

Full Length Research Paper

Hepatoprotective activity of polyherbal formulation against carbon tetrachloride-induced hepatotoxicity in rats

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Accepted 18 June, 2010

The ethanolic extracts of the polyherbal medicinal plants (*Asteracantha longifolia*, *Cyperus rotundus* and *Bryophyllum pinnatum*) were evaluated for hepatoprotective activity in carbon tetrachloride induced liver damage in rats. The ethanolic extract of polyherbal formulation at 250 mg/kg b.w. exhibited a significant protective effect by lowering serum and liver activities of aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), serum bilirubin, serum cholesterol and serum total protein when compared with standard silymarin. The hepatoprotective activity of the extracts may be attributed to increased regeneration of hepatocytes and inhibitory effects on microsomal enzymes.

Key words: Polyherbal formulation, carbon tetrachloride, silymarin.

INTRODUCTION

Liver, the largest organ in vertebrate body, plays a major role in metabolic activities like detoxification and excretion of many exogenous and endogenous compounds. Liver injury caused by toxic chemicals and certain drugs, has been recognized as a toxicological problem. In the absence of reliable liver protective drugs in modern system of allopathic medical practice, herbal drugs are playing important roles in health care programmes world wide and there is a resurgence of interest in herbal treatment of various hepatic ailments (Shivkumar et al., 2006).

Survey conducted in tribal areas of Maruthamalai hills, Coimbatore, Tamilnadu revealed the uses of these plants in treatment of jaundice and various kidney diseases.

Apart from the tribal uses of these plants in treatment of liver and kidney diseases, no systematic scientific study

has been undertaken to evaluate the hepatoprotective activities of the plants. Hence attempt was made to assess these plants for hepatoprotective activity against CCl₄ induced hepatotoxicity in rats.

MATERIALS AND METHODS

Collection of the plant materials

The plant materials used for the polyherbal formulation (PHF) preparation were *Asteracantha longifolia* Nees., *Cyperus rotundus* Linn. and *Bryophyllum pinnatum* Kurz. The plants were collected from Vallaiyar, Rajapalayam and Salem district of Tamilnadu, India respectively. They were identified and authenticated by Taxonomist Dr. V. Balasubramanian of Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimens were deposited at the herbarium collection of the department of Botany.

Extraction and preparation of polyherbal formulation

The plant parts were washed, shade dried and powdered. In order to prepare the polyherbal formulation, about 75 g (50%) of *A. longifolia*, 45 g (30%) of *C. rotundus* and 30 g (20%) of *B. pinnatum* plant powders were soaked overnight in 750 ml of 95% ethanol.

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Abbreviations: AST, Aspartate transaminase; ALT, alanine transaminase; ACP, acid phosphatase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; PHF, polyherbal formulation.

Table 1. Composition of plant parts used for the preparation of polyherbal formulation (PHF).

S/N	Name of the Plant	Plant part used	Composition (%)
1	<i>Asteracantha longifolia</i> Nees.	Whole plant	50%
2	<i>Cyprus rotundus</i> Linn.	Bulbs	30%
3	<i>Bryophyllum pinnatum</i> Kurz.	Leaves	20%

Table 2. Experimental design.

Group	Experimental design
I	Control rats- received normal pelleted diet
II	Toxic rats- carbon tetrachloride was induced (1.0ml/ kg b.w.) as single dose with 1:1 volume/ volume of liquid paraffin by intraperitoneal administration for two consecutive days (Manna <i>et al.</i> , 2006)
III	Standard drug treated rats – Silymarin (25mg / kg / b.w.) by oral administration for 30 days
IV	Polyherbal formulation (PHF) treated rats– (250 mg / kg b.w.) by oral administration for 30 days
V	Protective group - normal rats received polyherbal formulation extract (250 mg / kg b. w.) by oral administration for 30 days

This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvent were evaporated in a rotary evaporator, to get a yield of 22 g. This extract was dissolved in distilled water and this was administered orally to the rats. The percentage composition of the plant parts used for the ethanolic extract of polyherbal formulation preparation is shown in Table 1.

