1. INTRODUCTION
1.1 LIVER AND ITS FUNCTIONS

The liver, the largest gland in the body has multi-faceted functions, both exocrine (secreting bile into the duodenum) and endocrine (synthesizing a variety of substances that are released directly into the blood stream). The liver being interposed between the intestinal tract and the general circulation receives a variety of substances, both wanted and unwanted. It receives from the portal vein, all the material (except bulk of the lipid) absorbed from the intestinal tract which it metabolises and transforms for further use in the body. It may also receive toxic substances, both from the intestine and the general circulation, several of which it is capable of degrading. It synthesizes several important components of the blood plasma and exercises control over the general metabolism by virtue of its capacity to store carbohydrates as glycogen and to release glucose to maintain its normal concentration in the blood (Bloom and Fawcett, 1975).

According to Guyton (1975), the major metabolic functions of the liver include lipid, protein and carbohydrate metabolism.

Lipid metabolism includes a very high rate of beta oxidation of fatty acids and formation of acetoacetic acid, formation of lipoproteins and large quantities of cholesterol and phospholipids and conversion of large quantities of proteins and carbohydrates to fat.

Protein metabolism includes deamination of amino acids, formation of urea, formation of plasma proteins and interconversions among the different amino acids.

Carbohydrate metabolism includes glycolysis
(oxidation of glucose and glycogen to pyruvate and lactate), glycogenesis (the synthesis of glycogen from glucose),
glycogenolysis (the breakdown of glycogen to glucose) and
 gluconeogenesis (the formation of glucose or glycogen from non
 carbohydrate sources).

1.2 LIVER DISEASES

The liver is capable of degrading several of the toxic organic and inorganic substances the body is exposed to in day to day life. If, however, it fails to do so, several disorders result. These hepatic anomalies occur either due to hepatotoxins or viruses. It is on record, that haemolytic jaundice, hepatocellular jaundice, obstructive jaundice and acute parenchymal disease of the liver are caused mainly by toxic agents like carbon tetrachloride; while viral hepatitis, acute infective hepatitis, serum hepatitis and acute massive liver necrosis are caused by different viruses (Davidson and Macleod, 1975).

Liver disorders may be broadly classified into four categories; hepatitis (inflammation of the liver), hepatosis (non inflammatory disorders or regeneration of liver parenchyma), chronic hepatitis and liver cirrhosis (Wagner, 1980).

However, no strict hepatological delineation is found between the different manifestations. Recently, Sherlock (1986) has classified anomalies resulting from hepatic drug reaction into 14 categories, depending on the toxicant.

1.3 HEPATOTOXINS

The very large number of hepatotoxins makes the analysis and cure of liver diseases quite complex. Agents like α-amanitine, phalloidine, pyrrolizidine alkaloids, ethyl alcohol
and aflatoxins cause human toxicoses. Other hepatotoxins identified are chloroform, carbon tetrachloride, thioacetamide, D-galactosamine, α-naphthylisocyanate, ethionine, beryllium salts, allyl alcohol, allyl formate, alkyl nitrosamine, bentonite, dimethyl formamide, tannins, histamine, isonicotinic acid hydrazide, yellow phosphorus, sulfonamides and 6-mercaptopurine. As the mode of action of these chemicals varies greatly, it is quite difficult to apply uniform therapeutic regimen.

In most cases of hepatotoxic drug reaction, hepatocellular injury seems to be the primary event. This is rarely due to the drug itself. A toxic metabolite is usually responsible. The drug metabolising enzymes activate chemically stable drugs to produce potent alkylating, arylating or acylating agents (Sherlock, 1986).

1.3.1 Carbon Tetrachloride

1.3.1.1 Distribution

Carbon tetrachloride (CCl₄) is a hepatotoxin which is popularly used for inducing hepatic damage in test systems as it has a very wide range of effects on the liver. Moreover, human beings are exposed to this chemical in one way or the other. CCl₄ is widely distributed in many kinds of food stuffs and in the atmosphere at parts per billion levels (Cornell et al., 1975 and Fishbein, 1976). Atmospheric pollution by CCl₄ has been reported from all over the world, including Germany (Dueszeln and Thiemann, 1985), USSR (Isidorov and Zenkevich, 1985) and the USA (Olexsey, 1984, Staley et al., 1983).

Bull (1985) on analysing drinking water in the USA found that it contained CCl₄ and attributed its presence to the coal tar and paints used in the water storage and distribution systems. Similar results have been reported in N. Italy (Ziglio
et al., 1984) and Tokyo (Kotou et al., 1984). CCl₄ is also present in surface waters, even in non-industrialised areas (Agazzatti and Predieri, 1984; Fahrni, 1984). Homa (1985) found that the ground waters in Japan were also not spared by this contaminant. Although the amount of CCl₄ present in these water reservoirs are usually small, it can prove to be a serious health hazard, as it is readily absorbed through the lung, gastrointestinal tract and skin (De Rosa et al., 1984) and metabolised into more hazardous components.

1.3.1.2 Toxicity

The toxic effects of CCl₄ include its effect on the mucosal membranes, in addition to the destruction it causes in the liver. Jaundice develops within 48 hours of its administration and the liver becomes enlarged and tender. Spontaneous haemorrhage reflects profound hypoprothrombinaemia caused by it (Sherlock, 1986). Over long periods, CCl₄ induces growth retardation and renal tubular impairment (Kluwe et al., 1982). At low levels, CCl₄ slows down the cells metabolic activity by lowering ATP levels. It drastically inhibits protein and DNA synthesis, causes considerable accumulation of fat and disturbances in ion transport, which in itself results in a cascade of degenerative changes. At higher doses, CCl₄ can induce cell death by causing irreversible disorganisation that leads to necrosis (Farber and El-Mofty, 1975). Death in the acute stages of CCl₄ poisoning is due to kidney failure. If the patient survives, there are late hepatic sequelae.

