RESEARCH PAPER

DEVELOPMENT, STANDARDIZATION AND PHARMACOLOGICAL EVALUATION OF PHF-HP5: *IN VITRO* ANTIOXIDANT AND *IN VIVO* HEPATOPROTECTIVE ACTIVITIES AGAINST DRUG- AND CHEMICAL-INDUCED LIVER INJURY

Tatheer Fatima[1]*, Zeashan Hussain[2], Devendra Kumar Katiyar[3]
[1*]Dr. A.P.J. Abdul Kalam Technical University, Lucknow, Uttar Pradesh, India, tatheerfatima010@gmail.com
[2]Mahatma Gandhi Institute of Pharmacy, Lucknow, Uttar Pradesh, India, zeashanmgip@gmail.com
[3]Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow, Uttar Pradesh, India
***Corresponding Author**
Email: tatheerfatima010@gmail.com
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ABSTRACT
Background: Drug and chemical-induced liver injury is frequently associated with oxidative stress, lipid peroxidation and disruption of hepatic antioxidant defense mechanisms. Polyherbal formulations containing diverse phytochemical constituents represent potential sources of hepatoprotective agents; however, their biological activity requires systematic analytical characterization and experimental validation.
Objective: The present study aimed to develop and characterize PHF-HP5 and investigate its *in-vitro* antioxidant activity and *in-vivo* hepatoprotective potential against rifampicin–isoniazid- and carbon tetrachloride-induced hepatic injury.
Materials and Methods: PHF-HP5 was prepared by combining five authenticated medicinal plant materials in equal proportions, followed by aqueous decoction according to the traditional procedure described in the Sarangadhara Samhita. The formulation was subjected to physicochemical and phytochemical evaluation, HPTLC fingerprinting, and SEM-EDS analysis. Its antioxidant potential was assessed using *in-vitro* assays, while acute oral toxicity was evaluated before pharmacological investigation. Hepatoprotective activity was subsequently examined at doses of 200 and 400 mg/kg in two experimental models: rifampicin–isoniazid-induced and carbon tetrachloride-induced hepatotoxicity. The assessment included serum biochemical parameters, hepatic oxidative-stress and antioxidant markers and histopathological examination of liver tissue.
Results: PHF-HP5 exhibited identifiable phytochemical constituents and an HPTLC fingerprint containing bands corresponding to chlorogenic acid and quercetin. The formulation demonstrated radical-scavenging activity in the *in-vitro* antioxidant assays. In both experimental hepatotoxicity models, administration of PHF-HP5 at 200 and 400 mg/kg attenuated alterations in serum biochemical parameters and hepatic oxidative-stress markers. Histopathological findings further indicated partial preservation of hepatic architecture compared with the respective toxicant-treated groups.
Conclusion: The findings demonstrate the *in-vitro* antioxidant activity and preliminary *in-vivo* hepatoprotective potential of PHF-HP5 against drug- and chemical-induced hepatic injury. Its physicochemical, phytochemical, HPTLC and SEM-EDS profiles provide an initial basis for formulation characterization. The observed protective effects across two experimental models support further investigation of PHF-HP5, including detailed standardization, mechanistic studies and confirmatory pharmacological evaluation.
Keywords: PHF-HP5; Polyherbal formulation; Hepatoprotective activity; Antioxidant activity; Drug-induced hepatotoxicity; Carbon tetrachloride.
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1 INTRODUCTION

Liver injury is frequently associated with oxidative stress, lipid peroxidation, disruption of cellular membrane integrity, and inflammatory responses. Oxidative stress plays an important role in the pathogenesis of drug and chemical-induced hepatotoxicity, contributing to cellular damage and alterations in hepatic function [1]. Carbon tetrachloride (CCl_4), in particular, induces hepatic injury through the generation of reactive free radicals and subsequent lipid peroxidation [2]. Therefore, investigating antioxidant and hepatoprotective activities is important for evaluating the pharmacological potential of medicinal plants and polyherbal formulations.

Phyto therapeutic Rationale

Medicinal plants contain diverse phytoconstituents, including flavonoids, phenolic compounds,

alkaloids, terpenoids, and other bioactive molecules that may contribute to antioxidant and hepatoprotective activities. The present study focuses on five selected botanicals: *Artocarpus lakoocha, Lagenaria siceraria, Lactuca serriola, Portulaca oleracea,* and *Cichorium endivia*, which were combined in equal proportions to develop a multi-component polyherbal formulation.

