RESEARCH ARTICLE

Polyherbal Formulation Development, Evaluation and Pharmacological Screening for Hepatoprotective Activity

Hindole SS*, Vijayendraswamy SM, Sakhare RS

Channabasweshwar Pharmacy College (Degree), Latur, SRTMU Nanded, Maharashtra, India.

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ABSTRACT

The current research work investigates the hepatoprotective action ofpolyherbal formulation prepared from ethanolic extracts of *Azadirachta indica* A. Juss, *Moringa oleifera* Lam, *Cymbopogon citrates* (DC.) Stapf, *Tinospora cordifolia* (Thumb.) Miers, *Ricinus communis* L., against experimental hepatotoxicity. The World Health Organization (WHO) is promoting herbal remedies derived from traditional medicinal plants in several countries. The main goal of this investigation was to create and evaluate the polyherbal formulation to protect albino Wistar rats against the hepatotoxic effects of CCl_4. Three potential suspensions, polyherbal formulations A, B, and C, were formulated by trituration method using ethanolic extracts of specific medicinal plant components with different sodium carboxy methyl cellulose concentrations 1, 1.5, and 2%, respectively and evaluated the same for both certain physiochemical parameters, including organoleptic characteristics, pH, viscosity, sedimentation volume, redispersibility, density, specific gravity, zeta potential, and accelerated stability studies for three months and for hepatoprotective activity also. Evaluation parameters for Polyherbal Formulation-C have shown good results that met the specifications, with pleasant texture and appearance. Along with other physiochemical qualities, like pH, viscosity, flow rate and sedimentation, were all unchanged and showed significant hepatoprotective effects by reducing elevated serum enzyme levels SGOT, SGPT, ALP, total and direct bilirubin. It was discovered that each quality control parameter in the polyherbal suspension C formulation was thoroughly adequate and improved the recovery from hepatotoxicity caused by CCl4. These findings suggest that the produced polyherbal suspension C (containing ethanolic extracts of *A. indica* A. Juss, *M. oleifera* Lam, *C. citrates* (DC.) Stapf, *T. cordifolia* (Thumb.) Miers, *R. communis* L. may be stable, secure, and effective for use and exhibited notable hepatoprotective effects, possibly resulting from the combined impact of all these extracts.

Keywords: Polyherbal formulation, Hepatoprotective, Stability testing, Physiochemical, Standardization.

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INTRODUCTION

Because of increased public concern about the toxicity and side effects of allopathic pharmaceuticals, natural commodities will participate in an increasingly key role in the public health system. Herbal medicine has risen tremendously in recent decades as a result of increased global interest in natural, non-synthetic medications produced from plants or herbs due to improved tolerance and lower risk of adverse drug reactions.[1] In modern medicine, there are currently no viable treatments for liver illnesses. Herbal medicines are safe and effective, particularly for treating chronic illnesses that require long-term care. Combinations are more typically employed for giving plant medicines than single forms in order to maximize the benefit of their combined ability to reduce one another's negative effects. Keeping the aforementioned facts in mind, an indigenous polyherbal remedy was developed.[2,3] Leaves of *Ricinus communis* L., *Moringa oleifera* Lam., *Azadirachta indica* A. Juss, *Cymbopogon citrates* (DC.) Stapf and leaves and stems of *Tinospora cordifolia* (Thumb.) Miers are used to treat a wide range of disorders, including scurvy and respiratory ailments, antiviral, anti-inflammatory, hepatoprotective, analgesic, skin diseases, hypoglycemic, hypolipidemic, antimicrobial, antispasmodic, anti-inflammatory, diabetes, urinary tract infections, expectorant, carminative, digestive, antistress and aphrodisiac, antipyretic, diuretic and sedative, etc.[4-7] Thus, the current study was conducted to design and assess polyherbal suspension formulations from ethanolic extracts of above-selected plants against experimental hepatotoxicity which demonstrating strong hepatoprotective

Author for Correspondence: sshindole19@gmail.com

action and comparing these effects with LIV-52, a standard marketed medication.

