallyl disulphide, also found in Garlic oil, causes a redistribution of cell-cycle growth phases, induces apoptosis, and enhances butyrate-induced apoptosis in colorectal adenocarcinoma cells (HT-29)) [56]. Garlic sulfur components may activate phosphorylation of Rb by cyclin-bound cyclin dependent kinases (CDK) in late G1 and induce dissociation of Rb protein and permits E2F-mediated transcription of S-phase-promoting genes [66]. INK4 and Kip/Cip family inhibitors control CDK activity and prevent entry into S-phase after. DNA damage activates response pathways through ATM/ ATR and Chk1/2 kinases to block CDK activity [67], leading to cell cycle arrest and DNA repair or cell death [58] (Figure 1 and Figure 2). DATS suppress the viability of cultured human pancreatic cancer cells (Capan-2) and it increases the proportion of cells in the G2/M phase and induced apoptotic cell death. DATS enhance the expression of Fas, p21, p53 and cyclin B1, but down regulate the expression of Akt, cyclin D1, MDM2 and Bcl-2) [30]. DATS induces cell cycle inhibition and elevated levels of cyclin B1 and p21, and reduced levels of cyclin D1 in Capan-2 cells and H6C7 cells) [30]. DATS reduce mitosis in tumors, decrease HDAC activity, increase acetylation of H3 and H4, and inhibit cell cycle progression. It decrease pro-tumor markers (e.g., survivin, Bcl-2, c-Myc, mTOR, EGFR, VEGF) and promote apoptotic factors (e.g., bax, mcalpian, active caspase-3), and induced DNA fragmentation. There also occurs an increase in p21Waf1 expression, which is correlated with increased p53 expression and MDM2 degradation following DATS treatment [14]. DATS also enhance the expression of Fas, p21, p53 and cyclin B1, but down regulated the expression of Akt, cyclin D1, MDM2 and Bcl-2) [14]. DATS induce cell cycle inhibition and elevate levels of cyclin B1 and p21, and reduce levels of cyclin D1 in Capan-2 cells and H6C7 cells [30]. DATS causes cytotoxicity which is mediated by the generation of ROS and subsequent activation of the ASK1-JNK-Bim signal transduction pathway in human breast carcinoma MDA-MB-231 cells [44]. S-benzyl-cystein also-mediate cell cycle arrest and operate apoptosis that involves activation of mitochondrial-dependent caspase cascade through the p53 pathway in human gastric cancer SGC-7901 cells [50]. Tumor cells lacking p53 may continue to replicate damaged DNA and do not undergo apoptosis. Finally, cell cycle stalls in the cells with the damaged DNA.

DAS treatment of cultured cell also invokes changes typical of apoptosis such as morphological changes, DNA damage and fragmentation, dysfunction of mitochondria.

It also induce cytochrome c release and increase expression of pro-caspase-3 and -9. DAS also promote the release of AIF and Endo G from mitochondria in HeLa cells [64]. In fact G2/M checkpoint prevents cells containing damaged DNA from entering mitosis (M). Activated CDK1 (cdc2) bound to cyclin B promotes entry into M-phase. Wee1 and Myt1 kinases and cdc25 phosphatase competitively regulate CDK1 activity; Wee1 and Myt1 inhibit CDK1 and prevent entry into M-phase, while cdc25 removes inhibitory phosphates. DNA damage activates multiple kinases activity [68] mainly phosphorylate kinases Chk1/2 and tumor suppressor protein p53. Chk1/2 kinases stimulate Wee1 activity and inhibit cdc25C, preventing entry into M-phase [69]. Phosphorylation of p53 promotes dissociation between p53 and MDM2 and allows binding of the transcription factor to DNA (Figure 1 and Figure 2). SFN Sulforaphane (SFN) and NaHS activated p38 mitogen-activated protein kinases (MAPK) and c-Jun N-terminal kinase (JNK) [70]. Pre-treatment of PC-3 cells with methemoglobin decreased SFN-stimulated MAPK activities. DATS inhibits phosphatidylinositol 3'-kinase/Akt activation that, in turn, results in modulation of Bcl-2 family proteins, leading to enhanced apoptosis of T24 cells) [38]. Crushed garlic inhibit the proliferation of tumour cells by inducing G(2)/M cell cycle arrest and apoptosis) [71]. Similarly, ajoene also inhibit WHCO1 cell growth, inducing G(2)/M cell cycle arrest and apoptosis by caspase-3 activation. Here, vinyl group serves to enhance the anti-proliferation activity manifold [71].

2.6. Pro-inflammatory Cytokine Production

DADS inhibit the activation and nuclear translocation of p65, p50, and c-Rel subunits of nuclear factor (NF)-B and other transcription factors, such as c-fos, activated transcription factor-2, and cyclic adenosine monophosphate response element-binding protein, in B16F-10 melanoma cells [72]. In also does down regulation of pro-inflammatory cytokine production and gene expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF). DADS induces caspase-dependent apoptosis through a mitochondria-mediated intrinsic pathway in B16F-10 melanoma cells by activating p53 and caspase-3 gene expression and suppressing pro-inflammatory cytokines and NF-B-mediated Bcl-2 activation [72]. DADS treatment of colonic adenocarcinoma cells (HT-29) initiates a cascade of molecular events characteristic of apoptosis. These include a decrease in cellular proliferation, translocation of phosphatidylserine to the plasma-membrane outer-layer, activation of caspase-3 and

-9, genomic DNA fragmentation, and G(2)/M phase cell-cycle arrest [56]. Short-chain fatty acids (SCFAs), particularly butyrate (abundantly produced in the gut by bacterial fermentation of dietary polysaccharides), enhance colonic cell integrity but, in contrast, inhibit colonic cancer cell growth [56]. Combining DADS with butyrate augmented the apoptotic effect of butyrate on HT-29 cells. More likely, anti-cancerous properties of DADS are of greater benefit when supplied with other favorable dietary factors (short chain fatty acids/polysaccharides) that likewise reduce colonic tumor development [56].

DATS-mediated G2/M phase cell cycle arrest was found to be independent of reduced complex formation between cdk1 and cyclin B1, but it is correlated with delayed nuclear translocation of cdk1. DATS-mediated G2/M phase cell cycle arrest in DU145 cells results in differential kinetics of nuclear localization of cdk1 and cyclin B1 [73]. DAS treatment increased the accumulation of sub-G1 DNA which also increases the proportion of cells in the G2/M phase in a dose-dependent manner. In addition, DAS-induced apoptosis shows a decrease in the level of Bcl-2 expression and an increase in the level of Bax expression, and cytochrome c was remarkably released from mitochondrial into the cytosol by DAS. Furthermore, caspase-9 and caspase-3 were activated by DAS, and DAS cleaved PARP. DAS decreases cell proliferation and induce apoptosis via mitochondrial signaling pathway in ATC cells [74]. Here, both p38MAPK and caspase-3 are seems to be involved in the process of DADS-induced apoptosis in human HepG2 cells and interact with each other [75]. Diallyl trisulfide inhibits phorbol ester-induced tumor promotion, activation of AP-1, and expression of COX-2 in mouse skin by blocking JNK and Akt signaling [76].