Artocarpus lakoocha Roxb. (Moraceae), represented by its leaves in the present formulation, has traditionally been used for jaundice and gastrointestinal ailments. It contains bioactive constituents, including oxyresveratrol, stilbenoids, and flavonoids, which have been associated with antioxidant and hepatoprotective properties [3,4].

Lagenaria siceraria (Molina) Standl. (Cucurbitaceae), represented by the seed kernel, is reported to contain various phytoconstituents, including sterols, terpenoids, flavonoids and other bioactive compounds. Different parts of this plant have demonstrated antioxidant and hepatoprotective activities in experimental studies [5].

Lactuca serriola L. (Asteraceae), represented by its seeds, has been investigated for its phytochemical composition, including fatty acids and volatile constituents. Its reported phenolic constituents and free-radical-scavenging potential provide a basis for further pharmacological investigation [6].

Portulaca oleracea L. (Portulacaceae), represented by its seeds, is known to contain diverse phytochemicals, including flavonoids, alkaloids, terpenoids, and fatty acids. Several studies have reported antioxidant and anti-inflammatory properties of this species, supporting its potential contribution to the biological activity of polyherbal preparations [7].

Cichorium endivia L. (Asteraceae), represented by its seeds, contains phytoconstituents such as inulin, esculetin, and chlorogenic acid, with reported hepatoprotective and other pharmacological activities [8].

The diverse phytochemical profiles of these selected botanicals provide a scientific rationale for investigating their combined use in a polyherbal preparation. However, synergistic interactions among the individual plant constituents were not directly established in the present study.

Despite the traditional use and reported pharmacological properties of these botanicals, systematic characterization of their combined formulation and evaluation in experimental models of hepatic injury remain important areas of investigation. Accordingly, the present study aimed to prepare and characterize PHF-HP5, an aqueous polyherbal formulation, through physicochemical and phytochemical evaluation, HPTLC fingerprinting, and SEM-EDS analysis. Its *in-vitro* antioxidant activity and *in-vivo* hepatoprotective potential were further investigated using rifampicin–isoniazid- and carbon tetrachloride-induced hepatotoxicity models. Serum biochemical parameters, hepatic antioxidant status, and histopathological changes were evaluated to assess its protective effects. The study provides preliminary analytical and pharmacological evidence regarding PHF-HP5, while the underlying molecular mechanisms were not directly investigated.

2 MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

The plant materials were identified and authenticated by the Department of Pharmacognosy, Mahatma Gandhi Institute of Pharmacy, Lucknow, as documented in the institutional authentication letter dated 18 February 2020 (reference no. MGIP/02/20/1573). The authenticated materials were *Lactuca serriola L*. (seeds; Asteraceae), *Portulaca oleracea L*. (seeds; Portulacaceae), *Lagenaria siceraria* (kernel of seed; Cucurbitaceae), *Cichorium endivia L*. (seeds; Asteraceae), and *Artocarpus lakoocha Roxb*. (leaves; Moraceae). Plant parts are reported as recorded in the authentication certificate.

2.2 Preparation of Extract and Standardization

The authenticated plant materials were washed, shade-dried, and pulverized using a mechanical grinder. Powders were sieved through mesh 25 and 44 to obtain uniform particle size and then blended in equal proportions (1:1:1:1:1 w/w). A decoction was prepared according to the Sarangadhara Samhita method: 100 g of the composite powder was boiled in 800 mL of distilled water until one-fourth volume remained (≈ 200 mL). The filtrate was concentrated under reduced pressure (rotary evaporator, 40 °C) to yield a dry aqueous extract (21.54%w/w). The extract (PHF-HP5) was standardized following WHO and Ayurvedic Pharmacopoeia of India guidelines for colour, odour, taste, consistency, pH, and solubility.

2.3 Physicochemical and Phytochemical Evaluation

The PHF-HP5 formulation was subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, proteins, amino acids, fixed oils, fats, gums, mucilage, alkaloids, phytosterols, flavonoids, tannins and phenolic compounds, saponins, triterpenoids. Moisture content, ash values, and extractive values were determined by standard procedures (API Vol. I, 1989); The measured constants are summarized in Table 1.

