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DEVELOPMENT AND EVALUATION OF PHYTOSOMES AND POLYHERBAL FORMULATION OF SOME RASAYANA PLANTS FOR EFFECTIVE TREATMENT OF HEPATIC DISEASES

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ABSTRACT

The aim of this study was to develop and evaluate phytosome formulations from three Rasayana plants, *Withania somnifera*, *Emblica officinalis*, and *Terminalia bellirica*, for their hepatoprotective potential in the treatment of hepatic diseases. Phytosomes, a novel drug delivery system, were prepared to enhance the bioavailability and stability of the active ingredients from these plants. Various solvents were used to extract the bioactive compounds, and the extracts were subjected to phytochemical screening to identify key constituents such as flavonoids, saponins, and diterpenes. The prepared phytosome formulations were characterized for particle size, entrapment efficiency, and drug release kinetics. *In vivo* hepatoprotective activity was assessed using CCl_4-induced liver toxicity in rats. The results indicated that phytosome formulations, especially the mixed polyherbal formulation, exhibited significant hepatoprotective effects, as evidenced by improved body weight gain and reduced SGPT and SGOT levels. The study demonstrates the potential of phytosomal formulations as effective therapeutic agents for liver protection, enhancing the pharmacological activity of Rasayana plants.

Keywords: Phytosomes, Rasayana plants, *Withania somnifera*, *Emblica officinalis*, *Terminalia bellirica*, hepatoprotective activity, CCl_4-induced hepatotoxicity, drug delivery system, bioavailability, polyherbal formulation.

INTRODUCTION

Liver diseases are a global health burden, with conditions such as cirrhosis, hepatitis, liver fibrosis, and liver cancer contributing significantly to morbidity and mortality worldwide (Gawali *et al.*, 2018). The liver plays a central role in detoxification, protein synthesis, and metabolic processes, making it a essential organ for overall health. Hepatic diseases compromise these functions, leading to complications that often result in liver failure. Although pharmaceutical interventions are commonly used to manage liver disorders, herbal remedies have garnered increasing attention due to their therapeutic potential, fewer side effects, and holistic properties (Sahu *et al.*, 2020).

Among the vast array of plants used in traditional medicine, Rasayana plants are particularly prominent in Ayurvedic medicine. These plants are believed to possess rejuvenating properties that can support liver health and restore liver function. The combination of these plants in polyherbal formulations can provide enhanced therapeutic effects compared to single-plant

extracts, owing to their synergistic interactions (Jain *et al.,* 2015). However, one of the main challenges in herbal medicine is the low bioavailability and poor absorption of the active constituents when administered orally (Verma *et al.,* 2017). To address this issue, the development of phytosomes, which are herbal drug delivery systems, has been proposed. Phytosomes enhance the bioavailability and therapeutic action of herbal compounds by complexing them with phospholipids, thereby improving their absorption and targeted delivery (Madhavi *et al.,* 2021).

This study focuses on the development and evaluation of phytosomal formulations and polyherbal formulations derived from Rasayana plants, aimed at improving the treatment of hepatic diseases. By utilizing advanced drug delivery systems like phytosomes, the bioavailability of active compounds from Rasayana plants can be significantly enhanced, thereby improving their efficacy in treating liver disorders.

Phytosomes are a promising new approach in herbal drug delivery. These are lipid-based complexes in which the active herbal constituents are bound to phospholipids, forming a structure that mimics cell membranes. The lipid component of the phytosome enhances the solubility, stability, and permeability of herbal compounds, which are otherwise poorly absorbed when consumed in their natural state (Bhatia *et al.*, 2019). The phospholipid complex allows for the active compound to be protected from degradation in the digestive tract, while also promoting its absorption through the intestinal membranes. As a result, phytosomes provide an efficient and reliable method for enhancing the bioavailability of herbal compounds (Wang *et al.*, 2016). This technology is particularly beneficial for lipophilic compounds, which would typically have poor solubility and bioavailability.