Mitochondrial enzyme activity has been found increased in the blood of persons exposed to CCl₄ (Novakova et al., 1973). Lyubchenko et al. (1974) and Svabova and Mencik (1983) studied the liver functions of workers having occupational
contact with CCl₄ and observed considerably elevated levels of serum GPT and GOT. It is thus apparent that CCl₄ causes a wide range of toxic effects, especially, in the liver.

1.3.1.3 Mechanism of action of CCl₄

There are several hypothesis regarding the mechanism of action of CCl₄. Historically, the first of these is the mitochondrial hypothesis of Christie and Judah (1954), which proposes that mitochondrial degeneration caused by CCl₄ leads to toxic liver necrosis. Recknagel (1967) and Berger et al. (1986) have reported that the plasma membrane is probably responsible both directly (early injury to cellular membrane) and indirectly (functional disturbances) for CCl₄ induced damage. Still other workers have reported that both the in vitro and the in vivo irreversible binding of CCl₄ metabolites to cell constituents (DNA, proteins and lipids) maybe a contributing factor to the acute hepatotoxicity of the compound (Villarruel et al., 1975; Diaz Gomez and Castro, 1980; Ansari et al., 1986).

It has also been implicated that the mechanism of CCl₄ action entails homolytic cleavage of a carbon-chloride bond by the drug metabolising enzyme system of the endoplasmic reticulum to a reactive free metabolite CCl₃ which then triggers peroxidative breakdown of membrane-structural lipids (Recknagel and Glende, 1973; Recknagel et al., 1974; Castro and Diaz Gomez, 1976; Dianzani, 1984; Tomasi et al., 1987).

It is also known that the CCl₄ toxicant is a carbon-halogen bond cleavage, probably by one-electron reduction of CCl₄ by a particular ferrous cytochrome P450. Chloride ion and trichloromethyl radical (CCl₃) are the major initial products (Noguchi et al., 1982a, 1982b; Tjalve and Lofberg, 1983; Utsumi et al., 1985).
According to Rasmussen (1981), CCl$_4$ causes a myriad of hepatotoxic effects by grossly depressing Ca$^{2+}$ sequestration by the endoplasmic reticulum. This causes physiologically unacceptable alterations in cytosolic free calcium levels which have far reaching consequences as calcium plays a regulatory role in all aspects of intracellular motion and is involved in the regulation of intracellular movement of very low density lipoproteins and ultimate secretion of triglycerides by liver cells. A toxigenic rise in free calcium also has profound effects on lipid and carbohydrate metabolism and protein synthesis (Lowrey et al., 1981; Casini and Farber, 1981; Mandl et al., 1982; Younes et al., 1983; Recknagel, 1983; Rochelle and Moore, 1986).

1.3.1.4 Histological changes induced by CCl$_4$

It is well established that after the in vivo administration of CCl$_4$, the earliest morphological alterations appear within 15 minutes and involve the endoplasmic reticulum of hepatocytes. These changes are followed within the first hour by structural alterations in the golgi apparatus, plasma membrane and mitochondria (Rouiller, 1964). Light microscopy reveals that CCl$_4$ causes vacuolar necrosis of hepatic cells, cellular infiltration, increase of fat droplets and decrease of glycogen particles (Ichiro et al., 1973). Prendergast et al. (1967), reported morphological liver damage in dogs, monkeys, guinea pigs, rabbits and rats exposed to 10 ppm of CCl$_4$ continuously for 90 days.

In mice, CCl$_4$ causes degeneration and necrosis of hepatic cells of the central and midzonal region of the lobules. In 24 hours, cells of these two regions undergo complete necrosis and are soon invaded by inflammatory cells. Other
changes include sinusoidal congestion and hydropic and fatty degeneration (Kim et al., 1972). Bertelli et al. (1986) found that the most important lesions in the liver caused by CCl₄ are steatosis, together with focal necrosis, kupffer cell reaction and signs of phagosis and fibroblastic proliferation.

1.3.1.5 Ultrastructural changes

Scanning electron microscopic studies have revealed that 12 hours after CCl₄ administration, hepatocytes near portal tracts show normal dense cytoplasm. The intermediate zone hepatocytes are swollen and foamy with numerous vesicles which correspond to the dilated cisternae of rough endoplasmic reticulum. Besides vesicles, the foamy hepatocytes have some vacuoles which represent extremely dilated rough endoplasmic reticulum. Hepatocytes near the central vein show necrotic changes. Most membranous structures are disrupted except fat droplets and the nucleus. Nucleoplasm becomes loose and nuclear pores disappear (Itoshima et al., 1981). Perrissoud et al. (1981) observed that the plasma membrane of CCl₄ treated cells show fewer scattered microvilli.

The transmittance electron microscope reveals that CCl₄ causes bleb like formations on the surface of hepatocytes. The smooth endoplasmic reticulum migrates into these blebs. The endoplasmic reticulum looses its parallel orientation and becomes dispersed and disrupted. Ribosomes are lost from the rough endoplasmic reticulum. The diameters of endoplasmic reticulum tubules and vesicles are increased 2-3 fold when swelling is present (Berger et al., 1987). A degeneration and decrease in the number of endoplasmic reticulum has been seen by Ohnishi et al. (1974).
1.3.1.6 Hepatic circulation and CCl₄

Hepatic circulation is also effected by CCl₄ induced necrosis. The diameter of blood vessels in the liver remains the same, though velocity of erythrocytes in the veinules is significantly faster (Liang et al., 1975).