Preliminary qualitative screening revealed the presence of carbohydrates, proteins, amino acids, alkaloids, flavonoids, phenolic compounds, diterpenoids, and phytosterols; saponins and triterpenoids were absent

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2.4 Chromatographic and Elemental Characterization

2.4.1 HPTLC Fingerprinting

High-Performance Thin-Layer Chromatography was performed using silica-gel 60 F_{254} plates (Merck). The solvent system ethyl acetate: methanol: glacial acetic acid (5:4:1 v/v/v) afforded optimum resolution.[9] Plates were visualized at 254 nm and 366 nm and visible light after derivatization with anisaldehyde-sulphuric acid reagent (**figure 1**). The plate was then scanned densitometrically at 217 nm and the profile is presented in **figure 2.** Also, the R_f values and the relative percentage of area in each peak in the extract applied was also calculated and are presented in **table 1.**

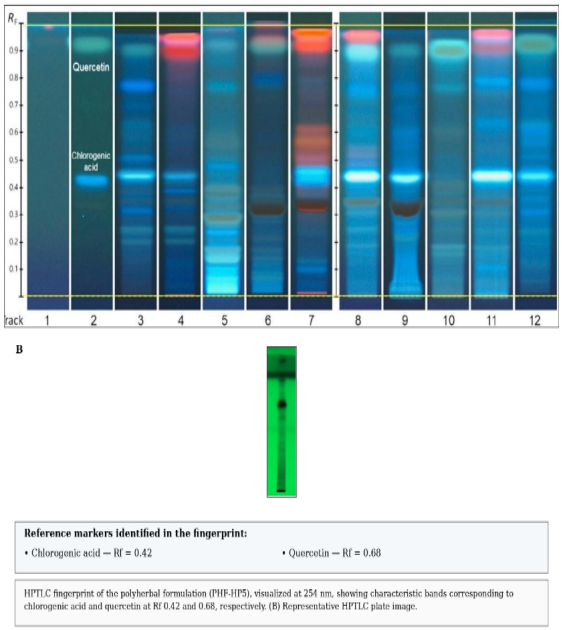


Figure 1: HPTLC fingerprint of PHF-HP5 showing characteristic marker bands

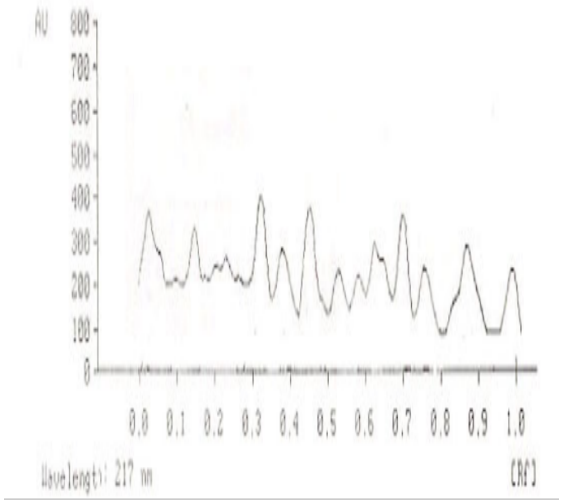


Figure 2: HPTLC fingerprint profile of the PHF-HP5

Table 1 R_f values and the relative percentages of each band of PHF-HP5 in TLC

Peaks	Rf	% Area
1	0.02	10.39
2	0.08	0.53
3	0.14	4.36
4	0.25	0.26
5	0.33	15.11
6	0.35	9.30
7	0.42	14.54
8	0.56	3.42
9	0.51	2.65
10	0.60	7.54
11	0.69	14.33
12	0.79	10.23
13	0.96	3.71

2.4.2 Scanning Electron Microscopy (SEM)

Morphological and elemental characterization was performed using SEM-EDS. SEM was used to assess particulate morphology and surface characteristics, while EDS was used to characterize the elemental composition of analyzed regions. No heavy-metal elements were detected in the analyzed spots under the measurement conditions. Representative morphology and elemental analysis are shown in Figs. 3 and 4, respectively.