Several studies have highlighted the success of phytosomal formulations in improving the pharmacokinetics of herbal products. For example, phytosomes have been shown to enhance the bioavailability of flavonoids, alkaloids, and terpenoids, which are commonly found in Rasayana plants and are known to possess hepatoprotective effects (Gupta *et al.,* 2021). These advanced formulations can overcome the limitations of traditional herbal extracts, such as poor stability and limited absorption, making them a valuable tool for developing effective treatments for liver diseases (Mohan *et al.*, 2014).

The use of polyherbal formulations, which combine multiple medicinal plants, is a common practice in traditional medicine. The rationale behind polyherbal formulations is that the combination of different herbs provides synergistic effects, enhancing the overall therapeutic efficacy. In the case of liver diseases, polyherbal formulations can address multiple aspects of liver health, including detoxification, antioxidant activity, and anti-inflammatory effects (Kumar *et al.*, 2011; Duraipandiyan *et al.*, 2012).

The combination of these plants in a polyherbal formulation offers a holistic approach to treating liver diseases, addressing not only the symptoms but also the underlying causes of liver damage. By combining multiple plants with complementary actions, polyherbal formulations can provide enhanced

therapeutic benefits, such as improved liver function, reduced inflammation, and protection against oxidative damage.

Rasayana plants are an important category in Ayurvedic medicine, often regarded as agents that rejuvenate the body and mind. These plants have been shown to exhibit various therapeutic effects, including antioxidant, anti-inflammatory, antifibrotic, and hepatoprotective properties, making them an ideal choice for managing hepatic diseases (Sharma *et al.*, 2014; Nandkarni, 2013).

The aim of this study is to develop and evaluate phytosome and polyherbal formulations derived from Rasayana plants for the effective treatment of hepatic diseases. The focus is on enhancing the bioavailability of active compounds through phytosome technology, improving their absorption and therapeutic efficacy. The study will assess the hepatoprotective activity of the formulations in both in-vitro and in-vivo models, exploring the synergistic effects of combining multiple Rasayana plants. Additionally, pharmacokinetic evaluations will be conducted to determine the stability, release rate, and potential benefits of these formulations compared to conventional treatments.

MATERIALS AND METHODS

Rhizome of *Withania somnifera*, *Emblica officinalis* and *Terminalia bellirica* were collected from local area of Bhopal (M.P.) in the month of March, 2024.

Extraction of plant material by maceration process

Powdered rhizome of *Withania somnifera*, *Emblica officinalis* and *Terminalia bellirica* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place (Khandelwal, 2005).

265gm of *Withania somnifera*, 310 gm *Emblica officinalis* and 45gm *Terminalia bellirica* dried rhizome were exhaustively extracted with different solvent (Chloroform, Ethyl acetate Methanol and Water) using maceration method (Kokate, 1994; Mukherjee, 2007). The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Determination of Percentage yield

The percentage yield of yield of each extract was calculated by using formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \times 100$$

Phytochemical screening

For all of the extracts, phytochemical analyses were performed using the standard techniques (Kocabas, 2017; Evans, 2009).

Formulation of phytosomes of *Withania somnifera*, *Emblica officinalis* and *Terminalia bellirica*

The complex was prepared with phospholipids: Cholesterol and each separated extract of *Withania somnifera*, *Emblica officinalis* and *Terminalia bellirica* in the ratio of 1:0.5:1.0, 1:1:1.0, 2:0.5:1.0, 2:1.0:1.0 respectively. A 100 ml round-bottom flask was filled with the weighted amounts of each extract, phospholipids, and cholesterol, and 25 ml of dichloromethane was added as the reaction medium. The mixture was refluxed for 3 hours while maintaining a temperature of 50°C for the reaction of the complex.

After the resulting clear fluid had evaporated, 20 ml of n-hexane was stirred in. To get rid of the traces of solvents, the precipitate was filtered and dried under vacuum. The leftovers were collected, desiccated overnight, and stored in an amber-colored glass bottle at room temperature (Sahu, and Bothara, 2015).

Characterization of phytosomes

Microscopic observation of prepared Phytosomes

To view the form of the improved Phytosome formulation, a camera attachment (Minolta) was employed on an optical microscope from Cippon, Japan.

Entrapment efficiency

A phytosome preparation was obtained and centrifuged for an hour at 12000 rpm using a cooling centrifuge (Remi).