Selection of animals

Healthy adult male wistar albino rats weighing about 150 to 200 g were procured from the animal breeding centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The animals were housed in spacious cages. The animals were maintained for 12 h in light and dark cycle at $28 \pm 2^\circ\text{C}$ in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India and provided with water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by the Institutional animal ethics committee (IAEC).

Experimental induction of hepatotoxicity

Hepatic damage was induced in experimental rats by intra-peritoneal administration of carbon tetrachloride at dose of 1.0 ml per kg body weight in 1:1 (v/v) of liquid paraffin, which served as a vehicle.

Experimental design of animals

The rats were divided into five groups of six animals each as given in Table 2.

Collection of serum sample

After the experimental regimen, the animals were sacrificed by cervical decapitation under mild ether anesthesia. Blood was collected and

centrifuged for 10 min at 2500 rpm. The serum was collected and then diluted in the ratio of 1:10 with saline. Aliquot of the diluted serum was used for the estimation of serum constituents and serum enzyme activities.

Collection of liver samples

Liver was removed immediately and washed with ice cold saline. 10% tissue homogenate was prepared by 0.1M tris HCl homogenizing buffer at pH 7.5. The homogenate was used for the assay of various biochemical parameters.

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Estimation of biochemical parameters

The serum and liver tissue homogenate was used to assayed the marker enzymes and serum constituents like aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), bilirubin, total protein and cholesterol according to the method of Reitman and Frankel (1957), King (1965), King and Armstrong (1934), Malloy and Evelyn (1998), Lowry et al. (1951) and Parekh and Jung (1970).

Statistical analysis

Values were represented as the mean of six values \pm S.D. The results were statistically analyzed using the statistical package, MINITAB, version 14. One way analysis of variance was employed for comparison among the six groups followed by Fisher's test. Statistical significance was set at $p < 0.05$ (Daniel, 2006).

RESULTS

From Tables 3 and 4 as well as Figures 1, 2 and 3, the

Table 3. Effect of PHF on marker enzymes in serum of control and experimental rats.

Groups	AST [#]	ALT [#]	ACP [§]	ALP [§]
I	79.21±0.93	34.76±1.05	66.03±0.73	84.96±0.75
II	170.64±1.02a*	149.45±2.36a*	183.25±1.04a*	166.79±0.64a*
III	90.53±0.78b*	52.00±2.09b*	80.27±2.71b*	95.15±2.92b*
IV	55.44±1.45c*,e ^{ns}	55.44±1.45c*,e ^{ns}	82.16±2.14c*,e ^{ns}	94.51±1.72c*,e ^{ns}
V	34.07±1.18d ^{ns}	34.07±1.18d ^{ns}	65.79±1.46d ^{ns}	85.05±1.17d ^{ns}

Values are expressed as mean ± SD of six animals. Statistical comparisons: a, Group II and I; b, Group III and II; c, Group IV and II; d, Group V and I; e, Group IV and III; * P < 0.05; ns; not significant; #, μ moles of phenol liberated/L; §, μ moles of pyruvate liberated/L.

Table 4. Effect of PHF on LDH, in liver of control and experimental in rats.

Groups	LDH [#]
I	42.88±1.27
II	82.59±0.63a*
III	50.77±1.45b*
IV	50.00±1.51c*,e ^{ns}
V	42.88±0.41d ^{ns}

Values are expressed as mean ± SD of six animals. Statistical comparisons are as in Table 3. #, μ moles of phenol liberated/L.

activities of marker enzymes AST, ALT, ACP, ALP, LDH were significantly increased ($p < 0.05$) in serum and liver of CCl₄ induced hepatic damaged rats. After the treatment with polyherbal formulation, the values showed near normal range in Group IV rats in serum and liver. The standard drug silymarin treated group (Group III) also showed normal activities. The Group V rats, which were treated with polyherbal formulation alone, showed protective effect without any side effect.