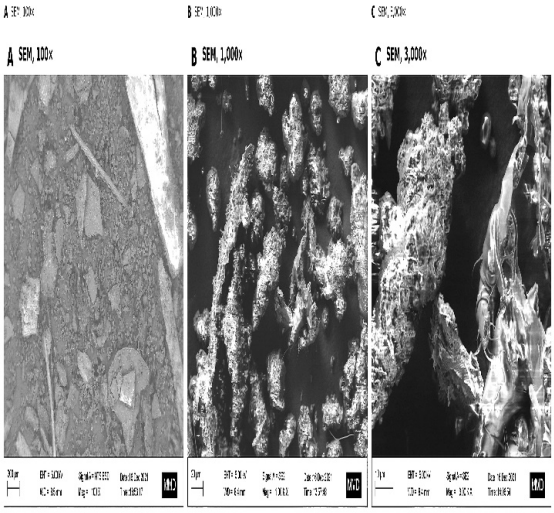


Figure 3. Representative scanning electron micrographs of PHF-HP5 showing surface morphology and particulate microstructure at different magnifications. Original SEM scale bars are retained

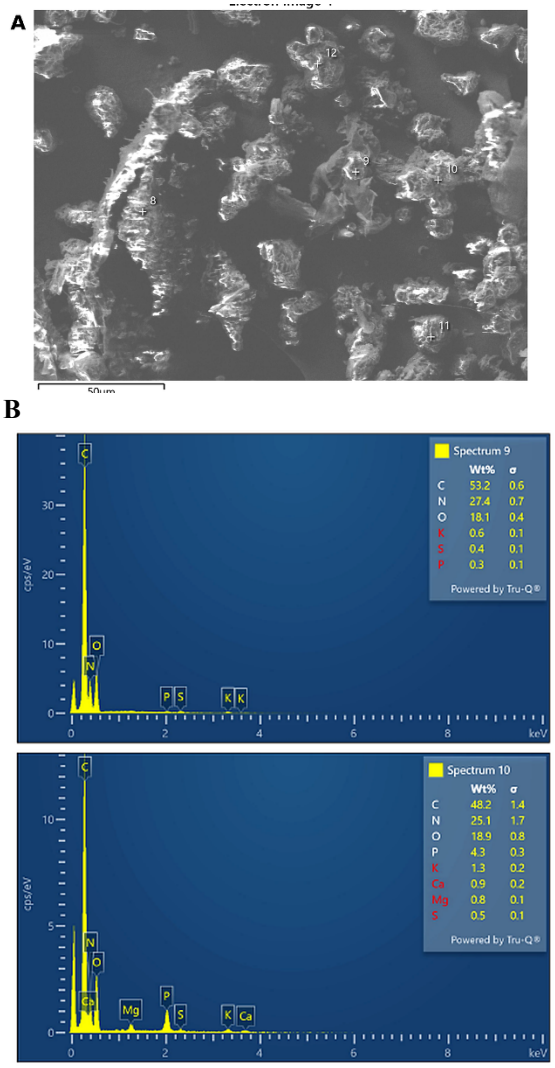


Figure 4 Representative SEM-EDS elemental characterization of PHF-HP5. (A) SEM image of the analyzed region showing the EDS analysis spots; and (B) representative EDS spectra (Spectra 9 and 10) with corresponding elemental weight percentages. Carbon, nitrogen and oxygen were the predominant detected elements, with smaller amounts of phosphorus, sulfur and potassium and, in Spectrum 10, calcium and magnesium. No heavy-metal elements were detected in these analyzed spots under the measurement conditions.

2.5 *In-vitro* Antioxidant Assays

2.5.1 DPPH Free Radical Scavenging

Aliquots of PHF-HP5 (100 µg/mL) were mixed with 0.1 mM DPPH solution; absorbance was measured at 517 nm after 30 min incubation.[10] Ascorbic acid served as standard. % Inhibition = $[(A_0 - A_1)/A_0] \times 100$. Result: PHF-HP5 = 83.13 ± 1.59 %; Ascorbic acid = 96.12 ± 0.38 %.

