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Hepatoprotective and Antioxidant Activity Evaluation of PHF08 on Carbon Tetrachloride Induced Hepatotoxicity

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Abstract

A Polyherbal formulation (PHF08) comprising of phytoconstituents with potential hepatoprotective activity was evaluated for its hepatoprotective and antioxidant activity using carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. The treatment effect was evaluated by determining the marker enzymes (SGOT, SGPT and ALP), albumin (Alb) and total proteins (TP) in serum whereas lipid peroxidation (LPO) and superoxide dismutase (SOD) were estimated in liver homogenates to evaluate antioxidant activity. PHF08 showed significant hepatoprotective activity as indicated by a decrease in serum marker enzymes (SGOT, SGPT & ALP) and LPO and elevated levels of SOD, Alb and TP in a dose dependant manner. Histopathological studies further confirmed the hepatoprotective activity of PHF08. The present findings are indicative of the hepatoprotective effects of PHF08 against CCl_4 induced oxidative damage being related to its antioxidant and free radical scavenging activity.

Key words: PHF09, Antioxidant, CCl_4, SOD, LPO

Introduction

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds. Any impairment to its function may lead to many implications on one's health. Management of liver disease is still a challenge to the modern scientific community [1].Conventional or synthetic drugs used in the treatment of liver diseases are often inadequate and can have serious adverse effects. As a result, there is a worldwide trend to go back to traditional medicinal plants. Many natural products of herbal origin are in use for the treatment of liver ailments [2].

Carbon tetrachloride (CCl_4) is a potent hepatotoxin producing centrilobular hepatic necrosis, liver cirrhosis, tumors, fatty liver and also kidney damage on chronic exposure[3]. The Polyherbal formulation (PHF08) is composed of extracts of *Tinospora cordifolia, Emblica officinalis, Withania somnifera, Curcuma longa, Glycyrrhiza glabra, Bacopa monnieri, Terminalia chebula, Asparagus racemosus, Terminalia arjuna, Aloe barbadensis,* plants rich in polyphenolic compounds known to be excellent antioxidants *in vitro* and have the capacity to scavenge free radicals and potentiate antioxidant defenses. Studies have been carried out on all the above herbs with antioxidant approach to manage various diseases [4].

The aim of the present study was to investigate the effects of PHF08 on liver function in CCl_4 induced hepatotoxicity and to make an attempt to understand the probable mechanism involved in producing hepatoprotective effects.

Materials and Methods

Experimental Animals:
The animals used for experimentation were albino wistar rats weighing between 100-200gm obtained from the registered breeder Bharat Serum and Vaccines, Thane. They were housed in clean polypropylene cages under standard conditions of temperature (25 ± 2^0C) and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed, Pune, India) and water *ad libitum.* All animals were handled with humane care. Experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No. 25/1999/CPCSEA).

Drugs and Chemicals:
Silymarin, Epinephrine and Trichloro Acetic Acid (TCA) were purchased from Sigma Chemical Co., St Louis, MO, USA. Thiobarbituric acid (TBA) was purchased from Hi-media Laboratories. All other chemicals and kits were obtained from local sources and were of analytical grade.

Preparation of test and Reference drug solutions:
PHF08 powder and Silymarin were suspended in an aqueous solution of 1% carboxymethyl cellulose (cmc) daily prior to administration.

Experimental:
CCl_4 induced hepatotoxicity:-
Albino wistar rats weighing in the range of 100- 150gm were divided into five groups of 6 animals each. Rats were intraperitoneally injected with 1:1(v/v) mixture of CCl_4 (0.7ml/kg, i.p.) and liquid paraffin on 1st, 4th and 7th day to all groups except normal control, one hour after drug treatment.

Group I - Served as Normal Control and received standard diet daily for 9 days.
Group II - Served as Toxicant Control.
Group III - Received PHF08 powder (200 mg/kg, p.o.) once daily for 9 days.
Group IV - Received PHF08 powder (600 mg/kg, p.o.) once daily for 9 days.
Group V - Received Silymarin (100 mg/kg, p.o.) once daily for 9 days.

