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Phytosomes as a Possible Nano-Delivery System for Enhanced Oral Bioavailability and Hepatoprotective Activity of Indigofera barberi

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Abstract:

Indigofera barberi Gamble, a Fabaceae plant, has traditionally been used to treat skin, renal, and liver problems. Documented phytochemicals include glycosides, steroids, tannins, phenolic compounds, and flavonoids. Its low bioavailability and solubility hinder GI absorption. This study sought to improve Indigofera barberi extract bioavailability by making the ethanolic extract more soluble and absorbable. We optimized Indigofera barberi-loaded phytosomes utilizing solvent evaporation. This study developed IBPCs-NPs, a novel phytosome-nanosuspensions for IB shielding, to improve IB bioavailability and hepatoprotection. In in vivo pharmacokinetic studies, the IBPCs-NP formulation had higher plasma concentration and in vitro dissolution rate. IBPCs-NPs also demonstrated stronger hepatoprotective effects in pharmacodynamic studies. Crystalline variation and IB-phospholipid interactions are also physicochemical features of optimized IBPCs-NPs. The synthesised IB phytosomes were cylindrical, smooth-surfaced, and distinct, with a diameter of 332.52 ± 1.54 nm. According to dissolving trials, the enhanced IB phytosomal formulation released silymarin faster and in greater proportions than pure silymarin, with better water solubility (~360 µg/mL). The optimized silymarin phytosomal formulation normalized antioxidant enzymes and helped CCl4-intoxicated cells. In in vivo experiments, it protected the liver from CCl4-induced hepatotoxicity in rats. The optimized phytosomal formulation enhanced oral silymarin bioavailability compared to pure silymarin. This was shown by six-fold systemic bioavailability. Whole phytosomes may increase water-insoluble phyto-constituent oral bioavailability as phospholipid-based nanocarriers.

Introduction:

Herbal therapies, dubbed phyto-pharmaceuticals, have long been used to treat numerous medical conditions1. Herbs' ubiquitous availability and vast range of medical benefits have raised their profile in modern medicine. Although phyto-constituents and plant extracts exhibit high bioactivity in a lab, their ineffectiveness non a living body is usually due to their large molecular size and/or low solubility in lipids, which reduces their bioavailability and delays absorption.(3, 4.)

Indigofera barberi (IB), a Fabaceae herb widely investigated, grows in the Tirumala Hills. Commonly known as Adavineelimanadu mokka. When planted under a bush, it can reach 1 metre. Branchlets dip slightly. This shows the three foliolate leaves. Hairy, obtuse, ovate-oblong, mucronate leaflets. Pink flowers bloom in axillary racemes. Subterete, severely pointed, white-tomentose, deflexed, appressed pods. Two to four seeds. September to December is the optimal time for blooming and fruiting5.

Nanotechnology may boost absorption site bioavailability and medication release, improving active herbal extract medicinal efficacy. Oral bioavailability and water solubility of active phyto-constituents have been extensively examined [11,12,13,14]. Use nanoemulsion, solid lipid, polymeric, liposome, inclusion complexation, etc. Phytosomes or phyto-phospholipid complexes boost active phyto-constituent bioavailability. Phytosomes may enhance non-water-soluble drug absorption [15]. They arise by complexing phospholipids with phyto-constituents [3]. Instead of residing in the vesicular membrane's aqueous core or phospholipid bilayers, bioactive phyto-constituents are hydrogen-bonded to the phospholipid polar head. Phytosomes chemically interact with the amphiphile molecule's polar head and phytoconstituent to improve bioavailability, stability, and drug encapsulation [16]. Increased absorption requires fewer active substances for pharmacological efficacy. Telange et al. [17] found that phytosomal formulations with apigenin had higher oral bioavailability, water solubility, and hepatoprotective effects. Apigenin failed. A study by Rathee et al. [18] evaluated polyherbal extract-loaded phytosomes for diabetic suppression Polyherbal phytosomes and metformin decreased diabetes in streptozotocin-nicotinamide-induced rats.

This work formulated IB in a phytosomal nanocarrier system to improve oral bioavailability and absorption. Nanoparticles in the optimized formulation have porous, virtually smooth surfaces. Additionally, the optimized recipe enhanced loaded IB's water solubility compared to pure IB. In rats with CCl4-induced liver damage, the optimized IB phytosomal formulation surpassed standard IB in hepatoprotective effectiveness and oral bioavailability.

Materials & Methods:

Sources of drugs and chemicals

Donors from Lipoid GmbH in Ludwigshafen, Rhineland-Palatinate, Germany, are phospholipid Phospholipon® 90H, which is 90% hydrogenated soy phosphatidylcholine. The chemicals utilised to prepare the phytosomes were of analytical quality, and they included methanol, chloroform, ethyl acetate, and dichloromethane, all of which were acquired from Ranchem Private Limited from Mumbai.

Collection and authentication of plant material

Prof. K. Madhava chetty of Sri Venkateswara University in Tirupathi verified the authenticity of the IB Gamble leaves after they were acquired in Chengalpattu (Dt), Tamil Nadu, India. There was a deposit of the plant specimen for future reference, with voucher number 2023/0519.

