Chapter-VII
Summary and Conclusions
7.1. Preamble

Worldwide cancer burden has got doubled between 1975 and 2000, and, is anticipated to get doubled again by the year 2020 and virtually tripled by 2030 with projected numbers being 20-26 million new diagnoses and 13-17 million deaths from cancer in the year 2030 (Globocan, 2008; IARC, 2008). With the growing body of evidence linking environmental exposures to cancer, it is becoming apparent that unacceptably huge burden of cancer is resulting from environmental and occupational exposures. According to an estimate, as many as two-thirds of all the cancer cases can be accredited to environmental causes (NCI, 2004). One of such environmental hazards are pesticides whose exposure has been associated with breast, lung, ovarian, pancreatic, kidney, testicular, colon, and stomach cancers, as well as Hodgkin and non-Hodgkin lymphoma, multiple myeloma, and soft tissue sarcoma (Reuben, 2010). Though regulatory agencies like Central Insecticides Board & Registration Committee (CIB & RC) in India and Environmental Protection Agency (EPA) in US have banned some pesticides due to their health risks but due to inadequacy of evidences many harmful pesticides are listed in Report on Carcinogens as likely to be cancer-causing, rather than as known carcinogens and are being used unrestrictedly. Same is the case with pesticide mancozeb which has many animal and epidemiology reports indicating its carcinogenic potential and thus is listed on the most hazardous pesticide list by PAN (PAN HHP, 2009). But due to lack of required amount of evidences it is referred to as class B2 probable human carcinogen by US EPA (2005a) and is very much in use with an annual consumption of ~5.6 million pounds (USEPA, 2005b). Thus, in this research work we have tested mancozeb’s neoplastic potential in human cells.

Uncovering pathways underlying pesticide-induced toxicity are very crucial as this mechanistic information can be combined with other data such as exposure levels and thereby will aid in rational risk appraisals of the pesticide. RNAi-based gene silencing technique for specific inhibition of protein expression may be a valuable tool for elucidating the relationships between phenotypic changes and target gene functions in response to xenobiotic-induced cytotoxicity. Thus, in this research work underlying mechanism of mancozeb-induced neoplastic alterations has been deduced by employing RNAi technique and various protein-inhibitors. Furthermore, this mechanistic information can be very instrumental in discovering antidotes against pesticide-induced damages.
Natural products present in food consumed by human population, which have lately been proved to have cancer chemopreventive and health promoting properties, are very promising candidates for attenuation of pesticide-induced toxicities. One such agent, [6]-gingerol, a pharmacologically active component of ginger (*Zingiber officinale*), which is reported to have chemopreventive properties, has been explored in this research work for its preventive potential against mancozeb-induced genotoxic and neoplastic effects.

While prevention and timely diagnosis can help in reducing the global cancer burden, there is also an urgent need to find better and effective therapeutic approaches for 28 million people around the world suffering with cancer (IARC, 2008), can win their battle for life. Chemotherapy is a treatment mode which due to its systemic nature holds an edge over local treatment mode surgery and radiotherapy and thus is a mainstay of cancer therapy. But despite success in inhibiting or eliminating cancer at preclinical level, clinical applications of chemotherapeutic agents are frequently limited due to the toxic side effects associated with anticancer drugs. The level of a drug needed to successfully eliminate all malignant cells is often intolerable by the patients while the levels that can be tolerated are not sufficient therapeutically. Moreover, lately oncologists have begun to surmise that chemotherapy has attained a plateau of effectiveness as a primary treatment modality, even if the dose-limiting toxicity and side effects can be efficiently restrained. That’s the reason novel targeted therapies that can interfere with specific molecular signaling pathways affecting cancer cell survival are being discovered as prospective treatment modes to make cancer cells more sensitive to chemotherapy. Actually, cancer cells can stimulate multiple pro-survival signaling cascades to maintain their viability in the situations of the DNA damage and oxidative damage inflicted by chemotherapy; use of targeted agents that disrupt these pro-survival signaling cascades can potentiate tumor cell killing, concomitantly lowering the amounts of required drug doses, thus, reducing the toxic side effects and enhancing the destruction of resistant cells while avoiding healthy cells where there is little or no expression of the targeted entity. Among such novel targeted therapies, RNAi mechanism, due to its superior attributes like high sensitivity, high efficiency, high specificity, ability to operate upstream of protein level, safe because of being an endogenous biological pathway, capability to target any mRNA coding for proteins irrespective of the protein structure and localization, has shown tremendous potential at preclinical and clinical levels. The concurrent use of cancer drugs and RNAi-mediated gene silencing is a paradigm providing strategies to inactivate essential genes promoting neoplastic growth. Plk1, which performs key functions in the regulation of mitotic progression, including mitotic entry, spindle formation, chromosome segregation, and cytokinesis, is an excellent molecule for
targeted therapy through RNAi. Several reports have shown that Plk-1 is overexpressed in various cancers including squamous cell carcinoma and prostate cancer and that expression levels of Plk-1 were highly correlated with histological grades of tumors, clinical stages, and prognosis of cancer patients.

