4. DISCUSSION
Liver is the prime organ concerned with various states of metabolic and physiological homeostasis of the organism. In modern medicine there is no specific cure for diseases like infective hepatitis and liver cirrhosis. Treatment of many liver diseases is symptomatic and often disappointing. There is, however, a plethora of herbal drugs in the indigenous system of medicine which are said to be useful in the treatment of these diseases. These are composed of several plant ingredients. Three of these plants have been studied during the course of the present investigation and found effective in varying degrees against hepatotoxicity caused by carbon tetrachloride and paracetamol.

A drug interfering with the action of either of these hepatotoxins on the liver would have to act at different levels; on absorption and distribution, metabolism, covalent binding to lipids and proteins, cytochrome P-450 or lipid peroxidation. A drug with such a wide range of effects is not easy to come by. An ideal drug would need to have both a preventive and a curative action. It should have a minimum of side effects and be effective even at small doses.

The action of a drug depends on the toxicant which has caused the liver ailment, as different toxicants act via widely ranging routes. For example, carbon tetrachloride and paracetamol attack the liver through different mechanisms. Despite the difference in the mechanism of action of these two compounds, they compete for the same enzymes which carry out their activation (Flaccavento et al., 1980). A drug which could successfully cure/prevent the damage caused by these hepatotoxins would indeed be of immense use.
In order to assess the activity of a drug, both in \textit{vitro} and in \textit{vivo} systems are used. Primary cultured rat hepatocytes are used for \textit{in vitro} studies. These isolated hepatocyte preparations obtained from rat livers have advantages over whole liver preparations or isolated organelles as the organization of intracellular organelles is preserved. \textit{In vitro} studies give a better comparison between different test groups as all the cells under test are derived from a common population. Deviations found between different experimental animals are thus avoided. Once the activity of a drug is established \textit{in vitro}, it is subjected to \textit{in vivo} studies.

Hepatocyte drug reaction has been monitored \textit{in vitro} by estimating the activity of the transaminases (GOT and GPT) released into the tissue culture media. The \textit{in vivo} studies conducted include detailed estimations of various enzymes, including those involved in carbohydrate, lipid and protein metabolism, as well as morphological, histological and histochemical studies, so as to get a complete and confirmed profile of the changes taking place within the liver of both intoxicated and drug treated animals.

4.1 MORPHOLOGY

The overdosing of both CCl\textsubscript{4} and paracetamol results in gross hepatic necrosis which is evident morphologically. It has been seen that the liver of rats administered either of these toxins are enlarged, pale and soft on gross examination, suggestive of fatty infiltration. These observations support those reported by Rege \textit{et al.} (1984) with CCl\textsubscript{4}. The swelling of the liver could be as a result of an increased intake of water by the liver cells (Bassi, 1960). It has also been suggested to be the specific functional consequence of direct membrane injury by
CCl₄ in increasing the calcium ion concentration within the cell (Thiers et al., 1960 and Judah et al., 1984).

The different plant extracts under study during the course of the present investigation exhibited their protective role morphologically, as the liver of animals injected with the toxin and certain plant extracts (especially the methanolic and chloroform soluble extracts of S. chirata and the methanolic extract of P. kurroa with CCl₄ and the chloroform soluble fraction of S. chirata with paracetamol) revealed a liver structure similar to the control liver.

It is conjectured that the plant extracts which are able to restore CCl₄ damaged livers morphologically, probably exert their effect by inhibiting the increase in calcium ion concentration to varying degrees, thus, preventing the cascade of changes resulting from this influx, which ultimately lead to an altered liver appearance. Studies on the protective effects of certain pharmacologically active compounds towards CCl₄ support this view (Judah and Rees, 1959; Rees et al., 1961; Rees and Spector, 1961).

The rate of accumulation and final concentration of a toxic metabolite might explain the various forms of cell injury seen in paracetamol lesion (Dixon et al., 1974). In the earlier stages, a relatively low concentration of free metabolite could result in a net catabolism of macro molecules and failure of the sodium "pump". This could account for a rise in osmotic pressure within the cell, leading to swelling of the cytoplasmic matrix. In contrilobular cells, which are richest in drug metabolising enzymes (Koudstaal and Hardonk, 1969, 1970), there is a rapid accumulation of the metabolite with widespread destruction of membranes and in particular, loss of permeability of
the plasma membrane. When the build up of free metabolite is slower and insufficient to destroy the semi permeability of the plasmalemma, water accumulation predominates, producing hydropic vacuolation. Such aqueous swelling may be an expression of sub lethal injury, which, elsewhere, is manifested by fatty change. This event could account for the structure of the paracetamol injured liver. Walker et al. (1985) observed that the initial increase in the size of the liver was a result of plasma accumulation due to erythrocyte vacuolation of hepatocytes and Disse space enlargement in centrilobular regions. Further increase in the liver size is a consequence of erythrocyte and additional plasma sequestration within the damaged liver.

The chloroform soluble fraction of *S. chirata* prevented the liver from becoming swollen and fatty. The liver retained its normal morphology. This leads us to assume that this extract of *S. chirata* prevents the build up of the free metabolite of paracetamol, preventing water and fat accumulation and subsequently impaired blood circulation due to erythrocyte and plasma sequestration within the damaged liver.

**4.2 HISTOLOGY AND HISTOCHEMISTRY**

CCl₄ reaches maximum concentrations in liver parenchyma within two hours. Should this hepatotoxin act directly on parenchymal cells of liver of intact animals, all lipid containing structural components of the cell might be affected by the presence of this lipid soluble, non polar, lipid solvent. Thus compositional, functional and morphological changes in all cytoplasmic membrane systems of the liver cells would be expected to occur during times of maximum concentration of CCl₄ in the liver (Reynolds, 1963).

The results obtained during the current
investigations reveal that CCl₄ causes massive centrilobular necrosis, disruption of hepatocyte membranes, accompanied by vacuolisation, kupffer cell infiltration, hepatic congestion, Disse space enlargement, increase in the number of binucleate cells and a general collapse and condensation of liver histoarchitecture. These findings have been substantiated by several workers earlier (Smuckler et al., 1962; Ichiro et al., 1973).

During the present studies, the necrotic areas have been found devoid of glycogen. A general fall in glycogen content in also observed with CCl₄ intoxication in agreement with the observations of Ohnishi et al. (1974). A depletion of proteins especially in the necrotic areas and an increase in the total lipids with large deposits in the necrotic regions has also been observed during the current investigation. These changes can be explained by the grossly depressed Ca²⁺ sequestration caused by CCl₄ which results in the markedly altered histology and histochemistry observed.

The chloroform soluble fraction of the methanolic extract of S.chirata and the methanolic extract of P.kurroa could, to a large extent, prevent these changes, followed by the methanolic extract of S.chirata. The chloroform insoluble extract of S.chirata and the methanolic extract of R.communis were not significantly effective against CCl₄ intoxication. In fact, these two extracts were toxic at high concentrations.

These results lead to the conclusion that the extracts of S.chirata and P.kurroa are capable of preventing CCl₄ entry into the cells, as even the presence of this toxin is enough to damage all lipid containing structures, being a strong lipid solvent. It can thus be presumed that these active components
attach to the same binding sites as CCl$_4$, thus preventing this toxin from entering the liver to a large extent.

Paracetamol is believed to alter permeability of the plasma membrane, which results in early potassium ion loss and enzyme leakage (Buttar et al., 1976 and Legros, 1976). Walker et al. (1980) reported endocytic vacuolation and disappearance of microvilli, coupled with terminal hydropic degeneration, suggesting an important role for the plasma membrane in the development of paracetamol toxicity.

The current studies reveal that centrilobular necrosis, accompanied by congestion predominate in paracetamol induced injury. Extensive vacuolisation, necrosis and nuclear pyknosis is also rampant. Hepatocyte membranes are damaged and the uniform hepatic histoarchitecture is broken down. Similar results have been reported earlier (Mitchell et al., 1973a, 1973b; Walker et al., 1980; Zimmermann, 1981; Walker et al., 1983; Flaks and Flaks, 1983 and Walker et al., 1985).

During the course of the present investigations, it was observed that the necrotic areas are devoid of glycogen, while the surrounding hepatocytes show a marked glycogen depletion. The midzonal and periportal cells, have, in the main, a normal glycogen content. A general protein depletion is also found. The necrotic areas and the hepatocytes around the central canal, however, reveal a normal protein content. The total lipids are increased many fold in the midzonal and peripheral hepatocytes. However, the necrotic regions are devoid of total lipids. The surrounding hepatocytes exhibit a high lipid content. Dixon et al. (1974) have reported enhanced hepatic lipids with paracetamol intoxication.

Congestion is a recognized feature of paracetamol
hepatotoxicity in mice (Piperno et al., 1978), rats (Dixon et al., 1975a), Dogs (Gazzard et al., 1975) Pigs (Miller et al., 1976) and hamsters (Chiu and Bhakthan, 1978). It appears to precede the development of necrosis. This congestion is not generalized throughout the body and its primary cause appears to be in the liver itself and related to the trapping of red blood cells (Walker et al., 1985).

Only the chloroform soluble fraction of the methanolic extract of *S. chirata* and the methanolic extracts of *S. chirata* and *P. kurroa* could significantly repair paracetamol induced anomalies in the histoarchitecture of the liver, the chloroform soluble fraction being the most effective. This may be due to the protective effect of these plant extracts towards the plasma membrane. By preventing an alteration in its permeability, these extracts would prevent the increased influx of ions. The red blood cells leave the sinusoids which form the endothelial cells and accumulate within the Disse space. This efflux of RBC's is prevented by the methanolic extract and chloroform soluble fraction of *S. chirata* and the methanolic extract of *P. kurroa*, possibly by preventing the entry of the toxins into the hepatocytes, either by binding to paracetamol itself or by competitive binding with paracetamol for the same binding site.