Methods for IB extract preparation

The Indigofera barberi Gamble leaves were washed, dried, and ground into a coarse powder. To acquire the crude extract, the powder was further extracted using a Soxhlet apparatus using ethanol as the solvent. The syphon tube filled with a colourless solvent as the extraction process came to a close. After filtering away the solvent, the mixture was concentrated to dryness in a Rotavapor apparatus with controlled temperature and lowered pressure. A value was extracted from the data [28].

Preparation of IB-Phospholipid Complex

At room temperature, 300 mg of phospholipid was added to 60 ml of acetone used to dissolve 300 mg of IB raw material into 100 ml RBF while stirring. After 48 hours, a vacuum at 35 degrees Celsius strained and condensed the liquid above the

cells to 5 millilitres. The liquid above was treated with 30 millilitres of n-hexane the next day and precipitated overnight. After suction-filtering, a few millilitres of cooled n-hexane rinsed the precipitants. Some solvent residue was removed by leaving samples in vacuum desiccators overnight. Dry materials were softly pulverized with a pestle and mortar to pass through a 150-m-pore 100 mesh sieve. IB-phospholipid complexes (IBPCs) were stored at -20 °C in a plastic bag immediately after production.

Synthesis of Phytosome-Nanosuspensions (NS)

High-pressure homogenization helped make phytosome nanosuspensions. To dissolve Poloxamer188, 50 cc bidistilled water was mixed with an agitator. A high-speed dispenser (XHF-D, Ningbo Scinentz Biotechnology Co. LTD., China) at 12,000 rpm for three minutes carefully dispersed the IBPCs complex into 50 ml of bidistilled water. Three minutes of agitation mixed the IBPCs complex solution with poloxamer 188. The ATS Nano-homogenize machine (AH-2010, ATS Engineering Inc., USA) homogenized the mixture. A pre-milling process at 50 and 100 bars, five cycles at 500 and 1000 bars, and fourteen final cycles at 1500 bars produced the final product. To prevent freeze-drying, phytosome-nanosuspensions were stored at -60 °C for 24 hours with 5% mannitol. A freeze dryer at -57o lyophilized materials for 48 hours after pre-freezing. Characterization studies were conducted on solid powders stored at -20 °C.

Eliminating Wasteful Variables in Formulation and Processing

High-pressure complexation and homogenization of IBPCs-NP. IB concentration, phospholipid molar ratio, reaction duration, temperature, number of high pressure homogenisation cycles, and stabilisers may affect IBPCs-NP synthesis. When choosing processing and formulation parameters, design of experiments (DOE) considered these issues. An optimisation research controlled all other factors while studying one parameter. The concentration experiment used 1:1 IB-phospholipid molar ratios. A study on IB/phospholipid ratio maintained the right IB concentration. The screen indicated particle size, PDI, and complexation efficiency (F%) as the most important biomarkers. Data was handled using IBM®SPSS® Statistics (version 22, International Business Machines Corp.) after duplicate measures.

Evaluation of IB Phytosomes

Particle Dimensions , Polydispersity Index, and ZP

The Malvern Zetasizer Nano-ZS (ZEN3600, Malvern Instrument Ltd., Malvern, UK) was used to evaluate the average size of the particles, polydispersity index (PDI), and potential for zeta of the phytosomal formulations in this work.

Analysis of Surface morphology

Transmission and scanning electron microscopy studied optimal IB phytosomal formulation surface topography. A JSM-6380LA scanning electron microscope (JEOL Ltd., Tokyo, Japan) revealed phytosomal powder on double-sided sticky tape on an aluminum stub. TEM was performed by sonicating a 1-to-20 distilled water-phytosomal powder dilution for three minutes with a vibra-cell sonicator (Vibra-CellTM Sonicator, Newtown, CT). After casting onto a 300 mesh carbon type-B copper grid, the sample was sonicated, filtered, and colored with 2% w/v uranyl acetate. Later, it was vacuum-dried overnight. The cloudy liquid film was exposed using a 200 kV JEOL-JEM-100S transmission electron microscope from Tokyo, Japan.

Estimation of Drug Content

The phytosomal complex measured IB spectrophotometrically. In conclusion, 5 mg phytosomal complex was accurately weighed and dispensed in 5 mL chloroform. IB didn't break, but phytosomes did. The non-complexed IB solid residue was filtered, dried, and redissolved in methanol. The Shimazu UV-visible spectrophotometer in Tokyo, Japan measured free, non-complexed IB at λmax of 286 nm. Percentage-based medication formula: Drug content as a percentage equals total IB minus free IB. Divide total IB by 100.

FTIR Spectroscopic studies

Alpha Bruker, Berlin, Germany- Fourier transform infrared spectrometer for the analysis of phytosomal component chemical interactions was utilised for infrared (IR) spectra matching. Between 4000 and 500 cm−1, the infrared spectra of both the pure IB and the enhanced IB phytosomal formulation were examined.

Calorimetry using Differential Scanning (DSC)

The TGA/DSC-SDT Q600 thermograms for the IB and improved IB phytosomal formulation were acquired with the use of a differential scanning colorimeter from TA Instruments in New Castle, DE, USA. A covered pan was used to heat 2 milligrammes of each sample from 25 to 400 a nitrogen generator heated to a temperature of 10 degrees Celsius/min purge of 60 millilitres per minute, in order to examine the thermal behaviour.