MATERIAL AND METHODS

Plants Collection and Identification

The chosen plant materials, including RCL, MOL, AIL, TCLS, and CCL, needed for the current study were sourced from various locations within the districts of Latur and Osmanabad, Maharashtra, India. Extra care was taken to ensure that only healthy plant materials were gathered and that alien elements were kept out. Plant herbarium sheets were created and sent for verification to the Botanical Survey of India's Department of Botany. Famous botanist Prof. D. L. Shirodkar of the Botanical Survey of India's Department of Botany further identified and verified the plants possessing authentication voucher number BSI/WRC/Iden.Cer./2021/3108210000452. Following collection, the plant samples were verified and properly cleaned with distilled and running tap water. They were then left to air dry at room temperature for a while. The plant material was then cleaned and allowed to dry for three to four weeks in the shade. An electric grinder was used to roughly smash the dried plant material. The color, flavor, texture, and taste of the powdered plant material were assessed. This dried plant material was kept in an airtight container so that it might be used in later research.

Chemical Reagents

In this study, analytical grade chemicals and solvents were used.

Extraction

Plant materials that had been dried in the air and roughly powdered were first defatted with petroleum ether using a soxhlet device. They were then extracted using a series of solvents in increasing order of polarity, such as ethanol, chloroform, and ethyl acetate. A rotary flash evaporator was used to concentrate the extracts running at low pressure, and the residue was then dried over sodium sulfite in a desiccator. The appropriate extracts were weighed after drying, the yield percentage was determined, and they were then sealed in an airtight container.[8-10]

Formulation

Dried ethanolic extracts of RCL, MOL, AIL, TCLS, and CCL were combined to create a 100 mL polyherbal suspension by trituration method (Table 1).

Table 1: Polyherbal formulation composition

S. No.	*Name of ingredient*	*Quantity*
01	Bio-active extract	1–5 g
02	Tween 80	0.1% v/v
03	Sodium carboxymethylcellulose (CMC)	2%
04	Methyl paraben	0.2% w/v
05	Lemon oil	0.01%
06	Distilled water	up to 100 mL

Table 2: Polyherbal formulation composition (Batchwise)

S. No.	*Name of ingredient*	*Polyherbal formulation*		
		PHF-A	*PHF-B*	*PHF-C*
01	Ethanolic extract of R.C. (g)	0.5	0.5	0.5
02	Ethanolic extract of M.O. (g)	0.5	0.5	0.5
03	Ethanolic extract of A.I. (g)	0.5	0.5	0.5
04	Ethanolic extract of T.C. (g)	0.5	0.5	0.5
05	Ethanolic extract of C.C. (g)	0.5	0.5	0.5
06	Tween80 (% v/v)	0.3	0.2	0.1
07	Sodium carboxy methylcellulose (CMC) (%)	1	1.5	2
08	Methyl paraben (% v/v)	0.2	0.2	0.2
09	Lemon oil (% v/v)	0.01	0.01	0.01
10	Distilled/Purified water (mL)	up to 100	up to 100	up to 100

Development of Polyherbal Suspension Dosage Form

The formula presented in Table 1 can be used to create a 100 mL suspension of ethanolic extracts from RCL, MOL, AIL, TCLS, TCL. To make the polyherbal suspension, dry ethanolic extracts of certain plant materials were triturated in a mortar and pestle using a suitable suspending agent (Tween 80) and sodium carboxymethyl cellulose (CMC)in a 1:1:1[2, 3] ratio together with various excipients. Every substance mentioned above was triturated using water. It was then necessary to add the preservative and flavoring ingredient (lemon oil) while continuously triturating the previously mentioned aqueous medium in a mortar and pestle. 1, 1.5, and 2% aqueous sodium CMC solution were used to create PHF-A, PHF-B, and PHF-C, three potential suspension formulations. In order to achieve a homogenous result, the remaining amount was increased to 100 mL *via* continuous trituration after the addition of distilled water (Table 2). All polyherbal formulations made from extracts of RCL, MOL, AIL, TCLS, CCL (Figure 1) were further evaluated in accordance with standards.