Therefore in this research study, RNAi-mediated silencing of Plk1 gene has been applied to chemosensitize skin cancer and prostate cancer cells towards chemotherapeutic drugs, and, the research work was taken up with the following objectives:

- To determine the genotoxic and neoplastic potential of mancozeb in human keratinocyte HaCaT cells and in mouse skin
- To unravel the underlying mechanism behind mancozeb-induced neoplastic alterations
- To explore [6]-gingerol as the preventive dietary antidote against mancozeb-induced genotoxicity and neoplastic alterations alterations in HaCaT cells and in mouse skin.
- To potentiate the sensitivity of cancer cells towards chemotherapy by utilizing RNAi mechanism

7.2. Mancozeb-induced genotoxicity and its inhibition by [6]-gingerol

Here we showed the genotoxic potential of mancozeb in human skin keratinocyte (HaCaT) cells and skin of Swiss albino mice along with its affect on oxidative status. Furthermore, the anti-genotoxic potential of [6]-gingerol against mancozeb-induced genotoxicity has been evaluated.

Cancer results when cells accumulate genetic errors and multiply without control. So if we succeed in inhibiting the genotoxicity by mancozeb then we can hope to combat mancozeb-induced cancer also. To achieve this objective it is imperative to understand the underlying mechanism behind this mancozeb-induced genotoxicity.

In many studies pesticides have been reported to induce oxidative stress, here we instigated the work with assessment of affect of mancozeb on redox status in HaCaT cells and in mouse skin. 24 h mancozeb exposure in HaCaT cells (0.05, 0.1, 0.25 and 0.5µg/ml) and mouse skin (100 mg/kg b wt.) was able to induce oxidative stress as evident by the increase in lipid peroxidation and ROS generation with a concomitant decline in intracellular GSH levels and the activities of various antioxidant enzymes Cu, Zn-SOD, CAT, GR and GST.

Then, two phytochemicals [6]-gingerol (10, 20 and 30 µM), and bromelain (50, 75 and 100 µg/ml), were evaluated for their protective potential against these mancozeb-induced
redox alterations in HaCaT cells. Further, their protection efficacy was compared with two known antioxidants vitamin C (100µM) and NAC (1mM). Both pre- and post-treatment schedules of these compounds with mancozeb were employed, out of which pre-treatment was found to be more efficacious than the post-treatment after mancozeb exposure. Another major finding was that the efficacy was in the order of [6]-gingerol> NAC> vitamin C> bromelain against mancozeb-inflicted redox imbalance. Out of the two tested phytochemicals [6]-gingerol and bromelain, [6]-gingerol was found to be better prospective as preventive antidote against mancozeb-induced oxidative stress and thus [6]-gingerol was selected for further studies henceforth. Protective efficacy of topical application of [6]-gingerol (20, 30 and 40 µM/animal) and NAC (30 and 40 µM/animal) against oxidative stress generated by mancozeb (100 mg/kg b wt.) was then validated in in vivo mouse skin. Both [6]-gingerol and NAC afforded dose-dependent protection but the protection afforded by [6]-gingerol was better than that by NAC.

