CHAPTER 2  LITERATURE REVIEW

2.1. Background

Modern day living is bereft with susceptibility to diseases including cancer. The knowledge, diagnostics, treatment and prognosis of diseases have improved tremendously during the past decade. Basically cancer is a disease of genes that control the proliferation, differentiation and death of cells. Most cancers are derived from single somatic cell and their progeny, as a result favoured selection of mutated clones (Hanahan et al. 2000).

The term “cancer” is used synonymous with the term malignant neoplasia and describes a disease, which is characterized by uncontrolled, abnormal growth of cells (proliferation). The process of conversion of a normal cell to a malignant state is called carcinogenesis. Carcinogenesis is a complicated, multistage process; essentially, a small population of abnormal cells is generated which then increases in abnormality, as a result of a series of mutations and change in patterns of gene expression. Cancer may result as a combination of chemical, physical, biological and genetic factors to individual cells and can develop at any age (Minamoto et al. 1999).

2.2. Chemotherapy

Cancer chemotherapy is the treatment of cancer by anticancer drugs especially targeting rapidly dividing cancerous cells. Chemotherapy is one of the important treatment strategies of cancer since it destroys cancer cells anywhere in the body. It even kills cells that have broken off from the main tumor and travelled through the blood or lymph systems to other parts of the body. Chemotherapy may be used alone for some types of cancer or in conjunction with other therapy such as radiation or surgery (Reichardt et al. 2003). Often, a combination of chemotherapy drugs is used to fight a specific
cancer. Certain chemotherapy drugs may be given in a specific order depending on the type of cancer it is being used to treat (Brennan et al. 1991). While chemotherapy can be quite effective in treating certain cancers, the agents do not differentiate normal healthy cells from cancer cells (Picci 2000). Because of this, there can be many adverse side effects during treatment.

2.3. History of cancer chemotherapy

The era of cancer chemotherapy began in the 1940s with the first use of nitrogen mustards and folic acid antagonist drugs. Cancer drug development has exploded since then into a multi-billion dollar industry. The targeted therapy revolution has arrived, but many of the principles and limitations of chemotherapy discovered by the early researchers still apply. Time since the discovery of chemotherapy, various types of therapeutic strategies has been evolved. Of which, combination chemotherapy using cisplatin along with other anti cancer molecules seems to be more effective (Flaherty et al. 1993; Buzaid et al. 1994).

2.4. Cisplatin

Cisplatin is an effective chemotherapy drug, used to treat variety of cancers, including lung cancer, ovarian cancer, head and neck cancer, testicular cancer, cervical cancer and lymphoma (Schrier 2002). It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum complexes react in vivo, binding to cellular DNA and causing crosslinks, which ultimately triggers apoptosis (programmed cell death). The cisplatin molecule (Figure 2.1) consists of a platinum atom surrounded by two chloride and two ammonia ligands, configured in cis-position (Basu and Krishnamurthy 2010). Unlike most cancer therapy drugs, which are usually complex organic compounds, cisplatin is a simple inorganic molecule.
Figure 2.1. Chemical Structure of Cisplatin (2D & 3D)

(Courtesy: cisplatin@3dchem.com)

- **Common name**: Cisplatin, cisplatinum, or cis–diammine dichloro platinum (II) (CDDP)
- **IUPAC name**: (SP-4–2)-diamminedichloridoplatinum
- **Structural name**: cis - Diamminedichloroplatinum
- **Trade names**: Platinol and Platinol- AQ
- **Molecular weight**: 300.05
- **Molecular formula**: Cl₂H₆N₂Pt or (NH₃)₂PtCl₂
- **Colour**: Deep yellow solid
- **State/Form**: Solid

It is soluble in water (0.253 g/100 g at 25° C), sodium chloride and dimethylformamide but insoluble in most common solvents. It decomposes at 270° C and slowly changes from the cis to the trans form in aqueous solution (Budavari 1989; McEvoy 1992 ).
2.4.1. Origin

The compound cisplatin (cis-\(\text{PtCl}_2(\text{NH}_3)_2\)) was first described by M. Peyrone in 1845 and known for a long time as Peyrone's salt (Peyrone 1844). The structure was deduced by Alfred Werner in 1893 (Stephen 2005). In 1965, Barnett Rosenberg \textit{et al.} (1965) discovered that electrolysis of platinum electrodes generated a soluble platinum complex which inhibited binary fission in \textit{Escherichia coli} (Rosenberg \textit{et al.} 1965). The octahedral Pt (IV) complex \(\text{cis}\ \text{PtCl}_4(\text{NH}_3)_2\), but not the \textit{trans} isomer, was found to be effective at forcing filamentous growth of \textit{E. coli} cells. The square planar Pt (II) complex, \(\text{cis}\ \text{PtCl}_2(\text{NH}_3)_2\) turned out to be even more effective at forcing filamentous growth (Rosenberg \textit{et al.} 1967). This led to the finding that \(\text{cis}\ \text{PtCl}_2(\text{NH}_3)_2\) was indeed highly effective at regressing the mass of sarcomas in rats (Rosenberg \textit{et al.} 1969). Nowadays, in cancer chemotherapy, cisplatin is considered to be one of the most remarkable successes in ‘the war on cancer.’ Since the accidental discovery nearly five decades ago, cisplatin has been widely used for chemotherapy (Pabla and Dong 2008).

