Research Article

Hepatoprotective effect of Poly herbal formulation containing indigenous medicinal plants against various hepatotoxic agents in rats

B. Sreshta*, S. Ravindra Babu

Department of Pharmacology, Malla Reddy Institute of Pharmaceutical Sciences, Secunderabad India.

https://doi.org/10.31024/ajpp.2018.4.2.22

Received: 18 March 2018 Revised: 19 March 2018 Accepted: 26 March 2018

Abstract

Objective: To evaluate the protective effect of PHF, a polyherbal formulation, against two experimentally induced hepatotoxicity models in rats. **Materials and methods**: Hepatoprotective activity of the PHF containing two indigenous medicinal plants extracts *Tinospora cordifolia* and *Curculigo orchioides*, was screened against CCl_4 and paracetamol induced hepatotoxicity in rats. **Results**: Administration of hepatotoxins (CCl_4 and paracetamol) shows significant morphological, biochemical and histopathological deteriorations in the liver of experimental animals. Pretreatment with PHF had significant protection against hepatic damage by maintaining the morphological parameters within normal range and normalizing the elevated levels of biochemical parameters (SGPT, SGOT, ALP and total bilirubin), which were evidently showed in histopathological study. **Conclusions:** The PHF has highly significant hepatoprotective effect at 200 and 400 mg/kg, p.o. on the liver of all the two experimental animal models. The liver protection due to combined action of all plant extracts along with their phytoconstituents.

***Keywords*:** Hepatoprotective, Polyherbal, CCl_4, Paracetamol

Introduction

The liver is a metabolically active organ responsible for many vital life functions. The primary functions of the liver are excretion of bilirubin, cholesterol, hormones, and drugs, metabolism of fats, proteins, and carbohydrates, enzyme activation, blood detoxification and purification. Due to these important activities, the liver is exposed to a number of insults and is one of the body's organs most subject to injury. Almost all types of liver injuries may lead to hepatic failure and ultimately death. Thus liver diseases are one of the most fatal diseases in the world today (Dinesh et al., 2014).

Hepatotoxicity is one of the major health problems in developed countries. Hepatotoxicity is caused by various toxicants such as paracetamol, certain chemotherapeutic agents, carbon tetrachloride, thioacetamide, chronic alcohol consumption and microbes. Several different substances can be toxic to the liver such as Viruses (Hepatitis A, Hepatitis B, Hepatitis C, Hepatitis D), Obesity, Chronic or long-term alcohol use, Genetic defects (Haemochromatosis), Drugs like PCM, Aspirin, Ibuprofen, amiodarone, isoniazid, methotrexate, valproic acid, rifampin, etc (Dirgha et al., 2013).

Till date available modern drugs have not been able to come up with a satisfactory answer for liver disorders because of high cost and additional adverse effects. It is therefore necessary to search for alternative drugs for the treatment of liver diseases to replace the currently used drugs of doubtful efficacy and safety (Dinesh et al., 2014). There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. Thus there are some marketed products in india [Silybon(microlabs), LIV 52(Himalaya)] are available as hepatoprotectives. At the same time surprisingly, we do not have readily available plant drugs/formulations to treat liver diseases. However a number of medicinal plants have been advocated in traditional system of medicine, especially in Ayurveda, for treating liver disorders (Suneetha et al., 2014).

The polyherbal formulation (PHF) under this study contains plant ingredients *Tinospora cordifolia* and *Curculigo orchioides*. These medicinal plants used for centuries in the Ayurvedic system of medicine. These drugs are used for the treatment of various diseases such as fever, diabetes, dyspepsia, jaundice, aphorodisiac, immunostimulant, hepatoprotective, antioxidant, anticancer, heart disease, leprosy, and helmenthiasis.The present study was undertaken to explore the hepatoprotective effects of PHF

**Address for Corresponding Author:*
B. Sreshta
Mall Reddy Institute of Pharmaceutical Sciences, Secunderabad-500100, Telangana state, India
Email:sreshtasamuel7@gmail.com
Mobile No: 9010822053

against CCl_4 and paracetamol induced hepatotoxicity in rats as well as to identify the active constituents responsible for hepatoprotection.

Materials and methods

Collection and preparation of plant extract

The plant materials used for the PHF preparation were *Tinospora cordifolia* and *Curculigo orchioides*. The plants were collected Tirumala Hills, Tirupati, India. They were identified and authenticated by Dr. Madhava Chetty, Professor of Botany, Sri Venkateshwara University, Tirupati and voucher specimen of the plant were preserved at institute herbarium library, for future reference.