2.7. Transcriptional Induction of p21

DADS treatment causes significant transcriptional induction of p21 in early hours of treatment, which is due to increased nuclear translocation of NF-κB and its specific binding to p21 promoter. It also induce transient G2/M phase arrest, which in later hours is lost leading to apoptosis via intrinsic mitochondria-mediated pathway through generation of reactive oxygen species followed by changes in mitochondrial membrane potential [47]. DADS induces reversible G2/M arrest through NF-κB mediated pathway in human leukemic cell lines, like U937, K562, and Jurkat, lacking wild type p53. However, failure of G2/M arrest make the system incapable of the damage repair system that leads to apoptosis [47]. Similarly, BGE and radiotherapy combination significantly induce Lewis cells' apoptosis in G2/M stage which decreases the expression of bcl-2, and up-regulated the expression of bax [10]. BGE sensitize the lung cancer Lewis cells to ionizing irradiation. This effect might be probably caused by changing the cell cycles and affecting expressions of bax and bcl-2 [10].

Spindle check points [77] are important to repair DNA damage and cancer therapy [78] (Figure 1 and Figure 2). These checkpoints ensure proper chromatid attachment prior to progression from metaphase to anaphase. Similarly, both SCF and APC/C protein complexes play prominent roles, with APC-cdc20 initiating the entry into anaphase by promoting ubiquitin-mediated degradation of multiple substrates, including cyclin B [79]. It indicates that regulatory proteins have important role in repairing of DNA level defects and occurrence of mutation in these proteins induce stressed in cancer prone cells. It is true that proteins like SCF and APC/C ubiquitin ligases also control cell growth [80]. There occurs a mutual regulation between the spindle check point and APC/C [81]. Organosulfur compounds modulate the activity of several metabolizing enzymes that activate (cytochrome P450s) or detoxify (glutathione S-transferases) carcinogens and inhibit the formation of DNA in different tissues [19].

3. Inhibition of Production of Reactive Oxygen Species

Reactive oxygen species (ROS) are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis [82]. These are also formed due to environmental stress mainly due to heat exposure, ionizing radiation [83], pollutants, tobacco, smoke, drugs and other agents. Their levels get increased dramatically if no antioxidant is used in the diet [82]. ROS reactive intermediates cause significant damage to cell structure and function. ROS are also formed intracellularly through multiple mechanisms and effect NADPH oxidase (NOX) complexes found in cell membranes, mitochondria, peroxisomes, and endoplasmic reticulum [84,85]. Cancer cells show extremely high ROS levels that shows weaker antioxidant defense system. Further, cellular ROS stress level elevated due to over drug dosage and cut down tolerability threshold, a induce cell death. [86] (Kong et al., 2006). On the other hand, normal cells show lower cellular stress and show very high capacity to fight ROS and its related changes [87]. Garlic chemical constituents showed very multiple therapeutic efficacy and are proved useful for preventing diseases associated with reactive oxygen species [88]. Aged garlic extract scavenges superoxide radicals [91] and induce apoptosis in cancer cells [89,90]. Diallyl trisulfide does inhibition of cell proliferation and migration in cancer cells and acts as chemopreventive drug [91]. The DATS-induced apoptosis in MDA-MB-231, MCF-7, and BRI-JM04 cells was found to be associated with reactive oxygen species (ROS) production [91]. However, over expression of Cu, Zn-superoxide dismutase (Cu,Zn-SOD) as well as Mn-SOD conferred significant protection against DATS-induced ROS production and apoptotic cell death in MDA-MB-231 and MCF-7 cells. The DATS treatment caused ROS generation, but not activation of Bax or Bak, in MCF-10A cells. Furthermore, the DATS-mediated inhibition of cell migration was significantly attenuated by Cu,Zn-SOD and Mn-SOD over expression in association with changes in levels of proteins involved in epithelial-mesenchymal transition. The DATS-mediated induction of heme oxygenase-1 was partially attenuated by overexpression of Mn-SOD. There seems a critical role for ROS in anticancer effects of DATS [91]. DATS also increases reactive oxygen species (ROS) production in primary colorectal cancer cells [43]. Diallyl tetrasulfide acts independently of reactive oxygen

species and tubulin represents one of its major cellular targets [42] (Kelkel et al., 2012). It induces production of reactive oxygen species (ROS) in normal cells similar to cancer cells in a time and dose dependent manner [92]. This is the main reason that both garlic and its derivatives are used as conventional drug in many countries for clinical treatment of cancer [93].

Garlic derivatives such as ajoene, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB) [94] and show antiproliferative effects) [95]. Allicin a sulfur compound breaks into diallyl disulfide (DADS) (66%), diallyl sulfide (DAS) (14%), diallyl trisulfide (9%), and sulfur dioxide [96]. Allicin easily reacts with amino acids and proteins, creating a -SH group. It binds to protein and fatty acids in the plasma membrane, and is trapped before absorption. It circulate in the blood stream [97] but did not detect in the blood sample after the ingesting raw garlic or pure allicin [94]. Allicin is metabolized into allyl methyl sulfoxide (AMS) and released into the breath after dietary intake [97]. Allicin shows antitumoral activity in murine lymphoma L5178Y [98] while DATS-induced apoptosis of human CNE2 cells [75] (Table 1). Allicin acts as an antioxidant in the blood and remove out all reactive oxygen species generated due to metabolic stress.

Garlic-derived compound S-allylmercaptocysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH(2)-terminal kinase 1 activation [99]. These components also show induction of apoptosis in breast cancer cell lines [100], and did attenuation of cell migration and induction of cell death in rat sarcoma cells [101]. The garlic-derived organosulfur component ajoene decreases basal cell carcinoma tumor size by inducing apoptosis [102]. Z-ajoene, a natural compound purified from garlic shows antimitotic and microtubule-interaction properties [103]. A protein fraction from aged garlic extract enhances cytotoxicity and proliferation of human lymphocytes mediated by interleukin-2 and concanavalin A [104]. More specifically, garlic preparations were found active against human tumor cell proliferation [105] and show antitumor [106] and anti-cancer effects [107]. *Allium* vegetables in the daily diet play important role in the prevention of cancer [108] (Table 1) (Figure 5b).

4. Control of DNA Damage

There are so many extracellular agents such as ionizing radiation, UV light, environmental chemicals and alkyltaing agents which cause DNA damage in cells that finally induce carcinogenesis. Many potential therapies are used to target carcinogenesis through a series of alternatives including plant-derived pharmaceutical agents. A large number of these herbal remedies are particularly derived from garlic and are used to treat cancer patients mainly for inhibition of cancer cell proliferation and growth. DATS inhibits cell growth of human melanoma A375 cells and basal cell carcinoma (BCC) cells by increasing the levels of intracellular reactive oxygen species (ROS) and DNA damage. It also induce G2/M arrest, endoplasmic reticulum (ER) stress, and mitochondria-mediated apoptosis, including the caspase-dependent and -independent pathways [109].

