Chapter – V

Pharmacology
1. Introduction

1.1 Introduction

Liver, largest organ in the body is being evolved to maintain the body’s internal milieu and also protect itself from the challenges it faces during its functioning. It is the vital organ of metabolism and excretion. Liver disease is still a worldwide health problem. Unfortunately synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects\(^1\). In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders\(^2\). In India, about 40 poly herbal commercial formulations reputed to have hepatoprotective action are being used. It has been reported that 160 phyto constituents from 101 plants have hepatoprotective activity\(^3\).

Drug-induced liver injury is a major health problem that challenges healthcare professionals. Drug induced liver injury accounts for more than 50% of acute liver failure, including hepatotoxicity caused by overdose of acetaminophen (39%) and idiosyncratic liver injury triggered by other drugs (13%)\(^4\). Tuberculosis is one of the most common infectious diseases. In India, pulmonary tuberculosis is one of the major causes for adult deaths\(^5\). Isoniazid (INH) and Rifampicin (RIF) the first line drugs used for tuberculosis chemotherapy are associated with hepatotoxicity\(^6\). The rate of hepatotoxicity has been reported to be much higher in developing countries, like India (8 to 30 %.) compared to that in advanced countries (2 to 3%). With a similar dose schedule\(^7\) oxidative stress as one of the mechanism for INH +RIF induced hepatic injury\(^8\).
Majority of normally formed free radicals is removed by the action of reduced glutathione. In circumstance where there is reduction in glutathione results in the initiation of lipid peroxidation (LPO) resulting in tissue injury. Hepatotoxicity can affect hundreds of millions of people worldwide. It is the common non neoplastic cause of death among hepatobiliary and digestive disorders. Serious side effects, the cost of the modern medicine and improper channel of treatment and competitive efficacy of natural products made the persons through the world to look for classical plant drugs for the treatment of hepatotoxicity. In view of the biological properties and chemical constituents of plant from *Stephania wightii* Dunn, it was decided to study the plant *Stephania wightii* Dunn which is widely used in folk medicine.
2. Review of literature

In India more than 87 medicinal plants were used in different combinations in the preparation of 33 patented herbal formulations for liver disorder. Some of the most commonly used plants in herbal formulations were *Andrographis paniculata*, *Apium graveolens* *Eclipta alba*, *Embelia ribes*, and *Trachyspermum ammi*. Some of the plant constituents possessing hepatoprotective activity were Andrographolide from *Andrographis paniculata*, Silybin from *Silybum marianum*, Picroside I from *Picrorhiza kurroa*, curcumin from *Curcuma longa* and Fumaric acid from *Sida cordifolia*\(^{10}\). The hepatoprotective activity of *Psidium guajava* in acute experimental liver injury induced by paracetamol was studied. The effects observed were compared with a known hepatoprotective agent, Silymarin. In the acute liver damage induced by paracetamol, *Psidium guajava* leaf extracts (500mg/kg, po) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, protein and bilirubin, some antioxidant enzymes, reduced glutathione, Glutathione peroxidase, superoxide dismutase and catalase activities, were also evaluated in the rats liver homogenate. The higher dose of the extract (500mg/kg, po) showed to be more effective than the lower dose (250mg/kg, po). Histological examination of the liver tissues supported the hepatoprotection. It is concluded that the aqueous extract of leaves of guava plant possesses good hepatoprotective activity.\(^{11}\)

The suspensions of chloroform extract of leaves in 0.3% carboxy methyl cellulose (CMC) was evaluated for hepatoprotective activity in Wistar albino rats by inducing hepatic injury with d-galactosamine (400 mg/kg). The chloroform extract of the Indian medicinal plant *Polygala arvensis* at an oral dose of 200 mg/kg and 400 mg/kg exhibited a considerable protection effect\(^{12}\). The hydroalcoholic extract of *Aerva lanata* (600mg/kg) was administered orally to the animals with hepatotoxicity induced by
paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. The test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin\(^{13}\). Hepatoprotective activity of Ethanolic extract of *Stachytarpheta indica* L. (Vahl) was evaluated by carbon tetrachloride induced toxicity with standard hepatoprotective drug as Liv-52. Ethanolic extract of *Stachytarpheta indica* L. (Vahl) produced decrease in CCl4, induced elevated levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), SALP and Serum bilirubin and reversed total protein in rats indicating hepatoprotective activity at the dose of 200mg/kg body weight and was comparable to that of standard drug Liv-52(1ml/kg body weight)\(^{14}\). The hepatoprotective activity of the aqueous-methanolic extract of *Ambrosia maritima* was investigated against acetaminophen (paracetamol, 4-hydroxy aceta

