The present study involves the investigations for the effect of Allicin, an organosulfur compound of garlic, on cervical cancer HeLa cell line and monocytes from cervical cancer patients. First of all, keeping in mind the previous reports of antiproliferative and apoptotic properties of Allicin on different cancer cell lines, various experiments were carried out to check the efficacy of Allicin to inhibit the proliferation of cervical cancer HeLa cells. In addition to this, anti-inflammatory property of Allicin was also verified by performing different experiments on monocytes isolated from the blood of cervical cancer patients. The results obtained are described below.

**Allicin Inhibited Proliferation of Cervical Cancer HeLa Cells**

Cervical cancer HeLa cells (5x10³ cells/well) were incubated with increasing concentrations of Allicin (0.3 to 3 μM) for growing time periods up to 48 h and their viability was determined by the MTT and Trypan blue dye exclusion assays. Our results have shown that Allicin inhibited proliferation of HeLa cells in a concentration and time dependent manner. A low magnitude of inhibition of cell proliferation, of about 14.79% (p<0.001) and 24.82% (p<0.001) compared to control, was observed in the presence of 0.3 and 0.6 μM Allicin respectively after 24 h of treatment (Fig. 11). In the presence of 1.5 μM Allicin, HeLa cells exhibited about 47.35% (p<0.001) inhibition of proliferation after 24 h. At 3 μM allicin, after 24 h, the rate of inhibition reached 56.57% (p<0.001) (Fig. 11). At the dose of 0.3 μM Allicin, a small amount of inhibition of proliferation (27.89%) was observed after 48 h of treatment (Fig. 12). Allicin, at the dose of 0.6 μM, 1.5 μM and 3 μM, showed marked inhibition of proliferation of about 41.53% (p<0.001), 59.89% (p<0.001) and 68.66% (p<0.001) respectively after 48 h of treatment (Fig. 12).

Similarly, we confirmed our above results with Trypan blue dye exclusion assay. A low magnitude of inhibition of cell proliferation, of about 12.62% (p<0.001) and 24.53% (p<0.001), were observed after 24 h of treatment in the presence of 0.3 and 0.6 μM Allicin respectively (Fig. 13). In the presence of 1.5 μM allicin, HeLa cells exhibited about 44.61% (p<0.001) inhibition of proliferation after 24 h. At 3 μM allicin, after 24 h, the rate of inhibition reached 53.84% (p<0.001) (Fig. 13). At the dose of 0.3 μM Allicin, a small amount of inhibition of proliferation (21.68%) was observed after 48 h of treatment (Fig. 14).
Figure 11: Dose dependent inhibition of growth of cervical cancer HeLa cells treated with Allicin (0.3-3 μM) for 24 hour assessed by MTT assay: NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 12: Dose dependent inhibition of growth of cervical cancer HeLa cells treated with Allicin (0.3-3 μM) for 48 hour assessed by MTT assay: NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 13: Dose dependent inhibition of growth of cervical cancer HeLa cells treated with Allicin (0.3-3 µM) for 24 hour assessed by Trypan Blue dye exclusion assay: NT denotes not treated (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 14: Dose dependent inhibition of growth of cervical cancer HeLa cells treated with Allicin (0.3-3 μM) for 48 hour assessed by Trypan Blue dye exclusion assay: NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Allicin, at the dose of 0.6 μM, 1.5 μM and 3 μM, showed marked inhibition of proliferation of about 36.51% (p<0.001), 54.69% (p<0.001) and 65.59% (p<0.001) respectively after 48 h of treatment (Fig. 14). These results indicated that Allicin inhibited cell growth in HeLa cells as a function of dose and time. Inhibition of cell proliferation could be the result of the induction of cell cycle arrest or apoptosis. Therefore, we hypothesized that Allicin induced inhibition of cell proliferation was due to alterations in cell cycle control and programmed cell death.

**Allicin Induced Cell Cycle Arrest in Cervical Cancer HeLa Cells**

To determine whether suppression of cell proliferation by Allicin results from inhibition of cell cycle progression, HeLa cells were treated with 1.5 and 3 μM Allicin for 24 hours and subjected to flow cytometric analysis of DNA fluorescence in propidium iodide (PI) stained cells. Allicin treatment over 24 h caused a significant cell cycle arrest in HeLa cells at Go/G1 phase (Fig. 15). The percentage of Go/G1 cells started to increase when the Allicin concentration was 1.5 μM. Until 3 μM, cells at Go/G1 reached over 1.56 fold of that of the control (Fig. 15). With increasing Allicin concentration, the percentage of cells at S and G2/M phase decreased and it seems that most of the cells at Go/G1 came from these phases. HeLa cells treated with 1.5 μM allicin resulted in a reduction in the S (from 26.3% to 20.3%) and G2/M fractions (32.5% to 28.1%) and these were associated primarily with accumulation of cells in the Go/G1 phase (41.2% to 51.6%) (Fig. 16). A further increase in allicin concentration to 3 μM resulted in a more pronounced reduction in the S (from 26.3% to 18.2%) and G2/M fractions (32.5% to 17.3%) with a concomitant increase in the percentage of cells in the Go/G1 (41.2% to 64.5%) (Fig. 17). Furthermore, a sub-G0/G1 population was also observed along with Go/G1 phase arrest in HeLa cells incubated with 1.5 and 3 μM Allicin suggesting that certain amount of cells underwent apoptosis after 24 h of treatment. Since, in addition to cell cycle arrest, the growth inhibition induced by Allicin treatment could also be due to programmed cell death, hence, we investigated whether Allicin induced apoptosis in HeLa cells.

