Chapter Six

General Discussion

Exposure to toxins is prevalent in our age of industrialisation, and the entry of chemicals into the body produces many deleterious effects in man and other beings. The liver is one of the important organs which is severely damaged as a result of chronic exposure to toxins, which may be a drug, a pesticide, an industrial effluent or any other chemical entity. Viral hepatitis is yet another common disease in the world, especially in the developing countries. However, there are no effective drugs for the treatment of most of the hepatic ailments. Thus, there is an urgent need to develop potent hepatoprotective agents against such hepatic dysfunctions. In recent years a little progress has been made in this direction, and a considerable amount of research has been carried out in this regard. Attempt to harness the potency of plants to develop new hepatoprotective drugs has become the need of the hour.

Many medicinal plants used thousands of years ago are still being used today, and their rationale could be scientifically justified by modern pharmacology and medicine.¹⁵⁸ These plants may serve as a potential source for new drugs, especially with the help of modern scientific knowledge.

In the past two decades, many crude drugs have been developed as an alternative medicine for the treatment of various liver diseases. There is an explosion of global awareness concerning the efficacy of many a plants to protect the liver from injuries induced by hepatotoxins.
The use of traditional medicine is widespread and plants still present a large source of structurally novel compounds that might serve as leads for the development of new drugs. That is why a large section of the world’s population relies on traditional remedies to treat a plethora of diseases, including liver ailments. These medicinal plants are of immense value due to its low cost, easy access and ancestral experience. It is high time for us to explore the possibility of identifying our traditional therapeutic knowledge and interpret it according to the recent advances, in order to give it a deserving place.

In order to efficiently metabolize drugs, during the process of evolution, the liver has developed “drug metabolizing enzymes” which are different from the enzymes of intermediary metabolism. Most of these enzymes are largely located in the hepatic microsomes. Biotransformation of a drug or a xenobiotic compound following its exposure can alter its distribution and action leading to its detoxification and excretion, or enhance its toxicity due to the biochemical disruption caused by reactive metabolites arising from biotransformation. Biotransformation of xenobiotics usually occurs in two phases. Phase I metabolism (detoxification) involves oxidative, reductive and or hydrolytic reactions that cleave substrate molecules to produce a more polar moiety. Phase II reactions (synthetic reactions) involve conjugation of certain endogeneous molecules to the products of phase I reactions. Cytochrome P-450 enzymes are responsible for the metabolic conversion of many drugs to the polar metabolites via phase I and phase II reactions to earlier excretion.

Liver injury induced by CCl₄ is a commonly used model for the screening of hepatoprotective drugs. Since the changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis, CCl₄-mediated hepatotoxicity was taken here as the experimental model for liver injury. CCl₄ is a potent hepatotoxin producing centriflobular hepatic necrosis which causes liver injury.
Serious attention is now paid to the cytotoxicity of active oxygen / free radicals as the root cause of various pathological conditions. Numerous pathological changes observed in CCl₄-intoxicated rats can be ascribed to the insult by free radicals produced during the metabolism of CCl₄. These highly reactive and unstable 'CCl₃ radicals can elicit subsequent lipid peroxidation chain reaction culminating in liver damage.

It has been observed that a majority of Ayurvedic preparations prescribed in diseased conditions now being explained to be mediated through oxidative stress, possesses strong anti-radical properties. Hepatoprotective medicinal plants too are not exceptions. Most of the diseased conditions are being considered caused primarily due to the imbalance between the pro-oxidant and antioxidant homeostasis. Antioxidant principles from natural resources possess multifacetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance. Therefore, much attention is being directed to harness and harvest the antioxidant principles from natural resources.

