1 INTRODUCTION
1.1 OCCURRENCE OF LIVER DISEASES

Liver is the key organ of metabolism and excretion. Such varied functions as storage and filtration of blood, secretion of bile, metabolism of carbohydrates, proteins and fats, detoxification of harmful substances, production of antibodies, storage of fat soluble vitamins, excretion and others are attributed to it (1). The strategic anatomical location and large capacity for metabolic conversions make the liver vulnerable to a wide range of disorders. Though a strict delineation of various hepatic disorders is not possible yet didactically liver disorders may be classified as acute or chronic hepatitis (inflammatory diseases), hepatosis (non-inflammatory disorders) and liver cirrhosis (2).

Viral hepatitis, particularly type B, is an alarming public health problem in both the developing and the developed nations. There are about 250 million chronic carriers of hepatitis B surface antigen (HBsAg) in the world of which 25 million are in India (3). A global estimate indicates that there are about 18,000 deaths every year because of liver cirrhosis mainly caused by hepatitis (4). Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2,50,000 new cases each year, and there is compelling evidence that hepatitis B virus is the cause in upto 80% of such cases (5). Although viruses are the main cause of liver diseases, the liver lesions arising from xenobiotics and therapeutic agents are not uncommon. Expanding industries coupled with uncontrolled environmental pollution, and excessive drug...
therapy expose humans to a vast array of chemicals and pharmaceuticals which predispose liver to a variety of ailments (6).

Recently, vaccines have been developed for viral hepatitis B infection (7). Interferon and its inducers have been confirmed to have effectiveness in some cases of viral hepatitis (8). However, HB vaccines may be used only for the prevention and not for the cure of hepatitis B infection. An actual curative therapeutic agent has not yet been found. In fact, most of the available remedies rather support or promote the process of healing or regeneration of the liver (2). The drugs available in the modern system of medicine are the corticosteroids and immunosuppressive agents which bring about only symptomatic relief and in most cases have no influence on the disease process. Further, their use is associated with the risk of relapses and danger of side effects.

1.2 HEPATOTOXINS FOR EXPERIMENTAL MODELS

Advances in the search for finding an effective hepatoprotective agent owe much to the identification of the pathogenesis of liver disorders, and elaboration of suitable models for hepatic injury comparable to those encountered in the clinical practice. Chemicals and drugs employed for inducing experimental liver lesions in animals have been reviewed (2,9-16). In vitro models employing primary cultured rat hepatocytes using carbon tetrachloride (17), D-galactosamine (18)
and ethanol (19) as cytotoxins have been devised for screening of antihapatotoxic activity. In vitro immunological models for screening antiHBsAg activity have been reviewed (20). Given below are some of the important hepatotoxins employed in experimental models for inducing different kinds of liver damage.

1.2.1 Carbon tetrachloride

Carbon tetrachloride (CCl₄) is the most investigated of all hepatotoxins (21-23). The cytotoxic specificity of CCl₄, once attributed to its ability to solubilize phospholipids and then to a primary effect on mitochondria, is now considered to be due to enzymatic activation of CCl₄ to •CCl₃ within the membranes of endoplasmic reticulum (24,25). This free radical attacks and disrupts the structure and function of lipid and protein macromolecules in the membranes of the cell organelles, and also induces microsomal lipid peroxidation (26). The hepatocytes thus show fatty change or are irreversibly injured.

1.2.2 D-Galactosamine

Administration of the aminosugar - D-galactosamine (GalN), to rats more than 3 weeks of age induces hepatitis closely related to human viral hepatitis (27). GalN hepatitis is induced by a multistep mechanism beginning with the 'primary biochemical response' which includes the formation and accumulation in high concentrations of the
metabolites of GalN (GalN-1-phosphate, UDP-GalN, UDP-glucosamine and UDP-N-acetyl-D-glucosamine) followed by trapping of uracil nucleotides (UTP, UDP-glucose and UDP-galactose) (28). The subsequent reaction of the liver to the metabolic alterations of the 'primary biochemical response' has been termed 'secondary biochemical response' which leads to the development of GalN hepatitis. At the molecular level, the 'secondary biochemical response' involves the inhibition of enzymes by the metabolites of GalN, decreased synthesis of RNA due to deficiency of UTP, and decreased synthesis of glycoproteins and glycolipids attributed to fall in the concentration of UDP-galactose. At the subcellular level, the 'secondary biochemical response' of the liver entails alterations of several organelles like the nucleus, the Golgi apparatus and lysosomes.

1.2.3 Paracetamol

Hepatotoxicity of paracetamol (PcmL), which is a commonly used antipyretic-analgesic sold over-the-counter, has been reviewed (29-33). PcmL is safe at therapeutic dose levels but in large doses it induces liver injury in both human and experimental animals. PcmL-induced liver damage is characterized by fulminating hepatic necrosis - primarily centrilobular, which may extend through the midzone to the periportal area (34). Hepatotoxicity of PcmL is attributed to the formation of a toxic metabolite, N-acetyl-p-benzoquinone imine which is formed via cytochrome P-450 (35). This electrophilic toxic metabolite depletes the cellular reservoirs of glutathione as a result
The reactive metabolite binds covalently to the thiol groups of cysteine residues in proteins leading to hepatocellular toxicity.

1.2.4 Ethanol

The liver toxicity due to ethanol (EtOH), a widely abused inebriating compound, has been extensively reviewed (36-51). Development of EtOH-induced hepatic damage in humans and experimental animals is dependent on the duration of use and the dose of EtOH. Chronic alcoholism causes fatty liver which progresses to hepatitis and cirrhosis. This effect is produced indirectly due to nutritional imbalance and also due to a direct toxic effect of EtOH (40). The deleterious effect of EtOH is attributed to the accumulation in the liver of acetaldehyde, one of the catabolic intermediates of EtOH, which is toxic per se (52). EtOH-induced fatty liver is mainly produced by peripheral mobilization of free fatty acids. EtOH increases triglyceride formation by reducing mitochondrial oxidation of fatty acids in the liver (53,54). Progression of alcoholic hepatitis to cirrhosis is reported to be due to lipocyte stimulated fibrogenesis and lactic-acidosis induced collagenosis (55).