Similarly, DADS was found to be effective in inhibiting BaP-induced cell proliferation, cell cycle transitions, reactive oxygen species, and DNA damage in a normal cell line. It also inhibits environmentally induced breast cancer initiation [110]. Diallyl polysulfides play potential role as chemopreventive and therapeutic agents in cancer treatment due to their selective antiproliferative effects.

Some herbal medicines including garlic show potentially beneficial effects against cancer progression and ameliorate chemotherapy-induced toxicities [111]. As most of the anticancer drugs undergo Phase I and/or II metabolism and are substrates of P-glycoprotein, breast cancer resistance protein, multidrug resistance associated proteins, and/or other transporters. Therefore, induction and inhibition of these enzymes seems to be essential and mechanism of action of transporters is important for herb-anticancer drug interactions and action. However, endogenous mechanisms causing DNA damage such as spontaneous chemical hydrolysis, intracellular interaction with reactive oxygen groups and errors in replication and recombination can be restored by identifying correct and appropriate bio-molecules from garlic in form of a formulation (Figure 4). In addition chemical carcinogen induced changes such as depurination, de-amination, and reactive oxygen species including superoxide anion (an ion and a free radical) formation cab ne controlled by highly permeable oxygen bearing molecules from garlic. These compounds conjugated with certain cofactors and vitamins can finish reactive oxygen species formed by the effect of ionizing radiation. Both garlic oil and sulfur rich compounds can minimize the formation of free radicals due to oxidant reactions with proteins and other biomolecules. Diallyl sulfide (DAS), one of the main active constituents of garlic, causes growth inhibition of cancer cells *in vitro* and promotes immune responses *in vivo* [64].

Moreover, garlic based drugs or crude extracts prepared green garlic cloves induce apoptosis through the generation of reactive oxygen species in Hep3B human hepatocarcinoma cells [112]. These also target DNA repair mechanisms, and do restoration of a series of metabolic and signaling pathways. Temperature- and pressure-treated green garlic does inhibition of ENNG-induced pyloric stomach and small intestinal carcinogenesis in mice [113]. DNA damage also occurs due to mistakes in DNA replication or recombination cause strand breaks to be left in DNA. An incorrect proof reading also results in incorporation of mismatched bases. When damage occurs due to effect of carcinogens it remain repairable but long term natural genetic changes cannot be reverted. Possible potential anti-cancer agents from garlic such as diallyl trisulfide can check these events in eukaryotic cells due by imposing external stress on "checkpoints" of cell cycle. Drug may target effectively on proper timing of cellular events that can stop cell cycle or delay it. Thus, passage of anticarcinogenic effect through a checkpoint from one cell cycle phase will also require coordinated set of proteins that monitor cell growth and DNA integrity. Using natural products/molecules in a state of deficiency of transcription and translation of cell cycle proteins may activate DNA synthesis and protein translation. Garlic products can stop uncontrolled cell division or propagation of damaged DNA and save cells

from genomic instability that give rise tumorigenesis (Figure 3). Diallyl trisulfide does transcriptional repression and inhibition of nuclear translocation of androgen receptor in human prostate cancer cells [114]. It also inhibits estrogen receptor-α activity in human breast cancer cells [115] (Table 1). Thus garlic based herbal medicines have shown potentially beneficial effects on cancer progression and may ameliorate chemotherapy-induced toxicities (Figure 4).
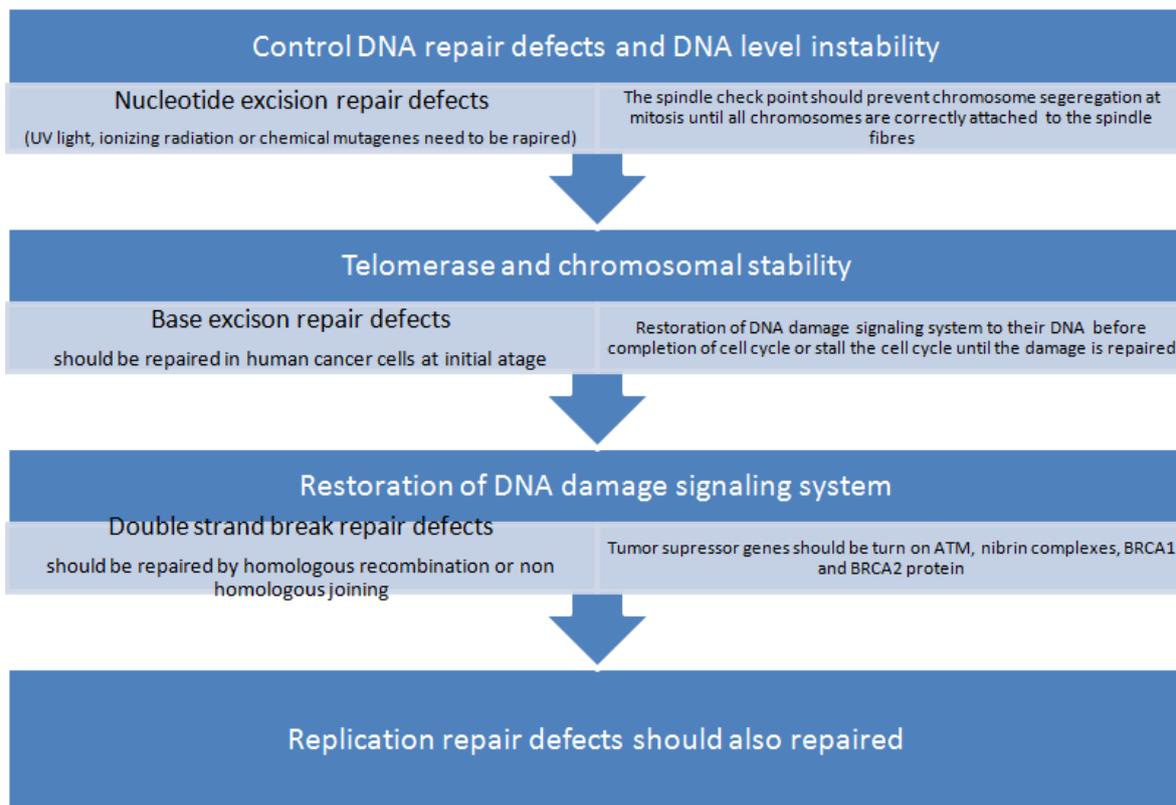
Figure 3. showing long strategy to repair DNA level defects and occurrence of mutation induced changes in stressed and cancer prone cells