Antioxidants are ubiquitous in natural products. The putative therapeutic impression of many traditional medicines appears to be attributed to the presence of antioxidant principles. Better understanding of the structure - activity - relationship of these phytochemicals and their relative importance in different mechanisms may provide deeper insight in finding out better and safer therapeutics. Identification and isolation of anti-free radical / antioxidant principles from such natural resources are simultaneously presenting enormous scope for their better therapeutic application. It is under this context lies the relevance of the present study. The aim of the study was to examine if treatment of drugs from C. fenestratum / C. ochrioides was capable of eliciting hepatoprotective and antioxidant effects in CCl₄-intoxicated rat model of hepatotoxicity.
Chronic CCl₄ intoxication was found leading to many cellular and tissue abnormalities. As a result of these abnormalities, alterations were observed in several biochemical constituents in experimental animals. In this study, CCl₄ exposure has been found to result in alteration in the activities of conventional hepatic marker enzymes, i.e., AST, ALT, ALP and GGT.

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentrations in cytoplasm, and AST in particular, also exists in the mitochondria. In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of enzymes through the plasma membrane, thereby causing an increased enzyme level in serum. If injury involves organelles such as mitochondria, soluble enzymes like AST normally located there will also be similarly released. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver.

Administration of drugs causes decrease in the activity of the above enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄. This is supported by the view that serum levels of transaminases return to normal, with the healing of hepatic parenchyma and regeneration of hepatocytes.

These findings also indicate changes in the permeability of the plasma membrane caused due to peroxidation of lipid by the generation of free radicals. As a result, the lipid profiles were found to be enhanced in liver tissue followed by a simultaneous depletion in the level of protein.

The free radicals generated during the metabolism of CCl₄ bind to proteins and hepatocyte membrane and lead to diminution of protein and impairment of mitochondrial glutathione redox status. LPO reactions have resulted in a decline in the activities of antioxidant enzymes such as SOD, CAT, GPX etc. Lipid
peroxides produced from unsaturated fatty acids are measurable in the form of TBARS and CD. Exposure to CCl₄ has resulted in an increment in the activity of LDH in serum and a decline in the activity of G-6-PD in liver. The liver architecture too in CCl₄-intoxicated rats showed a distorted look with severe steatosis, ballooning degeneration, nodule formation and fibrosis. The consequence of such damage was a significant reduction in the liver functions.

Co-administration of phyto-drugs has improved the situation much, thus indicating the protective effect of these drugs against hepatopathy due to CCl₄. All the altered biochemical parameters observed in CCl₄-treated rats were found attaining near-normalcy in drug co-administered rats. An assessment of percentage of hepatoprotection offered by the various extracts of the plants was also carried out.

In the pilot study, the maximum hepatoprotective effect with respect to hepatic marker enzymes was offered by the *C. fenestratum* stem powder at the dose of 800 mg/kg bw, and powdered *C. orchioides* rhizomes at the dose of 750 mg/kg bw. (Fig 6.1) Hence, these doses of the drugs can be deemed as the most potential doses.

Figure 6.1 Overall hepatoprotection by powdered *Coscinium fenestratum* stem and *Curculigo orchioides* rhizomes.

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase; PSCF: Powdered stem of *Coscinium fenestratum* 800 mg/kg body weight; PRCO: Powdered rhizomes of *Curculigo orchioides* 750 mg/kg body weight.
When different extracts of *C. fenestratum* stem at the dose of 75 mg / kg bw were co-administered to CCl₄-intoxicated rats, the maximum % of hepatoprotection was shown by the methanol extract in respect of the activities of liver marker enzymes (Fig 6.2). Similar observations were made in the case of methanol extracts of *C. orchioides* rhizomes too (Fig 6.2).

**Figure 6.2 Overall hepatoprotection by different extracts of *C. fenestratum* and *C. orchioides***

![Graph showing overall hepatoprotection](image)

PETR : Petroleum ether extract ; Benz : Benzene extract ; CHLO : Chloroform extract ; METH : Methanol extract ; Wat : Water extract.  
CF : *Coscinium fenestratum* ; CO : *Curculigo orchioides*

A dose dependent study with methanol extracts of *C. fenestratum* (doses 40, 60 and 80 mg / kg bw), and that of *C. orchioides* rhizomes (doses 40, 70 and 100 mg / kg bw) showed that both are effective in combating hepatotoxicity due to CCl₄, and also seen that methanol extract of *C. fenestratum* is more effective as compared to that of *C. orchioides* (Fig 6.3 and 6.4).
Figure 6.3 Percentage of hepatoprotection by different doses of MECF in respect of liver marker enzymes