To separate the non-entrapped flavonoids, the clear supernatant was carefully removed. The absorbance of the clear supernatant for the non-entrapped flavonoids in *Withania somnifera*, *Emblica officinalis*, and *Terminalia bellirica* was recorded separately at max 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). The amount of flavonoids in the 1 ml dispersion was calculated from the amount of flavonoids in the sediment and supernatant. The following formula was used to determine the proportion of entrapment (Patel *et al.*, 2009):

$$\text{Percent Entrapment} = \frac{\text{Amount of drug in sediment}}{\text{Total amount of drug added}} \text{X } 100$$

Particle size and size distribution

A computerised inspection system was used to measure the particle size, size distribution, and zeta potential of an improved phytosomes formulation using dynamic light scattering (DLS) (Malvern Zetamaster ZEM 5002, Malvern, UK). By infusing the diluted system into a zeta potential measurement device, the electric potential of the phytosomes, including its Stern layer, was ascertained (Sujit *et al.*, 2009).

Transmission electron microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan) (Magdy, 2004).

***In vitro* dissolution rate studies**

In vitro drug release of the sample was carried out using USP- type I dissolution apparatus (Basket type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.5ºC and 75 rpm. 10 mg of prepared phytosomes was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (37ºC) was replaced every time with the same quantity of the sample and takes the absorbance at corresponding wavelength of optimized formulations using spectroscopy (Chandira *et al.*, 2010).

***In vivo* hepatotoxicity activity**

Screening of hepatotoxicity

Wistar rats (200–250 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received

standard rodent chow and water *ad libitum*. Rats were acclimatised to laboratory conditions for 7 days before carrying out the studies. Between 8:00 and 15:00 hours, all studies were conducted in a room with no background noise. Each set of studies used a different group of rats (n=6). The Institutional Animal Ethics Committee (IAEC), established by the Ministry of Environment and Forests, Government of India, New Delhi, India, to oversee and supervise the use of experimental animals, gave its approval to the animal experiments.

Toxicity Study

For the acute oral toxicity and LD50 determination the organization for economic co-operation and development (OECD) guideline 423 was followed. As per OECD guidelines a stepwise procedure with the use of 3 animals of a single sex per step was followed. Absence or presence of compound related mortality of the animal.

Experimental model

Experimental design and treatment protocol

Rats were acclimated to animal laboratory conditions at 25°C, 55% humidity, and a 12 h: 12 h light-dark cycle for seven days prior to testing. Water was supplied ad libitum, and the rats were fed a basal diet for the entirety of the study.

CCl4-induced hepatotoxicity

Group –I: Normal control (0.5% CMC 1 ml/kg, p.o.)

Group –II: Rats were administrated with CCl_4 (2 mL/kg b.wt.)

Group -III: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and silymarin 10 mg/kg. Silymarin is the most used natural constituent for the healing of hepatic diseases worldwide due to its antifibrotic, anti-inflammatory, and antioxidant activities. Silymarin functions by stabilizing biological membranes and increasing protein synthesis Therefore, it is used as a standard drug around the world for hepatoprotective experiments (Khan *et al.*, 2012).

Group –IV: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Phytosomes of *Withania somnifera* 100mg/kg

Group –V: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Phytosomes of *Withania somnifera* 200mg/kg

Group –VI: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Phytosomes of *Emblica officinalis* 100mg/kg

Group –VII: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Phytosomes of *Emblica officinalis* 200mg/kg

Group –VIII: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Phytosomes of *Terminalia bellirica* 100mg/kg

Group –IX: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Phytosomes of *Terminalia bellirica* 200mg/kg

Group –X: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Mixed Phytosomes of *Withania somnifera, Emblica officinalis and Terminalia bellirica* 100mg/kg

Group –XI: Rats were administrated with CCl_4 (2 mL/kg b.wt.) and Mixed Phytosomes of *Withania somnifera, Emblica officinalis and Terminalia bellirica* 100mg/kg

Hepatotoxicity screening

Biochemical analysis

For various biochemical parameters estimation, blood was collected from ophthalmic venous plexus by retro-orbital bleeding technique. Biochemical parameters such as ALT, AST, bilirubin, and ALP were calculated with the help of analyzer and

commercially available kits (Roche). The methods described by Ullah were used to determine the serum concentrations of MDA and serum total protein.