2.4.2. Mechanism of action

Following administration of cisplatin, one of the chloride ligands is slowly displaced by water (an aqua ligand), in a process termed aquation. The aqua ligand in the resulting \([\text{PtCl(H}_2\text{O})(\text{NH}_3)_2]^+\) is itself easily displaced, allowing the platinum atom to bind to guanine residues of DNA. Subsequent to formation of \([\text{PtCl(guanine-DNA)(NH}_3)_2]^+\), crosslinking can occur via displacement of the other chloride ligand, typically by another guanine (Stephen 2005). Cisplatin crosslinks DNA in several different ways, interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible (Pruefer \textit{et al.} 2008).
Most notable among the changes in DNA are the 1,2-intrastrand crosslinks with purine bases. These include 1,2-intrastrand d(GpG) adducts which form nearly 90% of the adducts and the less common 1,2-intrastrand d(ApG) adducts. Intrastrand d(GpXpG) adducts occur at 1,3 position but are readily excised by the nucleotide excision repair. Other adducts include inter-strand crosslinks and nonfunctional adducts that have been postulated to contribute to cisplatin's activity. Interaction with cellular proteins, particularly HMG (hydroxy methyl guanine) domain proteins, has also been advanced as a mechanism of interfering with mitosis, although this is probably not its primary method of action (Stephen 2005).

2.5. **Drawbacks in cisplatin chemotherapy**

Although cisplatin has been a mainstay for cancer therapy, its use is mainly limited by two factors: acquired resistance to cisplatin and severe side effects in normal tissues (Florea *et al.* 2011). It is also a potential human carcinogen and develops secondary malignancies in patients who have been treated with cisplatin (Greene 1992).

2.5.1. **Cisplatin resistance**

As cisplatin chemotherapy is the cornerstone for treatment of many cancers, resistance to cisplatin is also becoming a frequent mechanism of treatment failure. Cisplatin-induced drug resistance is known to involve a complex set of cellular changes whose exact molecular mechanism remains elusive (Chen *et al.* 2007). Decreased intracellular concentration due to decreased drug uptake, increased reflux or increased inactivation by sulfhydryl molecules such as glutathione, increased excision of the adducts from DNA by repair pathways, increased lesion bypass and altered expression of regulatory proteins involved in signal transduction pathways that control the apoptotic
pathway can cause resistance to cisplatin (Kartalou and Essigmann 2001; Strodal 2007).

2.5.2. Toxic side effects of cisplatin

The therapeutic efficacy of cisplatin is limited due to the development of various side effects in the hosts. Over dose and over use of cisplatin may lead to the following side effects.

- Nephrotoxicity (Fillastre and Raguenez-Viotte 1989; Yao *et al.* 2007)
- Neurotoxicity (Gregg *et al.* 1992; Alberts and Noel 1995)
- Hematotoxicity (Khynriam and Prasad 2001)
- Nausea and Vomiting (Hesketh *et al.* 1997)
- Electrolyte disturbance (Windsor *et al.* 2012)
- Hepatotoxicity (Lu and Cederbaum 2006)
- Gastrointestinal toxicity (Yanez *et al.* 2003)
- Ototoxicity (Kohn *et al.* 1988; Ravi *et al.* 1995)
- Mutagenicity (Kartalou and Essigmann 2001; Prasad and Khynriam 2002)
- Peripheral neuropathy (Hamers *et al.* 1991)
- Embryotoxicity (Keller and Aggarwal 1983)

These drawbacks in cisplatin chemotherapy led to the urge in search for alternate therapies.

2.6. Natural products in cancer therapy

Plants have been used for treating various diseases of human beings and animals since time immemorial. They maintain the health and vitality of individuals and also cure diseases, including cancer without causing toxicity.
More than 50% of all modern drugs in clinical use are of natural products and many of which have the ability to control cancer cells. According to World Health Organization (WHO), more than 80% of people in developing countries depend on traditional medicine for their primary health needs. In a survey, it was found that more than 60% of cancer patients use vitamins or herbs as anticancer agent (Sivalokanathan et al. 2005; Madhuri and Pandey 2008).

Epidemiological data available strongly correlates the dietary intake of food, vegetables and medicinal plants reduced the risk of cancer in experimental animals and humans. Plant derived foods contain thousands of chemically dissimilar phytochemicals, many of which have been investigated using \textit{in vitro} and \textit{in vivo} studies to determine their effects on cancer and their related mechanism of action (Morse and Stoner 1993). In this regard, naturally occurring biologically active polyphenols derived from common dietary sources are gaining increasing interest as potential cancer therapeutics (Steinmetz et al. 1991). Moreover, several plant phenolics are known to be potent inhibitors for mutagenesis and carcinogenesis (Srinivasan et al. 2007). Polyphenols have invited great interest in cancer prevention and treatment because of their possible beneficial implications on human health (Bravo 1998).

\textbf{2.7. Chemosensitizer}

A chemosensitizer is a drug that makes tumor cells more sensitive to the effects of chemotherapy. By making tumor cells more sensitive, lower doses of chemotherapy can be used which may decrease the side effects of treatment. In the recent past, research has been focused to explore chemical substances present in plants (phytochemicals) as sensitizers of chemotherapy.
2.7.1. Phytochemicals in chemotherapy

Phytochemicals, defined as bioactive non-nutrient plant compounds present in fruits, vegetables, grains and other plant foods, have been linked to risk reduction of major chronic diseases including cancer. Therefore, phytochemicals are the most promising chemopreventive and treatment options for the management of cancer (Kushi et al. 2006).