- AST: Aspartate aminotransferase
- ALT: Alanine aminotransferase
- ALP: Alkaline phosphatase
- GGT: Gamma glutamyl transpeptidase

MECF: Methanol extract of *C. fenestratum*

Figure 6.4 Percentage of hepatoprotection by different doses of MECO in respect of liver marker enzymes

- AST: Aspartate aminotransferase
- ALT: Alanine aminotransferase
- ALP: Alkaline phosphatase
- GGT: Gamma glutamyl transpeptidase

MECO: Methanol extract of *C. orchioides*
Fig 6.5 depicts % of overall hepatoprotection shown by MECF and MECO in respect of liver marker enzymes, protein as well as lipid profiles in serum of experimental animals. In this case too, MECF was found evoking maximum hepatoprotection. When hepatoprotective efficacy of MECF and MECO was evaluated with respect to the lipid profiles in the liver (Fig 6.6) and kidney (6.7), the maximum activity has been exhibited by MECF.

Figure 6.5 Overall hepatoprotection shown by MECF (60 mg/ kg) and MECO (70 mg/ kg) with respect to different biochemical parameters in serum

LM enz : Liver marker enzymes; T. protein : Total protein; T. lipids : Total lipids; Cholrl : Cholesterol; P. lipids : Phospholipids; TGL : Triglycerides; MECF : Methanol extract of *C. fenestratum*; MECO : Methanol extract of *C. orchioides*. 
Figure 6.6 Overall hepatoprotection shown by MECF (60 mg/kg) and MECO (70 mg/kg) with respect to different biochemical parameters in liver.

T. lipid: Total lipids; Cholesterol; P. lipids: Phospholipids; TGL: Triglycerides; MECF: Methanol extract of *C. fenestratum*; MECO: Methanol extract of *C. ochioides*.

Figure 6.7 Overall hepatoprotection shown by MECF (60 mg/kg) and MECO (70 mg/kg) with respect to different biochemical parameters in kidney.

T. lipid: Total lipids; Cholesterol; P. lipids: Phospholipids; TGL: Triglycerides; MECF: Methanol extract of *C. fenestratum*; MECO: Methanol extract of *C. ochioides*.
Figures 6.8 and 6.9 show the % of hepatoprotection offered by MECF and MECO in respect of the antioxidant parameters in liver and kidney of experimental rats. As in the former case, here too MECF was found to be more active.

The decline in the activity of hepatic microsomal G-6-P-D can be attributed to the damage caused to the microsomal membrane due to CCl₄ as a result of LPO reactions. Attainment of near-normalcy in this regard is a clear indication of the protective effect offered by the drugs on microsomal membranes. An enhancement in the activity of serum LDH is indicative of hepatic dysfunctions, and its retrieval towards normalcy can be deemed as the protective effect of a drug. On evaluating the % of hepatoprotection shown by MECF and MECO with regard to the activities of G-6-PD in liver and LDH in serum (Fig 6.10), it was found that MECF evoking enhanced activity as compared to MECO.

**Figure 6.8 Overall hepatoprotection by MECF and MECO in respect of antioxidant parameters in the liver of rats.**

TBARS: Thiobarbituric acid reactive substances; CD: Conjugated dienes; GSH: Reduced glutathione; SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase; GR: Glutathione reductase; GST: Glutathione-S-transferase. MECF: Methanol extract of *C. fenestratum*; MECO: Methanol extract of *C. ochrooides*. 
Figure 6.9 Overall hepatoprotection by MECF and MECO in respect of antioxidant parameters in the kidney of rats.

TBARS : Thiobarbituric acid reactive substances; CD : Conjugated dienes; GSH : Reduced glutathione; SOD : Superoxide dismutase; CAT : Catalase; GPX : Glutathione peroxidase; GR : Glutathione reductase; GST : Glutathione -S- transferase.MECF : Methanol extract of C. fenestratum; MECO : Methanol extract of C. orchioides.

Figure 6.10 Percentage of hepatoprotection offered by MECF and MECO in respect of activities of G-6-PD (in liver) and LDH (in serum).

