CHAPTER – I
INTRODUCTION

The liver is one of the largest and vital organ in the body. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, plasma protein synthesis, hormone production and detoxification. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. Thus, to maintain a healthy liver is a crucial factor for overall health and well-being. But it is continuously and variedly exposed to environmental toxins and abused by poor drug habits, alcohol and prescribed as well as over-the-counter drugs which can lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver diseases. Thus, liver diseases are some of the fatal diseases in the world today.

The liver is a major target organ for toxicity of xenobiotics and drugs, because most of orally ingested chemicals and drugs first go to liver where they are metabolized into toxic intermediates. A large number of xenobiotics are reported to be potentially hepatotoxic (Ajith et al., 2007). Hepatocytes, which make up the majority of the liver structure, are very active in the metabolism of exogenous chemicals, and this is one of the major reasons why the liver is a target for toxic substances (Timbrell, 2001). During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which cause oxidative stress (Kohen and Nyska, 2002) leading to hepatic damage.

Liver injury or liver dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Liver cell injuries caused by various toxic chemicals like certain
antibiotics, chemotherapeutic agents, carbon tetrachloride (CCl₄), thioacetamide (TAA) etc, excessive alcohol consumption and microbes are well studied. The available synthetic drugs to treat liver disorders in this condition also cause further damage to the liver.

The liver can be damaged in a variety of ways.

- Cells can become inflamed (such as in hepatitis).
- Bile flow can be obstructed (such as in cholestasis).
- Cholesterol or triglycerides can accumulate (such as in steatosis).
- Blood flow to the liver may be compromised.
- Liver tissue can be infiltrated by abnormal cells.

**Hepatotoxicity** implies chemical-driven liver damage. Certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Other chemical agents, such as those used in laboratories, industries and natural chemicals (e.g., microcystins) can also induce hepatotoxicity. The human body identifies almost all drugs/chemicals as foreign substances (i.e. xenobiotics) and subjects them to various chemical processes (i.e. metabolism) to make them suitable for elimination. This involves chemical transformations to (a) reduce fat solubility, and (b) to change biological activity. Although almost all tissues in the body have some ability to metabolize chemicals, smooth endoplasmic reticulum in the liver is the principal "metabolic clearing house" for both endogenous chemicals (e.g., cholesterol, steroid hormones, fatty acids, proteins) and exogenous substances (e.g., drugs, alcohol) (Donald *et al.*, 2006).

Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. About 75% of
blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins that bring drugs and xenobiotics in near-undiluted form. Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, intracellular organelles that produce energy. Its dysfunction releases excessive amount of oxidants that, in turn, injure hepatic cells. Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress (Jaeschke et al., 2002). Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside the liver. This promotes further liver damage (Patel et al., 1998). Non-parenchymal cells such as Kupffer cells, fat storing stellate cells, and leukocytes (i.e. neutrophil and monocyte) also have a role in the mechanism.

Drug metabolism is usually divided into two phases: phase 1 and phase 2. Phase 1 reaction is thought to prepare a drug for phase 2. However many compounds can be metabolized by phase 2 directly. Phase 1 reaction involves oxidation, reduction, hydrolysis, hydration and many other rare chemical reactions. These processes tend to increase water solubility of the drug and can generate metabolites that are more chemically active and potentially toxic. Most of phase 2 reactions take place in cytosol and involve conjugation with endogenous compounds via transferase enzymes. Chemically active phase 1 products are rendered relatively inert and suitable for elimination by this step.

Acetaminophen (paracetamol) is usually well tolerated in prescribed dose, but overdose is the most common cause of drug-induced liver disease and acute liver failure worldwide (Keeffe and Friedman, 2004). Non-steroidal anti-inflammatory drugs (NSAIDs) have emerged as a major group of drugs exhibiting hepatotoxicity.
Both dose-dependent and idiosyncratic reactions have been documented (Manov et al., 2006). Aspirin and phenylbutazone are associated with intrinsic hepatotoxicity; idiosyncratic reaction has been associated with ibuprofen, sulindac, phenylbutazone, piroxicam, diclofenac and indomethacin. Glucocorticoids promote glycogen storage in the liver. An enlarged liver is a rare side-effect of long-term steroid use in children (Iancu et al., 1986). The classical effect of prolonged use both in adult and paediatric population is steatosis (Alpers et al., 1982). Isoniazide (INH) is one of the most commonly used drugs for tuberculosis; it is associated with mild elevation of liver enzymes in up to 20% of patients and severe hepatotoxicity in 1-2% of patients (Sarich et al., 1999). Natural products include many amanita mushrooms, aflatoxins and industrial toxins include arsenic, carbon tetrachloride, and vinyl chlorides are producing hepatotoxicity.

**Epidemiology of liver diseases**

Liver disease is one of the major causes of morbidity and mortality in public, affecting humans of all ages. Chronic liver damage is a worldwide common pathology characterized by inflammation and fibrosis that can lead to chronic hepatitis, cirrhosis and cancer (Tessitore and Bollito, 2006; Kohle et al., 2008). National Liver Foundation has estimated that liver diseases are among the top ten killer diseases in India, causing lakhs of deaths every year. According to the National Centre for Health Statistics (NCHS), chronic liver disease and cirrhosis is the 12th leading cause of death in the United States. According to the latest WHO data published in April 2011, liver disease deaths in India reached 208,185 or 2.31% of total deaths. The age adjusted death rate is 23.59 per 100,000 of population ranks India 27 in the world. Some of the commonly known disorders are viral hepatitis, alcoholic liver disease,
non-alcoholic fatty liver disease, autoimmune liver disease, metabolic liver diseases; drug induced liver injury, gallstones, etc. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2, 50,000 new cases each year (Gupta and Misra, 2006). According to WHO estimates, globally 170 million people are chronically infected with hepatitis C alone and every year 3-4 millions are newly added into the list. Also, there are more than 2 billion infected by hepatitis B virus (HBV) and over 5 million are getting infected with acute HBV annually (Negi et al., 2008).

More than 900 drugs have been implicated in causing liver injury (Friedman, 2003) and it is the most common reason for a drug to be withdrawn from the market. Drug-induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures. (McNally, 2006; Ostapowicz et al., 2002).