Phytochemical Screening[11, 12]

The phytochemical screening of the developed preparation detected the presence of proteins, carbohydrates, alkaloids, glycosides, steroids, flavonoids, terpenoids, phenolic compounds, and other components (Table 3).

Evaluation Parameters of the Polyherbal Suspension[13-16]

A variety of characteristics, including aesthetic qualities, pH, viscosity, sedimentation volume, re-dispersibility, zeta potential, crystal growth, etc., were evaluated for all three polyherbal formulations.

Figure: 1 Photographs of polyherbal formulations

Table 3: Phytochemical evaluation

S. No.	*Phytoconstituents*	*Polyherbal formulation*		
		PHF-A	*PHF-B*	*PHF-C*
01	Carbohydrate	+	+	+
02	Glycoside	+	+	+
03	Protein	+	+	+
04	Steroids	++	++	++
05	Terpenoids	+	+	+
06	Tannins	+	+	+
07	Saponin	-	-	-
08	Phenols	+++	+++	+++
09	Alkaloids	+	+	+
10	Flavonoids	+++	+++	+++

Organoleptic Properties/Aesthetic Characteristics

The following criteria, such as color, flavor, taste and texture, were used to assess the organoleptic characteristics of the polyherbal suspension (Table 4).

Accelerated stability studies

For polyherbal compositions containing bioactive components, accelerated stability tests were carried out by incubating the samples for three months at room temperature (8°C) and 45°C ± 2 with 75% ± 5% humidity. At intervals of one to three months, a variety of characteristics, including sedimentation volume, viscosity, re-dispersibility, pH, zeta potential, and crystal development, was observed for an optimal formulation.

Sedimentation Volume

The ratio of final sediment height to beginning suspension height when suspension settles in a cylinder under suitable normal circumstances is known as the sedimentation volume. A measured volume of suspension was kept in an undisturbed state in a graduated cylinder for a predetermined amount of time in order to evaluate it.

It should be noted that sedimentation volume is indicated as the maximum height.

$$F = Hu/Ho$$

Table 4: Organoleptic evaluation

S. No.	*Parameter*	*PHF-A*	*PHF-B*	*PHF-C*
01	Nature	Liquid suspension	Liquid suspension	Liquid suspension
02	Color	Greenish black	Greenish black	Greenish black
03	Odor	Characteristic	Characteristic	Characteristic
04	Taste	Slight bitter	Slight bitter	Slight bitter

Redispersibility

The suspension was given time to settle using a measurement cycle. A calculation was done to find out how many inversions would be required to achieve a uniform suspension once the cylinder's mouth was closed and turned 180º.

Rheology

Using the following formula, it was determined how long it would take for each suspension sample to go through a 10 mL pipette.

Flow rate = Volume of Pipette (mL)/Time (seconds)

pH

A Eutech pH meter was used to measure the pH of the polyherbal formulation.

Determination of Viscosity

With a spindle 2 run at 250 rpm, a Brookfield viscometer type III was used to measure the viscosity of the polyherbal formulation. All computation results yielded mean values, and each calculation was repeated three times.

Particles Size Analysis

The particle size distribution inside a suspension formulation is a key factor in its stability. Optical microscopy was utilized to determine particle size distribution in diluted samples.

Crystal Growth

Crystal formation, which is often caused by temperature fluctuations during storage and results in smaller dimensions, may further degrade the stability of polyherbal suspensions. The crystal formulations at room temperature (8 and 45°C) were determined.

Zeta Potential Determination

To understand the behavior of a dispersive system, the dynamic light scattering (DLS) method and the Malvern Zeta sizer Nano-ZS series automated inspection system at 25 ± 0.5 were used to analyze the zeta potential of suspension (Figure 2).