Extraction and preparation of PHF

The dried plants was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh). 100gm dried powder of whole plants of *Tinospora cordifolia* and *Curculigo orchioides* were separately defatted by treating with pet-ether and then extracted with ethanol by using soxhlet apparatus till solvent was colorless. The extract was dried till constant weight was obtained. 50mg of each extract was mixed together and dissolved in 10 ml water, boiled in water bath for 5minutes, cooled and centrifuged at 4000 rpm for 10 minutes. The aqueous extracts of leaf and stem bark were prepared according to the procedure of Dinesh et al. (2014) and Padmanabhan et al. (2014). The clear supernatant obtained was labeled as herbal preparation.

Preliminary phytochemical screening

Phytochemical analysis of the extract was performed for the identification of phytochemicals such as alkaloids, carbohydrates, proteins and amino acids, tannins, flavanoids, steroids, resins (Kokate et al., 1996).

Drugs and chemicals

Silymarin procured from Micro labs, Banglore and all biochemical kits were purchased from Himedia Laboratories Pvt. Ltd Mumbai. All other chemicals and reagents were of analytical grade and purchased from local firms.

Experimental animals

Male wistar albino rats of 200-250 g were acclimatized for 7 days under standard husbandry conditions, i.e., room temperature of (23 ± 2)°C, relative humidity of 45- 55% and light: dark cycle of 12:12 h. All the experimental protocols were approved by the institutional Animal Ethics Committee (IAEC) of Malla Reddy Institute of Pharmaceutical sciences (Reg. No: 1662/PO/Re/S/12/CPCSEA). They were allowed to take standard pellet food (Anjali Animal Food, Indore) and water ad libitum. All animal experiments were performed according to the Committee for the Purpose of the Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Acute toxicity study

Acute toxicity test was performed according to OECD guideline 423. Swiss albino mice were divided into test group comprising of six animals in each group. The animals were subjected for acute toxicity study using both plant extracts at a dose of 2000 mg/kg. The mice were observed continuously for 1 h and then half hourly for 4 h for any gross behavioral change and general motor activities like writhing, convulsion, response to tail pinching, gnawing, pupil size, fecal output, feeding behavior, etc., and further up to 72 h for any mortality (OECD, 2001).

Experimental Procedure

Carbon tetrachloride (CCl_4) induced acute hepatotoxicity

Wistar rats of 200-250 g were divided in to five groups with six animals each (n=6). Group-1 and group-2 were served as normal control and disease control respectively. Group 3, 4 and 5 corresponded to reference standard (Silymarin-100mg/kg/day, p.o., PHF-200mg/kg/day, p.o. and PHF-400mg/kg/ day, p.o. respectively). The treatment lasted for 7 days and on the seventh day's night all the animals were fasted for 12 h. Then all the animals except those in groups-1 were treated with 1 mL of CCl_4 in liquid paraffin (1:1). The 24 h after CCl_4 administration, body weight recorded and blood samples were collected for the estimation of biochemical parameters, namely SGPT, SGOT, ALP and total bilirubin (Sunil Mistry et al., 2012). All the animals were sacrificed and the liver weight was recorded. Liver tissues collected were subjected to histopathology.

Paracetamol induced hepatotoxicity

Wistar rats of 200-230 g were divided into five groups with six each (n=6). Group 1 and group 2 were served as normal control and disease control respectively. Group 3, 4 and 5 corresponded to reference standard (Silymarin -100mg/kg/day, p.o., PHF-200 mg/kg and PHF-400mg/kg respectively). The treatment was carried out for 7 days and on seventh day's night all the animals were fasted for 12 hrs and all the animals except those in group 1 were treated with paracetamol (2 g/kg, p.o.) in sucrose solution (40% v/v) in three divided doses. 48 hrs after paracetamol administration, body weight was documented and blood samples were collected for the estimation of biochemical parameters, namely, SGPT, SGOT, ALP and total bilirubin (Devaraj et al., 2011). All the animals were sacrificed and the liver weight was recorded. Liver tissues collected were subjected to histopathology.

Histopathological Studies

For histological studies, the liver tissues were fixed with

10% phosphate buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (5M) were cut and stained with routine hematoxylin and eosin (H & E) stain for photo microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue.

Statistical analysis

Statistical analysis Values were expressed as mean±SEM. Statistical difference in mean was analyzed using one way ANOVA and followed by Turkey's multiple comparison tests, P< 0.05 were considered statically significant.

Results

Preliminary Phytochemical Screening

Phytochemical screening showed the presence of glycosides, flavonoids, alkaloids, proteins, carbohydrates and phenolic compounds.