### BIOCHEMICAL STUDIES

#### In Vitro

*In vitro* studies were conducted on primary cultured rat hepatocytes in order to monitor the direct effect of the test sample on hepatocytes, as blood flow does not remove any added test substance from the cells, unlike in the *in vivo* system.

*CCl₄* is known to damage hepatocyte membranes, thus releasing several membrane bound enzymes. Drug efficacy of the
plant extracts was thus tested by measuring activity of the transaminases, GOT and GPT in the tissue culture media.

4.3.1.1 Transaminases

Transaminases are soluble enzymes operating the reversible exchange of amino acids between $\alpha$ amino and $\alpha$ keto acids. GOT and GPT function at the junction between the metabolism of protein and carbohydrate (Sobotka and Stewart, 1964). CCl$_4$ damage to the cultured hepatocytes resulted in significantly enhanced levels of GOT and GPT in the culture media. Similar observations have been reported by several workers (Hikino et al., 1984a, 1984b, 1984c; Hikino, 1985; Kiso et al., 1984, 1985a; Adzet et al., 1987).

The transaminases act as the final common steps in the elimination of nitrogen from the various amino acids as well as a source of the keto acids for the Kreb's cycle and for gluconeogenesis. The transaminase reactions feed the products of amino acids into the carbon pool as $\alpha$ ketoglutarate, pyruvate and oxaloacetate from which glucose is made (Gavasto et al., 1957). CCl$_4$ induced changes in transaminase activity would thus effect both protein and carbohydrate metabolism. A drug which prevents/repairs this anomaly would thus be highly useful. Out of eighteen commercial herbal formulations subjected to in vitro screening model of antihepatotoxic activity, eight preparations exhibited a most significant lowering effect on the GPT activity elevated by CCl$_4$ in the tissue culture media of primary cultured rat hepatocytes. These were M-Liv, Livomyn, Liva, Livergen, Livarin, Livokin, Livex and Liv 52. Livotone and Tefroli proved ineffective.

Two preparations, Liverin and Livotone revealed no
significant activity against CCl$_4$ induced GOT leakage. Livarin, Livokin, Livergen, M-Liv, Livex and Hepa 10 and Bonliv were significantly antihepatotoxic.

The methanolic extract of *S. chirata* was effective in depressing GPT levels in the tissue culture media even at a dose of 0.25 mg/ml. When the enzyme under study was GOT, this extract was effective only at 2 mg/ml. The activity of the methanolic extract was increased with subsequently higher doses.

The chloroform soluble fraction of the methanolic extract of *S. chirata* had significantly higher activity than the methanolic extract of *S. chirata* and its chloroform insoluble fraction.

The GPT leakage induced by CCl$_4$ was depressed by both the petroleum ether soluble and the petroleum ether insoluble fractions of the chloroform soluble fraction of *S. chirata*, although the petroleum ether soluble part was more effective. This latter fraction was effective in lowering media GOT activity at 2mg/ml, while the activity of the petroleum ether insoluble fraction fell at this concentration.

The aqueous extract of *S. chirata* had limited activity in restoring GPT levels and was ineffective in preventing CCl$_4$ induced GOT leakage from the cultured rat hepatocytes.

The in vitro screening experiments thus revealed that the chloroform soluble fraction of the methanolic extract of *S. chirata* is the most active in rectifying CCl$_4$ induced hepatotoxicity.

The methanolic extract of *P. kurroa* roots and rhizomes brought CCl$_4$ induced elevated GPT levels in the media back to control levels at the 2 mg/ml dose level. GOT activity
was significantly decreased over the CCl$_4$ group even with 0.1 mg/ml of this extract. Pandey and Chaturvedi (1969) have also reported this plant's hepatoprotective potential towards CCl$_4$ induced toxicity.

The methanolic extract of the leaves of $R$. communis showed maximum hepatoprotective activity towards GPT at 2 mg/ml, although its activity was significant even at lower doses. GOT levels were significantly restored with both the 1mg/ml and 2mg/ml doses.

On comparing activities of the methanolic extracts of the three plants under study, it is apparent that $P$. kurroa was the most active in lowering CCl$_4$ enhanced GPT and GOT activity in the media, followed closely by $S$. chirata. $R$. communis, though effective, had significantly lower antihepatotoxic activity. It is known that CCl$_4$ binds irreversibly to various cell constituents (Ansari et al., 1986). It therefore appears that certain constituents of $S$. chirata and $P$. kurroa have similar binding sites on the cell membranes as CCl$_4$ and thus prevent the toxin from exerting its effect in full.

4.3.2 *In Vivo*

The extracts found hepatoprotective *in vitro* were further subjected to *in vivo* studies. Serum enzymes were used as an index of assessing hepatocyte damage and repair *in vivo*.

4.3.2.1 Transaminases

The transaminases are amongst the important, specific liver-released enzymes. During the course of the present investigation, it was found that both CCl$_4$ and paracetamol cause drastic elevations in the levels of GOT and GPT in the serum.

Similar results have been reported by several authors with CCl$_4$ (Teschke *et al.*, 1983; Hayes *et al.*, 1986) and
Several authors have used these two enzymes in the serum as an index to measure the hepatoprotective or curative effects of various plant extracts (Pao et al., 1974; Han et al., 1979; Adzet et al., 1987).

4.3.2.1.1 SGPT

The levels of GPT in the serum which were significantly enhanced with CCl₄ dosing decreased with 200 and 400 mg/kg of the methanolic extract of *S. chirata*. As in the *in vitro* experiments, here too, the chloroform soluble fraction of the methanolic extract revealed the most significant activity, while the chloroform insoluble fraction was ineffective. The latter extract proved toxic in combination with CCl₄ as at a dose of 400 mg/kg, half of the experimental animals died. Paracetamol induced significant elevations in the activity of SGPT levels. This enhancement could only be rectified by the chloroform soluble fraction of *S. chirata*.

*P. kurroa* proved promising in depressing SGPT levels elevated by CCl₄ even at 100 mg/kg. At 400 mg/kg, this enzyme's activity in the serum reached close to control levels. This methanolic extract however, exhibited insignificant activity against paracetamol induced hepatic necrosis. These observations are in conformity with those of Pandey and Chaturvedi (1969). It has recently been reported that kutkin, a mixture of equal amounts of iridoid glycosides, picroside I and kutkoside, along with a small amount of other minor glycosides isolated from the alcoholic extract of *P. kurroa* rhizomes prevents changes in serum biochemistry induced with paracetamol (Dhavan, 1988).

The methanolic extract of *R. communis* leaves
significantly reduced GPT activity at 100 and 200 mg/kg dose levels when the toxin was CCl$_4$, thus confirming the results of Viseswaram and Sant (1985). However, at 500 mg/kg, it proved toxic in combination with CCl$_4$ as three of the six experimental animals died. This plant extract was ineffective at lowering sGPT activity against paracetamol intoxication.

It thus appears that the plant extracts under study are capable of inhibiting/repairing toxin induced alterations in membrane fluidity to different extents, thus preventing enzyme leakage from the liver.

4.3.2.1.2 sGOT

The activity of this transaminase increased significantly after both CCl$_4$ and paracetamol administration. The methanolic extract of *S. chirata* was effective in restoring sGOT levels which were raised with CCl$_4$ and paracetamol. The chloroform soluble fraction of *S. chirata* showed significant activity against CCl$_4$ and was more effective than the methanolic extract and its chloroform insoluble fraction. The elevated activity of sGOT due to paracetamol was lowered by the chloroform soluble fraction of *S. chirata*, the chloroform insoluble fraction proving ineffective.

The methanolic extract of *P. kurroa* roots and rhizomes was significantly active in lowering CCl$_4$ induced elevated sGOT activity, even at a dose of 50 mg/kg. This plant extract failed to restore the paracetamol induced elevations in the activity of this enzyme in the serum.

*Ricinus communis* significantly lowered sGOT levels elevated by CCl$_4$ and offered no protection against paracetamol damage.

The three plant extracts studied during the course
of the present investigation have a protective effect on the hepatocyte membranes against CCl$_4$ damage. However, the paracetamol induced membrane damage was rectified only with the chloroform soluble fraction.

4.3.2.2 sLDH

The biochemical studies conducted revealed that serum LDH activity was significantly enhanced with both CCl$_4$ and paracetamol induced intoxication.

Any alteration in hepatic LDH quantities would cause drastic alterations in hepatic physiology as LDH is the terminal enzyme of the glycolytic sequence. It is responsible for the conversion of lactate to pyruvate and vice versa under different physiological conditions of respiration. Elevated serum LDH activity has been reported by CCl$_4$ (Schroeder et al., 1976 and Nakamura et al., 1985). It has also been reported that paracetamol overdosage results in elevated sLDH activity (Vonen and Moerland, 1984; Acosta and Bruckner, 1985).

The sLDH values were found significantly elevated with both CCl$_4$ and paracetamol during the course of the present investigation. This increase was rectified with the methanolic extract and the chloroform soluble fraction of _S.chirata_. The chloroform insoluble fraction was inactive against paracetamol damage. Only the chloroform soluble fraction of the methanolic extract exhibited activity in this direction.