Diffraction analysis

A Rigaku micro flex 600 X-ray diffractometer was used to examine the molecular crystallinity of IB and its enhanced phytosomal formulation. The instrument was located in Hokkaido, Japan. The device was set up using a 40 kV tube voltage, a 40 mA tube current, a 2θ scanning angle, and a 1°/min step width.

Analysis of Solubility

A sealed glass container containing excess IB and the optimised IB phytosomal formulation was subjected distilled water or n-octanol, up to 5 millilitres at 25 ± 1 °C in order to determine the sample's solubility. After stirring the mixture for 24 hours, we spun it in a centrifuge at 5000 rpm for 30 minutes. Spectrophotometry was performed using a solvent-dilution of 1 mL of filtrate to a final volume of 10 mL, with a maximum wavelength set at 286 nm.

Investigating Dissolution in a Vitro

The dialysis bag technique was used to investigate the IB phytosomal complex dissolution profile, which allowed for an evaluation of the formulation's in vitro drug release. There was a certain amount of pure IB (10 mg) and an enhanced IB phytosomal formulation (a particular weight) in the dialysis bags. The 100 mL of phosphate buffer, which had a pH of 7.4, was put in glass vials with the bags. Stirring the glass vials required a temperature of 37 ± 1 °C and a rotational speed of 50 rpm. Two millilitres (mL) of buffer was added to glass vials at regular intervals to maintain a steady sink state.

In Vivo Studies

Animals

The rats utilised in this investigation were male Wistar rats weighing between 175 and 200 grammes. An animal ethics commission in Uppal, Hyderabad, approved all of Jeeva Life Sciences's studies on local animals. More details may be found in NGSMIPS/IAEC/140. Ten days before to the studies, the rats were housed in plastic tubs covered with stainless steel, in groups of seven or eight. A light-dark cycle lasting 12 hours was instituted, with the relative humidity kept at 50 ± 10% and the temperature at 22 ± 2 °C.

Liver-Shielding and Antioxidant Activity Investigations

Six Wistar rats were randomly allocated to four groups. Negative control group received 1% v/v Tween 80 oral solution for seven days. The positive control group got 1.5 mL/kg of CCl4 and olive oil intraperitoneally on day seven after seven days of oral 1% v/v Tween 80. Third group: 100 mg/kg IB orally once daily for seven days via intravenous solution. On day seven, they received CCl4 and olive oil intraperitoneally. After consuming an optimized IB phytosomal solution orally for seven days at 100 mg IB/kg/day, the last group received an intraperitoneal injection of CCl4 and olive oil on the seventh day. Two days after CCL4 poisoning, blood was centrifuged. A series of liver function tests included total bilirubin, SGPT, SGOT, and SALP. Animals were slaughtered for liver antioxidant enzyme biochemical assays after blood samples. After being taken from the corpse, ivers were immersed in ice-cold saline and blended with pH 7.4 Buffered with 0.1M Tris HCl. Centrifuged liver tissue supernatants are tested for GRD, GSH, GPx, GST, SOD, and catalase. TBARS detected lipid peroxidation in liver homogenates by measuring MDA [30]. MDA and TBA produce pink TBARS at 530 nm. We quantify MDA in nM/mg protein.

Histopathological Studies

Using 10% neutral buffered formalin, animal livers were prepared for histopathology. Zeiss Primo Star microscopy (Carl-Zeiss, Oberkochen, Germany) was used to examine liver slices stained with haematoxylin and eosin.

Data Analysis by Statistic

All the numbers are shown as the average plus or minus the standard deviation. A portion of the statistical study included the students' t-test and a one-way analysis of variance. Significant P values were under 0.05.

Results & Discussion

Optimization of IB-Phospholipid Complex Preparation

IB-phospholipid complex (IBPCs) production is the most important step in IBPCs-NP preparation. Standard solvent evaporation/anti-solvent recipitation produced IB-phospholipid complex (Khan et al., 2013). Precipitated from organic solvent utilizing acetone as an antisolvent and n-hexane to prevent proton exchange mechanism disruption. After adding phospholipid to acetone, its polar head would weakly bind with IB (Zhao et al., 2019). Slow-reducing phospholipid created more acetone complexes. N-hexane filters this mixture's supernatant for IBPCs.