Acute toxicity studies

No signs and toxic symptoms were observed during the acute toxicity study using the ethanolic extract after oral administration of dose up to 2000 mg/kg indicating that the formulation was non-toxic and safe. Thus 1/10th of the maximum dose (200mg/kg) and double of the dose (400mg/kg) were selected for the present study.

Effect PHF against CCl_4 induced hepatotoxicity

CCl_4 treated animals showed significant elevation of serum biochemical parameters, such as SGPT, SGOT, ALP and total bilirubin.The pathological lesion of the liver was evident. Pretreatment with silymarin (100mg/kg, p.o.) and PHF at 200 and 400 mg/kg, p.o. for 7days, had produced significant protective effect on CCl_4 induced hepatic damage by maintaining the morphological changes and normalizing the elevation of serum biochemical parameters (SGPT, SGOT, ALP and total bilirubin), and therefore inhibited the histopathological abnormalities caused by CCl_4. PHF showed dose dependent protection against CCl_4 induced hepatic damage (Table 1 and Figure 1).

Effect of PHF against paracetamol-induced hepatotoxicity

Administration of the paracetamol at a dose of 2 g/kg, p.o. showed cetrilobular necrosis in histopathological studies in animal and its association with elevation of serum biomarkers for liver function, such as SGPT, SGOT, ALP and total bilirubin.. Pretreatment with PHF at 200 mg/kg and 400mg/kg, p.o. for 7days offered significant protection against paracetamol induced hepatic damage by inhibiting the morphological changes and maintaining the serum biochemical parameters (SGPT, SGOT, ALP and total bilirubin). Histopathological analysis demonstrated that the pathological lesion caused by paracetamol were very minimal in PHF pretreated groups. PHF showed dose dependent protection against paracetamol induced hepatic damage and the protective effect of PHF -400 mg/kg, p.o. was comparable with silymarin-100 mg/kg, p.o. (Table 2 and Figure 2).

Discussions

Hepatotoxicity is a common ailment resulting in serious debilities ranging from severe metabolic disorders to even mortality. About 20,000 deaths are reported every year due to liver disorders. The mortality from liver failure is 80% with 1/3rd of the patients under 25 years of age (Hand, 2013).

In CCl_4-induced hepatotoxicity model, upon administration of CCl_4 to animals, it undergoes enzymatic activation, majorly by CYP2E1, into the trichloromethyl free radical

Table 1. Effect PHF on CCl_4- induced hepatotoxicity in rats

Groups	Serum biochemical parameters (U/L)			
	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Bilirubin(mg/dl)
Group I (2ml distilled water, p.o, once daily	89.43±3.12	76.14±1.25	42.9±.3.85	0.59±0.12
Group II (CCl_4 1ml /kg, i.p on day6)	149.3±2.65^a	106.4±2.36^a	130.2±7.82^a	3.27±0.11^a
Group III (Silymarin 100mg/kg, p.o once daily+CCl_4 1ml /kg, i.p on day6)	104.3±3.62^b	73.6±3.01^b	58.4±3.67^b	0.72±0.18^b
Group IV(PHF 200mg/kg, p.o, once daily+CCl_4 1ml /kg, i.p on day6)	136.9±2.43^b	86.4±3.14^b	75.5±3.57^b	1.49±0.11^b
Group V (PHF 400mg/kg, p.o, once daily+CCl_4 1ml /kg, i.p on day6)	129.4±3.21^b	79.3±3.62^b	62.8±3.50^b	0.89±0.15^b

Values are expressed as mean ±SEM for six animals; a= p<0.05 as compared to normal control; b= p<0.05 as compared to CCl_4 treated group; PHF=Polyherbal formulation.

Table 2. Effect of PHF on Paracetamol-induced hepatotoxicity in rats

Groups	Serum biochemical parameters			
	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Bilirubin(mg/dl)
Group I (2ml distilled water, p.o, once daily	92.23±2.16	73.0±1.72	59.09±.3.95	0.32±0.11
Group II (Paracetamol 2g/kg, p.o)	151.7±2.16[a]	105.6±2.14[a]	103.2±6.59[a]	2.23±0.25[a]
Group III (Silymarin 100mg/kg, p.o once daily)	106.3±2.25[b]	68.52±1.54[b]	56.9±3.19[b]	0.65±0.17[b]
Group IV (PHF 200mg/kg, p.o, once daily)	132.0±3.54[b]	76.48±2.54[b]	72.7±3.37[b]	0.85±0.08[b]
Group V (PHF 400mg/kg, p.o, once daily)	122.3±6.61[b]	72.70±1.54[b]	62.5±3.92[b]	0.78±0.11[b]

Values are expressed as mean ±SEM for six animals;a= p<0.05 as compared to normal control; b= p<0.05 as compared to paracetamol treated group; PHF=Polyherbal formulation.