**Allicin-Induced Apoptosis in Cervical Cancer HeLa Cells**

To determine whether the loss of viability in HeLa cells induced by Allicin was due to apoptosis, HeLa cells were treated with 1.5 and 3 μM Allicin for 24 hours and phosphatidylserine (PS) externalization was measured by fluorescence activated cell
Figure 15: Cell cycle analysis of Allicin-treated HeLa cells: Cells were treated with 0.6, 1.5 and 3 μM Allicin for 24 hour and stained with propidium iodide in the presence of RNase A as described in Materials and Methods. Cell cycle analysis was carried out with a flow cytometer. The data shown here are from a representative experiment repeated three times with almost similar results.
Figure 16: Cell cycle analysis of Allicin-treated HeLa cells: Cells were treated with 1.5 μM Allicin for 24 hour and stained with propidium iodide in the presence of RNase A as described in Materials and Methods. Cell cycle analysis was carried out with a flow cytometer. NT denotes no treatment (Control). The data shown here are from a representative experiment repeated three times with almost similar results.
Figure 17: Cell cycle analysis of Allicin-treated HeLa cells: Cells were treated with 3 μM Allicin for 24 hour and stained with propidium iodide in the presence of RNase A as described in Materials and Methods. Cell cycle analysis was carried out with a flow cytometer. NT denotes no treatment (Control). The data shown here are from a representative experiment repeated three times with almost similar results.
sorting (FACS) after Annexin-V FITC/PI staining. Our results showed that Allicin induced significant amount of apoptosis in HeLa cells of about 17.1% and 31.4% after 24 h of treatment with 1.5 and 3 μM Allicin respectively (Fig. 18, 19). Moreover, the percentage of apoptotic cells did not coincide with the percent of viable cells, which could be due to a cell cycle arrest at G0/G1 phase. In order to explore the mechanism(s) by which Allicin induces apoptosis, we investigated the alterations in the expression of selected proteins that are involved in the complex processes of apoptosis.

Altered Bax and Bcl-2 Expressions in Allicin treated HeLa Cells

The mitochondrial apoptotic pathway (intrinsic pathway) is largely mediated through Bcl-2 family proteins, which include both proapoptotic members such as Bax and Bak 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). The ratio of the proapoptotic Bax and antiapoptotic Bcl-2 is critically balanced during cell proliferation such that an increase in the levels of Bax or a decrease in the level of Bcl-2 can shift the ratio and trigger a signal initiating apoptosis. We hypothesized that Allicin induces apoptosis in HeLa cells through mitochondrial pathway. Therefore, we investigated the effect of Allicin on protein expression of Bax and Bcl-2 by Western blot analysis which was carried out after treating HeLa cells with 1.5 and 3 μM Allicin for 24 h. Our Western blot results showed an upregulated Bax expression and a downregulated Bcl-2 expression in Allicin treated HeLa cells (Fig. 20). Thus, an increased Bax/Bcl-2 ratio in HeLa cells treated with Allicin indicated the initiation of apoptosis.

Allicin-Induced Cytochrome c Release from Mitochondria

Cytochrome c is a component of the electron transport chain in mitochondria and plays an important role in initiation of apoptosis. In response to pro-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). This release of cytochrome c in turn activates initiator caspase-9, a cysteine protease. Caspase-9 further activates executioner caspase-3 and -7, which are responsible for destroying the cell from within. Therefore, we investigated whether there was a release of cytochrome c in the cytosol of
Figure 18: FITC-Annexin V Assay for Measurement of Apoptosis in Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour and stained with FITC-Annexin V and Propidium Iodide. Percent apoptosis was measured using a Flow cytometer. The data shown here are from a representative experiment repeated three times with almost similar results.
Figure 19: Percent Apoptosis in Allicin-treated HeLa cells: Cells were treated with 1.5 and 3 μM Allicin for 24 hour and stained with FITC-Annexin V and propidium iodide as described in Materials and Methods. Percent apoptosis was determined by a flow cytometer. NT denotes no treatment (Control). Data represent mean ± S.E.M of three independent experiments.
Figure 20: Modulation of Bax and Bcl-2 Levels in Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from total cell lysates was subjected to SDS-PAGE and Western blotting using Bax and Bcl-2 antibodies.
HeLa cells treated with Allicin. For that reason, HeLa cells were treated with 1.5 and 3 μM Allicin for 24 h and release of cytochrome c was analyzed by Western blotting. Our result showed an increased amount of cytochrome c in the cytosol of HeLa cells treated with Allicin after 24 h (Fig. 21). Thus, the release of cytochrome c from mitochondria of HeLa cells treated with Allicin indicated the initiation of the apoptotic cascade induced by the said compound.

