5. SUMMARY
The present investigations embody histological, histochemical and biochemical studies with the extracts of *Swertia chirata*, *Picrorhiza kurroa* and *Ricinus communis* against CCl₄ and paracetamol hepatotoxicity. Biochemical assessment of the enzymes of major metabolic pathways have been given primary importance.

It is an established fact that hepatic dysfunction is the result of alterations in the various metabolic pathways in the liver. Both CCl₄ and paracetamol cause widespread necrosis and membrane breakdown, accompanied by enhanced hepatic lipid concentrations and decreased protein content. They also interfere with the metabolism of lipids, carbohydrates and proteins in the liver.

To investigate the antihepatotoxic potential of *S. chirata*, *P. kurroa* and *R. communis*, both *in vitro* and *in vivo* experiments were conducted.

*In vitro* experiments were carried out using primary cultured rat hepatocytes. Initially, 18 commercial herbal formulations were screened for their antihepatotoxic potential, using carbon tetrachloride as the toxicant. CCl₄ damages the hepatocyte membranes, causing an efflux of certain membrane bound enzymes into the culture media. The media GOT and GPT levels were monitored as the basis of assessing hepatocyte damage and repair. The results obtained showed that practically all the formulations studied could prevent enzyme leakage to varying extents.

The *in vitro* studies conducted on the various plant extracts were primarily focused on the methanolic extract of *S. chirata* which exhibited significant antihepatotoxic potential and was thus further fractionated into chloroform soluble and
chloroform insoluble fractions. The former was found highly effective. The water soluble extract of *S. chirata* was also mildly hepatoprotective. The methanolic extracts of *P. kurroa* and *R. communis* also revealed significant activity. The methanolic extract of *P. kurroa* proved more active than the methanolic extract of *S. chirata* which was more effective than the extract of *R. communis*.

Histological profile of the livers of animals treated with CCl₄ reveals that this hepatotoxin caused a wide spread breakdown of membranes, although the nuclear membranes remained intact. Necrosis was rampant as was mononuclear cell infiltration. Swollen balloon cells were seen at the periphery. In fact, there was a general breakdown of the liver histoarchitecture. Paracetamol intoxication too resulted in similar damage, in addition to hepatic congestion.

These anomalies with CCl₄ were significantly decreased with the methanolic extracts of *P. kurroa* and *S. chirata* and the chloroform soluble fraction of *S. chirata*. The chloroform insoluble fraction of *S. chirata* and the methanolic extract of *R. communis* caused very little recovery at lower concentrations (100 and 200 mg/kg) and were toxic at higher dose levels (400 and 500 mg/kg). Under paracetamol intoxication only the chloroform soluble fraction of *S. chirata* revealed significant hepatoprotective activity. The methanolic extracts of *S. chirata* and *P. kurroa* also improved the liver histoarchitecture, although to a lesser extent. The other two extracts i.e. the methanolic extract of *R. communis* and the chloroform insoluble fraction of *S. chirata* proved ineffective.

Histochemical studies revealed enhanced total lipids and depressed protein concentrations in the liver treated with
both the hepatotoxins. CCl4 caused marked depletion of hepatic glycogen, whereas the change was less obvious with paracetamol. All the extracts studied caused some improvement in hepatic proteins. Total lipids were highly depressed by the methanolic extract of S.chirata and its chloroform soluble fraction and the methanolic extract of P.kurroa against CCl4 and by the extract of R.communis and the chloroform soluble fraction of S.chirata when the toxin was paracetamol. Glycogen concentrations were significantly enhanced by the chloroform soluble fraction and the methanolic extract of S.chirata and the methanolic extract of P.kurroa.

Biochemical studies revealed that CCl4 and paracetamol effected nearly all the major metabolic pathways in the liver, causing drastic changes in enzyme activities and in quantities of lipids, proteins and also glycogen.

The overall picture presented by the plant extracts under study reveals that the 400 mg/kg dose of the methanolic extract of P.kurroa and the chloroform soluble fraction of S.chirata prove to be the most effective against CCl4 induced hepatotoxicity, followed by the 200 mg/kg dose of these two plant extracts and the methanolic extract of S.chirata. When the toxin administered is paracetamol, the chloroform soluble fraction of S.chirata exhibited highest hepatoprotective activity followed by the methanolic extracts of S.chirata and P.kurroa. The methanolic extract of P.kurroa is ineffective at 50mg/kg and shows maximum activity at 400 mg/kg. The plant extracts with the least antihepatotoxic activity are the chloroform insoluble fraction of S.chirata and the methanolic extract of R.communis leaves.

Studies on the serum enzymes GOT, GPT, LDH and alkaline
phosphatase reveal that the methanolic extract of \textit{P. kurroa} has the highest normalizing effect against CCl\textsubscript{4} followed by the chloroform soluble fraction of the methanolic extract of \textit{S. chirata}. This latter fraction has significant activity against paracetamol-induced hepatotoxicity as well. The methanolic extract of \textit{S. chirata} reveals maximum activity at 200 mg/kg. The chloroform insoluble fraction of \textit{S. chirata} and the methanolic extract of \textit{R. communis} have the least hepatoprotective effect in normalizing serum enzymes elevated by both CCl\textsubscript{4} and paracetamol.

While studying the lipids (total lipids, total phospholipids, phosphatidyl choline, phosphatidyl inositol, phosphatidyl serine, phosphatidyl ethanolamine, sphingomyelin, total cholesterol, cholesterol esters, free cholesterol, triacylglycerides, triglycerides, mono and diglycerides and free fatty acids) and certain enzymes involved in lipid metabolism (malate dehydrogenase, glucose 6-phosphate dehydrogenase and HMG CoA reductase), the chloroform soluble fraction of \textit{S. chirata} was found to be the most effective against both the toxins, followed by the methanolic extracts of \textit{S. chirata} and \textit{P. kurroa}. The chloroform insoluble fraction of \textit{S. chirata} and the methanolic extract of \textit{R. communis} exhibited the least hepatoprotective activity with respect to parameters involved in lipid metabolism.

The enzymes of carbohydrate metabolism (glucose 6-phosphatase, glucose 6-phosphate isomerase, glycogen phosphorylase, fructose 1,6-diphosphatase, amylase and hexokinase) and glycogen are also restored to the maximum extent with the chloroform soluble fraction of \textit{S. chirata}, which is most effective at 400 mg/kg, followed by the 200 mg/kg dose. The antihepatotoxic activity of the methanolic extract of \textit{P. kurroa} is a close second, followed by the methanolic extract of \textit{S. chirata}. The extract of
R. communis and the chloroform insoluble fraction of S. chirata exhibit very mild activity in normalizing carbohydrate metabolism against CCl₄ and are ineffective against paracetamol intoxication.

Protein metabolism abnormalities (studied on the basis of hepatic proteins, GOT and GPT), caused by CCl₄ induced necrosis were restored most significantly by the methanolic extract and chloroform soluble fraction of S. chirata, followed by the methanolic extract of P. kurroa. The abnormalities in protein metabolism induced by paracetamol hepatotoxicity were restored most significantly by the methanolic extracts of P. kurroa and S. chirata and the chloroform soluble fraction of the methanolic extract of S. chirata. The methanolic extract of R. communis revealed the lowest profile of activity in this regard.

The present studies thus reveal that while the methanolic extract of R. communis has limited activity, S. chirata and P. kurroa are promising candidates as hepatoprotectants of the future.