Table 2. Nutritional value of garlic (*Allium sativum*) and its components

Garlic, raw	Nutritional value per 100 g (3.5 oz)	Metabolic functions
Nutrient	Types	Energy provider
Carbohydrates	33.06 g	Play key roles in the immune system, fertilization, preventing pathogenesis, blood clotting and development
Sugars	1gm	Sugar good for human health
Dietary fiber	2.1 gm	production of healthful compounds, increase bulk, soften stool, and shorten transit time through the intestinal tract
Fat	0.5 gm	Membrane synthsis, tissue
Protein	6.36 gm	Build body tissues
Vitamins		
Thiamine B1	17% (0.2 mg)	Synthesis of acetylcholine, carbohydrate metabolism
Riboflavin (B2)	(9%) (0.11gm)	Forms the coenzyme FAD
Niacin (B3)	5% (0.7gm)	Forms the coenzyme NAD
Pantothenic acid (B5)	12% (0.596)	Forms conezymes involved in amino acid metabolism
Vitamin B6	96% (1.235 mg)	Coenzyme in many chemical reactions
Folate (B9)	1% (3 μg)	Induce DNA synthesis
Vitamin C	38% (31.2 mg)	Promotes protein synthesis
Trace metals		
Calcium	18% (181 mg)	Mtrix component of bone tissue, cofactors of coagulation enzyme
Iron	13% (1.7 mg)	Constituent of hemoglobin
Magnesium	7% (25mg)	Activates ATPase
Manganese	80% (1.672)	Cofactor of kinases and isocitric decarboxylase
Phosphorus	22% (153mg)	Contituent of lipids, proteins, nucleic acids, sugar phosphates
Sodium	1% (17mg)	Membrane transporter
Zinc	12% (1.16 mg)	Co-factor of enzyme
Selenium	14.2 μg	Cofactor of glutathione peroxidase
Sulfur	16%	Antimcrobial

*μg = micrograms, mg = milligrams, IU = International units, Percentages are roughly approximated, **Garlic bulbs contain approximately 84.09% water, 13.38% organic matter, and 1.53% inorganic matter, while the leaves are 87.14% water, 11.27% organic matter, and 1.59% inorganic matter.

Table 3. Biological activity of chemical constituents isolated from Garlic (*Allium sativum)* and its associating species