Figure 4 and Table 5 represent the levels of serum bilirubin, serum total protein and serum cholesterol. Levels of bilirubin and cholesterol were significantly ($p < 0.05$) increased in Group II while the total protein level was significantly decreased. This reduction in biochemical parameters by ethanolic extract of polyherbal formulation (Group IV) was similar when compared to that of silymarin (Group III).

DISCUSSION

The liver is an important metabolic organ involved in the synthesis of large number of metabolites. It contains large amount of marker enzymes (Singh et al., 2005). Hepatocellular necrosis leads to very high level of AST

and ALT in blood released from liver to blood. However, alanine transaminase is a better index of injury, as its activity represents 90% of total enzymes present in the body. The decrease in serum transaminase concentration indicates the stabilization of plasma membrane and protection of hepatocytes against the damaged caused by carbon tetrachloride (Chandrashekar et al., 2004).

ALP activity on the other hand is related to the functioning of hepatocytes and increase in its activity is due to its increased synthesis in the presence of increased pressure (Manjunatha et al., 2005). Acid phosphatase is a marker enzyme use to assess lysosomal changes *in vivo* as it is localized almost exclusively in particles and its release parallel to that of lysosomal hydrolyases (Emmanuel et al., 2001).

Bilirubin, an endogenous organic anion binds reversibly to albumin and it is transported to the liver, and then conjugated with glucuronic acid and excreted in the bile. Hepatobiliary disease is indicated when conjugated fraction of bilirubin exceeds the upper limit of normal, even if the total serum bilirubin is normal or near normal (Raghavendren et al., 2004).

The decline in total protein content can be deemed as a useful index of the severity of cellular dysfunction in chronic liver disease. The lower level of total protein recorded in the serum of CCl₄ treated rats reveals the severity of hepatopathy. The attainment of near normalcy in total protein content of serum of polyherbal treated rats conforms the hepatoprotective effect of the selected herbs (Venukumar and Latha, 2002)

Carbon tetrachloride is one of the most commonly used hepatoprotection. It is well documented that carbon tetrachloride is biotransformed under the action of cytochrome p₄₅₀ in the microsomal compartment of the liver to trichloromethyl radicals which readily reacts with molecular oxygen to form trichloromethyl peroxy radicals (Raucy et al., 1993). Both radicals can bind covalently to the macromolecules and induce peroxidative of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides followed by pathological changes such as