In order to investigate mancozeb-induced genotoxicity, we conducted DNA alkaline unwinding assay (DAUA) in HaCaT cells (0.5, 2 and 5 µg/ml) and in mouse skin (topical dose 100 mg/kg b wt.). In HaCaT cells mancozeb was recorded to induce significant dose-dependent increase in strand breaks at 2 and 5 µg/ml while apparently no damage was seen at the lowest dose of 0.5µg/ml (p<0.05). In mouse skin also significant augmentation in DNA strand breaks was recorded (p<0.05).

[6]-Gingerol which has the potential to confer commendable protection against mancozeb-induced oxidative stress was then employed to study its anti-genotoxic ability against mancozeb-induced genotoxic damage and it was observed that [6]-gingerol pre-treatment at doses 10, 20 and 30 µM in HaCaT cells and at doses 20, 30 and 40 µM/animal in mouse skin, respectively, could provide dose-dependent protection. NAC pre-treatment in HaCaT cells (1mM dose) and in mouse skin (40 µM dose) also protected against mancozeb-induced genotoxicity, as assessed through DAUA assay, but the protection efficacy of [6]-gingerol was better than that by NAC. These results showed that [6]-gingerol is effective against mancozeb-induced genotoxicity.

Further, in order to assess whether orally adminstered [6]-gingerol can also be effective in protecting from mancozeb’s genotoxicity, 10mg/kg b wt. dose of [6]-gingerol was orally administered to animals 1h prior to mancozeb exposure (2.5 mg/kg b. wt. i.p.). Micronuclei induction and chromosomal aberration assays carried out in mouse bone marrow revealed that orally administered [6]-gingerol can provide significant protection against genotoxicity inflicted by mancozeb (p<0.05). Thus, in in vivo, protection from mancozeb-induced genotoxicity was observed in both orally as well as topically [6]-
gingerol administered animals. Vitamin C (10mg/kg b wt) when given prior to mancozeb in mancozeb-exposed animals, could protect against the mancozeb-induced genotoxicity but the protection by [6]-gingerol was superior to that by vitamin C.

The observation that known antioxidants NAC and vitamin C could afford protection against mancozeb-induced genotoxicity indicated that oxidative stress indeed was playing a role in this genotoxicity inflicted by mancozeb.

Conclusively, results of this study show that mancozeb has the potential to induce oxidative stress-mediated genotoxicity in HaCaT cells and in mouse skin. Additionally, this study also demonstrates that the effectiveness of [6]-gingerol as an antioxidant and antigenotoxic agent is an attribute that can be effectively used in reducing mancozeb-induced damage.

7.3. Evaluation of mancozeb's neoplastic potential and its underlying mechanism in human keratinocyte HaCaT cells and mouse skin: [6]-gingerol as an antidote

In this chapter the neoplastic potential of mancozeb in HaCaT cells and its underlying molecular mechanism involving oxidative stress generation, Nuclear Factor-κB (NF-κB), Extracellular signal Regulated Kinase ½ (ERK1/2) and Polo like kinase 1 (Plk1) signaling pathways has been illustrated. Then, we have validated the involvement of these molecules in underlying mechanism of mancozeb-induced hyperproliferation in in vivo system of swiss albino mouse skin also. Mancozeb has been shown to possess carcinogenic potential in one of the previous study done in our lab using Swiss albino mice skin carcinogenesis model (Shukla et al., 1990). Furthermore, the potential of [6]-gingerol to inhibit mancozeb-induced neoplastic alterations has also been demonstrated in this chapter.