Evidences showed that flavonoids modulate the efficacies of chemotherapeutic drugs such as cisplatin (Cipak et al. 2003). Several phytochemicals, like capsaicin from chilli peppers, epigallocatechine gallate (EGCG) extracted from green tea (Tacchini et al. 2000; Suzuki et al. 2004), genistein found in soybeans and other legume species (Takada and Aggarwal 2003; Tainton et al. 2004) and curcumin from Indian turmeric were widely employed in cancer research and their effects were studied along with chemotherapy and radiation. They are capable of enhancing the efficacy of chemotherapy and radiotherapy in various in vitro and in vivo cancer models. They act predominantly by modulating intracellular cell signaling pathways, abrogating drug resistance and diminishing systemic toxicities (Milas 2001; Terakado et al. 2004). Due to their wide range of biological and pharmacological effects, especially with regard to chemoprevention and the lack of toxicity in animal and human models, they might also act as possible agents for enhancing the effects of chemotherapy.

Various strategies have been explored to overcome tumor drug resistance, among which the combination of chemotherapy with plant polyphenols as a chemosensitizer has emerged as a promising one (Garg et al. 2002). Furthermore, these plant polyphenols may enhance the tumoricidal effects of chemotherapy and radiotherapy, protect normal cells from therapy induced damage, and increase systemic bioavailability of chemotherapeutic agents (Garg et al. 2005).
2.8. **Enhancement of cytotoxicity by chemosensitizers**

Majority of chemotherapeutic drugs are cytotoxic in nature. More the cytotoxicity of a chemotherapeutic drug, more intense will be its effect on the cancer cells. Previous studies have shown that many phytochemicals do have cytotoxic action towards cancer cells and were proven to improve the cytotoxicity of chemotherapy in preclinical studies. For example, caffeic acid, a dietary polyphenol was observed to induce cytotoxicity in human fibrosarcoma, HT-1080 malignanat cell line (Prasad et al. 2011). Similarly, camellin B (a hydrolysable tannin dimer), showed cytotoxic action towards HeLa cell lines (Wang et al. 2001). In a study by You et al. (2010), plant polyphenol gallic acid, induced cytotoxicity and apoptosis in cervical cancer cells. These evidences have illustrated that phytochemicals particular polyphenols, possessed cytotoxicity against cancer cells. Hence, polyphenols with cytotoxic activity towards cancer cells are coupled with chemotherapy would result in the further enhancement of the cytotoxicity of chemotherapeutic drugs. Schwartz et al. (1997) have also reported that flavopiridol potentiated the cytotoxic effects of mitomycin c by promoting drug induced apoptosis in breast and gastric cancer cells. Similarly, genistein and diadzein enhanced the cytotoxicity of cisplatin in squamous cell carcinoma cells (Ali et al. 2009).

2.9. **Prooxidant activity of polyphenols in cancer cells**

Plant polyphenols are generally recognized as strong physiological antioxidants. Besides serving as antioxidants, they also exhibit prooxidant properties *in vitro* in the presence of transition metal ions such as iron and copper (Bhat et al. 2007). Polyphenols may behave as prooxidants depending upon the cellular environment. Cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may
trigger a series of redox-dependent reactions (Forman et al. 2002; Halliwell et al. 2005). Caffeic acid (a dietary polyphenol) showed prooxidant induced DNA breakage in human peripheral lymphocytes through the involvement of endogenous copper ion (Bhat et al. 2007). Prasad et al. (2011) also reported that caffeic acid expressed its prooxidant nature against human fibrosarcoma cancer cells.

Both antioxidant and prooxidant effects of polyphenols have been described, with contrasting effects on cell physiological processes. As antioxidants, polyphenols may improve cell survival of normal cells but as prooxidants, they may induce apoptosis in cancer cells and prevent tumor growth (Lambert et al. 2005). However, the biological effects of polyphenols may extend well beyond the modulation of oxidative stress. So, a detailed understanding of the molecular events underlying these various biological effects of polyphenols is necessary for the evaluation of its overall impact on cancer.

2.10. Role of antioxidants and reactive oxygen species in chemotherapy

Reactive oxygen species (ROS) are produced or its production is increased in cancer cells upon treatment with cytotoxic agents such as cisplatin. Tumor cells have higher levels of ROS than their normal counterparts and are therefore more sensitive to the additional oxidative stress generated by anticancer agents (Trachootham et al. 2009). Elevated ROS levels inhibit cancer progression through the stimulation of pro-apoptotic signals, leading to the death of cancer cells (Park et al. 2008). They are closely involved in stress-induced apoptosis. Research evidence suggests that ROS also induced programmed cell death in several cancer cells (Park et al. 2008). Moreover, ROS production can enhance the cell sensitivity to cisplatin through activation of signalling pathways (Benhar et al. 2001; Acharya et al.
Recently, many phenolic phytochemicals have been shown to induce apoptosis in cancer cells by the generation of ROS (Fan et al. 2009).