1.3.1.7 Chromosomal changes

CCl₄ has been found to cause a series of aberrations in the chromosomes of rats and mice, mainly in the form of sub chromatid breaks, gaps etc. CCl₄ causes hypodiploidy, polarisation and centromeric dysfunction (Sharma and Anand, 1984). Lans et al. (1984) reported that CCl₄ administration to rats caused liver neoplasms. Apparently, this CCl₄ induced damage is under mutagenic control by certain genes Biesel et al., 1984). It elevates the frequency of sister chromatid exchanges in human lymphocyte cells in vitro (Sobti, 1984).

1.3.2 Paracetamol

Paracetamol (acetamide, N-4-hydroxyphenyl), a widely used analgesic and antipyretic is another common hepatotoxicant. Although safe at therapeutic doses, an overdose results in marked hepatic damage and in several cases, death in both laboratory animals and man.

1.3.2.1 Toxicity of paracetamol

Paracetamol did not enter regular clinical use till the 1950's (Spooner and Harvey, 1976). Within a few years of its entry into popular use, reports of suicidal overdose leading to hepatic necrosis began to appear (Proudfoot and Wright, 1970; Rumack and Matthew, 1975; Davis et al., 1976; Rumack, 1978). The incidence of this drug being used in suicide attempts has been on the increase over the last two decades (Prescott et al., 1971;
Clark et al., 1973; Arabi and Shahid, 1982; Ortega et al., 1985; Bidault et al., 1987). Almost all cases of paracetamol induced hepatic necrosis result from single large overdose taken in suicide attempts (Hamlyn et al., 1978). Unwittingly too, chronic liver disease can occur due to smaller amounts being ingested over a long period (Bonkowsky et al., 1978; Olsson, 1978).

Multiple studies have clarified the epidemiologic (Hamlyn et al., 1978), toxicologic (Clark et al., 1973; Wright and Prescott, 1973) and clinical features (Davis et al., 1976) of paracetamol poisoning as well as histological (Dixon et al., 1971; Portmann et al., 1975) and biochemical features (James et al., 1975).

1.3.2.2 Mechanism of action of paracetamol

Paracetamol is metabolized at therapeutic doses by sulphation and glucuronidation but as the dose is increased, these pathways get saturated and a greater proportion of the drug is available for oxidation by the microsomal cytochrome P-450 system (Hinson, 1980) to yield a reactive product, probably N-acetyl-p-benzoquinoneimine (NAPQI) (Mitchell et al., 1973a; Dahlin et al., 1984). NAPQI is highly reactive and is detoxified in the liver, either by conjugation at the meta position with glutathione (GSH) or reduction to the parent compound, paracetamol, which also consumes GSH (Albano et al., 1985). After GSH depletion, NAPQI reacts with sulphydryls on cellular macromolecules, resulting in the oxidation and/or arylation of cellular components, particularly, proteins (Streeter et al., 1984). Arylation may contribute to the disruption of intracellular calcium homoeostasis (Moore et al., 1985). Depletion and/or oxidation of cellular GSH also results in lipid peroxidation and membrane damage (Fairhurst et al., 1982). Thus, paracetamol toxicity results when the rate
of production of the reactive metabolite exceeds the glutathione conjugating capacity and the free radical metabolite binds to cellular molecules (Mitchell et al., 1973b).

1.3.2.3 Histological changes induced by paracetamol

Vacuolisation along the sinusoidal margins of centrilobular cells is the earliest lesion in paracetamol poisoning. These are followed by centrilobular congestion (Walker et al., 1980). Single cell necrosis is characterised by swollen "balloon" cells. Centrilobular necrosis is widespread in the pericentral region, characterised by hydropic cells with pyknotic nuclei. The vacuoles formed occasionally communicate with the space of Disse. Hinson (1980) described a fulminating necrosis which is primarily centrilobular and may also extend through the midzone towards the peripheral area.

1.3.2.4 Ultrastructural changes induced by paracetamol

Scanning electron microscopy studies on paracetamol injured liver reveal numerous cytoplasmic lesions in centrilobular hepatocytes, the first among which are large pores. There is an endocytic vacuolisation at the lateral and sinusoidal margins of centrilobular hepatocytes, loss of microvilli, Disse space enlargement, dilation of the canaliculi and disappearance of the star-like projections from hepatocyte lateral surfaces (Walker et al., 1983).

Paracetamol damage as observed by transmittance electron microscopy reveals enlargement and pallor of mitochondria and disaggregation of polyribosomes. Plasma membrane damage also plays an important role. The necrotic areas are devoid of glycogen, while the midzonal and perportal areas have a normal glycogen content (Dixon et al., 1975a). Chiu and Bhakhthan (1978), Poulsen et al. (1981) and Walker et al. (1985) have reported a
progressive loss of the structural integrity of the endoplasmic reticulum, lipid infiltration, vacuolisation, sinusoidal congestion and myeloid figure formation.

1.3.2.5 Hepatic circulation and paracetamol

Paracetamol induced hepatic congestion results in blood and plasma volumes falling significantly, leading to hypovolemic shock and impaired circulation within the congested liver (Walker et al., 1985).

