Discussion
Cervical cancer is the number one cancer in Indian women (Gajalakshmi et al., 2001) with a five year survival rate of 47% in Kerala (Sankaranarayanan et al., 1995), 41% in Bangalore (Kumaraswamy et al., 1998) and 60% in Chennai (Gajalakshmi et al., 2000), posing a major public health problem. The standard first line treatment for invasive cervical cancer is usually cisplatin (Platinol) and 5-fluorouracil (5-FU, Adrucil, Efudex) used in combination and in addition to radiation. Recurrent and late stage cervical cancers are often treated with a combination of platinum, bleomycin, methotrexate, and 5-FU. Chemotherapy is administered intravenously, through injection, or in pill form and side effects often accompany treatment and may be severe; which can include nausea, vomiting, diarrhea, and leukopenia (low white blood cell count). Most of these side effects are a result of drug toxicity to healthy tissues such as skin, hair follicles, and epithelial cells that line the digestive tract and patient tumors can develop resistance to these therapies as well (Stewart and Viswanathan, 2006; Muggia et al., 2004). Hence, there is a need to develop alternate, safer, less toxic, and more specific therapies for uterine cervical cancer.

Some of the most successful currently used anticancer drugs are extracts or derivatives of compounds from plants or other natural products. These include the advance class of drugs known as taxanes (Paclitaxel, Docetaxel) that come from the yew tree (Taxus baccata), and the vinca alkaloids (Vincristine, Vinblastine, Vinorelbine) which are derivatives from the periwinkle plant (Catharanthus roseus). These compounds affect the growth of all rapidly dividing cells, whether normal or malignant, and like other drugs, are associated with significant toxicity and side effects. Millions of research dollars each year go to the discovery of new natural products with limited side effects that may be used as effective antitumor agents. Epidemiological observations and laboratory studies, both in cell culture and animal models have indicated anticarcinogenic potential of garlic and its organosulfur constituents, which has been traditionally used for varied human ailments around the world.

Garlic (Allium sativum L.) is a widely consumed herb in foodstuffs and medicines. It has been reported to reduce chemically induced esophageal, skin, pulmonary, stomach, breast, uterine cervix, colon, mammary and lung tumor (Hussain et al., 1990; Hong et al., 2000; Milner, 2001; Nakagawa et al., 2001; Takezaki et al., 2001; Limuro et al., 2002; Ku
et al., 2002). The anticarcinogenic/antitumorgenic effects of garlic and its organosulfur compounds have been attributed not only to the modulation of the antioxidation and/or drug-metabolizing enzyme system, but also to the reduction of cell proliferation and induction of apoptosis in tumor cells (Dirsch et al., 1998; Kwon et al., 2002; Pinto et al., 2001; Shirin et al., 2001; Sigounas et al., 1997; Ledezma et al., 2004; Knowles and Milner, 2001; Oommen et al., 2004; Robert et al., 2001; Wu et al., 2001, 2002; Milner, 2001; Siegel et al., 2004; Tsao and Yin, 2001; Kwon et al., 2003).

Allicin, the major component present in freshly crushed garlic, is one of the most biologically active compounds of garlic (Ali et al., 2000; Hasan et al., 2006; Hasan et al., 2007). Allicin is formed from alliin by the action of allinase and gets metabolized rapidly into diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), Ajoene, S-allylmercaptopcysteine (SAMC), S-allylcysteine (SAC) and vinyl dithiines (Dirsch et al., 1998; Hirsch et al., 2000; Sigounas et al., 1997; Sundaram and Milner, 1993; Welch et al., 1992). Allicin is readily permeable through phospholipid membranes aiding its biological activity, but its unstable nature raises the question whether all the effects of Allicin observed are due to Allicin itself or its metabolites (Hirsch et al., 2000; Miron et al., 2000). Indeed, its metabolites, SAMC (Sigounas et al., 1997; Pinto et al., 1997), DAS, DADS, DATS (Hageman et al., 1997; Sundaram and Milner, 1993), ajoene (Scharfenberg et al., 1990; Dirsch et al., 1998), and SAC (Welch et al., 1992), have been shown to possess an anticancer activity, which varies depending on the agent and the experimental model. There is evidence that Allicin is formed from its metabolite, DADS, in human liver microsomes indicating that Allicin may also be acting intracellularly (Teyssier et al., 1999).