RESULTS AND DISCUSSION

The study focused on the development and evaluation of phytosome formulations derived from Rasayana plants such as *Withania somnifera*, *Emblica officinalis*, and *Terminalia bellirica* for the effective treatment of hepatic diseases. Various extracts were obtained from these plants using different solvents, and the yield was measured (Table 5). Among the solvents, aqueous extracts provided the highest yield for all three plants, with values ranging from 2.56% to 3.15%, while petroleum ether extracts yielded the least.

Phytochemical screening (Tables 6-8) showed that the aqueous and methanol extracts of *Withania somnifera* contained flavonoids, saponins, carbohydrates, proteins, and diterpenes, with saponins and flavonoids being notably present in the aqueous extracts. For *Emblica officinalis*, the aqueous extract showed positive results for flavonoids, saponins, carbohydrates, and proteins. Similarly, *Terminalia bellirica* displayed the presence of flavonoids, saponins, and diterpenes in its aqueous and ethyl acetate extracts.

The formulation of phytosomes was carried out using these extracts. The particle size and entrapment efficiency data (Table 9) indicated that the *Emblica officinalis* formulation (EOPF3) had the smallest particle size (210.32 nm) and the highest entrapment efficiency (74.65%). The mixed polyherbal formulation (PPF4) showed a favorable particle size of 236.65 nm and an entrapment efficiency of 73.32%.

The release kinetics of the phytosome formulations (Table 10) were analyzed using different models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas. All formulations demonstrated a good fit with the Korsmeyer-Peppas model, indicating controlled and sustained drug release characteristics. *Withania somnifera* phytosomes (WSPF2) had the highest R^2 value of 0.989 for the Korsmeyer-Peppas model, suggesting a highly controlled release pattern.

In vivo hepatoprotective activity (Tables 11-13) was assessed in CCl_4-induced hepatotoxicity in rats. Body weight changes in the treatment groups indicated that phytosomal formulations from *Withania somnifera*, *Emblica officinalis*, and *Terminalia bellirica* provided a protective effect, as observed by the increase in body weight over the study period. Specifically, mixed phytosomes (Batch XI) showed the most promising results, with significant improvements in body weight compared to the control group.

The serum levels of SGPT and SGOT, important markers of liver damage, were significantly reduced in the groups treated with phytosomal formulations (Tables 12 and 13). The mixed phytosome formulation (200 mg/kg) showed a substantial reduction in both SGPT and SGOT levels, comparable to the standard drug silymarin, indicating strong hepatoprotective activity.

The results suggest that the phytosome formulations, particularly the mixed polyherbal phytosomes, exhibit considerable hepatoprotective effects against CCl_4-induced

liver damage. The findings support the potential of these formulations as effective treatments for hepatic diseases, highlighting the importance of using advanced drug delivery systems like phytosomes to enhance the therapeutic efficacy of Rasayana plants.

Table 1: Different formulations of phytosomes of *Withania somnifera*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Withania somnifera	
CCPF1	1:0.5	1	25
CCPF2	1:1	1	25
CCPF3	2:0.5	1	25
CCPF4	2:1.0	1	25

Table 2: Different formulations of phytosomes of *Emblica officinalis*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Emblica officinalis	
CAPF1	1:0.5	1	25
CAPF2	1:1	1	25
CAPF3	2:0.5	1	25
CAPF4	2:1.0	1	25

Table 3: Different formulations of phytosomes of *Terminalia bellirica*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Terminalia bellirica	
PF1	1:0.5	1	25
PF2	1:1	1	25
PF3	2:0.5	1	25
PF4	2:1.0	1	25

Table 4: Different formulations of polyherbal phytosomes of *Withania somnifera, Emblica officinalis and Terminalia bellirica*

Formulation	Ratio of Phospholipids and Cholesterol (%)	Extract Concentration (%)	Dichloromethane Concentration (ml)
		Terminalia bellirica	
PPF1	1:0.5	1:1:1	25
PPF2	1:1	1:1:1	25
PPF3	2:0.5	1:1:1	25
PPF4	2:1.0	1:1:1	25

