

Formulation and Evaluation of Hepatoprotective Polyherbal Formulation containing Indigenous Medicinal Plants

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Abstract: Hepatoprotective activity of polyherbal formulation (PHF) was evaluated and compared statistically after inducing hepatotoxicity in rats by subcutaneous administration of carbon tetra chloride (CCl₄) with olive oil as a diluent in 1:1% v/v on 2nd and 3rd day. The liver damage was confirmed by estimation of elevated levels of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP) and serumbilirubin. Polyherbal formulation (PHF) pretreatment (300mg/kg/b.w.) significantly reduces the CCl₄ induced elevated serum levels of SGOT, SGPT, ALP and serum bilirubin.

Key words: CCl₄ induced hepatotoxicity, polyherbal formulation (PHF), Hepatoprotective activity.

INTRODUCTION

The liver is a vital organ which regulates many important metabolic functions and is responsible for maintaining homeostasis of the body. A number of chemical agents and drugs which are used on a routine basis produce cellular as well as metabolic liver damage¹. Liver diseases are mainly caused by toxic chemicals, excess consumption of alcohol, infections and autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages². No effective measures are available for the treatment of liver disease in modern medicine so far. Herbal drugs, used in Indian systems of medicine are however claimed to be effective and safe in such ailments. These drugs are considered benign and are of particular value in the treatment of

chronic disease requiring prolonged therapy. Plant medicines are more often used in combination rather than in a single in order to get maximum benefit from their combined strength.

The polyherbal formulation (PHF) under study contains plant ingredients *Cocciniaindica*, *Sidacordata* and *Scopariadulcis*. These drugs are used in Indigenous and Ayurvedic system of medicine for the treatment of diseases such as liver, diabetes, gonorrhoea, abdominal pain, diarrhoea, fever and inflammation³⁻⁶. The ingredients and content in dose is based on the traditional knowledge on these plants. The present study was undertaken to explore the hepatoprotective effects of polyherbal formulation (PHF) 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 *Cocciniaindica*, *Sidacordata* and *Scopariadulcis*. The plants were collected from Raipur district of Chhattisgarh, India. They were identified and authenticated (CNH/I-304/2010/Tech. II/255) by office of the additional Director, Central National Herbarium, Govt. of India, Howrah.

Extraction and preparation of polyherbal formulation: The plant parts were washed, shade dried and powdered. In order to prepare the polyherbal formulation (PHF), about 75 gms of *Sidacordata*, 45 gms of *Cocciniaindica* and 30 gms of *Scopariadulcis* plant powders were soaked overnight in 750 ml of 95% ethanol. 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 20 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 polyherbal formulation preparation is shown in Table 1.

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

S/N	Name of Plant	Plant part used	Composition (%)
1.	<i>Cocciniaindica</i>	Leaves	50%
2.	<i>Sidacordata</i>	Leaves	30%
3.	<i>Scopariadulcis</i>	Whole	20%

Chemicals: Silymarin (Microlabs, Mumbai), CCl₄ (Qualigen's, Mumbai), Liquid paraffin (Qualigen's, Mumbai), Estimation of marker enzymes for liver functions such as SGOT, SGPT, ALP, Total bilirubin, Total protein were performed by using kits provided by Span Diagnostic Ltd., (Surat, India), Semi-autoanalyser (MERCK Microlabs-300) was used as instrument for parameter testing. All other chemicals were purchased from SD fine chemicals, Mumbai and were of analytical grade.

Selection of animals: Healthy adult male wistar albino rats weighing about 150 to 200 g were procured from the animal house, Gayatri College of Pharmacy, Sambalpur (Registration No. 1339/ac/10/CPCSEA). The animals were housed in spacious cages. The animals were maintained for 12 h in light and dark cycle at 28 ± 2°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 Anjali foods, Indore, 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).

Induction of hepatotoxicity and drug administration schedule: Hepatoprotective activity was carried out using Male Wistar Rat (150-200 gm). The animals were grouped into 5 (five) groups of 5 (five) animals each group and maintained on standard diet and water ad libitum⁷⁻¹⁰.

Normal Group I: Serve as normal control, 4% gum acacia 1.0 ml/kg/b.w. orally for 5 days with 1.0 ml olive oil given subcutaneously on 2nd and 3rd day.

Positive Control Group II: Serve as toxicant receiving 4% gum acacia 1.0ml/kg/b.w. orally for 5 days with 1:1 CCl₄ in olive oil 1.0 ml/kg/b.w. subcutaneously on 2nd and 3rd day.

Test standard Group III: Serve as standard receiving Silymarin 25mg/kg/b.w. orally for 5 days with 1:1 CCl₄ in olive oil 1.0 ml/kg/b.w. subcutaneously on 2nd and 3rd day.

Test Group IV: Received polyherbal formulation (PHF) at 300mg/kg/b.w. orally for 5 days with 1:1 CCl₄ in olive oil 1.0 ml/kg/b.w. subcutaneously on 2nd and 3rd day.

Test Group V: Received polyherbal formulation (PHF) at 500mg/kg/b.w. orally for 5 days with 1:1 CCl₄ in olive oil 1.0 ml/kg/b.w. subcutaneously on 2nd and 3rd day.

Rats were anesthetized with anesthetic ether on 6th day and blood was collected from retro-orbital plexus and then sacrificed by cervical dislocation. The liver was carefully isolated and preserved in 10% formalin. The weight of each liver was recorded and then subjected to histopathological studies.