2.5.2 Total Antioxidant Capacity (Phosphomolybdenum Assay)

The assay mixture contained PHF-HP5 extract (100 µg/mL), 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Absorbance was read at 695 nm; expressed as ascorbic-acid equivalents. PHF-HP5 = 1.45 ± 0.22 AAE µmol/g. [11]

2.5.3 Thiobarbituric Acid (TBA) Method

Lipid peroxidation inhibition was determined by colour intensity of MDA–TBA complex at 552 nm. PHF-HP5 = 2.08 ± 0.95 µmol MDA eq./g, indicating strong peroxidation suppression. [12]

2.5.4 Hydroxyl Radical Scavenging (Deoxyribose Assay)

Degradation of deoxyribose by hydroxyl radicals generated from an Fe^{3+}–EDTA–H_2O_2 system was quantified at 532 nm [13]. PHF-HP5 demonstrated hydroxyl radical-scavenging activity, with inhibition values of 25.01 ± 2.64 at 200 µg/mL and 24.45 ± 1.24 at 400 µg/mL, comparable to gallic acid (24.22 ± 0.55).

2.5.5 Total Phenolic Content

Determined by Folin–Ciocalteu reagent; expressed as mg gallic acid equivalents per g extract. PHF-HP5 200 = 0.27 ± 0.13; PHF-HP5 400 = 0.31 ± 0.14 mg GAE/g.

2.6 Experimental Animals

The animal study was conducted under institutional IAEC project proposal No. MGIP/05/2020-21.

2.7 Acute Oral Toxicity Study

Acute toxicity was evaluated according to OECD Test Guideline 423 (2002) [14]. Female mice were administered PHF-HP5 orally at 5, 50, 300 and 2000 mg/kg b.w. and observed for behavioural, neurological and autonomic changes for 14 days. No mortality or overt toxicity was observed up to 2000 mg/kg. Based on this acute-toxicity assessment 200 and 400 mg/kg were selected for pharmacological evaluation. [15]

2.8 Hepatoprotective Studies

2.8.1 Rifampicin + Isoniazid Induced Hepatotoxicity

Rats were divided into five groups (n = 6): I – Control (Normal saline 5 mL/kg); II – Toxin (Rifampicin + Isoniazid 50 mg/kg each); III – Standard (Silymarin 50 mg/kg + RIF + INH); IV – PHF-HP5 200 mg/kg + RIF + INH; V – PHF-HP5 400 mg/kg + RIF + INH. The RIF + INH model is consistent with the established oxidative-stress model described by Sodhi et al. [15]

Treatments were given orally for 13 consecutive days. Blood samples were collected for estimation of serum SGOT, SGPT, ALP and bilirubin; liver tissues were used for biochemical assays and histopathology.

2.8.2 Carbon Tetrachloride (CCl_4) Induced Hepatotoxicity

The animals were grouped into five groups (n = 6): I – normal saline control; II – CCl_4 1 mL/kg *i.p.*; III – silymarin 25 mg/kg p.o.; IV – PHF-HP5 200 mg/kg *p.o.*; and V – PHF-HP5 400 mg/kg p.o. CCl_4 was prepared in liquid paraffin (1:2), administered once every 72 h for 14 days, and PHF-HP5 treatment was initiated 10 days before CCl_4

administration and continued until the end of the experiment. Biochemical and enzymatic parameters were assessed at the end of the exposure period.[2]

2.9 Biochemical and Antioxidant Parameters

Serum SGOT, SGPT, ALP and bilirubin were analysed using standard kits (Erba Diagnostics, India). Liver homogenates were used to quantify malondialdehyde (MDA), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST), and superoxide dismutase (SOD) activities by spectrophotometric methods. [12,16,17,18,19].

2.10 Histopathological Studies

Liver tissues were collected from experimental animals and fixed in 10% neutral buffered formalin. The tissues were dehydrated through graded alcohol concentrations (80%, 95%, and absolute alcohol), cleared with xylene, and embedded in paraffin wax. Sections of approximately 5 µm thickness were cut using a microtome, mounted on albumin-coated glass slides, and stained with hematoxylin and eosin (H&E). The stained sections were dehydrated, cleared with xylene, and mounted using DPX mounting medium.[20]

2.11 Statistical Analysis

The results of the study were expressed as mean ± SEM (Standard error of the mean). The student t-test and analysis of variance (ANOVA) were used to analyze the experimental data for its significance. Graphs were plotted in GraphPad Prism v9.0.

3 RESULT AND DISCUSSION

3.1 Physicochemical and Phytochemical Analysis

The polyherbal formulation (PHF-HP5) was a dark-brown amorphous powder, readily soluble in water and exhibiting a mildly acidic pH (4.4 ± 0.1). Moisture content (8.6 %), total ash (5.6 %), and alcohol-soluble extractive (21.4 %) were recorded as physicochemical characteristics of the extract. Organoleptic and basic physicochemical characteristics are provided in Table 1.