Many changes occur in cancer cells as they become tumorigenic, including release from growth factor regulation, loss of contact inhibition, increased proteolysis, avoidance of immune surveillance, acquisition of immortality, promotion of angiogenesis and metastasis (Hartwell, 1997) and it is classically defined as uncontrolled cellular division. The cell cycle is controlled by the temporally and spatially fluctuating activities of protein complexes and its progression requires the regulation of different cyclins, cyclin-dependent kinases (Cdks), Cdk inhibitors and dephosphorylation of several regulatory proteins (Murray and Hunt, 1993; Morgan, 1995; Molinari, 2000; Murray, 2004).
Cellular stresses may activate signal transduction pathways, referred to as checkpoints, which lead to cell cycle arrest and these checkpoints ensure completion of phase specific events and protect against genomic instability or, in cases where the damage is too severe, switch the cell fate to programmed cell death (Molinari, 2000; Murray, 2004). Many anticancer treatments initially cause perturbations in cell cycle progression and the interrupted phase depends on the genetic background of the cell as well as the mode of action of a given treatment. Earlier studies have shown that garlic-derived OSCs can suppress growth of cancer cells of different anatomical locations in association with cell cycle arrest (Knowles and Milner, 2000; Xiao et al., 2005; Herman-Antosiewicz and Singh, 2005; Herman-Antosiewicz, 2007; Antosiewicz, 2006). Garlic-derived OSCs have also been shown to modulate a number of key elements in cellular signal transduction pathways linked to the apoptotic process (Hong et al., 2000; Nakagawa et al., 2001; Xiao et al., 2004; Xiao et al., 2006; Karmakar et al., 2007; Kim et al., 2007).

So, in the present study, first we investigated the effect of Allicin on HPV 18 positive cervical cancer HeLa cell line. Our results illustrated that Allicin strongly suppressed the proliferation of HeLa cells in a dose- and time-dependent manner. Furthermore, we also observed Allicin-induced cell cycle arrest in HeLa cells at G0/G1 phase along with a sub-G0/G1 population suggesting that certain amount of cells underwent apoptosis which was further confirmed by the results of FITC-Annexin V Assay. Thus, antiproliferative action of Allicin in HeLa cells could be attributed to cell cycle arrest and apoptosis. Therefore, the above combined results demonstrated that Allicin treatment caused a significant decrease in the percentage of viable cell along with a concurrent induction of apoptosis in cervical cancer HeLa cells; however, the percentage of apoptotic cells did not coincide with the percent of viable cells. This difference in cell viability and apoptosis could be due to cell cycle arrest at G0/G1 phase induced by Allicin.

Previous studies have already shown the effect of Allicin and its metabolites on cancer cell proliferation, cell cycle regulation and apoptosis (Sundaram and Milner, 1993; Zheng et al., 1997; Hirsch et al., 2000; Iciek et al., 2001). Allicin has been reported to induce growth inhibition, apoptosis and cell cycle arrest at G2/M phase in human promyelocytic leukemia-derived HL60 cells and human myelomonocytic U937 cells (Miron et al., 2008). In vitro studies on human mammary MCF-7 cancer cells revealed that
antiproliferative activity of Allicin is accompanied by accumulation of cells in the regulatory checkpoints, G0/G1 and G2/M, of the cell cycle (Hirsch et al., 2000). Allicin can inhibit the cell proliferation of erythroleukemia cell lines, HL-60 and K562, and arrest their cell cycle at S phase (Zheng et al., 1997). Allicin can also exhibit the antiproliferation effect on human mammary (MCF-7), endometrial (Ishikawa) and colon (HT-29) cancer cells, and accumulate cells at the G0/G1 and G2/M phases of the cell cycle in MCF-7 cells (Hirsch et al., 2000). Interestingly, other ally sulfur compounds also cause similar effects (Knowles and Milner, 2001). Allicin metabolite DADS could significantly inhibit the proliferation of HepG2 cells (Iciek et al., 2001). DAS, DADS and DATS significantly suppressed colon tumor cell proliferation (Knowles and Milner, 1998). DADS significantly increased the cell cycle arrest at G2/M phase of human colon tumor cells (HCT-15) via the inhibition of Cdc2 (cyclin-dependent kinase 1, Cdk1) activity (Knowles and Milner, 1998). DADS also induced cell cycle arrest and apoptosis in human A549 lung carcinoma cells (Wu et al., 2005). DADS and DATS significantly modulated the expressions of cyclin and Cdk to induce J5 cells arrested at G2/M phase (Wu et al., 2004). Ajoene and Diallyl sulfide blocked HL-60 human myeloid leukemia cells at the G2/M phase (Dirsch et al., 1998; Zheng et al., 1997). Allicin has been shown to induce apoptosis in gastric cancer SGC-7901 cells and the cause of apoptosis was related to decreased telomerase activity, an enzyme which Allicin inhibits in a time- and dose-dependent manner (Sun and Wang, 2003). Allicin-induced apoptosis has also been reported in human cervical cancer SiHa cells and mouse fibroblast-like L-929 cells, manifested through the appearance of characteristic apoptotic morphological changes in apoptotic bodies, through DNA fragmentation, and activation of caspases-8, -9 and -3 (Oommen et al., 2004). Allicin was also shown to induce apoptosis in human epithelial carcinoma through a caspase-independent pathway, mediated by the release of apoptotic-inducing factor (AIF) from mitochondria and protein kinase A (PKA) activation (Park et al., 2005).