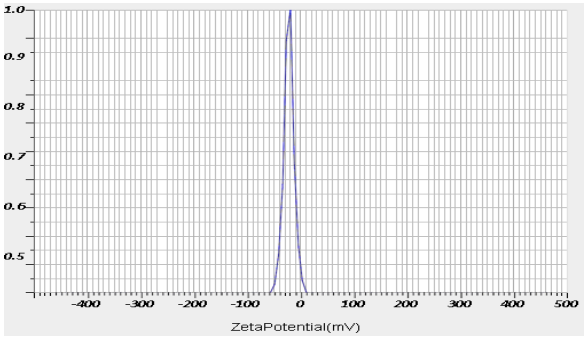

Figure 2: Graph of Zeta Potential

Based on the above evaluation study results PHF-C is selected for hepatoprotective screening.

Experimental Animals

Albino mice and rats (Wistar) of either sex were chosen for the Hepatoprotective studies obtained from Channabasweshwar Pharmacy College (Degree), Latur. The animals were maintained at conventional laboratory diet and water *ad libitum*. This protocol received acceptance from CPCSEA having Ref. No. CBPCL/IAEC/2024-25/722.

Acute Toxicity Study

The acute toxicity tests were conducted utilizing up and down procedures in compliance with the OECD's revised draft standards 423 from October 2000.The doses were chosen depending upon this research to evaluate the hepatoprotective activity[2].

CCl4 Induced Hepatotoxicity in Rat

The hepatoprotective properties of polyherbal formulations were tested on wistar rats of either sex employing the CCl4 induced hepatotoxicity technique. Six groups of six rats each were used in this experiment.
Group I (control) - Rats were received 1% Tween 80 p.o.as a vehicle in distilled water.
Group II (Toxic) - Rats were given intraperitonial injections of0.7 mL/kg CCl4.
Group III (Standard)- Rats were administered an oral dose of 1 mL/kg Liv-52.
Group IV (PHF-C1) Rats were received 100 mg/kg bw PHF-C1 orally,
Group V (PHF-C2) Rats were received 200 mg/kg bw of PHF-C2 orally,
Group VI (PHF-C3) Rats received 400 mg/kg bw of PHF-C3 orally.

The above-mentioned doses were administered over ten days. On the third, sixth, and tenth days, all animals except group I received 0.7 mL/kg of CCl4 intraperitoneally to induce liver damage. On the tenth day, one hour after the final injection of CCl4, rats were sacrificed by cervical dislocation, the carotid artery was used to draw blood, and centrifugation was used to separate the serum, which was further used to analyze biochemical markers such as SGOT, SGPT, SALP, total and direct bilirubin. The livers were then carefully removed, cleaned of extraneous tissue, rinsed with alcohol, and weighed based on body weight per 100 grams before being placed in a neutral formalin solution of 10% and examined histopathologically.

Statistical Analysis

The one-way ANOVA followed by Dunnett's t-test was used to analyze the data. Results were presented as Mean ± SEM for each biochemical parameter from six animals per group.

Statistical significance was determined at $p < 0.001$ in all situations.[2, 17,18]

RESULT AND DISCUSSION

Polyherbal suspensions were produced as per (Tables 1 and 2) shown in Figure 1 as follows;

Formulated polyherbal suspensions were further analyzed for different parameters as follows as[19-21]

Organoleptic Properties of Polyherbal Suspension

It was discovered that the three formulations, PHF-A, PHF-B, and PHF-C, have similar morphological properties, such as a greenish-black color, a slightly bitter taste with a distinct odor, and a liquid nature.