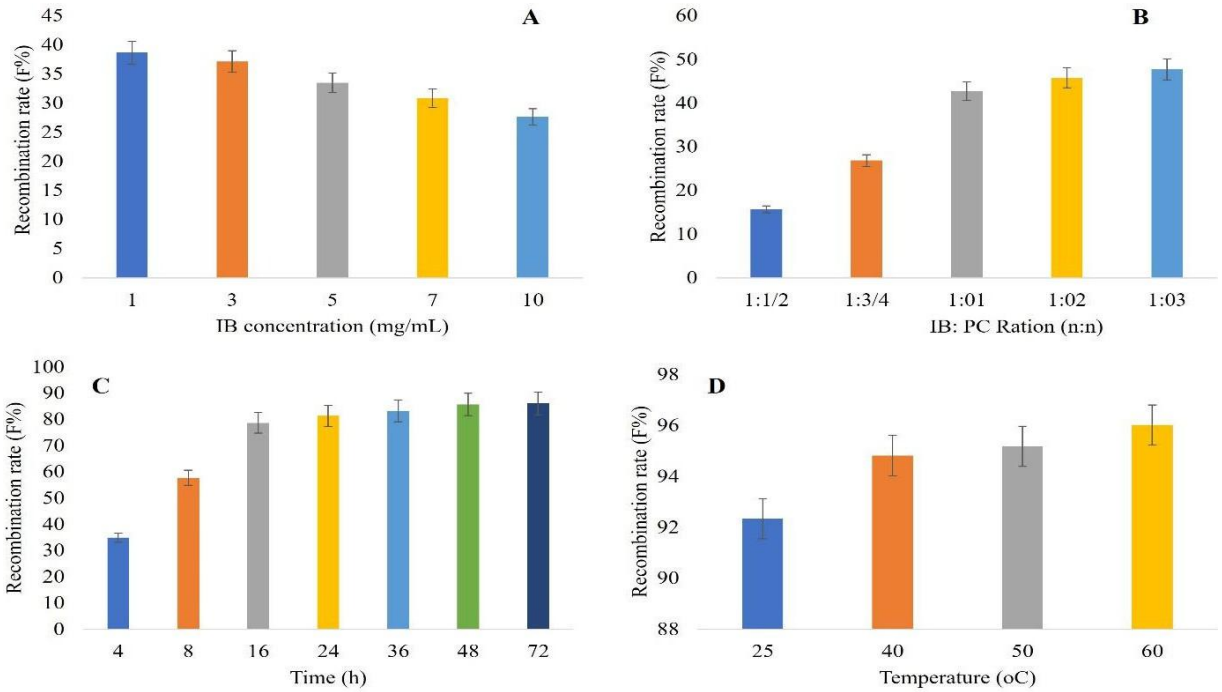


Fig. 1 Improved IB-phospholipid complex formation. How much IB (A) is present influences how well complexations work. Section B: The effect of the S/P molar ratio on the complexation efficiency. (C) How reaction time affects complexation efficiency.(D) Thermal effects on complexation efficiency. The data (n = 3) are presented as the mean plus or minus the standard deviation.

IBPC complexation efficiency (F%) may affect phytosome-nanosuspensions drug loading. Thus, IBPC optimization variables such IB concentration, IB-phospholipid molar ratio, reaction duration, and temperature were examined. IBPC complexation efficiency decreased with IB concentration, perhaps due to IB's limited solubility (Fig. 1A). Complexation was greatest at 1 mg/ml IB. Phospholipid concentration increased complexation efficiency (Fig. 1B). A 1:1 IB/phospholipid IB complexation efficiency plateaued. Figs. 1C and 1D demonstrated proper reaction time and temperature. Complexation efficiency plateaued at 48 h, the ideal reaction period for IBPC compounds. Also, reaction temperature had little effect on complexation efficiency.

Optimization of IBPCs-NPs Preparation

The smaller size and higher surface energy of nanoparticles make them metastable and higher Gibbs free energy. In spite of its presence, phospholipid cannot stabilize the system. Stabilizing phytosome nanosuspension reduces agglomeration and increases activation energy and stability. Poloxamer 188, polysorbate 80, hydrophobic polyvinyl chloride, and propylene glycol stabilized IBPCs-NPs. HPMC-stabilized nanosuspensions had the tiniest particles, however drying induced irreversible nanocrystal agglomeration and inhomogeneous precipitation in the coarse suspension (Muller and Peters, 1998). Without precipitation, poloxamer 188 had low PDI and particle size. Jain asserts Poloxamer 188 significantly adsorbs on nanoparticle surfaces, changing their characteristics. Poloxamer 188 on nanoparticle surfaces decreases Gibbs free energy and hydrophilicity, enhancing steric repulsion and stability (Jain et al., 2013). Poloxamer 188 stabilizes phytosome-

nanosuspension best. The lowest IBPCs-NP and PDI were obtained at 4:1 weight ratio (IBPCs/poloxamer 188). At 400 mg, IBPCs-NP particle size and PDI were lowest. Future research could use the 400 mg nanosuspension with poloxamer 188 stabiliser and 1–4 IBPCs. Create IBPCs-NP with homogenization pressure and cycle count. Pressure reduced IBPCs-NP particle size. IBPCs-NP particles shrank under 1500 bar homogenization. Nanoparticles are metastable and have a higher Gibbs free energy, hence 14 homogenization cycles at 1500 bar were optimum for IBPCs-NP synthesis In spite of its presence, phospholipid cannot stabilize the system. Stabilizing phytosome nanosuspension reduces agglomeration and increases activation energy and stability. Poloxamer 188, polysorbate 80, hydrophobic polyvinyl chloride, and propylene glycol stabilized IBPCs-NPs. HPMC-stabilized nanosuspensions had the tiniest particles, however drying induced irreversible nanocrystal agglomeration and inhomogeneous precipitation in the coarse suspension (Muller and Peters, 1998). Without precipitation, poloxamer 188 had low PDI and particle size. Jain asserts Poloxamer 188 significantly adsorbs on nanoparticle surfaces, changing their characteristics. Poloxamer 188 on nanoparticle surfaces decreases Gibbs free energy and hydrophilicity, enhancing steric repulsion and stability (Jain et al., 2013). Poloxamer 188 stabilizes phytosome-nanosuspension best. The lowest IBPCs-NP and PDI were obtained at 4:1 weight ratio (IBPCs/poloxamer 188). At 400 mg, IBPCs-NP particle size and PDI were lowest. Future research could use the 400 mg nanosuspension with poloxamer 188 stabiliser and 1–4 IBPCs. Create IBPCs-NP with homogenization pressure and cycle count. Pressure reduced IBPCs-NP particle size. IBPCs-NP particles shrank under 1500 bar homogenization. Best for IBPCs-NP preparation were 14 homogenization cycles at 1500 bar.