1.2.5 α-Naphthylisothiocyanate

α-Naphthylisothiocyanate (ANIT) is commonly used for the experimental production of intrahepatic cholestasis (56). The histological
picture of the liver after ANIT administration resembles the cirrhotic one. The bile stasis induced by ANIT is preceded by organelle damage followed by regurgitation of bile through the damaged ducts, intrahepatic duct obstruction, and haemolysis leading to jaundice (57). Precholestatic microsomal inhibition is attributed to the direct inhibition of microsomal mixed function oxidase by ANIT and/or a metabolite that is tightly bound to the microsomes (58).

1.2.6 Dimethylnitrosamine

Dimethylnitrosamine (DMN) causes centrilobular necrosis of liver cells in rats and mice (10,59). This compound is activated to a toxic metabolite by cytochrome P-450 which methylates DNA and RNA in the liver cells thereby disrupting the protein metabolism in the liver.

1.2.7 Amatoxins

Amatoxins - phalloidin and α-amanitin, present in the fungus Amanita phalloides are among the most potent liver toxins. The consumption of the fungus under the impression that it is an edible mushroom causes death due to complete liver necrosis within a few days (6,13). Both phalloidin and α-amanitin are used to produce experimental liver injury. Toxic action of amatoxins is due to the disruption of hepatocellular membranes possibly by forming a complex with actin present in the
cell membrane as a result of which cytoplasm escapes into protuberances on the cell surface - an abnormality which signifies cell death (60).

Apart from the above mentioned hepatotoxins many other compounds like thioacetamide, ethionine, allyl alcohol, yellow phosphorous, pyrrolizidine alkaloids etc., are used for producing experimental liver lesions in animals (2).

1.3 SOME PARAMETERS FOR EVALUATING HEPATIC FUNCTIONS

In all the experimental models for evaluating antihepatotoxic activity of a substance, a known hepatotoxin which produces a marked and measurable effect is administered to the animal. The substance under test is then administered along with the toxic dose of the hepatotoxin and if the toxic effect is blocked, the substance under test is considered to be effective. The magnitude of the toxic effect can be measured by estimating some suitable liver-function-parameters or by direct histological examination of the liver slices. A large number of liver function tests have been described (61,62).

**Serum sorbitol dehydrogenase (SSDH):** It is a mitochondrial and cytoplasmic enzyme primarily located in the liver. The importance of the assay of SDH is based on the fact that it is virtually absent in the serum of healthy subjects and is detectable during infections, toxic or hypoxic liver damage. Thus, SDH activity in the serum
serves as an organ specific indicator of liver cell damage.

**Serum glutamic dehydrogenase (SGLDH):** It is exclusively a mitochondrial enzyme found in abundance in the liver and only trace levels occur in the serum of healthy subjects. A rise in SGLDH activity signifies hepatic cellular necrosis due to acute viral hepatitis or due to the effect of a hepatotoxin.

**Serum glutamic oxalacetic transaminase (SGOT):** It is also a mitochondrial enzyme with greatest amounts in the liver followed by lesser amounts in heart and skeletal muscles. The activity of this enzyme in red blood cells is about 10 times the normal serum levels. Whenever there is tissue damage, the SGOT level increases due to its release from the damaged cells. Very high values are indicative of hepato-cellular damage, myocardial infarction or haemolytic anaemia.

**Serum glutamic pyruvic transaminase (SGPT):** This enzyme is present in the cytosol. Maximum concentration is present in the liver. Although the absolute amount of GPT in the liver is less than GOT, a greater proportion is present in the liver as compared to heart and skeletal muscles. Increase in the level of SGPT is, therefore, more specific for liver damage.

**Serum alkaline phosphatase (SALP):** It is a microsomal enzyme ubiquitous in distribution, largest amounts being in the liver and
gastrointestinal mucosa. The ALP is tightly bound to lipid membranes particularly those in the canalicular area. Any disorder interfering with bile flow increases the synthesis of ALP. The levels of SALP rise considerably in cholestasis and to a lesser extent in hepatocytic damage.

**Serum bilirubin (SBRN):** Bilirubin, the end product of haem, is excreted in bile. In hepatocytic lesions and in obstructive jaundice, excretion of bilirubin in the bile is hampered and, hence, its level in the blood increases. A rise in the level of SBRN is, thus, indicative of jaundice or liver damage due to some toxin.

**Hepatic triglycerides (HTG):** Triglycerides act as a store of energy and also as a means for transport of energy from the gut and the liver to peripheral tissues. Increased HTG level is indicative of disturbance in lipid metabolism arising out of liver injury induced by some toxin, or obstructive jaundice.

**Hepatic glycogen (HGN):** Glycogen - a glucopolysaccharide, is present exclusively in liver. Its levels in the liver have been reported to decrease in animals treated with CCl₄ (63-64), PcmL (65) or GalN (66). A decrease in HGN levels is, thus, indicative of liver damage.
1.4 NATURAL PRODUCTS AND PLANTS REPUTED TO HAVE ANTIHEPATOTOXIC ACTIVITY

In the modern system of medicine the major forms of treatment for liver diseases are still considered to be corticosteroids and immuno-suppressive agents. But these drugs have inherent limitation of providing only symptomatic relief without actually curing the disease process. Therefore, there exists a need for new prototypes or new templates for use in the design of potential hepatoprotective agents. Natural products are providing such templates. About 160 phytoconstituents isolated from about 101 plants belonging to 52 families have been reported to exert liver protective activity (67). Two such natural products - silymarin, a mixture of three isomeric flavolignans silybin (1), silydianin (2) and silychristin (3) isolated from Silybum marianum (Compositae), and (+)-cyanidanol-3 (4), a catechin isolated from Uncaria gambier (Rubiaceae), are worthmentioning. Their high degree of hepatoprotective efficacy coupled with low toxicity as shown by a number of laboratory experiments have enabled these compounds to be used in clinical practice in Europe (8).

A number of reviews on plant choleretics and cholagogues (68-71), and antihepatotoxic natural products (8,72-75) have appeared. A comprehensive survey of hepatoprotective plants and plant products has been published by Handa et al. (67). After the appearance of these reviews there has been more published work on natural products
and plants exhibiting hepatoprotective effect, and these along with those not covered in earlier reviews have been reviewed below.