Apoptosis plays a crucial role in the regulation of the development and homeostasis of multicellular organisms (Yan and Shi, 2005; Steller, 1995). Balance between cancer cell proliferation and spontaneous cell death via apoptosis has an important role in the regulation of cancer cell growth (Brown and Attardi, 2005; Makin and Hickman, 2000;
Thompson, 1995). Anticancer drugs also function by inducing cancer cell death via induction of apoptosis in sensitive cells (Roninson, 2003; Johnstone et al., 2002; Kaufmann and Earnshaw, 2000; Chen et al., 1996; Zhu et al., 1997). Apoptosis may be triggered by extracellular death signals, deprivation of survival signals, and genetic or toxicological damage (Brune, 2002). A common observation in response to these stimuli is the activation of caspases, a group of cysteine proteases that serve as main effectors of programmed cell death. There are two major pathways of caspase activation: ligand-dependent or receptor-induced activation (extrinsic pathway), through death receptors such as Fas (CD95) or the members of tumor necrosis factor receptor (TNF-R) superfamily, and mitochondria-dependent activation (intrinsic pathway), via cytochrome c (cyt c) release from mitochondria induced by stress, irradiation, or inflammation (Budihardjo et al., 1999, Li et al., 1997).

The mitochondrial apoptotic pathway (intrinsic pathway) is largely mediated through Bcl-2 family proteins, which include both proapoptotic members such as Bax, Bak and BNIP3 that promote mitochondrial permeability and antiapoptotic members such as Bcl-2 and Bcl-xL that inhibit their effects, or inhibit the mitochondrial release of cytochrome c (Korsmeyer et al., 1993; Antonsson et al., 1997). Another important component is the tumor suppressor protein p53, which simultaneously suppresses Bcl-2 and activates Bax. The ratio of Bax to Bcl-2 is critical to cell survival which determines whether a cell undergo apoptosis (Oltavi et al., 1993). Bcl-2 family members possess one or more of four Bcl-2 homology (BH) domains. The precise complement of these structural domains determines the pro or antiapoptotic activity of Bcl-2-related proteins (Tsujimoto, 1998). Family members that possess exclusively BH3 domains are proapoptotic, and have multiple mechanisms of action. Some BH3-only proteins, such as Bad and Bik, promote the intrinsic death pathway by sequestering Bcl-2 and inhibiting its antiapoptotic effects by displacing Bid and Bim (Puthalakath et al., 1999; Letai et al., 2002). Bid and Bim can act directly to activate Bax, a multidomain proapoptotic Bcl-2 family member (Marani et al., 2002). In response to undefined apoptotic signals, Bax translocates to the mitochondria where it oligomerizes into a heteromeric protein channel. This putative Bax channel inserts into the outer mitochondrial membrane and releases apoptotic factors, such as cytochrome c (Wolter et al., 1997). Cytochrome c leakage supports the formation
of an apoptosome complex by binding to apoptotic protease activating factor-1 (Apaf-1), which activates the caspase-9 molecules (upon cleavage of the bound zymogen procaspases-9), which in turn activate caspase-3 (Korsmeyer, 1995; Vodovotz et al., 2004) to initiate apoptosis. Caspases are synthesized as inactive proenzymes and their activation during apoptosis results in cleavage at specific aspartate cleavage sites (Cohen, 1997; Thornberry et al., 1997). While initiator caspases-8 and -9 undergo autocatalytic activation, executioner pro-caspase-3 is processed by initiator caspases. Caspase-3 is one of the key executioners of apoptosis, being responsible either partially or totally for the proteolytic cleavage of many key proteins, such as the nuclear enzyme PARP, followed by DNA fragmentation (Enari et al., 1998; Nagata, 2000).