Potential of zeta, size of particles, and polymer dispersity index

Optimised IBPCs-NP were assessed for size and zeta potential using DLS. The measured particle size was 332.52 ± 1.54 nm and the PDI was 0.284 ± 0.11. ZETA's potential determines the stability of nanosuspensions. Stable nanosuspension systems have zeta potentials of -30 mV for electrostatically stabilised systems and -20 mV for sterically stabilised systems (Bajaj et al., 2012 The zeta potential of phytosome-nanosuspension was -28.94 ± 1.24 mV, indicating better stability and less agglomeration. These studies indicated stable, uniform phytosome-nanosuspensions.

FTIR Spectroscopic studies

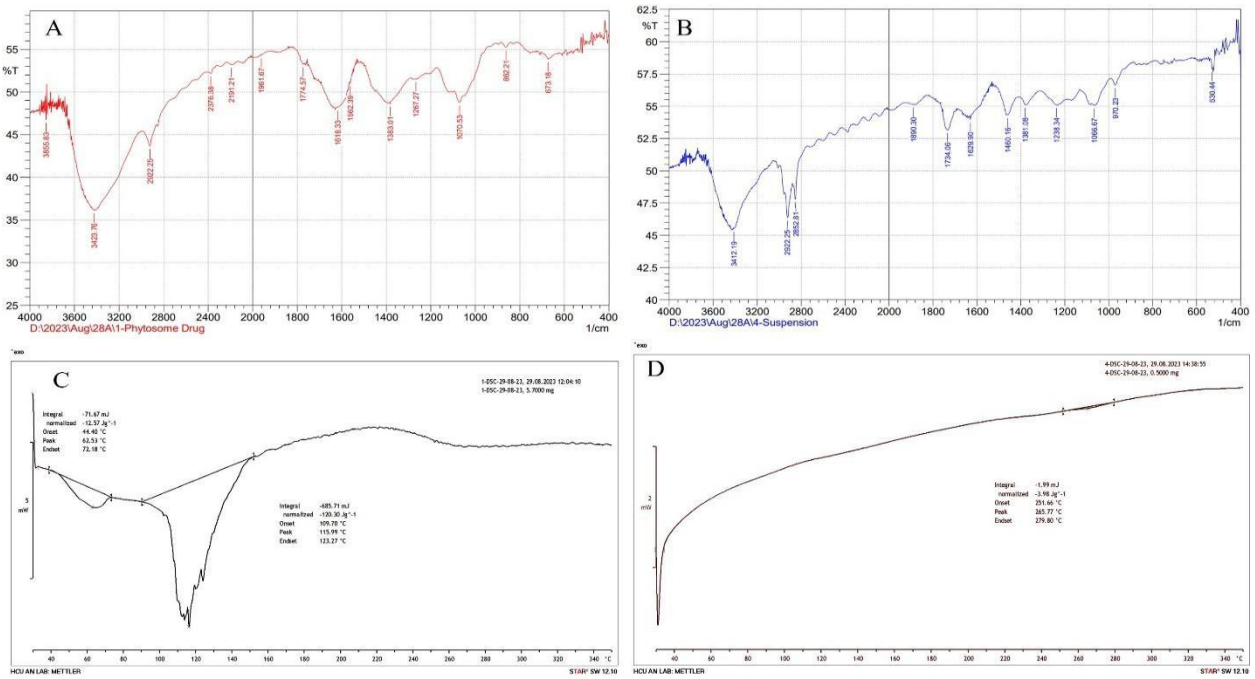
Figure 3 shows that extensive bonding occurred at 1643 cm−1, the dialiphatic CH2 group at 2965 cm−1, and TQ at 1615 cm−1. The two amino group salts, on the other hand, were identified at 2849 and 2923 cm−1, respectively, by Phospholipon® 90H. confirming the presence of the ketone group at 1650 and 1740 cm-1. Reduced peak strength of main groups and disappearance of Phospholipon® 90H peak (3017 cm−1) in phytosomes suggest TQ and Phospholipon® 90H function groups work

cooperatively [70].