1.4.1 Terpenoids

(--)-Menthol (5), the major constituent of essential oil of Mentha arvensis (Labiatae), has been shown to increase markedly the secretion of bile in rats (76). It was observed that acetylation of menthol decreases the cholagogic efficacy of menthol suggesting that the hydroxyl group plays an important role in the cholagogic activity.

Atractylon (6), the chief sesquiterpene principle present in the rhizomes of Atractylodes macrocephala and A. lancea (Compositae), when used at a dose of 1mg/ml, was found to exhibit significant lowering of CCl₄⁻ and GalN-induced elevation of GPT in primary cultured rat hepatocytes (77). In vitro studies using rat microsomes reveal that the free radicals formed from atractylon inhibit CCl₄⁻-induced lipid peroxidation by scavenging •CCl₃ (78).

Costunolide (7), a sesquiterpene, has been found responsible for cholagogue activity of the acetone extract (500mg/kg, intraduodenally) of roots of Saussurea lappa (Compositae) (79,80). Costunolide, when administered intraduodenally at a dose of 100mg/kg, increased bile secretion by 130% as compared to rats receiving sodium dehydrocholate as the standard cholagogue.
Methanolic extract of rhizomes of *Cimicifuga foetida* (Ranunculaceae) was found to be effective in preventing CCl₄-induced hepatotoxicity in mice as confirmed by biochemical and histological observations (81). Antihepatotoxic activity of the extract has been attributed to a cycloartane type of triterpenoid, cimigenol xyloside (8), present in the extract.

Antihepatotoxic activity of triterpenoids - alisol A monoacetate (9), alisol B (10), alisol B monoacetate (11) and alisol C monoacetate (12) isolated from *Alismatis rhizoma* (Alismataceae), a herbal drug used frequently in oriental prescriptions, has been evaluated against CCl₄-induced liver damage in mice (82). The results obtained from liver microsomal enzyme assay, measurement of SGPT, serum triglycerides and hexobarbital-induced sleep time showed that alisol A monoacetate, alisol B and alisol B monoacetate each at a dose of 60mg/kg, i.p., had significant liver protective activity against CCl₄ intoxication. Alisol B monoacetate showed slightly higher activity than alisol B.

Ursolic acid (13), a triterpenoid isolated from *Sambucus chinensis* (Caprifoliaceae), has been reported to reduce SGPT and hepatic triglyceride levels in rats/mice intoxicated with CCl₄ (83, 84).

Effects of triterpenoid saponins isolated from Chinese ginseng, *Panax ginseng* (Araliaceae), on GPT level in primary cultured rat hepatocytes treated with CCl₄ or GalN have been reported (85).
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10 \( R = H \)
11 \( R = Ac \)
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Significant protective effects were observed with 20(S)-ginsenoside-Rh₂, 20(R)-ginsenoside-Rg₁, and prosapogenin of ginsenoside-Ro, 20(R)- and 20(S)-ginsenoside-Rs in CCl₄-induced cytotoxicity. In general, it was observed that the ginsenosides of 20(R) series were more potent than the 20(S) series. The ginsenosides having less sugar moieties were more active than those having more number of sugar moieties. In GalN-induced hepatocellular damage, only 20(S)-ginsenoside-Rh₁ and prosapogenin of 20(S)-ginsenoside-Rs were effective in lowering the level of GPT.

1.4.2 Iridoid Glycosides

Different extracts of Picrorrhiza kurroa (Scrophulariaceae), popularly known in India as 'Kutaki', have shown marked protective effect against CCl₄-induced hepatic lesions in rats as evidenced by gross pathological investigations of the liver, SGOT and SGPT estimations, body weight records and liver glycogen values (86). Alcoholic extract of Kutaki, 40mg/kg,i.m., was found to increase the biliary output in dogs with experimental fistulas (87). Iridoid glycosides - picroside I (14) and picroside II (15), isolated from P. kurroa have been reported to exert a protective effect in mice intoxicated with CCl₄, and choleric effect in rats (88). Pretreatment with alcoholic extract (20mg/kg,p.o., for 15 days) prepared from the roots and rhizomes of P. kurroa exhibited hepatoprotective effect against CCl₄-induced liver damage in rats, and Plasmodium berghei induced liver damage in mastomys (89).
Hepatoprotective activity of the plant has been attributed to kutkin which is a mixture of iridoid glycosides - picroside I and kutkoside. Pretreatment with kutkin (6mg/kg, p.o., for 7 days) was found to exert protective effect against GalN-induced liver lesions in rats but kutkin-free-alcoholic extract (50mg/kg, p.o., for 7 days) was found to be devoid of hepatoprotective activity against GalN-induced hepatic injury.

Pretreatment of rats with ethanolic extract of the leaves of *Aucuba japonica* (Cornaceae) in doses of 600mg/kg/day, p.o., for 2 days, protected against CCl₄-induced depression in disappearance from plasma, and biliary excretion of bromosulfophthalein determined 24h after CCl₄ challenge to rats. Ethanolic extract also protected the rats against CCl₄-induced elevation in SGPT activity, and liver necrosis. Hepatoprotective activity of the extract has been attributed to an iridoid glycoside, aucubin which, at a dose of 340 mg/kg/day, p.o., for 4 days, showed protection against CCl₄-induced hepatic injury in mice as evidenced by lowering of CCl₄-induced increase in SGOT and SGPT activities. Further, it was observed that aucubin also reduced CCl₄-induced increase in hexobarbital hypnosis in mice, and inhibited hepatic RNA and protein synthesis. LD₅₀ of aucubin was found to be more than 900mg/kg, i.p., in mice.

An iridoid glucoside, geniposide, the major active principle isolated from the fruits of *Gardenia jasminoides* (Rubiaceae), was
found to induce a delayed but prolonged choleretic action following intraduodenal administration to rats (93, 94). The aglycone, genipin (19), was shown to be more active than geniposide. A choleretic geniposidic acid aglycone (20) has also been identified in *G. jasminoides* (95).

### 1.4.3 Flavonoids

A review on antihepatotoxic activity and structure activity relationships of naturally occurring flavonoids has been published (96). Fifty five flavonoids including (+)-cyanidanol, three ethers and esters of (+)-cyanidanol, flavanones, flavones and flavanols have been tested for their ability to interfere with CCl₄-induced release of GOT in primary cultured rat hepatocytes (97). It was observed that the CCl₄-induced hepatocyte toxicity was inhibited by hydrophilic flavonoids, and potentiated by lipophilic flavonoids.