Antioxidants are potent scavenger of free radicals and of particular importance in protection against cancer (Collins et al. 1994). The antioxidants superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione are the backbone of cellular antioxidant defense mechanisms (Branco et al. 2006). Many studies suggested that antioxidant systems are critical in protecting against tumor promoting agents. Interestingly, cell malignancy or transformation is often accompanied by a decrease in activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which increases the cell sensitivity to pro-oxidant compounds (Sergediene et al. 1999). As a result, the susceptibility of tumor cells to chemotherapy is associated with decreased levels of antioxidants (Dal-Pizzol et al. 2003). Bhosle et al. (2005) showed that ellagic acid decreases antioxidant enzymes like SOD, CAT and GPx in mice treated with radiation. On the other hand, ellagic acid shows protection against radiation induced ROS in normal cells. This shows a differential action of ellagic acid in tumor cells and its normal counterpart. Thus, it can be ascribed that a single molecule can differentially act in cancer and normal cells. According to Manoharan et al. (2002), the activities of antioxidant enzymes were decreased in cervical cancer patients as compared to healthy subjects. The low activity of antioxidant enzymes observed in cancer patients may be due to increased accumulation of free radicals which damage all proteins (Estrela et al. 1992). Hence, decreased levels of antioxidants in cancer cells may finally lead to cancer cell death.

Recently published reports have suggested that the changes in the antioxidants, ROS and lipid peroxidation levels when polyphenols are treated against cancer cells (Park et al. 2008; Fan et al. 2009; Bandugula and Prasad
Similarly Karthikeyan et al. (2011) reported that, polyphenol ferulic acid brought notable changes in the antioxidants, lipid peroxidation and ROS levels through its prooxidant action in cervical cancer cells.

2.11. DNA damage, mitochondrial membrane potential and other cellular changes leading to apoptosis in chemotherapy

Apoptosis is a normal physiological process which occurs during embryonic development as well as during the maintenance of tissue homeostasis. It is a tightly controlled cell suicide that occurs under a range of physiological and pathological conditions. Apoptosis in cancer cells is characterized by a number of features such as morphological alterations, nuclear condensation, nuclear fragmentation giving definite pattern in gel electrophoresis and imbalance of cellular signaling machinery (Khosravi-Far and Esposti 2004). There is strong evidence that tumour growth is not only a result of uncontrolled proliferation but also of reduced apoptosis (Tamm et al. 2001). Thus inducing cancer cell apoptosis has been one of the key strategies in anticancer therapy.

Earlier reports have shown that polyphenols induce or promote apoptosis in cancer cells. Recently, Prasad et al. (2011) showed that caffeic acid, a plant polyphenol can cause mitochondrial transmembrane potential change in fibrosarcoma cells and thereby leading them to apoptosis. 2’ nitroflavone a synthetic flavonoid, brought about apoptosis characterized by nuclear condensation and morphological changes in cervical cancer cells (Cardenas et al. 2008). According to Murugan et al. (2010), black tea polyphenols induced intrinsic apoptosis in HepG2 malignant cells through NF-kappa B signaling. Bandugula and Prasad et al. (2012) reported that FA can cause DNA damage and thereby inducing apoptosis in non-small cell lung carcinoma cell lines.
Apoptosis is characterized by distinct morphological changes including nuclear and cytoplasmic condensation, DNA fragmentation, phosphatidylserine externalization and plasma membrane blebbing (Burz et al. 2009). Induction of apoptosis by polyphenols can cause these morphological changes inside the cells. Karthikeyan et al. (2011) and Bandugula and Prasad (2012) showed that treatment of FA caused these morphological changes in lung and cervical cancer cells indicating apoptosis.

2.12. Role of signaling proteins involved in apoptosis in chemotherapy

Gaining further knowledge on the role of apoptotic signalling pathways may lead to novel strategies to enhance chemotherapy. Cellular changes due to apoptosis are triggered by the proteolytic activity of a family of cysteinyll aspartate-specific proteases, known as caspases. They dismantle the cell by cleaving and thus inactivating key cellular proteins including the DNA repair enzyme poly(-ADP-ribose) polymerase (PARP) (Juge et al. 2007). This cellular destruction results in the formation of apoptotic bodies that are subsequently eliminated by phagocytosis (Bucur et al. 2001; Khosravi-Far and Esposti 2004). Caspases are synthesized as inactive proenzymes, which are activated by cleavage at specific aspartate residues to activate enzymes comprising large (p20) and small (p10) units (Keum et al. 2004). A subset of caspases, termed initiator caspases interact with specific adapter molecules that facilitate their autoprocessing (Keum et al. 2004).

Upon activation, initiator caspases process a second class of caspases, termed effector caspases, which act on key cellular proteins, resulting in the dissolution of the cell (Nicholson 1999). At present, two major apoptosis pathways have been identified: the death receptor or extrinsic pathway and the mitochondrial or intrinsic pathway. The extrinsic pathway is activated through cell surface death-receptors binding their respective cytokine ligands, followed
by induction of the initiator caspase-8 and subsequent activation of the effector caspase-3 (Stennicke and Salvesan 2000).

The intrinsic pathway depends on mitochondrial membrane permeabilization, which causes the release of apoptogenic factors, such as cytochrome c from the intermembrane space to the cytoplasm (Meier and Vousden 2007). Once released, cytochrome c directly activates Apaf-1 and in the presence of dATP or ATP, induces the formation of a multimeric apoptosome complex, resulting in the activation of the caspase-9 followed by the cleavage and activation of caspase-3 and -7.