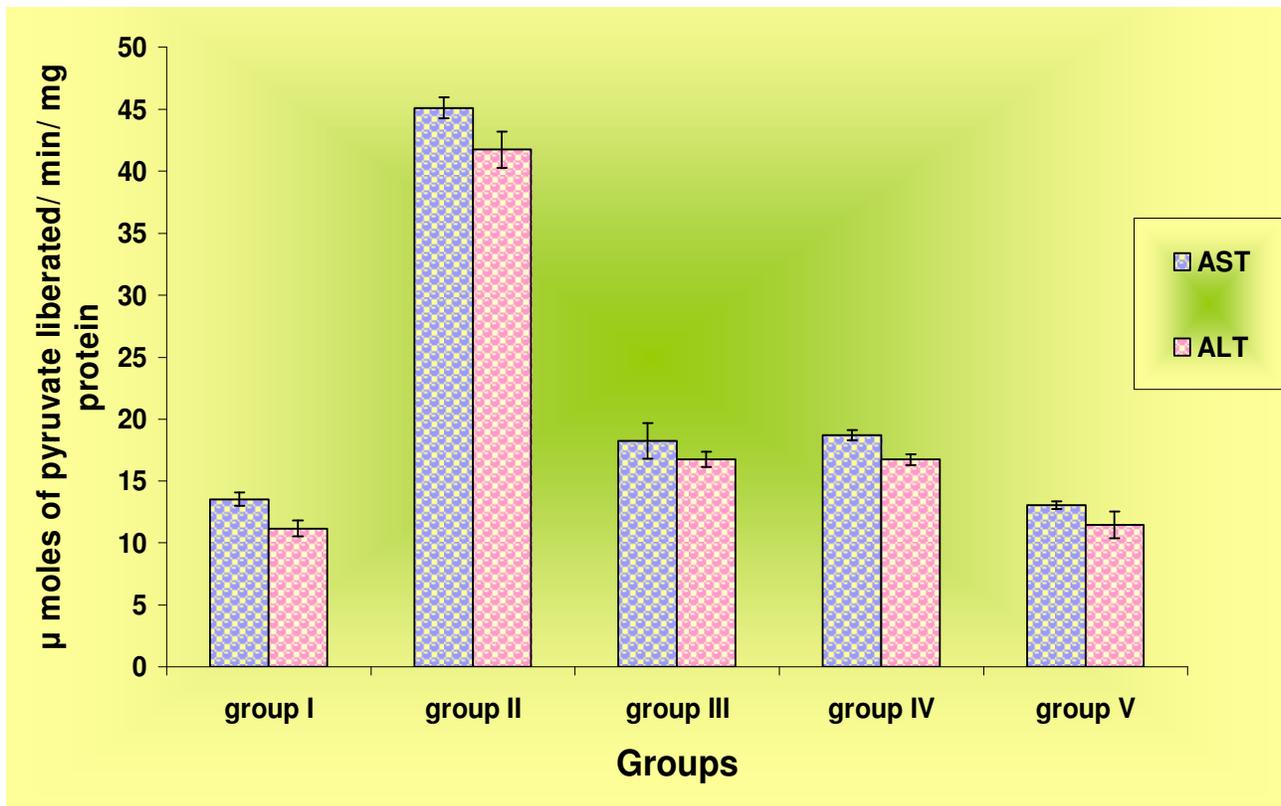


Figure 1. Effect of PHF on AST and ALT levels in liver of control and experimental rats.