**Allicin-Induced Activation of Caspases during Apoptosis**

The downstream signals during apoptosis are transmitted via caspases, which, upon conversion from pro to active forms, mediate the proteolytic cleavage of many key proteins, such as the nuclear enzyme PARP, followed by DNA fragmentation (Enari et al., 1998; Nagata, 2000). We were keen to know whether caspases play some role in the apoptosis of HeLa cells induced by Allicin. Therefore, we determined the caspase activities in HeLa cells treated with Allicin. As described previously, the cell growth inhibition by Allicin was strongly dose and time dependent; we examined the dose and time dependence of caspase activation as well. HeLa cells were treated with different doses of Allicin (0.3-3 μM) for 24 and 48 h. Cytosolic proteins were extracted and assayed for caspase activities by incubation with a chromogenic substrate. LEHD-pNA (for caspase-9) or DEVD-pNA (for caspase-3). Our results showed a significant induction of caspase-9 and -3 activities in HeLa cells after treatment of Allicin at 1.5 and 3 μM concentrations (p<0.001) (Fig. 22, 23). Insignificant amount of caspase-9 and -3 activities were observed at 0.3 and 0.6 μM concentration of Allicin after 24 and 48 h of treatment (Fig. 22, 23). Thus, the caspase activities in Allicin treated HeLa cells were found to be dose and time dependent and maximal at the dose of 3 μM Allicin after 24 and 48 h of treatment. Furthermore, we confirmed our above results by Western blot analysis of caspase-9 and -3 in total cell lysate of HeLa cells prepared after 24 h of treatment with 1.5 and 3 μM of Allicin. Our western blot results showed that treatment of HeLa cells with Allicin led to cleavage and activation of initiator caspase-9 which was evident after 24 h of treatment (Fig. 24). The activation of initiator caspase-9 resulted in cleavage and activation of downstream effector caspase-3 which was also marked after 24 h of treatment with 1.5 and 3 μM Allicin (Fig. 25). Thus, activation of caspase-9 and -3 in
Figure 21: Mitochondrial Release of Cytochrome c by Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from cytosolic cell lysate was subjected to SDS–PAGE and Western blotting using cytochrome c antibody.
Figure 22: Dose and time dependent activation of caspase-9 in Allicin-treated HeLa cells: HeLa cells were treated with different concentrations of Allicin (0.3-3 μM) for 24 and 48 h. Cells were harvested and lysed in lysis buffer. Enzymatic activity of caspase-9 was determined by incubation of 50 μg of total protein with substrate LEHD-pNA for 2 h at 37 °C. The release of p-NA was monitored spectrophotometrically at 405 nm. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 23: Dose and time dependent activation of caspase-3 in Allicin-treated HeLa cells: HeLa cells were treated with different concentrations of Allicin (0.3-3 μM) for 24 and 48 h. Cells were harvested and lysed in lysis buffer. Enzymatic activity of caspase-3 was determined by incubation of 50 μg of total protein with substrate DEVD-pNA for 2 h at 37 °C. The release of p-NA was monitored spectrophotometrically at 405 nm. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 24: Cleavage and Activation of Caspase-9 in Allicin-treated HeLa Cells:
HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from
total cell lysates was subjected to SDS–PAGE and Western blotting using cleaved
caspase-9 antibody.
Figure 25: Cleavage and Activation of Caspase-3 in Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from total cell lysates was subjected to SDS–PAGE and Western blotting using cleaved caspase-3 antibody.
HeLa cells treated with Allicin clearly indicated the involvement of intrinsic or mitochondrial pathway during Allicin-induced programmed cell death.

**Caspase Inhibitors Abrogated Apoptosis Induced by Allicin**

The above results clearly indicate that caspase-9 and -3 proteases are activated in response to the apoptosis induced by Allicin. To determine whether the activation of caspase-9 and -3 is required for the induction of cell death by Allicin, HeLa cells were pretreated with cell permeable caspase inhibitors, Z-LEHD-FMK (caspase-9 inhibitor), Z-DEVD-FMK (caspase-3 inhibitor) or Z-VAD-FMK (general caspase inhibitor), followed by Allicin (3μM) for 24 h and subsequently caspase activities and cell viabilities were measured. Our results showed that pretreatment of Z-LEHD-FMK and Z-DEVD-FMK abolished caspase-9 and caspase-3 activities respectively in HeLa cells induced by Allicin (p<0.001) and respective caspase activities were found to be similar to the control value (no significant difference) (Fig. 26, 27). Similarly, Z-LEHD-FMK, Z-DEVD-FMK and Z-VAD-FMK also abrogated the cell growth inhibition and in turn apoptosis in HeLa cells induced by Allicin (p<0.001) and the respective cell viabilities were found to be similar to the control value (no significant difference) (Fig. 28, 29, 30). Thus, the above combined results showed that the activation of caspases is a crucial event during Allicin-induced apoptosis in HeLa cells.

**Allicin-Induced Cleavage of Poly (ADP-Ribose) Polymerase (PARP) during Apoptosis**

Caspase-3 plays an important role in apoptosis as an executioner caspase and mediates the cleavage of Poly (ADP-Ribose) Polymerase (PARP), a nuclear enzyme involved in DNA repair. Therefore by Western blot analysis, we then determined whether there is a PARP cleavage in HeLa cells after 24 h of treatment with 1.5 and 3 μM Allicin. Our results revealed PARP cleavage in Allicin treated HeLa cells and its cleaved product of 89 KDa was obvious in the Western blot (Fig. 31). Thus, the induction of caspase-9 and -3 activities and cleavage of PARP is a specific biochemical event, brought about by Allicin during apoptosis.