The mitochondrial pathway is controlled by members of the Bcl-2 family regulating mitochondrial membrane permeability and subsequent release of proapoptotic factors. They can be divided into a pro- and antiapoptotic group (Reed 2006). Bcl-2 comes under the antiapoptotic subclass, act on the outer mitochondrial membrane by neutralizing the killer proteins. In addition to their role in executing apoptosis, caspases also play important signaling roles in nonapoptotic processes, including regulation of actin dynamics, innate immunity, cell proliferation, differentiation, and survival. Under such conditions, caspase activation does not lead to cell death. The required regulation of caspase activity is partly mediated by members of the inhibitor of apoptosis (IAP) protein family. The IAPs are a family of caspase inhibitors that directly bind caspase-3, -7 and/or -9 and thereby impair the activity of these critical effectors of apoptosis (Lamkanfi et al. 2007).

Several studies have suggested the involvement of molecular pathways in chemosensitization of chemotherapeutic drugs. In a study by Sharma et al. (2005), quercetin chemosensitized cisplatin in human head and neck cancer cells by modulating molecular pathways. Quercetin with cisplatin down regulated Bcl-2 and upregulated caspases. Similarly, curcumin enhanced vinorelbine mediated apoptosis by the modulation of mitochondrial pathway

2.13. Polyphenols

Polyphenols are the most abundant antioxidants in the diet. Their total dietary intake could be as high as 1 g/day, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants. For perspective, this is ~10 times higher than the intake of vitamin C and 100 times higher that the intakes of vitamin E and carotenoids. Their main dietary sources are fruits and plant-derived beverages such as fruit juices, tea, coffee, and red wine. Vegetables, cereals, chocolate, and dry legumes also contribute to the total polyphenol intake. Some polyphenols are also ingested as food supplements which may improve the health status (Scalbert et al. 2005).

2.14. Chemosensitization by polyphenols

Several reports have highlighted the enhanced efficacy of chemotherapy when it is combined with plant polyphenols.

Goel and Aggarwal (2010) have stated that the chemopreventive agent curcumin (derived from turmeric) have the ability to sensitize many human cancers to chemotherapy and radiation, as well as afford protection against the toxicity of these treatment regimens. The soy isoflavone genistein synergistically combines with 5-fluorouracil to induce apoptosis by modulating signaling pathways in chemoresistant cancer cells (Hwang et al. 2005). Sharma et al. (2005) showed the flavanoid quercetin sensitizes the head and neck cancer cells for cisplatin treatment by modulating the molecular pathways involved in apoptosis. Similarly, an isoflavone mixture containing
genistein and diadzein found to sensitize squamous cell carcinoma cell lines to cisplatin therapy (Ali et al. 2009).

The inhibitory effects on the combination of chemopreventive agents were studied on prostate cancer cells *in vitro* and *in vivo* (Adhami et al. 2007). In another study, it was found that curcumin and quercetin when combined with cisplatin increased cisplatin induced apoptotic cell death in Hep-2 cells via mitochondrial pathway. It was also reported that, priming of curcumin or quercetin minimized the side effects associated with cisplatin therapy (Kuhar et al. 2007). Similarly, green tea polyphenols and curcumin together with celecoxib and vinorelbine, enhanced apoptosis in cancer cells (Sen et al. 2005; Adhami et al. 2007). According to Javvadi et al. (2008) curcumin exhibited radiosensitizing effects by increasing radiation induced ROS production and Mitogen-Activated Protein Kinase Pathway (MAPK) in cervical cancer cells.

Research evidences have suggested that the amino acid theanine, a major component of green tea, has a synergistic effect with chemotherapeutics drugs including cisplatin for a variety of cancers (Sugiyama and Sadzuka 1998; Sugiyama and Sadzuka 2003). They have also reported that tea polyphenols reversed multi drug resistance in P388 leukemia bearing mice (Sadzuka and Sugiyama 2000). Similarly, epigallo catechin-3- gallate (EGCG) and other polyphenols may enhance chemotherapy induced antitumor activity and increase the concentration of chemotherapy drugs in tumors by inhibiting the efflux (Garg et al. 2005).

In a study by Tamura et al. (2003), genistein (a soy isoflavone) potentiated the effects of cisplatin chemotherapy such as cell growth inhibition and apoptosis. Li et al. (2004) found that, treatment with genistein before docetaxel or cisplatin administration enhanced tumor cell death compared with chemotherapeutic drug alone, in pancreatic cancer cell line.
Additionally, studies on soy iso flavone genistein increased the antiproliferative effects and apoptosis inducing action of cisplatin and other chemotherapeutic drugs in lung cancer in vivo and in vitro (Lei et al. 1999, Wietrzyk 2001). Similarly Khoshyomn et al. (2000) showed that, genistein potentiated growth inhibition and cytotoxicity of cisplatin, in meduloblastoma cells.

In a study by Kubota et al. (2003), pretreatment with resveratrol sensitized paclitaxel treatment by significantly enhancing its antiproliferative effects (Garg et al. 2005). Resveratrol also sensitized non- Hodgkin’s lymphoma and multiple myeloma cell lines to paclitaxel induced apoptosis (Jazirehi and Bonavida 2004).