Non-alcoholic fatty liver disease (NAFLD) is a distinct hepatic condition characterized by abnormal fat accumulation in liver cells; histologically resembling alcohol induced liver damage. Non-alcoholic fatty liver disease (NAFLD) is a distinct hepatic condition and one of the most common causes of chronic liver disease globally. Prevalence of the disease is estimated to be around 9-32% in the general Indian population, with a higher incidence rate amongst obese diabetic patients (Kalra et al., 2013).

Liver cancer is the sixth most common cancer worldwide (El-Serag, 2002; Lenhard et al., 2001) accounting for 5.7% of the overall incident cases of cancer. There is wide geographic variability in incidence with a majority of the cases occurring in developing countries compared with developed countries (Parkin et al., 2005). In fact, liver cancer is the third most common cancer in developing countries.
among men after lung and stomach cancer. It is also between two and eight times more common in men than in women (Parkin et al., 2005; McGlynn et al., 2001; Kew et al., 2002; Fong, et al., 2001).

Among the different liver cancers, hepatocellular carcinoma (HCC) is the most common. Although the most common risk factors associated with HCC are HBV and HCV infections, other risk factors like alcohol use, smoking, and aflatoxin exposure also contribute significantly to the burden of this disease, particularly in developing countries. Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for 75% of the cases of primary liver cancer worldwide, with an even higher proportion in developing countries (Parkin et al., 2005).

Alcoholic liver disease accounts for a significant proportion of primary liver cancer cases in the United States and Europe, and both alcohol and tobacco use have significant effects in Asia and Africa (Bosch et al., 2004). Aflatoxin exposure also accounts for a significant number of cases in Asia (Bosch et al., 2004), especially China and Taiwan.

**Carbon tetrachloride:**

The liver injury induced by carbon tetrachloride (CCl₄) (Table 1.1 and Fig. 1.1) is the best characterized system for xenobiotic - induced hepatotoxicity and is commonly used as model for screening of the anti-hepatotoxic and hepatoprotective activities of drugs. Carbon tetrachloride (CCl₄) has been used extensively to study hepatotoxicity in animal models by initiating lipid peroxidation, thereby causing injuries to kidney, heart, testis and brain (Tirkey et al., 2005; Preethi and Kuttan, 2009; Khan et al., 2010), in addition to liver pathogenesis (Murugesan et al., 2009).
Carbon tetrachloride is one of the xenobiotics that has been reported to induce acute and chronic tissue injuries (Ogeturk et al., 2005; Jaramillo-Juarez et al., 2008) through bioactivation of the phase I cytochrome P450 system to form reactive metabolic trichloromethyl radicals (\(^{\cdot}\text{CCl}_3\)) and peroxyl trichloromethyl radicals (\(^{\cdot}\text{OOCCl}_3\)). The double allelic hydrogen bonds of polyunsaturated fatty acid (PUFA) are susceptible to abstraction by free radicals; CCl\(_4\) exposure induces an increase in lipo peroxide and free peroxide radical concentrations that are highly reactive and cause injury or necrosis (Weber et al., 2003; Miyazaki et al., 2009). It is a well-known hepatotoxin that catabolizes radical induced lipid peroxidation, damage the membranes of liver cells and organelles and causes swelling and necrosis of hepatocytes. Carbon tetrachloride can induce liver damage through the formation of reactive free radicals that can bind covalently to cellular macromolecules forming nucleic acid, protein and lipid adducts; through the induction of hypomethylated ribosomal RNA, resulting in inhibition of protein synthesis. These injuries are mediated through the formation of reactive intermediates such as trichloromethyl (\(^{\cdot}\text{CCl}_3\)) free radicals and reactive oxygen species.

The Indian system of holistic medicine known as “Ayurveda” uses mainly plant-based drugs or formulations to treat various ailments, including cancer. Ayurveda is primarily a spiritual and traditional way of curing a disease. The Indian traditional medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials. Medicinal plants play a key role in the human health care. About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials (WHO, 1993). Ayurveda is a native Indian healthcare system which is currently used by millions of people in India, Nepal and Sri Lanka for their day-to-day healthcare needs (Cooper, 2008). Plants have great
potential uses, especially as traditional medicine and pharmacopoeial drug. A number of natural products have been used as lead compounds because of specific activity and low toxicity (Sanda et al., 2011). Medicinal plants have provided the modern medicine with numerous plant derived therapeutic agents (Evans, 2000; Oladunmoye et al., 2009; Pandey et al., 2010). Many plants contain a variety of phytochemicals (Agrawal and Singh 1993; Agrawal and Singh, 2010; Hemlata et al., 2006; Pandey et al., 1995, 1997; Pandey and Shukla, 2001) which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases (Newman et al., 2003; Gilani and Rahman, 2005; Srivastava et al., 2011). The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or the other for this purpose. It is an alternative treatment, which helps to cure certain chronic diseases that cannot be totally cured using allopathic medicines.

The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver diseases by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization of herbal products and randomized placebo controlled clinical trials to support clinical efficacy. Of the at least 877 small-molecule drugs introduced worldwide between 1981 and 2002, the origins of most (61%) can be traced to natural products (Newman and Cragg, 2007). Many of these natural products have pharmacological or biological activity that can be exploited in pharmaceutical drug discovery and drug design.
Medicines derived from plants have played a pivotal role in the health care of many cultures, both ancient and modern (Newman et al., 2003; Butler, 2004; Balunas and Kinghorn, 2005; Gurib-Fakim, 2006; Newman and Cragg, 2007). Scientific evaluation of plants has often shown that active principles in these are responsible for therapeutic success. A number of herbal preparations are available in the market. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles (Srinivas et al., 2011).

Allopathy has been introduced recently; and is widely accepted and practiced across the world. The objective of allopathic treatment is to provide instant relief by destroying the germs, bacteria, virus etc.; that caused the sickness. However, it cannot ensure that the disease will be cured permanently. Most of the allopathic medicines are synthetically prepared and hence they have some or the other side-effect. On the other hand, Ayurvedic medicines are basically natural drugs which are mostly harmless to our body. Ayurvedic medicines mainly concentrate on the root cause of the problem to cure the specific system of our body; and hence we can maintain good health for a long time.