Table 2. Physicochemical parameters of PHF-HP5 extract

Parameter	Result
Colour	Dark brown
Odour	Pungent
Taste	Tasteless
Consistency	Solid, smooth
% Yield	21.54 %w/w
Solubility	Water
pH	4.4

Preliminary qualitative screening revealed the presence of flavonoids, phenolic compounds, alkaloids, glycosides, proteins, carbohydrates, diterpenes, and phytosterols; saponins and triterpenoids were not detected under the screening conditions.

The measured physiochemical constants are summarized in Table 2 & 3.

Table 3. Physicochemical constants of PHF-HP5

Parameter	Result (% w/w)
Moisture content	8.6
Foreign matter	0.48
Total ash	5.62
Acid-insoluble ash	0.35
Water-soluble ash	0.60
Alcohol-soluble extractive	21.4
Water-soluble extractive	16.15

3.2 Acute toxicity studies

No behavioral or physiological abnormalities were observed up to 2000 mg/kg body weight in the acute toxicity study. These findings indicate no overt acute toxicity under the conditions tested.

3.2.1 Hepatotoxicity model

Histopathology of the RIF + INH model showed hepatic injury in the toxin control and comparatively preserved architecture in the silymarin- and PHF-HP5-treated groups (Fig. 5). Histopathology from the CCl_4 model is presented in Fig. 6

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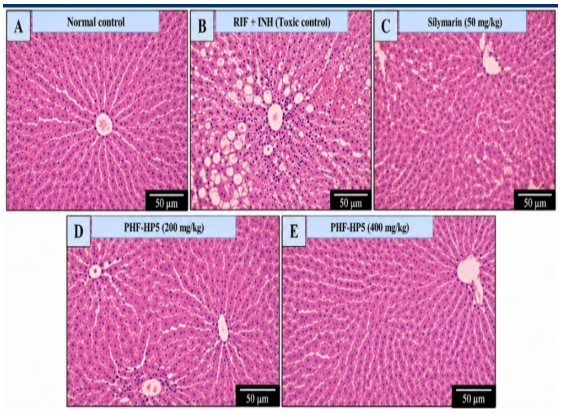


Figure 5 Histopathological sections of rat liver in the RIF + INH-induced hepatotoxicity model (H&E staining, 400×). (A) Normal control showing preserved hepatic architecture (B)RIF + INH toxic control showing hepatic injury; (C) silymarin 50 mg/kg showing attenuation of hepatic injury; (D) PHF-HP5 200 mg/kg; and (E) PHF-HP5 400 mg/kg.

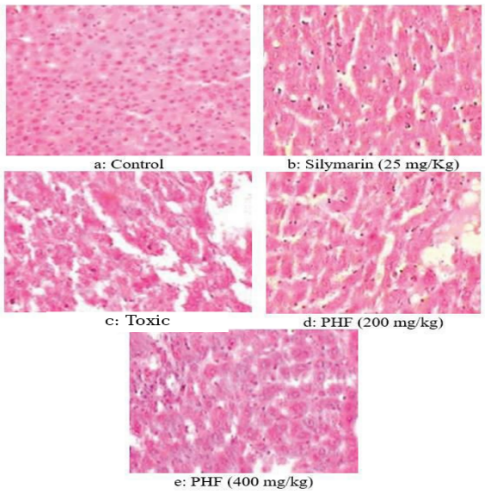


Figure 6 Histopathological sections of rat liver in the CCl_4-induced hepatotoxicity model (a) Normal Control (b) silymarin 25 mg/kg (c) CCl_4 toxic control (d) PHF-HP5 400 mg/kg (e) PHF-HP5 200 mg/kg

The observed hepatoprotective effects may be associated with the antioxidant and phytochemical characteristics of PHF-HP5; specific molecular mechanisms were not directly investigated in this experiment. Hepatic antioxidant results for the RIF + INH model are summarized in Table 6.

Administration of rifampicin and isoniazid increased serum AST (SGOT), ALT (SGPT), ALP, and total bilirubin compared with the normal control group. Treatment with PHF-HP5 at 200 and 400 mg/kg attenuated these alterations (Table 4). The reductions in AST and ALT were comparable between the two PHF-HP5 doses, while total bilirubin showed a greater reduction at 400 mg/kg. The corresponding group means are plotted in Fig. 7.