**Cyclosporin A (CsA) Suppressed the Cytotoxic Effect of Allicin on the Cell Viability**

Mitochondrial permeability transition (mPT) is an increase in the permeability of the mitochondrial membranes to molecules of less than 1.5 KDa and results from opening of
Figure 26: Suppression of Allicin-induced caspase-9 activation by caspase-9 inhibitor (Z-LEHD-FMK): HeLa cells were pretreated for 2 h with caspase-9 inhibitor (Z-LEHD-FMK) and then treated with 3 μM of Allicin for 24 hour. Cells were harvested and lysed in lysis buffer. Enzymatic activity of caspase-3 was determined by incubation of 50 μg of total protein with substrate LEHD-pNA for 2 h at 37 °C. The release of p-NA was monitored spectrophotometrically at 405 nm. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 27: Suppression of Allicin-induced caspase-3 activation by caspase-3 inhibitor (Z-DEVD-FMK): HeLa cells were pretreated for 2 h with caspase-3 inhibitor (Z-DEVD-FMK) and then treated with 3 μM of Allicin for 24 hour. Cells were harvested and lysed in lysis buffer. Enzymatic activity of caspase-3 was determined by incubation of 50 μg of total protein with substrate DEVD-pNA for 2 h at 37 °C. The release of p-NA was monitored spectrophotometrically at 405 nm. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 28: Abrogation of Allicin-induced growth suppression of HeLa cells by caspase-9 inhibitor (Z-LEHD-FMK): HeLa cells were pretreated for 2 h with caspase-9 inhibitor (Z-LEHD-FMK) followed by 3 μM of Allicin for 24 hour and cell viability was determined by MTT assay. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 29: Abrogation of Allicin-induced growth suppression of HeLa cells by caspase-3 inhibitor (Z-DEVD-FMK): HeLa cells were pretreated for 2 h with caspase-3 inhibitor (Z-DEVD-FMK) followed by 3 μM of Allicin for 24 hour and cell viability was determined by MTT assay. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 30: Abrogation of Allicin-induced growth suppression of HeLa cells by general caspase inhibitor (Z-VAD-FMK): HeLa cells were pretreated for 2 h with general caspase inhibitor (Z-VAD-FMK) followed by 3 μM of Allicin for 24 hour and cell viability was determined by MTT assay. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 31: PARP Cleavage in Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from total cell lysates was subjected to SDS–PAGE and Western blotting using PARP antibody.
protein pores called mitochondrial permeability transition pores (mPTP) which are formed in the membranes of mitochondria under certain pathological conditions (pro-apoptotic stimuli) which can lead to mitochondrial swelling and play an important role in apoptosis (Zamzami and Kroemer, 2001). 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). The role of mPTPs in the cytotoxicity of Allicin was gained by using Cyclosporin A (CsA), an inhibitor of Cyclophilin D which is a structural part of mPTP. Cyclosporin A 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. We hypothesized that Allicin-induced cytochrome c release in cytosol from mitochondria took place after Bax translocation to mitochondria and it could be suppressed by blocking mPTPs with CsA. Thus in other words, CsA could block the antiproliferative and apoptotic effect of Allicin. Accordingly, we investigated the effect of pretreatment of CsA on Allicin treated HeLa cells. HeLa cells were pre-incubated with 5 μM CsA for 1 h and then treated with allicin (1.5 and 3 μM) for 24 h. Our results have shown that CsA inhibited the detrimental effects of allicin on cell viability as determined by the MTT assay. Cell viabilities were found to be 51.21% and 40.35% when HeLa cells were treated alone with 1.5 and 3 μM Allicin respectively but pretreatment with CsA (5 μM) significantly ameliorated the growth suppressive effect of Allicin and cell viabilities increased to 74.31% and 65.14% respectively, compared to control (p<0.001) (Fig. 32, 33). There was no significant difference between nontreated and CsA-treated cells. No significant difference was found between CsA alone and CsA followed by Allicin treated cells. Thus, the effect of pretreatment of CsA on HeLa cells suggested the involvement of mPTPs in Allicin-induced apoptosis.

Cyclosporin A (CsA) Suppressed Allicin-Induced Apoptosis
Since CsA prevents the mPTPs from opening and thus inhibiting cytochrome c release, we hypothesized that it could also suppress the Allicin-induced apoptosis. Fluorescence activated cell sorting (FACS) analysis of HeLa cells treated with 3 μM Allicin for 24 h showed that 31.79% of the cells were apoptotic, whereas CsA pretreatment reduced the
Figure 32: Effect of pretreatment of Cyclosporin A (CsA) on cell viability of Allicin-treated HeLa cells: Cells were treated with 1.5 μM Allicin for 24 h pretreated with 5 μM CsA and cell viability was measured by the MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 33: Effect of pretreatment of Cyclosporin A (CsA) on cell viability of Allicin-treated HeLa cells: Cells were treated with 3 μM Allicin for 24 h pretreated with 5 μM CsA and cell viability was measured by the MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
apoptotic fraction to 15.53% (p<0.001) (Fig. 34). The fraction of HeLa cells at the G0/G1, S and G2/M phase were 41.2%, 26.3% 32.5% respectively for nontreated cells and 42.4%, 27.3% and 30.3% respectively for cells treated with CsA only (the difference was not significant). Cell cycle arrest at G0/G1 in Allicin-treated cells with or without CsA pretreatment was found to be 64.5% and 65.1%, respectively (the difference was not significant) (Fig. 35). Thus it is obvious from this result that CsA suppressed only Allicin-induced apoptosis and did not affect cell cycle distribution altered by Allicin.

**Allicin-Induced Intracellular Glutathione (GSH) Depletion**

Glutathione is the major regulator of the thiol-disulfide redox state of living cells (Gilbert, 1990; Powis, 1995; Sies, 1999; Moran et al., 2001). Allicin has been shown to react with sulfhydryl (-SH) containing molecules e.g. GSH (Rabinkov et al., 1998). Reduction in intracellular GSH levels has been associated with apoptotic cell death in various cell types (Liu et al., 1998; Singh et al., 1998; Meister, 1995; Lash, 2006). Therefore, we set out to investigate whether a decrease in the GSH level in the Allicin-treated HeLa cells can account for the strong cell growth inhibitory and apoptotic activity of this compound. Accordingly, GSH level was measured in HeLa cells treated with Allicin (1.5 and 3 μM) after different time intervals (0-24 h) and the effect of Allicin treatment on GSH levels was compared with that on cell proliferation. Our results showed that treatment of HeLa cells with 1.5 and 3 μM Allicin resulted in a drop in GSH concentration to 46% and 37.71% of the control level respectively, within 4 h (p<0.001) (Fig. 36, 37). This decrease was transient and was followed by a rapid recovery of the basal GSH level within 24 h. This is in accordance with a previous report, where GSH-Allicin conjugate (S-allylmercaptoglutathione) is formed immediately after Allicin penetration in red blood cells (Miron et al., 2000). Minimal GSH level was also measured in HeLa cells after treatment with different concentrations of Allicin (0.3-3 μM) for 4 h. The minimal GSH level was found to be 81.91%, 64.79%, 46% and 37.71% of the control value (without treatment) when HeLa cells were treated with 0.3, 0.6, 1.5 and 3.0 μM Allicin respectively. Hence, the extent of the Allicin-induced decrease in GSH level in HeLa cells and the growth inhibition induced by this compound demonstrated similar concentration dependence (Fig. 38).
Figure 34: Effect of Cyclosporin A (CsA) on Allicin-induced apoptosis in HeLa cells: Cells with or without CsA pretreatment (5 μM) were incubated in absence or presence of 3 μM Allicin for 24 hour and stained with FITC-Annexin V and propidium iodide as described in Materials and Methods. Percent apoptosis was determined by a flow cytometer. NT denotes no treatment (Control). Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 35: Effect of Cyclosporin A (CsA) on cell cycle distribution of Allicin-treated HeLa cells: Cells with or without CsA pretreatment (5 μM) were incubated in absence or presence of Allicin (3 μM) for 24 hour and stained with propidium iodide in the presence of RNase A as described in Materials and Methods. NT denotes no treatment (Control). The data shown here are from a representative experiment repeated three times with almost similar results.
Figure 36: Effect of Allicin on Glutathione (GSH) content of HeLa cells: Cells were treated with 1.5 μM Allicin and at each time point cells were harvested and washed twice with PBS. Cell pellets were extracted with 5% metaphosphoric acid and analyzed for GSH content and protein concentration. Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 37: Effect of Allicin on Glutathione (GSH) content of HeLa cells: Cells were treated with 3 μM Allicin and at each time point cells were harvested and washed twice with PBS. Cell pellets were extracted with 5% metaphosphoric acid and analyzed for GSH content and protein concentration. Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 38: Minimal Glutathione (GSH) level in Allicin-treated HeLa cells: Cells were treated with Allicin (0.3-3 μM) for 4 hour (maximum GSH depletion time point), harvested and washed twice with PBS. Cell pellets were extracted with 5% metaphosphoric acid and analyzed for GSH content and protein concentration. NT denotes no treatment (Control). Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Allicin-Induced Depletion of Free Sulfhydryl (-SH) Contents of Cytosol and Mitochondria