Garlic component	Characteristics/attributes	Biological activity
	Chemo-preventive and anticancer	
Allicin	Multiple therapeutic agent	Found in raw garlic opens thermo-transient receptor potential channels that are responsible for the burning sense of heat in foods
Allicin derivatives	major contributors to the characteristic odor of garlic	Anti-mutagenic and anti-proliferative properties
Alliin	A sulfur-containing compound found in garlic	Flavor and aroma, as well as its potential health benefits, Prevents LPS-induced inflammation in 3T3-L1 adipocytes.
Vinyldithiins	A sulfur-containing compound found in garlic	Strong antioxidant , control several signaling pathways, including the inflammatory and apoptotic ones, inhibit matrix metalloproteinase activities and tightening tight junctions
Proteins, minerals, saponins, flavonoids, enzymes, B vitamins	Non sulfur compounds	Anticarcinogenic
Allixin mainly phytoalexin	A nonsulfur, with a γ-pyrone skeleton structure	Antioxidant, show antimicrobial antitumor promoting effects, inhibition of aflatoxin B2 DNA binding, and neurotrophic effects, cancer prevention.
Allicin	Organosulfur compound	Strong odor a stinking rose, repellent action
Allyl methyl sulfide	After food intake garlic's strong-smelling sulfur compounds are metabolized, forming allyl methyl sulfide.	Abundant sulfur compounds in garlic responsible for turning garlic green or blue during pickling and cooking. Act as mosquito repellent.
	Growth inhibitors of cancer cells	
Diallyl sulfide	A garlic-derived organosulfur compound is used to prevent growth of pancreatic cancer cells	Prevents tumor progression and promotes apoptosis in ectopic glioblastoma xenograft, prevent growth of pancreatic cancer cells, promotes cell-cycle arrest through the p53 expression. It also triggers induction of apoptosis via caspase- and mitochondria-dependent signaling pathways in human cervical cancer Ca Ski cells. It is found more toxic to prostate cancer cells PC-3, human retina pigment epithelial cells (ARPE-19) and HCT116 cells It induces apoptosis in MCF7 human breast cancer cells.
diallyl trisulfide (DATS),	Cytotoxic to prostate cancer cells	Highly cytotoxic to prostate cancer cells, inhibits cell proliferation by triggering either cell cycle arrest or apoptosis, shows pro-apoptotic activity regulated by a caspase-dependent cascade through the activation of both intrinsic and extrinsic signaling pathways, or mediated through the blocking of PI3K/Akt and the activation of the JNK pathway
Diallylpolysulfides	organosulfur compound	diallylpolysulfides induce growth arrest and apoptosis in cells
diallyltetrasulfide (DATTS)	organosulfur compound	Induce mitotic arrest to apoptosis
gamma-glutamylcysteines, Allylcysteine sulfoxide (alliin)	organosulfur compound	Generate hot odor
Allyl sulfides	organosulfur compound	Inhibit cell growth of skin cancer cells through induction of DNA damage mediated G2/M arrest and apoptosis.
S-allylcysteine	organosulfur compound	acts on human ovarian cancer cells *in*
S-allylmercaptocysteine	organosulfur compound	induce cell cycle arrest and reduce the risk of various types of human cancer.
S-alkenylmercaptocysteine	organosulfur compound	Induce apoptosis in pancreatic cells
Garlicnins B(1), C(1), and D	Sulfur containing compounds	Highly toxic to cancer cells
S-allylmercaptocysteine	active organosulfur compounds	Highly toxic to cancer cells
S-allylcysteine,	active organosulfur compounds	Suppresses proliferation and induces apoptosis in human ovarian cancer cells in vitro. reduced the migration of A2780 cells and decreases the protein expression of Wnt5a, p-AKT and c-Jun proteins which are involved in proliferation and metastasis
Polysulfanes	Sulfur containing compounds	Possess antimicrobial, chemopreventive and anticancer properties.
	Cell-cycle regulation/cell cycle arrest / apoptosis	
Garlic oil	Steam distillation of garlic bulbs, leaf oil is also extracted	Inhibits the proliferation of AsPC-1, PANC-1, and Mia PaCa-2 cells and induced programmed cell death, cell cycle arrest, and show pro-apoptosis effects on AsPC-1 cells in a dose and time dependent manner *in vitro*
Garlic water extract	Maceration of garlic bulbs	causes cell-cycle dysregulation and control signal transduction pathways leading to cell cycle arrest and apoptosis induction in cancer cells
Black garlic extracts (BGE)	Homogenization of garlic bulbs	check proliferation of lung cancer while green garlic possesses enough potential to control colorectal cancer and other types of carcinoma
Aged black garlic extract (ABGE)	Long storage effect on garlic bulbs	Highly beneficial in preventing or inhibiting oncogenesis
S-benzyl-cysteine	Sulfur containing compounds	SBC exerts cytotoxic activity involving activation of mitochondrial-dependent apoptosis through p53 and Bax/Bcl-2 pathways in human gastric cancer SGC-7901 cells
DATS, DADS, ajoene, and S-allylmercaptocysteine (SAMC)	Sulfur containing compounds	induce cell cycle arrest and apoptosis, regulating ion channels, modulating Akt signaling pathways, histone deacetylase inhibition, and cytochrome P450 inhibition
Ajoene	Sulfur containing compounds	shows antimitotic and microtubule-interaction properties. induces apoptosis in human promyeloleukemic cells,
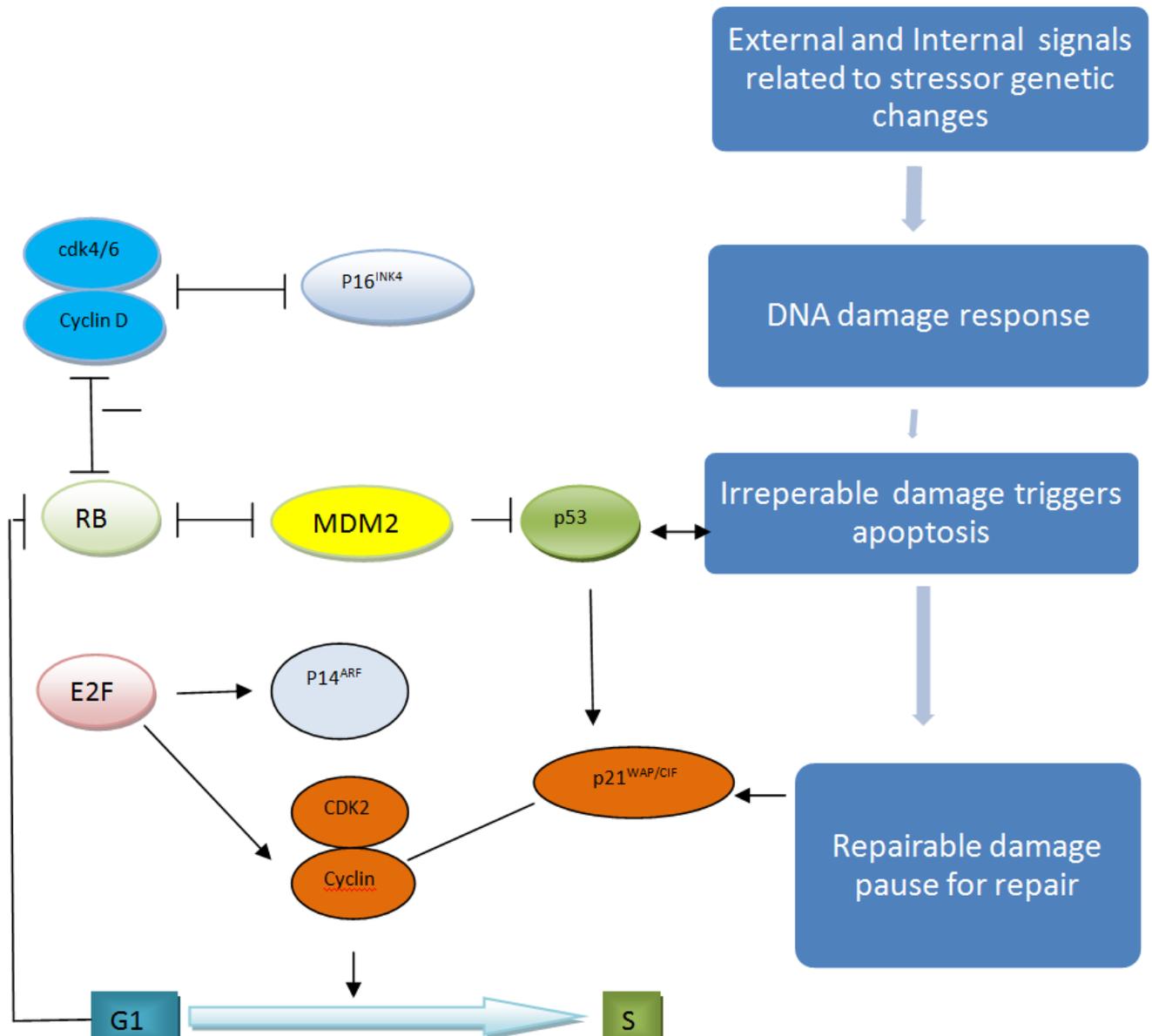
Figure 4. Showing control of cell cycle progression and various steps of DNA damage due to External and Internal signals related to stressor genetic changes

DNA damage resulted in an impaired cellular response due to hypersensitivity generated by carcinogenic agents. If this DNA damage remains defective for longer period it results in mutations that lead to altered protein functions and generate impaired cellular responses. Normal cells react to DNA damage by stalling progress through the cell cycle at a check point until the damage has been repaired or triggering apoptosis if the damage remains un-reparable. All this is initiated by ATM protein briefly which sense DNA damage and relays the signal to the p53 protein which is synthesized under regulation of p53 gene that is known as the guardian of the genome [116]. It plays a critical role in intrinsic tumor suppression via two mechanisms either through cell cycle arrest or by induction of apoptosis. A variety of triggers such as DNA damage, oncogene activation, and telomere erosion can lead to the activation of p53. An increase in p53 transcription inhibits cell cycling. Possibly p53 may be knocked out by mutation or by the action of an inhibitor such as the MDM2 gene product which binds p53 and targets for its degradation. S-Allylmercaptocysteine

(CySSA) from garlic increases in phospho-p53, Bax and Bad levels which indicate that apoptosis occurs via the mitochondrial pathway [13] (Figure 4). SAMC-induced apoptosis is associated with the Id-1 pathway and that the inactivation of Id-1 enhances the ability of SAMC to inhibit the survival, invasion and migration of bladder cancer cells [117].