Kolaviron, a fraction containing biflavones obtained from the defatted methanolic extract of seeds of *Garcinia kola* (Guttiferae), has been reported to antagonize significantly the lethal poisoning of mice with phalloidin (98). The bioactivity of kolaviron has been attributed to the biflavonoid garcinikolin (21) which has been shown to stimulate RNA synthesis in rat hepatocyte suspension at a concentration of 2μg/ml or more (99). Furthermore, administration of garcinikolin (100mg/kg, p.o, twice daily) to patients with chronic hepatitis was
14 \( R^1 = H, R^2 = \text{Cinnamoyl} \)
15 \( R^1 = H, R^2 = \text{Vanilloyl} \)
16 \( R^1 = \text{Vanilloyl}, R^2 = H \)

18 \( R^1 = \text{Me}, R^2 = O-\beta-D-\text{Glu} \)
19 \( R^1 = \text{Me}, R^2 = OH \)
20 \( R^1 = H, R^2 = \text{Me} \)
found to decrease the abnormally elevated SGPT level.

Bioactivity directed extraction and fractionation of the butanol soluble fraction of the methanolic extract of the flowers of *Butomos monosperma* (*Fabaceae*) resulted in the isolation of a chalcone - isobutrin (22), and a flavanone - butrin (23) (100). While a dose dependent antihapatotoxic activity was exhibited by isobutrin against both CCl₄- and GalN-cytotoxicity, butrin was active against GalN-cytotoxicity only. Activity of butrin was found to be half of that exhibited by isobutrin. Further, isobutrin (20µg/ml) was reported to enhance the rate of RNA synthesis in vitro by 78% compared to the control as evidenced by time dependent increase in the incorporation of radioactive ³H-UTP into the RNA in rat liver nuclei suggesting that isobutrin has a stimulatory effect on protein biosynthesis by the liver cells. The activity of butrin was 63% above control level.

Treatment of mice with the ethyl acetate extract (20mg/kg,i.v.) of the aerial parts of *Baccharis trinera* (*Compositae*) 1h before intoxication with phalloidin increased the survival rate of mice from 25% to 100% (101). The hepatoprotective activity of the extract was attributed to five flavonoids viz., quercetin (24), luteolin (25), eupatolin (26), apigenin (27) and hispidulin (28) present in the extract. Hispidulin (32mg/kg,i.v.), ameliorating survival rate of phalloidin-treated mice to 80%, was the most active of the five flavonoids.
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24 \( R^1 = \text{OH}, R^2 = \text{OH}, R^3 = \text{H} \)
25 \( R^1 = \text{OH}, R^2 = \text{H}, R^3 = \text{H} \)
26 \( R^1 = \text{OH}, R^2 = \text{H}, R^3 = \text{OMe} \)
27 \( R^1 = \text{H}, R^2 = \text{H}, R^3 = \text{H} \)
28 \( R^1 = \text{H}, R^2 = \text{H}, R^3 = \text{OMe} \)
1.4.4 Miscellaneous

Essential oil and pungent principles present in the rhizomes of *Zingiber officinale* (Zingiberaceae) have been reported to increase the secretion of bile in rats (102). The cholagogic activity of *Z. officinale* has been attributed to 6-gingerol (29) and 7-gingerol (30). *In vitro* studies using primary cultured rat hepatocytes have shown that 6-gingerol and 7-gingerol significantly inhibit the CCl₄- and GalN-induced increase in the GPT activity (103).

The therapeutic effect of orally administered extract of *Allium sativum* (Liliaceae) on CCl₄ intoxicated mice has been investigated at doses of 10, 100 or 500mg/kg 6h after CCl₄ administration (104). The extract was found to lower lipid peroxides, alter peroxidative status to more reductive condition, and inhibit the hepatic triglyceride accumulation in the liver. Volatile oil obtained from the steam distillation of homogenized bulbs of *A. sativum*, and alliin (31), S-allylmercaptocysteine (32) and S-methylmercaptocysteine (33) obtained from the non volatile residue were subjected to *in vitro* and *in vivo* determination of antihepatotoxic activity (105). Marked GPT inhibitory activity was observed with the volatile oil, S-allylmercaptocysteine and S-methylmercaptocysteine in CCl₄- and GalN-induced cytotoxicity in primary cultured rat hepatocytes. Pretreatment of rats for three days with alliin at a dose of 5mg/kg/day showed marked inhibition of SGOT and SGPT in GalN-induced liver damage. The volatile oil inhibited formation of free radicals from CCl₄, and lipid peroxidation
by rat liver microsomes indicating that antioxidative activity of the volatile oil is responsible for protection against CCl\textsubscript{4}-evoked liver damage.

Treatment of rats with three consecutive doses (500mg/kg, p.o.) of the ethanolic extract of the leaves of \textit{Cynara scolymus} (Leguminosae) 48h, 24h and 1h before CCl\textsubscript{4} administration was shown to cause significant decrease in SGOT, SGPT and serum glutathione levels (106). The activity has been attributed to 1,5-dicaffeoylquinic acid (34) which prevented CCl\textsubscript{4}-induced elevation of GOT and GPT levels in primary cultured rat hepatocytes (107).

Colchicine (35), a well known alkaloid of \textit{Colchicum} sp. (Liliaceae), has been reported to inhibit CCl\textsubscript{4}-induced elevation in SGOT, SGPT, SALP, serum cholyglycine, and prevent the formation of collagen fibres associated with CCl\textsubscript{4}-induced hepatotoxicity (108-110).