In the current study, keeping in mind the previous reports on garlic and its organosulfur compounds, we further explored the involvement of mitochondrial apoptotic pathway in Allicin-induced apoptosis in HeLa cells. Our Western blot results showed that Allicin induced the mitochondrial release of cytochrome c in the cytosol. Moreover, Allicin treated HeLa cells also exhibited an augmented caspase-9 and -3 activities which were found to be dose dependent. Likewise, Western blot results also showed cleavage and activation of procaspase-9 and -3 which further confirmed our finding of enhanced caspase-9 and -3 activities. Pretreating HeLa cells with caspase inhibitors Z-VAD-FMK, Z-DEVD-FMK (caspase-9 inhibitor) and Z-LEHD-FMK (caspase-3 inhibitor) completely abrogated Allicin-induced caspase activation and cell proliferation. Furthermore, we also demonstrated Allicin-induced PARP cleavage in HeLa cells and cleaved product of 89 KDa was found to be evident in Western blot. Similarly, results of Western blot analysis also revealed an upregulated Bax and downregulated Bcl-2 protein expressions in Allicin-treated HeLa cells. Therefore, our above combined results established the contribution of mitochondrial or intrinsic pathway in Allicin-induced apoptosis.

The majority of garlic-derived compounds activate the so called intrinsic or mitochondria-mediated pathway in the execution of apoptosis, which involves loss of mitochondrial membrane potential and release of apoptogenic molecules from the mitochondria to the cytosol (Thornberry and Lazebnick, 1998; Hengartner, 2000). Garlic-derived OSCs are believed to trigger apoptosis by modulating the levels of Bcl-2 proteins. Allicin has been reported to induce apoptosis through mitochondrial pathway
followed by significant release of cytochrome c and activation of caspase-9 and -3 in human promyelocytic leukemia-derived HL60 cells and human myelomonocytic U937 cells (Miron et al., 2008). PARP cleavage has been reported in human mammary (MCF-7) cancer cells during Allicin-induced apoptosis (Hirsch et al., 2000). DAS or DADS treatment increased the ratio of Bax/Bcl-2 in SH-SY5Y neuroblastoma cells, as well as in H460 and H1299 lung cancer cells compared with untreated controls (Hong et al., 2000; Karmakar et al., 2007). A time-dependent upregulation of Bax protein level and concomitant downregulation of Bcl-xL protein level was observed in DADS treated MDA-MB-231 breast cancer cell line (Nakagawa et al., 2001). The Z-ajoene-induced apoptosis in HL-60 cells was associated with caspase-mediated cleavage of Bcl-2 (Li et al., 2002). The DATS induced apoptosis in prostate cancer cells correlates with a decrease in Bcl-2 level as well as with hyperphosphorylation of this protein, which reduces Bcl-2/Bax interaction and activates the mitochondrial pathway of apoptosis (Xiao et al., 2004). The DATS-induced apoptosis in LNCaP cells correlated with a modest increase in protein levels of pro-apoptotic Bcl-2 family members Bax and Bak (Kim et al., 2007).