1.3.2.6 Chromosomal changes caused by paracetamol

Milam and Byard (1985) reported that paracetamol is not genotoxic towards rat primary hepatocyte cultures. Amo and Matsuyama (1985) fed mice with large amounts of paracetamol for two and a half years and found that it was not a carcinogen. Flaks and Flaks (1983) reported paracetamol to be tumorigenic in mice. Sasaki and Hiraga (1983) observed that this drug increases the mutagenic effects of UV radiation in cultured Chinese hamster ovary cells.

1.3.3 Biochemical Changes Due to CCl₄ and Paracetamol

1.3.3.1 Serum enzymes

Serum enzymes are severely effected by both the toxins under study. This is so, because the site of CCl₄ and paracetamol damage is the membrane, both internal and external. Certain membrane bound as well as cytoplasmic enzymes which are released into the blood by the action of these hepatotoxins are mentioned below.

1.3.3.1.1 Transaminases

The transaminases, Glutamate oxaloacetate transaminase (GOT) and Glutamate pyruvate transaminase (GPT) are estimated in both the in vitro (where they are analysed in the tissue culture media) and the in vivo (where they are estimated
in animal serum) systems, as a parameter for estimating liver damage and repair. The activity of both GOT and GPT has been found to rise with CCl₄ induced hepatotoxicity (Popov, 1975; Schroeder et al., 1976; Choudhari et al., 1984; Ohta et al., 1985; Nakamura et al., 1985). A similar trend is observed with paracetamol, where GOT and GPT activity has been found elevated in mice, rats, isolated rat hepatocyte cultures and in man (Buttar et al., 1976; Strubelt et al., 1978, 1981; Linscheer et al., 1980; Zimmerman, 1981; Wendel and Jarschke, 1983).

1.3.3.1.2 Lactate dehydrogenase

Serum lactate dehydrogenase (LDH) activity increases with both the hepatotoxins. An increase in this enzyme activity with CCl₄ poisoning has been observed (Gordeeva, 1973; Hayes, 1986). This trend has also been observed with paracetamol intoxication. Harman and Camish (1986) found elevated LDH levels in mice serum. LDH leakage has been observed from rat hepatocytes challenged in vitro with this analgesic (Acosta et al., 1980; Goethals et al., 1984; Milam and Byard, 1985).

1.3.3.1.3 Alkaline phosphatase

An increase in serum alkaline phosphatase (Alk Pase) has been reported (Schroeder et al., 1976; Sharma et al., 1984; Melen et al., 1985). Han et al. (1983) however, reported a drop in this enzymes activity with CCl₄ induced damage. Paracetamol treatment to mice results in elevated alkaline phosphatase activity in the serum (Banerjeee et al., 1976; Skakun and Shman'ko, 1984).

1.3.3.1.4 Sorbitol dehydrogenase

Serum sorbitol dehydrogenase activity has been found elevated with CCl₄ (Matsumoto et al., 1975; Gingold and Pasquale, 1976).
CCl₄ intake also results in increased serum glutamate dehydrogenase (Teschke et al., 1983; Adzet et al., 1986) as well as serum ornithine carbamyl transferase activity (Baumann and Bierauer, 1985).

Certain other serum enzymes, the levels of which are elevated as a reaction with paracetamol are isocitrate dehydrogenase (Devalia et al., 1982); alanine transferase (Vonen and Moerland, 1984; Skakun and Shman'ko, 1984) and aspartate dehydrogenase (Miranda et al., 1983).

1.3.3.2. Bilirubin

Bilirubin is the major pigment of bile and tends to rise in most forms of hepatitis (Gubskii, 1974; Melen et al., 1985).

1.3.3.3 Lipids

Lipid quantities and composition are also altered significantly by both CCl₄ and paracetamol. CCl₄ produces fatty degeneration in the liver due to an impairment of protein synthesis with subsequent failure of lipoprotein formation and secretion (Robinson and Seakins, 1962). The cytotoxic specificity of CCl₄ has been related to its properties as a lipid solvent (Reynolds, 1963). Bartsch and Gerber (1975) reported that CCl₄ causes an increase in the concentrations of both total and neutral lipids, while total phospholipids decrease in the liver.

The phospholipids of major importance in the liver include lysophosphatidyl choline, cardiolipin, phosphatidyl ethanolamine, phosphatidyl choline, sphingomyelin, phosphatidyl serine, phosphatidic acid and phosphatidyl inositol. Hepatic phospholipids are altered both quantitatively and qualitatively with CCl₄. Total phospholipid levels get depressed (Coleman, 1973; Lamb and Schwertz, 1982; Horiuchi and Shigenu, 1984; Gebhart and Brabec, 1985). Plasma phospholipids also decrease with CCl₄.
CCl₄ causes a decrease of hepatic phosphatidyl ethanolamine and phosphatidyl choline as well as inhibition of the conversion of the former to the latter (Shuji et al., 1975; Villarruel et al., 1987). Bennedetti et al. (1974) had, however, reported an increase in phosphatidyl ethanolamine. Vengerovskii et al. (1987) also observed increases in quantities of lysophosphatidyl choline and cardiolipin and no changes in phosphatidyl inositol, sphingomyelin and phosphatidyl serine with CCl₄. Almatov et al. (1986) found that in hepatitis, phosphatidyl inositol, cardiolipin, sphingomyelin and lysophosphatidyl ethanolamine decrease in the mitochondria of liver cells while phosphatidyl ethanolamine, lysophosphatidyl choline, lysophosphatidic acid and lysocardiolipins increase.