The methanolic extract of _P.kurroa_ was more active than the corresponding extract of _S.chirata_ against CCl$_4$ intoxication. It, however, proved ineffective against paracetamol induced damage with respect to LDH activity. The methanolic extract of _R.communis_ was ineffective.
4.3.2.3 Alkaline phosphatase

This phosphodiesterase is distributed in almost all the animal tissues and catalyzes a number of reactions. Alkaline phosphatase has relatively broad specificity and it is capable of acting on a number of structurally related substrates.

The present investigation revealed that there is an increase in the levels of serum alkaline phosphatase content with both CCl₄ and paracetamol intoxication. Similar observations have been made for CCl₄ (Melen et al., 1985) and paracetamol (Banerjee et al., 1976).

The results obtained with the plant extracts revealed that the methanolic extract of S. chirata was able to restore the activity of serum alkaline phosphatase in a dose dependent manner. The chloroform soluble fraction of S. chirata had higher activity than the methanolic extract. The chloroform insoluble fraction proved ineffective. A similar trend was observed against paracetamol induced toxicity.

The methanolic extracts of P. kurroa and R. communis proved ineffective against the two toxins under study.

Alkaline phosphatase is a lysosomal enzyme which is loosely bound to the cell substrates and is released into the blood when the lysosomal membrane is broken down (Lehninger, 1978). The restoration in the levels of alkaline phosphatase by the plant extracts under study could thus be conjectured to be due to their preventing the breakdown of the lysosomal membranes to varying extents, thus preventing alkaline phosphatase leakage into the blood.

4.3.2.4 Bilirubin

When haemoglobin is destroyed in the body, the protein and iron portions are reused but the porphyrin portion
is ultimately converted to bilirubin, which is the major pigment in bile. Hepatotoxicity caused by both CCl₄ and paracetamol resulted in elevated serum bilirubin concentrations. Similar results have been reported by Baumann and Bierauer (1985) and Ortega et al. (1985). Hepatic jaundice is caused by liver dysfunction resulting from damage to the parenchymal cells by liver poisons like CCl₄. Jaundice could result from a failure of a damaged liver to excrete the bile produced in normal amounts (Lehninger, 1978).

The methanolic extract of *S. chirata* significantly depressed the CCl₄ induced elevations in serum bilirubin levels, though it failed to do so when the hepatotoxin administered is paracetamol. The chloroform soluble fraction of *S. chirata* was however, found to be effective while the chloroform insoluble fraction was ineffective. The methanolic extracts of *R. communis* and *P. kurroa* also failed to restore the levels of bilirubin raised by paracetamol.

The formation of conjugated bilirubin within the liver is apparently a necessary condition for the excretion of bile (Lehninger, 1978). The hepatoprotective activity of *S. chirata* towards CCl₄ intoxication could be attributed to its ability to restore the parenchymal cell functions, thus enabling the liver to excrete bilirubin in the bile.

4.3.2.5 Lipids

The current studies reveal that both CCl₄ and paracetamol cause a profound disturbance of lipid metabolism, resulting in escalation in the concentrations of hepatic lipids, mainly due to the enhancement in the amounts of triglycerides and cholesterol. The action of CCl₄ on lipids is based on its prooxidant effect (Vengerovskii et al., 1985) while paracetamol
appears to act as an antioxidant (Reiter and Wendel, 1983).

4.3.2.5.1 Total lipids
The total lipid content in the livers of both CCl$_4$ and paracetamol treated animals was significantly enhanced over the control values.

The methanolic extract of *S. chirata* had significant activity against both CCl$_4$ and paracetamol induced hepatic necrosis. This extract prevented lipid retention in the liver at 200 mg/kg. The hepatoprotective activity of the chloroform soluble fraction was even more marked while the chloroform insoluble extract proved ineffective. 200 mg/kg of the methanolic extract and chloroform soluble fraction of *S. chirata* were also effective against paracetamol induced fatty liver formation.

The methanolic extract of *P. kurroa* also depressed grossly elevated CCl$_4$ and paracetamol induced total lipid concentrations. The methanolic extract of *R. communis* which was only slightly effective at the 200 mg/kg dose level against CCl$_4$ was significantly effective against paracetamol induced fatty infiltration.

It is quite possible that the methanolic extract and chloroform soluble fraction of *S. chirata* and the methanolic extracts of *P. kurroa* and *R. communis* which are effective have an antioxidant effect on CCl$_4$ and a pro-oxidant effect on paracetamol as they reversed the effects of these two toxins.

4.3.2.5.2 Phospholipids
The phospholipids are integral components of all cell membranes. CCl$_4$ and paracetamol administration was found to induce both qualitative and quantitative changes in hepatic phospholipids. The loss in hepatic phospholipids observed with
CCl₄ was, however, more marked than that observed with paracetamol.

Hepatic phospholipids have also been found depressed by several workers with both CCl₄ (Comporti and Benedetti, 1973; Negishi and Aizawa, 1975; Lamb et al., 1984) and paracetamol (Lohmann et al., 1984) induced damage.

It is believed that CCl₄ exposure in vitro causes a rapid Ca²⁺ dependant rise in the degradation of plasma membrane phospholipids (Lamb and Schwertz, 1982). This reduction may be an important event in many forms of chemical injury to the liver cell. The fluid mosaic model of biological membranes proposed by Singer and Nicholson (1972) states that a phospholipid bilayer is the major structural component. The functional and structural capacity of the liver cells may be dependant on their capacity to maintain the phospholipid content of the plasma membrane.

The abnormal phospholipid metabolism in paracetamol-induced hepatic injury is manifested at the fine structural level by myeloid body formation (Chiu and Bhakthan, 1978). The changes in phospholipids with paracetamol may be due to significant increases in lecithin (Lohmann et al., 1984).

CCl₄ induced liver cell injury is a consequence of enhanced phospholipid degradation. Therefore, the hepatocytes ability to survive may be dependant on the cells capacity to replace the degraded phospholipids by forming new phospholipids. The phospholipase C mediated reaction in the hepatocyte membrane phospholipids is difficult to reverse because of the cell's reduced ability to generate the phospholipids that are necessary to repair the damaged membrane.

The methanolic extract of S. chirata enhanced CCl₄ depressed phospholipid concentrations at all the three dose
levels studied (100, 200 and 400 mg/kg) but was ineffective against paracetamol. The chloroform soluble fraction was effective against both the toxins. The chloroform insoluble fraction showed slight activity (P<0.05) against only CCl₄ induced depressions in hepatic phospholipid concentrations.

The methanolic extract of P. kurroa elevated CCl₄ depressed hepatic phospholipid levels at only the 400 mg/kg dose and could do so at 200 mg/kg when paracetamol was the toxin. The extract of R. communis was significantly active when the toxin administered was CCl₄ and was ineffective against paracetamol.

It is conjectured that CCl₄ induced raised Ca²⁺ levels in the hepatocytes are prevented by the plant extracts which are effective in normalising hepatic phospholipid concentrations. Thus the intracellular phospholipase C mediated degradation of hepatocellular phospholipids is inhibited by these extracts and qualitative and quantitative changes in hepatic phospholipids are minimised. This is based on the evidence that Ca²⁺ may be a key mediator in chemical induced cell injury. Hepatotoxin activated phospholipase C may result in degradation of plasma membrane phospholipids. This membrane alteration could result in an influx of extracellular Ca²⁺ into the cell, further disrupting the ability of the mitochondria and endoplasmic reticulum to sequester calcium (Tyson et al., 1976; Moore et al., 1976; Green et al., 1980).

4.3.2.5.2.1 Phosphatidyl serine and phosphatidyl inositol

Phosphatidyl serine is a cephalin and phosphatidyl inositol is a lipositiol. During the present studies, different phospholipid classes were separated by TLC. Phosphatidyl serine and phosphatidyl inositol may or may not separate and were therefore estimated together in most of the experiments. CCl₄
depressed concentrations of these two phospholipids significantly. Paracetamol intoxication resulted in elevated quantities of phosphatidyl serine and phosphatidyl inositol in the liver. Vengerovskii (1987) reported no change in these phospholipids with CCl₄. Almatov et al. (1986) reported that in rat liver mitochondria, phosphatidyl inositol decreased in CCl₄ induced hepatitis. Phosphatidyl serine represents a major functional component of plasma membranes (Mc Murray and Magee, 1972). The significance of the effect of CCl₄ on phosphatidyl serine, which it preferentially degrades could be due to the high concentration of this lipid in the plasma membrane.

The methanolic extract of *S. chirata* and its chloroform insoluble fractions were ineffective in rectifying CCl₄ and paracetamol-induced anomalies in these lipids. However, the chloroform soluble fraction showed significant hepatoprotection against both these hepatotoxins at 200 mg/kg. The methanolic extracts of *P. kurroa* and *R. communis* too could not decrease the significantly elevated concentrations of phosphatidyl inositol and phosphatidyl serine. The chloroform soluble fraction of *S. chirata* shows hepatoprotective activity as it can restore the lipid composition of the membrane to a significant extent, phosphatidyl serine being one of the major membrane components.

4.3.2.5.2.2 Sphingomyelin

Sphingomyelins are phospholipids containing a complex amino alcohol, sphingol. The current studies revealed that both CCl₄ and paracetamol significantly decreased concentrations of sphingomyelins in the liver. This decrease has also been reported with CCl₄ by Almatov et al. (1986). Vengerovskii et al. (1987) however, failed to detect any change
with this haloalkane.