The present review provided the status report on the scientific approaches made to herbal preparations used in Indian systems of medicine for the treatment of liver diseases. In spite of the availability of more than 300 preparations for the treatment of jaundice and chronic liver diseases in Indian systems of medicine using more than 87 Indian medicinal plants, only four terrestrial plants had been scientifically elucidated *Glycyrrhiza glabra* was shown to be hepatoprotective and capable of inducing an indigenous interferon. *Picrorhiza kurroa* was proved to be anti-inflammatory, hepatoprotective and immunomodulatory. *Phyllanthus amarus* was
effective against hepatitis B and C viruses, hepatoprotective and immunomodulating, as well as possessing anti-inflammatory properties\textsuperscript{16}.

The roots of \textit{Boerhaavia diffusa} \textit{L.}, commonly known as ‘Punarnava’, were used by a large number of tribes in India for the treatment of various hepatic disorders. The hepatoprotective activity of roots exhibited marked protection of a majority of serum parameters, i.e. glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), acid phosphatase (ACP) and ALP, but not GLDH and bilirubin, the aqueous form of drug (2 ml/kg) administration had more hepatoprotective activity than the powder form\textsuperscript{17}. \textit{Scoparia dulcis} \textit{L.} (family: Scrophulariaceae) is a glagabrious under shrub with small white flowers, which was widely used in the indigenous system of medicine for treating liver ailments The hepatoprotective activity of 1:1:1 petroleum ether, diethylether, and methanol (PDM) extract of \textit{Scoparia dulcis} \textit{L.} against carbon tetrachloride-induced liver injury in mice was studied. 800mg/kg, p.o. concentration of PDM significantly prevented the carbon-tetrachloride induced elevation serum Aspartate amino transferase, (ASAT), alanine amino transferase,(ALAT), alkaline phosphatase (ALP), and Total proteins, (TP) levels and prevented the decrease in superoxide dismutase (SOD), glutathione reductase (GSHR), and Glycogen. Standard silymarin at a dose of 100mg/kg p.o. also significantly prevented all these changes\textsuperscript{18}. The tribal groups of Western Ghats use stem bark extract of \textit{Pterocarpus santalinus} in treating diabetes, fever, snakebite and jaundice. The hepatoprotective ctivity of crude aqueous and ethanol stem bark extracts of \textit{Pterocarpus santalinus} (Fabaceae) using carbon tetrachloride induced hepatic damage in male Wister albino rats was studied. The animals treated with aqueous and ethanol stem bark extracts exhibited decrease in Total bilirubin., Aspartate transferase (AST), alanine transferase(ALT), and, alkaline phosphatase (ALP)\textsuperscript{19}. 


V.Gujrati et al investigated the hepatoprotective activity of alcoholic and aqueous extracts of leaves of Tylophora indica (Asclepiadaceae), a traditionally medicinal plant against ethanol-induced hepatotoxicity. The alcoholic and aqueous leaf extracts of pretreated animals had significantly reduced AST, ALT and ALP levels and increased total protein and serum albumin levels, indicating their hepatoprotective effect against alcohol-induced liver cell damage. Eugenia jambolana Lam. (Myrtaceae) popularly known as jamun was being used to liver dysfunctions and diabetes by the traditional practitioners. Administration of (doses 100, 200, and 400mg/kg p.o.) significantly prevented carbon tetrachloride induced elevation of serum SGOT, SGPT, ALP, ACP and bilirubin level. The results were comparable to that of standard positive control Liv.52. V.P.Raj et al studied the in vitro and in vivo hepatoprotective effects of the total alkaloid fraction of Hygrophila auriculata (Acanthaceae) leaves against carbon tetrachloride – induced toxicity in freshly isolated rat hepatocytes, HepG2 cells, and animal models. Hygrophila auriculata is an erect semi woody plant, in homeopathy it is being used to treat jaundice. Treatment with total alkaloid fraction of Hygrophila auriculata at 80mg/kg body weight showed a significant decrease in ASAT, ALAT, ALP, total bilirubin, lactate dehydrogenase (LDH) and significant elevation in the triglycerides (TGL), total proteins and albumin levels in serum. Standard silymarin at 250mg/kg b.w. also exhibited similar results.

