INTRODUCTION
1. INTRODUCTION

Human health care

Health is wealth. Health is the most precious gift to human being. In the modern world a foetus inside the womb of mother ought to take vaccination. Because of the industrialization, human beings are continuously exposed to pollutants as in the form of air pollution, water pollution, soil pollution, food adulteration etc. They have to take unlimited number of allopathic medicines which gives a temporary relief but it results in the initiation of many side effects.

Now-a-days people are going to seek remedy from herbal medicines for their ailments. Medicinal plants used in the Indian Traditional Medicine play a key role in world health care systems (Bajaj and Williams, 1995) have attracted the attention of scientists the world over (Kamboj, 2000). About 75% to 90% of the rural people in the world rely on herbal medicine for their primary health care (WHO, 2005).

According to World Health Organization, of the 119 plant derived pharmaceutical medicines, about 74% are being used in modern medicine in many ways that are directly correlated with their traditional use as plant medicines by the native people. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Sidha and Unani. These medicinal 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.

The use of herbal medicines can be traced back to 2100 B.C. in ancient China at the time of Xia dynasty, and in India during the Vedic period. The first written reports are date back to 600 B.C. with Charaka Samhita of India, and in China the same became systematic by 400 B.C. (Schuppan et al., 1999). The basic concept in these medicinal systems is that the disease is a manifestation of a general imbalance of the dichotomous energies that govern life as a whole and human life in particular, and they focus on medicine that can balance these energies and maintain good health.

Ayurveda is based on the principle of “maintaining the health of a healthy person and relieving the patient from diseased condition”. In Indian Ayurvedic system, the forces are said to be ‘agni’ (strength, health and innovation) and ‘ama’ (weakness, disease and intoxication). Like Ayurveda, Sidha medicines believe in a perfect balance of three ‘doshas’ known as ‘Vatha’ (space and air elements), ‘Pitta’ (fire and water elements) and ‘Kapha’ (water and earth elements). All these Indian systems of medicine have primarily claimed to have a curative potential for their medicinal preparations for all kinds of liver diseases (Thyagarajan et al., 2002).

Many species of plants have medicinal properties that have been shown to produce beneficial effects in the treatment of various diseases
(Burkill, 1985). Although herbal medicines are effective in the treatment of various ailments and very often these drugs are unscientifically explored and/or improperly used. Therefore, these plant drugs deserve a detailed study in the light of modern science.

**The Liver – A great chemical factory of human body**

Human body is composed of untold billions of living cells, all are very much alive, and all are carrying out their particular functions. Liver, which weighs about 1500 g is tucked up under the ribs in the right upper part of the abdominal cavity. The liver is rather simple in appearance, but it is involved in several important functions, such as metabolism, secretion, digestion, storage and detoxification of drugs. The main functions of liver are as follows:

- Blood coming from the intestinal system, charged with the products it has absorbed, arrives at the liver via the portal vein and is being processed by the liver.
- The hepatic artery supplies the liver itself with blood and the blood leaves the liver through the hepatic vein to go on to the heart and the general body circulation.
- Liver manufactures bile salts which are important in assisting the absorption of fats from the blood.
- Liver produces a number of important proteins such as transferin which combines with iron and binds with other elements and assist both absorption and their subsequent
use. It also produces the proteins which are essential for blood clotting.

- The liver is able to breakdown the fat to produce energy.
- It also helps for the breakdown of certain hormones, drugs, diuretics to a form in which they are finally eliminated from the body.
- Many enzymes are manufactured by the liver, and they play an important part in drug metabolism, the breakdown and subsequent use of the drugs.
- The liver is an important storehouse for many substances, such as iron, folic acid and vitamin B_12, all are essential for the normal production of blood.

Bile acids are needed for the absorption of fat from intestine, prothrombin, which is essential for clotting of blood, urea, and which forms an important component of urine are produced within the liver.