Both the methanolic extract and the chloroform soluble fraction of S.chirata (200mg/kg) rectified this anomaly caused by either of the toxins. The chloroform insoluble fraction was active against only paracetamol induced hepatic sphingomyelin losses. The methanolic extracts of P.kurroa and R.communis also showed significant activity towards sphingomyelin at a dose level of 200 mg /kg against paracetamol damage.

4.3.2.5.2.3 Phosphatidyl choline

Phosphatidyl cholines are lecithins which have both metabolic and structural functions. The current observations indicate that both CCl$_4$ and paracetamol grossly depress hepatic phosphatidyl choline concentrations, although CCl$_4$ proves more toxic than paracetamol.

CCl$_4$ toxicity results in decreased concentrations of hepatic phosphatidyl choline, probably due to the decreased synthesis of phosphatidyl choline from the diglycerides or because of the decreased conversion of phosphatidyl ethanolamine to phosphatidyl choline (Sugano et al., 1970). The former possibility is compatible with the observation that CCl$_4$ brings about degeneration of endoplasmic reticulum, where most, if not all, synthesis of phospholipid occurs (Bennedetti et al., 1974).

The depression caused by these two hepatotoxins in phosphatidyl choline concentrations can be successfully redeemed with 200 mg/kg of the methanolic and chloroform soluble extracts of S.chirata. The chloroform insoluble fraction of this plant, however proved ineffective as did the extracts of P.kurroa and R.communis.

4.3.2.5.2.4 Phosphatidyl ethanolamine

This class of phospholipids are cephalin which
contain glycerol, fatty acids, phosphoric acids and ethanolamine. The current results show that both CCl₄ and paracetamol toxicity results in significant decreases in phosphatidyl ethanolamine. Phosphatidyl ethanolamine levels decrease with CCl₄ poisoning due to decreased synthesis from the diglycerides. Shuji et al. (1975) reported a decrease in this phospholipid with CCl₄. Bennedetti et al. (1974), however, found an increase.

The fall in concentrations of this phospholipid were restored by all the three extracts of S. chirata under study. The methanolic extract of P. kurroa was effective when the toxin administered was paracetamol, while the extract of R. communis revealed no activity at a dose of 200 mg/kg.

It is known that CCl₄ caused decreased synthesis of phosphatidyl ethanolamine from diglycerides (Shuji et al., 1974). S. chirata and P. kurroa thus probably exert their hepatoprotective effect by enhancing synthesis of this lipid from diglycerides, or, conversely, preventing toxin induced inhibition of phosphatidyl ethanolamine synthesis from diglycerides.

4.3.2.5.3 Triacylglycerides

Triacylglycerides are the most abundant family of lipids and the major components of depot or storage lipids in hepatic cells. These glycerides comprise three lipid classes; the monoglycerides, diglycerides and triglycerides. The present studies revealed enhanced hepatic triacylglyceride concentrations with both CCl₄ and paracetamol. Similar results with CCl₄ have also been reported by Gravela et al. (1979). It has been demonstrated that paracetamol induced hepatic necrosis is due to a chemically reactive arylating metabolite which covalently binds to vital hepatocellular macromolecules (Jollow et al., 1973).

During the course of the present investigation, it
was observed that the CC14 and paracetamol elevated hepatic triacylglyceride concentrations were brought down with the methanolic extract and chloroform soluble fraction of *S. chirata*, the latter proving more effective. The chloroform insoluble fraction of *S. chirata* revealed no hepatoprotective potential. The extracts of *P. kurroa* and *R. communis* proved significantly antihepatotoxic in combating paracetamol induced hepatic damage with respect to triacylglycerides.

In the CC14 poisoned hepatocytes, the triacylglyceride quantities are more severely effected than protein secretion. This fact, without ruling out the possibility of a direct impairment of microtubular function by CC14 suggests that either specific damage to the triacylglyceride secreting sites of the plasma membrane occur, or other changes in steps preceding lipoprotein transport are involved. This is probably due to depressed intracellular calcium levels (Gravela et al., 1979).

Triacylglycerides are water soluble lipids. These are carried out of the liver into the blood in combination with proteins. Triacylglyceride accumulation in the liver is probably largely prevented by the active extracts as they release the block in the lipoprotein secretory pathway which is caused by the two toxins studied. The hepatoprotective action of these extracts is possibly because they inhibit large concentrations of calcium from accumulating in the hepatocytes and thus effect the triacylglyceride secretory sites.

4.3.2.5.4 Triglycerides

Triglycerides represent a major class of lipids in the liver. The levels of triglycerides in the liver were found to be significantly enhanced with both the toxins studied. A rapid
accumulation of fats in the liver has been reported with both CCl$_4$ (Dianzani et al., 1975; Cagen and Klaasen, 1979; Gravela et al., 1979; Rolando et al., 1984) and paracetamol (Fleurstein and Wendel, 1979; Olinescu et al., 1982).

In CCl$_4$ or paracetamol induced fatty liver, the earliest derangement involves mechanisms other than inhibition of protein synthesis e.g. coupling of triglycerides, phospholipids and lipid-acceptor protein to produce lipoprotein or the intracellular transport of lipoproteins into secretory vesicles and its discharge outside the liver cells through the microtubular system. These toxins block triglyceride secretion through two different mechanisms; inhibition of protein synthesis with a subsequent failure of lipoprotein formation and impairment of secretory activity.

200 mg/kg of the methanolic extract of *S. chirata* and its chloroform soluble fraction rectified CCl$_4$ induced elevated triglyceride concentrations but had no significant activity against paracetamol. The chloroform insoluble fraction of *S. chirata* proved ineffective. Extracts of *P. kurroa* and *R. communis* also proved significantly hepatoprotective against both these toxins in depressing hepatic triglyceride concentrations. The methanolic extract of *R. communis* proved more effective than the methanolic extracts of *S. chirata* and *P. kurroa* in combating paracetamol induced elevations in hepatic triglycerides.

The enhanced triglyceride levels as a result of CCl$_4$ intoxication are primarily due to decreased lipoprotein secretion (Pencil et al., 1983; Rehman et al., 1983; Kato and Nakazawa, 1986). It is therefore possible that these plants extracts have the ability to release this blockage.

4.3.2.5.5 Mono and Diglycerides

The monoglyceride and diglyceride quantities were
also increased in the liver when the experimental animals were subjected to CCl₄ or paracetamol intoxication. The responses of the different glycerides seems to be reflected in the extent of the modification in functioning of the enzymatic systems forming the two glycerides. These may in turn indicate that the metabolic pool(s) of diglycerides available for the synthesis of triglyceride and phospholipid are substantially disturbed by CCl₄.

The current studies reveal that none of the extracts of *S.chirata* under study were significantly effective when CCl₄ was the toxicant. The methanolic extract and its chloroform soluble fraction, however, proved active against paracetamol intoxication. The methanolic extracts of *F.kurroa* and *R.communis* significantly reduced the levels of hepatic mono and diglycerides elevated by paracetamol.

Extracts of the three plants under study were capable of preventing the breakdown of these two glycerides by paracetamol and were thus capable of protecting the liver from becoming necrotic.

4.3.2.5.6 Free fatty acids

The levels of free fatty acids in the liver fell significantly with CCl₄ induced hepatic damage. Paracetamol, however, had no significant effect. CCl₄ induced steatosis is due to a disturbance of mitochondrial function, inhibition of β-oxidation of fatty acids and delay of the transport of lipoproteins from the liver to the blood. CCl₄ causes a fall in the quantities of hepatic free fatty acids (Valcazar *et al.*, 1980).

Only the chloroform soluble fraction of the
methanolic extract of *S. chirata* was able to restore CCl₄ depressed, hepatic free fatty acid quantities to a significant extent. The other extracts proved ineffective.

The observation that CCl₄ exposure decreases polyunsaturated fatty acids in the cellular debris fraction suggests that CCl₄ might be metabolised to free radicals in the plasma membrane and the endoplasmic reticulum. The plasma membrane is thought to be the site of sizeable fatty acid loss. As polyunsaturated fatty acid groups of microsomal phospholipids undergo chain scission, portions of the membrane detach from the microsome resulting in proportional losses of total proteins and lipid (Weddle et al., 1976).

It is conjectured that the chloroform soluble fraction of *S. chirata* is capable of restoring mitochondrial functions and possibly promoting the β-oxidation of fatty acids, thus preventing the fall in the quantities of free fatty acids caused by CCl₄.

4.3.2.5.7 **Cholesterol**

Cholesterol is a steroid alcohol containing a hydroxyl group. Treatment with both CCl₄ and paracetamol resulted in an increase in the levels of hepatic total cholesterol. Cholesterol is the precursor of many steroids in animal tissues, including the bile acids which aid in emulsification and absorption of lipids in the intestine. The major pathway of cholesterol degradation is conversion to bile acids which are released into the duodenum. An increase in cholesterol quantities in the liver with CCl₄ has been reported (Chaudhary et al., 1984).

The methanolic extract of *S. chirata* and its chloroform soluble fraction were able to revert the increase in cholesterol induced by both CCl₄ and paracetamol. The chloroform
insoluble fraction also revealed significant potential when the hepatotoxin under test was paracetamol. The methanolic extract of *P. kurroa* significantly lowered hepatic cholesterol even at a dose of 100 mg/kg against CCl₄ and at 200 mg/kg against paracetamol-induced elevations. The extract of *R. communis* was not active when the toxin under test was CCl₄ but had marked antihepatotoxic potential when paracetamol was the intoxicant. In fact, the methanolic extract of *R. communis* was more effective than either of the other methanolic extracts as far as hepatic cholesterol concentrations were concerned.