Cissus quadrangularis Linn (Vitaceae) was an edible plant found in hotter parts of India used for treatment of bone fracture, piles, chronic ulcers, constipation and blindness. The hepatoprotective activity of methanol extract of Cissus quadrangularis against isonizid-induced hepatotoxicity in rats was studied. Isoniazid- treated animals showed marked increase in the level of enzymes AST, ALT,ALP and bilirubin total and direct. Pretreatment of rats with methanol extract of Cissus quadrangularis reduced the
level of marker enzymes and bilirubin significantly\textsuperscript{23}. Gnanaprakash and \textit{et al} used aqueous extract of \textit{Flacourtia indica} to prevent Carbon Tetrachloride Induced hepatotoxicity in rat. Treatment of aqueous extract of \textit{Flacourtia indica} leaves (250 & 500 mg/kg) exhibited a significant protective effect by altering the serum levels of AST, ALT, ALP, Total Protein, and Total Bilirubin\textsuperscript{24}. Nazneen \textit{et al} studied the petroleum ether, ethyl acetate and methanol extracts of the aerial parts of \textit{Flacourtia indica} (Burm.f.) Merr., against hepato-protective properties. In paracetamol-induced hepatic necrosis in rat models, all extracts were found to reduce serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum alkaline phosphatase (SAP). The most significant reduction of the serum level of SGOT, SGPT was exhibited by petroleum ether and ethyl acetate extracts\textsuperscript{25}.

Tetrandrine (TET) is the major pharmacologically active compound of Chinese herb \textit{Stephania tetrandra} S Moore, which has been used traditionally for the treatment of rheumatic disorders, silicosis and hypertension. TET was able to prevent T-cell-mediated liver injury \textit{in vivo}. The beneficial effect may depend on suppressing the production of various inflammatory mediators in the liver through inhibiting of NF-κB activation\textsuperscript{26}. 
3. Materials and Methods

3.1 Preparation of plant extracts

The powder of *Stephania wightii* Dunn was dried in the shade. The shade-dried powder 750g was extracted first with ethanol and water by continuous hot percolation, using soxhlet apparatus. The extraction was continued for 72 hours. The resulted dark–brown extract was concentrated upto 100ml on Rota vapor under reduced pressure. The concentrated crude extract were lyophilized in to powder and used for the study.

3.2 Animals

Male Wistar albino rats weighing 150 – 200g body weight were used in this study obtained from institute animal center, KM College of Pharmacy, Madurai. The protocol was approved by the Institutional Animal Ethical Committee. Animals were kept in animal house at an ambient temperature of 25°C and 45 – 55% relative humidity, with 12hours each of dark and light cycles. Animals were fed pellet diet and water *ad libitum*. Committee for the purpose of control and supervision on experiments animals (CPCSEA) guidelines for laboratory animal facility (IJP2003; 35: 257 – 274)) was followed. Body weights of these rats were monitored sequentially in control and experimental animals for a period of 28 days.

3.3 Chemicals

Isoniazid and rifampicin as a pure were purchased from Micro labs, India. Bilirubin,Total Protein, Alkaline phosphatase (ALP) Alanine transaminases (ALT), Aspartate transaminases (AST), and Gamma glutamate transpeptase (GGTP) were assayed by using kits from Ranbaxy diagnostic New Delhi.
3.4 Induction of experimental hepatotoxicity

Isoniazid and rifampicin (100mg/kg body weight) solution was prepared separately in sterile distilled water. All the test drugs were administered orally by suspending in 1 % Carboxy methyl cellulose (CMC). Rats were treated with isoniazid, co-administered with rifampicin for 21 days by \textit{ip} route\textsuperscript{28}. In order to study the effect of ethanolic and aqueous extract of \textit{Stephania weightii} Dunn in rat 350mg/kg body weight\textsuperscript{29} and 300mg/kg body weight\textsuperscript{30}, were used respectively. Silymarin (2.5mg/kg body weight) was used as a standard drug in this study\textsuperscript{31}. Animals were divided into five groups as control (n =6), INH + RIF (n=6), INH+RIF+ethanolic extract of \textit{Stephania weightii} Dunn (n=6), INH+RIF+aqueous extract of \textit{Stephania weightii} Dunn (n=6), and INH+RIF+Silymarin (n=6), where n is the number of animals included in this study (Table 33).