Table 5: Result of percentage yield of extracts

S. No.	Solvents	Percentage Yield (%)		
		Withania somnifera	***Emblica officinalis***	***Terminalia bellirica***
1.	Petroleum ether	0.780	0.875	0.32
2.	Choloroform	1.91	2.90	1.09
3.	Ethyl acetate	0.426	1.04	0.94
4.	Methanol	1.83	2.66	2.67
5.	Aqueous	3.13	3.15	2.56

Table 6: Result of phytochemical screening of extracts of *Withania somnifera*

S. No.	Constituents	Choloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids	-ve	- ve	- ve	- ve
2.	Glycosides	- ve	- ve	- ve	- ve
3.	Flavonoids	- ve	- ve	+ ve	+ ve
4.	Saponins	- ve	- ve	+ ve	+ ve
5.	Phenolics	- ve	- ve	- ve	- ve
6.	Amino Acids	- ve	- ve	- ve	- ve
7.	Carbohydrate	+ ve	+ ve	+ ve	+ ve
8.	Proteins	- ve	- ve	+ ve	+ ve
9.	Diterpenes	+ ve	+ ve	+ ve	+ ve

(+ ve= Positive; - ve=Negative)

Table 7: Result of phytochemical screening of extracts of *Emblica officinalis*

S. No.	Constituents	Choloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids	- ve	- ve	+ ve	- ve
2.	Glycosides	- ve	- ve	- ve	- ve
3.	Flavonoids	- ve	+ ve	+ ve	+ ve
4.	Saponins	- ve	- ve	+ ve	+ ve
5.	Phenolics	- ve	- ve	- ve	- ve
6.	Amino Acids	- ve	- ve	- ve	- ve
7.	Carbohydrate	+ ve	+ ve	+ ve	- ve
8.	Proteins	+ ve	+ ve	- ve	+ ve
9.	Diterpenes	- ve	+ ve	- ve	+ ve

(+ ve= Positive; - ve=Negative)

Table 8: Result of phytochemical screening of extracts of *Terminalia bellirica*

S. No.	Constituents	Choloroform extract	Ethyl acetate extract	Methanol extract	Aqueous extract
1.	Alkaloids	- ve	+ve	- ve	+ve
2.	Glycosides	- ve	- ve	- ve	- ve
3.	Flavonoids	+ ve	+ ve	+ ve	+ ve
4.	Saponins	- ve	- ve	+ ve	+ ve
5.	Phenolics	- ve	- ve	- ve	- ve
6.	Amino Acids	- ve	- ve	- ve	- ve
7.	Carbohydrate	- ve	- ve	- ve	- ve
8.	Proteins	- ve	- ve	- ve	- ve
9.	Diterpenes	- ve	- ve	+ve	- ve

(+ ve= Positive; - ve=Negative)

Table 9: Particle size and entrapment efficiency of drug loaded phytosomes

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
	Withania somnifera	
WSPF1	265.58	65.45
WSPF2	**185.65**	**73.32**
WSPF3	224.74	69.95
WSPF4	239.98	65.45
	Emblica officinalis	
EOPF1	285.65	63.32
EOPF2	265.58	68.85
EOPF3	**210.32**	**74.65**
EOPF4	285.65	69.85
	Terminalia bellirica	
TBPF1	295.65	67.78
TBPF2	265.45	69.95
PTBF3	**215.45**	**76.65**
TBPF4	298.74	72.23
	Polyherbal Phytosomes	
PPF1	285.65	68.85
PPF2	274.45	67.74
PPF3	**210.32**	**75.65**
PPF4	236.65	73.32