The liver has another very important function, that of detoxifying or destroying many harmful poisons and even certain medicines that are taken in to the body for other purposes. When the liver is sick, these numerous activities are impaired, and soon the whole body suffers. All these endless activity means that the liver cells eventually become worn out and must be replaced. In most people the breaking down and building up process continues without any apparent difficulty.
Toxicity may accrue through accumulation of parent drug or, via metabolic activation, through formation of a chemically reactive metabolite, which, if not detoxified, can effect covalent modification of biological macromolecules (Fig. 1.1). The identity of the target macromolecule and the functional consequence of its modification will dictate the resulting toxicological response in the living systems.

Fig. 1.1 Relationship between drug metabolism and toxicity.

Liver diseases – A global health problem

Liver diseases are considered as one of the most serious health problems as the liver is an important organ for detoxification and deposition of endogenous and exogenous substances. Liver diseases may be classified as acute or chronic hepatitis (inflammatory liver diseases),
heptoses (non inflammatory diseases) and cirrhosis (degenerative disorder resulting in fibrosis of the liver).

Liver diseases are mainly caused by some toxic chemicals for example, certain antibiotics, chemotherapeutics, peroxidised oil, alfatoxin, carbon tetrachloride chlorinated hydrocarbons etc. and also due to excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver (Recknagel, 1983; Hiroshi et al., 1987; Wendell et al., 1987; Dianzani et al., 1991). Enhanced lipid peroxidation produced during the liver, microsomal metabolism of ethanol may result in hepatitis and cirrhosis (Smuckler, 1975).

It has been estimated that about 90% of the acute hepatitis is due to viruses. The major viral agents involved are Hepatitis B, A, C, D (delta agents), E and G. Of these, Hepatitis-B infection often results in chronic liver diseases and cirrhosis of liver. Primary liver cancer has also been shown to be produced by these viruses. Hepatitis-B causes a global health problem. Patients with chronic hepatitis-B (CHB) carry a significant risk to eventually develop cirrhotic liver disease (Lavanchy, 2004; Mailliard and Gollan, 2006). Although an effective vaccine has been available for more than 20 years, it is estimated that there are 350 million people with CHB and that as many as two billion people
worldwide have been infected with hepatitis-B virus (HBV), (Tran and Martin, 2004).

Hepatitis-C virus (HCV) will continue to be a serious global health threat for many years to come because of the chronic nature of the infection, its high prevalence and the significant morbidity of the resulting disease. The hepatitis-C virus (HCV) infects approximately three percent of the world’s population (Shoukry et al., 2004). Hepatitis-E has a worldwide distribution and causes substantial morbidity and mortality in some developing countries, particularly among pregnant women (Mast and Krawczynski, 1996). Among the many viruses that are known to infect the human liver, hepatitis-B virus (HBV) and Hepatitis-C virus (HCV) are unique be of their prodigious capacity to cause persistent infection, cirrhosis, and liver cancer etc. (Guidotti and Chisari, 2006).

**Treatment – Hepatoprotective plants**

In spite of the tremendous advances made in allopathic medicine, no effective hepatoprotective medicine is available. Plant drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities. Nearly 150 phytoconstituents from 101 plants have been claimed to have hepatoprotective activities (Handa et al., 1989; Doreswamy and Sharma, 1995). In India more than 87 medicinal plants are used in different combinations in the preparation (of 33
patented) herbal formulations for liver diseases (Hikino and Kiso, 1988; Handa et al., 1989; Sharma et al., 1991; Evans, 1996).