Ayurvedic medicines are highly effective in curing chronic illness, especially diseases related with our liver; as compared to allopathic treatment. These days, many people are in favour of using Ayurvedic medicines. Hence they are in great demand across the world, especially in India. This is mainly due to the fact that, Ayurvedic medicines hardly have any side effects, as they are prepared from natural resources like plants, herbs, etc. These medicines are now used widely in the developing as well
as developed countries. Besides this, the harmful side effects of some allopathic medicines, has also resulted in the rising popularity of Ayurvedic medicines.

It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants (Pushpangadan et al., 1995). Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi ingredient plant formulations. In spite of the tremendous advances made, no significant and safe hepatoprotective agents are available in modern therapeutics. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases. Medicinal plants may serve as a vital source of potentially useful new compounds for the development of effective therapy to combat a variety of liver problems. Many herbs have been proven to be effective as hepatoprotective agents while many more are claimed to be hepatoprotective but lack any scientific evidence to support such claims. Developing a satisfactory herbal therapy to treat severe liver diseases requires systematic investigation of properties like anti-hepatotoxicity (antioxidants), stimulation of liver regeneration and choleretic activity. The therapeutic values were tested against a few chemicals-induced subclinical levels of liver damages in rodents. Development of such medicines with standards of safety and efficacy can revitalise treatment of liver disorders and hepatoprotective activity.
These biologically active chemical substances known as secondary metabolites in medicinal plants, form the foundations of modern prescription drugs (Sofowora, 1993). Phytochemicals are natural bioactive compounds found in plants, including the medicinal plants, fruits, vegetables, flowers, leaves, roots and fibres and they act as a defense system against diseases or more accurately protect plants against diseases (Krishnaiah et al., 2009).

The therapeutic potentials, including antioxidant, antimicrobial and anticarcinogenic properties of higher plants are due to the presence of secondary metabolites (Canigueral et al., 2008; Kaur and Arora, 2009). The medicinal values of these plants lie in bioactive phytochemical constituents that produce definite physiological actions on the human and animal body. Some of the most important bioactive phytochemical constituents are the glycosides, alkaloids, flavonoids, tannins, steroids, terpenoids, essential oils and phenolic compounds (Harbone, 1984; Edeoga et al., 2005; Okwu, 2005).

There are several plants possessing hepatoprotective activities. Based on ethnopharmacological information, detailed information were collected on hepatoprotective usage of several traditional medicinal plants. As for example, *Andrographis lineata, Andrographis paniculata, Azadirachta indica, Boerhavia diffusa, Careya arborea, Cassia fistula, Cleome viscosa, Eclipta Alba, Fumaria indica, Morinda citrifolia, Phyllanthus amarus, Phyllanthus polyphyllus, Phyllanthus reticulates, Picrorhiza kurroa, Polygala arvensis, Pterocarpus santalinus, Pterospermum acerifolium, Solanum nigrum, Swertia Chirata*, etc. In our study, we have selected two indigenous and widely distributed Indian medicinal plants – *Eclipta*
alba (Family: Asteraceae) and Boerhavia diffusa – (Family: Nyctaginaceae), to evaluate the hepatoprotective activity.

**Eclipta alba**

In India, the plant is known as bhangra, "bhringaraj" or bhringraja. Another plant Widelia calendulacea is also known by the same name, but Eclipta has white flowers so called white bhangra and Widelia has yellow flower so it is called yellow Bhangra (Puri, 2003). The branches are hairy, reddish brown and can grow up to 40 cm height. The roots are found growing at the thickened nodal points. The leaves are opposite, lance like with toothed edge and hairy. The flowers are white, small and arranged in small clusters. The flowering stalk arises from the axis of the leaf. The dry fruit is formed by fusion of two carpels, which do not break open and each has just one seed. Root well developed, cylindrical and greyish (Chopra et al., 1966) (Fig. 1.2).

**Scientific classification**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>(unranked)</td>
<td>Angiosperms</td>
</tr>
<tr>
<td>(unranked)</td>
<td>Eudicots</td>
</tr>
<tr>
<td>(unranked)</td>
<td>Asterids</td>
</tr>
<tr>
<td>Order</td>
<td>Asterales</td>
</tr>
<tr>
<td>Family</td>
<td>Asteraceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Eclipta</td>
</tr>
<tr>
<td>Species</td>
<td>E. alba</td>
</tr>
<tr>
<td>Binomial name</td>
<td><em>Eclipta alba</em> (L.) Hassk.</td>
</tr>
<tr>
<td>Synonyms</td>
<td><em>Eclipta erecta, Eclipta prostrate, Verbesina alba, Verbesina prostrate</em></td>
</tr>
</tbody>
</table>
Vernacular names:

Sanskrit - Bhringaraj; Hindi - Bhangra; English - Bhringaraj; Bengali - Kesuti; Marathi - Maka; Tamil – Garuja, Karisalnkanni; Unani - Bungrah; Chinese - Lichang; Japanese – Takasaburo; Kannada- Garuga; Malayalam – Kayyoni; Telugu – Guntagalagaraku.

Distribution:

_Eclipta alba_ Linn. occurs throughout the whole of India. It is widely distributed throughout India, China, Thailand, and Brazil. _Eclipta alba_ (L.) has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. There are three kinds or _Eclipta alba_ - the white - flowering, the yellow - flowering, and the black - fruiting, but all three grow throughout India by marshes, rivers, and lakes or on the foothills of the Himalayas. It is found in other eastern countries including Indonesia, Sri Lanka, Philippines, Nepal and Malaysia where it grow well in clay and moist ground - bunds, paddy fields, water courses, tanks, both in plains and hilly regions.

Traditional uses:

In Ayurveda the plant is considered as a rasayana for longevity and rejuvenation. Plant is bitter, hot, sharp, dry in taste and is used in Ayurveda and "Siddha" for the treatment of kapha and vata imbalances. _Eclipta alba_ Linn. has been traditionally used for blackening, promoting hair growth and strengthening the hair. It is useful in inflammations, hernia, eye diseases, bronchitis, asthma, leucoderma, anaemia, heart, skin diseases and syphilis etc. It is popularly used to enhance the memory and has a reputation as an anti-aging agent. The leaf juice is also effective when applied externally to treat minor cuts, abrasions, and burns. The leaves of _Eclipta alba_ have been also used in the treatment of scorpion stings. Charaka advises
taking the juice of *Eclipta alba* with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies. The expressed leaf juice is applied along with honey is a popular remedy for catarrh in infants. Plant is rubbed on the gums in toothache and applied with a little oil for relieving headache and with sesame oil in elephantiasis. Roots of *Eclipta alba* are emetic and purgative. A complete symptomatic relief in epigastric pain, nausea and vomiting in ulcer patients has also been observed (Puri, 2003). In Taiwan, entire plant is used as a remedy for the treatment of bleeding, haemoptysis, haematuria, itching, hepatitis, diphtheria and diarrhoea. In China, it is used as a cooling and restorative herb, which supports the mind, nerves, liver and eyes. A black dye obtained from *Eclipta alba* is also for dyeing hair and tattooing. *Eclipta alba* also has traditional external uses, like athlete foot, eczema and dermatitis, on the scalp to address hair loss and the leaves have been used in the treatment of scorpion stings. It is used as anti-venom against snakebite in China and Brazil (Mors, 1991). The fresh juice of leaves is used for increasing appetite, improving digestion (Cheryl Lans., 2007) and as a mild bowel regulator.