On the 10th day, animals in all groups were anesthetized with light ether and 4ml of blood was withdrawn by retro-orbital/cardiac puncture route and serum was separated for biochemical parameters. Following blood withdrawal, the animals were humanely sacrificed liver was aseptically removed for histopathological and biochemical parameters [5]

Estimation of Marker Enzyme Assay:
The lysosomal enzymes SGOT, SGPT, ALP, Total protein (TP), Albumin (Alb) were assayed in serum using standard kits supplied by Span Diagnostics. The results were expressed as IU/l for SGOT, SGPT and ALP, whereas g/dl for TP and Alb.

Estimation of antioxidant enzyme level:
Liver was carefully washed to remove blood residue with 0.9% cold saline solution and 10% liver homogenate was prepared using 0.1M phosphate buffer solution (pH 7.4) .The SOD estimation on liver homogenates was carried out by Sun & Zigman method [6]. This method is based upon the ability of SOD to inhibit auto-oxidation of epinephrine to adrenochrome and other derivatives in alkaline pH. The absorbances at 320nm were read continuously over a period of 5 mins. The result was expressed in terms of u/mg protein. LPO was determined Beaug & Aust method [7]. This method is based on absorbance of Thiobarbituric acid Reactive Substances (TBARS) measured at 560 nm, the result was expressed in % inhibition of LPO.

Histopathological Studies:
Animals were sacrificed on the day of withdrawal of blood and the livers were aseptically removed, sliced and washed with ice cold saline. Liver sections were fixed in 10% formalin solution. After dehydration, the pieces of liver were embedded in paraffin wax, cut into 4–6 µm thick sections and stained using haematoxylin and eosin. They were observed under a microscope for histopathological changes in liver architecture and photographed.

Statistical Analysis:
The results of hepatoprotective and antioxidant activities are expressed as mean ± SEM. The statistical analysis of the results were carried out with Graph Pad Prism 4 program and based on Analysis of Variance (ANOVA), one way ANOVA followed by Dunnet's test.

Results & Discussion

Following CCl_4 induced hepatotoxicity, a marked increase was observed in the serum SGOT, SGPT and ALP levels with a significant decrease in Alb and TP levels, treatment with PHF08 showed a significant reduction in serum SGOT, SGPT and ALP levels and an increase in TP level in dose dependant manner as shown in **table 1**.

Following CCl_4 induced hepatotoxicity; a marked increase in levels of LPO and decrease in levels of SOD was observed. Treatment with PHF08 showed an increase in SOD levels and reduction in LPO levels in dose dependant manner as shown in **table 2**.

Table 1: Effect of PHF08 on biochemical serum parameters in Carbon tetrachloride-induced hepatotoxicity

Treatment groups and dose (mg/kg)	SGOT (IU/l)	SGPT (IU/l)	ALP (IU/l)	Total Protein g/dl	Albumin g/dl
Control	221.00 ±4.13	35.20 ±1.65	107.80 ±6.62	7.35 ±0.23	4.15 ±0.16
Toxicant	355.5 ±18.32 a**	79.60 ±4.67 a**	199.00 ±8.31 a**	5.65 ±0.16 a**	3.117 ±0.13 a**
PHF08-200	296.50 ±5.45 b**	64.80 ±2.34 b*	147.50 ±5.56 b**	6.40 ±0.18 b*	3.533 ±0.18
PHF08-600	269.3 ±6.27 b**	56.80 ±4.44 b**	128.80 ±7.66 b**	6.85 ±0.21 b**	3.867 ±0.08 b**
Silymarin-100	265.30 ±15.67 b**	43.80 ±1.88 b**	130.8 ±5.82 b**	6.70 ±0.12 b**	3.75 ±0.11b*

N = 6, Each data suggest Mean ± SEM; One-way ANOVA followed by Dunnet's test is applied for statistical analysis,;a = toxicant groups was compared with control group, b = treated groups were compared with toxicant group;∗ Significant at $p < 0.05$, ∗∗ Significant at $p < 0.01$

Table 2: Effect of PHF08 on biochemical liver enzymes in Carbon tetrachloride-induced hepatotoxicity