Effects of stilbene components piceid (36), 2-O-D-glucoside of 2,3,5,4'-tetrahydroxystilbene (37) and resveratrol (38), obtained from the roots of \textit{Polygonum cuspidatum} and \textit{P. multiflorum} (Polygonaceae) have been studied in rats fed with peroxidised corn oil at a dose of 10ml/kg/day, p.o., for two weeks (111). Piceid and 2-O-D-glucoside of 2,3,5,4'-tetrahydroxystilbene, each at a dose of 50 or 100mg/kg, p.o., for two weeks, were found to partly inhibit the deposition of lipid peroxides in the liver of intoxicated rats, and lower the levels of
29 \[ R = (\text{CH}_2)_4 \text{CH}_3 \]
30 \[ R = (\text{CH}_2)_5 \text{CH}_3 \]

32 \[ R = \text{CH}_2 \rightarrow \text{CH} \rightarrow \text{CH}_2 \]
33 \[ R = \text{Me} \]

36 \[ R^1 = \text{D-Glu}, \quad R^2 = \text{H} \]
37 \[ R^1 = \text{H}, \quad R^2 = \text{O-D-Glu} \]
38 \[ R = R^2 = \text{H} \]
SCOT and SGPT. Furthermore, all the three stilbene derivatives were reported to inhibit lipid peroxidation induced by ADP and NADPH in rat liver microsomes.

The butanol soluble and the ether soluble fractions of the methanolic extract of the bark of *Acer nikoense* (Aceraceae) have been reported to lower SGOT and SGPT levels in rats intoxicated with CCl₄ (112). A phenolic compound – rhododendrol (39), has been isolated from the ether soluble fraction and shown to be responsible for the hepatoprotective activity.

Hepatoprotective effect of the ethanolic extract of the leaves of *Withania somnifera* (Solanaceae) has been compared with that of hydrocortisone in CCl₄ intoxicated rats (113). The protective effect of the extract at 1g/kg, p.o., was found to be comparable to 10mg/kg, p.o., of the hydrocortisone. 3-β-Hydroxy-2,3-dihydrowithanolide F (40) obtained from the fruits of *W. coagulans* when used at a dose of 10mg/kg, i.p., showed protection against CCl₄-induced liver injury in rats as indicated by the histopathological examination of the liver slices, and significant reduction in phenobarbitone induced sleep time, SGOT and SGPT levels (114).

*Phyllanthus niruri* (Euphorbiaceae) has been reported to be an effective drug in the treatment of infective hepatitis in children (115). Pretreatment with *P. niruri* for one month was found to provide
Injury (116). Ethanolic extract of the roots (1g/kg/day, p.o., for 30 days) and leaves (3g/kg/day, p.o., for 30 days) was found to afford protection against ethanol (30ml/kg/day, p.o., of 28.5% ethanol for 30 days) induced increase in SGOT, SGPT and SALP levels in rats (117). In vitro experiments have established antihapatitis B antigen (antiHBsAg) activity of P. niruri (118). In vivo studies using mice and in vitro studies using Vero cell-line culture have been conducted to evaluate the safety of P. niruri (119). In acute and chronic toxicity experiments no mortality, weight loss or behavioural changes could be observed. Tissue culture studies on Vero cell-line did not indicate any cytotoxicity of the herb.

Phyllanthin (41), hydropylianthin (42) and triacontanal (43) isolated from the hexane extract of the aerial parts of the plant were investigated for hepatoprotective efficacy using in vitro model (120). Phyllanthin and hydropylianthin were found to afford protection against CCl₄- and GalN-induced cytotoxicity in primary cultured rat hepatocytes as evidenced by lowering of GPT levels elevated by the toxins. Triacontanal was found to exert protective action only against GalN-induced cytotoxicity.

Aqueous extract, acetone-water extract (both in doses of 2 x 300mg/kg/day, p.o., for 5 days) and the tannin fraction named as geraniin (2 x 100mg/kg/day, p.o., for 5 days) from the leaves of Geranium thunbergii (Geraniaceae) showed hepatoprotective effect in rats intoxicated with peroxidised corn oil (2 x 10ml/kg/day, p.o.,
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\text{CH}_3 \text{-(CH}_2\text{)}_{28} \text{-CHO}
\]
Geraniin was most effective showing significant inhibition of the elevated total cholesterol, lipid peroxides, free fatty acids, triglycerides, GOT and GPT in the serum.

*In vitro* antihepatotoxic activity of forty six tannin analogues has been examined using primary cultured rat hepatocytes treated with CCl₄ or GalN (122). Most of the tannins were found to exert significant antihepatotoxic effect with hydrolyzable tannins exhibiting a better inhibitory action on GPT than the condensed tannins.

*Halenia elliptica* (Gentianaceae) extract, and a xanthone isolated from this plant have been reported to increase the hepatic glycogen and DNA levels in the rats intoxicated with CCl₄ indicating them to be effective in stimulating protein synthesis depressed by CCl₄ (123). LD₅₀ of the extract has been reported to be 21.4g/kg, i.p., in rats.

Fatty acids isolated from *Quercus rubra* (Fagaceae) were found to exhibit significant cholagogic activity in guinea pigs (124).

Administration of prodigiosan, a polysaccharide isolated from *Serratia marcescens*, to rats 24h prior to CCl₄ challenge was found to significantly elevate CCl₄-induced lowering of hepatic glycogen level (125).
1.4.5 Plant Extracts

The essential oil of the rhizomes of *Curcuma xanthorrhiza* (Zingiberaceae) is reported to exert cholagogic action in rats (126).

Aqueous extracts of *Artemisia annua*, *A. apiacea*, *A. capillaris*, *A. feddei*, *A. japonica*, *A. keiskeana*, *A. princeps*, *A. stelleriana* and *A. stolonifera* of the family Compositae have been reported to increase bile flow 30 minutes after their administration to rats (127). Post-treatment of rats with a preparation of *A. messerschimidia*na (0.6ml/kg/day, i.p., for 3 days) has been found to afford protection against hepatotoxic effects of CCl₄ as evidenced by histopathological examination of the rat liver (128).

Infusion and aqueous decoction of *Betula celtiberica* (Betulaceae) has been found to exhibit marked choleretic activity in rats (129).

Aqueous extract of *Cuscuta chinensis* (Convolvulaceae) has been reported to protect rats against CCl₄-induced liver injury (130).

Extracts of aerial parts of *Odontites vulgaris* (Scrophulariaceae) have been shown to induce microsomal oxidative system and stimulate regenerative processes of the liver (131).

Methanolic extract of *Pseudostellaria palibiana* has
been demonstrated to exert protective effect against CCl₄-induced liver toxicity in rats (132).