Most commonly used 12 plants in herbal formulations are *Andrographis paniculata*, *Boerhaavia diffusa*, *Eclipta alba*, *Picorrhiza kurroa*, *Oldenlandia corymbosa*, *Asteracantha longifolia*, *Apium graveolens*, *Cassia occidentalis*, *Cichorium intybus*, *Curcuma longa*, *Embolia ribes*, *Tinospora cordifolia* and *Trachysermum ammi* (Treadway, 1998; Luper, 1999; Thyagarajan et al., 2002). The anti-hepatitis virus activities of the traditional plants are not studied in experimental animals expect in a few plants. This is mainly due to lack of ideal “in vivo” test systems. *Andrographis paniculata*, *Eclipta alba*, *Glycyrriza glabra*, *Picorrhiza kurroa*, *Phyllanthus amarus* and *Silybum marianum* are reported to have activity against jaundice producing Hepatitis-B virus (Saxena et al., 1993; Doreswamy and Sharma, 1995; Wang et al., 1996; Chrungo et al., 1997; Karunakar et al., 1997; Rawat et al., 1997; Scott Luper, 1999).

Picroliv (*Picorrhiza kurroa*), Andrographolide (*Andrographis paniculata*), Punarnavine (*Boerhaavia diffusa*), Silymarin (*Silybum marianum*), Glycyrrhizin (*Glycyrrhiza glabra*), Fumaric acid (*Sida cordifolia*), Ursolic acid (*Eucalyptus spp.*) etc. are some of the plant constituents responsible for hepatoprotective activity (Cruz et al., 1992; Asha, 1996; Kumar and Mishra, 1997; Scott Luper, 1999). Studies carried out at Tropical Botanic Garden and Research Institute (TBGRI)
has shown that *Trichopus zeylanicus*, *Phyllanthus madraspatensis*, and *P. kozhikodianus* are extremely active against paracetamol induced liver damage in rat (Asha and Pushpangadan, 1998; Subramoniam *et al.*, 1998). Ursolic acid which occurs in many plants also showed promising hepatoprotection against paracetamol and carbontetrachloride induced liver damage in rats (Shukla *et al.*, 1992; Saraswati *et al.*, 1996). The hepato-protective activity of *Boerhaavia diffusa* roots were shown by Rawat *et al.* (1997) in thioacetamide intoxicated rats.

**Dietary prevention of liver damage**

Dietary modifications can, to a large extent, prevent toxic environmental, chemicals-induced liver damage. Most of the hepatotoxins damage liver directly or indirectly by oxidative damages.

Antioxidants can protect experimental animals and human from oxidant mediated liver damages. Antioxidant nutraceuticals are those which contain vitamin E, vitamin C, vitamin A and beta carotene. They are present in certain vegetables (e.g. Carrot), turmeric, fixed oils, fruits and fishes. Antioxidants present in such food are those compounds which either prevent the formation of oxygen free radicals or trap them (Kokate, 2004). It has been shown that carrot juice can protect mice from CCl₄ - induced hepatotoxicity (Bishayee *et al.*, 1995). *In vitro* experiments have demonstrated strong antihepatotoxic action of curcumin present in turmeric (Evans, 1996).
The liver rejuvenative herb – Boerhaavia diffusa

The plant Boerhaavia diffusa was named in honour of Hermann Boerhaave, a famous Dutch Physician of the 18th Century (Chopra, 1969). The whole Boerhaavia plant, fresh as well as dried is the genuine source of the drug Punarnava. One of the well documented hepatoprotective plants Boerhaavia diffusa, L. is commonly known as "Punarnava". The genus Boerhaavia has several species, and is distributed in the tropical, sub tropical and temperate regions of the world (Heywood, 1978). It is found in Australia, China, Egypt, Pakistan, Sudan, Sri Lanka, South Africa, USA and in several countries of Middle East. Out of 40 species of this genus, 6 species are found in India, they are: Boerhaavia diffusa, B. chinensis, B. erecta, B. repens, B. rependa and B. rubicunda (Chopra, 1969; CSIR, 1988). It is also indigenous to India, it is found throughout the warmer parts of the country up to an altitude of 2000 m in the Himalayan region. It grows well on wastelands and in fields after the rainy season (Chopra, 1969). The plant is also cultivated to some extent in West Bengal (CSIR, 1988). It is a diffusely branched prostrate perennial herb found throughout the wasteland of India. It is also known by several vernacular names (Nadkarani, 1982)