Several studies have shown that curcumin sensitizes cells to chemotherapy (Bharti et al. 2003). Curcumin potentiated the cytotoxic effects of chemotherapeutic drugs in prostate cancer cells (Hour et al. 2002; Chuang et al. 2002). These findings supported the fact that curcumin with chemotherapeutic drugs may have synergistic effects (Garg et al. 2005). Emodin, a natural anthroquinone polyphenol, may sensitize cancer cells to chemotherapy by generation of ROS (Yi et al. 2004). It also enhanced the inhibitory effects of cisplatin in lung cancer cells (Zhang and Huang 1996).

Flavopiridol and silymarin may potentiate the effects of chemotherapy on cancer cells (Zhang and Morris 2003). Another study demonstrated the synergistic effects of silybin plus cisplatin or doxorubicin in breast cancer and ovarian cancer cells (Scambia et al. 1996).

Thus, a vast array of research evidence has proved the usage of polyphenols as chemosensitizers of chemotherapy.
2.15. Protection of normal cells from chemotherapy by polyphenols

Chemopreventive polyphenols were found to protect normal cells from radiation or chemotherapy induced damage when given along with chemotherapeutic drugs. The protective actions of some of the important phytochemicals are discussed below.

Addition of chemopreventive agents with chemotherapy was found to reduce the side effects of cisplatin. Reports have shown that curcumin was a potent protector against chemotherapeutic drugs and other toxic chemicals. In a study by Greggi Antunes et al. (2001), the antioxidant curcumin reduced cisplatin induced kidney damage in rats by reducing the oxidative stress caused by cisplatin. It protected cells from doxorubicin-induced renal injury, cardiotoxicity and gastrointestinal injury. These protective effects could be secondary to the suppression of oxidative stress and inflammatory damage (Garg et al. 2005). Similarly, oral administration of curcumin (200 µmol/kg) significantly reduced lung toxicity by reducing lipid peroxidation in rats treated with whole-body radiation (Thresiamma et al. 1996). In an in vivo model of human breast cancer, dietary supplementation with curcumin significantly inhibited cyclophosphamide-induced tumor regression. From this, it was concluded that dietary curcumin could inhibit chemotherapy-induced apoptosis by inhibiting reactive oxygen species (Garg et al. 2005).

Ferulic acid protected lymphocytes and animal models from the damage of toxic chemicals like nicotine and carbon tetrachloride (Srinivasan et al. 2005; Sudheer et al. 2007). In the year 2004, Ozen et al. proved that pretreatment of caffeic acid phenyl ester (CAPE), an active component of propolis, reduced cisplatin induced renal damage in rats due to its free radical scavenging action. Ginger may prevent chemotherapy and radiotherapy induced nausea in shrews, rats, and dogs (Sharma et al. 1997; Sharma et al. 1998).
All these reports strongly suggested that introduction of polyphenols in chemotherapy could greatly inhibit the side effects resulted by a chemotherapeutic drugs.

2.16. Ferulic acid

Perusal of literature survey revealed that polyphenols are excellent molecules in enhancing the therapeutic efficacy and reducing the side effects of chemotherapeutic drugs. In the recent past, the health benefits of ferulic acid have received a lot of attention among polyphenols pertaining to cancer research.

Ferulic acid (4-hydroxy, 3-methoxy cinnamic acid, FA) (figure 2.2) is a ubiquitous phenolic compound of plant tissues and thus constitutes a bioactive ingredient of many foods. This hydroxy cinnamic acid derivative is found in the plant cell wall components such as arabinoxylans as covalent side chains. As a component of lignin, FA is a precursor in the manufacture of other aromatic compounds. The etymology is from Ferula, referring to the giant fennel (Ferula communis). FA is rich in variety of dietary substances with a wide range of properties (Nyaradzo et al. 2009).

![Chemical Structure of Ferulic acid](image)

Figure 2.2. Chemical Structure of Ferulic acid
2.16.1. IUPAC Name
- (E)-3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid

2.16.2. Other Names
- 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid
- 3-methoxy-4-hydroxycinnamic acid
- 4-hydroxy-3-methoxycinnamic acid
- Ferulate
- Coniferic acid
- trans-ferulic acid

2.16.3. Sources

Ferulic acid (FA), together with dihydroferulic acid, is a component of lignocellulose, serving to crosslink the lignin and polysaccharides, thereby conferring rigidity to the cell walls (Shahadi et al. 2004). It can be extracted from wheat bran and maize bran using concentrated alkali. Biosynthesis of FA is by the action of the enzyme O-methyl transferase on caffeic acid (Shahadi et al. 2004). In plants, FA (right) is derived from phenylalanine, which is converted to 4-hydroxycinnamic acid (left) and then caffeic acid (figure 2.3 and figure 2.5).
FA is rich in rice bran, whole grain foods, citrus fruits, coffee, apple, artichoke, peanut, orange, pineapple, banana, beet root, cabbage, spinach and broccoli (figure 2.4) (Nyaradzo et al. 2009).