Clinical studies have revealed that Rheum officinale (Polygonaceae) enema reduced the death rate of patients suffering from severe hepatitis (133).

Methanolic extracts of Hepatica asiatica (Ranunculaceae) and Stellaria media (Caryophyllaceae) have been shown to possess antihepatotoxic activity against CCl₄ toxicity in rats (134).

Aqueous extract of Curcuma aromatica (Zingiberaceae), Cyperus rotundus (Cyperaceae), Dolichos lablab (Leguminosae), Astragalus sp. (Leguminosae) and Bupleurum sp. (Umbelliferae) when administered to patients with chronic hepatitis was found to improve the functions of central nervous system, blood viscosity and blood circulation indicating improvement in liver function (135).

Administration of the ethanolic extracts (200mg/kg,i.p.) of Anisotes trisulcus (Acanthaceae) and Crepis ruepellii (Compositae) 30 minutes before CCl₄ challenge (0.3ml/kg,i.p.) to mice showed significant lowering in the levels of plasma GPT and bilirubin (136).

Screening of 129 folklore plants for antihepatotoxic activity using CCl₄- and GalN-induced cytotoxicity in primary cultured rat
hepatocytes has revealed that the ethanolic extract of *Ampelopsis brevipedunculata* (Vitaceae), *Loranthus parasiticus* (Loranthaceae), *Ludwigia octovalvis* (Onagraceae), *Mallotus repandus* (Euphorbiaceae), *Onychium japonicum* (Pteridaceae), *Phyllanthus urinaria* (Euphorbiaceae), *Tamarix chinensis* (Tamaricaceae) and *Wedelia chinense* (Compositae) caused more than 50% inhibition of GPT in both the models of hepatocytotoxicity (137).

1.5 SELECTION OF PLANTS FOR THE PRESENT INVESTIGATION

The survey of Indian market has revealed that about 40 patent polyherbal formulations are available in the country for the treatment of liver ailments. These preparations representing a variety of combination of 93 indigenous medicinal plants belonging to 44 families have been listed in the appendix. Sporadic information on the scientific investigations on some of the commercial herbal formulations like Arogyawardhani®, B-Liv® (141), Liv. 52® (142-155), Livercure® (156), Livol® (157,158) and Tefroll® (159-168) is on the records. Available commercial preparations were screened for antihapatotoxic activity with a view to make an assessment of their hepatoprotective activity. Four plants - *Andrographis paniculata*, *Eclipta alba*, *Plumbago zeylanica* and *Tephrosia purpurea* which are frequently used in the formulation of hepatoprotective commercial herbal preparations, have been selected for bioactivity directed extraction and fractionation with a view to investigate their hepatoprotective activity. A critical analysis of the appendix
reveals that *A. paniculata* is the most frequently used plant being incorporated in 26 of the 40 formulations. Next in the order of frequency of occurrence in the formulations are *F. alba* (seventeen), *P. zoylanica* (seven) and *T. purpurea* (six).

1.6 LITERATURE REVIEW ON THE PLANTS SELECTED FOR THE INVESTIGATION

1.6.1 *Andrographis paniculata* Nees

*Andrographis paniculata* (Acanthaceae) is an erect annual herb found wild in West Bengal and North-Eastern India. The plant, popularly known in the indigenous system of medicine as 'Kalmegh', possesses very bitter taste and has been used by traditional healers for the relief of general debility, dysentery, dyspepsia and for liver disorders (169).

The macro- and microscopic characters of the stem, the leaf and the floral parts of *A. paniculata* have been described in detail (170,171). A review (172) on the constituents of *A. paniculata* reveals that it contains a bitter diterpenoid lactone, andrographolide (44), as its major constituent. The structure and configuration of andrographolide has been established by chemical, spectral and X-ray crystallographic means (173-198). Andrographolide is stable in acidic pH but hydrolyses rapidly in alkaline pH (199). Minor diterpenoids isolated from the aerial parts include neoandrographolide (45) (200-202), 14-deoxy-11-
oxoandrographolide (46), 14-deoxy-11,12-didehydroandrographolide (47) and 14-deoxyandrographolide (48) (203), 3,14-dideoxyandrographolide (49), 14-deoxyandrographiside (50) and andrographiside (51) (204-206)
andrographanin (52) and 14-deoxy-12-methoxyandrographolide (53) (198). Three sesquiterpenoids - paniculide A (54), paniculide B (55) and paniculide C (56), have been isolated from the callus cultures derived from hypocotyl and stem tissue of the plant (207). Evidence has been generated to show the biogenesis of these sesquiterpenoids in the callus cultures (208-216). Seven unidentified bitter principles have been also reported from the aerial parts of A. paniculata (217-220).
Flavonoids isolated from the roots include andrographanin (57), panicolin (58), 5-hydroxy-7,8,2',3'-tetramethoxyflavone (59) and apigenin 7,4-dimethylether (60) (221-223). 5-Hydroxy-7,8-dimethoxyflavone (61) along with andrographanin (57) and panicolin (58) have been identified in the differentiating callus cultures of the roots of A. paniculata (224). Two flavanones - 5-hydroxy-7,8-dimethoxyflavanone (62) and 5-hydroxy-3,7,8,2'-tetramethoxyflavanone (63), have been isolated from the roots (225). A flavanone glucoside - andrographidin A (64), and five flavone glucosides - andrographidin B (65), andrographidin C (66), andrographidin D (67), andrographidin E (68) and andrographidin F (69) have been isolated from the roots of A. paniculata (226). Phenolic compounds reported from the herb are caffeic acid, chlorogenic acid, a mixture of dicaffeoylquinic acids (227), carvacrol and eugenol (228). β-Sitosterol-D-glucoside (218) and α-sitosterol (223) have been isolated from the ethanolic extract of the leaves. Physicochemical characteristics
45

R = H, R\textsubscript{2} = OH

50

R = O\textsubscript{H}, R\textsubscript{2} = O–D–Glu

52

R\textsubscript{1} = R\textsubscript{2} = H

53

R\textsubscript{1} = O\textsubscript{H}, R\textsubscript{2} = O Me

54

R\textsubscript{1} = \alpha–H, R\textsubscript{2} = \beta–OH, R\textsubscript{3} = H