Figure 2: A&B. FTIR spectrum of Pure IB and Optimized formulation of phytosomes,

Differential scanning colorimetric (DSC)

In DSC thermogrammes, extracts and phospholipids had identical endothermic melting transitions without interaction (Fig). The PC DSC thermogram indicates endothermic peaks at 150.4ºC, 172.7ºC, and 180.8ºC. RN's endothermic activity peaks at 175.6C. The endothermic maximum for the PC/RN combination and each component is approximately 1ºC lower at 149.57ºC and 174.89ºC, respectively. Neither the 172.7ºC nor 180.8ºC maxima were observed in the physical combination. At temperatures lower than the physical mixture, phytosomes have two endothermic peaks on their DSC thermogram: 145.8oC and 171.31oC. The PC and RN summits are gone. The transition temperatures for complex phases were lower than PC. PC and RN may interact, according to thermogram data. Hydrogen bonds and van der Waals forces can cause such contact, however Xu et al. (2009) observe that they



C&D DSC thermogram of Pure IB and Optimized IB phytosomes

do not form a new molecule. In Figure 4, the endothermic peak and phase transition temperature of the phosphatidylcholine carbon-hydrogen chain were 180.8ºC and 180.8ºC, respectively, due to weak bonding between RN and PC, reducing phospholipid polarity and aliphatic hydrocarbon chain sequence.

XRD studies

IB and optimised phytosome XRD patterns are in Figure 3. Sharp diffraction peaks confirmed pure DPD's structural structure. PP3 retained DPD's distinctive diffraction peaks, albeit with reduced intensity, suggesting vesicle encapsulation caused partial crystalline to amorphous transition. The single sharp peak of PPR and PC may be related to PP3's diffraction peaks. XRD verifies FTIR observations that crystalline DPD partially amorphizes.

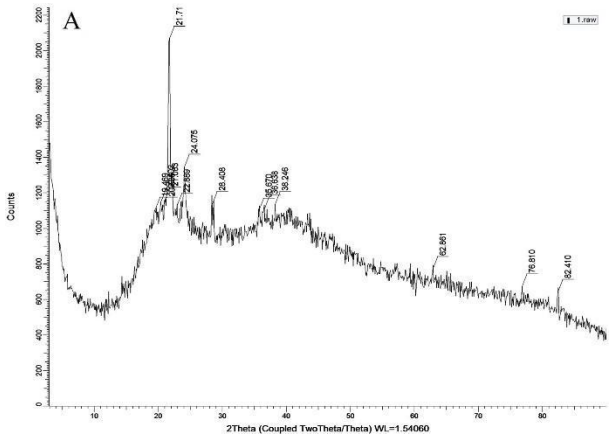


Figure 3: Powdered X-ray diffractogram of Pure IB and Optimized IB phytosomes

Solubility Study

Solubility and partition coefficients determine oral drug fate in vivo. Oral drugs cannot be excessively lipophilic since they absorb poorly. Hydrophobic IB has reduced bioavailability due to limited water solubility [26]. Table 2 shows pure IB and IB phospholipid phytosomal complex water solubility. Table 2 shows that IB has low aqueous solubility in distilled water (35.12 ± 0.95µg/mL). However, the IB phytosomal complex had a much higher water solubility (485.16 ± 1.3µg/mL) than pure IB ($p < 0.001$) IB phytosomal formulation's improved solubility may be owing to its amphiphilic character and drug crystallinity converting to amorphous upon phospholipid complexation, which is explained by diffraction studies.

Table 2. Phosphate buffer and water solubility of optimised IB phytosomal formulation and pure IB at p^H 7.4.

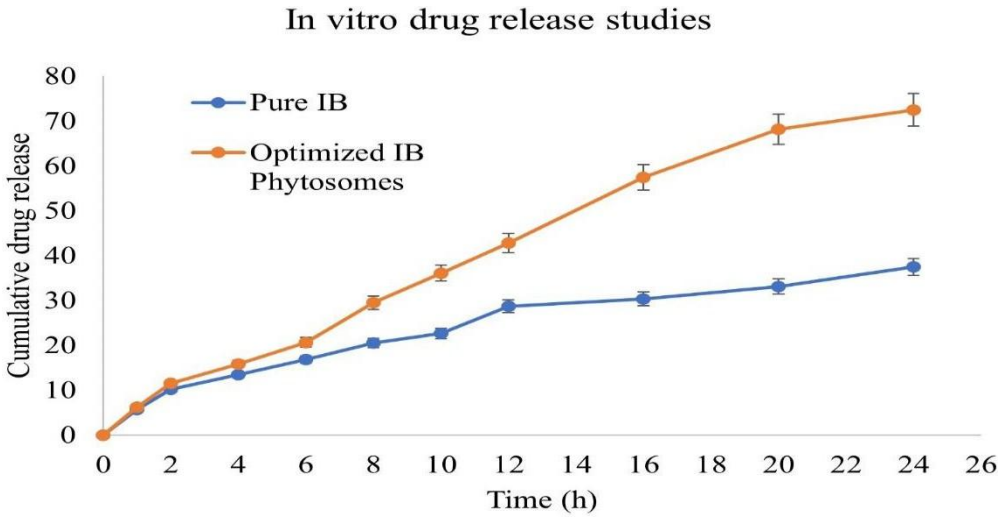
Medium	Solubility (µg/m

	Pure IB	Op
Water	35.12 ± 0.95	25
n-octanol	112.57 ± 0.32	48

The results show the average and standard deviation of three separate tests.