An elevation in hepatic total cholesterol levels with CCl₄ damage has been observed (Bartsch and Gerber, 1975; Choudhary et al., 1984). Rehman et al. (1983) observed increases in free cholesterol while Bonora et al. (1976) found enhancement of cholesterol esters with CCl₄ damage. There is a significant decrease in serum cholesterol levels (Wakasugi et al., 1985) and also in plasma cholesterol (Chandrasekharan and Juggi, 1975). CCl₄ toxicity increases hepatic triglycerides (Akahori, 1975; Harisch and Meyer, 1985; Adzet et al., 1986).

The free fatty acids decrease in the liver with the onset of CCl₄ poisoning (Valcazer et al., 1980 and Vengerovskii et al., 1987). CCl₄ causes fatty accumulation in the liver within the first hour of poisoning (Schotz and Recknagel, 1960). Rao and Recknagel (1968) measured elevated levels of conjugated dienes in lipids isolated from liver microsomes within minutes after CCl₄ poisoning. They found that fatty acids radicalised by the free
radical \( \text{CCI}_3 \) attack can in turn undergo polymerisation reaction with a second \( \text{CCI}_3 \).

1.3.3.4 Lipoproteins

Lipids are mainly carried from the liver in combination as lipoproteins (Seakins and Robinson, 1963). Any alteration in liver physiology will change this transport. There are three major classes of lipoproteins. The very low density lipoproteins (VLDL), the low density lipoproteins (LDL) and the high density lipoproteins (HDL). VLDL are decreased in the serum with \( \text{CCI}_4 \) intoxication (Lombardi and Ugazio, 1965; Marinari et al., 1985; Becker et al., 1987). A decrease in the concentration of plasma HDL and LDL has also been reported (Comporti and Benedetti, 1973; Cottalasso et al., 1984, Konevalova and Chirkin, 1987).

Paracetamol overdosage too results in an increase in the hepatic total lipids (Dixon et al., 1974) and especially the triglycerides (Buttar et al., 1976). Fat accumulation in the liver has been noted by Rudd et al. (1981). Wendel et al. (1984) and Skakun and Shman’ko (1984) have found increased lipid peroxidation in the rat liver, while Lisa et al. (1985) have reported no change in hepatic levels of lipid peroxidation products in isolated hamster hepatocytes. Banerjee et al. (1976) observed increase in serum cholesterol on paracetamol damage. Phospholipids are also changed with a significant increase in lecithin (Lohmann et al., 1984). Chiu and Bhakthan (1978) have reported that the abnormal phospholipid metabolism is manifested at the fine structural level by myeloid body formation.

1.3.3.5 Lipid enzymes

1.3.3.5.1 Malate dehydrogenase

Malate dehydrogenase (MDH) is a lipogenic enzyme.
which catalyse the reversible reaction between L-malate and oxaloacetate. Gazzaniga (1975) and Matyuschichev et al. (1979) reported a decrease in this enzymes activity when challenged with CCl₄. Serum malate dehydrogenase activity decreases and reaches a peak at 24-36 hours on paracetamol treatment (Zieve et al., 1985).

1.3.3.5.2 Glucose 6-phosphate dehydrogenase

Glucose 6-phosphate dehydrogenase (G6PD) functions in both lipogenesis (where it converts oxaloacetate to L-malate) and the hexose monophosphate shunt (where it can account for the complete oxidation of glucose). G6PD activity increases when it is challenged with CCl₄ (Taketa et al., 1976; Watanabe et al., 1976,1978; Rana and Tayal, 1986). Paracetamol causes elevations in G6PD activity when incubated with microsomes of RBC (Bloom et al., 1983).

1.3.3.6 Glycogen

Liver glycogen is largely concerned with the maintenance of blood glucose levels. A change in glycogen levels in the liver would thus result in an imbalance in carbohydrate metabolism. CCl₄ treatment results in drastic decrease in hepatic glycogen levels (Aksenova, 1972; Hornbrook, 1974; Jung and Lee, 1984; Dolak et al., 1985). Contradictory reports have been received concerning hepatic glycogen levels in paracetamol induced hepatotoxicity. Jepson et al. (1987) have reported an early increase in histochemically demonstratable glycogen content in periportal hepatocytes. Dixon et al. (1974) earlier reported that the necrotic areas are devoid of glycogen but the midzonal and periportal areas have a normal glycogen content. Hinson et al. (1983) have found histochemically demonstratable centrilobular glycogen depletion. Chiu and Bhakthan (1978) however, have observed a general glycogen depletion after paracetamol feeding.
1.3.3.7 Carbohydrate enzymes

It has been ascertained that glycogen levels are altered with both CCl₄ and paracetamol. It follows that the enzymes of gluconeogenesis and glycogenolysis also exhibit certain changes in their activity.

1.3.3.7.1 Glucose 6-phosphatase

Glucose 6-phosphatase (G6Pase) is the terminal hydrolytic enzyme for both the glycogenolytic and the gluconeogenic pathways. This enzyme exhibits early loss with CCl₄ induced damage (Gubskii, 1974, 1982; Reynolds and Moslen, 1975; Hortelano et al., 1979; Poli et al., 1981; Marinari et al., 1985; Hatta et al., 1986). This enzyme has also been found significantly diminished in the liver in case of paracetamol injury (Chiu and Bhakthan, 1978; Sharma et al., 1983).

1.3.3.7.2 Hexokinase

Glucose enters the glycolytic pathway by phosphorylation to glucose 6-phosphate. This is accomplished by the glycolytic enzyme hexokinase, the function of which is to ensure a supply of glucose to the tissues even in the presence of low blood glucose concentrations. Hexokinase also loses a large portion of its activity with CCl₄ intoxication. Korneiko and Nikandeov (1974) and Taketa et al. (1976) have reported an increase in low Km hepatic hexokinase activity with CCl₄, while Sorokin and Yakobson (1979) observed decreases in high Km hexokinase activity.