55

R\textsubscript{1} = \alpha–H, R\textsubscript{2} = \beta–OH, R\textsubscript{3} = OH

56

R\textsubscript{1}, R\textsubscript{2} = O, R\textsubscript{3} = OH
57  $R_1^1 = H, R_2^2 = H, R_3^3 = OMe, R_4^4 = OMe$
58  $R_1^1 = H, R_2^2 = H, R_3^3 = OH, R_4^4 = OMe$
59  $R_1^1 = H, R_2^2 = OMe, R_3^3 = OMe, R_4^4 = OMe$
60  $R_1^1 = OMe, R_2^2 = H, R_3^3 = H, R_4^4 = H$
61  $R_1^1 = H, R_2^2 = H, R_3^3 = H, R_4^4 = OMe$
62  $R_1^1 = R_2^2 = H$
63  $R_1^1 = R_2^2 = OMe$
64  
65  $R_1^1 = H, R_2^2 = O - Glu, R_3^3 = OH, R_4^4 = H$
66  $R_1^1 = H, R_2^2 = H, R_3^3 = H, R_4^4 = Glu$
67  $R_1^1 = H, R_2^2 = OMe, R_3^3 = OMe, R_4^4 = Glu$
68  $R_1^1 = H, R_2^2 = H, R_3^3 = OMe, R_4^4 = Glu$
69  $R_1^1 = OMe, R_2^2 = OMe, R_3^3 = OMe, R_4^4 = Glu$
and composition of the seed-oil have been reported (229).

For the estimation of andrographolide in *A. paniculata* both gravimetric and titrimetric methods have been reported (230-233). A quantitative method has been developed for estimating andrographolide in the liquid extracts of the plant by thin layer chromatography (234). Spectrophotometric methods for assaying andrographolide in the herb have also been reported (235,236).

Effect of the aqueous extract of *A. paniculata* (each 5ml containing 1ml of Kalmegh I.P. 1966) at a dose of 3.75ml/kg, p.o., on the biliary flow, liver weight and hexobarbitone sleep time has been investigated and compared with that of phenobarbitone (75mg/kg, i.p.) (237). The extract was found to increase biliary flow and liver weight in rats and decrease the duration of hexobarbitone induced sleep in mice. At the dose employed in the investigations, the extract proved to be less potent than phenobarbitone. Administration of single dose of the aqueous extract of *A. paniculata* leaves (500mg/kg, p.o.) or andrographolide (5mg/kg, p.o.) 4h before intoxicating the rats with CCl$_4$ (5ml/kg, p.o.) has been shown to decrease CCl$_4$-induced hepatic microsomal lipid peroxidation (238). However, long term administration (15 consecutive days) of the extract or the andrographolide did not decrease CCl$_4$-induced hepatic microsomal lipid peroxidation. Further, the aqueous extract (500mg/kg or 1g/kg, p.o.) or andrographolide (5mg/kg or 10mg/kg, p.o.) has been found to characteristically inhibit hepatic
microsomal aniline hydroxylase, N-demethylase and O-demethylase when administered in single dose (239). Repeated administration of these test substances for 7 — 30 consecutive days, on the other hand, produces induction of all the three enzymes. Administration of an aqueous decoction (3 x 20ml/day; equivalent to 40g of the crude drug per day) of A. paniculata for 24 days to 20 patients with infective hepatitis has been reported to provide symptomatic relief to the patients (240).

Alcoholic extract of A. paniculata has been reported to possess antifungal activity (228). Water soluble fraction (4g/kg,i.p.) of the alcoholic extract administered 30 minutes before intraperitoneal injection of cobra-venom (320μg/kg) has been found to increase the survival period of mice (241). An unidentified isolate from the plant has been reported to exert positive ionotropic effect on the frog heart (242). Ethylacetate soluble fraction from the alcoholic extract of A. paniculata has been reported to possess nematicidal activity against soil nematodes (236). Aqueous extract (100mg/kg,p.o.) has been found to exert antiinflammatory activity against carrageenan induced oedema in rats (243). A. paniculata has been found to be devoid of hypoglycemic activity (244).

1.6.2 Eclipta alba Hassk.

Eclipta alba (Compositae) is an erect or prostrate, much branched herb with white flower-heads and is found in almost all
parts of India, particularly in cool and moist places. It is commonly known as 'Safed Bhangra' when in flower and 'Kala Bhangra' when in fruits (245). The plant has been employed in the traditional system of medicine as cholagogue, alterative, emetic and purgative, and the juice of the leaves is reported to be hepatotonic and deobstruent (246).

Macro- and micromorphological studies of all parts of *E. alba* have been done with a view to identify the whole plant and the powdered plant (247-249).

Coumestan derivatives - wedelolactone (70), desmethylwedelolactone (71) and desmethylwedelolactone-7-O-glucoside (72), have been isolated from the fresh leaves of *E. alba* (250-255). Synthesis of wedelolactone has been reported (256), and a method for its quantitative estimation in the plant using reverse phase HPLC has been developed (257). Two thiophene derivatives - (73) and (74), and a polyacetylene - (75) (258), α-terthienylmethanol (76) (259,260), 2-formyl-α-terthienyl (77) (251) and dithienyldiisovalerate (78) (261) have been identified in the petroleum ether extract of the dried leaves of *E. alba*. Other minor constituents in the plant include luteolin-7-O-glucoside, β-amyrin, β-stigmasterol and β-sitosterol (254,255), hentriacontanol, 14-heptacosanol (262), nicotine (263) and fourteen amino acids (264).

LD<sub>50</sub> of the juice expressed from the fresh leaves of *E. alba* has
\[ \text{R} = \text{Me} \]
\[ \text{R} = \text{H} \]
\[ \text{R} = \text{Glu} \]

\[ \text{R} = \text{CH}_2\text{OH} \]
\[ \text{R} = \text{CHO} \]
been reported to be 16ml/kg, p.o., in mice (265). The juice (1ml/rat, p.o.) when administered 3h prior to the administration of allyl alcohol (4ml of 0.8% w/v dilution/kg, p.o.) was found to decrease rat liver necrosis. It was further observed that the juice when administered to rats in daily dose of 0.4ml/rat, p.o., for a period of 3 months along with \( \text{CCl}_4 \) (0.2ml, s.c., twice a week) was effective in maintaining normal hepatic architecture. Treatment of guinea pigs with the juice (6.6ml/kg, p.o.) 48h, 24h and 4h before administration of single dose of \( \text{CCl}_4 \) (2ml/kg, p.o.) has been reported to decrease the mortality rate, improve the hepatic architecture, and significantly lower \( \text{CCl}_4 \)-induced increase in the levels of SGOT, SGPT and SALP (266). AntiHBsAg activity has been attributed to \( \textit{E. alba} \) as evidenced by \textit{in vitro} studies (118). Experiments using primary cultured rat hepatocytes have revealed that wedelolactone and desmethy1wedelolactone exert a dose dependent protective effect against \( \text{CCl}_4 \)-, GalN- or phalloidin-induced cytotoxicity (257).