Studies on Drug release

Pure IB and optimised IBPCs-NP dissolving profiles in pH 7.4 phosphate buffer are presented in Figure 6. The enhanced IB phytosomal formulation and pure IB released drugs similarly for 24 h. Pure IB plateaued at 8 h, yielding 37.52% medicine after 24 h. The increased IB phytosomal formulation released 70.8% of medicine after 24 h, unlike pure IB. Compared to pure IB, complexing the medication with phospholipid boosted its solubility in vitro, which may explain the optimised



phytosomal formulation's greater drug release. By fitting release data onto several kinetic models, the release kinetics of IB phytosomal formulations were investigated. Plantosomal medication release in vitro was regulated by diffusion according to the Higuchi model.

Figure 4: Invitro release studies of Phytosomes and pure drug solution

The Influence of an IB-Phospholipid Phytosomal Complex on Liver Hepatoprotection

Main metabolic and excretion organ, liver, detoxifies. Many hepatotoxins cause significant liver disease. Animal studies use hepatotoxin CCl4. Hepatic CYP450 enzymes convert CCl4 into harmful reactive oxidants that peroxidize cellular membranes and limit blood antioxidant enzyme activity[28]. hepatotoxicity CCl4-induced animals were tested for pure IB or IB-phospholipid phytosomal complexes to normalize hepatic marker levels. Table 3 shows that rats poisoned with CCl4 had significantly elevated liver enzyme levels (SGPT, SGOT, SALP, and total bilirubin) ($p < 0.01$), suggesting liver damage. Pre-treatment with pure IB for a week reduced blood marker enzyme levels in rats given CCl4 as a toxin, protecting their livers. After one week of pre-treatment with IB-phospholipid phytosomal complexes, the control group had no blood hepatic marker rise (Table 3). These findings suggest that IB phytosomes provide better hepatoprotection than pure IB.

Hepatic Antioxidant Enzyme	Group—I (Normal Control)	Group—II (CCl4-Int Rats)
SGPT (U/L)	42.77 ± 1.82 **	134.37 ±
SGOT (U/L)	38.22 ± 2.71 **	97.76 ± 3.38
SALP (U/L)	141.53 ± 2.26 **	267.64 ± 3.29
Total bilirubin (mg/dL)	0.66 ± 0.03 **	1.41 ± 0.02

Data are mean ± SD (n = 6). The significance levels are (*) $p < 0.05$ and ** $p < 0.01$ when compared to rats that were intoxicated with CCl4. test for alkaline phosphatase, glutamate pyruvate, and glutamate oxaloacetate in the blood.

Protective Effects of an IB-Phospholipid Phytosomal Complex on Live Cells

IB is a popular natural hepatic dysfunction treatment because it is anti-inflammatory, antifibrotic, and antioxidant [29]. Rat liver samples were used to measure antioxidant enzymes. IB-phospholipid phytosomal complex antioxidant activity was assessed in animals treated with CCl4 or left untreated. The enzymes were GRD, GSH, GST, GPx, CAT, SOD. Table 4 shows the antioxidant biochemical effects of pure IB or IB-phospholipid phytosomal complexes. CCl4 poisoning significantly reduced antioxidant enzyme levels ($p < 0.01$) in liver homogenates compared to controls. Compared to CCl4, pure IB pre-treatment significantly reduced GSH, GPx, and CAT loss after 7 days ($p < 0.05$). Pre-treatment with enhanced IB phytosomes protected rats from CCl4-induced antioxidant enzyme reductions. Similar to the naïve negative control group, liver homogenates showed similar antioxidant enzyme levels (GSH, GPx, GST, GRD, SOD, and CAT). Besides replenishing plasma antioxidant enzymes, customized IB phytosomes may protect the liver from CCl4 damage.

Hepatic Antioxidant Enzyme	Group—I (Normal Control)	Group—II (CCl4-Intoxicated Rats)	Gr (Pl
GSH	49.16 ± 3.99 **	18.86 ± 1.28	29.

(nmol/mg protein)		
GPx (nmol/mg protein)	332.23 ± 4.91 **	193.76 ± 3
GST (nmol/mg protein)	296.43 ± 4.73 **	165.28 ± 3
GRD (nmol/mg protein)	21.16 ± 1.54 **	7.89 ± 0.9
SOD (U/mg protein)	7.41 ± 0.11 **	4.21 ± 0.1
CAT (U/mg protein)	212.85 ± 2.87 **	95.46 ± 3.