1.3.3.7.3 Fructose 1,6-diphosphatase

Fructose 1,6-diphosphatase is an important rate limiting enzyme essential in maintaining equilibrium between glycolysis and gluconeogenesis. Gazzaniga (1975) did not find a significant change in the activity of this enzyme, while Taketa
et al. (1976) and Faus et al. (1978) have observed significant losses of FDPase activity with CCl₄.

1.3.3.7.4 Glycogen phosphorylase

Glycogen phosphorylase is a glycogenolytic enzyme which initiates the breakdown of glycogen to glucose 1-phosphate. Hornbrook (1974) reported an increase in this enzyme's activity while Long and Moore (1986) found a depression in glycogen phosphorylase activity with CCl₄. Modified glycogen phosphorylase activity has been observed with paracetamol (Krack et al., 1980). Jepson et al. (1987) have reported an early increase in this enzyme's activity in the perivenous hepatocytes with paracetamol intoxication.

1.3.3.7.5 Amylase

Amylase is a glycolytic enzyme which catalyzes the conversion of maltose to D-glucose. Gingold and Pasquale (1976) have reported a decrease in serum amylase activity on CCl₄ treatment.

1.3.3.7.6 Lactate dehydrogenase

LDH catalyzes the conversion of pyruvate to lactate. This enzyme's activity in the liver decreases with CCl₄ damage (Gordeeva, 1973; Faus et al., 1978).

1.3.3.8 Proteins

An inhibition in protein synthesis in CCl₄ injured livers has been observed (Dianzani and Gravela, 1974; Deo et al., 1975; Gravela et al., 1979; Head et al., 1981). Younes and Siegers (1980) have found that paracetamol binds covalently to liver microsomal proteins. Protein synthesis is inhibited and hepatic protein levels fall. Similar observations have been made by Jollow et al. (1973), Pessayre et al. (1980), Smith et al. (1984) and Lisa et al. (1985).
1.3.3.9 Enzymes of protein metabolism

1.3.3.9.1 Transaminase

The transaminases function at the junction between the metabolism of proteins and carbohydrates. The activity of hepatic GOT and GPT have been found to fall with the onset of CCl₄ induced liver damage (Moran et al., 1961; Bengmark and Olsson, 1962). Hepatic GOT levels have also been reported to be enhanced with CCl₄ intoxication (Matsumoto et al., 1975).

1.3.3.10 Alkaline phosphatase

CCl₄ causes significant fall in the activity of hepatic alkaline phosphatase (Kolesnikova, 1973; Gazzaniga, 1975). Alkaline phosphatase has a relatively broad specificity and is capable of acting on a number of different structurally related substrates. A fall in its activity thus results in a wide variety of functional alterations.

1.3.3.11 Acid phosphatase

Acid phosphatase is a key enzyme having wide spread distribution in the cells and is present in different isozymic forms (Mann and Mann, 1981). These two phosphodiesterases (acid and alkaline phosphatase) are distributed in almost all the animal tissues, including the liver and catalyse a number of reactions. The effect of CCl₄ on acid phosphatase activity in the rat liver is controversial. Kolesnikova (1973) has reported a decrease while Gazzaniga (1975) found no significant change in its activity. Alpers and Isselbacher (1966) and Korolenko et al. (1975) observed an increase in the hepatic acid phosphatase activity.

1.3.3.12 5'nucleotidase

5' nucleotidase catalysis the hydrolysis of ribonucleoside 5'-monophosphates and deoxynucleoside 5'-monophosphates to their corresponding nucleosides and orthophosphates. Reports of
this enzyme's activity when challenged with CCl$_4$ are controversial. Masuda et al. (1975) reported no change; Kamath and Rubin (1974) found depressed activity and Hanczycowa and Wozniak (1980) observed an increase in 5' nucleotidase activity in the CCl$_4$ injured liver.

1.3.3.13 Adenosine triphosphatase

Adenosine triphosphatase (ATPase) are a group of enzymes having wide spread distribution in cellular systems. These membrane bound enzymes are actively involved in the transport of cations across the membranes and regulate cellular respiration. A change in ATPase activity would thus result in alterations in nearly all aspects of cellular function. CCl$_4$ damages the energy conversion system to the extent where ATP synthesis is most markedly decreased (Akahori, 1975). Histochemically, Smuckler et al. (1962) observed decreased ATPase activity in the CCl$_4$ cirrhotic liver. Biochemically, this decrease has been seen by Masuda et al. (1973) and Kamath and Rubin (1974). Masuda et al. (1975) report that while Mg$^{2+}$ — ATPase decreases, Na$^+$K$^+$ ATPase is not effected by CCl$_4$. Rufeger and Frimmer (1976) and Yahuaca et al. (1985) found that ATPase decrease with CCl$_4$ and Gubskii (1982) has reported enhanced activity of H$^+$ dependant ATPase. Ca$^{2+}$ ATPase activity is inhibited in plasma membranes with paracetamol damage (Moor et al., 1985). Intracellular ATP levels in the liver also fall (Vonen and Moerland, 1984).

1.3.3.14 Cytochrome P450

Cytochrome P450 is essential in the energy production process. Its levels in the liver also get depressed to a significant extent when challenged with CCl$_4$ (Reynolds and Moslen, 1975; Hatta et al., 1986). Cytochrome P450 levels are drastically decreased in the liver with paracetamol intoxication.
Cytochrome P450 catalyses the activation of paracetamol (Moldeus, 1978; Straat et al., 1986). Steele et al. (1983) however, reported that this catalysis was undertaken by cytochrome P448 and not cytochrome P450.