According to Wakasugi *et al.* (1985), the increased cholesterol content was not due to liver hypertrophy but due to the suppressed secretion of this moiety from the liver. The extracts with promising results, probably exerted their effect by allowing the free flow of cholesterol from the liver into the blood. This conjecture was further substantiated by the results obtained with serum cholesterol levels. Another possibility is, that a feedback mechanism could possibly be activated by these plant extracts, resulting in depressed cholesterol synthesis. This was substantiated by the results obtained while monitoring hepatic HMG CoA reductase activity. Normalisation of the cholesterol content in response to these plant extracts might be considered as an expression of the functional improvement of hepatocytes.

4.3.2.5.7.1 Free cholesterol

This class of hepatic cholesterol has also been found increased with both CCl₄ and paracetamol. Similar results with CCl₄ intoxication have been reported by Rehman *et al.* (1983). Only the chloroform soluble fraction of the methanolic extract of *S. chirata* could depress free cholesterol
levels in the liver.

4.3.2.5.7.2 Cholesterol esters.

Hepatic cholesterol ester concentrations have been found enhanced with CCl$_4$ and paracetamol intoxication during the course of the present investigations. The cholesterol esters increased not as a result of enhanced production but due to suppressed release into the blood as evidenced by reduced plasma cholesterol concentrations (Bonora et al., 1976).

The methanolic extract of *S. chirata* and its chloroform soluble fraction were significantly effective in lowering hepatic cholesterol concentrations against both the toxins, the chloroform soluble fraction proving more effective. The chloroform insoluble fraction could lower only CCl$_4$ elevated cholesterol ester quantities. The methanolic extracts of *P. kurroa* and *R. communis* proved ineffective in lowering concentrations of this moiety when the toxin under test was paracetamol.

4.3.2.5.7.3 Scholesterol

Serum levels of cholesterol in experimental rats administered CCl$_4$ and paracetamol were found significantly decreased. Wakasugi et al. (1985) also reported similar findings with CCl$_4$. Plasma cholesterol levels have been reported to decrease with paracetamol-induced hepatic damage (Chandrasekharan and Juggi, 1975).

The methanolic extract of *S. chirata* and its chloroform soluble and insoluble fractions showed marked activity in elevating scholesterol against CCl$_4$. The chloroform insoluble fraction was however, ineffective when the toxin administered was paracetamol. The methanolic extracts of *P. kurroa* and *R. communis* also significantly enhanced serum cholesterol concentrations.

The hepatoprotective action of the plant extracts
under study could be due to their ability to release the CCl₄ and paracetamol induced inhibition of cholesterol secretion. This was further confirmed when hepatic cholesterol concentrations were monitored.

4.3.2.5.8 Lipoproteins

All the three classes of plasma lipoproteins under study (HDL, LDL and VLDL) were found significantly decreased after CCl₄ and paracetamol treatment.

Plasma lipoproteins are complexes in which the lipids and proteins occur in relatively fixed ratio. It is now generally accepted that the early onset of hepatic damage by CCl₄ is dependant upon a block in the lipoprotein secretory pathway. Lipoprotein transport is accomplished by microtubules (Gravela et al., 1979 and Dianzani et al., 1981).

CCl₄ is able to impair both secretory and the formative sides of the golgi apparatus which play a key role in lipoprotein secretion (Poli et al., 1979).

4.3.2.5.8.1 Very low density lipoprotein (VLDL)

These contain four different types of polypeptide chains having distinctive amino acid sequences. VLDL represent the major vehicle by which triglycerides are transported from the liver into the blood compartments. VLDL are the lipoprotein family most involved in CCl₄ induced impairment of lipoprotein secretion which resulted in a fast increase in liver triglycerides (Marinari et al., 1985; Becker et al., 1987; Vengerovskii et al., 1987).

The current studies indicate that both CCl₄ and paracetamol actively depress serum VLDL concentrations. The methanolic extract and chloroform soluble fraction of S. chirata showed significant activity against both the toxins, while the
chloroform insoluble fraction proved ineffective. *P. kurroa* and *R. communis* extracts significantly elevated paracetamol-depressed serum VLDL concentration.

4.3.2.5.8.2 Low density lipoproteins (LDL)

This class of lipoproteins also falls in the plasma when challenged by either CCl₄ or paracetamol. Dianzani and Poli (1984) reported that CCl₄ induces impairment of the lipoprotein secretory pathway by its covalent binding to cell structures. Benedetti *et al.* (1974), Vengerovskii *et al.* (1987) and Konevalova and Chirkin (1987) reported reduced plasma low density lipoproteins with CCl₄.

The chloroform soluble and chloroform insoluble fractions of *S. chirata* and the methanolic extract of this plant elevated plasma levels of low density lipoproteins significantly against CCl₄ intoxication. The chloroform soluble fraction being the most effective and the chloroform insoluble fraction the least active. However, the chloroform soluble fraction of *S. chirata* did not bring about any change in LDL levels elevated by paracetamol. The methanolic extracts of *P. kurroa* and *R. communis* at doses of 200 mg/kg exhibited lowering effect on low density lipoproteins.

4.3.2.5.8.3 High density lipoproteins (HDL)

This class of lipoproteins has two different types of polypeptide chains. Plasma HDL concentrations monitored during the current investigations were found to drop significantly with CCl₄ administration. Poli *et al.* (1985) and Becker *et al.* (1987) have also made similar observations. HDL concentrations in the plasma have been found increased with the methanolic extract of *S. chirata* even at a concentration of 100 mg/kg. Its chloroform soluble and insoluble fractions also exhibited significant
activity when the toxin was CCl₄. When paracetamol was the hepatotoxin, the chloroform insoluble fraction of *S. chirata* did not reveal significant activity. The methanolic extract and chloroform soluble fractions of *S. chirata* significantly elevated depressed plasma HDL concentrations. The methanolic extracts of *F. kurroa* and *R. communis* were significantly active in elevating HDL levels when paracetamol was the toxicant.

The results obtained on monitoring the three classes of lipoproteins after administering the various plant extracts along with the toxins lead us to conclude that the extracts which elevated plasma lipoproteins did so either by releasing the block in the microtubule mediated lipoprotein secretory pathway or by promoting the combining of lipids and proteins to form the secretory lipoproteins.

4.3.2.6 Enzymes of lipid metabolism

4.3.2.6.1 Malate dehydrogenase

This enzyme catalyses the reversible reaction between L-malate and oxaloacetate in the presence of NAD⁺. The net flux of this reaction is in the direction of oxaloacetate. Both CCl₄ and paracetamol dosing significantly depressed MDH activity. MDH is localised in the cytosol and mitochondria and is one of the enzymes of the TCA cycle. It also plays a major role in the maintenance of reducing equivalents in the intra and extra mitochondrial compartments of the cell via the malate-aspartate shuttle system. This system is bidirectional and can transport electrons from extra mitochondrial NADH into the mitochondria or from intramitochondrial NADH to cytosol (Lehninger, 1978). A decrease in this enzyme's activity in the liver with CCl₄ has been reported (Matyuschichev et al., 1979). Paracetamol poisoning has been found to elevate serum MDH
activity (Zieve et al., 1985). Inhibition of MDH activity could be related to alterations in the respiratory system of the cell (Storey and Kayne, 1977).

The methanolic extract of *S. chirata* and its chloroform soluble fraction exhibited significant activity, the latter proving more effective. The chloroform insoluble fraction of *S. chirata* was ineffective. The methanolic extract of *P. kurroa* proved active even at 100 mg/kg. *R. communis* revealed its activity only against paracetamol.

Malate dehydrogenase in the mitochondria is very loosely bound and its decreased activity is probably related to a mitochondrial permeabilisation in agreement with the findings of Rees and Sinha (1960) and Dinman et al. (1963). Thus, these extracts probably alter the flux of the reaction initiated by the toxins (CCl₄ and paracetamol) and by some as yet undeciphered mechanism, prevent mitochondrial permeabilisation and allow the respiratory system of the cell to function smoothly, thereby preventing the loss of malate dehydrogenase.

4.3.2.6.2 Glucose 6-phosphate dehydrogenase

G6PD is a mitochondrial enzyme which catalyses the dehydrogenation of glucose 6-phosphate to 6-phosphogluconate via the formation of 6-phosphogluconolactone with NADP as the hydrogen ion acceptor. This can account for the complete oxidation of glucose. This enzyme supplies the NADPH needed for lipogenesis. Thus, there is a direct correlation between lipogenesis and oxidation of glucose via the shunt pathway.

A decrease in this enzyme's activity with CCl₄ poisoning has been reported by Gazzaniga (1975) whereas Matyuschichev et al. (1979), Watanabe and Nagashima (1983) and Rana and Tayal (1986) have observed an increase. Our observations
agree with the former view of Gazzaniga (1975). However, paracetamol overdosage grossly elevates the activity of this enzyme.

The chloroform soluble fraction of *S. chirata* could elevate CCl$_4$ depressed G6PD activity at a dose of 400 mg/kg. The other extracts of this plant proving ineffective. Paracetamol induced damage was also effectively combated by this extract at a dose of 200 mg/kg. The methanolic extract of *P. kurroa* showed significant activity against CCl$_4$ (at 100mg/kg) and paracetamol induced anomalies (at 200 mg/kg). The extract of *R. communis* was significantly protective when the toxin was paracetamol but was ineffective against CCl$_4$ induced depressed G6PD activity.