Estimation of Biochemical Parameters: Blood was collected from retro-orbital plexus 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 estimation of serum constituents and serum enzymes like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (ALP), Serum Bilirubin and Serum Total Protein. The enzyme levels were assayed using standard kits obtained from Span Diagnostic Ltd¹¹⁻¹⁴.

Statistical analysis: One-Way analysis of variance (ANOVA) was applied for each parameter in order to estimate significant intergroup differences.

Fig 1. Effect of polyherbal formulation (PHF) compared to Silymarin (Standard) on CCl₄-induced rise in SGOT, SGPT and ALP. The values represents Mean±S.D., n=5.

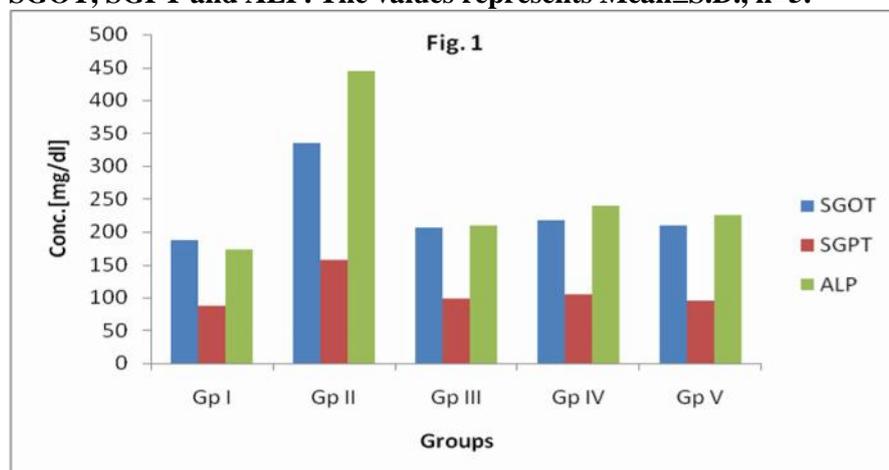
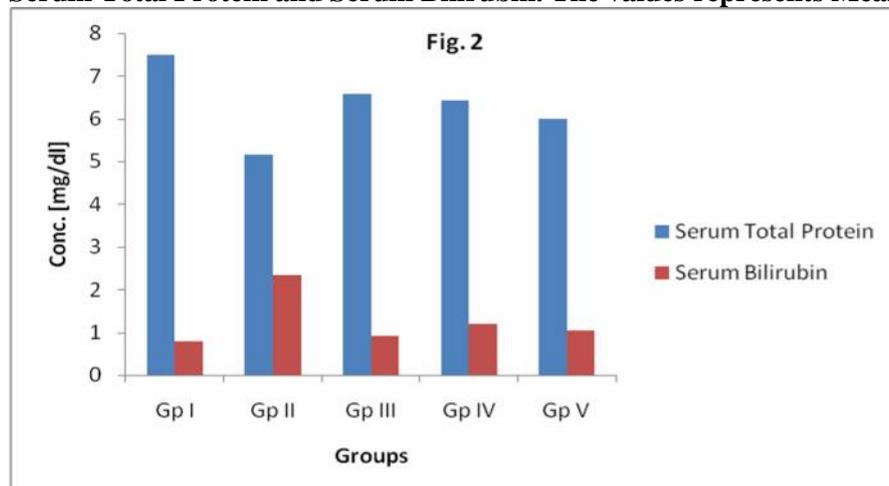


Fig 2. Effect of polyherbal formulation (PHF) compared to Silymarin (Standard) on CCl₄-induced rise in Serum Total Protein and Serum Bilirubin. The values represents Mean±S.D., n=5.



RESULTS AND DISCUSSION

On subcutaneous administration of CCl₄ in dose of 1.0 ml/kg/b.w. produced acute hepatic damage in positive control (CCl₄ treated) when compared with normal control. All the groups except the normal group were treated with CCl₄ exhibits significant increase in level of enzymes SGOT, SGPT, ALP and serum bilirubin.

In case of test groups treated with polyherbal formulation (PHF) at dose of 300mg/kg and 500mg/kg/b.w. were administered simultaneously

with CCl₄. Significant difference in enzyme levels (Fig.1 & Fig.2) in normal, positive and test groups indicate the hepatoprotective activity of polyherbal formulation (PHF). The rise of SGOT levels was significantly reduced at lower dose (300mg/kg), further increase in dose levels (500mg/kg) do not show dose dependent activity.

In the assessment of liver damage by CCl₄ the determination of enzyme levels was largely used. Serum SGPT, SGOT, ALP and bilirubin are the most sensitive markers employed in the diagnosis of

hepatic damage because these are cytoplasmic in location and are released into the circulation after cellular damage. In this study, an increase in the activities of SGPT, SGOT, ALP and bilirubin in serum evidenced the CCl₄ induced hepatocellular damage¹⁵⁻¹⁸.

CONCLUSION:

The polyherbal formulation (PHF) designed and optimized its dose as a hepatoprotective agent for

the treatment of liver diseases. Our findings clearly state that polyherbal formulation (PHF) is potent hepatoprotectant, seems to be highly promising agent in protecting hepatic tissue against CCl₄ induced liver damage.

ACKNOWLEDGEMENT

The authors are thankful to Director, Gayatri College of Pharmacy for providing the requirements to complete the work.

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