**Phytochemistry:**

The herb *Eclipta alba* contains mainly coumestans i.e. wedelolactone (I) and demethylwedelolactone (II), polypeptides, polyacetylenes, thiophene-derivatives, steroids, triterpenes and flavonoids. Coumestans are known to possess estrogenic activity (Bickoff *et al.*, 1969). Wedelolactone possesses a wide range of biological activities and is used for the treatment of hepatitis and cirrhosis (Wagner, 1986), as an antibacterial and anti-hemorrhagic (Kosuge, 1985) (Table 1.2 and 1.3).
PHARMACOLOGICAL PROPERTIES:

Crude extract:

The crude extract has been found to have wound healing properties. The fresh plant is used as self-medication by AIDS patients in Southern Thailand and showed potential as a therapeutic agent against Giardia intestinalis infections (Sawangjaroen et al., 2005; Tewtrakul et al., 2006 and Cheryl lens, 2007). The leaf extract showed hypolipidemic activity in atherogenic diet induced hyperlipidemic rats (Dhandapani, 2007). It has antimicrobial and antioxidant properties (Karthikumar et al., 2007). Three percent extract of Eclipta alba is used in pilex formulation with other ingredients. It has been reported to decrease bleeding time (Mukherjee and Poddar, 1976). Leaf extract has been used in oedema. It is used in the treatment of paronychia (Khan, 2008).

Antihepatotoxic properties:

Hepatoprotective activity of methanolic extract and subfractions of leaves and the chloroform extract and subfractions of roots of Eclipta alba was carried out using carbon tetrachloride - induced liver damage and lysosomal enzymes level in Wistar albino rats. The methanolic extract of leaves and the chloroform extract of roots of Eclipta alba showed significant activities and respectively causing 72.8% and 47.96% reduction of lysosomal enzyme (Lal et al., 2010).

Antihyperlipidemic properties:

It has been reported that in the atherogenic diet - induced hyperlipidemic model, the aqueous leaf extract of the Eclipta prostrata was given orally to the rats which significantly reduced total cholesterol, triglycerides, total protein. There was a
significant elevation in the high density lipoprotein cholesterol levels (Dhandapani, 2007).

**Antioxidant properties:**

The antioxidant effects of *Eclipta prostrata* was reported when 50 mg/kg and 100 mg/kg body weight dose were fed orally into Charles River Sprague - Dawley CD rats which reduced serum hydroxyl radical and serum lipid peroxide levels compared to untreated group. Antioxidant activity of *Eclipta prostrata* was determined by FRAP, radical scavenging activity, reducing activity, and DPPH assay. The antioxidant capacity was increased by increasing the concentration of the extracts from 25 to 100 mg/ml (Rao et al., 2009). The antioxidant activity of the hexane, ethyl acetate, ethanol and water extracts of *Eclipta prostrata* were determined by ferric thiocynate (FTC). Ferric thiocynate method was used to determine the amount of peroxide formed and that react with ferrous chloride (FeCl$_2$) to form a reddish ferric chloride (FeCl$_3$) pigment. Hexane, ethyl acetate, ethanol and water extract at various concentration (50, 100, 250 and 500 in µg/mL), showed antioxidant activities in a concentration - dependent manner (Karthikumar, 1976).

**Immunomodulatory activities:**

It has been reported that protection of neuronal tissues may be possibly due to the immunomodulatory action of *Eclipta alba*. Therefore, *Eclipta alba* can serve as a potential memory modulator (Banji et al., 2007). Experimentation made to assess the immunomodulatory activity of methanolic extracts of whole plant of *Eclipta alba* (1.6% wedelolactone) at five dose levels (dose - response relationship) ranging from 100 to 500 mg/kg body weight using carbon clearance, antibody titer and cyclophosphamide immunosuppression parameters significantly increased phagocytic index, antibody titer; the F ratios of the phagocytic index and WBC count were also
significant (Jayathirthaa and Mishraa, 2004). The aqueous leaf extract of *Eclipta alba* was fed into a fish (tilapia, *Oreochromis mossambicus*) at 0, 0.01, 0.1 or 1% levels as a diet for 3 weeks. After each week, non-specific humoral (lysozyme, antiprotease and complement) and cellular (myeloperoxidase content, production of reactive oxygen and nitrogen species) responses and disease resistance against *Aeromonas hydrophila* were noted which resulted in increased activity of non-specific immune parameters. The results indicate that dietary intake of *Eclipta alba* aqueous leaf extract enhances the non-specific immune responses and disease resistance of *Oreochromis mossambicus* against *Aeromonas hydrophila* (Christybapita *et al.*, 2007).

**Analgesic and anti-inflammatory activity:**

Albino Wistar rats were used to investigate anti-inflammatory activity in which methanolic extract was administered orally. Administration of 100 and 200 mg/kg body weight of extract showed significant anti-inflammatory activity in carrageenan - and egg white - induced hind paw oedema in rats which was compared with indomethacin (10 mg/kg) and cyproheptadine (8 mg/kg) (Arunachalam *et al.*, 2009). Analgesic effect was studied on albino mice using ethanolic and alkaloidal extract of *Eclipta alba*. Standard experimental models such as the tail clip method, the tail flick method and the acetic acid - induced writhing response were used which showed both the ethanolic extract as well as the total alkaloids produced good analgesic activity in all the different models of analgesia used. The total alkaloidal fraction was the most efficacious in all models tested (Singh *et al.*, 2008 and Sawant *et al.*, 2004).
**Antidiabetic activity:**

Leaf suspension of *Eclipta alba* (2 and 4 g/kg body weight) orally in alloxan-induced diabetic rats resulted in reduction in blood glucose level and glycosylated hemoglobin. There was decreased activity of glucose-6 phosphatase and fructose1,6-bisphosphatase, and an increase in the activity of liver hexokinase. Thus oral administration of *Eclipta alba* suspension possess potent antihyperglycemic activity (Ananthi *et al*., 2003). *Eclipta alba* as an ingredient in polyherbal formulation Pan-five were scientifically and clinically proved to possess antidiabetic and diuretic activity by acting upon pancreas by restoration and regeneration of pancreatic β-cell activity (Hemalatha *et al*., 2006).

