1. Introduction

1.1 Chemotherapy

Chemotherapy is the treatment of cancer with one or more anti-neoplastic drugs. The anti-neoplastic agents can be used along with other treatments, such as surgery or radiation therapy. Chemotherapeutic drugs act by killing cells that divide rapidly as the main properties of cancerous cells. Furthermore, chemotherapy can harms the cells that divide rapidly under normal conditions.

The first anti-cancer agent (arsphen-amine) discovered in 1909 and used to treat syphilis [1]. During the world-war I, Mustard gas was discovered to be a potent agent for suppressor of hemato-poiesis [2]. It was reasoned that an agent damaged the white blood cells might have a similar effect on cancerous cells. Therefore, in December 1942, several patients with advanced lymphomas were treated [3]. However, the first chemotherapy drug developed from this line of research was Mustine. Since then, many other drugs have been developed to treat cancer, although the principles of chemotherapy discovered by the researchers still apply [4].
1.1.1 Anti-neoplastic Agents

The decision on the use chemotropic agents are depends on the type of cancer and also stage of malignancy. However, chemotropic agents can be grouped by mechanism of action into the mitotic inhibitors, alkylating agents, anti-neoplastic, antibiotics, anti-metabolites and hormonal agents [5]. From these agents, alkylating agents are the oldest group of chemo-therapeutics in use today. The covalent bonds between alkylating agents and biological molecules, in particular DNA, could disturbing the protein synthesis process and also cellular division [6]. In the alive cells, the groups sensitive to alkylating agents are COOH, OH, HS, NH, NH₂, which are in a linear chain or could be in a ring like pyrimidine and purine bases, as well as phosphates bonds [7].

The primary cause, for the anti-cancer properties of alkylating agents is the ability of them to have the covalent binds to DNA. The alkylating agents are able to either bind twice to one strand (intra-strand crosslink) or may bind once to both strands (inter-strand crosslink) of DNA. This leads to a form of programmed cell death called apoptosis [6]. The most effective members of alkylating agents are platinum-based complexes such as Cisplatin, Carboplatin and Oxaliplatin [8]. Cisplatin (cisdiamminedichloro platinum II) is the first platinum-based compelexe of alkylating agents which introduced in treatment of cancerous cells. The anti-tumor properties of platinum-based drugs were discovered in 1965 for the first time on inhibition the growth of Escherichia coli [9]. However, these observation led Rosenberg to study anti-tumor properties of Cisplatin, particularly for the cure of testicular cancer. Cisplatin have a structure with platinum in the center, which are linked by four ligands, two nitrogen ligands and two leaving ligands of oxygen or chloride. Nowadays, Cisplatin is one of the major chemotropic agents, commonly used for the treatment of solid tumors such as ovarian, testicular, cervical, lung, and bladder cancers [10].
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Figure 1-1: The platinum anticancer drugs: Cisplatin, Carboplatin and Oxaliplatin [7].

1.1.2 Cisplatin: Mechanism of action and side effects

The biochemical mechanisms of Cisplatin involve the binding of the drug to DNA and non-DNA targets and induction of apoptosis and necrosis in cells. In an aqueous medium, Cisplatin is converting to reactive derivatives. This structure is inhibited in the presence of a high chloride concentration, which decreases toxicity and efficacy of Cisplatin [11]. The chloride ions are the potent sites in structure of Cisplatin. In normal condition, inside the cell, chlorides sites of Cisplatin will replace by water molecules. These loosely bounds allowing the platinum to attack other bimolecular, such as DNA. Because of these bonds, Cisplatin easily forms cross-links between DNA bases. Most of these cross-links are formed at the DNA sites where guanine and adenine are next to each other in the same strand [12]. These cross links cause severe problems in cases of DNA replication.

Figure 1-2: The main binds of Cisplatin to DNA. (A) intrastrand cross-link, (B) interstrand cross-link, (C) monofunctional adduct, and (D) protein-DNA cross-link [11,12].
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The exact mechanism of Cisplatin cyto-toxicity is not well understood, but several studies have suggested, the importance of the impaired antioxidant defense system as the main mechanisms of Cisplatin toxicity [13]. The mitochondria has reported as the first target for Cisplatin toxicity, resulting in a reduction of the mitochondrial membrane potential [14].