Treatment groups and dose (mg/kg)	SOD u/mg protein	Lipid Peroxidation (% Inhibition)
Control	4.50± 0.10	56.51 ±4.76(63.7)
Toxicant	2.48± 0.07a**	155.7 ±2.89(0.00)
PHF08- 200	3.12± 0.13	125.4 ±6.95(19.47)
PHF08-600	3.74± 0.32 b**	98.47 ±8.04(36.76)
Standard	3.85± 0.25 b**	89.3 ±3.40(42.65)

N = 6; Each data suggest Mean ± SEM ; One-way ANOVA followed by Dunnett's test is applied for statistical analysis, ; a = toxicant groups was compared with control group, b = treated groups were compared with toxicant group; ∗ Significant at $p < 0.05$, ∗∗ Significant at $p < 0.01$

Biochemical observations were further substantiated by histopathological studies. The liver sections of animals treated with CCl_4 **(Figure 2)** showed mild to marked multifocal centrilobular necrosis and diffuse granular degeneration with minimal multifocal individual cell pyknosis, mild diffuse lymphocytic infiltration. Compared to the lesions observed in the toxicant group, the lesions noted in livers of PHF08-200 group **(Figure 4)** showed moderately multifocal moderate centrilobular necrosis and minimal diffuse granular degeneration. PHF08- 600 group **(Figure 5)**

showed mildly multifocal minimal degree centrilobular necrosis and minimal diffuse granular degeneration, the results being almost comparable with Silymarin treated group **(figure 3).**