Sanskrit - Punarnava, Shothaghni
English - Spreading Hogweed
Hindi - Beshkapore; Gadhaparna; Thikri; Sant.
Bengali - Gandhapurna; Swetapoorna; Punernaba
Punjabi - Itsit
Gujarathi - Vakha - Khaparo
In India it is found all over, very commonly the way-side, wastelands even in very poor soil, river banks, etc., the plant prefers acidic, neutral and basic (alkaline) soils. It cannot grow in the shade. It requires dry or moist soil and can tolerate drought and well flourishes during rainy season. The aerial parts then disappear but revive or sprout.

**Taxonomic description**

The systematic position of *B. diffusa* is as follows (Bentham and Hooker, 1862)

- **Class** - Dicotyledons
- **Sub-class** - Monochlamyldae
- **Series** - Curvembryae
- **Family** - Nyctaginaceae
- **Genus** - Boerhaavia
- **Species** - diffusa

It has a large root system. The root stock is stout, fusiform and woody with many erect or procumbent branches. They are elongated and grow vertically downwards striking deep into the soil. The stems are creeping, often purplish, swollen at their nodes. The leaves are thick,
long petioled in unequal pairs, ovate, oblong or sub-orbicular, acute or obtuse, entire or sinuate, glabrous above, whitish beneath and with somewhat undulate margin, (up to 2 inch long, 1-25 inch broad).

The flowers are small, pink in colour, in panicles, axillary and terminal. Perianth funnel-shaped, ovoid below, narrowed above the ovary, 5-lobed, lower part prominently 5-ribbed. The stamens are 2 or 3; filaments 3mm; stamens as long as perianth tube; anthers didynamous. Ovary is oblique, stipitate, ovules are erect and stigma is peltate. The fruits are indehiscent, hard, club-shaped anthocarps, which are glandular hairy and 5-ribbed. They are slimy and swelling in water. The fruits are very viscid, easily detached and so adherent to clothes or the fur of animals. *B. diffusa* is a very variable according to soil and climate. It is self-pollinated and is propagated through seed.

**Chemical constituents**

A large number of interesting plant chemicals have been found in *B. diffusa*, including flavonoids, steroids and alkaloids many of which drive its biological activities.

The air dried plant was found to contain unusually large quantities of potassium nitrate (Nadkarani, 1982). The main plant chemicals in this plant include alanine, aspartic acid, β-D-galactopyranoside, boeravinone, boerhaavic acid, boerhavine, campesterol, daucosterol, beta ecdysone, eupalitin, flavone, hentriacontane, liriodendrin, lignin, oxalic acid, oleaic acid,
punarnavine, punarnavoside, stigmasterol, syringaresinol, triacontane ursolic acid, etc. (Adesina, 1979; Aynechi, 1982; Jain, 1984; Coimbra and Raul, 1994; Crunz, 1995; Ferreres et al., 2005; Pandey et al., 2005).

**Medicinal properties**

*B. diffusa* is used in traditional medicine for its diuretic, anti-inflammatory, antimitotic, anti-bacterial, antinoceptive, immunomodulatory, antiproliferative, antifibrinolytic, nephritic, anticonvulsant and hepatoprotective properties. The roots of *B. diffusa* have held an important place in herbal medicine in both India and Brazil for many years. In Brazil, *B. diffusa* is considered as a plant medicine of great importance, extraordinarily beneficial in the treatment of liver disorders (Cruz, 1995). It is an important and effective tribal plant resource which is deserving of much more use and interest here in the United States.

*B. diffusa* was listed in TRAMIL, a research project on the medicinal plant resources of the Caribbean (Robineu and Soejarto, 1996), because it has been used in Martinican folk medicine as an agent for the treatment of pain in general and of sore throat in the form of juice or decoction of fresh leaves (Robineau, 1995).