The mitochondrial apoptotic pathway (intrinsic pathway) is also regulated by the mitochondrial permeability transition pore (mPTP) (Hirsch et al., 1997). The mPTP is thought to be a large protein complex composed of the voltage-dependent anion channel (VDAC), the adenine nucleotide translocator (ANT), and cyclophilin D (Martinou et al., 1999). The components of the mPTP are associated with the mitochondrial inner membrane and form a pore that is permeable to low molecular weight (<1.5 KDa) solutes and ions, ultimately leading to mitochondrial membrane depolarization and swelling of the mitochondrial matrix (Zamzami and Kroemer, 2001) causing cell death. mPTP opening also acts as an initiating event to trigger Bax translocation to mitochondria, ultimately leading to cytochrome c release and caspase activation (Precht et al., 2005).

Cyclosporin A (CsA) is an inhibitor of cyclophilin D and blocks the formation of the mPTP and prevents its opening which could lead to abrogation of mitochondrial membrane depolarization and Bax translocation to mitochondria. This ultimately suppresses release of cytochrome c from mitochondria and thus caspase activation which play important role in apoptosis. Therefore, in the current study, the effect of CsA
pretreatment on Allicin-induced suppression of proliferation and apoptosis of HeLa cells was also investigated. Our results showed that CsA pretreatment of HeLa cells inhibited Allicin-induced growth suppression and cell death, corroborating the central role of mitochondria in the apoptotic process induced by Allicin. Furthermore, Allicin induced a cell cycle arrest at G0/G1 in HeLa cells. Upon CsA pretreatment, the percentages of cells at G0/G1 remain unchanged. So, it can be deduced from this result that the effect of Allicin on cell cycle arrest was independent of its proapoptotic activity. Furthermore, the above effect of CsA on HeLa cells suggested the involvement of mPTPs in Allicin-induced apoptosis. A recent report has demonstrated that CsA pretreatment of human promyelocytic leukemia-derived HL60 cells inhibited Allicin-induced cell death (Miron et al., 2008).

Glutathione (GSH), cysteine and accessible free sulfhydryl (-SH) groups of various proteins and peptides are rapidly oxidized by Allicin to their respective Allyl disulfide derivatives (Rabinkov, 2000; Rabinkov, 1998). The mixed disulfide formed is less active than Allicin, yet it can easily react with free -SH in thiol-disulfide exchange reactions, and produce, at the end of a series of reactions, the oxidized form of glutathione (GSSG), the oxidized form of cysteine (cystine) and glutathiolated S-proteins. Glutathione is the major regulator of the thiol-disulfide redox state of living cells (Gilbert, 1990; Powis, 1995; Sies, 1999; Moran et al., 2001). Alterations in cellular free thiols have already been described as a key event in the generation of apoptosis (Meister, 1995; Lash, 2006).

In the present study, the effect of Allicin on cellular GSH content and total free thiol (-SH) content of cytosol and mitochondria was also examined. Our results showed that Allicin induced an initial drop in GSH levels that was followed by a regeneration process, occurring several hours after treatment. This may be due to decreased feedback inhibition of GSH biosynthetic enzymes such as γ-glutamylcysteine synthetase (γ-GCS) by GSH (Lash, 2006). In addition, Allicin also caused GSH depletion concomitantly in the cytosol and mitochondria in HeLa cells. The rate of free -SH depletion from the cytosol and the mitochondria was similar, indicating that Allicin penetrated the mitochondria and the cell membrane equally well and reacted with free -SH in both compartments. Furthermore, in BSO-pretreated cells, there was no recovery of GSH after its Allicin-induced depletion, as BSO is a direct inhibitor of γ-GCS. Since BSO-induced depletion of GSH levels
caused neither mitochondrial permeability transition nor apoptosis (no effect on cell survival), it is likely that BSO treatment depletes GSH mainly from the cytosol, whereas Allicin induced depletion of mitochondrial GSH trigger the apoptotic process. Previous studies have already shown that Allicin strongly depleted the cytosolic and mitochondrial glutathione level, which plays very important role in maintaining the cellular and mitochondrial redox potential, and thus sensitized the cells to undergo apoptosis (Hirsch et al., 2000; Miron et al., 2008). The following Allicin-induced apoptotic scenario can be deduced from this study: Allicin penetrates the cells and readily reacts with any exposed -SH present in its vicinity. As GSH is the most abundant thiol molecule in the cytosol and the mitochondria, it is the main target for Allicin reaction. The reaction with glutathione leads to an immediate change in the ratio GSH/GSSG. The increased concentration of GSSG and the depletion of GSH bring about a decrease in the cellular reduction potential state. Concomitantly, mitochondrial damage occurs, its enzymatic activity decreases and the cytochrome c is released to the cytosol. Upon its release, cytochrome c participates in the activation of the caspase-9, followed by activation of caspase-3.

