CHAPTER 9  CONCLUSION AND SCOPE OF THE STUDY

Chemotherapy is the most common non-surgical treatment for all stages of cancers. Cisplatin is one of the standard chemotherapeutic drugs used for the treatment of wide variety of tumors for more than 30 years. Besides its advantages the big obstacle in cisplatin therapy is the cisplatin resistance developed by the cells and toxic side effects to the host. Chemoresistance is observed in the treatment of variety of solid tumors including cervical cancer. To circumvent this problem, there is a need for a substance which could enhance the effects of chemotherapy and decrease the dose limiting side effects of it. It is really a good thing, if a single compound satisfies both the needs.

Dietary phytochemicals have been increasingly recognized in prevention and treatment of human diseases including cancer. Chemosensitizers are substances that can sensitize cancer cells to chemotherapeutic drugs. There exists enormous prospect for the evaluation of phytochemicals to increase sensitization of cancer cells during chemotherapy. Results of various studies have suggested that phytochemicals especially polyphenols, sensitize cancer cells to chemotherapy. They also showed protective action against the effects of toxic chemicals in experimental animals. In this regard, the plant polyphenol ferulic acid (FA), with a variety of beneficial biological effects, is in focus of cancer treatment nowadays. FA, with both antioxidant and prooxidant properties is proved to possess radiosensitizing effects and also showed protective effects in cancer cells in animal models.
Hence it was hypothesized that FA may have the potency to augment chemotherapeutic effects by sensitising cancer cells and protect normal cells during cisplatin chemotherapy in cell lines and animal models.

In the present study, the chemosensitizing effect of ferulic acid on two types of cancer cell lines, cervical cancer (HeLa and SiHa) and lymphoma (Dalton’s lymphoma) cell lines was evaluated. The protection effected when ferulic acid was combined with chemotherapy was also studied on normal human lymphocytes and tumor induced animal models.

The cancer cells, HeLa, SiHa and DLA cells in culture condition showed increased toxic response to chemotherapy in the presence of ferulic acid. The results of the current study shows that ferulic acid-treatment prior to cisplatin chemotherapy significantly increased the levels of TBARS, conjugated dienes and lipid hydroperoxides than cancer cells treated with cisplatin alone. The prooxidant mechanism of ferulic acid might be the reason for the enhanced lipid peroxidation during combined treatment. Phenolics could behave as both antioxidants and prooxidants, depending on its concentration, free radical source and cellular environment.

The susceptibility of tumor cells to chemotherapy is associated with decreased levels of antioxidants. It was observed that ferulic acid treatment prior to chemotherapy significantly decreased the activities of antioxidant enzymes such as SOD, CAT and GPx in HeLa, SiHa and DLA cells when compared to cells treated with cisplatin alone. Enhanced lipid peroxidation and oxidative damage occurred during ferulic acid plus cisplatin treatment may be the reason for decreased activities of antioxidant enzymes.

Further, ferulic acid-treatment prior to cisplatin significantly decreased the levels of GSH in HeLa, SiHa and DLA cells when compared to cisplatin alone treatment. The detoxifying antioxidant GSH, might conjugate with
cisplatin molecule resulting in the increased efflux of GSH conjugated cisplatin and thereby decreasing the concentration of cisplatin in cancer cells. Polyphenols depletes GSH via increasing the oxidation of GSH and inhibiting GSH recycling mechanisms. It is implicated that FA might act synergistically with cisplatin and thereby depletes GSH to sensitize tumor cells to cisplatin therapy.

Many chemotherapeutic drugs including cisplatin increase the levels of ROS in cancer cells and thereby promoting them to apoptotic cell death. Hence, increased ROS is believed to induce cancer cell death. In the present study, FA primed cisplatin treated cancer cells showed increased intracellular ROS levels when compared to cisplatin alone treated cells which may be due to the prooxidant activity of FA and ROS generating effect of cisplatin.

The enhancement of chemotherapy induced DNA damage in ferulic acid pretreated cancer cells was seen in the present study. A significant increase in the levels of DNA damage accompanied by increase in % tail DNA, tail length, tail moment and Olive tail moment in ferulic acid plus cisplatin treated cancer cells was observed. Polyphenols have been shown to induce DNA damage. Moreover, increased ROS levels can induce DNA damage. Decreased antioxidant status, increased ROS and lipid peroxidation during ferulic acid treatment may augment chemotherapy induced DNA damage in HeLa and SiHa cells.