DADS induces apoptosis in the MCF-7 breast-cancer cell line through interfering with cell-cycle growth phases in a way that increases the sub-G(0) population and substantially halts DNA synthesis [56]. It also induces phosphatidylserine translocation from the inner to the outer leaflet of the plasma membrane and activates caspase-3 [56]. It also modulates the cellular levels of Bax, Bcl-2, Bcl-xL, and Bcl-w in a dose-dependent manner, and show involvement of Bcl-2 family proteins in apoptosis [56]. Diallyl trisulfide (DATS) induces apoptosis and inhibits the growth of many cancer cell lines. Mainly it induces apoptotic cell death in human primary colorectal cancer cells through a mitochondria-dependent caspase cascade signaling pathway through a significant

decrease of the anti-apoptotic Bcl-2 that resulted in up-regulation of the ratio of Bax/Bcl-2 and the activity of caspase-3, -8, and -9 [44,46]. It also increases in the levels of cytochrome c, Apaf-1, AIF and caspase-3 and caspase-9 activity [44]. Eventually, DATS induce the apoptosis and inhibit the cancer cell proliferation in a concentration- and time-dependent manner [46]. Exposure to DATS additionally induced endogenous endoplasmic reticulum stress markers and intracellular Ca^{2+} mobilization, upregulation of Bip/GRP78 and CHOP/GADD153, and activation of caspase-4 [46]. It also makes an increase in reactive oxygen species (ROS) production in primary colorectal cancer cells but decrease in the level of $\Delta\Psi m$ was associated with an increase in the Bax/Bcl-2 ratio which led to activation of caspase-9 and -3. No doubt, DATS can offer protection against chemically-induced neoplasia as well as oncogene-driven spontaneous cancer development in experimental animals. DATS do alteration in carcinogen-metabolizing enzymes, operate cell cycle arrest, and impose induction of apoptotic cell death, suppression of oncogenic signal transduction pathways, and inhibition of neoangiogenesis [118]. DATS did not affect the activity of sulfurtransferases and lowered sulfane sulfur level in HepG2 cells. But is possible that sulfane sulfur containing DATS may be bioreduced in cancer cells to hydroperthiol that leads to H_2O_2 generation, thereby influencing transmission of signals regulating cell proliferation and apoptosis [119]. Similarly, allicin also inhibit the cell viability of U87MG human glioma cells in a dose- and time-dependent manner. Allicin-induced inhibition of cell viability was due to apoptosis of cells. The mechanisms of apoptosis were found to involve the mitochondrial pathway of Bcl-2/Bax, the MAPK/ERK signaling pathway and antioxidant enzyme systems [37] (Figure 4).

DATS also exerts chemopreventive potential via ER stress and the mitochondrial pathway in BCC cells [41]. DAS, DADS, and DATS induce down regulation expression of PI3K, Ras, MEKK3, MKK7, ERK1/2, JNK1/2, and p38 and then lead to the inhibition of MMP-2, -7, and -9. DAS, DADS, and DATS inhibited NF-κB and COX-2 for leading to the inhibition of cell proliferation [120]. DADS inhibit the activities of matrix metalloprotease (MMP)-2 and -9 in AGS cells in dose-dependent manner. It decreases expression of their mRNA and proteins and repressed the levels of claudin proteins (claudin-2, -3, and -4 [121]. In addition, both diallyl tri- and tetrasulfide are reported as strong inducers of an early mitotic arrest and subsequent apoptosis [42]. Diallyl tetrasulfide acts independently of reactive oxygen species and tubulin represents one of its major cellular targets. Tubulin depolymerization prevents the formation of normal spindle microtubules, thereby leading to G2/M arrest. Moreover, c-jun N-terminal kinase, which is activated early in response to diallyl tetrasulfide treatment, mediates multisite phosphorylation and subsequent proteolysis of the anti-apoptotic protein B-cell lymphoma 2 [42] (Figure 4).

SAC suppress the proliferation of PC-3 cells and led to cell cycle arrest at the G0/G1 phases, as well as inducing cell apoptosis which was accompanied by the decreased expression of Bcl-2 and increased expression of Bax and caspase 8 [122]. This chemopreventive effect of SAC may

be associated with the suppression of carcinogenesis factors such as N-methylpurine DNA glycosylase and OPN. SAC also significantly suppresses the phosphorylation of Akt, mammalian target of rapamycin, inhibitor of κBα and extracellular signal-regulated kinase 1/2 in tumour tissues [122]. But SAC-mediated suppression of cyclin D1 protein was associated with an augmented expression of the cell-cycle inhibitor p16(Ink4). SAC also inhibit expression of cyclo-oxygenase-2, vimentin and NF-κB p65 (RelA) [123]. In addition, both SFN Sulforaphane (SFN) and NaHS activate p38 mitogen-activate protein kinases (MAPK) and c-Jun N-terminal kinase (JNK) [124]. Thus, pre-treatment of PC-3 cells with methemoglobin decreases SFN-stimulated MAPK activities. Suppression of both p38 MAPK and JNK reversed H(2)S- or SFN-reduced viability of PC-3 cells. H(2)S mediates the inhibitory effect of SFN on the proliferation of PC-3 cells. Therefore, H(2)S-releasing diet or drug might be beneficial in the treatment of prostate cancer [124]. SAC also hinder the migration and invasion of MHCC97L cells corresponding with up-regulation of E-cadherin and down-regulation of VEGF. It also significantly induced apoptosis and necrosis of MHCC97L cells through suppressing Bcl-xL and Bcl-2 as well as activating caspase-3 and caspase-9) [124,125]. In addition, SAC could significantly induce the S phase arrest of MHCC97L cells together with down-regulation of cdc25c, cdc2 and cyclin B1 [125]. Similarly, phytoalexins show specific targets in cancer cells that include signal transduction pathways, transcription factors, cell cycle checkpoints, intrinsic and extrinsic apoptotic pathways, cell invasion and matrix metalloproteinase, nuclear receptors, and the phase II detoxification pathway [126]. Thiacremonone inhibits tumor growth accompanied with the reduction of PRDX6 expression and glutathione peroxidase activity, but its shows an increase in expression of cleaved caspase-3, -8, -9, Bax, p21 and p53) [21]. Garlic extract induce cytotoxic and apoptotic effects in HL-60 cells that is mediated by oxidative stress [127], phosphatidylserine externalization, caspase-3 activation, and nucleosomal DNA fragmentation and formation of MDA, a by-product of lipid peroxidation and biomarker of oxidative stress [127] (Figure 4).

5. Inhibition of Carcinogenesis

Diallyl trisulfide inhibits benzo(a)pyrene-induced precancerous carcinogenesis in MCF-10A cells [128] and induce apoptosis in human basal cell carcinoma cells via endoplasmic reticulum stress and the mitochondrial pathway [39]. Diallyl sulfide, diallyl disulfide and diallyl trisulfide affect drug resistant gene expression in colo 205 human colon cancer cells *in vitro* and *in vivo* [120] (Table 1). Diallyl trisulfide (DATS) is used to prevent growth of pancreatic [31] and prostate cancer cells PC-3) [129], and HCT116 cells [143]. Diallyl trisulfide (DATS) inhibits mouse colon tumor in mouse CT-26 cells allograft model in vivo) [64]. Diallyl disulfide inhibits the proliferation of HT-29 human colon cancer cells by inducing differentially expressed genes [130]. Diallyl sulfide inhibited murine WEHI-3 leukemia cells in BALB/c mice *in vitro* and *in vivo* [131] and induces molecular

mechanisms which target cancer chemoprevention [132]. It also induces Ca2+ mobilization in human colon cancer cell line SW480 [133] (Table 1).

