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Formulation and Evaluation of Rutin Phytosomes

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Abstract: *Rutin phytosomes were developed to enhance the antioxidant and hepatoprotective potential of rutin. Three preparation methods—solvent evaporation, salting out, and lyophilization were evaluated, with lyophilization yielding a stable and dry rutin–phospholipid complex. Optimization using a quadratic response surface model identified the rutin:SPC ratio as the primary factor influencing particle size and drug content. The optimized phytosomes exhibited suitable particle size, low PDI, negative zeta potential, and high drug incorporation. Characterization by DSC, FTIR, SEM, and TEM confirmed successful complex formation and uniform vesicular nanostructures. In vitro, the optimized phytosomes retained strong free radical–scavenging activity. In vivo, CCl_4-induced hepatic damage was effectively attenuated by the optimized phytosomes, as evidenced by restoration of liver function markers, antioxidant enzymes, TBARS levels, and near-normal histological architecture, outperforming free rutin. Overall, lyophilized rutin phytosomes demonstrated enhanced stability, antioxidant activity, and hepatoprotective efficacy, highlighting their potential as an improved oral delivery system for rutin.*

Keywords: *Rutin phytosomes*

I. INTRODUCTION

Rutin, a naturally occurring flavonoid glycoside widely present in fruits, vegetables, and medicinal plants, has attracted considerable attention due to its potent antioxidant, anti-inflammatory, and hepatoprotective properties. Despite its promising pharmacological potential, the clinical application of rutin is significantly limited by its poor aqueous solubility, low permeability, and consequently reduced oral bioavailability. These limitations hinder its therapeutic efficacy and necessitate the development of advanced delivery systems to enhance its pharmacokinetic and pharmacodynamic performance.

Phytosome technology has emerged as a promising approach to improve the bioavailability of poorly soluble phytoconstituents. In this system, bioactive compounds form molecular complexes with phospholipids, enhancing their solubility, membrane permeability, and systemic absorption. Compared to conventional formulations, phytosomes offer improved stability and better interaction with biological membranes, thereby facilitating efficient drug delivery. Several studies have demonstrated that phytosomal formulations significantly enhance the therapeutic effectiveness of flavonoids and other plant-derived compounds.

In the present study, rutin phytosomes were developed using different preparation techniques, including solvent evaporation, salting out, and lyophilization, with the aim of identifying a stable and efficient delivery system. A systematic optimization approach using response surface methodology was employed to evaluate the influence of formulation variables on critical parameters such as particle size and drug content. The optimized formulation was further characterized using physicochemical and morphological techniques.

Furthermore, the study investigated the in vitro antioxidant activity and in vivo hepatoprotective potential of the optimized rutin phytosomes in a CCl_4-induced hepatotoxicity model. The findings of this study are expected to provide valuable insights into the development of an effective phytosomal delivery system for improving the therapeutic potential of rutin.

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Materials and method

Rutin was procured from Yucca Enterprises,Mumbai, India. Soybeanphosphatidylcholineand Lecithinsoya-30% procured from Lipoid®, Ludwigshafen, Germany and HiMedia Laboratories, Mumbai, India respectively. All the chemicals, solvents, and reagent susedin the study were of analytical grade.

Preparation of rutin phytosomes

In the preliminary studies, the rutin phytosomes were prepared by three differentmethods, namely solvent evaporation, salting out, and lyophilization method and compared. Phytosomes were prepared by taking the rutin and SPC in1:2 molar ratio. The SPC used was Lipoid® S 100.

Solventevaporationmethod

The rutin phytosomes were prepared by solvent evaporation technique as per the method described by Freag MS *et al.,* rutinand SPC (in 1:2 molar ratio) were accuratelyweighed andrefluxed for 5hat 45±5ºCbyplacing into a 250 ml round bottom flask containing 20 ml of dioxane: methanol mixture (7:3) and then the solvent was evaporated under the vacuum using a rotary evaporator. The dried rutin phytosomal complex product was collected.[1]

Saltingout method

Inthesaltingout method, rutin andSPCweretakenin1:2 molar ratio anddissolved in35 ml ofa mixture ofDMSO, dehydrated ethanol, and chloroform(2:2:3). This solution was stirred on a magnetic stirrer overnight, andn-hexane (75ml) was added until precipitation formation. The precipitate was filtered and dried under vacuum to get dried rutin phytosomal complex product.[2]