Hydroxyl radical is a highly reactive member among the oxygen radicals. Along with releasing of hydroxyl radicals, lipid per-oxidation produced by changes in the fluidity of membranes [15]. The cell membrane lipid per-oxidation will be along with tissues damage in patient treated with Cisplatin [16]. According to this toxicities, pervious studies demonstrated the importance of supplementation of anti-oxidants in reduction of toxicity in patients treated with Cisplatin [17].

Upon the administration of Cisplatin, drug distributed throughout the body, and makes a interacting with both cancerous and healthy tissues. Along with inducing apoptosis in cancerous cells, Cisplatin undergoes many non-selective reactions with a variety of bio-molecules, such as proteins and phospholipids [13]. These interactions are the reason of dose-limiting usage of Cisplatin [14]. Despite the success of the drug, Cisplatin has several sever side effects such as severe renal tubular damage, peripheral neuro-toxicity and liver damage. These side effects limited the applied doses of Cisplatin in patients.

1.1.2.1 Neuro toxicity

The neurologic complications of Cisplatin occur in most cases. Cisplatin neuropathies are seen as flexia, and loss of vibratory sensation. Therefore, neuro-toxicity can be approached as an avoidable side effect of Cisplatin [18,19]. The amount of DNA cross-links and also different plasma concentrations in neurons, are significantly correlated with the degree of neuro-toxicity induced by Cisplatin [20,21,22].
1.1.2.3 Nephrotoxicity

The acute renal toxicity is the most important side effect of Cisplatin, even following a single dose [23, 24, 25]. Cisplatin is metabolized to a nephro-toxin through intermediates of the GSH-conjugates. These conjugates may be formed in either the liver or the kidney [26, 27]. Inhibiting the conjugation of platinum-GSH has been shown to reduce Cisplatin nephro-toxicity [28, 29]. Furthermore, generation of free radicals in nephrons has reported as the cause of acute renal failure induced by Cisplatin [29].

1.1.2.4 Hepatotoxicity

Neuro and nephro-toxicity, has been recognized as the most important dose-limiting factors of Cisplatin. However, severe liver damage can occur when the drug is administered at high dosages [29]. The damage to the structure of liver after Cisplatin treatment, is commonly assessed by the determination of serum aminotransferases (ALT and AST) activities [30]. Oxidative stress plays an important role in Cisplatin induced hepato-toxicity [29]. For example, high doses of vitamin E protected oxidative damage in liver and also enzymatic antioxidants have the important protective responses against Cisplatin toxicity in the livers of tumour-bearing mice [31, 32].

1.2 Reactive oxygen species (ROS)

The first theory of ROS induced aging, was reported by Harman in 1956, [33]. According to Harman theory free radicals produced during the biological reactions and are leading to oxidative stress. This theory highlights a loss of the protective mechanisms that reduce the ability to oxidative challenges [34].

The ROS are the natural byproduct of the metabolism of oxygen and have important roles in cell signaling and homeostasis. Furthermore, free radicals are the important contributor of pathological conditions including degenerative
diseases. However, during the environmental stress, the levels of ROS can increase dramatically; this will resulted in significant damage to cell structures [35]. The ROS could affect many cellular functions by oxidizing proteins, damaging nucleic acids and lipid per-oxidation. it is important to note that whether ROS will act as damaging, signaling or protective factors depends on the equilibrium between production and scavenging of ROS at the proper site of action [34]. Oxidative stress occurs in case of this critical balance due to depletion of antioxidants or accumulation of ROS in different body organs.

Most reactive oxygen species are by-products of mitochondrial electron transport. Atomic oxygen has two unpaired electrons in separate orbits. This electron structure makes oxygen capable to radical formation. The reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including: superoxide, hydrogen peroxide, hydroxyl radical, hydroxyl ion, and nitric oxide.

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<th>Hydroxyl ion</th>
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Table 1-1: The structure of common reactive oxygen species. The X designates an unpaired electron.
1.2.1 ROS and the biological systems

A number of defense mechanisms of body are evolved to provide a balance between production and removal of ROS in biological systems.