**Hair growth and alopecia:**

*Eclipta alba* is used in hair oil preparations since it promotes hair growth and maintains hair black. A 10% w/v of *Eclipta alba* was main ingredient in the preparation of herbal formulation for hair growth (Thorat *et al*., 2009) Alopecia is a dermatological disorder with psychosocial implications on patients with hair loss. In the reported work petroleum ether and ethanolic extracts were incorporated into oleaginous cream (water in oil cream base) and applied topically on shaved denuded skin of albino rats. The time (in days) required for hair growth initiation as well as completion of hair growth cycle was recorded. Minoxidil 2% solution was applied topically and served as positive control for comparison. The result of treatment with 2 and 5% petroleum ether extracts were better than the positive control minoxidil 2% treatment (Roy *et al*., 2008).

**Anticancer activity:**

Methanolic extract of *Eclipta alba* was evaluated for its anticancer activity against Ehrlich ascites carcinoma (EAC) in Swiss albino mice. On day 1, the extract
of *Eclipta alba* at a dose of 250 and 500 mg/kg body weight were administered orally and continued for 9 consecutive days. The anticancer activity was examined by determining the tumor volume, tumor cell count, viable tumor cell count, nonviable tumor cell count, mean survival time and increase in life span in experimental animal models. The extract increased the life span of EAC treated mice and restored the haematological parameters as compared with the EAC bearing mice. Thus, study revealed that the methanolic extract of *Eclipta alba* showed anticancer activity in the tested animal models (Gupta *et al.*, 2005). Coumestans are also known to act as phytoestrogens. These compounds are present in soyabean and clover. In many countries it is used as diet which acts as chemopreventive agent in breast and prostate cancer (Basu *et al.*, 2008).

Dasycyphins - C (saponins) a newer isolated compound from *Eclipta prostrata* reported to have anticancer - cytotoxic activity (Khanna, 2008). It was tested under *in vitro* conditions in HeLa (Human cervical carcinoma) and vero cell lines. At the concentration of 50 µg/ml it showed a good anticancer - cytotoxic activity on HeLa cells (Khanna, 2008). A rat hepatic stellate cell line (HSCs) was used as an *in vitro* assay system. The methanolic extract of aerial parts of *Eclipta prostrata* showed significant inhibitory activity on HSCs proliferation (Lee *et al.*, 2008).

**Combination therapy:**

*Eclipta alba* (whole plant), *Mimosa pudica* (whole plant), *Vitex negundo* (whole plant), and *Solanum nigrum* (aerial parts) possessed styptic and anti-inflammatory properties and help in regeneration of the vascular endothelium (Sahu and Srivastava, 2001). Combination of Herbs like *Anethum sowa* (Shatapushpa), *Piper longum* (Pippali mool), *Valeriana wallichii* (Tagar), *Cassia fistula* (Aragvadh), *Withania somnifera* (Ashwagandha) and *Triphala* (A herbal combination of three
fruits) with *Eclipta alba* (Bhringaraj) pacify the aggravated vata dosha and combination with *Elaeocarpus ganitrus* (Rudraksha), *Herpestris monniera* (Brahmi) showed a tranquilizer effect (Haveliwala, 1963). Herbal mixture containing *Phyllanthus nigrum*, *Picrorrhiza kurroa*, *Zingiber officinale*, *Boerhaavia diffusa*, *Andrographis paniculata*, *Cichorium intybus*, *Embelia ribes*, *Terminalia chebula*, *Terminalia arjuna*, *Piper longum* with *Eclipta alba* is used as a good digestive tonic (Bruce *et al*., 2000).

**Other pharmacological activities:**

Ethanolic and ethyl acetate fractions of *Eclipta prostrata* were tested for its antibacterial activities against *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* (Karthikumar *et al*., 2007). *Eclipta prostrata* is combined with a non-plant material which is used to bath children suffering from malnutrition for 9 days.

**Boerhavia diffusa**

*Boerhavia diffusa* (Nyctaginaceae), commonly known as ‘Punarnava’ in the Indian system of medicine is a perennial creeping herb. *Boerhavia diffusa* is a widely studied plant and has a long history of uses by the tribal people and in Ayurvedic and Unani medicines. Pharmacological studies have demonstrated that *Boerhavia diffusa* known to possess anticonvulsant, diuretic, anti-inflammatory, antifibrinolytic, antibacterial, antihelminthic, antileprosy, antiasthmatic, antiurethritis, antilymphoproliferative, antimetastatic, antidiabetic, immune-modulation, anti-nociceptive, nephroprotective, antiurolithiatic and antioxidative activities.
It is widely distributed throughout India and flourishes during rainy seasons. The aerial parts then disappear but revive or sprout again next year (Sivarajan and Balchandran, 1985). It is a diffusely branched pubescent or glabrous, prostrate herbs, abundantly occurring as a weed throughout India, up to an altitude of 2000 m in the Himalayas (Fig. 1.3).

**Stem:** Greenish purple, stiff, slender, cylindrical, swollen at nodes, minutely pubescent or nearly glabrous, prostrate divericately branched, branches from common stalk, often more than a metre long.

**Root:** Well developed, fairly long, somewhat tortuous, cylindrical, 0.2 - 1.5 cm in diameter, yellowish brown to brown coloured, surface soft to touch but rough due to minute longitudinal striations and root scars, fracture, short, no distinct odour, taste, slightly bitter, sweet, pungent.