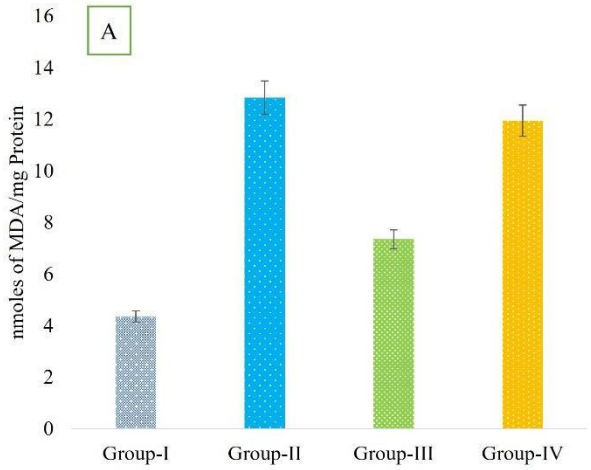
To test IB-phospholipid phytosomal complex antioxidant activity, TBARS quantified malondialdehyde (MDA), a major lipid peroxidation product [30]. Figure 7 shows a significant increase in lipid peroxidation ($p < 0.01$) in rats exposed to CCl4 poisoning compared to controls. IB did not protect against CCL4-triggred lipid peroxidation because the positive control group had equivalent malondialdehyde (MDA) levels, as were rats pretreated with IB and then exposed to CCL4, and rats in the experimental group that received no IB. However, pre-treatment with IB-phospholipid phytosomal complexes significantly reduced CCl4-induced MDA levels ($p < 0.01$). Hepatic parenchymal monooxygenases convert CCl4 to CCl3 radicals [31]. CCl3 may alkylate cellular proteins and create lipid peroxides from polyunsaturated fatty acids to damage the liver and alter marker enzyme levels [32,33]. The modified IB phytosomal formulation reduced all CCl4-induced cellular abnormalities, this study found. After CCl4 enhanced liver-impaired enzyme levels in mice, the improved IB phytosomal lowered MDA and corrected all these markers. Additionally, antioxidant enzyme loss paused. In rats, IB phytosomal formulation's ROS scavenging lowers CCl4-induced oxidative damage.

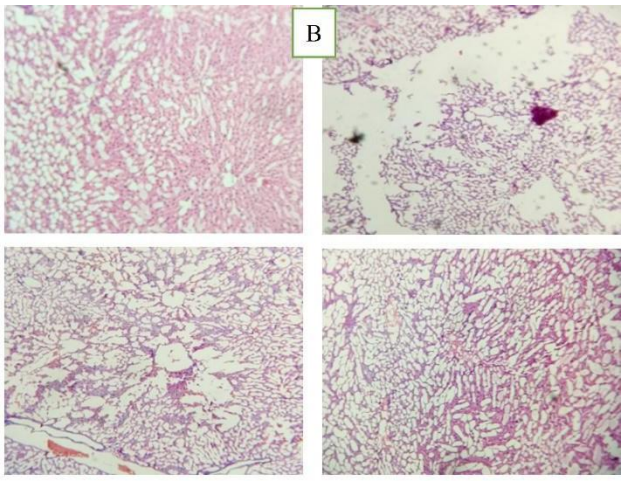
Histopathological Studies

After CCl4-induced symptoms, researchers used histology to assess liver damage in rats treated with pure IB and the improved IB phytosomal formulation. In sober rats treated with CCl4, liver cells had clean cytoplasm and were healthy (Figure 8). However, CCl4-intoxicated rats showed hepatotoxicity with central lobular destruction and parenchymal cell and fatty tissue degradation. Pure IB pre-treatment protected the liver by reducing fatty tissue deterioration and parenchymal cell damage. Improvements in IB phytosomal formulation protected liver tissue from CCl4 hepatotoxicity. Rats' normal hepatic cells' cytoplasm and central vein were recovered after IB phytosome therapy. The improved IB phytosomal formulation may rebuild hepatic cell architecture due to its

antioxidant capabilities.

Figure 5: Purified IB and an optimised IB phytosomal structure as functions of lipitase activity (nmoles of MDA released/g tissue). One group of rats served as a control, receiving 1.5 mL/kg of olive oil and 1:1.5 mL/kg of CCl4. Another group of rats was given plain IB while still under the influence of CCl4. Finally, there was a group of rats





given an optimised IB phytosomal formulation while still under the influence of CCl4. B. Histological micrographs of liver sections from rats exposed to different compounds: (A) rats given a negative control (1% v/v Tween 20), (B) rats administered a mixture of 1.5 mL/kg of olive oil and 1 mL/kg of CCl4 (C) rats given plain IB after being intoxicated with CCl4, and (D) rats given an optimised IB phytosomal formulation after being intoxicated with CCl4. magnified one hundred times.

Conclusion:

IB's limited solubility and bioavailability hindered preclinical use, hence this work produced phytosome-nanosuspensions for IBPCs complex. Weak intermolecular interactions between IB's hydroxyl and phospholipids' polar heads created IBPCs. We observed that homogenising IBPCs increased solubility and gastrointestinal absorption without diminishing molecular interaction. Compared to current products, IBPCs-NP improve IB phytosome hydrophily, stability, and bioavailability. IBPCs-NP, A reliable technique of pharmaceutical administration was created by merging phytosome and nanosuspension technology. This method achieved high intravenous bioavailability in both test tubes and living creatures. In CCl4-induced mice of oxyhepatitis, the pharmacodynamic assessment of IBPCs-NP showed that it improved liver protection and was safer. enabling phytosome-nanosuspension methods to synthesise IB and other IB-like polyphenol medications in preclinical and clinical contexts We found 76% IBPCs-NP yield, which needs to be increased in future experiments.

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