Superoxide dismutase (SOD) catalyzes the conversion of two superoxide anions into a molecule of hydrogen peroxide (H$_2$O$_2$) and oxygen (O$_2$).

\[ \cdot 2 \text{O}_2 + 2 \text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

In the peroxisomes, the enzyme CAT converts H$_2$O$_2$ to water and oxygen, and completes the detoxification initiated by SOD.

\[ 2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \]

There are also a number of non-enzymatic antioxidants molecule that play a role in detoxification in oxidative stress cases. Glutathione is the most important intracellular defense member against the reactive oxygen species. This tri-peptide provides an exposed sulphhydryl group, which serves as an abundant target for attack. The ratio of the GSSG and the GSH is a dynamic indicator of the oxidative stress of an organism [35]. Glutathione peroxidase (GPx) is a group of enzymes containing selenium, which also catalyze the degradation of hydrogen peroxide, as well as organic peroxides to alcohols.

![Diagram](image)

**Figure 1-3:** Removal of H$_2$O$_2$ from the cells and its conversion to H$_2$O by the GPx enzyme.
1.2.2 Measuring of the Oxidative stress

The measurement of oxidative stress such as free radical production and oxidative damage is possible. Measurement is depends on the type of oxidative stress and the kind of sample available. At the animal level the effects of oxidative stress could measured from blood products, urine samples or from the tissue homogenates [36]. The ratio of GSH to GSSH is also a good indicator of oxidative stress in different organs. Total glutathione can be determined colorimetrically by reacting GSH with DTNB in the presence of glutathione reductase [37, 38].

Lipid peroxidation is one of the most important indicators of oxidative stress. Unsaturated fatty acids present in cellular membranes are a common target for ROS. The reaction occurs where a free radical will capture hydrogen from an unsaturated fatty acid to form water. Lipid peroxides are unstable and decompose to form a complex series of compounds, such as malondialdehyde (MDA) [39]. However, measurement of lipid peroxidation has relied on the detection of thiobarbituric acid reactive compounds such as MDA generated [40].
Superoxide detection is based on the interaction of superoxide with the other measurable compounds. The reduction of ferricytochrome c to ferrocytochrome c has been used to measure the rate of superoxide formation. Hydrogen peroxide is another member of reactive oxygen species. A number of colorimetric substrates such as tetramethyl-benzidine and phenol red are used to measure hydrogen peroxide levels.

\[
\text{Fe}^{+3} \text{cytochrome c} + \cdot \text{O}_2 \rightarrow \text{Fe}^{+2} \text{cytochrome c} + \text{O}_2
\]

### 1.3 Plant as the natural antioxidants

Natural antioxidants are presence in all part of plants. These components belonged to the classe of phytochemicals such as phenols, carotenoids and flavonoids, which are able to scavenge free radicals such as superoxide or lipid peroxides [41].

The most current research on natural antioxidants has been focused on polyphenolic compounds such as flavonoids [42]. Fruits, plant extracts and vegetables are reach sources of polyphenols, such as flavonoids and carotenoids, whose activities have been established in recent years. The anti-oxidative potential of plant-based antioxidants resulted from the action of lesser-known compounds or from the action of
the cocktail of antioxidants present in plants [43]. However, results from studies on single compounds such as vitamins C or β-carotene have not supported too much protective effect against oxidative stress [44].

Polyphenols are a class of natural chemicals characterized by the presence of phenol in their structural [45]. These structures underlie the unique biological potential of polyphenols [46]. The recently studies on polyphenols has suggested the role of polyphenols in the prevention of degenerative diseases, and cancers in human [47]. Although, the antioxidant properties of polyphenols have been studied, but it has become clear that the mechanisms of action of polyphenols go beyond the modulation of oxidative stress [48].