**Leaves:** Opposite in unequal pairs, larger ones 25 - 37 mm long and smaller ones 12 - 18 mm long ovate - oblong or sub orbicular, apex rounded or slightly pointed, base subcordate or rounded, green and glabrous above, whitish below, margin entire or subundulate, dorsal side pinkish in certain cases, thick in texture, petioles nearly as long as the blade, slender.

**Flowers:** Very small, pink coloured, nearly sessile or shortly stalked, 10 - 25 cm, in small umbels, arranged on slender long stalks, 4 - 10 corymb, axillary and in terminal panicles, bracteoles, small, acute, perianth tube constricted above the ovary, lower part greenish, ovoid, ribbed, upper part pink, funnel - shaped, 3 mm long, tube 5 lobed, stamen 2 - 3.

**Fruit:** One seeded nut, 6 mm long clavate, rounded, broadly and bluntly 5 ribbed, viscidly glandular.
Scientific classification

Kingdom: Plantae
(unranked): Angiosperms
(unranked): Eudicots
(unranked): Core eudicots
Order: Caryophyllales
Family: Nyctaginaceae
Genus: Boerhavia
Species: B. diffusa
Binomial name: Boerhavia diffusa

Vernacular names:

Sanskrit: Kahtilla, Sophaghni, Sothaghni, Varshabhu; Assamese: Ranga Punarnabha;
Bengali: Rakta Punarnava; English: Horse Purslane, Hog Weed; Gujrati: Dholisaturdi, Motosatodo; Hindi: Gadapurna, Lalpunarnava; Kannada: Sanadika, Kommeberu, Komma; Kashmiri: Vanjula Punarnava; Malayalam: Chuvanna Tazhutawa; Marathi: Ghetuli, Vasuchimuli, Satodimula, Punarnava, Khaparkhuti; Oriya: Lalapuiruni, Nalipuruni; Punjabi: Khattan; Tamil: Mukurattai (Shihappu); Telugu: Atikamamidi, Erra galijeru; Bengali: Punarnnava

Distribution:

Boerhavia diffusa is widely dispersed, occurring throughout India, the Pacific, and southern United States. Genus Boerhaavia, consisting of 40 species is distributed in tropical and sub-tropical regions and warm climate. It is found in Ceylon, Australia, Sudan and Malay Peninsula, extending to China, Africa, America and Islands of the
Pacific. Among 40 species of Boerhavia, 6 species are found in India, namely *Boerhavia diffusa*, *Boerhavia erecta*, *Boerhavia rependa*, *Boerhavia chinensis*, *Boerhavia hirsute* and *Boehavia rubicunda*. *Boerhaavia diffusa* in India is found in warmer parts of the country and throughout up to 2,000 m altitude in the Himalayan region. It is a perennial, spreading hogweed, commonly occurring abundantly in waste places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal (Ahmad AND Sharma, 2008).

**Traditional uses:**

*Boerhavia diffusa* has a long history of uses by indigenous and tribal people and in Ayurvedic or natural herbal medicines (Dhar *et al*., 1968). The root, leaves, aerial parts or the whole plant of *Boerhaavia diffusa* have been employed for the treatment of various disorders in the Ayurvedic herbal medicine (daily used by millions of people in India, Nepal, Sri Lanka and indirectly through it being the major influence on Unani, Chinese and Tibetan medicines). The root is mainly used to treat gonorrhoea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers (Kirtikar and Basu, 1956). It cures corneal ulcers and night blindness (Gupta *et al*., 1962), and helps restore virility in men. People in tribal areas use it to hasten childbirth (Shah *et al*., 1983). The juice of *Boerhaavia diffusa* leaves serves as a lotion in ophthalmia. It is also administered orally as a blood purifier and to relieve muscular pain (CSIR, 1988). The roots are reputed to be diuretic and laxative and are given for the treatment of anasarca, ascites and jaundice (Rawat *et al*., 1997).
**Phytochemistry:**

The plant has gained lot of importance in the field of phytochemistry because of its various pharmacological and biological activities such as immunomodulatory effects, immunosuppressive activity, antimetastatic activity, antioxidant activity, antidiabetic activity antiproliferative and antiestrogenic activity, analgesic and anti-inflammatory activity, antibacterial activity, antistress and adoptogenic activity, antilymphoproliferative activity, nitric oxide scavenging activity, hepatoprotective activity, anti-viral activity, bronchial asthma, antifibrinolytic activity, chemopreventive action, genetic diversity analysis, anticonvulsant activity. The *Boerhavia diffusa* plant contains a large number of such compounds as flavonoids, rotanoids, alkaloids, steroids, triterpenoids (Kadota *et al*., 1989; Lami *et al*., 1990; Jain and Khanna, 1989).

In a preliminary screening, plant revealed presence of sterols (Singh and Udupa, 1972), β-sitosterol (Srivastava *et al*., 1972; Desai *et al*., 1973) and alkaloids (Garg *et al*., 1980). Presence of steroids, sugars and alkaloids were also reported (Shukla, 1982). It contains about 0.04% of alkaloid known as punarnavine (C\textsubscript{17}H\textsubscript{22}N\textsubscript{2}O, mp 236-237°C) (Surange and Pendse, 1972) and punarnavoside, an antifibrinolytic agent. It also contains about 6% of potassium nitrate, an oily substance and ursolic acid (Kokate *et al*., 2005). The green stalk of the plant has also been reported to contain boerhavin and boerhavic acid. Many rotanoids have been isolated from the roots of the plant (Kadota *et al*., 1989; Lami *et al*., 1990). These include a series of boerhavinones viz., boerhavinone A, boerhavinone B, boerhavinone C, boerhavinone D, boerhavinone E and boerhavinone F. Four new compounds were isolated from *Boerhaavia diffusa* namely (i) eupaltin 3-O-β-D-galactopyranosyl-(1”>2”)-O-β-D-galactopyranoside, (ii) 3,3’5-trihydroxy-7-methoxyflavone (iii) 4’,7-
dihydroxy-3’-methylflavone and (iv) 3,4-dimethoxyphenyl-1-O-β-D-apiofuranosyl-(1”>3’)-O-β-D-glucopyranoside (Maurya et al., 2007).

PHARMACOLOGICAL PROPERTIES:

Analgesic activity:

The analgesic property of aqueous extracts obtained from *Boerhavia diffusa*, mainly from the leaf juice of the plant. The data also confirmed the traditional indications. The mechanism underlay this analgesic effect remains unknown but the aqueous extract obtained from leaf juice is endowed with an apparently morphinomimetic central analgesic property (Hiruma-Lima et al., 2000).