Thus, it can be seen that both CCl₄ and paracetamol toxicity is multifaceted and has several manifestations.

1.4 TEST SYSTEMS

Both in vitro and in vivo test systems are employed for the study of antihepatotoxic activity. Screening studies for antihepatotoxic activity have been conducted by in vitro experiments on hepatocytes in culture by several workers (Hikino et al., 1984a, 1984b; Kiso et al., 1984, 1985b; Adzet et al., 1987). Despite the differences in the systems, the in vitro mechanism can be compared with the in vivo (Garrison and Haynes, 1973). According to these authors, the isolated hepatocytes from a perfused liver can be taken as representatives of an intact liver. Isolated hepatocytes are capable of metabolically activating the drugs, a pre-requisite for many drugs before they exert their activity. In the in vitro system, blood flow is also not a factor in loss of measurable product. The principle advantages are that in cultured hepatocytes, the intact cell is studied with preservation of permeability barriers, of defence mechanisms and of the organization of intracellular organelles (Weddle et al., 1976).

1.5 ANTIHEPATOTOXIC ACTIVITY

There are about forty commercial herbal formulations in the Indian market for liver disorders (Handa et al., 1986). A few of these like Liv 52 (Banerjee et al., 1976; Singh et al., 1978; Subbarao and Gupta, 1979) and Livol (Pandey et al., 1982)
have been subjected to scientific evaluations but a majority of the other formulations have not been evaluated. The drugs used in the Ayurvedic system of medicine are mixtures of extracts of various plants. The antihepatotoxic activity of some plants and plant constituents have also been a subject of scientific research.


1.6 LITERATURE ON THE PLANTS UNDER INVESTIGATION

1.6.1

*Swertia chirata* (Buch-Ham.)

*S.chirata* is indigenous to the temperate Himalayas and grows wild in Kashmir, Simla hills, Nepal extending upto Bhutan and Khasi hills at an altitude above 4,000 feet.

*S.chirata* is an annual herb with a straight, quadrangular stem, upto one meter long. The stem is round and purple at the base but angular and yellowish brown above. The upper portion of the stem is much branched as compared to the lower part. The leaves are sessile, opposite decussate, oval,
oblong or broadly lanceolate acuminate having 5 to 7 veins. The flowers show calyx and corolla of the same size and there are two very distinct glandular depressions without hair at the base of each petal. The fruit is an ovate capsule. The root is primary tapering and slightly twisted with longitudinal wrinkles. It has a very bitter taste but no characteristic odour.


*S.chirata* was introduced into medicinal practice in England in 1936. It is official in Indian Pharmacopoeia (1966). This plant is a popularly used bitter in Indian medicine. The drug is derived from the whole plant. In West India, it enjoys a special reputation as a remedy for bronchial asthma and liver disorders. *S.chirata* is a constituent of two of the thirty three Indian herbal market preparations for liver disorders available (Biligen of 'Standard Pharma Remedies'; Calcutta and Livex of 'Bhartiya Aushadh Nirmanshala', Gujarat) as well as a compound powder 'Sudarshan Churana'.

*S.chirata* has antimalarial activity. Goyal *et al.* (1985) and Shetty *et al.*, (1985) have found it to be effective in a rodent test system infected with *Plasmodium vivax*. Its antifeeding effects against Anomis sabulifera have been reported (Mallick *et al.*, 1985). Okada (1976, 1978) has reported insect repellant properties of this plant. The ethanolic extract of
S.chirata is effective in lowering the blood glucose in rats (Mukherjee and Mukherjee, 1987).

Work done on S.japonica (Hikino et al. 1984b, 1985) indicates this plant to be antihepatotoxic and found its active constituents as amarogentin, amarosverin, swertiamarin, swertiamarin acetate, bellidifolin, swertia japonin, swerisin, methyl bellidifolin, methyl swertianin and oleanolic acid. All these appear to contribute to the prevention of CCl₄ and galactosamine induced damage to primary cultured rat hepatocytes.

S.japonica has diverse properties. It is an effective insect repellant (Okada,1976b). It reportedly promotes hair growth (Yamamoto et al., 1983) and is used as a constituent of some cleansing agents (Ichihara, 1978). Swertiamarin, isolated from S.japonica depresses the central nervous system, has antiulcerogenic actions and inhibits gastric secretion (Yamahara et al., 1978). Kanamori et al.(1984a, 1984b) carried out studies on the mutagenicity of S.japonica and attributed it to its xanthone derivatives. When this methanolic extract was treated with nitrite, it was mutagenic to Salmonella typhimurium and the active principles were identified as amarogentin and amaroswerin (Kanamori et al., 1986).

Mangiferin isolated from S.mussoti exhibited hepatoprotective activity (Guo and Chen, 1980). S.davidi was found useful in the treatment of bacillary dysentry (Tian and Zhang, 1986). The tetraoxygenated and pentaoxygenated xanthones of S.purpurascens produced significant central nervous system stimulant actions (Ghosal et al., 1975).

1.6.2 
Picrorhiza kurroa (Royle and Benth.) 
P.kurroa grows wild at an altitude of 3,000 to 5,000
meters in the alpine Himalayas ranging from Kashmir to Sikkim. It is a perennial herb with a large woody root stock. The rhizomes are cylindrical or slightly curved pieces. The surface bears longitudinal wrinkles. A few root scars are also seen. The rhizomes have an unpleasant odour and are very bitter in taste.