Researchs on polyphones and specially flavonoids and their antioxidant properties began after 1995 [49]. Dietary polyphenols constitute one of the most numerous groups of natural products in the plant kingdom. More than 8000 phenolic structures are known, and among them over 4000 flavonoids have been identified [50, 51]. Polyphenols are classified by their source, chemical structure, and biological functions. In the current introduction, classification of polyphenols will be done according to their chemical structures as follows:

Flavonoids are the most common group of polyphenolic compounds in the human diet. The major sources of flavonoids include fruits, tea, wine and plants extracts [52]. The main groups of flavonoids are flavonols, flavones, isoflavones, flavanones, flavanols and anthocyanidins. The biological importance of flavonoids have been attributed to their reducing capacities on the intracellular conditions [53].

Flavonoids display a potent antioxidant capacity in vitro [54]. During the absorption, flavonoids are metabolised in a significant alteration in their reducing capacities [55]. In the human body, flavonoids may undergo at least three types of intracellular metabolism, oxidative metabolism, P450-related metabolism and conjugation with thiols, particularly GSH. Circulating of flavonoid metabolites, such
as sulphates and conjugated O-methylated forms, or intracellular metabolites like flavonoid-GSH adducts, have greatly reduced anti-oxidative potential [56].

The most common subgroup of flavonoid is ubiquitous. Flavones and flavonols, including their glycosides, methoxides and other acylated products, make them as the largest subgroup among all polyphenols. The most common flavonol, aglycones, quercetin and kaempferol, alone have at least 279 and 347 different glycosidic combinations, respectively [57]. Flavanols are found in many fruits, particularly in the skins of grapes, apple and blueberries [58]. Pro-anthocyanidins are also known as strong antioxidants, which have been associated with several protective potentials against degenerative diseases [57]; and finally Anthocyanidins are the principal components of the pigments of the plants, and certain special varieties of grains [57, 58].

In addition to the phenolic acids, flavonoids and phenolic amides, there are several non-flavonoid polyphenols found in plants that are considered important to human health. Among these, resveratrol, ellagic acid and its derivatives are found in berry fruits, and in the skins of different nuts.

![Figure 1-6: Structure of the most important groups of polyphenols [57, 58].](image-url)
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A major problem in studies of flavonoids is the limitation of methods for measurement in biological samples [59, 60, and 61]. The conjugation pathway for flavonoids begins in intestinal cells. The flavonoid is then bound to albumin and transported to the liver [62]. The liver, extend the conjugation of the flavonoids by adding a sulfate group or a methyl group. The addition of these groups to flavonoids increases the circulatory elimination time and also decreases their toxicity.

Flavonoids are playing an important role on vascular system because of their anti-oxidative properties. Free radicals can injure the endothelial wall. The pervious clinical studies have reported the importance of flavonoid intakes against endothelial disease [63]. In these conditions, antioxidant defense systems of body are inadequate, and damage from ROS is involved in degenerative disease [64]. Free radicals can damage DNA, and disturb division of cells with un-repaired or mis-repaired damage leads to mutations. If these changes appear in critical genes, such as tumor suppressor genes, initiation or progression may result. [65]. Some flavonoids such as fisetin, are stated to be potent inhibitors of cell proliferation [66]. A large clinical study suggested the presence of an inverse association between flavonoid intake and the subsequent incidence of cancerous cells [67].

1.4 Herbal products

Herbs are defined as any part of a plant that is used as a drug for their protective properties. Herbs have identified as sources of various phytochemicals and many of them possess powerful antioxidant activity [68]. A variety of diseases has reported to cure with herbs, from degenerative disease, infections to AIDS and even malaria. Nowadays, about 35% of medicines are of plant origin and their properties have reported as equal to chemical drugs [68]. The benefits of herbs are including their low price, availability and lesser side effects compared to chemical drugs [69]. The main bioactive components of herbs are alkaloids, glycosides, flavonoids, vitamins and mineral. It was proved that some herbs contain anti-oxidative properties which are more active than those of vegetables and fruits [70]. The level of bioactive compounds
and the antioxidant activity of a herbal extract is depends on extraction method [71]. The most often solvents techniques for plant extraction are ethanol, methanol, ethyl acetate, acetone, and boiling water based methods [72].