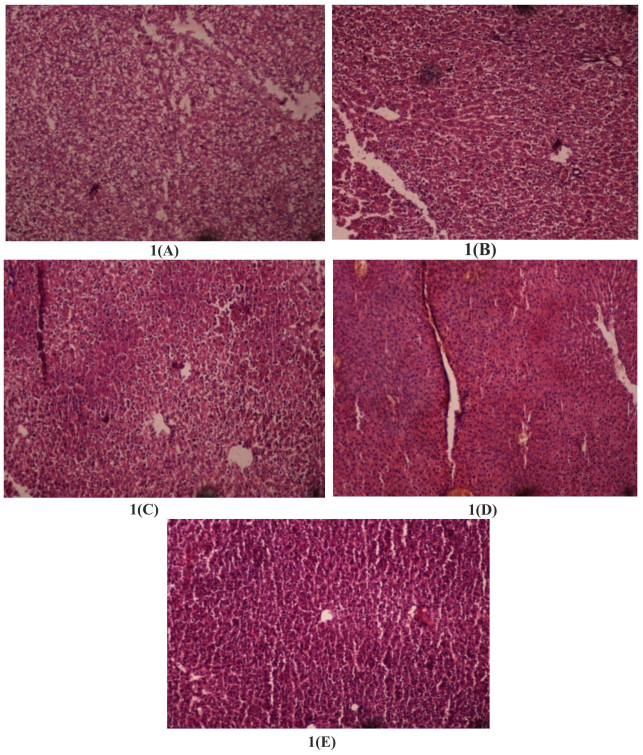

Figure 1. Effect of PHF on CCl_4-induced hepatotoxicity in rats. Photomicrograph showed, 1A: Liver section of normal rat (group-1): No fatty change, normal hepatocyte.; 1B: Liver section of CCl_4-treated rat (group-2): Loss of hepatic architecture with intense, Peripheral central vein necrosis, apoptosis, Hepatocellular vascuolization, fatty changes.; 1C: Liver section of rats treated with $CCl_4$and 100mg/kg of Silymarin (group-3):Apparently normal hepatocyte.; 1D: Liver section of rats treated with CCl_4 and 200mg/kg of PHF (group-4):Swelling of hepatocyte, hepatocyte atrophy, mild fatty change with central vein damage.; 1E: Liver section of rats treated with CCl_4 and 400mg/kg of PHF (group-5):Normal hepatic architecture was seen with only, Moderate accumulation of fatty lobule.