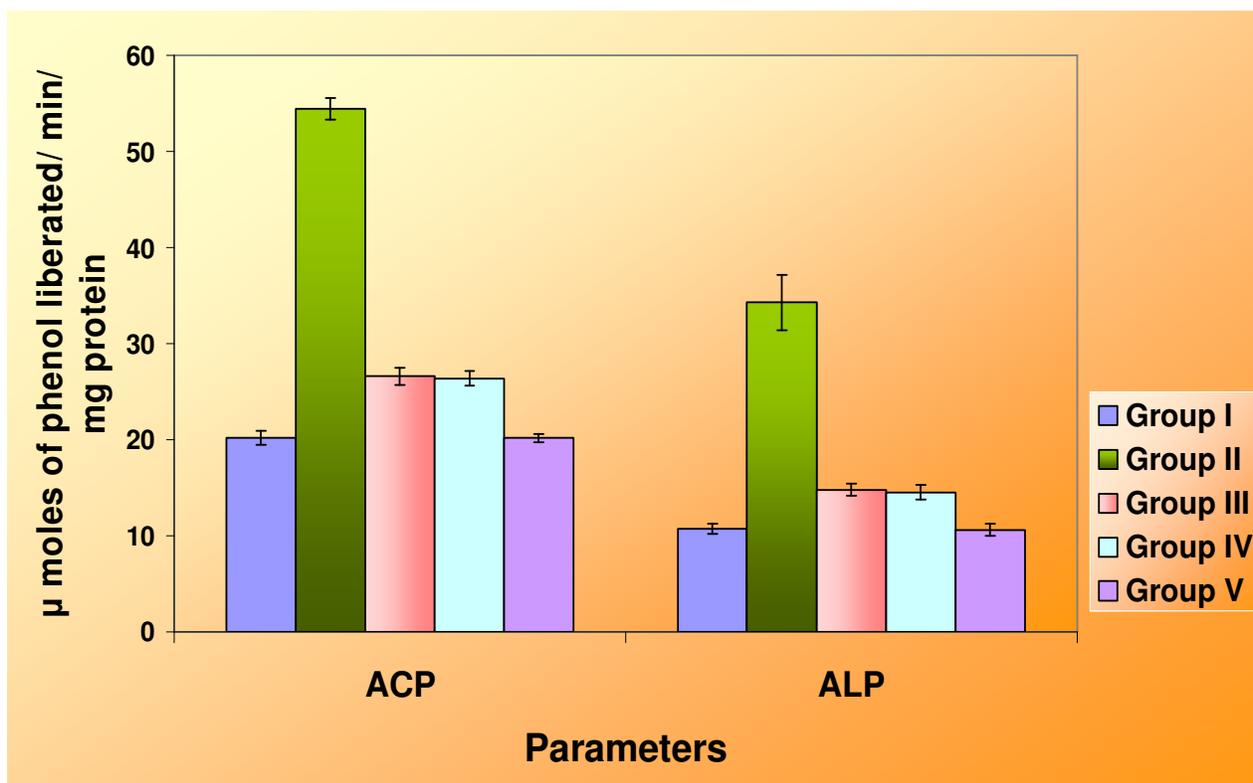


Figure 2. Effect of PHF on ACP and ALP levels in liver of control and experimental rats.

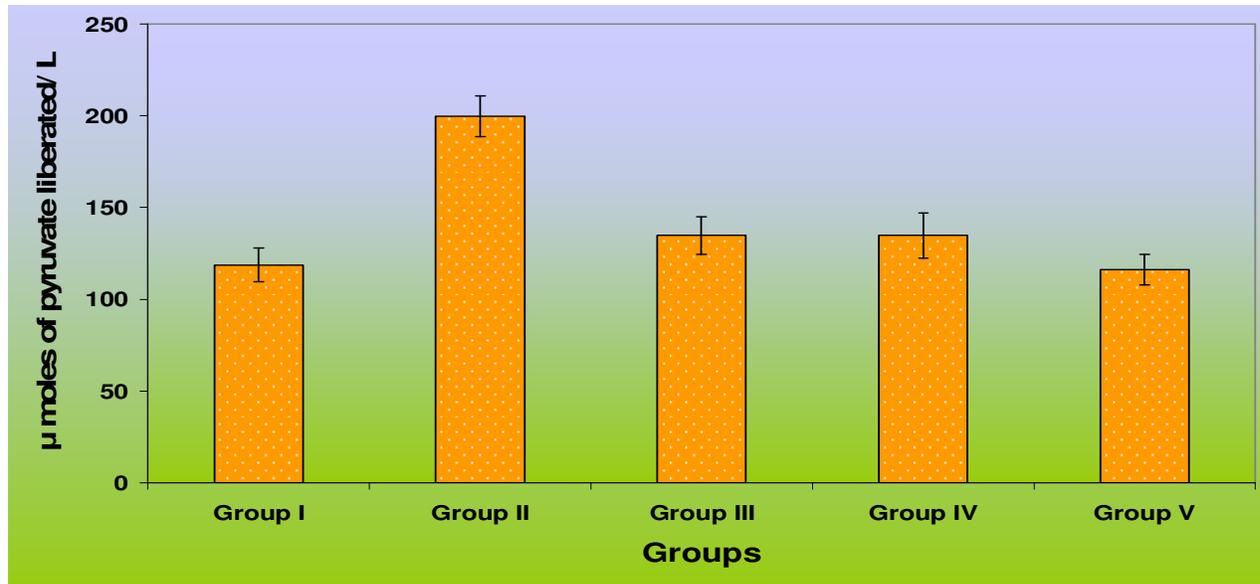


Figure 3. Effect of PHF on LDH in serum of control and experimental rats.