Anti-inflammatory activity:

The aqueous and acetone extracts of the root, showed significant anti-inflammatory activity against carrageenan - induced oedema and formaldehyde - induced arthritis in albino rats. The aqueous extract and an alkaloid significantly inhibited the increased serum amino transferase activity in arthritic animals similar to hydrocortisone. Liver ATP phosphohydrolase activity was also increased by aqueous extract and the alkaloid (Bhalla et al., 1971).

The water insoluble alcoholic extract of different parts of the plant viz., root, stem, leaves and flowers of plant was studied for its anti-inflammatory activity against carrageenan - induced oedema in rats and for diuretic activity. The root and leaves were found to be most active (Mudgal, 1974) and the activity was found maximum during rainy season (Mudgal, 1975). The effect of extract obtained from the root was studied on experimental acute pyelonephritis in rats. It reduced the inflammatory changes as well as the abscess formation in kidneys of animals infected with
inoculation of *Escherichia coli*. It also reduced the bacterial count in the urine samples of infected animals (Singh *et al.*, 1988).

**Hepatoprotective activity:**

The hydro-alcoholic extract of roots of *Boerhavia diffusa* exhibited a significant protective action of liver evident by a reduction in elevated levels of serum lysosomal enzymes namely serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and alkaline phosphate (ALP) are in both CCl₄ and rifampicin-isonizid - induced hepatotoxicity (Desai *et al.*, 2008).

**Diuretic:**

The effect of extracts of red and white varieties of the plant was studied on diuresis and renal enzymes. Both the varieties exhibited diuretic activity in toads. Red variety showed comparatively less activity. It inhibited the activity of kidney’s succinic dihydrogenase but showed stimulatory effect on it in lower doses. Inhibition produced by red variety was less than that of white variety. It depressed kidney tissue slice respiration but had no effect on kidney phosphatase. It stimulated the activity of kidney d-amino acid oxidase. The activity was more in white variety (Chowdhury and Sen, 1955). The petroleum ether extract of plant exhibited diuretic activity associated with increased sodium excretion in rats when given in a dose of 250 mg/ kg body weight orally. The results were compared with polythiazide (Gaitonde *et al.*, 1974).

**Antistress activity:**

The extract improved the stress tolerance by significantly increasing the swim duration and reducing the elevated WBC, blood glucose and plasma cortisol. Immunomodulatory activity was evaluated by carbon clearance and delayed hypersensitivity test (Sumantha and Mustafa, 2007).
**Immunomodulatory effect:**

In a study to evaluate the adaptogenic potential of root, the aqueous extract of the root powder was studied for its effect on *Escherichia coli* - induced abdominal sepsis, macrophage phagocytic activity in mice and on cold and forced swimming stress in rats. Pre-treatment with root powder extract at a dose of 200 mg kg/body weight/ day orally for 15 days prior to *Escherichia coli* challenge produced significant leucocytosis with reduction in mortality in rats and also significantly increased macrophage phagocytic activity in mice. The plant extract reversed the stress - induced elevations in the levels of glucose, cholesterol, SGPT, BUN and reduction in triglycerides (Mungantiwar et al., 1997). The alkaloidal fraction isolated from the root was investigated for its effect on plasma cortisol, adrenal cortisol and humoral response in stressed rats. It exhibited restorative activity against stress - induced changes in plasma and adrenal cortisol levels. It also significantly augmented the antibody production in stressed rats as compared to control (Mungantiwar et al., 1997).

**Antifertility:**

The ethanolic extract of root in a dose of 250 mg/kg body weight (daily) p.o. to pregnant albino female rats during the entire period of gestation did not show any teratogenic effects, as litter size and survival rate of foetuses were the same as for the normal control group and no foetal abnormality was detected (Singh et al., 1991).

**Immuno suppressive activity:**

A research was also carried out to evaluate the immunomodulatory properties of this plant extract on various *in vitro* tests such as human natural killer (NK) cell cytotoxicity, production of nitric oxide (NO) in mouse macrophage cells. RAW 264.7, interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), intracytoplasmic interferon-g
(IFN-Y) and expression of various cell surface markers on human peripheral blood mononuclear cells (PBMCs). Ethanolic extracts of Boerhavia diffusa roots inhibited human NK cell cytotoxicity in vitro, production of NO in mouse macrophage cells, IL-2 and TNF-α in human PBMCs. Intracytoplasmic IFN and cell surface markers such as CD16, CD25 and HLA-DR did not get affected on treatment with Boerhavia diffusa extract. Hence, it demonstrates immunosuppressive potential of ethanolic extract of Boerhavia diffusa (Mehrotra et al., 2002).

**Antidiabetic activity:**

A study was carried out to investigate the effects of daily oral administration of aqueous solution of Boerhaavia diffusa L. leaf extract (200 mg/kg body weight) for 4 weeks on blood glucose concentration and hepatic enzymes in normal and alloxan - induced diabetic rats. A significant decrease in blood glucose and significant increase in plasma insulin levels were observed in normal and diabetic rats treated with Boerhavia diffusa leaf extract (Pari and Satheesh, 2004). Chloroform extract of Boerhavia diffusa leaf produced dose-dependent reduction in blood glucose in streptozotocin - induced non-insulin dependent diabetes mellitus (NIDDM) rats comparable to that of glibenclamide. The results indicate that the reduction in blood glucose produced by the extract is probably through rejuvenation of pancreatic beta-cells or through extra pancreatic action (Nalamolu et al., 2004).

**Radioprotective activity:**

In a study on the effect of the plant in radiation - induced haemopoietic injury in albino mice, pre-treatment (in the dose of 260 mg/kg body weight orally for 21 days) to mice exposed to total body irradiation (6 Gy) for 3 min showed significant increase in haemoglobin (Hb) and total red blood cells (RBC) count. After irradiation,
there was no fall in RBC count and Hb unlike in controls. The study indicated that the plant had selective effect on the erythroid compartment (Thali et al., 1998).