Physicochemical Properties of Polyherbal Suspension

Polyherbal suspensions were prepared and evaluated for many characteristics, including sedimentation volume, redispersibility, pH, viscosity, flow rate, zeta potential, crystal growth, and phytochemical analysis. Phytochemical screening of polyherbal formulations contained phytoconstituents like phenols, alkaloids, flavonoids, tannins, steroids, glycosides, and terpenoids, but not Saponin, as shown in Table 4. In PHF-A, the density varied from (1.032), the sedimentation volume ranged from (0.98), the pH ranged from (4.46), the viscosity was (13.87), the zeta potential was (-24.0), and the rapid flow rate was (18 seconds) per 5 mL of formulation (Table 5). In comparison to PHF-A, it was discovered that the sedimentation volume in PHF-B ranged from (0.98). This was attributed to an increase in the concentration of Sodium CMC, which also had an impact on the viscosity (26.09 cP), pH (5.09), a decrease in formulation flow rate (39 seconds) per 5 mL of formulation, particle size (12.85 um), zeta potential (-19.0), and density (1.037) (Table 5). When kept at room temperature (RT) between 8 and 45°C, PHF-C has a greenish-black color, a slightly bitter taste, and a distinct odor. This suspension's pleasant appearance and texture remained unchanged as temperatures varied. After storage, the pH of the suspension is (5.80), indicating that no notable changes occurred. The viscosity of the PHF-C suspension was determined to be adequate, with a coefficient of (101.22) cP, a zeta potential of (-14.0), and a flow rate of (65) seconds per 5 mL, suggesting satisfactory rheological behavior. Because the sedimentation

Table 5: Physicochemical evaluation of polyherbal suspensions

Formulation	*Sedimentation volume*	*Redispersibility*	*Flow rate mL/sec*	*pH*	*Particle size (µm)*	*Viscosity (cP)*	*Crystal Growth*	*Zeta Potential*	*Density*
PHF-A	0.98	Poor	5/18	4.46	13.61	13.87	No	-24.0	1.032
PHF-B	0.98	Good	5/39	5.09	12.85	26.09	No	-19.0	1.037
PHF-C	0.99	Excellent	5/59	5.80	12.90	97.24	No	-14.0	1.092

Table 6: Accelerated stability study of optimized batch PHF-C at RT 8 and 45°C

Time in days	*8°C*									*45°C*								
	Sedi-mentation volume	*Redis-persibility*	*Flow-rate*	*pH*	*Particle size (µm)*	*Viscosity (cP)*	*crystal growth*	*Zeta potential*	*Density*	*Sedi-mentation volume*	*Redis-persibility*	*Flow-rate*	*pH*	*Particle size (µm)*	*Viscosity (cP)*	*crystal growth*	*Zeta potential*	*Density*
0	0.99	Excellent	5 mL /59sec	5.80	12.90	97.24	No	-14.0	1.092	0.99	Excellent	5 mL /59sec	5.80	12.90	97.24	No	-14.0	1.092
30	0.99	Excellent	5 mL /59sec	5.80	12.90	98.24	No	-14.0	1.093	0.99	Excellent	5 mL /59sec	5.80	12.90	98.24	No	-14.0	1.093
45	0.99	Excellent	5 mL /61sec	5.80	12.93	99.24	No	-13.0	1.094	0.99	Excellent	5 mL /61sec	5.80	12.93	99.24	No	-13.0	1.094
60	0.98	Excellent	5 mL /62sec	5.80	12.93	99.24	No	-13.0	1.094	0.98	Excellent	5 mL /62sec	5.80	12.93	99.24	No	-13.0	1.094
90	0.98	Excellent	5 mL /63sec	5.80	12.98	101.2 2	No	-12.0	1.095	0.98	Excellent	5 mL /63sec	5.80	12.98	101.2 2	No	-12.0	1.095

Table 7: Comparative Hepatoprotective evaluation in rats of polyherbal formulation

Group	*Normal*	*CCl_4*	*Liv-52*	*PHF-C1*	*PHF-C2*	*PHF-C3*
SGOT(IU/L)	41.02 ± 0.8794	81.46** ± 3.461	50.32*** ± 3.387	56.61** ± 2.373	52.79** ± 2.119	71.71 ± 1.534
SGPT(IU/L)	37.60 ± 2.370	168.0** ± 9.587	49.75*** ± 2.612	88.95*** ± 4.084	58.37*** ± 2.949	121.1** ± 6.214
ALPK.A Units)	155.9 ± 2.631	237.0** ± 15.74	163.6*** ± 3.668	176.5* ± 4.771	161.2** ± 3.958	182.7 ± 14.29
Total bilirubin (mg/dl)	0.7167 ± 0.0823	1.793** ± 0.0375	0.8850*** ± 0.0343	1.165** ± 0.0836	0.9450** ± 0.0758	1.402 ± 0.1013
Direct bilirubin (g/dl)	0.8000 ± 0.0432	1.890** ± 0.1083	0.8300** ± 0.0432	0.7783*** ± 0.05582	0.4767*** ± 0.0281	0.8900** ± 0.0236
Liver weight (g)	4.398 ± 0.0707	6.200** ± 0.1807	4.463*** ± 0.0672	4.873** ± 0.067	4.597*** ± 0.0573	5.103** ± 0.064