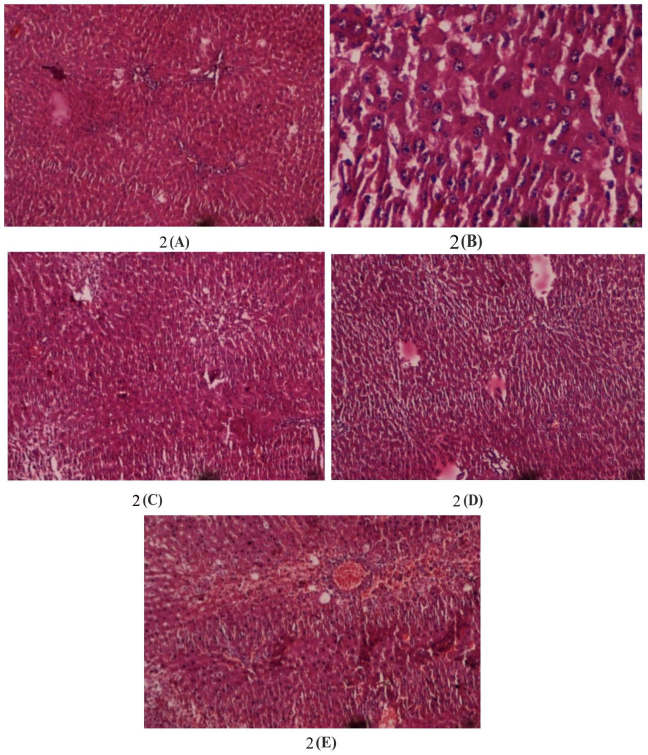

Figure 2. Effect on histopathological studies of liver tissue in paracetamol induced hepatotoxic rats. Photomicrograph showed, 2A: Liver section of normal rat (group-1): Hepatocytes showed the normal lobular architecture of the liver with hepatocyte arranged in single cords.; 2B:Liver section of paracetamol-treated rat (group-2): Hepatocytes showed granular degeneration, Cell injury in centrilobular zone, vacuolation of cytoplasm as a feature of ballooning degeneration, apoptosis, dropout necrosis, bridge necrosis and inflammation.; 2C: Liver section of rats treated with paracetamol and 100mg/kg of Silymarin (group-3): Hepatocytes showed minimal inflammation with less granular degeneration and piecemeal necrosis.; 2D: Liver section of rats treated with paracetamol and 200mg/kg of PHF (group-4):Swelling of hepatocyte, hepatocyte atrophy, mild fatty change with central vein damage.; 2E: Liver section of rats treated with paracetamol and 400mg/kg of PHF (group-5):Normal hepatic architecture was seen with only, Moderate accumulation of fatty lobule.

($CCl_3^{\cdot}$) within the membrane of the endoplasmic reticulum. This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic reticulum membrane phospholipids. These processes are known as lipid peroxidation leading to functional and structural disruption of hepatocytes (Sunil Mistry et al., 2012).

Administration of paracetamol at a dose of 1-3 g/kg/day, p.o. results in hepatic damage. The toxic metabolite N-acetyl-p-benzoquineimine is an oxidative product of paracetamol formed by the action of cytochrome P-450 and it reacts with reduced glutathione (GSH) to yield nontoxic-3-GS-yl-paracetamol. Depletion of GSH causes the remaining quinone to undergo covalent bonding with cellular macromolecules (sulphydryl groups of protein) and leads to cell death. Histopathology of the liver shows necrosis of the

centrilobular hepatocytes characterized by nuclear pyknosis, eosinophilic cytoplasm and large excessive hepatic lesions (Sunil Mistry et al., 2012).

The present study revealed that serum SGOT, SGPT, ALP, total bilirubin and triglycerides levels were significantly elevated in rats intoxicated with CCl_4 and paracetamol in comparison with normal group. Oral administration ethanolic extract of PHF to rats caused a decrease in the activity of the liver enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl_4 and paracetamol. This is supported by the view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes.

Pretreatment with silymarin (100mg/kg, p.o.), PHF (200 and 400 mg/kg, p.o.) for 7 days offered significant protection against the CCl_4 and Paracetamol-induced hepatic damage. Both the doses of PHF prevented the histological changes caused by CCl_4 and Paracetamol. The possible mechanism of action may be associated with inhibition of CYP2E1 activity or scavenging of free radicals responsible for CCl_4 toxicity (Sunil Mistry et al., 2012).

Depletion of elevated bilirubin level and lipid parameters were observed with the preadministration of extract of PHF suggesting the possibility of the extract being able to stabilise biliary dysfunction of rat liver during chronic injury with CCl_4 and mechanism of lipid lowering effects of extracts might be attributed due to inhibitory activity on microsomal acyl coenzyme A: cholesterol acyltransferase. This enzyme is responsible for acylation of cholesterol to cholesterol esters in liver (Matsuda, 1994).

The hepatoprotective activity observed with this plant polyherbal formulation extract may be due to the presence of flavonoids, lactones, tannins and alkaloids as the preliminary phytochemical investigations revealed the presence of these compounds. Earlier studies with flavonoids, lactones, tannins and alkaloids established the hepatoprotective activity of these compounds (Kanai et al., 1998).

In present study, histopathological sections of CCl_4 and Paracetamol induced hepatotoxicity comprised of piecemeal necrosis, apoptosis, focal inflammation, portal inflammation and fibrosis in the liver tissue (fig 1B &2B).The PHF administration via extractives forms significantly prevented above histological degenerative changes in the liver and showed significant dose dependent hepatocellular integrity protective activity (fig 1D,1E, 2D, 2E), much comparable to standard hepatoprotective drug Silymarin, much being used in medical practice.

Conclusion

In conclusion, our findings clearly state that PHF extract, at 200mg/kg and 400mg/kg dose level offers significant dose dependent protection against experimentally induced hepatotoxic models. The resulting hepatoprotective activity of PHF could be attributed to phytochemicals like flavonoids, tannins and alkaloids.

Acknowledgement

The authors are thankful to the management and principal of Malla Reddy Institute of Pharmaceutical sciences, Secunderabad, for providing necessary facilities to carry out the present research work.

Conflict of interest

We declare that we have no conflict of interest.

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