Cancer cell proliferation was significantly inhibited by ferulic acid and cisplatin. Ferulic acid was found to be cytotoxic in vitro at the concentration of 10 µg/mL in cancer cells. A combination of 5 µg/ml of cisplatin with 10 µg/ml of ferulic acid augmented the cell growth inhibition more effectively than a cisplatin dose alone or ferulic acid-treatment alone. Further, the therapeutic synergy between ferulic acid and cisplatin was observed in the present study. It was also observed that FA and cisplatin not only increased cytotoxicity in
cancer cells but also decreased the number of cells with reproductive capacity in clonogenic cell survival assay.

The apoptotic morphology was observed in ferulic acid and cisplatin treated cervical cancer cells. The percentage apoptosis was high in ferulic acid plus cisplatin treated cells when compared to ferulic acid or cisplatin treatment alone.

Mitochondrial membrane depolarization occurs during the early stage of apoptosis. It was found that FA pretreated HeLa and SiHa cells were characterized by increased mitochondrial membrane depolarization than cisplatin alone treated cancer cells, depicting the preparation of cancer cells for apoptosis due to sensitization by FA.

During apoptosis chromatin condensation occurs. FA and cisplatin treated cancer cells showed clear signs of nuclear condensation and fragmentation suggesting that the cancer cells sensitized with FA were prepared for apoptotic cell death.

Apoptosis is the result of co-ordinated action of pro-apotic and anti-apoptotic proteins like caspase 3, 8 and 9, p53 and Bcl-2. FA primed cisplatin treated HeLa cells showed an upregulation in the expression of apoptotic proteins and a down regulation in the expression of anti-apoptic proteins. Hence it was clearly understood that FA sensitization is accompanied by the involvement of apoptotic signaling pathways.

From the results of the present study, it was summarized that FA enhanced cisplatin therapy by increasing cell proliferation, decreasing antioxidant status and clonogenic cells, increasing lipid peroxidation, DNA damage, apoptosis, nuclear condensation, and apoptotic protein expression in cervical cancer cells. There appears to be a therapeutic synergy between FA and cisplatin. Also in DLA cells the therapeutic synergy was seen.
The sensitization of cancer cells to chemotherapy by FA is mainly due to the prooxidant activity of FA. This prooxidant induced cytotoxicity is unfit for the normal cells to survive. Hence, the effect of FA on cytotoxicity was tested in normal human lymphocytes. It was found that FA did not induce cytotoxicity in cancer cells and also it protected normal cells from the cisplatin induced cytotoxicity. This confirmed that sensitization of FA does not affected normal cells.

Studies on Dalton’s lymphoma induced solid and ascitic tumor in mice potentiated the treatment effects of cisplatin and also protected the normal host tissues from therapy induced toxicities. Thus, ferulic acid enhanced the effects of cisplatin and also reduced the side effects of cisplatin in animal models also. Moreover, reduced the side effects of cisplatin.

On the whole, the current research findings revealed the potential chemosensitizing and chemoprotective effects of ferulic acid on cancer chemotherapy in vitro and in vivo. Thus, FA not only augmented the therapeutic efficacy of cisplatin by sensitizing cancer cells but also it protects the host from toxic side effects. Ferulic acid treated along with Cisplatin enhanced the therapeutic effects of the chemotherapeutic drug and decreased the side effects to the host in preclinical studies. Moreover, FA is widely available in plant and food sources and it is already in use as a dietary supplement. FA having apoptotic activity and potential synergy with standard cytotoxic drug cisplatin will allow reduction of doses as well as multidrug regimens of toxic chemotherapeutic agents while maintaining or even enhancing chemotherapeutic efficacy.

Therefore, further mechanistic studies would potentiate the development of ferulic acid as a therapeutic agent along with anticancer drugs in chemotherapy in clinical trials. If FA is clinically proven as a therapeutic
and protective agent, it would be of great help to the humanity in the fight against cancer.