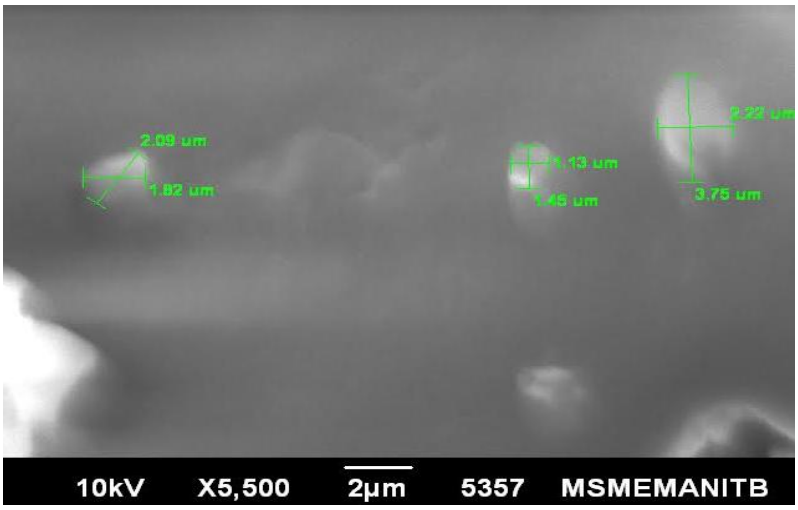

Figure 1: TEM image of phytosomes formulation WSPF2

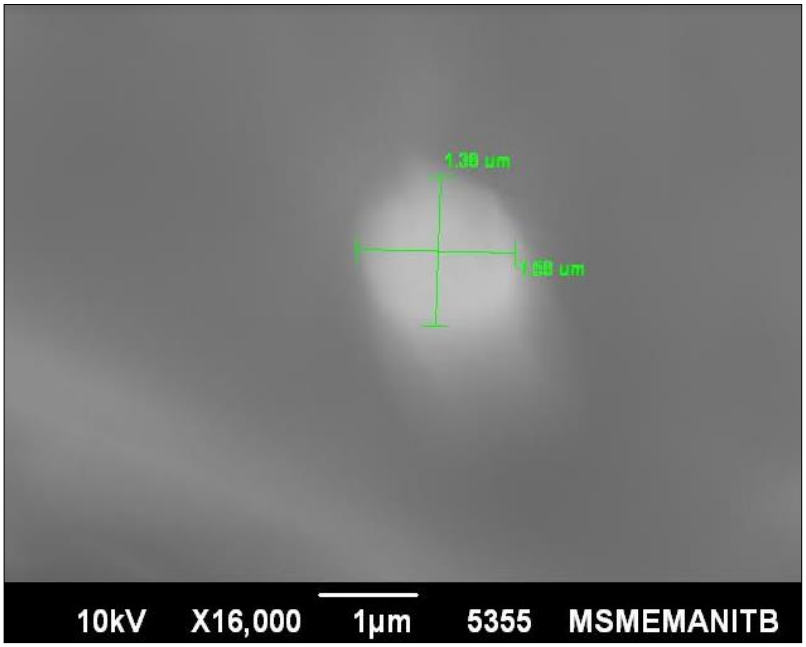

Figure 2: TEM image of phytosomes formulation EOPF3

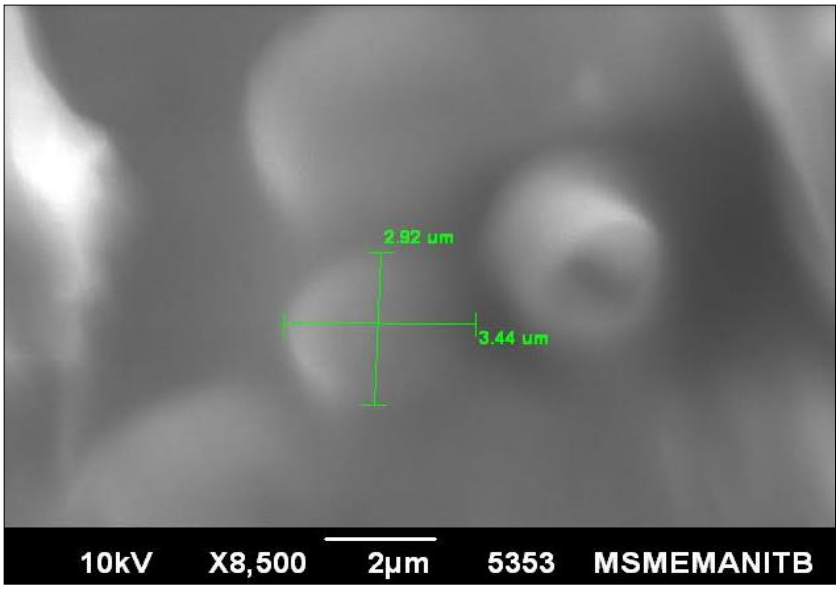

Figure 3: TEM image of phytosomes formulation TBPF3

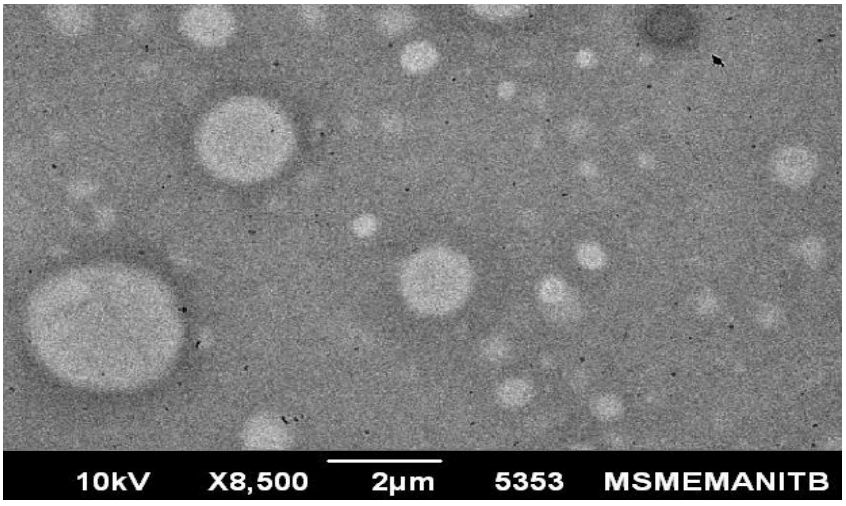

Figure 4: TEM image of phytosomes formulation PPF4

Table 10: Regression analysis data of optimized formulations

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
WSPF2	0.955	0.881	0.994	**0.989**
EOPF3	0.920	0.924	**0.986**	0.984
TBPF3	0.916	0.933	0.985	**0.988**
PPF4	0.956	0.847	0.987	**0.989**

Results of *in vivo* hepatoprotective activity

Table 11: Mean Body Weight Change

Groups	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal Control	0.5% CMC 1 ml/kg, p.o.	210.00±2.10	229.00±2.50
II	Control (CCl_4)	2 mL/kg b.wt.	223.00± 1.60	225.00± 1.70
III	CCl_4+Silymarin	10 mg/kg p.o.	238.00± 2.35	242.00± 2.52