Weinger et al. (1977) reported presence of picrosides 6'-cinnamoylcatapol, 6'-vanilloylcatapol and 6'-catapol, in addition to a mixture of cinnamoyl-β and 6'-cinnamoyl-β-D-glucopyranose in the rhizomes of P. kurroa. Laurie et al. (1985) isolated a novel cucurbitacin glycoside bitter principle from root extracts of P. kurroa and found it to be 25- acetoxy-2-β-glucosyloxy-3,16,20-trihydroxy-9-methyl-19-norlanosta-5,25-diene-2-one.

The drug is a bitter tonic and is used as a substitute for gentian. It is one of the ingredients of several herbal formulations with a claim towards liver protecting activity. These include Acilvan (Aciss Labs, Kanpur), Hepex (Anglo French Drug Co., Bombay), Livarin (Patiala Ayur Pharm., Sirhind), Lüwerin (Herbs Era Pharm., W. Bengal), Livertone (Herbo Med, Calcutta) Livotrit (Zandu Pharm. Works, Bombay) and Vimliv (Solumiks, Bombay).

Kloss and Schwabe (1972) have reported that two iridoid glycosides from P. kurroa, benzoyl and vanilloylcatalpol, showed protective effects against liver intoxication of mice with CCl₄ and choleretic activity in rats.

"Kutkin" has been extracted from the roots of P. kurroa (Dhawan, 1988). It is a mixture of equal parts of iridoid glycosides, picroside I and kutkoside along with a small amount of other minor glycosides. It prevents in a dose dependent manner changes caused by paracetamol and Plasmodium berghei in...
serum and liver biochemistry. Kutkin also has a dose dependant choleretic activity in anaesthetised or conscious rats and guinea pigs and it antagonises the cholestatic effect of ethinyl estradiol. In vitro studies with hyperimmune system of HBsAg carriers demonstrate a reversible concentration dependant inactivation of the virus (Dhawan, 1988).

P. kurroa has choleretic actions and is considered useful in jaundice and hepatitis. Pandey and Chaturvedi (1969) studied the effect of different extracts of P. kurroa on experimentally induced abnormalities in the liver and reported that the drug actively regresses the raised serum transaminase values and elevates the depressed liver glycogen content caused by CCl4 hepatotoxicity in rats.

1.6.3 Ricinus communis (Linn.)

R. communis is indigenous to India and is widely cultivated in Andhra Pradesh, Gujarat and Karnataka. This plant is also found in Brazil, China, Russia and Thailand.

R. communis is an annual or perennial bush or occasionally a soft wooded small tree upto six meters or more. The leaves are green or reddish green, 30-60 cms in diameter, palmately, 5-11 lobed. The leaves are serrate and the petioles have conspicuous glands. The flowers are monoecious, in spikes, 30-60 cms long with staminate flowers on the lower and pistillate flowers on the upper part of the axis. The fruit is a capsule covered with soft, spine-like processes. The seeds are oblong, smooth, variously coated, mottled and varying in size.

The medicinal properties of the seeds of R. communis are well known for their cathartic effect internally and embollient effect externally. R. communis is used in folk medicine in Asia and Africa for a variety of disorders. In India, the roots
and leaves are regarded as being particularly useful in the
treatment of lumbago, rheumatism, sciatica, pleurodynia and
certain skin diseases (Singh, 1956). In Africa, an infusion of
leaves of this plant is a Zulu remedy for stomach ache. In
Vietnam, this plant is used as a diaphoretic and diuretic (Nguyen
and Vialard, 1953). The leaf is said to be an emmenagogue and
also useful for rheumatism (Bally, 1938). In Libya and Somalia,
the leaves are used in the treatment of Sramboesia (Cortesi,
1936). It has also been used to relieve headache (Beyer, 1927).
The leaves of R. communis are also considered useful in jaundice
(CSIR, 1972).

Viseswaram and Sant (1985) have reported that
R. communis has antihepatotoxic potential against CCl4 damage in
rabbits.

1.7 RESEARCH ENVISAGED
In the modern system of medicine, corticosteroids
and immunosuppressants are employed for alleviating liver
disorders. These drugs, however, have several side effects. A
number of drugs in the indigenous Indian system of medicine
reported to have hepatoprotective action include combination of
extracts of various plants. There are about forty such patent
herbal formulations available in the Indian market and these
incorporate about 100 plants belonging to 40 families. Three
plants viz Swertia chirata (Family Gentianaceae), Picrorhiza
kurroa (Family Scrophulariaceae) and Ricinus communis (Family
Euphorbiaceae) which feature prominently in traditional Indian
medicine have been selected for the present investigations. For
the determination of antihepatotoxic activity, both in vitro and
in vivo models have been employed. CCl4 has been selected to
induce toxicity in primary cultured rat hepatocytes. Use of
isolated hepatocytes facilitates screening of a large number of extracts and formulations in a shorter period of time saving a large number of intact animals. The plant extracts found effective in the in vitro system have been subjected to extensive in vivo studies to further confirm their activity. For the in vivo studies, paracetamol and CCl₄ were used as the hepatotoxins. The in vivo experiments include histological, histochemical and biochemical studies. The histochemical studies comprise screening of hepatic lipids, glycogen and proteins. The biochemical studies include in addition, estimation of serum enzymes which are liver specific and serum bilirubin. Several hepatic enzymes, including those involved in the metabolism of lipids, carbohydrates and proteins have also been studied.