When we exposed HaCaT cells to mancozeb (0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 µg/ml) in order to assess its probable carcinogenic potential in human skin, dose-dependent increase in cell proliferation was recorded with the maximum increase of ~2 fold at dose 0.5 µg/ml, thus this dose was selected for all further assessments. Mancozeb (0.5 µg/ml) exposure in HaCaT cells was found to increase cell proliferation (increased BrdU incorporation and PCNA expression) and neoplastic transformation (colony formation and anchorage independent growth assays). In in vivo topical exposure of mancozeb (100mg/kg b wt.) on skin of Swiss albino mice induced hyperproliferation as evident by increase in BrdU incorporation, S-phase fraction of cell cycle and epidermal hyperplasia. Further studies were conducted in order to identify the affected pathways, which would significantly improve the understanding of molecular pathomechanisms of mancozeb-
induced cancer and provide a fundamental platform for the creation of effective preventive strategies. In Chapter IV of this dissertation we have illustrated mancozeb’s ability to generate oxidative stress and its involvement in genotoxic damage inflicted by mancozeb. Thus, we initiated this pursuit of understanding the mechanism with the same line of thought and used NAC which is a known antioxidant in order to discern the involvement of oxidative stress in mancozeb-induced proliferation. NAC could inhibit hyperproliferation induced by mancozeb in both HaCaT cells (1mM) and mouse skin (40 µM/animal), thus establishing the pivotal role of oxidative stress in this process.

Deregulated inflammatory pathways have a causal relationship with cancer. In this study also proteins p-ERK1/2, p-NF-κBp65 and Plk1, that are involved in mediating the inflammation and proliferation, were found up-regulated on mancozeb exposure in both HaCaT cells and mouse skin. ERK pathway is typically activated by growth factors and has a major role in cell proliferation and tumorigenesis because expression of constitutively active forms of components of this pathway can transform cells. In the present study, pharmacologic inhibition of the ERK pathway decreased mancozeb-induced neoplastic transformation in HaCaT cells, thus validating that the ERK pathway is important for mancozeb-induced neoplastic transformation in humans. Transcription factor NF-κB, a chief regulator of many inflammatory genes, is constitutively active in majority of cancers and is stimulated by carcinogens, tumor promoters, carcinogenic viral proteins, chemotherapeutic agents, and γ–irradiation. In this study, we showed that the pharmacological inhibition of NF-κB by Bay11-7082 inhibited mancozeb-induced DNA synthesis, cell growth and anchorage-independent growth in HaCaT cells. We, therefore, validated that NF-κB activation was certainly required for neoplastic transformation induced by mancozeb in HaCaT cells. Plk1, which is a key controller of the G2/M checkpoint, is found to be up-regulated in many cancers and its overexpression can lead to dysregulated cell proliferation which is typical of the early stages of neoplastic transformation. In this study, specific inhibition of Plk1 by using siRNA for Plk1 could inhibit mancozeb-induced proliferation and transformation, thus we validated the involvement of this protein in mancozeb-induced neoplastic transformation in HaCaT cells.

ERK and NF-κB are important redox sensors of the cell that engage in extensive crosstalk with other signaling pathways, serving as a ‘signaling hub’ for an increasing list of growth factors, cytokines and inflammatory mediators. In the present study also proliferation related proteins downstream of NF-κB and ERK1/2 viz. Cyclin D1, COX-2, Elk1, S100 A6 and S100A9 proteins, were found upregulated on mancozeb exposure. Cyclin D1 acts as a sensor in response to extracellular stimuli like growth factors and
stress. Aberrant cyclin D1 expression has been observed early in carcinogenesis and its over-expression has been reported in several human cancers. COX-2, the inducible isoform of prostaglandin H synthase, is found overexpressed in practically every premalignant and malignant condition. Elk1 is an important protein of Ets oncogene family, also known to positively regulate COX-2 promoter activity. Both S100A6 and S100A9, proteins belonging to S100 family of proteins which has been linked to various cancers, are known to be regulated via redox modifications. Thus, proteins p-NF-κBp65, p-ERK1/2, Plk1, Cyclin D1, COX-2, Elk1, S100 A6 and S100A9 are involved in the inflammatory proliferation induced by mancozeb in HaCaT cells.