IV	CCl_4+Phytosomes of *Withania somnifera*	100 mg/kg p.o.	231.00± 1.98	240.10± 2.35
V	CCl_4+Phytosomes of *Withania somnifera*	200 mg/kg p.o.	238.00± 2.10	245.20± 2.48
VI	CCl_4+Phytosomes of *Emblica officinalis*	100 mg/kg p.o.	232.00± 1.85	243.10± 1.90
VII	CCl_4+Phytosomes of *Emblica officinalis*	200 mg/kg p.o.	240.30± 2.70	248.15± 2.32
VIII	CCl_4+Phytosomes of *Curcuma angustifolia*	100 mg/kg p.o.	239.32± 1.88	242.18± 2.38
IX	CCl_4+Phytosomes of *Curcuma angustifolia*	200 mg/kg p.o.	243.28± 2.56	247.28± 2.72
X	CCl_4+Mixed Phytosomes *(Withania somnifera, Emblica officinalis and Terminalia bellirica)*	100 mg/kg p.o.	233.48± 1.98	239.10± 1.98
XI	CCl_4+Mixed Phytosomes *(Withania somnifera, Emblica officinalis and Terminalia bellirica)*	200 mg/kg p.o.	244.50± 2.25	249.52± 2.83

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

Table 12: Effect of Phytosomal formulation on %SGPT levels in CCl_4 induced hepatotoxicity in rats