We further investigated the effect of Allicin on total free sulfhydryl (-SH) contents of cytosol and mitochondria of HeLa cells. Accordingly, total free sulfhydryl (-SH) contents were measured in the cytosolic and mitochondrial fractions of HeLa cells treated with Allicin (3 μM) after different time intervals (0-8 h). The decrease in free -SH in the cytosol (Fig. 39) was accompanied by a decrease of free -SH in the mitochondria (Fig. 40). Since mitochondrial GSH is supplied from the cytoplasm and upon Allicin treatment it was depleted in both cellular compartments, it could follow by a substantial decrease in the cell-reducing potential. Consequently, depolarization of the mitochondrial membrane could occur and trigger a chain of apoptotic events leading to cell death.

Buthionine sulfoximine (BSO) Irreversibly Augmented Allicin-Induced Intracellular Glutathione (GSH) Depletion

DL-Buthionine-(S,R)-sulfoximine (BSO) is a selective inhibitor of γ-glutamyl cysteine synthetase (γ-GCS), an enzyme in the glutathione biosynthetic pathway. We hypothesized that Allicin-induced GSH depletion could not be recovered to basal level in HeLa cells pretreated with BSO and thus cells could become more susceptible to undergo apoptosis. Accordingly, we first investigated the effect of pretreatment of BSO in Allicin treated HeLa cells. When HeLa cells were treated only with 100 μM BSO for 24 h, the GSH level was found to be decreased to 32.37% (p<0.001) of the control value (without BSO treated cells) (Fig. 41). Moreover, Allicin (1.5 and 3 μM) applied to HeLa cells pretreated with 100 μM BSO for 24 h caused an additional GSH decrease to 15.5% (p<0.001) and 11.91% (p<0.001) of GSH of only BSO treated cells respectively (Fig. 42). Here, there was no recovery of GSH as in the case of HeLa cells treated with Allicin alone.

Buthionine Sulfoximine (BSO) Enhanced the Allicin-Induced Cell Growth Inhibition

We also investigated whether BSO altered the Allicin-induced cell growth suppression besides augmenting Allicin-induced GSH depletion in HeLa cells. Hence, HeLa cells were pretreated with 100 μM BSO followed by 1.5 and 3 μM Allicin for 24 h and their viability was determined by MTT assay. HeLa cells pretreated with BSO followed by 1.5
Figure 39: Effect of Allicin on free Thiol (-SH) content of cytosolic fraction of HeLa cells: Cells were treated with Allicin (3 μM) and at different time intervals, cells were harvested and washed twice with PBS. Cell fractionation was done and total free -SH and protein concentration were determined. Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 40: Effect of Allicin on free Thiol (-SH) content of mitochondrial fraction of HeLa cells: Cells were treated with Allicin (3 μM) and at different time intervals, cells were harvested and washed twice with PBS. Cell fractionation was done and total free-SH and protein concentration were determined as described earlier. Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 41: Time dependent decrease of Glutathione (GSH) level in Allicin-treated HeLa cells pretreated with Buthionine sulfoximine (BSO): Cells were pretreated with or without 100 μM BSO for 24 hour and further treated with 1.5 μM Allicin. At each time point, cells were harvested and washed twice with PBS. Cell pellets were extracted with 5% metaphosphoric acid and analyzed for GSH content and protein concentration. Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 42: Time dependent decrease of Glutathione (GSH) level in Allicin-treated HeLa cells pretreated with Buthionine sulfoximine (BSO): Cells were pretreated with or without 100 μM BSO for 24 hour and further treated with 3 μM Allicin. At each time point, cells were harvested and washed twice with PBS. Cell pellets were extracted with 5% metaphosphoric acid and analyzed for GSH content and protein concentration. Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
and 3 μM Allicin showed a further decrease in viability to 31.47% and 23.18% respectively in comparison to only Allicin treated cells (50.13% and 41.24% respectively) (p<0.001) (Fig. 43). HeLa cells treated only with BSO showed no significant difference in viability (95.21%) compared to control (98.14%) (Fig. 44). Thus it is clear from this result that an irreversible depletion in GSH content caused due to BSO pretreatment further sensitized Allicin treated HeLa cells to undertake death. 