G-6-PD : Glucose-6-phosphate dehydrogenase; LDH : Lactate dehydrogenase
MECF : Methanol extract of C. fenestratum; MECO : Methanol extract of C. orchioides.

In all our studies MECF was found to be more effective than MECO in combating liver damage due to CCl₄ intoxication. This prompted us to purify the active hepatoprotective principles from the stem of C. fenestratum. Assessment of
% of hepatoprotection elicited by different doses (5, 10 and 15 mg / kg bw) of the purified sample (berberine) from *C. fenestratum* showed that 10 mg is the most effective dose with regard to the activities of liver marker enzymes and antioxidant enzymes (Fig 6.11).

**Figure 6.11** Percentage of hepatoprotection offered by the purified compound from *C. fenestratum* in respect of different parameters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>5 mg</th>
<th>10 mg</th>
<th>15 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPX</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase
ALP: Alkaline phosphatase; GGT: Gamma glutamyl transpeptidase
SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase

Free radical-induced LPO has gained much importance, because of its involvement in several pathological conditions, such as liver disorders. LPO involves the formation of lipid radicals, oxidation of unsaturated lipids and the eventual destruction of membrane lipid producing a variety of break down products and deleterious effects. These injurious processes can be prevented by reducing or destroying the formation of free radicals which are continuously formed in a biological system. In normal tissues, endogenous enzymes (SOD, CAT, GPX and GST etc.) are there that counteract the free radicals. Situations in which pro-oxidant mechanisms within the body are more active than the antioxidant mechanism
Oxidative stress predispose and contribute to the pathogenesis of several ailments in various organs of the body. Antioxidants are a small group of substances that protect living cells from the destructive consequence of powerful oxidising intermediates that can be formed from oxygen.

Studies on the hepatoprotective experimental model indicated that the biotransformation of CCl₄ occurs in the endoplasmic reticulum and is mediated by Cyt P-450, the principal isoform implicated as the catalyst being Cy P2E1. Cyt P-450 is inhibited suicidally by the reactive metabolites of CCl₄. CCl₃ radical initially formed being relatively unreactive, reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl₃OO·), the probable initiator of lipid peroxidation. The free radical brings about autooxidation of the fatty acids present in the cytoplasmic membrane phospholipids, and causes functional and morphological changes in the cell membrane. Furthermore, influx of extra-cellular calcium ions into cell is claimed to be an important step leading to cell death. Therefore, the examination of the preventive action in liver damage caused by CCl₄ may give an indication of the liver protective action of drugs in general.

The results of the present study suggest that C. fenestratum and C. orchioides contain free radical-scavenging activity, which could exert a beneficial action against pathological alterations caused by CCl₄ exposure. The anti-hepatotoxic activity of these plants may be due to:

1. inhibitory effects on microsomal enzymes or on lipid peroxidation.
2. stimulatory effects on hepatic regeneration
3. free radical scavenging effects
4. membrane stabilisation
5. altered mode of detoxification of compounds
(6) activation of reticulo-endothelial system, or

(7) enhancement of protein synthesis.

Thus, the present data provide a rationale for the use of *C. fenestratum* and *C. orchioides* as a suitable herbal treatment for the management of hepato-biliary disorders.

**Significance and the future prospects**

In this study, *C. fenestratum* and *C. orchioides*, the two medicinal plants used for the treatment of jaundice in the traditional system of medicine were subjected to scientific evaluation with the help of modern medicinal chemistry and pharmacology. Both were found to be highly effective in resisting induced hepatopathy due to CCl₄ exposure. As CCl₄-intoxicated liver injury simulates viral hepatitis, the role of these medicinal plants for combating viral hepatitis may not be dispensed with.

Future studies in this regard may be earmarked towards improvised techniques to synthesise the active principle from the stem of *C. fenestratum*, so as to combat effectively the ever increasing incidence of hepatitis. It is also advisable to explore the possibility of isolating and purifying the active hepatoprotective principles from the methanol extract of *C. orchioides*. Ample chances are awaiting the researchers in this direction.