3.3. *In-vitro* Antioxidant Activity

CCl_4 exposure increased hepatic MDA and reduced GSH, GPx, GR, GST and SOD compared with the normal control group. PHF-HP5 attenuated these changes at both tested doses, although the magnitude of response varied among individual parameters (Table 7). Serum biochemical results for this model are shown in Table 5; hepatic antioxidant parameters are presented in Table 7.

Table 4 Effect of PHF-HP5 on serum biochemical parameters in RIF + INH-induced hepatotoxicity.

These findings support an association between improved hepatic antioxidant status and the observed hepatoprotective effects. Group-mean relationships between antioxidant parameters and MDA are illustrated in Figs. 9 and 10 for the RIF + INH and CCl_4 models, respectively.

The PHF-HP5 exhibited measurable radical-scavenging activity across the assays performed. The DPPH and other antioxidant assay results are described in the following text.

DPPH assay: PHF-HP5 (100 µg/mL) showed 83.13 ± 1.59 % inhibition, compared with ascorbic acid (96.12 ± 0.38 %).

Hydroxyl radical assay: PHF-HP5 demonstrated hydroxyl radical-scavenging activity, with inhibition values of 25.01 ± 2.64 at 200 µg/mL and 24.45 ± 1.24 at 400 µg/mL [13]

Total phenolic content: 0.31 ± 0.14 mg GAE/g.

The *in-vitro* findings demonstrate measurable antioxidant activity of PHF-HP5 across the assays performed.

From an Ayurvedic perspective, the formulation may be discussed in relation to traditional concepts such as Rasayana and Pittashamaka activity. The experimental findings provide contemporary evidence relevant to the biological effects investigated; however, direct equivalence between these experimental outcomes and Ayurvedic concepts should be interpreted cautiously.

Proposed Pharmacological Interpretation:

Specific molecular pathways and enzyme targets were not directly measured in the present study. The proposed pharmacological interpretation should therefore be regarded as preliminary and require further experimental validation.

The integrated findings support further investigation of PHF-HP5 as a standardized multi-component polyherbal preparation.

Group	Treatment	AST/SGOT (U/L)	ALT/SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dL)
I	Normal control	27.11± 4.53	69.52± 1.16	75.46± 1.23	7.23±0.52
II	RIF + INH toxic control	80.30± 7.49***	124.05 ±3.67***	156.59 ±8.02***	44.57± 0.93***
III	Silymarin 50 mg/kg	42.89± 5.08***	84.78± 1.246***	95.73± 0.86***	24.07± 0.505***
IV	PHF-HP5 200 mg/kg	46.76± 4.64**	84.07± 1.6**	90.20± 0.52**	16.92± 0.355**
V	PHF-HP5 400 mg/kg	44.81± 4.22**	82.92± 3.21**	94.73± 0.63**	6.913± 0.145**

*Data are expressed as the means ± SEM, Number of animals in each group (n=6), P value: *<0.05, **<0.01, ***<0.001 when compared with control*

Table 5 Effect of PHF-HP5 on serum biochemical parameters in CCl_4-induced hepatotoxicity.

Group	Treatment	AST/SGOT (U/L)	ALT/SGPT (U/L)	ALP (U/L)	Total bilirubin (mg/dL)
I	Normal control	58 ± 0.97	71 ± 0.51	64 ± 1.72	0.70 ± 0.03
II	CCl_4 toxic control	161 ± 2.95***	145 ± 0.58***	120 ± 0.08***	2.42 ± 0.17***
III	Silymarin 25 mg/kg	76 ± 1.46***	82 ± 1.08***	76 ± 1.06***	0.80 ± 0.03***
IV	PHF-HP5 200 mg/kg	98 ± 1.39*	66 ± 1.75*	95 ± 1.30*	0.70 ± 0.02*
V	PHF-HP5 400 mg/kg	86 ± 0.58**	76 ± 0.089***	84 ± 1.32**	0.67 ± 0.06**

*Data are expressed as the means ± SEM, Number of animals in each group (n=6), P value: *<0.05, **<0.01, ***<0.001 when compared with control*

Table 6 Effect of PHF-HP5 on hepatic antioxidant parameters in RIF + INH-induced hepatotoxicity.