N-acetylcysteine (NAC) Abolished Allicin-Induced Inhibition of Cell Proliferation

Our above results have already shown that Allicin treatment transiently reduced the GSH level and in turn altered the redox balance of HeLa cells which could sensitize them to undergo apoptosis. Since N-acetylcysteine (NAC) is a cysteine prodrug which replenishes intracellular GSH level; therefore, we investigated whether NAC treatment could abrogate the Allicin-induced inhibition of cell proliferation, cell cycle arrest and apoptosis of HeLa cells. Accordingly, HeLa cells, pretreated with NAC (0.1–1.0 mM) for 1 h, were further treated with Allicin (0.3–3 μM) for 24 h and the cell viability was measured by MTT assay. Our results showed that HeLa cells treated with Allicin (0.3, 0.6, 1.5 and 3.0 μM) in the presence of NAC (0.1, 0.5 and 1.0 mM) recovered to normal viability pattern similar to control (difference was not significant) (Fig. 45, 46, 47). So, it can be concluded from this result that NAC sequestered the antiproliferative activity of Allicin in HeLa cells by replenishing intracellular GSH level.

N-acetylcysteine (NAC) also Abrogated Allicin-Induced Cell Cycle Arrest and Apoptosis

Our above results have clearly revealed that 3 μM Allicin induced significant apoptosis (30.31%) in HeLa cells (Fig. 18, 19). However, pretreatment of the HeLa cultures with 1 mM NAC reduced the apoptosis induced by Allicin to 8.73% (Fig. 48). Likewise, 3 μM Allicin induced significant G0/G1 arrest (64.5% cells in G0/G1 phase) (Fig. 15, 17). However, pretreatment of NAC (1 mM) completely abrogated the cell cycle arrest induced by Allicin and the percentage of G0/G1 cells in Allicin exposed cells recovered to a level similar to the control (41.7%) (Fig. 49).

Allicin Suppressed the Expression of HPV E6 and E7 Protein

HPV oncogenes E6 and E7 have been reported to be vital for carcinogenesis, progression, invasion and metastasis of cervical cancer (Munger and Howley, 2002). So in this study,
Figure 43: Effect of pretreatment of Buthionine sulfoximine (BSO) on cell viability of Allicin-treated HeLa cells: Cells were pretreated with or without 100 μM BSO for 24 hour followed by 1.5 μM Allicin for 24 h and cell viability was determined by MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 44: Effect of pretreatment of Buthionine sulfoximine (BSO) on cell viability of Allicin-treated HeLa cells: Cells were pretreated with or without 100 μM BSO for 24 hour followed by 3 μM Allicin for 24 h and cell viability was determined by MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 45: Effect of N-acetyl cysteine (NAC) on cell viability of Allicin-treated HeLa cells: Cells were treated with Allicin (0.3-3 μM) for 24 h in the absence or presence of 0.1 mM N-acetyl cysteine and cell viability was measured by the MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 46: Effect of N-acetyl cysteine (NAC) on cell viability of Allicin-treated HeLa cells: Cells were treated with Allicin (0.3-3 μM) for 24 h in the absence or presence of 0.5 mM N-acetyl cysteine and cell viability was measured by the MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 47: Effect of N-acetyl cysteine (NAC) on cell viability of Allicin-treated HeLa cells: Cells were treated with Allicin (0.3-3 μM) for 24 h in the absence or presence of 1 mM N-acetyl cysteine and cell viability was measured by the MTT assay. NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 48: Effect of N-acetyl cysteine (NAC) on Allicin-induced apoptosis in HeLa cells: Cells with or without NAC pretreatment (1 mM) were incubated in absence or presence of 3 μM Allicin for 24 hour and stained with FITC-Annexin V and propidium iodide as described in Materials and Methods. Percent apoptosis was determined by a flow cytometer. NT denotes no treatment (Control). Data represent mean ± S.E.M of three independent experiments. p<0.001 was considered significant.
Figure 49: Effect of N-acetyl cysteine (NAC) on cell cycle distribution of Allicintreated HeLa cells: Cells with or without NAC pretreatment (1 mM) were incubated in absence or presence of Allicin (3 μM) for 24 hour and stained with propidium iodide in the presence of RNase A as described in Materials and Methods. NT denotes no treatment (Control). The data shown here are from a representative experiment repeated three times with almost similar results.
we also examined the effect of Allicin on E6 and E7 oncoprotein expression by Western blot analysis which was performed after treating HPV 18 positive HeLa cells with 1.5 and 3 μM Allicin for 24 h. Our results showed that HPV E6 and E7 protein expressions were significantly downregulated in HeLa cells after the treatment (Fig. 50). Since HPV E6 and E7 oncoproteins play important role in cervical carcinogenesis, downregulation of these proteins by Allicin indicated that it could be a promising chemotherapeutic agent in prevention of cervical carcinogenesis.

**Allicin Upregulated the Expression of p53 Tumor Suppressor Protein**

HPV E6 protein deregulate cell cycle control by neutralizing cellular p53 tumor suppressor protein (Munger and Howley, 2002; Mantovani and Banks, 2001; Scheffner and Whitaker, 2003). So in this study, we also investigated the effect of Allicin on the expressions of p53 protein by Western blot analysis which was carried out after treating HeLa cells with 1.5 and 3 μM of Allicin for 24 h. Our results showed that p53 protein expression was significantly upregulated in HeLa cells after 24 h of treatment (Fig. 51). Thus, the result indicated that Allicin could restore the function of p53 by disrupting the E6/p53 pathway in cervical cancer cells. 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.