**Micropropagation**

The culturing of plant cells, tissues or organs grown in *in vitro*, conditions using artificial medium is known as plant tissue culture. It is
an art and an indispensable tool. It requires unique facilities and special skills. Specific growth regulators are supplied to control growth and development. Tissue culture is an ideal method for the production of large number of homogenous plants from a piece of plant part within short duration. It is based on the principle of totipotency in which each living cells of an organism has the capacity and ability to develop into an entire organism.

In the present study, an attempt had been made to achieve direct organogenesis from shoot tip and nodal explants and indirect organogenesis from leaf and cotyledonary explants of B. diffusa using different concentrations and combinations of growth hormones. Mass root regeneration from leaf explants have also been tried with different concentrations of auxins. In vitro flowering, in vitro seed setting, ex vitro flowering and ex vitro seed setting have also been obtained.

**Pharmacognosy**

Pharmacognosy may be defined as a branch of bioscience deals with the details of medicinal and related products of crude drugs from natural sources which are treated scientifically. The development of science of phytopharmaceuticals and the hopes for remedies in chronic diseases generated new enthusiasm in the research workers to develop herbal medicines. A Pharmacognostic study includes biological, geographical sources, history, cultivation, collection, processing for market and commerce of crude drug and macroscopical, microscopic or
histological studies of the crude drug. It also includes chemical constituents and quality chemical tests. In the modern age of advancement in instrumentation technology, sophisticated techniques like nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) have markedly reduced the time and effort required for structural determination. In the present study, morphological and anatomical studies of both in vitro and in vivo plant parts and phytochemical analysis of in vivo parts of B. diffusa have been studied.

**Pharmacological evaluation**

Pharmacology can be defined as the study of the effects of chemical substances on the function of living systems. For standardization or evaluation of herbal drugs, assessment of biological efficiency is found to be the most assuming method. The assessment of antihepatotoxicity of Boerhaavia diffusa has been studied by using in vitro cell lines and in vivo animal model test systems. For testing hepatoprotective activity cultured hepatocytes were used in in vitro method. The leaf and root extract effect of B. diffusa on hepatotoxin induced cultured heatocytes and of control was studied by means of serum parameters, biochemical parameters and histopathological studies.

**Bioinformatic studies**

Bioinformatics is a new branch of science which deals with the application of computer knowledge in processing the biological data to
get the meaningful information. Biological data are being stored in the biological data bank. Likewise, there is a protein data bank where all the information about the proteins is stored.

**Scope of the present investigation**

The liver plays an astonishing array of vital functions in the maintenance and performance of the body. Through a vast network of biochemical reactions, the liver controls a major portion of body's internal environment. Liver diseases have become a threat to the medicinal world. Acute or chronic liver diseases may lead to cirrhosis and then liver cancer. As the tenth most common cause of death, chronic hepatitis-B (CHB) is a preventable global health problem of enormous magnitude whereas, the hepatitis-C and hepatitis-E are incurable.

There are no effective allopathic medicines, vaccines, steroids or other methods of prevention for hepatitis, so, the investigation of crude drug from medicinal herbs play a revitalizing role in liver protection. The present study was undertaken with the following objectives:

1. To standardize the protocol for micro propagation of the important medicinal plant *B. diffusa* from various explants for direct and indirect organogenesis.

2. To study the Pharmacogonsy aspects of the *in vitro* and *in vivo* plant parts of *B. diffusa* including phytochemical analysis of *in vivo* and in vitro grown plants.
3. To study the hepatoprotective activity of the leaf and root extracts of *B. diffusa* through pharmacological evaluation using *(in vitro)* cell line and *(in vivo)* animal models.

4. To study the nature and properties of the important enzymes involved in the hepatoprotective analysis such as ASAT, ALAT and ALP by Molecular Visualization Tools like RASMOL, JMOL etc.