Fresh garlic extract induces growth arrest and morphological differentiation of MCF7 Breast cancer Cells [134]. Sulfide compounds found in dietary garlic supplements [135] increase C reactive protein concentration [136] and enhance therapeutic potential against infectious agents [137]. These effect cell proliferation, caspase 3 activities, thiol levels and anaerobic sulfur metabolism [119] and modulate peroxisome proliferation activated receptor gamma co-activator 1 alpha in human hepatoblastoma HepG2 cells [57]. Garlic oil shows protective effects on hepatocarcinoma induced by N-nitrosodiethylamine in rats [138]. Similarly, S-allylcysteine (SAC), a garlic derivative suppresses proliferation and metastasis of hepatocellular carcinoma [128] and induces cell cycle arrest and apoptosis in androgen-independent human prostate cancer cells [122]. Oxidative species and S-glutathionyl conjugates aids in the apoptosis induction by allyl thiosulfate [139].

The aged garlic extract (AGE) contains S-allyl-L-cysteine (SAC) which is a potential therapeutic agents for Alzheimer's disease [140]. It is a major water- soluble compound found in aged garlic exerts cytotoxic activity by involving activation of mitochondrial-dependent apoptosis through p53 and Bax/Bcl-2 pathways in human gastric cancer SGC-7901 cells [44]. It inhibits tumor progression and the epithelial-mesenchymal transition in mouse xenograft model of oral cancer [123]. It also shows ameliorating effects against Aβ-induced neurotoxicity and cognitive impairment [141]. It induces inhibition of gastric cancer cell growth *in vitro* and *in vivo* [39]. Similarly, S-benzyl-cysteine (SBC) a structural analog of S-allylcysteine (SAC), mediates cell cycle arrest and apoptosis that involves activation of mitochondrial-dependent caspase cascade through the p53 pathway in human gastric cancer SGC-7901 cells [42]. S-allylmercaptocysteine (SAMC) shows potent therapeutic effect on human cancer cells by causing apoptosis [10,142]. Hydrogen sulfide mediates the anti-survival effect of sulforaphane on human prostate cancer cells [124]. Garlic-derived allyl sulfides cause inhibition of skin cancer progression [39] (Wang et al., 2012) and show modifying effect on colon carcinogenesis in C57BL/6J-ApcMin/+ mice [143].

However, intake of fruits and vegetable reduce the risk of gastric cancer [144,145] and restore health and reduces the chances of occurrence of physiological diseases [146]. Both garlic and garlic-derived organic sulfur compounds are used for controlling breast cancer [147,148]. Besides, sulfur compounds garlic also contains saponins, flavonoids vitamins, minerals, hence daily consumption of garlic vegetables daily can lower down the risk of hematologic malignancies and other lifestyle diseases [149,150,151]. Garlic dietary supplements also contain multivitamins and folic acid which show strong anticancer effects in breast cancer survivors [152]. These lower down oxidative stress and induce apoptotic mechanisms in acute promyelocytic leukemia [127,170]. Green garlic shows cardioprotective effects [153] (Alkreathy et al., 2012) and is used as a complementary and alternative medicines for treatment of breast cancer [154]. It shows potential beneficial effects in oncohematology [155] (Table 1).

Consumption of s-allylcysteine inhibits the growth of human non-small-cell lung carcinoma in a mouse xenograft model [156]. Similarly, S-allylmercaptocysteine effectively inhibits the proliferation of colorectal cancer cells under *in vitro* and *in vivo* conditions [157]. S-allylmercapto-L-cysteine shows anticancer activity in implanted tumor of human gastric cancer cell [44] (Table 1). S-allyl cysteine attenuates oxidative stress associated cognitive impairment and neurodegeneration in mouse model of streptozotocin-induced experimental dementia of Alzheimer's type [158]. There occurs a relationship between lipophilicity and inhibitory activity against cancer cell growth of nine kinds of alk(en)yl trisulfides with different side chains [159] (Table 1). Garlic oil suppressed the hematological disorders induced by chemotherapy and radiotherapy in tumor-bearing mice [160]. Thiacremonone, a sulfur compound isolated from garlic, attenuates lipid accumulation partially mediated via AMPK activation in 3T3-L1 adipocytes [124]. Artesunate combined with allicin shows synergistic anticancer effect in osteosarcoma cell line in vitro and *in vivo* [161]. Moreover, garlic components showed antioxidant activity [172] inhibit fibrosarcoma tumor growth in BALB/c mice positive [163].

Oil-soluble compounds such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS) and ajoene are more effective than water-soluble compounds in protection against cancer. DADS, a major organosulfur compound derived from garlic, decrease carcinogen-induced cancers in experimental animals and inhibit the proliferation of various types of cancer cells. Its mechanisms of action include: the activation of metabolizing enzymes that detoxify carcinogens; suppression of the formation of DNA adducts; antioxidant effects; regulation of cell-cycle arrest; induction of apoptosis and differentiation; histone modification; and inhibition of angiogenesis and invasion [164]. No doubt DAC inhibitory effects of garlic organosulfur compounds might play a role in primary cancer prevention at doses achievable by human diet. [165]. However, the decrease of the peripheral total white blood cells (WBCs) count induced by CTX/radiation was significantly suppressed by GO co-treatment. No doubt, GO consumption may benefit for the cancer patients receiving chemotherapy or radiotherapy [160].

5.1. Inhibition of Cancer Cell Proliferation and Invasion

Diallyl disulfide shows anti-invasive activity through tightening of tight junctions and display inhibition of matrix metalloproteinase activitiy in LNCaP prostate cancer cells [74]. Diallyl trisulfide does inhibition of cell proliferation and migration in cancer cells and acts as chemopreventive drug [91]. Similarly, natural tetrasulfides also show antiproliferative effect of in human breast cancer cells that is mediated through the inhibition of the cell division cycle 25 phosphatases [166]. Garlic reduces the risk of colorectal polyps [167] and accelerates red blood cell turnover and splenic erythropoietic gene expression in mice [168]. Boiled garlic does inhibition of 1, 2-dimethylhydrazine-induced mucin-depleted foci and O^6-methylguanine DNA adducts in the rat colorectum