Data are expressed as Mean ± SE*** p <0.001 when compared with normal control
SGPT: serum glutamate pyruvate transaminase**, SGOT:** serum glutamate oxaloacetate transaminase**, ALP:** Alkaline phosphatase

volume is close to the permissible limit of one, it did not vary significantly over time (Table 5).

The optimized PHF-C formulation was sealed, placed in a stability test chamber, and submitted to stability investigations under accelerated storage circumstances for three months in line with the International Conference on Harmonization (ICH) criteria at room temperature (8ºC and 45ºC). The suspension was tested for pH, viscosity, crystal formation, redispersibility, zeta potential, and particle size change at intervals of 0-3 months. Each result was compared to the final formulation shown in (Table 6). During the stability period, the PHF-C oral suspension formulation showed a small increase in particle size, although it remained well within the permissible limit as compared to other PHF-A and PHF-B formulations; hence, PHF-C was selected for further pharmacological screening for hepatoprotective activity. LD_{50} studies showed that up to a maximum dosage of 2,000 mg/kg BW, the animals were found to be safe. Regular behavioral patterns remained unchanged, and no evidence of toxicity or mortality was discovered. Therefore, for all ensuing in vivo studies, the PHF-C formulation's $1/20^{th}$, $1/10^{th}$, and $1/5^{th}$ doses—that is, 100, 200, and 400 mg/kg—were employed.

cP shows the effects of different doses of PHF-C1, PHF-C2, and PHF-C3 suspension formulations on various biochemical parameters. CCl4-induced hepatotoxicity resulted in significant (p <0.001) increases in blood SGPT, SGOT, ALP, total bilirubin, direct bilirubin, and liver weight compared to the control group of rats. PHF-C2 formulation (200 mg/kg) significantly (p <0.001) reduced blood SGPT, SGOT, ALP, total bilirubin, direct bilirubin, and liver weight in CCl_4-induced hepatotoxicity compared to the CCl_4-treated group alone. However, the PHF-C2 formulation had the highest hepatoprotective efficacy. PHF-C2 had the most hepatoprotective effect, followed by PHF-C3 and PHF-C1.

After conducting stability testing at various temperatures, the formulations are stable and adequate because there were no appreciable changes in sedimentation, viscosity, or other physicochemical properties. The physicochemical and organoleptic qualities were mostly unchanged.

CONCLUSION

The current study found that the polyherbal formulation comprised bioactive compounds like flavonoids, polyphenols, terpenoids, steroids, alkaloids, and glycosides. Since liquid medication forms can overcome swallowing issues, they are superior to solid dosage forms in youngsters and the elderly. The majority of Ayurvedic formulations are liquid in nature and frequently comprise more than two basic drugs. Suspension is among the most reliable and popular oral dosage forms due to its ease of swallowing and distribution adaptability. The polyherbal suspension was made by triturating ethanolic extracts of specific plants with a suitable suspending agent and other excipients. Significant differences in sedimentation, viscosity, and other physicochemical parameters were detected following a trial with different amounts of sodium CMC. Based on the current findings, we may assume that the results of PHF-C3's polyherbal suspension showed the presence of flavonoids and other essential phytochemicals and the macroscopic and physicochemical features of the polyherbal formulation did not change much after findings of accelerated stability trials also and all stability parameters were stable, acceptable, and ideal at all temperatures and aforesaid PHF-C2's Polyherbal suspension possesses a potent hepatoprotective impact as well, which may be related to the antioxidant capability of the flavonoids included in it.

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