As tumor promotion is closely related with inflammation and oxidative stress, compounds that have anti-inflammatory and antioxidative activities can act as good antitumor promoters as well. With this premise we tested [6]-gingerol which is a well reported antioxidant and we also showed its potential to afford a significant protection against oxidative stress generated by mancozeb (p<0.05). In this study [6]-gingerol not only significantly protected against mancozeb-induced hyperproliferative changes in HaCaT cells (10, 20 and 30µM) and in mouse skin (20, 30 and 40µM/animal) but also this protection afforded by [6]-gingerol was more effective than that by NAC(1mM in HaCaT cells and 40 µM/animal in mouse skin) (p<0.05).

At the molecular front mancozeb-induced upregulation of p-ERK1/2, p-NF-κB, p-Elk1, S100 A6, S100A9, Plk1, COX-2 and Cyclin D1 proteins was also reduced by [6]-gingerol pre-treatment in both HaCaT cells and mouse skin. These preventive effects of [6]-gingerol on mancozeb-induced oxidative stress and up-regulation of proteins linked with inflammation-mediated proliferation, correlate with the inhibitory effect of [6]-gingerol against mancozeb-induced proliferation and transformation. These results suggest that the primary effects of [6]-gingerol may be against inflammation, which may thereby result in the inhibition of mancozeb-inflicted proliferative damage.

Thus, it can be inferred from our study that mancozeb induced neoplastic effect in both HaCaT cells and mouse skin through involvement of ERK1/2, NF-κB and Plk1 signal transduction pathways (Figure 7.1). This study also shows that [6]-gingerol has commendable antioxidant and anti-inflammatory attributes against mancozeb-induced damage by restoring the normal levels of protein involved in inflammation and proliferation pathways (Figure 7.1).
Figure 7.1. A schematic representation of possible mechanisms for the mancozeb-induced neoplastic alterations. Signaling pathways and blocking mechanisms are indicated with green and red colored lines, respectively.
7.4. Silencing Plk1 gene through RNAi sensitizes human cancer cells towards phytochemicals/anti-cancer drugs

7.4.1. Chemosensitization of p53 mutant human epidermoid squamous carcinoma A431 cells to CDDP by Plk1 knockdown

In this study we have illustrated the effect of Plk1 knockdown on chemosensitivity of p53-mutant human epidermoid squamous cell carcinoma (SSC) A431 cells towards chemotherapeutic drug cisplatin (CDDP), and, two phytochemicals namely, [6]-gingerol and bromelain. Furthermore, the chemosensitization effect of Plk1 knockdown on etoposide chemosensitivity in both androgen-dependent and androgen-independent prostate cancer cells has also been illustrated here.

Worldwide, one in three cancers are skin-related (WHO, 2010) and thus, developing novel strategies to treat skin cancer represents an urgent goal. In order to explore the possibility of Plk1 as an effective therapeutic target in SCC A431 cells, we employed RNAi technique to silence endogenous Plk1 expression and then analyzed the phenotypic changes. On knocking down Plk1 by using Plk1-specific siRNA (siPlk1) significant inhibition of proliferation, strong G2/M arrest at 48hr and finally cell death at 72 hr were recorded (p<0.05). Plk1 downstream proliferation related pathways were found modulated with upregulation of proteins Cyclin B1 and p-Cdc2, and, reduction of p-Cdc25C. These results indicated that Plk1 can make an excellent molecular target for SCC therapy.

Then, in order to determine an effective combination for SSC therapy, we tested siPlk1 in combination with CDDP/ [6]-gingerol/ bromelain. CDDP, an established antineoplastic drug in the clinic, is most constant ingredient of chemotherapy regimens for many cancers including SCC but problems of drug resistance and side-effects hamper its wide usage. [6]-Gingerol and bromelain, two well reported chemopreventive phytochemicals, have many reports accrediting them with anti cancer potential in skin cancer. Plk1 knockdown conferred ~25%, ~33% and ~80% reduction in IC50s of bromelain, [6]-gingerol and CDDP in A431 cells. As among the three combinations tested, siPlk1 afforded maximum chemosensitization effect in the combination with CDDP, thus, this combination of siPlk1 and CDDP was assessed in further assays. On combination, significant cell death could be achieved at doses even 5 times lower than that of CDDP alone and this effect was amplified synergistically (p<0.05).