Group	Treatment	GSH (µmol/g)	GPx (µmol/g)	GR (µmol/g)	GST (µmol/g)	SOD (µmol/g)	MDA (nmol/mL)
I	Normal control	3.70 ± 0.32	3.30 ± 0.31	2.46 ± 0.24	47.12 ± 4.40	356 ± 7.10	34.12 ± 2.98
II	RIF + INH toxic control	2.45 ± 0.32**	2.10 ± 0.18**	1.58 ± 0.35***	22.24 ± 2.34**	235.25 ± 3.10***	69.20 ± 5.10***
III	Silymarin 50 mg/kg	3.48 ± 0.45**	2.84 ± 0.28**	2.45 ± 0.32**	38 ± 4.65***	324 ± 4.34**	39.46 ± 2.22**
IV	PHF-HP5 200 mg/kg	3.35 ± 0.25**	2.38 ± 0.45*	2.65 ± 0.18**	40.02 ± 3.47**	300.10 ± 5.10**	40.15 ± 2.75**
V	PHF-HP5 400 mg/kg	3.68 ± 0.22*	3.45 ± 0.32**	2.55 ± 0.38**	48 ± 2.55*	342.47 ± 5.42*	38.02 ± 3.44**

*Data are expressed as mean ± SEM (n = 6). ns = not statistically significant (p > 0.05); *p* < 0.05 = statistically significant; **p** < 0.01 = highly statistically significant; and ***p*** < 0.001 = very highly statistically significant.*

Table 7 Effect of PHF-HP5 on hepatic antioxidant parameters in CCl_4-induced hepatotoxicity.

Group	Treatment	GSH (µmol/g)	GPx (µmol/g)	GR (µmol/g)	GST (µmol/g)	SOD (µmol/g)	MDA (nmol/mL)
I	Normal	3.65 ±	3.40 ±	2.40 ±	43.81	357 ±	33.32 ±

	control	**0.30**	**0.37**	**0.22**	**± 3.35**	**7.15**	**3.20**
II	**CCl_4 toxic control**	**2.45 ± 0.23*** **	**1.74 ± 0.23*** **	**1.52 ± 0.25****	**22.31 ± 2.64*** **	**234.50 ± 4.22*** **	**65.20 ± 4.20****
III	**Silymarin 25 mg/kg**	**3.21 ± 0.34****	**2.46 ± 0.30****	**2.19 ± 0.36*** **	**36.10 ± 4.31*** **	**300 ± 4.80*** **	**39.15 ± 2.39*****
IV	**PHF-HP5 200 mg/kg**	**3.68 ± 0.21****	**2.55 ± 0.28****	**2.35 ± 0.29****	**31.15 ± 2.4**	**337.40 ± 4.8**	**39.02 ± 3.80****
	mg/kg	*	*		**7*** **	**6****	
V	**PHF-HP5 400 mg/kg**	**3.57 ± 0.16****	**3.00 ± 0.24*** **	**2.49 ± 0.33****	**43 ± 14*** **	**323.74 ± 6.24****	**39.46 ± 3.50*****

*Data are expressed as mean ± SEM (n = 6). ns = not statistically significant (p > 0.05); *p* < 0.05 = statistically significant; **p** < 0.01 = highly statistically significant; and ***p*** < 0.001 = very highly statistically significant.*

4 CONCLUSION

The present study provides preliminary pharmacognostic, physicochemical, HPTLC, and SEM-EDS characterization of PHF-HP5, supporting its initial standardization as a polyherbal formulation. Its *in-vitro* antioxidant activity and beneficial effects on biochemical and hepatic oxidative-stress parameters in rifampicin–isoniazid- and carbon tetrachloride-induced hepatotoxicity models indicate promising antioxidant and hepatoprotective potential. The HPTLC fingerprint also revealed bands corresponding to chlorogenic acid and quercetin. Further studies are required to validate these findings, elucidate the underlying mechanisms, assess the contribution of individual constituents, and establish batch-to-batch reproducibility before therapeutic applicability in humans can be considered.

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Ethical Approval

Animal procedures were conducted under institutional IAEC project proposal No. MGIP/05/2020-21.

Conflict of Interest

The authors declare no conflict of interest.

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