**Effect of Allicin on the Cell Viability of Monocytes**

Monocytes are the main effector cells of the immune system, and the regulation of their survival and apoptosis is essential for monocyte-involved immune responses. Allicin has been reported to have anti-allergic and anti-inflammatory activities, but its effect on monocytes has not yet been explored. In order to evaluate whether Allicin affects the viability of monocytes isolated from blood of cervical cancer patients, they were treated with varying concentrations of Allicin (0.3-3 μM) and their survival was determined by MTT assay. Our results showed that Allicin reduced the survival of monocytes in a concentration and time dependent manner. Insignificant amount of reduction in viability of monocytes of about 7.88% of control was observed in the presence of 0.3 μM Allicin after 24 h of treatment (Fig. 52). In the presence of 0.6 and 1.5 μM allicin, monocyte exhibited about 25.59% and 41.67% reduction in viability respectively after 24 h
Figure 50: Downregulation of HPV E6 and E7 Oncoprotein in Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from total cell lysates was subjected to SDS–PAGE and Western blotting using E6 and E7 antibodies.
Figure 51: Upregulation of p53 Tumor Suppressor Protein in Allicin-treated HeLa Cells: HeLa cells were treated with 1.5 and 3 μM of Allicin for 24 hour. Protein (50 μg) from total cell lysates was subjected to SDS-PAGE and Western blotting using p53 antibodies.
Figure 52: Dose dependent reduction in viability of monocytes, isolated from blood of cervical cancer patients, treated with Allicin (0.3-3 μM) for 24 hour assessed by MTT assay: NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
At 3 μM allicin, after 24 h, the rate of reduction reached 64.86% (p<0.001) (Fig. 52). At the dose of 0.3 μM Allicin, insignificant amount of decrease in survival (12.56%) was observed after 48 h of treatment. Allicin, at the dose of 0.6 μM, 1.5 μM and 3 μM, showed marked reduction in survival of monocytes of about 31.66% (p<0.001), 46.41% (p<0.001) and 71.77% (p<0.001) respectively after 48 h of treatment (Fig. 53). Thus it is obvious from this result that Allicin strongly reduced the viability of monocytes. 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 immunomodulatory effects. We, then, hypothesized that this marked reduction in monocyte viability is due to apoptosis and to confirm this we further investigated the activity of caspases in Allicin treated monocytes.

**Allicin-Induced Activation of Caspases during Apoptosis in Monocytes**

Since the activation different caspases in a cell is a hallmark of apoptosis, we determined the activities of caspases to ascertain our notion. Monocytes were treated with different doses of Allicin (0.3-3 μM) for 24 and 48 h. Cytosolic proteins were extracted and assayed for caspase activities by incubation with a chromogenic substrate, LEHD-pNA (for caspase-9) or DEVD-pNA (for caspase-3). Our results showed a significant induction of caspase-9 and -3 activities in monocytes after treatment of Allicin at 1.5 and 3 μM concentrations (p<0.001) (Fig. 54, 55). Insignificant amount of caspase-9 and -3 activities were observed at 0.3 and 0.6 μM concentration of Allicin after 24 and 48 h of treatment. The caspase activities were observed to be dose and time dependent and maximal at the dose of 3 μM Allicin after 24 and 48 h of treatment. Thus monocyte cultures treated with Allicin exhibited significantly higher activities of caspase-9 and -3, thereby indicating that the activation of caspases was associated with reduced cell survival and apoptotic death of Allicin-treated monocytes.

**Caspase Inhibitor Abolished Apoptosis in Monocytes Induced by Allicin**

The above results clearly indicate that caspase-9 and -3 proteases are activated in response to the apoptosis induced by Allicin. To determine whether the activation of caspases is required for the induction of cell death by Allicin, percent viability was determined in monocytes pretreated with cell permeable caspase inhibitor Z-VAD-FMK (general caspase inhibitor), followed by Allicin (3 μM) for 24 h. Our result showed that
Figure 53: Dose dependent reduction in viability of monocytes, isolated from blood of cervical cancer patients, treated with Allicin (0.3-3 μM) for 48 hour assessed by MTT assay: NT denotes no treatment (Control). Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
**Figure 54:** Dose and time dependent activation of caspase-9 in Allicin-treated monocytes: Monocytes were treated with different concentrations of Allicin (0.3-3 μM) for 24 and 48 h, harvested and lysed in lysis buffer. Enzymatic activity of caspase-9 was determined by incubation of 50 μg of total protein with substrate LEHD-pNA for 2 h at 37 °C. The release of p-NA was monitored spectrophotometrically at 405 nm. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 55: Dose and time dependent activation of caspase-3 in Allicin-treated monocytes: Monocytes were treated with different concentrations of Allicin (0.3-3 μM) for 24 and 48 h, harvested and lysed in lysis buffer. Enzymatic activity of caspase-3 was determined by incubation of 50 μg of total protein with substrate DEVD-pNA for 2 h at 37 °C. The release of p-NA was monitored spectrophotometrically at 405 nm. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
this caspase inhibitor abolished caspase activities and apoptosis induced by Allicin (p<0.001) (Fig. 56).

**Effect of Allicin on the Tumor Necrosis Factor-α (TNF-α) Expression in Monocytes**

Our above result on monocytes showed that Allicin could be a promising immunomodulatory compound. We further hypothesized that whether Allicin play some role in regulation of proinflammatory cytokines in monocytes. Consequently, we investigated the effect of Allicin on the expression of TNF-α secreted by monocyte culture. Our result showed time and dose dependent downregulation of TNF-α expression in monocytes treated with different doses of Allicin (0.3-3 μM) for 24 and 48 h. Secreted TNF-α in culture supernatant of healthy individual monocytes was 7.41 pg/ml (p<0.001, data not shown), whereas in monocytes isolated from blood of cervical cancer patient, devoid of any allicin, was 221.2 pg/ml (p<0.001). Thus, in comparison to healthy subjects, monocytes from cervical cancer patients exhibited a 29.85-fold augmented level of secreted TNF-α (Fig. 57). However, secreted TNF-α in culture supernatant of monocytes from cancer patients was suppressed to 195.2, 142.7, 98.2 (p<0.001) and 67.6 pg/ml (p<0.001) when cultures received 0.3, 0.6, 1.5 and 3 μM of Allicin for 24 h, respectively (Fig. 57). Similarly, secreted TNF-α in culture supernatant of monocytes from cancer patients was suppressed to 153.4, 126.1, 84.2 (p<0.001) and 52.4 pg/ml (p<0.001) when cultures received 0.3, 0.6, 1.5 and 3 μM of Allicin for 48 h, respectively (Fig. 58). These results showed that Allicin strongly inhibited the expression of secreted TNF-α in monocytes from cervical cancer patient.