N-acetylcysteine’s (NAC) primary function in vivo is to supply cysteine necessary for GSH synthesis and replenishment (Zafarullah et al., 2003). It can also reduce disulphide bonds in proteins (Ziment, 1988; Harada et al., 2004) and scavenge free radicals (Aruoma et al., 1989). The direct antioxidant activity of NAC has been proposed primarily based on data from in vitro studies, where NAC has been shown to reduce oxidant-induced cell damage and cell death by apoptosis (Zafarullah et al., 2003). Results of the present study demonstrated that simultaneous treatment of HeLa cells with NAC and Allicin abrogated the antiproliferative and apoptotic activity of Allicin. Furthermore, the cell cycle arrest induced by Allicin at G0/G1 phase was also shown to be abolished by NAC. The results observed could be due to NAC’s key role to provide cysteine for GSH synthesis and replenishment depleted by Allicin. In other words, NAC could play an important role in maintaining cellular redox environment destroyed by Allicin. Additionally, NAC could also sequester the harmful activity by competing with GSH for the reaction with Allicin. Previous reports have confirmed this observation that NAC could alter the antiproliferative and apoptotic activity of garlic organosulfur compounds. NAC has been
reported to eliminate the growth inhibitory effect of Allicin in human promyelocytic leukemia-derived HL60 cells and human myelomonocytic U937 cells (Miron et al., 2008). DADS-induced cell cycle arrest and apoptosis in human A549 lung carcinoma cells has also been reported to be abrogated by pretreatment of NAC (Wu et al., 2005).

HPV oncoproteins E6 and E7 can disturb cell cycle regulation and impede apoptosis through binding to two tumor suppressor proteins, p53 and pRb, respectively which results in cellular immortalization and transformation (Munger and Howley, 2002; Mantovani and Banks, 2001). The p53 tumor suppressor gene is critically involved in cell cycle regulation, DNA repair, and programmed cell death and RB and its family regulate the activity of E2F transcription factors, which control transcription of various genes required for cell cycle progression. The HPV E6 protein binds to p53, promoting its degradation through the ubiquitin pathway and thus abrogates its function and E7 protein binds to hypophosphorylated members of RB (retinoblastoma) family, resulting in their destabilization and the disruption of RB-E2F repressor complexes (Ferenczy and Franco, 2002; Gonzalez et al., 2001; Scheffner and Whitaker, 2003).

Most striking finding of this current study is that Allicin strongly suppressed the expressions of HPV18 E6 and E7 oncoproteins in cervical cancer HeLa cells. This downregulation of E6 and E7 expressions could result in cessation of cervical cancer cell growth. However, the underlying mechanism of the suppression of E6 and E7 oncoproteins of HPV by Allicin is not known and needs further extensive research. Furthermore, the expression of p53 tumor suppressor protein in Allicin treated HeLa cells was also found to be upregulated. In other words, Allicin could restore the function of p53 and disrupt the E6/p53 pathway which plays a vital role in cervical carcinogenesis. Since p53 simultaneously suppresses anti-apoptotic Bcl-2 and activates apoptotic Bax, its increased expression and restoration of function could also assist in programmed cell death induced by Allicin in cervical cancer cells.