1.5  Ficus species

The Ficus consists of over 800 species and is one of 40 genera of the mulberry family, Moraceae [73]. A number of Ficus species are used as for their medicinal properties in traditional medicine especially in India. These uses, however, are most widely found in the Middle East [74]. The greatest notable species of Ficus are Ficus religiosa (FR, the Bo tree), Ficus elastica, Ficus benghalensis (FB, the banyan tree) and Ficus racemosa. Many of these plants possess auxiliary aerial roots extending to the ground from their branches [75]. Phytochemical investigation on Ficus species revealed the phenolic compounds as the major components of this species and reported to have anti-oxidatav activity which attributed to the high phenolic content of them [73,74].

The benefits of Ficus species were performed in first time by Ullman [75], against cancer tumors. From the initial research of Ullman, a number of Ficus species have been evaluated for their biological activities such as anti-cancer and anti-inflammation, etc.
1.5.1 *Ficus religiosa* (FR)

*Ficus religiosa* (FR), grows throughout India and cultivated in south-east Asia. The tree is regarded as a sacred tree to both Hindus as well as Buddhists. The different extracts of FR has got medicinal properties in Indian culture since ancient times [75]. In Ayurveda, FR belongs to a class of rasayana. Rasayana are antioxidants, rejuvenators, and relieve stress in the body. FR emerged as a good source of traditional medicine for the treatment of asthma, diabetes, diarrhea, inflammatory disorders and sexual disorders [76]. The pharmacological bioactive metabolite studied in FR investigated for its potential against cancer, inflammatory disorders, cardiovascular disorders and oxidative stress related disorders [77].
1.5.2 *Ficus bengalensis* (FB)

Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Urticales*
Family: *Moraceae*
Genus: *Ficus*
Species: *bengalensis*

*Figure 1-8*: Tree and leaf of *Ficus bengalensis*

*Ficus bengalensis* (FB), commonly called as Banayan tree, is evergreen tree, with spreading branches and arial roots. It is found in tropical forests throughout the world and distributed all over India. In ayurveda, extracts obtained from various parts of the tree are reported to have demulcent and cooling properties. The latex is suggested to have an aphrodisiac action [76, 77]. Aqueous extract of FB Linn, leaf mixed with sugar and honey for cure diarrhea; and aerial roots, used in bleeding piles. Different extracts of FB also used as a blood purifier in urinary and urinogenital disorders [78]. Nalpamaram is an important group of ayurvedic formulation that constitutes the parts of FB plant which are, used in the treatment of skin diseases. The recently pharmacological studies suggested the potential of FR against, anti-diabetic, ameliorative activity and its Anti bacterial activities [79].
1.6 Objectives of the project

Indian ayurveda is one of the most important and wildly accepted branches of medicinal science. In ayurveda system, medicines prepared from the different parts of the plants are used to treat a variety of diseases. However, many facets of the medicinal activities of many plants are yet to be uncovered. The drugs used in allopathy treatments are known to show side effects on non target organs leading to toxicity and oxidative stress. In the ayurveda medicines references, leaves of FR and FB have been reported to possess the notable anti-oxidative potential. Therefore the aim of the present study was to investigate the potential of leaf extracts of FR and FB against Cisplatin induced oxidative stress. The objectives of the present study are as follows.

- To study the bioactive profiles of the leaf extracts, obtained from FR and FB.
- To identify of the potential of leaf extract of FR and FB to ameliorate the post chemotherapeutic changes accruing in the kidney, liver and the nervous system of mice.
- To study the antioxidant activity of FR and FB leaf extract on oxidative stress induced by Cisplatin.

1.7 Significance of study

The mechanism of Cisplatin side effects are documented to the combination of multi-ways, such as the generation of reactive oxygen species. The antioxidant action of polyphenols have been suggested to explain this beneficial properties already. A number of Ficus species as well as FR and FB are used for antioxidant properties in ayurveda. So we are interested to study the anti oxidative effects of the extracts obtained from leaf of current plants on the post chemotherapeutic changes occurring in the liver, kidney and the neuro-system of mice. So our aim is to provide a new treatment against the side effects induced by Cisplatin. The present work involves
screening the treatment of plants extracts to treat the Cisplatin side effects in mice model.