Figure 2.3. Biosynthesis of Ferulic acid
Figure 2.4. Common dietary agents rich in ferulic acid
Figure 2.5. Synthesis of ferulic acid and related compounds in plants (Kroon et al. 1997).
2.16.4. Properties of ferulic acid

Ferulic acid (FA) is a strong antioxidant besides that it possesses various other properties like,

- Antihyperlipidemic
- Antimicrobial
- Radioprotective
- Anticarcinogenic properties (Nyaradzo et al. 2009; Baskaran et al. 2010)

FA, like many phenols exhibits antioxidant effect in response to free radicals by donating hydrogen from its phenolic hydroxyl group. The free radical scavenging effect of FA has been reported to be similar to that of superoxide dismutase (Shanthakumar et al. 2012). Majority of the effects of FA in the protection and ailment of a disease were closely related to its antioxidant property. The antioxidant properties of FA have been reported in many studies (Sudheer et al. 2007; Srinivasan et al. 2007). In certain countries, FA has been approved as a food additive to prevent lipid peroxidation, because of its strong antioxidant and radical scavenging nature, in certain countries (Srinivasan et al. 2007).

FA has also been known to be effective against cancer, cold, flu, skin aging, muscle wasting, influenza and so on (Rukkumani et al. 2004). FA exhibits beneficial effects against various diseases like cancer, diabetes, cardiovascular and neurodegenerative disorders. FA suppressed carcinogenesis in the forestomach, lungs, skin, tongue and colon in experimental animal models (Mori et al. 1999; Kawabata et al. 2000; Alias et al. 2009; Baskaran et al. 2010). Previous studies have demonstrated the
chemopreventive efficacy of ferulic acid in oral carcinogenesis (Balakrishnan et al. 2010).

2.16.5. Protective effect of ferulic acid

Chemotherapy results in serious damages to the host cells. Compounds that are capable of negating reactive oxygen species mediated oxidative damage will have potential benefits in the therapy of many diseases including cancer. The oxidative damage in cancer can be modified by the phytochemicals (Valko et al. 2007). In the year 2007, Sudheer et al. reported that ferulic acid (FA) protected rat peripheral blood lymphocyte cultures from nicotine-induced DNA damage. When administered in rats induced with colon cancer, FA modified the effects of phase II detoxifying enzymes which are responsible for protecting the host from toxic effects of a carcinogen (Kawabata et al. 2000).

Srinivasan et al. (2005) have reported that ferulic acid protected carbon-tetrachloride induced hepatotoxicity in an experimental animal model, which ascribed to its antioxidant potential. Tanaka et al. (1993) reported the inhibitory effect of FA on tongue carcinogenesis. FA suppressed TPA-promoted skin tumorigenesis (Asanoma et al. 1994) as well as inhibited the occurrence of pulmonary cancers (Lesca 1983). Baskaran et al. (2010) reported that oral administration of FA protected the biochemical and molecular abnormalities of mammary cancer in 7,12 - dimethylbenz[a]anthracene (DMBA) induced rats.

FA showed a protective effect on mice induced with skin cancer by DMBA (Alias et al. 2009). It also prevented loss of bone fragility in ovariectomized rats (Sassa et al. 2003). Ramar et al. (2012) reported that, FA when administered together with resveratrol, protected diabetic mice induced by alloxan by reversing the antioxidant and lipid peroxidation status.
Shanthakumar et al. (2012) stated that FA protected mice from gamma radiation induced effects.

2.17. Experimental models to study the effects of polyphenols in chemotherapy

2.17.1. HeLa cells as an experimental model

HeLa isolated in 1951, is the oldest and one of the most well-known cell lines, commonly used in cancer research. HeLa cells are widely used in the scientific research and have the capability of growing both in suspension and as anchorage dependent (John 2002). This application shows a convenient method for continuous harvesting of large numbers of HeLa cells. It is the first continuous cancer cell line that have been a mainstay of cancer research ever since their isolation from the aggressive glandular cervical cancer of a young woman Henrietta Lacks' more than 50 years ago (Hannah 2000). Knowledge of almost every process that occurs in human cells has been obtained using HeLa cells. HeLa cells are termed "immortal" because they can divide an unlimited number of times in a laboratory cell culture as long as fundamental cell survival conditions are met (i.e. being maintained and sustained in a suitable environment). These cells can proliferate abnormally and rapidly, when compared to other cancer cells (Rahbari et al. 2009).

There are many strains of HeLa cells as they continue to evolve by being grown in cell cultures. It has been estimated that the total number of HeLa cells that have been propagated in cell culture far exceeds the number of cells in Henrietta Lacks' body (Terry Sharrer 2006). HeLa cells have an active version of the enzyme telomerase during cell division, which prevents the incremental shortening of telomeres that is implicated in aging and eventual cell death. In this way the cells circumvent the Hayflick Limit, which is the limited number of cell divisions that most normal cells can later undergo before becoming senescent (Ivankovic et al. 2007).
HeLa cells have been used in a number of cancer studies including those involving sex steroid hormones such as Estradiol, estrogen, and estrogen receptors along with estrogen like compound such as Quercetin and its cancer reducing properties (Bulzomi and Pamela 2012). There have also been studies on Hela cells on the effects of flavonoids and antioxidants on cancer cell proliferation and the fundamental mechanism of their anticancer activity (Kim et al. 2012). In 2011, HeLa cells were used in tests of novel heptamethine dyes IR-808 and other analogs which are currently being explored for their unique uses in medical diagnostics in cancer research (Tan et al. 2011). HeLa cells have also been used in in vitro cancer research to define cancer markers in RNA, and have been used to establish an RNAi Based Identification System and Interference of Specific Cancer Cells (Xie et al. 2011). Hence, HeLa cell lines have become a good experimental model for in vitro studies.