One of the possible explanations for the synergistic apoptotic effect recorded on combining Plk1 knockdown with CDDP treatment in p53 deficient A431 cells is a more
stringent G2/M arrest imposed on treatment with DNA damaging-agents in Plk1 depleted cells, the phase most susceptible to DNA damage, which ultimately led to chemosensitization.

In cancers where p53 is mutated as in A431 cells, p73 α, member of p53 family, is known to mediate significant apoptosis and unlike p53, it is rarely lost or mutated in cancer. These facts make it a very promising target for cancers where p53 is mutated. Recently, reports have demonstrated that Plk1 protects p53-deficient cells from p73α-mediated apoptosis by inhibiting p73α (Koida et al., 2008). In this study Plk1 knockdown in A431 cells led to a significant increase in the protein level of p73α (p<0.05). The mRNA levels of p73α transcription targets bax and gadd 45a increased while the p73α mRNA levels remained unchanged suggesting that the increase in the protein level of p73α was unlikely a transcriptional effect. CDDP treatment when combined with Plk1 knockdown increased p73α protein levels synergistically. Nuclear translocation and co-localisation of Plk1 with p73α on CDDP treatment and then synergistic upregulation of p73α on CDDP treatment in siPlk1 transfected cells indicate towards regulatory role of Plk1 in p73α protein level but the exact mechanism remains unknown. p73 is known to be linked to chemosensitivity in SCC cell lines including A431 where CDDP and many other chemotherapeutic drugs have been demonstrated to increase the endogenous p73 levels (Bergamaschi et al., 2003).

Conclusively, siPlk1 in combination with CDDP demonstrated a high efficacy in inhibiting proliferation of A431 cells by blocking cells in DNA damage sensitive G2- M phase and upregulating pro-apoptotic p73α protein (Figure 7.2). The data suggests that specific targeting of Plk1 can sensitize p53-mutant A431 cells to conventional chemotherapeutic agent CDDP, thereby reducing its side-effects by lowering the dosage.
Figure 7.2. Proposed mechanism of chemosensitization of p53 mutant human epidermoid squamous carcinoma A431 cells to CDDP by Plk1 knockdown.
7.4.2. Chemosensitization of prostate cancer cells LNCaP, PC-3 and DU145 to etoposide by Plk1 knockdown

Prostate cancer is one of the most common cancers in men. Existing treatment approaches and surgical intervention have been unable to effectively manage this dreaded cancer; therefore, efforts are ongoing to explore novel targets and strategies for its management. Silencing of Plk1 through siPlk1 led to significant inhibition of proliferation, accumulation of cells in G2/M phase, and finally apoptosis in all the three prostate cancer cells LNCaP, PC-3 and DU145 (p<0.05). Etoposide is widely used anticancer drug in many cancers including prostate cancer but drug resistance and dose limiting toxicities are the problems encumbering its wide usage. When etoposide treatment was given in Plk1-silenced cells, significant apoptosis could be achieved at doses even ~5, ~3 and ~3 times lower than that of etoposide alone in LNCaP, DU145 and PC-3 cells, respectively, and this effect was amplified synergistically (p<0.05). Cell cycle analysis revealed accumulation of cells in the G2/M phase preceding induction of apoptosis which suggested that apoptosis was associated with G2-M arrest following DNA damage. Thus, one plausible explanation for the synergistic apoptotic effect observed on combining Plk1 knockdown with etoposide treatment in prostate cancer cells is a more stringent G2/M arrest imposed by DNA damaging-agents in Plk1 depleted cells, the phase in which cells are most vulnerable to DNA damage, which ultimately enhanced chemosensitization.

Lately some reports have shown that Plk1 negatively regulates the activity of anti-apoptotic protein survivin, a member of the IAP family. In this study Plk1 depletion was accompanied with decreased expression of survivin which suggests that survivin downregulation can be involved in the observed chemosensitization effect of Plk1 knockdown in prostate cancer cells.