[169] while crude garlic extract and its fractions showed cytotoxic effect [170] on malignant and nonmalignant cell lines [171]. Garlic provides great protection against physiological threats [172] and lower downs risk of gastric cancer [10,173]. DAS also does growth inhibition and induce apoptosis in anaplastic thyroid cancer cells by mitochondrial signaling pathway[74] while allyl sulfur compounds causes cellular detoxification of carcinogens and is used in cancer therapy [174]. Garlic phytochemicals counteract the cardiotoxic side effects of cancer chemotherapy [175] (Table 1). SAMC inhibit the survival, invasion and migration of bladder cancer cells [117].
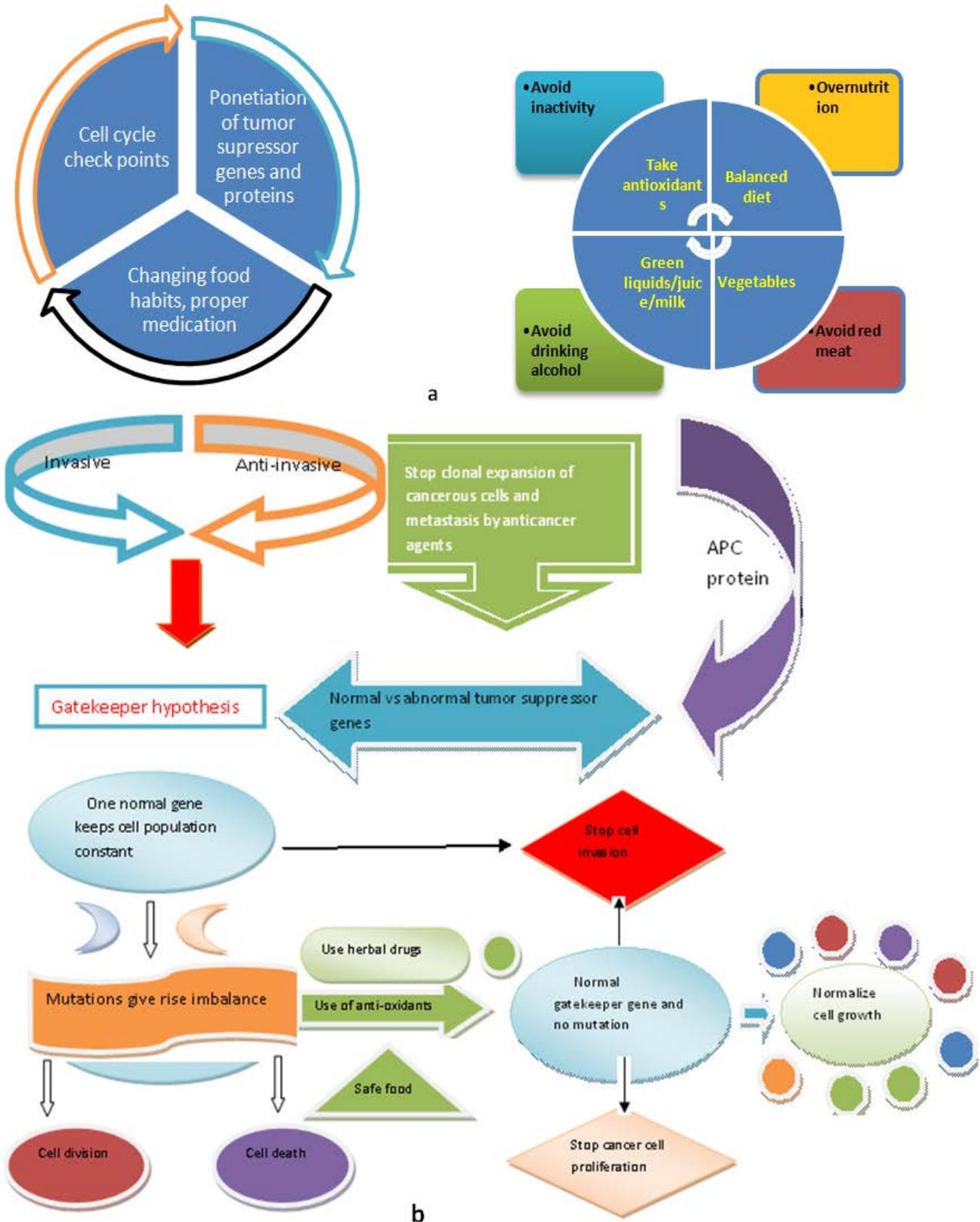

Figure 5. a. Control of cancer progression by genetic, molecular and dietary food habits, b Control of mutation by gatekeeper gene

5.2. Chemopreventive/antitumor Effects

Garlic derived natural products showed chemopreventive effects and successfully prevent colorectal, [11] and prostate cancer [176]. These also show onco-cardiological prevention [177] reduce the risk of invasive cervical) [75] and ovarian carcinoma [16]. Diallyl trisulfide induces Bcl-2 and caspase-3-dependent apoptosis via downregulation of Akt phosphorylation in human T24 bladder cancer cells) [39] (Wang et al., 2010). Garlic powder supplemented diet shows chemopreventive effects in diethylnitrosamine-induced rat hepatocarcinogenesis) [178] (Figure 5a). It also shows chemoprotection against cyclophosphamide toxicity in mice [179] and protects against adriamycin induced alterations in mouse red blood cells (Thabrew et al., 2000) [180]. Garlic inhibits methylcholanthrene-induced carcinogenesis in the uterine cervix of mice [181] and shows prevention of 4-nitroquinoline 1-oxide-induced rat tongue [182] and hamster cheek pouch carcinogenesis. It shows protective effects against bromobenzene toxicity to precision cut rat liver slices [183,184]. Diallyl disulfide shows prevention of chemically induced skin tumor development [185]. Organosulfur compounds from garlic and onions protect from benzo[a]pyrene-induced neoplasia and glutathione S-transferase activity in the mouse [186] (Table 1). Onion and garlic oils and extracts inhibit tumor promotion in experimental animals [187,188]. Diallyl sulfide, a flavor component of garlic (Allium sativum), inhibits dimethylhydrazine-induced colon cancer [189].

6. Conclusion

Organosulfur compound isolated from garlic have been shown to have anticancer activity both *in vitro* and *in vivo*. These are also used as pharmaceuticals for treatment of acute and chronic diseases and are thought to be powerful instrument in maintaining health as medicine and nutraceutical. Garlic components do restoration of cell death pathways via the targeting of mitochondrial proteins. These can normalize the central pathway which can frequently impair cancer cells and contributes to the development of resistance to conventional chemotherapy. Garlic derived molecules can be formulated and designed to target oxidants or pro-oxidants and to activate pro-apoptotic proteins as well as to block these proteins. Garlic based herbal products and targeted molecules can find good solutions for clinical management of various cancer types. Today garlic based herbal medicines are attracting public health authorities, pharmaceutical industries because of its larger use in prevention and treatment of so many diseases and disorders. Garlic derived dietary supplements are highly demanded by nutritionists, physicians, food technologists and food chemists. No doubt garlic and its derived herbal products are providing optimal health and quality of life.

Conflict of Interests

Author has no conflict of interests. The author alone is responsible for the content and writing of the paper.

Abbreviation

DAS=diallyl sulfide
DADS= diallyl disulfide
DATS= diallyl trisulfide
SBC=S-benzyl-cysteine
SAMC =S-allylmercaptocysteine
SAC =S-allylcysteine (SAC)
ROS =reactive oxygen species
BSO =buthionine sulfoximine
CySS=S-alkenylmercaptocysteine
caspase=cysteine-aspartic acid protease.

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