2.17.2. **SiHa cells as an experimental model**

More than 20 years ago, SiHa cells were established from a surgically removed carcinoma of the uterine cervix by researchers during their efforts to increase numbers of human cell lines potentially carrying hypothetical human tumor viruses. About 15 years later, SiHa cells became one of three main cervical carcinoma cell lines that had been cultured many years in laboratories namely, SiHa cells (from Japan), HeLa and CaSki cells (from non Asians). SiHa cells were found to have HPV 16 DNA integrated into the q21-q31 region of chromosome number 13. This simple arrangement of the viral DNA in SiHa cells has attracted increasing attention of researchers wishing to utilize the cells as an experimental model because of easier interpretation of yielded. Actually, in the 4 years starting from 1986, at least 18 international scientific reports, and in the 4 years from 1991, 69 reports have referred to SiHa cells in their titles or abstracts.
Several studies on phytochemicals and plant extracts have been carried out using SiHa cells as experimental models. Recently Chen and Tian (2013) showed that the effect of triptolide was enhanced by aspirin by using SiHa cell lines. The anticancer and apoptosis inducing properties of plant extracts and phytochemicals have been studied using SiHa cells as experimental models. Recently, Gupta et al. (2013) suggested the potency of Morinda citrifolia (Noni) extracts to induce mitochondrial mediated apoptosis in SiHa cells. Recent reports showed that SiHa cells were used as one of the experimental models to study the enhancing effects of tea polyphenols on cisplatin chemosensitivity through the induction of apoptosis (Singh et al. 2013). Thus, it was clear from the recent reports that SiHa cell lines were used as in vitro experimental models in cancer research.

2.17.3. Dalton’s lymphoma

Growing human and animal tumors as xenografts in animal models has become an important tool for cancer studies. The tumor Dalton’s lymphoma was originated in the thymus gland of a DBA/2 mouse at the National Cancer Institute, Bethesda, US in 1947. Subsequently, an ascites form was developed by repeated intraperitoneal transplantation of tumor (Chakrabarti et al. 1984).

Dalton’s ascites lymphoma xenografted mice serve as a good experimental model to study the biochemical, physiological and histological effects of a chemotherapeutic drug in vivo. The toxic profile or the protective effect of any compound of interest can be well studied using this model. The effects of phytochemicals on the side effects of chemotherapeutic drugs like cisplatin can be studied using this experimental model. Several studies were carried out using Dalton’s lymphoma tumor in mice. Ultrastructural and biochemical changes on treatment with cyclophosphamide and ascorbic acid were studied in Dalton’s lymphoma induced mice (Prasad et al. 2010). Different groups of drugs work in different ways to fight cancer cells and shrink tumors. Caffeine, EGCG, one of the important tea polyphenols, inhibit

Based on the above literature survey, we found that ferulic acid is a good plant polyphenol with strong antioxidant and anticarcinogenic activities. And more over, to the best of our knowledge, no studies have been carried out so far using the combination of ferulic acid and cisplatin for cancer treatment. So, the present study was designed to evaluate the sensitizing and protective effect of ferulic acid on cisplatin chemotherapy based on *in vitro* and *in vivo* approaches.

2.18. Relevance of the present study

Cancer is one of the major ‘killer diseases’ worldwide and every effort is being met to find a proper remedy. Chemotherapy is considered as one of the important treatment strategy of cancer but the wide usage of the chemotherapeutic drugs was limited because of its toxicity and resistance developed during treatment. Although the chemotherapeutic drug cisplatin offers a vital role in cancer therapy, host toxicity and resistance developed by cancer cells remains a major obstacle. The present study is based on sensitizing cancer cells and animal models to the chemotherapeutic drug cisplatin by the plant polyphenols ferulic acid and thereby aimed to increase its therapeutic effect and reduce the side effects to the host.

Ferulic acid possesses many cytotoxic, biological and pharmacological activities. This compound is a human dietary constituent and is non-toxic. So, the present study was carried out to study the chemosensitizing effect of ferulic acid on cisplatin chemotherapy.
2.19. Challenges and future directions

Treatment of various solid tumors such as renal cell carcinoma, prostrate cancer, head and neck cancers, breast cancers, lymphoma and cervical cancer is a challenge nowadays. Cervical cancer, which is of second largest incidence, amongst the female populations, is only modestly responsive or non-responsive to radiotherapy or chemotherapy. The success of chemotherapy, therefore, depends on increasing the sensitivity of the malignant cells to chemotherapy induced cell kill and decreasing their side effects to the host. Various dietary modulators and phytochemicals especially polyphenols, can work as excellent adjuvants to chemotherapy in a variety of cancers. These polyphenols (such as ferulic acid) work at increasing oxidative damage or by synchronizing the cells to a chemosensitive phase of cell cycle thus causing enhanced killing. They also may protect the normal cells of the host from adverse side effects elicited by chemotherapy. The future perspectives lie in identifying more such compounds and elucidating the mechanism through which they act for developing effective protocol for cancer chemotherapy.