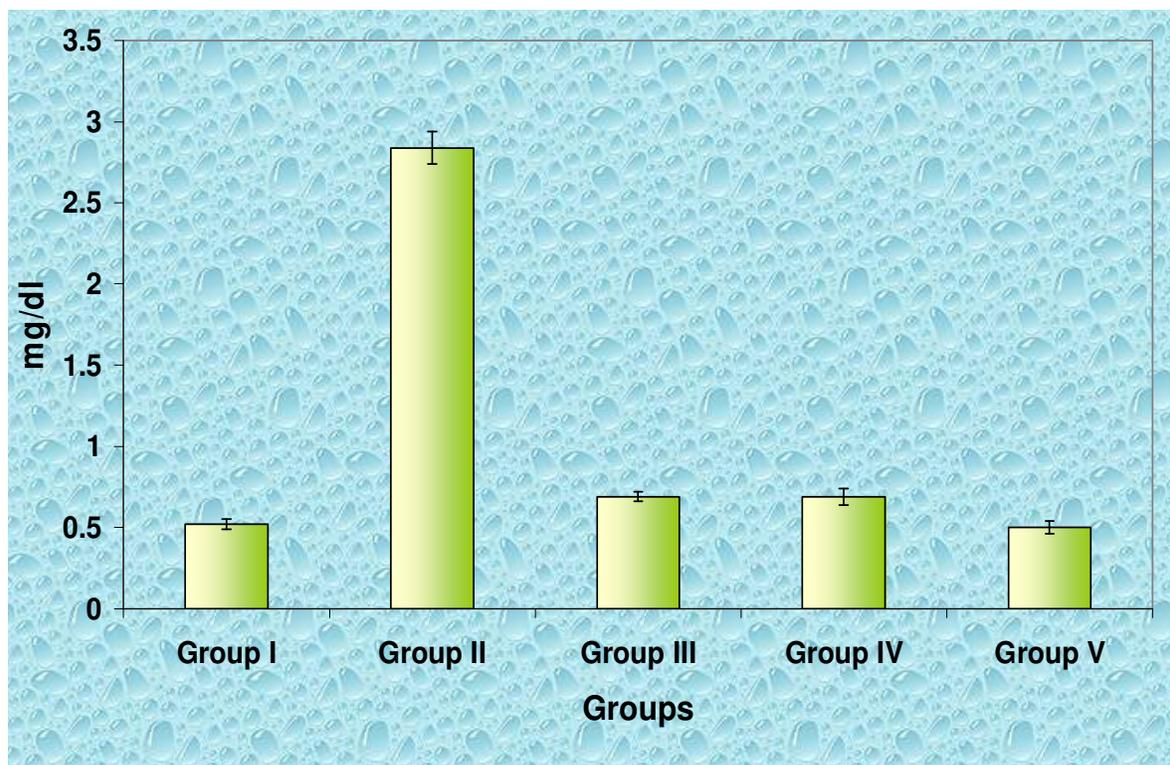


Figure 4. Effect of PHF on bilirubin in serum of control and experimental rats.

depression of protein synthesis (Mondal et al., 2005).

In conclusion, our findings clearly state that polyherbal formulation (PHF) extract, with its potent hepatoprotectant, seems to be a highly promising agent in protecting hepatic tissue against carbon tetrachloride induced liver

damage.

ACKNOWLEDGEMENT

Teacher fellowship to the second and third authors from

Table 5. Effect of PHF on total protein and cholesterol in serum of control and experimental rats.

Groups	Protein (mg/dl)	Cholesterol (mg/dl)
I	6.06±0.24	95.62±1.10
II	3.38±0.12a*	148.55±2.19a*
III	5.45±0.08b*	102.62±1.62b*
IV	5.41±0.07c*,e ^{ns}	105.83±2.84c*,e ^{ns}
V	6.08±0.15d ^{ns}	94.35±2.24d ^{ns}

Values are expressed as mean ± SD of six animals. Statistical comparisons are as shown in Table 3.

University Grants Commission UGC, New Delhi is gratefully acknowledged.

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