**Anti-metastatic activity:**

Administration of punarnavine (40 mg/kg body weight) prophylactically (95.25%), simultaneously (93.9%) and 10 days after tumor inoculation (80.1%) could inhibit the metastatic colony formation of melano main lungs. Survival rate of the metastatic tumor-bearing animals were increased significantly by the administration of punarnavine in all the modalities compared to the metastasis bearing untreated control. These results correlated with the biochemical parameters such as lung collagen hydroxylproline, uronic acid, hexosamine, serum sialic acid, serum glutamyl transpeptidase and serum vascular endothelial growth factor (VEGF) levels and histopathological studies. Punarnavine administration could suppress or down regulate the expression of MMP-2, MMP-signal-regulated kinase and VEGF in the lung tissue of metastasis - induced animals. Punarnavine could inhibit MMP-2 and MMP-9 protein expression in gelatin zymographic analysis of B16F-10 cells. These results indicate that punarnavine could inhibit the metastatic progression of B16F-10 melanoma cells in mice (Manu and Kuttan, 2009).

**Antioxidant activity:**

Ethanolic and methanolic extracts were prepared and screened for *in vitro* antioxidant activities using ferric reducing power and hydrogen peroxide scavenging activity. The activity was compared to standard antioxidant like ascorbic acid. Both the extracts showed strong antioxidant activity in both the methods. Between these two extracts, ethanolic extract has shown better antioxidant activity as compared to methanolic extract in both the activities (Rachh et al., 2009).
Antimicrobial activity:

The methanolic extract of *Boerhavia diffusa* leaves had significant *in vitro* antimicrobial activity. Hence, further results revealed that among several pathogenic bacteria, only *Staphylococcus aureus* was susceptible for *Boerhaavia diffusa*. In *Boerhaavia diffusa*, maximum inhibition was observed in *Staphylococcus aureus* followed by *Bacillus megaterium* and *Bacillus cereus*, respectively at 50 μL concentration (Girish and Satish, 2008).

Antiviral activity:

*Boerhavia diffusa* plant possess potent antiviral efficacy against phytopathogenic viruses. The antiviral agent isolated from this plant was found to be a glycoprotein with a molecular weight of 16-20 kDa. Administered by foliar spraying in the field, this antiviral agent could protect some economically important crops against natural infection by plant viruses (Awasthi and Verma, 2006). Root of *Boerhaavia diffusa* contains basal proteins which show high virus inhibitory activity against plant viruses. Root extract of this plant induce strong systemic resistance in susceptible host plant. (Lohani *et al.*, 2007). The aqueous extract of the leaves inhibited potato virus Y infection on chilli plants (Suriachandraselvan and Narayanasamy, 1987).

Chemopreventive action:

Cancer chemopreventive property of *Boerhavia diffusa* was evaluated on 7,12-dimethyl benz (a) anthracene (DMBA) induced skin papillomagnesis in male Swiss albino mice (6-7 weeks old). This leads to the supposition that the inhibition of tumorigenesis by the plant extract might have been executed either by preventing the formation of active carcinogens from their precursors or by augmenting detoxification
process, preventing promotional events in the mouse skin through free radical scavenging mechanism (Bharali et al., 2003).

**Antilymphoproliferative activity:**

It inhibited T cell mitogen phytohemagglutinin and concanavalin A-stimulated proliferation of human peripheral blood mononuclear cells (PBMC). It also inhibited purified protein derivative antigen-stimulated PBMC proliferation and human mixed lymphocyte culture. In addition, *Boerhavia diffusa* extract inhibited the growth of several cell lines of mouse and human origin, such as mouse macrophage cells (RAW 264.7), human macrophage cells (U937), human monocytic cells (THP-I), mouse fibroblast cells (L.929), human embryonic kidney cells (HEK293), mouse liver cells (BNLCL.2), African green monkey kidney cells (COS-I), mouse lymphoma cells (EL-4), human erythroleukemic cells (K562) and human T cells (Jurkat). (Mehrotra et al., 2002).

**Liv. 52**

The natural ingredients in Liv.52 exhibit potent hepatoprotective properties against infective hepatitis. Liv52 has been validated by 276 clinical trials and research studies. Liv. 52 maintain the functional efficacy of the liver. It detoxifies and protects liver cells from harmful toxins and supports the liver’s ability to regenerate itself. Liv. 52 promote optimum liver function and as a daily health supplement, it helps to improve appetite and the assimilation process. Liv. 52 also support the liver’s function when challenged by toxins. Liv. 52 facilitate rapid elimination of acetaldehyde, the toxic intermediate of alcohol metabolism and detoxify liver cells. Liv-52 is an indigenous multiherbal hepatotonic that has been widely used as a hepatoprotective agent in various liver disorders (Poli et al.,1985; Karandikar et al., 1963; Saini NR, Saini N.,1985; Dhumal et al.,1989) and moreover, it has shown protective effects in
hepatotoxicity induced by radiations. The oral administration of Liv. 52 to experimental animals have been reported to provide considerable protection against liver damage by carbon tetrachloride (CCl₄) (Ghosh, 2005) Hepatoprotective and anti-inflammatory effects of some of the individual ingredients of both formulations such as Liv-52 and Livomyn are also reported in literature. (Jindal et al., 1975; Reddy et al., 1993; Gilan and Janbaz, 1994; Sultan et al., 1995).

SCOPE OF THE THESIS

This thesis contains the study regarding mitigatory effect of Eclipta alba and Boerhavia diffusa – two Indian medicinal plants - against CCl₄-induced hepatotoxicity.

- Phytochemical analyses of the plants were carried out by standard qualitative and quantitative methods. Antioxidative potency of the plant extracts have been ascertained by using different chemical models. Quantification of the active constituents responsible for hepatoprotective activity of the plants has been carried out.

- The in vitro hepatoprotective potential of – Eclipta alba and Boerhavia diffusa plant extracts have been analysed against CCl₄ - induced toxicity.

- Mitigatory effects of the both the plant extracts – Eclipta alba and Boerhavia diffusa were tested against CCl₄ – induced hepatotoxicity in vivo conditions. Activities of various liver marker enzymes, the extent of lipid peroxidation, alterations in enzymatic and non-enzymatic antioxidants, DNA, RNA and other relevant biochemical parameters as well as histopathological examinations were taken into consideration in this study. The hepatoprotective
activities of both the plant extracts were compared with the standard polyherbal drug Liv. 52.

- The hepatoprotective properties of both the plant extracts have been compared on the basis of their hepatoprotective index.

- The results were statistically analysed and relevance of the present results are discussed with reference to recent development.