### 4.3.2.6.3 Hydroxymethylglutaryl-CoA reductase

HMG CoA reductase is an extramitochondrial enzyme which plays a very important role in cholesterol synthesis. Cholesterol is formed from mevalonate. HMG CoA is converted to mevalonate in a two stage reduction by NADPH, catalysed by HMG CoA reductase. It is likely that it is in the form of a cholesterol sterol carrier protein that cholesterol is converted to bile acids and participates in the formation of membranes and lipoproteins. It is also as a cholesterol sterol carrier protein that cholesterol might affect the activity of HMG CoA reductase. There is a proposed feedback mechanism, whereby this enzyme in the liver is inhibited by cholesterol (Harper *et al.*,1975). Thus, in cholesterol biosynthesis, HMG CoA reductase is considered to be the rate limiting enzyme in the liver (Nakayama *et al.*,1977).

The current studies revealed that paracetamol causes depressed hepatic HMG CoA reductase activity, possibly due to increased cholesterol quantities which leads to a feedback suppression of liver HMG CoA reductase. Cholesterol is, however,
not in itself the inhibitor of this enzyme (Lehninger, 1978). This pattern changed with CCl₄ which significantly enhanced this enzyme's activity.

With the exception of the chloroform insoluble extract of *G. chirata* (which was ineffective against CCl₄) all the extracts under study proved active against both CCl₄ and paracetamol in maintaining normal HMG CoA reductase activity.

It is probably due to the protective, rather than the curative properties of these drugs that they are able to minimise toxin induced damage in either direction. It is known that when CCl₄ and paracetamol are administered simultaneously, their hepatotoxic effect is not added. In fact, it is decreased as these two compounds compete for the same binding sites (Flaccavento *et al.*, 1980). It is conjectured that the plant extracts under study occupy the same binding sites as CCl₄ and paracetamol and thus prevent them from exerting their effect in full.

4.3.2.7 Glycogen

Liver glycogen is largely concerned with the maintenance of blood glucose. Concentration of hepatic glycogen was found grossly depressed after CCl₄ intoxication during the present study. The liver is well established to possess distinct and dynamic heterogeneity with the periportal and perivenous hepatocytes having different metabolic capacities (Jungermann and Katz, 1982). The periportal zone is predominantly gluconeogenic whereas the perivenous zone is mainly glycolytic. It has been proposed that the periportal zone is the major site of glycogen synthesis from 3-carbon precursors and the perivenous zone converts glucose to lactate and is a major site of FDP synthesis (Jungermann *et al.*, 1982; Pilkis *et al.*, 1985; Soley *et al.*, 1985).
CCl₄ is believed to selectively destroy perivenous hepatocytes. Studies conducted during the current investigation revealed that while CCl₄ significantly depressed hepatic glycogen, paracetamol had no discernable effect.

CCl₄ promotes net glucose release by the liver. Assuming that CCl₄ hepatotoxicity is largely confined to the perivenous hepatocytes, results imply that the perivenous zone is a major site of glycogen depletion. That CCl₄ causes glucose release suggests that it diverts glucose 6-phosphate away from glycogen synthesis into glucose production (Zimmermann, 1978).

Depletion of hepatic glycogen with the onslaught of CCl₄ damage was also observed by Hortelano et al. (1979) and Adzet et al. (1986). Similar observations were made with paracetamol poisoning (Krack et al., 1980; Rudd et al., 1981). However, Jepson et al. (1987) reported an increase in hepatic glycogen content with paracetamol.

The methanolic extract and chloroform soluble fraction of S.chirata acted in a dose dependant manner against CCl₄ toxicity in enhancing glycogen concentrations, as did the extract of P.kurroa. The chloroform insoluble fraction of S.chirata and the methanolic extract of R.communis were, however, ineffective. Similar results have been observed with P.kurroa by Pandey and Chaturvedi (1969). According to Jungermann and Katz (1982), CCl₄ induced breakdown of glycogen can be brought about either by diminished glycogenesis or increased glycogenolysis as a result of cAMP accumulation. It thus appears, that S.chirata and P.kurroa either enhance glycogenesis (by inhibiting the diversion of glucose 6-phosphate from glycogen synthesis) or depress glycogenolysis (by preventing cAMP accumulation) and thus inhibit the fall in glycogen concentrations to varying
6. BIBLIOGRAPHY
4.3.2.8 Enzymes of carbohydrate metabolism

The major function of carbohydrate in metabolism is as a fuel to be oxidised and provide energy for other metabolic processes. In this role, carbohydrate is utilised in cells, mainly in the form of glucose. Activities of certain key enzymes of glycolysis, glycogenolysis, hexose monophosphate shunt and gluconeogenesis in the liver of experimental animals have been studied.

4.3.2.8.1 Glucose 6-phosphatase

G6Pase is a microsomal enzyme which possesses pyrophosphatase activity. During the present investigation, it was observed that both CCl\textsubscript{4} and paracetamol intoxication inhibited hepatic G6Pase activity. This inhibition can lead to the accumulation of Glucose 6-phosphate instead of further breaking down to the glucose and phosphate moiety, as G6Pase removes phosphate from glucose 6-phosphate, enabling the free glucose to diffuse from the cell into the extracellular spaces, including the blood. G6Pase is the terminal hydrolytic enzyme for both the glycogenolytic and the gluconeogenic pathways and therefore an inhibition of this enzyme may block the production of glucose from other sources such as amino acids and oxaloacetate (Harper et al., 1977).

In vivo loss of G6Pase activity with CCl\textsubscript{4} is primarily due to lipid peroxidation (Masuda, 1981), CCl\textsubscript{4} induced G6Pase loss has been reported by several workers (Shampai and Gubskii, 1972; Orrego et al., 1976; Kanamura et al., 1981; Jung and Lee, 1984). Loss of G6Pase activity with paracetamol has also been reported (Chiu and Bhakthan, 1978; Sharma et al., 1983).

The methanolic extract of S.chirata and its
chloroform soluble fraction as also the methanolic extract of *P. kurroa* showed significant potential as far as elevating CCl₄ and paracetamol induced depressed hepatic G6Pase activity. The methanolic extract of *R. communis* was effective only against CCl₄. The chloroform insoluble fraction of *S. chirata* was ineffective.

The inhibition in the activity of G6Pase by the two toxins under study may result in the disruption of glucose metabolism. The hepatoprotective action of the plant extracts is possibly a result of the drugs depressing glucose 6-phosphate concentrations by promoting its breakdown to glucose and phosphate and thus preventing the inhibition of glucose 6-phosphatase activity which results with the accumulation of glucose 6-phosphate.

4.3.2.8.2 Hexokinase

Hexokinase is a glycolytic enzyme which catalysis the phosphorylation of glucose to glucose 6-phosphate. The activity of this enzyme was found grossly depressed with both CCl₄ and paracetamol-induced intoxication. Similar observations with CCl₄ have been made by Taketa *et al.* (1976) and Sorokin and Yakobson (1979).

Amongst the extracts of *S. chirata* studied the chloroform soluble fraction proved the most active offering protection against both the toxins. The methanolic extracts of *S. chirata* and *R. communis* proved ineffective.

Hexokinase is inhibited in an allosteric manner by glucose 6-phosphate production. The function of this enzyme is to ensure a supply of glucose to the tissues even in the presence of low blood glucose concentrations. A fall in hexokinase activity would thus inhibit the entire glycolytic pathway.

CCl₄ and paracetamol cause accumulation of glucose
6-phosphate in the liver. This accumulated moiety inhibits hexokinase activity. The hepatoprotective effect of the extracts is probably a result of promoting the utilisation of glucose 6-phosphate and thus preventing its accumulation. This would open the pathway for hexokinase to express its activity.

4.3.2.8.3 **Fructose 1,6-diphosphatase**

FDPase is a key rate limiting enzyme and it plays an important role in maintaining equilibrium between glycolysis and gluconeogenesis. During the course of the present investigation it was observed that FDPase activity was significantly depressed by both the toxins under study. Depressed hepatic FDPase activity has also been observed with CCl₄ intoxication (Faus *et al.*, 1978). Gazzaniga (1975) however reported no change with this toxicant.

The conversion of Fructose 1,6 diphosphate to fructose 6-phosphate, necessary to achieve a reversal of glycolysis is catalysed by FDPase. This is a key enzyme, in the sense that its presence determines whether or not a tissue is capable of synthesizing glycogen from pyruvate and tissue phosphates. FDPase also functions in the hexose monophosphate shunt in order to oxidise glucose to carbon dioxide completely. The inhibition of FDPase may apparently lead to the decreased breakdown of Fructose 1,6-diphosphate and consequently to the lesser generation of glucose by gluconeogenic pathway.

Amongst the extracts of *S.chirata* tested for hepatoprotective activity only the chloroform soluble fraction exhibited significant lowering of FDPase against CCl₄ intoxication. When the toxin used was paracetamol, the methanolic extract was also effective. The extract of *P.kurroa* significantly elevated CCl₄ depressed FDPase activity at a dose of 200 mg/kg but was ineffective at this dose level when paracetamol was used.
as the toxicant. The extract of *R. communis* proved ineffective.

It is conjectured that *S. chirata* and *P. kurroa* promote FDPase activity by inducing synthesis of the enzyme protein at certain critical dose levels, or alternately, the accessibility and utilization of the substrate may be enhanced. The effect of these two drugs could, on the other hand, be in blocking the binding sites occupied by CCl4 and paracetamol, thus preventing them from altering FDPase function.