Studies have reported that Plk1 regulates and inhibits p53 activity. As mentioned earlier, among the three prostate cancer cell lines LNCaP, PC-3 and DU145, etoposide chemosensitization effect by Plk1 knockdown was maximum in LNCaP cells. As one of the major differences in LNCaP (wild-p53), DU145 (mutant p53) and PC-3 (p53-null) cells lies in their p53 expression levels. Thus, to understand the mechanism behind achieving better chemosensitization in LNCaP cells as compared to DU145 and PC-3 cells, levels of p53 protein expression were measured in LNCaP cells and Plk1 knockdown was found to significantly enhance p53 protein’s level in LNCaP cells (p<0.05). Thus, one of the mechanisms for enhancing LNCaP cells’ chemosensitivity
towards etoposide is liberation of p53 from Plk1-induced suppression in Plk1-silenced cells.

Etoposide is an inhibitor Topoisomerase II- (topo II) α, enzyme that has indispensable role in cell proliferation and Plk1 plays a positive regulatory role in topo IIα functioning in cell cycle progression. Thus, it will not be unreasonable to believe that inhibition of topo IIα might be playing an important role behind this commendable antiproliferative and apoptosis induction potential of this combination of Plk1 knockdown and etoposide.

Conclusively, we provide evidence demonstrating that siPlk1 in combination with etoposide possesses a high efficacy in inhibiting proliferation of prostate cancer cells by blocking cells in DNA damage sensitive G2-M phase and downregulating anti-apoptotic survivin protein. This combination gave maximum response in LNCaP cells due to upregulation in pro-apoptotic p53 protein (Figure 7.3.). The data suggests that specific targeting of Plk1 can sensitize all three prostate cancer cell lines LNCaP, PC-3 and DU145 to conventional chemotherapeutic agent etoposide irrespective of their differences in PCA stage, p53 status and AR expression, thereby reducing etoposide’s side-effects by lowering its doses.
Figure 7.3. Proposed mechanism of chemosensitization of prostate cancer cells LNCaP, PC-3 and DU145 to etoposide by Plk1 knockdown
7.5. Conclusions

The following conclusions were drawn from this study:

- Mancozeb has genotoxic and neoplastic potential in both human keratinocyte HaCaT cells and mouse skin.
- Oxidative stress generation underlies mancozeb-induced genotoxicity.
- Causal mechanism underlying mancozeb-induced neoplastic alterations involves oxidative stress generation, p-NF-κBp65, p-ERK1/2, Plk1, Cyclin D1, COX-2, Elk1, S100 A6 and S100 A9 signaling pathways that are involved in mediating the inflammation and proliferation.
- [6]-Gingerol has provided effective protection against mancozeb-induced genotoxicity.
- Owing to its commendable antioxidant and anti-inflammatory attributes, [6]-gingerol can act as a potent antidote against mancozeb-induced neoplastic alterations in HaCaT cells and mouse skin by inhibiting mancozeb-induced upregulation of p-ERK1/2, p-NF-κB, p-Elk1, S100 A6, S100A9, Plk1, COX-2 and Cyclin D1 proteins.
- [6]-Gingerol can afford better protection against mancozeb-induced genotoxicity and neoplastic alterations in comparison to that provided by NAC.
- Plk1 knockdown can sensitize p53-mutant A431 cells to conventional chemotherapeutic agent CDDP and reduce its side-effects by lowering the required dosage by blocking cells in DNA damage sensitive G2- M phase and upregulating proapoptotic p73α protein.
- Plk1 knockdown can enhance etoposide chemosensitivity and reduce etoposide’s side-effects by lowering its doses in prostate cancer cell lines LNCaP, PC-3 and DU145 irrespective of their differences in PCA stage, p53 status and AR expression by blocking cells in DNA damage sensitive G2-M phase and downregulating antiapoptotic Survivin protein.
- Among the three prostate cancer cell lines LNCaP, PC-3 and DU145, Plk1 knockdown provided maximum enhancement in etoposide chemosensitivity in LNCaP cells due to upregulation in proapoptotic p53 protein.