**Effect of Allicin on the Interleukin-1α (IL-1α) Expression in Monocytes**

Subsequently, we investigated the effect of Allicin on the expression of IL-1α secreted by monocyte culture. This result also showed time and dose dependent downregulation of IL-1α expression in monocytes treated with different doses of Allicin (0.3-3 μM) for 24 and 48 h. Secreted IL-1α in culture supernatant of healthy individual monocytes was 3.44 pg/ml (p<0.001, data not shown), whereas in monocytes isolated from blood of cervical cancer patient, devoid of any allicin, was 50.44 pg/ml (p<0.001). Thus, in comparison to healthy subjects, monocytes from cervical cancer patients exhibited an augmented level of secreted IL-1α (Fig. 59). However, secreted IL-1α in culture supernatant of monocytes from cancer patients was suppressed to 46.22, 33.66, 26.34 (p<0.001) and 14.77 pg/ml
Figure 56: Abrogation of Allicin-induced reduction in viability of monocytes by general caspase inhibitor (Z-VAD-FMK): Monocytes were pretreated for 2 h with general caspase inhibitor (Z-VAD-FMK) followed by 3 μM of Allicin for 24 hour and cell viability was determined by MTT assay. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 57: Effect on TNF-α expression in monocytes treated with Allicin for 24 hour: Monocytes, isolated from blood of cervical cancer patients, were treated with 0.3-3 μM of Allicin for 24 hour and amount of TNF-α secreted in culture medium was determined by TNF-α ELISA Kit. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 58: Effect on TNF-α expression in monocytes treated with Allicin for 48 hour: Monocytes, isolated from blood of cervical cancer patients, were treated with 0.3-3 μM of Allicin for 48 hour and amount of TNF-α secreted in culture medium was determined by TNF-α ELISA Kit. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 59: Effect on IL-1α expression in monocytes treated with Allicin for 24 hour:
Monocytes, isolated from blood of cervical cancer patients, were treated with 0.3-3 μM of Allicin for 24 hour and amount of IL-1α secreted in culture medium was determined by IL-1α ELISA Kit. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
(p<0.001) when cultures received 0.3, 0.6, 1.5 and 3 μM of Allicin for 24 h, respectively (Fig. 59). Similarly, secreted IL-1α in culture supernatant of monocytes from cancer patients was suppressed to 37.13, 32.26, 21.51 (p<0.001) and 12.43 pg/ml (p<0.001) when cultures received 0.3, 0.6, 1.5 and 3 μM of Allicin for 48 h, respectively (Fig. 60). These results showed that Allicin strongly inhibited the expression of secreted IL-1α in monocytes form cervical cancer patient.

**Effect of Allicin on the Interleukin-6 (IL-6) Expression in Monocytes**

We also investigated the effect of Allicin on the expression of IL-6 secreted by monocyte culture. This result also showed time and dose dependent downregulation of IL-6 expression in monocytes treated with different doses of Allicin (0.3-3 μM) for 24 and 48 h. Secreted IL-6 in culture supernatant of healthy individual monocytes was 8.51 pg/ml (p<0.001, data not shown), whereas in monocytes isolated from blood of cervical cancer patient, devoid of any allicin, was 73.12 pg/ml (p<0.001). Thus, in comparison to healthy subjects, monocytes from cervical cancer patients exhibited an augmented level of secreted IL-6 (Fig. 61). However, secreted IL-6 in culture supernatant of monocytes from cancer patients was suppressed to 67.23, 44.32, 34.33 (p<0.001) and 19.23 pg/ml (p<0.001) when cultures received 0.3, 0.6, 1.5 and 3 μM of Allicin for 24 h, respectively (Fig. 61). Similarly, secreted IL-6 in culture supernatant of monocytes from cancer patients was suppressed to 64.28, 41.18, 27.11 (p<0.001) and 15.37 pg/ml (p<0.001) when cultures received 0.3, 0.6, 1.5 and 3 μM of Allicin for 48 h, respectively (Fig. 62). These results also showed that Allicin strongly inhibited the expression of secreted IL-6 in monocytes form cervical cancer patient. Thus, in the current study, we clearly demonstrated that Allicin strongly induced the apoptosis of monocytes from cervical cancer patient, suppressed the expressions of proinflammatory cytokines TNF-α, IL-1α and IL-6 in monocytes and consequently could act as a promising anti-inflammatory and immunomodulatory agent in cervical cancer.
Figure 60: Effect on IL-1α expression in monocytes treated with Allicin for 48 hour: Monocytes, isolated from blood of cervical cancer patients, were treated with 0.3-3 μM of Allicin for 48 hour and amount of IL-1α secreted in culture medium was determined by IL-1α ELISA Kit. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 61: Effect on IL-6 expression in monocytes treated with Allicin for 24 hour: Monocytes, isolated from blood of cervical cancer patients, were treated with 0.3-3 µM of Allicin for 24 hour and amount of IL-6 secreted in culture medium was determined by IL-6 ELISA Kit. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.
Figure 62: Effect on IL-6 expression in monocytes treated with Allicin for 48 hour: Monocytes, isolated from blood of cervical cancer patients, were treated with 0.3-3 μM of Allicin for 48 hour and amount of IL-6 secreted in culture medium was determined by IL-6 ELISA Kit. Data represent mean ± S.E.M. of three independent experiments. p<0.001 was considered significant.