Lyophilization method

Lyophilization method was performed as described by Freag MS *et al.,* in the first step, rutin was dissolved in DMSO (2.5 %w/v) and SPC in t-butyl alcohol (1.5 %w/v). Then, rutin solution was added to the SPC solution followed by 3 h stirring on a magneticstirrer. The complex formed between the rutin and SPC was then isolated by lyophilization. The complex containing solution was filled in vials, and the vials were frozenat –80ºCbykeeping indeep freezer for 4 h. Thefrozen vialswerethenplaced in a lyophilizer with condenser temperature of –70 ºC. Lyophilization was done at 40 mbar pressureand ashelftemperatureof–40 ºCfor 24hfollowed bysecondarydrying at 25ºC for another 24 h. Thenthe dried rutinphytosomalcomplexproduct was removed fromthe freeze drier.[3]

The dried phytosomal complex products obtained from three different methods werefilled in amber coloured glass containers and placed in a desiccator over fused calcium chloride at room temperature (20±2 ºC) until further use.

Below is your text rewritten with proper spacing, paragraph breaks, and formatting, while keeping the original content unchanged:

Characterization of Rutin Phytosomes

Differential Scanning Calorimetry (DSC)

The thermal behaviour of the rutin:SPC complex was assessed using differential scanning calorimetry. The samples were sealed in an aluminium crimp cell and heated at a rate of 10 °C/min from 25 °C to 500 °C in a nitrogen atmosphere (60 ml/min) [150]. Thermograms of pure rutin, SPC (Lipoid® S100), the physical mixture of rutin:SPC (1:2), and rutin phytosomes prepared by solvent evaporation, salting out, and lyophilization methods were obtained using a thermal analyzer.

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra matching approach was used for the detection of possible chemical interactions between the drug and polymer. Attenuated Total Reflection (ATR) method was used in which a small quantity of each sample was placed just below the probe, on to which the probe was tightly fixed and scanned in the wavenumber region 4000–500 cm^{-1} using an FTIR spectrometer [151]. Samples assessed included pure rutin, SPC (Lipoid® S 100), the physical mixture of rutin:SPC (1:2), and rutin phytosomes prepared by the lyophilization method.

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Selection of Formulation Factors

Based on results obtained after the preliminary preparation of the phytosomes by three different methods, one best developing technique was selected. For this technique, the influence of variable formulation factors such as selection of proper co-solvent (methanol, ethanol, chloroform, acetone or t-butyl alcohol), proper SPC type (Lipoid® S 100, Lipoid® S 75, Phospholipon® 90 G, or Lecithin soya–30%), and optimum rutin:SPC ratio (1:1, 1:2, and 1:3) were assessed.

Formulation of Rutin Phytosomes and Optimization

Formulation of Rutin Phytosomes: Rutin phytosomes were formulated by the lyophilization method as described by Freag MS et al., with slight modifications. First, rutin and SPC were accurately weighed in 1:1, 1:2, and 1:3 molar ratios; rutin was dissolved in DMSO and SPC in t-butyl alcohol. The rutin solution was then added to the SPC solution, followed by 3 h stirring on a magnetic stirrer for the formation of the phytosomal complex.

The solution containing the phytosomal complex was isolated by lyophilization with mannitol used as cryoprotectant (0.5–1.5% w/v). Before lyophilization, the solution was sonicated for 3 min. This solution was filled into vials and frozen at –80 °C for 4 h. The frozen vials were then placed in a lyophilizer with condenser temperature –70 °C. Lyophilization was carried out at 40 mbar and a shelf temperature of –40 °C for 24 h followed by secondary drying at 25 °C for 24 h. The dried rutin phytosomal complex was collected and stored in amber glass containers inside a desiccator over fused calcium chloride until further use.[3]

Design of Experiments (DoE): Preliminary studies identified drug:phospholipid ratio (w:w) and mannitol concentration (% w/v) as the main influencing formulation factors. A quadratic response surface design represented by a second-order polynomial model was used to investigate their effects on particle size (nm) and drug content (% w/w) of rutin phytosomes using Design Expert® software.

Response surface methodology (RSM) helps quantify relationships between parameters at different levels. The independent variables—drug:phospholipid ratio (A) and mannitol concentration (B)—were selected at three levels: low (–1), medium (0), and high (+1), resulting in a 3-level factorial randomized quadratic design with nine experimental trials.[4,5]

The quadratic equation used:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 + \ldots \text{ (Eq. 1)}$$

Where Y is the measured response, b_0 is the intercept, b_1 to b_{22} are regression coefficients, and X_1 and X_2 are coded levels of independent variables.