4.3.2.8.4 Glycogen phosphorylase

This is a glycogenolytic enzyme which catalyzes the reaction converting glycogen to glucose-1-phosphate. The observations made during the present studies revealed that the activity of glycogen phosphorylase increased significantly with CCl4 and decreased to a large extent when paracetamol was administered. Decrease in glycogen phosphorylase activity would lead to accumulation of glycogen in the organ. A decrease in phosphorylase activity may cause inhibition in glycogenolytic pathways and consequently the lack of adequate glucose source for glycolysis. Increase in glycogen phosphorylase activity would conversely, lead to depletion of glycogen in the liver. Hornbrook (1974) observed an increase, while Long and Moore (1986) reported a decrease in the activity of this enzyme with CCl4 intoxication. Jepson et al. (1987) observed an early increase in glycogen phosphorylase in the perivenous hepatocytes with paracetamol.

The methanolic extract of *S. chirata* proved capable of elevating paracetamol-depressed and in depressing CCl4 elevated hepatic glycogen phosphorylase activity at 200 mg/kg. The chloroform soluble fraction proved even more active, while the chloroform insoluble fraction was ineffective against both the toxins, as was *R. communis*. The extract of *P. kurroa* was highly...
effective against CCl₄ (even at a dose of 100 mg/kg) and paracetamol-induced aberrations in this enzyme's activity.

The methanolic extracts of Swertia chirata and P. kurroa and the chloroform soluble portion of the former extract elevated CCl₄ depressed hepatic glycogen levels, while correspondingly depressing glycogen phosphorylase activity in the liver. A paradox was observed when the toxin was paracetamol, as a decrease in this enzyme's activity would expectedly be accompanied by enhanced hepatic glycogen concentration. However, no significant change was observed in this moiety. This was probably due to the cumulative activity of the enzymes of carbohydrate metabolism. The glycolytic enzymes promoting glycogen breakdown and the decreased glycogen phosphorylase activity with paracetamol leading to glycogen accumulation. The resultant of these activities was, that no discernable change in the hepatic glycogen was found.

4.3.2.8.5 Amylase

The conversion of maltose to D-glucose is catalysed by amylase. The current studies revealed that hepatic amylase activity is grossly depressed with both CCl₄ and paracetamol. The decrease in amylase activity could be due to the non availability of polysaccharides for further breakdown and utilization. Inhibition of amylase activity may limit the option of maltose to be converted to D-glucose and subsequent utilization in energy production in the glycolytic pathway. Impaired amylase synthesis could also result in decreased serum amylase with CCl₄ intoxication (Gingold and Pasquale, 1976).

Swertia chirata (methanolic extract) enhanced hepatic amylase activity at the 200 mg/kg and 400 mg/kg dose levels. This extract could not repair paracetamol induced depressed
amylase activity. Chloroform soluble fraction proved the most effective at the same dose levels, while the chloroform insoluble fraction was ineffective in restoring hepatic amylase activity. The methanolic extract of *P. kurroa* was active at 200 mg/kg when either CCl₄ or paracetamol was used as the hepatotoxin. The extract of *R. communis* proved ineffective.

*S. chirata* and *P. kurroa* probably exerted their effect by making available the polysaccharides required for amylase activity to be expressed and thereby increased the activity of this enzyme.

4.3.2.8.6 Glucose 6-phosphate isomerase

G6Pase isomerase catalyzes the isomerisation of glucose 6-phosphate to fructose 6-phosphate. This reaction proceeds readily in either direction and is reversible in glycolysis and gluconeogenesis.

The activity of G6P isomerase was significantly decreased in the liver when challenged with either CCl₄ or paracetamol. The inhibition of this enzyme's activity leads to the reversal of glycolysis by blocking the formation of fructose 6-phosphate and then to fructose 1,6 diphosphate (Lehninger, 1978).

The methanolic extract of *S. chirata* at 200 and 400 mg/kg and its chloroform soluble fraction at the same dose showed significant activity in enhancing depressed enzyme levels. The chloroform insoluble fraction was ineffective against CCl₄. A similar trend was observed when paracetamol was administered instead of CCl₄. The methanolic extract of *P. kurroa* had markedly higher activity against CCl₄ but was ineffective against paracetamol. The methanolic extract of *R. communis* showed no protection towards this enzyme when the toxicant was paracetamol.
The depression in glucose 6-phosphatase activity resulted in an accumulation of glucose-6-phosphate. This accumulation should, in the normal course of events, enhance G6P isomerase activity. However, the opposite trend was observed. Therefore, it may be presumed that these toxins either act directly on the enzyme protein or inhibit the accessibility and utilization of the substrate. If this was the case, the hepatoprotective activity of *S. chirata* and *P. kurroa* must indeed be very wide, as they appear to have a large sphere of activity, not only necessarily occupying the same binding sites as the toxins, but, in addition, enhancing the enzyme's accessibility and utilization of the substrate and perhaps, enhancing enzyme synthesis as well.

4.3.2.8.7 Lactic dehydrogenase

Pyruvic, which is the normal end product of glycolysis under aerobic conditions is reduced under anaerobic conditions by the NADH to lactate, the reaction being catalysed by LDH. A fall in hepatic LDH activity on CCl₄ and paracetamol dosing was observed during the course of the present investigations. Similar results with CCl₄ have been reported by Gordeeva (1973) and Faus et al. (1978).

The methanolic extract and chloroform soluble fraction of *S. chirata* were effective in elevating both CCl₄ and paracetamol induced depressions in hepatic LDH activity. The chloroform soluble fraction proved more effective. The chloroform insoluble fraction was ineffective. The methanolic extract of *P. kurroa* had significant antihepatotoxic activity against both these toxins. The extract of *R. communis* was ineffective.

It is possible that the methanolic extracts of *S. chirata* and *P. kurroa* and the chloroform soluble fraction of
S. chirata prevent CCl₄ and paracetamol from binding with the enzyme molecules at the nucleotide binding sites, thus reducing their toxic effects.

4.3.2.9 Proteins

Proteins are the most abundant organic molecules in cells, consisting of 50% or more of their dry weight. They are found in every part of every cell since they are fundamental in all aspects of cell structure and function.

The present work reveals that both CCl₄ and paracetamol grossly depressed hepatic protein concentrations. Fatty acid degeneration induced in the liver by different toxins is related to an impairment of protein synthesis and a consequent fall of lipoprotein formation. A decrease in hepatic cellular proteins has been reported in both CCl₄ (Poli et al., 1979; Ito, 1981) and paracetamol induced hepatic injury (Hart and Timbrell, 1979; Nelson et al., 1980). Several investigators have reported that CCl₄ (Reiner et al., 1972; Uehleke et al., 1973 and Glende et al., 1976) and paracetamol (Younes and Siegers, 1980; Strubelt et al., 1981) bind covalently to liver microsomal proteins in the presence of NADH.

Smuckler and Benditt (1965) reported that an alteration in the microsomal function leading to a breakdown of polyribosomes is the earliest recognizable change produced by CCl₄ in rat liver cells. The decreased life span of mRNA, a rate limiting factor in the polysome cycle is said to account for this change (Gravela and Dianzani, 1979). The treatment of rats with CCl₄ produces an early defect in methylation of hepatocyte RNA which occurs concurrently with a defect in the protein synthetic capacity of isolated ribosomes. Methylation/Demethylation of 2'-0-ribosome sites in rRNA exposed on the surface of
cytoplasmic ribosomal subunits may represent an important cellular mechanism for controlling protein synthesis in quiescent hepatocytes and it appears that CCl₄ disrupts protein synthesis by inhibiting the 2'-0-ribose methylation (Clawson et al., 1987).

During the current investigation, it was observed that both CCl₄ and paracetamol depressed hepatic protein concentrations. This inhibition by CCl₄ is caused, not only by a metabolism-dependant (irreversible) pathway but a metabolism-independent (reversible) mechanism as well. Extracellular calcium is not required for CCl₄ inhibition of protein synthesis (Hegarty et al., 1984).

*S. chirata* administration to CCl₄ treated animals resulted in remarkably enhanced protein concentrations with all the extracts and at all the dose levels studied. With paracetamol-induced damage, however, only the chloroform soluble fraction was effective at 200 mg/kg. *P. kurroa* (methanolic extract) could reverse the effect of only CCl₄, while *R. communis* was ineffective.

It is conjectured that *S. chirata* and *P. kurroa* exert their hepatoprotective effect in elevating toxin-induced depressed protein concentrations by inhibiting the covalent binding of CCl₄ and paracetamol to the hepatic microsomal proteins, possibly by binding at these sites.

4.3.2.10 Enzymes of protein metabolism

4.3.2.10.1 Glutamate pyruvate transaminase

GPT is, as discussed earlier, one of the enzymes involved in protein synthesis. The current studies reveal that both the toxins under study depress hepatic GPT concentrations significantly. Dinman et al. (1963) reported that decreased GPT activity in the liver with CCl₄ was due to an inhibition of GPT
synthesis rather than its release from cell structures. Similar inhibition in GPT activity with CCl₂ has been reported (Fleisher and Wakin, 1956; Morrison et al., 1966). GPT transfers the amino group of alanine to α-ketoglutaric acid, forming glutamic and pyruvic salts (Sobotka and Stewart, 1964).

The methanolic extract of *S. chirata* and its chloroform soluble fraction (at doses of 200 and 400 mg/kg) elevated these depressed levels, while the chloroform insoluble fraction showed no significant change with CCl₂ challenge. However, when paracetamol was given to the experimental animals, only the chloroform soluble extract (at a dose of 200 mg/kg) exhibited protective activity. The methanolic extract of *P. kurroa* significantly elevated hepatic GPT activity after CCl₂ intoxication but proved ineffective when the toxin was paracetamol. The extract of *R. communis* proved ineffective.