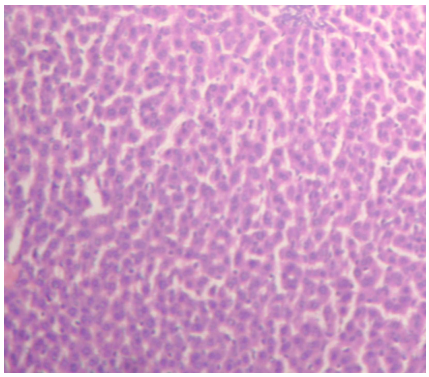

Fig-1: NORMAL CONTROL GROUP

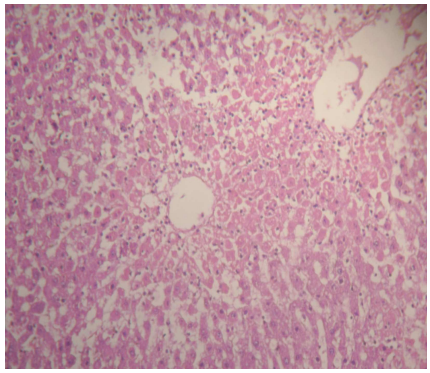

Fig-2: TOXICANT GROUP

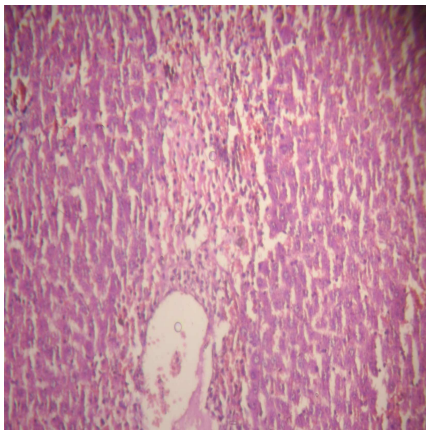

Fig-3: SILYMARIN TREATED GROUP

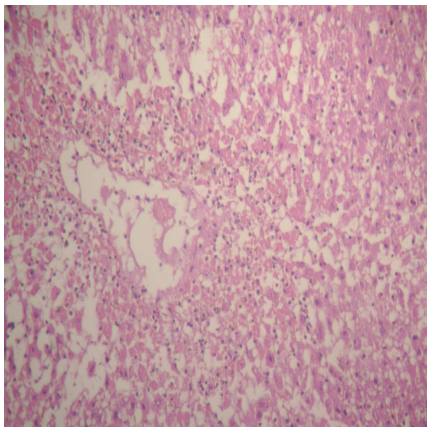

Fig-4: PHF08 (200MG/KG) TREATED GROUP

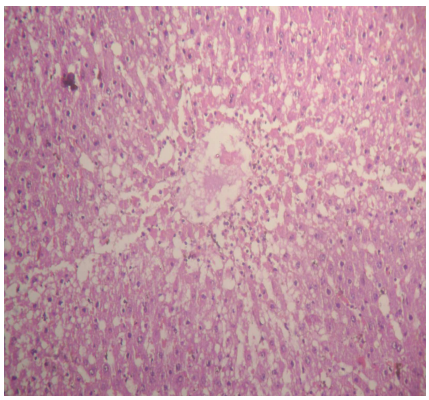

Fig-5: PHF08 (600MG/KG) TREATED GROUP

The efficacy of any hepatoprotective drug is essentially dependent on its capacity of either reducing the harmful effects or maintaining the normal physiologic function which has been disturbed by hepatotoxic agents [8].

CCl_4 is one of the most commonly used hepatotoxins in experimental hepatopathy, the changes associated with CCl_4-induced liver damage are similar to that of acute viral hepatitis. It is biotransformed by Cytochrome P-450 to its active metabolite, the trichloromethyl (CCl_3-) radical, which readily reacts with oxygen to form a trichloromethylperoxyl radical (CCl_3O_2-). These free radicals trigger cell damage through two mechanisms viz., covalent bonding to cellular macromolecules and peroxidative degradation of membrane lipids and endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn yield products like malondialdehyde (MDA), which cause loss of integrity of cell membranes and damage to hepatic tissue [9]. Assessment of liver function can be performed by estimating the activity of serum enzymes SGOT, SGPT and ALP, which are enzymes originally present in high concentrations in the cytoplasm. When there is hepatic injury, these enzymes leak into the blood stream in conformity with the extent of liver damage. The elevated levels of these marker enzymes in CCl_4-treated rats in the present study corresponded to the extensive liver damage induced by the toxin. Treatment with the test drug PHF08 (at both doses) as well as the reference drug silymarin significantly reduced the elevation in liver enzymes. Further, PHF08 treatment increases the levels of TP and Alb in the serum, which indicates hepatoprotective activity. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism that accelerates the regeneration process and the production of liver cells [10].

The body has an effective defense mechanism to prevent and neutralize the free radical-induced damage. This is accomplished by a set of endogenous antioxidant enzymes such as SOD, catalase and GPX, which constitute a mutually supportive team of defense against reactive oxygen species (ROS).The decrease in the activity of SOD in liver of CCl_4 treated rats may be due to increased lipid peroxidation or inactivation of the enzyme by cross linking with MDA. This will cause an increased accumulation of superoxide radicals, which could further stimulate lipid peroxidation[11]. Decrease in enzyme activity of SOD is a sensitive index in hepatocellular damage and is the most sensitive enzymatic index in live injury [12]. The drug-treated groups showed increase in the level of these enzymes as compared to toxicant group, which indicates the antioxidant activity of the PHF08. The level of lipid peroxide is a measure of membrane damage and alterations in structure and function of cellular membranes. In the present study, elevation of lipid peroxidation in the liver of rats treated with CCl_4 was observed, which was reduced by PHF08 treatment, which shows a dose dependant activity which can be comparable with standard drug, Silymarin.

The biochemical observations were further supported by histopathological examination of liver sections of the rat. CCl_4 administration leads to centrilobular necrosis, granular degeneration with leucocytic infiltration in necrotic zone. Treatment with PHF08 at both dose levels resulted in hepatoprotection with regeneration of liver cells further confirming the hepatoprotective activity of PHF08.

Conclusion

The above investigation shows that the PHF08 has promising hepatoprotective properties. Reduction of lipid peroxidattion and increase in levels of antioxidant enzyme SOD, indicates that its hepatoprotective activity is mainly due to its antioxidant mechanism. Further studies need to be carried out to determine other mechanisms of action that might be related to its

hepatoprotective action and detailed phytochemical analysis needs to be carried out to isolate the phytoconstituents responsible for this activity.

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