Monocytes play an important role in the initiation, development, and outcome of the immune response (van Furth, 1981; Meuret, 1981; Muller, 2001; Lee et al., 2002). Immune responses and inflammatory reactions mediated by monocytes are regulated not only positively by cellular recruitment, proliferation, and cross-talk, but also negatively by apoptosis of monocytes (Savill, 1997; Kiener et al., 1997). Monocytes generated in
the bone marrow circulate in the bloodstream for a few days, after which they are programmed to undergo apoptosis in the absence of specific survival signals. Several inflammatory cytokines, such as GM-CSF, TNF-α, and IL-1β have been reported to antagonize this spontaneous apoptosis of monocytes (Mangan et al., 1992; Mangan and Wahl, 1991; Mangan et al., 1991). LPS, a cell wall component of gram-negative bacteria, also has the potential to prevent monocytic apoptosis (Mangan et al., 1991). On the other hand, several reports have demonstrated that anti-inflammatory cytokines, such as IL-4 or IL-10, and glucocorticoids, such as dexamethasone, promote the apoptosis of monocytes (Mangan et al., 1992; Schmidt et al., 1999; Estaquier and Ameisen, 1997). Therefore, the homeostasis and apoptotic processes of monocytes were regulated both positively and negatively by multiple stimulations, and this regulation has been postulated to play a pivotal role in the monocytes-related inflammatory responses. Strikingly, findings of the current study demonstrated that Allicin strongly induced apoptosis in monocytes of cervical cancer patients. Because the regulation of monocytic apoptosis has a great importance in the regulation of inflammation, this might be one of the mechanisms of Allicin's anti-inflammatory effects.

Previous studies have shown that several proinflammatory cytokines (IL-1α, IL-6, or TNF-α) stimulate proliferation of carcinoma cell lines derived from several different tissues including cervix (Ito et al., 1993; Wu et al., 1993; Sugarman et al., 1986; Lewis et al., 1987, Wei et al., 2003). Thus, these proinflammatory cytokines might act as paracrine or autocrine growth factors in promoting malignant progression of cervical cancer. Surprisingly, results of the present study showed an augmented expression of proinflammatory cytokines TNF-α, IL-1α and IL-6 in monocytes from cervical cancer patients. Furthermore, expressions of these proinflammatory cytokines in monocytes were shown to be suppressed by Allicin treatment. So, our above results indicated that suppression of proinflammatory cytokines such as TNF-α, IL-1α and IL-6 by Allicin could alter the local microenvironment of cervical tumor leading to malignant progression.

Other biological pathways that might be implicated in anticancer approaches are related with the immune and the inflammatory system. Since both are strongly interdependent, organosulfur compounds affect both of them. Accordingly, garlic contents were described
to possess immunomodulatory and anti-inflammatory properties, by modulating lipopolysaccharide (LPS)-induced cytokine levels e.g. decrease of proinflammatory interleukin (IL)-1β and tumor necrosis factor (TNF-α) in human blood (Keiss et al., 2003), which resulted moreover in a reduction of the activity of the transcription factor NF-κB, which is strongly linked with inflammatory diseases like cancers (Aggarwal and Shishodia, 2004; Delhalle et al., 2004), in human embryonic kidney (HEK 293) cells, when exposed to the treated blood (Keiss et al., 2003).

Anti-inflammatory effects were also observed by prevention of a radiotherapy-induced up-regulation of the intercellular adhesion molecule-1 (ICAM-1) by Allicin (Son et al., 2006), or by a decrease of the level of cyclooxygenase-2 (COX-2), an enzyme responsible for the biosynthesis of prostaglandins and known to be induced in several cancers. The latter effect appeared to be correlated with an anti-neoplastic activity in vitro (Elango et al., 2004). In addition, stimulation of the immune defense (lymphocytes) by Allicin was demonstrated in vitro and supposed to be contributing to the compounds antitumoral potency in mice (Patya et al., 2004). Furthermore, apart from interactions with the immune system or inflammatory processes, induction of differentiation of human leukemia cells (HL-60) was shown by garlic and onion oils (Seki et al., 2000).

In summary, the above combined results of the present study established that Allicin, an organosulfur compound of garlic (Allium sativum), has a potent antiproliferative and apoptotic properties against cervical cancer HeLa cell line. Allicin-induced growth inhibitory action in HeLa cells involved the cell cycle arrest and apoptosis involved the activation of caspase-9 and -3 as well as release of cytochrome c from mitochondria, downregulation of antiapoptotic Bcl-2 and upregulation of apoptotic Bax and p53. Interestingly, Allicin also suppressed the expression of HPV E6 and E7 oncoproteins implicated in cervical carcinogenesis. In addition, Allicin also induced apoptosis and suppressed the augmented expression of inflammatory cytokines TNF-α, IL-1α and IL-6 in monocytes of cervical cancer patients. Thus, it could be concluded that in view of the above antiproliferative, apoptotic and anti-inflammatory properties, Allicin could be a promising chemotherapeutic agent in prevention of cervical cancer.