It is conjectured that the protective effect of *S. chirata* and *P. kurroa* is either by directly enhancing GPT synthesis or by preventing the toxins from exerting their effect in depressing this enzymes synthesis rather than by their effect on the hepatocyte membrane.

4.3.2.10.2 *Glutamate oxaloacetate transaminase*

This enzyme catalyses the reversible transfer of the amino groups of aspartic acid and α-ketoglutaric acid to glutamic and oxaloacetic acids. The activity of hepatic GOT was found to fall with CCl₂ damage. Paracetamol, however, elevated the activity of this transaminase. Similar results with CCl₂ were reported by Alibert et al. (1963) and Gazzaniga (1975). The decrease could be due to inhibited synthesis of this enzyme rather than to a release from subcellular particles (Recknagel, 1967). Molander et al. (1957) have however, reported enhanced GOT activity with CCl₂.
The current studies reveal depressed hepatic GOT activity with CCl₄ and elevated activity in the liver when the toxin administered is paracetamol. The methanolic extract of *S.chirata* and its chloroform soluble fraction significantly enhanced CCl₄ depressed hepatic GOT activity in the liver. The chloroform insoluble fraction was ineffective. When the toxin was paracetamol, only the chloroform soluble fraction of *S.chirata* could significantly depress GOT levels in the liver. The methanolic extract of *P.kurroa* was significantly active at 100 and 200 mg/kg against CCl₄ intoxication but was ineffective against paracetamol. The methanolic extract of *R.communis* was inactive in both the cases.

The protective action of *S.chirata* and *P.kurroa* is probably due to the ability of these extracts to bind at the same binding sites as the toxins and thus prevent their activity from being expressed in full in either direction.

### 4.3.2.11 Alkaline phosphatase.

The activity of this enzyme in the liver decreased with both CCl₄ and paracetamol intoxication. Similar results with CCl₄ have been reported by Kolesnikova (1973) and Gazzaniga (1975). The methanolic extract of *S.chirata* and its chloroform soluble fraction significantly elevated hepatic alkaline phosphatase activity, while the chloroform insoluble fraction revealed no activity when challenged with CCl₄ at the same dose levels. When the hepatotoxin was paracetamol, only the chloroform soluble fraction of *S.chirata* was significantly active at 200 mg/kg. The methanolic extract of *P.kurroa* significantly elevated alkaline phosphatase levels at 200 mg/kg when challenged with CCl₄. When paracetamol was the hepatotoxin, this drug was ineffective. The methanolic extract of *R.communis* also proved ineffective against
paracetamol hepatotoxicity at a dose of 200 mg/kg.

The wide range of activity of alkaline phosphatase makes it difficult to speculate the mode of action of these plant extracts.

4.3.2.12 Acid phosphatase

Acid phosphatase is a key enzyme having wide spread distribution in cells and is present in different isozyme forms. Acid phosphatase is capable of catalysing the hydrolysis of various phosphate esters at acid pH.

The current studies reveal that both CCl₄ and paracetamol depressed hepatic phosphatase activity. This depression was more pronounced in the case of CCl₄ than in paracetamol-induced hepatic dysfunction.

An increase in hepatic alkaline phosphatase activity with CCl₄ has been reported by Korolenka et al. (1975). A decrease was found by Kolesnikova (1973), while Gazzaniga (1975) observed no change in hepatic acid phosphatase activity with CCl₄. Alpers and Isselbacher (1966) reported an increase in the activity of the supernatant fraction of acid phosphatase on addition of CCl₄ which increases the release of lysosomal enzymes such that a greater percentage is found in the supernatant versus the particulate fraction.

Only the chloroform soluble fraction of the methanolic extract of *S. chirata* was effective at 200 mg/kg in elevating this enzymes activity against paracetamol and CCl₄ induced reduction in hepatic alkaline phosphatase. Methanolic extracts of *P. kurroa* and *R. communis* showed no significant elevations against either of these hepatotoxins.

The action of the chloroform soluble extract of *S. chirata* is difficult to discern due to the presence of this
enzyme in different isozyme forms, each having different activity.

4.3.2.13 5' nucleotidase

This enzyme catalyzes the hydrolysis of ribonucleoside 5'-monophosphates and deoxynucleoside 5' monophosphates to the corresponding nucleosides and orthophosphate. 5' nucleotidase is localized predominantly in the plasma membrane and is found in the hepatocytes in a distribution similar to alkaline phosphatase.

Both CCl₄ and paracetamol intoxication resulted in enhanced 5' nucleotidase activity in the liver. Agostini et al. (1980) reported no change in the activity of this enzyme with CCl₄. Kamath and Rubin (1974) found depressed activity and Hanczycowa and Wozniak (1980) observed an elevation in this enzyme's activity. Any disruption in 5'nucleotidase activity would result in manifold changes, as this enzyme regulates the concentration of 5'AMP, influences the overall activity of the glycolytic pathway and the citric acid cycle as the activities of the key enzymes concerned are allosterically modified by 5'AMP (Bodansky and Schwartz, 1968).

The methanolic extract of S. chirata and its chloroform soluble fraction significantly depressed the enzyme's activity at a dose of 200 mg/kg, when either CCl₄ or paracetamol were administered. The activity of the chloroform soluble fraction was significantly higher than that of the methanolic extract. The chloroform insoluble fraction of S. chirata was ineffective. The extracts of P. kurroa and R. communis significantly depressed enzyme activity at a dose of 200 mg/kg when the hepatotoxin was paracetamol.

The toxins under study caused wide spread devastation of the plasma membrane. Since this is the major site of localization of 5' nucleotidase, it follows, that with plasma membrane
disruption, the activity of this enzyme will be altered. The plant extracts found effective, prevented the disruption of the plasma membrane to different extents (this has also been evidenced histologically) and thus stabilised 5' nucleotidase activity.

4.3.2.14 Mg$^{2+}$ Adenosine triphosphatase

ATPase are a group of enzymes having wide spread distribution in cellular systems. The current investigations reveal that both CCl$_4$ and paracetamol drastically inhibit the activity of this enzyme. These membrane bound enzymes are actively involved in the transport of cations across the membranes and regulate cellular respiration by regulating ADP/Pi and ADP/ATP ratio in the cells, essentially required to maintain respiration in the mitochondria. Mg$^{2+}$ ATPase is present in bound form with mitochondria and hence control oxidative phosphorylation in these organelle (Lehninger, 1978; Mann and Mann, 1981). By maintaining the characteristic intracellular concentrations of ions, Mg$^{2+}$ ATPase determine the transmembrane electrochemical gradient (Skou, 1957). ATPase activity is highly dependant upon the lipid composition of the plasma membrane. Changes in membrane fluidity could result in specific degradation of ATPase (Yahuaca et al., 1985).

CCl$_4$ damages the ion transport system as it acts synergestically with the energy conversion system at the early stages. It also damages the energy conversion system resulting in markedly decreased ATP synthesis (Akahori, 1975). Several workers have reported decreased ATPase activity with CCl$_4$ (Dianzani et al., 1975; Luthra et al., 1984) and paracetamol (Vonen and Moerland, 1984 and Moore et al., 1985).

The methanolic extract of _S. chirata_ showed significant elevations of ATPase activity against CCl$_4$ at its 200 mg/kg dose.
It was ineffective at 100 mg/kg. The chloroform soluble fraction of the methanolic extract of *S. chirata* had significant activity while the chloroform insoluble fraction was ineffective. At 400 mg/kg, the chloroform soluble fraction brought CCl₄ depressed ATPase activity close to control levels. A similar trend was observed when the hepatotoxin used was paracetamol. The methanolic extract of *P. kurroa* exhibited significant activity against CCl₄ at 100 and 200 mg/kg. It was also highly effective at 200 mg/kg, when paracetamol was used as the hepatotoxin. The extract of *R. communis* proved ineffective.

It can thus be conjectured that *S. chirata* and *P. kurroa* enhanced hepatic ATPase activity after CCl₄ and paracetamol intoxication by restoring the lipid composition of the plasma membrane, thus, restoring the damaged ion transport system, since this had a direct effect upon ATPase.

4.4 CONCLUSION

The results obtained with *Swertia chirata* reveal it to be a promising candidate for further investigations with a view to the development of an antihepatotoxic drug. The chloroform soluble fraction of the methanolic extract of *S. chirata* was significantly hepatoprotective against both the toxins. The wide range of the activity of this plant extract can be assessed by the observed effects on lipid metabolism (where it acts as an antioxidant), carbohydrate metabolism (where, besides enhancing activity of all the carbohydrate enzymes in reversal of the effects of paracetamol, it also depresses CCl₄ enhanced glycogen phosphorylase activity, as well as elevating activity of the other enzymes of carbohydrate metabolism) and protein metabolism (in elevating protein concentrations and hepatic GOT and GPT activity when the toxin is CCl₄ and in depressing elevated GOT activity when
paracetamol is the toxicant).

The present studies have also revealed the efficacy of the methanolic extract of the roots of *P. kurroa* which exhibits wide ranging effects, significantly normalising hepatic functions against both carbon tetrachloride and paracetamol induced hepatotoxicity.

The methanolic extract of the leaves of *R. communis*, which did not prove significantly hepatoprotective against *CCl*<sub>4</sub> induced hepatotoxicity, prevents the destructive effect of paracetamol on lipid metabolism.