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Treatment protocol of the animals</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Group I</td>
<td>Normal control</td>
<td>Received 2ml of 1% carboxy methyl cellulose (CMC)</td>
</tr>
<tr>
<td>2</td>
<td>Group II</td>
<td>Hepatotoxic control</td>
<td>Received 50mg/kg bw INH+RIF for 21 days orally</td>
</tr>
<tr>
<td>3</td>
<td>Group III</td>
<td>Treatment control</td>
<td>were given INH+RIF+ alcoholic extract for 21 days orally. 250mg/kg bw suspended with 2ml of 1% CMC</td>
</tr>
<tr>
<td>4</td>
<td>Group IV</td>
<td>Treatment group</td>
<td>were given INH+RIF+aqueous extract for 21 days orally. 250mg/kg bw suspended with 2ml of 1% CMC.</td>
</tr>
<tr>
<td>5</td>
<td>Group V</td>
<td>Positive control (STD)</td>
<td>were given INH + RIF + Silymarin 70mg/kg bw orally for 21 days</td>
</tr>
</tbody>
</table>

Rats were treated as per the protocol. The protocol was approved by the IAEC. Body weights of these rats were monitored sequentially in control and experimental animals for a period of 21 days.
3.5 Bio Chemical Markers

Rats were sacrificed 1 hour after administration drug on day 21. The blood was collected by retro orbital artery bleeding. Blood samples were kept for 30 minutes without any disturbance in clot altrivator sample tubes. These blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum. ALP, ALT, AST, GGTP, Total bilirubin levels were estimated from the serum samples using auto analyzer. Results of biochemical analysis were given in Table 34. The results were expressed as Mean ± Standard error means (SEM). Statistical analysis was carried out by using one way ANOVA followed by Newman (1939) Keul’s (1952) multiple range tests\textsuperscript{32,33}.
4. Result and discussion

4.1 Biochemical Parameters

4.1.1 Control group (Group I)

The basal levels of liver enzymes (ALP, AST, ALT& GGTP) is control are 118.60±2.60, 120.40 ±3.60, 34.60 ± 1.14, 92.60 ± 1.68 respectively. Total bilirubin & Total protein levels were 0.58 ± 0.04 & 0.48 ± 0.03 respectively.

4.1.2 Hepatotoxic control (Group II)

There was significant increase in total bilirubin (1.96 ± 0.11) accompanied by significant decrease in level of total protein (0.32 ± 0.04) and also significant increase in ALP\textsuperscript{34,35} (343.00 ± 6.64) AST (462.8 ± 9.68), ALT (168.20 ± 3.46) and GGTP (182.80 ± 6.21) as compared to the control.

4.1.3 Ethnolic extract treated group (Group III)

There was a significant decrease in total bilirubiun (0.82 ± 0.06) accompanied by significant increase in level of total protein (0.40 ± 0.06) and also significant decrease in ALP (182.4 ± 5.80), AST (212.4 ± 2.84) ALT (64.8 ± 2.01) and GGTP (111.4 ± 4.22) as compare to the toxic control.

4.1.4 Aqueous extract of \textit{Stephania wightii} treated group (Group IV)

There was significant decrease in total bilirubin (0.80 ±0.08), accompanied by significant increase in level of Total Protein (0.41 ± 0.05) and also significant decrease in ASP (180.9 ± 6.26), AST (220.80 ± 3.90), ALT (66.40 ± 2.24) & GGTP (116.80 ± 3.92) as compare to the toxic control.
4.1.5 Positive control group

There was significant decrease in total bilirubin (0.74 ± 0.02), accompanied by significant increase in level of total protein (0.43 ± 0.09) and also significant decrease in ALP (172.4 ± 6.16), AST (168.4 ± 1.82), ALT (54.2 ± 1.96) & GGTP (102.60 ± 1.28) as compared to the toxic control.

Although *Stephania wightii* Dunn was reported to possess varied Medicinal properties such as analgesic\(^\text{36}\) effect and anticonvulsant activities, there is no previous report about the hepato protective activity of this plant. The present investigation reports the hepato protective effect of ethanolic and aqueous extract of this plant. In the present study hepatotoxic model in wistar rat is successfully produced by administering INH and RIF (50mg/kg/day) orally. The vast majority of hepatic dysfunction episodes should have occurred within 2 months of commencement of anti tuberculosis chemotherapy as generally reported\(^\text{37}\). It was also reported that isoniazid did not make more injuries than RIF and in this connection, it was the combination of these INH + RIF drugs that confer the additive or even synergistic, potential of liver toxicity than either agent alone, as conjectured\(^\text{38,39,40}\).