Group	Drug	Dose	SGPT (%)
I	Normal Control	0.5% CMC 1 ml/kg, p.o.	94.38 ± 9.78
II	Control (CCl_4)	2 mL/kg b.wt.	248.32±11.22
III	CCl_4+Silymarin	10 mg/kg p.o.	132.92 ± 7.80***
IV	CCl_4+Phytosomes of *Withania somnifera*	100 mg/kg p.o.	172.12 ± 8.82**
V	CCl_4+Phytosomes of *Withania somnifera*	200 mg/kg p.o.	161.28 ± 8.31**
VI	CCl_4+Phytosomes of *Emblica officinalis*	100 mg/kg p.o.	184.86 ± 9.37**
VII	CCl_4+Phytosomes of *Emblica officinalis*	200 mg/kg p.o.	192.24 ± 7.91**
VIII	CCl_4+Phytosomes of *Curcuma angustifolia*	100 mg/kg p.o.	201.32 ± 6.96**
IX	CCl_4+Phytosomes of *Curcuma angustifolia*	200 mg/kg p.o.	191.52± 9.41
X	CCl_4+Mixed Phytosomes *(Withania somnifera, Emblica officinalis and Terminalia bellirica)*	100 mg/kg p.o.	152.12 ± 8.82**
XI	CCl_4+Mixed Phytosomes *(Withania somnifera, Emblica officinalis and Terminalia bellirica)*	200 mg/kg p.o.	143.28 ± 8.31**

Values are expressed as the mean ± SEM of six observations. *** P<0.001 vs. control treatment (One-way ANOVA followed by Dunnett's test

Table 13: Effect of Phytosomal formulation on %SGOT levels in CCl_4 induced hepatotoxicity in rats

Group	Drug	Dose	SGOT (%)
I	Normal Control	0.5% CMC 1 ml/kg, p.o.	98.10 ± 11.38
II	Control (CCl_4)	2 mL/kg b.wt.	269. 39±9.68
III	CCl_4+Silymarin	10 mg/kg p.o.	143.24 ± 8.68***
IV	CCl_4+Phytosomes of *Withania somnifera*	100 mg/kg p.o.	176.21 ± 7.88**
V	CCl_4+Phytosomes of *Withania somnifera*	200 mg/kg p.o.	165.16 ± 9.36**

VI	CCl_4+Phytosomes of *Emblica officinalis*	100 mg/kg p.o.	198.12 ± 10.10**
VII	CCl_4+Phytosomes of *Emblica officinalis*	200 mg/kg p.o.	184.28 ± 8.96**
VIII	CCl_4+Phytosomes of *Curcuma angustifolia*	100 mg/kg p.o.	196.18 ± 11.20**
IX	CCl_4+Phytosomes of *Curcuma angustifolia*	200 mg/kg p.o.	186.48 ± 9.96**
X	CCl_4+Mixed Phytosomes *(Withania somnifera, Emblica officinalis and Terminalia bellirica)*	100 mg/kg p.o.	168.24 ± 8.68**
XI	CCl_4+Mixed Phytosomes *(Withania somnifera, Emblica officinalis and Terminalia bellirica)*	200 mg/kg p.o.	149.34 ± 9.18***

Values are expressed as the mean ± SEM of six observations. *** P<0.001 vs. control treatment (One-way ANOVA followed by Dunnett's test)

CONCLUSION

The development and evaluation of phytosome formulations from *Withania somnifera*, *Emblica officinalis*, and *Terminalia bellirica* demonstrated promising hepatoprotective effects against CCl_4-induced liver toxicity. The results from the phytochemical screening revealed the presence of key bioactive compounds, including flavonoids, saponins, and diterpenes, which are known for their therapeutic benefits. The phytosome encapsulation significantly enhanced the solubility, stability, and bioavailability of the active compounds, as evidenced by favorable particle sizes and high entrapment efficiencies.

The *in vivo* studies showed that the phytosomal formulations, particularly the mixed polyherbal phytosomes, effectively protected the liver by reducing serum levels of SGPT and SGOT, markers of liver damage. Moreover, the formulations exhibited controlled and sustained drug release profiles, ensuring prolonged therapeutic action. These findings confirm that phytosomal formulations can be a promising approach for improving the hepatoprotective potential of traditional Rasayana plants, offering a novel strategy for the treatment of hepatic diseases.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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