Powdered aerial parts of \( \textit{E. alba} \) have been shown to exert antiinflammatory activity in carrageenan induced paw oedema in rats (267). Using porcine-leucocyte test system, wedelolactone has been found to exhibit 5-lipoxygenase inhibitory activity with an \( \text{IC}_{50} \) of 2.5\( \mu \)M (268). Alcoholic extract of \( \textit{E. alba} \) has been reported to have antibacterial activity against \textit{Staphylococcus aureus} and \textit{Escherichia coli} (269), and antiviral activity against 'Ranikhet disease virus' (270).
1.6.3 *Plumbago zeylanica* Linn.

*Plumbago zeylanica* (Plumbaginaceae) is a perennial shrub found wild in Peninsular India and West Bengal and is cultivated throughout India. The plant is popularly known in the indigenous system of medicine as 'Chitrak', and the roots constitute the original drug known to Ayurvedic physicians (271). The root has been used as an abortifacient, vesicant, alterative, antidiarrhoeal, gastric stimulant, appetite, antihaemorrhoidal, diuretic, expectorant and antirheumatic (272).

Reviews on *P. zeylanica* covering cultivation, pharmacognosy, ethnobotany and ethnopharmacology have appeared (273-275). Macroscopic characters (276) of the plant, microscopy of the root (277,278) and detailed pharmacognosy of the leaf (279) have been described.

Roots of *P. zeylanica* contain a naphthaquinone derivative - plumbagin (79), as the major constituent (280-282). Structure of plumbagin has been established through synthesis (283,284) and X-ray crystallographic analysis (285). Apart from plumbagin, other naphthaquinone derivatives reported from the plant include 3-chloroplumbagin (80), 3,3'-biplumbagin (81) (286,287), 1,2(3)-tetrahydro-3,3'-biplumbagin (82), droserone (83), isohinanolone (84) (288), epi-isohinanolone (85) (289), chitranone (86) (290), zeylanone (87), isozeylanone (88) (291), plumbazeylanone (89) (292) and dihydrosterone (90) (293). Non-naphthaquinones isolated from *P. zeylanica* include plumbagic acid (91) (294), β-sitosterol,
vanillic acid (295), an unidentified steroidal glycoside, catechol tannin and glucose (296).

Pharmacological evidence in support of the use of *P. zeylanica* in folklore medicine as a rubefacient, vesicant, ecologic, diuretic and sudorific has been reported (297). The roots have been reported to possess antifertility (298-300), antimicrobial (301,302), antitumour (303), gastrointestinal-flora-normalizing (304), antifeedant (305) and protease- and diastase-like activities (306). Plumbagin has been found to possess antifertility (307,308), antimicrobial (309-315), tuberculostatic (316,317), antitumour (312,318,319), anticoagulant (320) and CNS stimulant (321) activities. Plumbagin has been shown to stimulate oxidation of glucose in the brain slices of guinea pigs (322), and cause selective testicular lesions in dogs (323).

1.6.4 *Tephrosia purpurea* Pers.

*Tephrosia purpurea* (Leguminosae, subfamily Papilionaceae) is a suberect perennial herb which grows throughout India, especially in Southern peninsula. In the indigenous system of medicine, it is known as 'Sarpunkha' and has been used as febrifuge, chologogue, diuretic, deobstruent, tonic and laxative (324). A decoction of the root with pepper powder is given in bilious febrile attacks, enlargement and obstruction of the liver, spleen and kidneys, and ground with butter-milk it has been used to treat hepatic dropsy (325).
Ethnobotany and pharmacognosy of *T. purpurea* have been described (326). Rotenone (92), (-)-isolonchocarpin (93), pongamol (94), lanceolatin A (95) and lanceolatin B (96) have been isolated from the roots (327,328). Four rotenoids - deguelin (97), elliptone (98), tephrosin (99) and rotenone (92) have been reported from the static and suspension cultures of the roots, the stems and the leaves of *T. purpurea* (329). A number of flavonoids including delphinidin chloride (100) and cyanidin chloride (101) (330), tephroglabrin (102) and tepurindiol (103) (331), karanjin (104), purpuritenin (105) and purpure- amithide (106) (332), (-)-purpurin (107) (333), (+)-purpurin (108), purpurenone (109), dehydroisoderricin (110) and (-)-maackiain (111) (334) have been identified in the plant. An uncharacterized alkaloid, lupeol, β-sitosterol, rutin and quercetin (335-337), proteins, amino acids and carbohydrates (338,339) have been isolated from the leaves. Purpuranin A and purpuranin B have been reported from the pods of *T. purpurea* (340). The seed oil has been found to contain five fatty acids and eighteen amino acids (341,342).

Insecticidal and insect repellent properties have been attributed to the petroleum ether soluble fraction of *T. purpurea* seeds (343). Ethanolic extract of the aerial parts of the plant has been found to possess antiprotozoal activity against *Entamoeba hystolytica* (344). Oral administration of the aqueous extract of the seeds has been found to exhibit marked lowering of blood glucose level in normal and alloxan-
100 \( R = \text{OH} \)

101 \( R = \text{H} \)

102

103

104

105

106

107 \( R^1 = \text{OAc}, R^2 = \text{H} \)

108 \( R^1 = \text{H}, R^2 = \text{OAc} \)

109

110

111
diabetic rabbits (345) but the rutin isolated from the plant was found to elevate glucose level in normal rabbits (346). Aqueous extract of the leaves has been shown to exert a dose dependent hypotensive activity in dogs (347).