During the metabolism of INH hydrazine was produced directly (from INH) and indirectly (from acetyl hydrazine). From earlier study, it was evident that hydrazine plays a role in INH produced liver damage in rats, which is consistent with the report by Garner *et al*\(^\text{41}\). The combination of INH and RIF is reported to result in higher rate of inhibition of biliary secretion and an increase in liver cell lipid peroxidation and cytochrome P450 is thought to be involved in the synergistic effect of RIF on INH\(^\text{42}\). However, its role in INH – induced hepatotoxicity was unclarified as INH itself is an inducer of is CYP 2E1\(^\text{43}\).
INH is metabolized into the bioactive metabolites hydrazine and acetylisoniazid followed by hydrolysis to acetylation which is oxidized into hepatotoxic intermediaries by CYP 450. ALT, AST, and ALP are well known diagnostic indictors of hepatic injury\textsuperscript{44}. Increased levels of these enzymes in serum of the toxic control group (Group2) indicate liver damage as these enzymes leak out from liver into blood due to tissue damage. Pretreatment with alcoholic and aqueous extract of \textit{Stephania wightii}, the levels of these markers were near normal. It may be due to the consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by INH + RIF. Hepatotoxicity is characterized by cirrhotic liver condition which in turn increased the bilirubin release, which was observed in toxic control group\textsuperscript{45}. Pretreatment with alcoholic and aqueous extract of \textit{Stephania wightii}, restored the level of bilirubin to near normal status, suggesting the possibility that the extracts stabilized the biliary dysfunction of rat liver, which is an indication of the improvement of the functions of the liver cells. The results obtained were comparable with those of the silymarin treated positive group.

The estimation of GGTP level was a valuable screening test with high negative predictive value for liver disease\textsuperscript{46}. A number of drugs and chemicals are known to increase GGTP activity by the induction of hepatic microsomal enzymes. Comparatively in ethanoloic extract of \textit{Stephania wightii} Dunn and aqueous extract of \textit{Stephania eightii} Dunn treated groups significant changes occurred in total bilirubin and GGTP levels. This suggests that the aqueous and alcoholic extracts of \textit{Stephania wightii} have good hepatoprotective effect. Purification of extracts and identification of active principle may yield a good hepatoprotective drug.
Table. 34 Effect of *Stephania weightii* Dunn in different biochemical parameters of INH+RIF induced hepatotoxic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Bilirubin</th>
<th>Total Protein</th>
<th>Alkaline phosphatase ALP</th>
<th>Aspartate Transaminase AST</th>
<th>Alanine Transamsmase ALT</th>
<th>GGTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0.58±0.04</td>
<td>0.48±0.03</td>
<td>118.60±2.60</td>
<td>120.40±3.60</td>
<td>34.60±1.14</td>
<td>92.60±1.68</td>
</tr>
<tr>
<td>Hepatotoxic control</td>
<td>1.96±0.11†</td>
<td>0.32±0.04†</td>
<td>343.00±6.64†</td>
<td>462.80±9.68†</td>
<td>168.20±3.46†</td>
<td>182.80±6.21†</td>
</tr>
<tr>
<td>Ethanolic Extract</td>
<td>0.82±0.06*</td>
<td>0.40±0.06*</td>
<td>182.40±5.80*</td>
<td>212.40±2.84*</td>
<td>64.80±2.01*</td>
<td>111.40±4.22*</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>0.80±0.08*</td>
<td>0.41±0.05*</td>
<td>180.90±6.26</td>
<td>220.80±3.90*</td>
<td>66.40±2.24*</td>
<td>116.80±3.92*</td>
</tr>
<tr>
<td>Positive Control</td>
<td>0.74±0.02</td>
<td>0.43±0.09</td>
<td>172.4±6.16</td>
<td>168.4±1.82</td>
<td>54.2±1.96</td>
<td>102.6±1.28</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM

Values are find out by using one way ANOVA followed by Newman Keul’s multiple range test.

†- values are significantly different from control at $P < 0.01$.

* – Values are significantly different from hepatotoxic control at $P < 0.01$. 
References