Independent and dependent variables with coded and actual levels

Factors	–1 (Low)	0 (Medium)	+1 (High)
A = Rutin:SPC ratio (w:w)	1:1	1:2	1:3
B = Mannitol concentration (% w/v)	0.5	1.0	1.5

Dependent variables:

R1 = Particle Size (nm); R2 = Drug Content (% w/w)

***In Vitro* Evaluation of Optimized Rutin Phytosomes**

Average Particle Size, PDI, and Zeta Potential: Mean particle size (PS) and PDI of all formulations including the optimized batch were determined by dynamic light scattering (DLS). Zeta potential (ZP) of optimized phytosomes was measured by electrophoretic light scattering (ELS) using Malvern Zeta Nano Sizer at 90° scattering angle and 25 ± 0.5 °C. Samples were diluted 1:10 with distilled water and sonicated before measurement . Triplicate analysis was performed.[6]

Drug Content: The amount of rutin incorporated into phytosomes was estimated using the spectroscopic method described by Tan Q et al. A weighed amount of phytosomes (~10 mg of rutin added) was dispersed in 5 ml chloroform. The complex and SPC dissolve, leaving free rutin to precipitate. After filtration and drying, free rutin was dissolved in methanol and analyzed at 360 nm [69].

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% Incorporated drug = (W added – W free) / W added × 100

Differential Scanning Calorimetry (DSC): Thermal response of optimized phytosomes was evaluated under similar conditions.

FTIR Spectroscopy: FTIR was used to determine interactions between rutin and SPC using ATR method for the samples described earlier.[7]

Surface Morphology

Scanning Electron Microscopy (SEM): SEM samples were prepared by lightly sprinkling pure rutin and optimized phytosomes on carbon tape mounted onto aluminum stubs. Images were captured.

Transmission Electron Microscopy (TEM): Optimized phytosomes were diluted (1:20 ratio), sonicated, and one drop placed on carbon-coated grids. Stained with ammonium molybdate (2% w/w) and viewed at 200 kV.

Proton NMR (^{1}H-NMR): ^{1}H-NMR spectra of rutin, SPC, and optimized phytosomes were obtained using DMSO as solvent.

In Vitro Drug Release: Drug release was evaluated using dialysis bags in pH 1.2 buffer for first 2 h, then pH 7.4 buffer. Samples were withdrawn periodically and analyzed at 360 nm.

In Vitro Drug Release Kinetics: Data was fitted to zero-order, first-order, Higuchi, and Korsmeyer-Peppas models. (Zero-order, first-order, Higuchi, and Korsmeyer-Peppas equations formatted as in original.)

In Vitro Stability: Optimized phytosomes (1 mg) were added to SGF (pH 1.2) and SIF (pH 7.4) and incubated at 37 °C for 2 h and 6 h, respectively. PS, PDI, and ZP were measured.[8-14]

In Vitro Antioxidant Activity: DPPH assay was performed for pure rutin, SPC, optimized phytosomes, and standard ascorbic acid using DPPH solution (0.1 mM) and measuring absorbance at 517 nm.[15]

In Vivo Evaluation of Optimized Rutin Phytosomes

Animals: Adult male Wistar rats (180–200 g) were used with ethical approval (Approval Nos. NGSMIPS/IAEC/MAY-2017/43 and NGSMIPS/IAEC/March-2019/140). Animals were housed at 20–25 °C, 45–55% RH with 12 h light/dark cycles.

In Vivo Hepatoprotective and Antioxidant Activity Studies

Dosing: Four groups of rats (n=6 each) received:

Group I: Normal control

Group II: CCl_4 intoxicated

Group III: Rutin (100 mg/kg)

Group IV: Optimized rutin phytosomes (~100 mg/kg rutin)

CCl_4 (50% v/v in olive oil, 5 ml/kg) was administered on day 7.

Estimation of Liver Marker Enzymes

After 7 days, blood was collected for ALT, AST, ALP, total and direct bilirubin assays using commercial kits.[16] Below is your entire text rewritten with proper spacing, headings, paragraph breaks, and formatting, while keeping the technical content unchanged.

To prepare the working reagent, 4 parts of R1 were mixed with 1 part of R2. To 100 µl of serum sample, 1000 µl of working reagent was added and mixed well. After 1, 2, and 3 minutes, absorbance was measured at 340 nm and 37 ºC using a semi-automatic analyzer.

ALT activity (U/L) = ΔA/min × F, where F = 1746.

Estimation of AST (SGOT)

In vitro quantitative determination of AST in rat serum was carried out using the modified IFCC method. To prepare the working reagent, 4 parts of R1 were mixed with 1 part of R2. To 100 µl of serum sample, 1000 µl of working reagent was added and mixed well. After 1, 2, and 3 minutes, absorbance was measured at 340 nm and 37 ºC.

AST activity (U/L) = ΔA/min × F, where F = 1746.

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Estimation of ALP

In vitro quantitative determination of ALP in rat serum was carried out using the modified AMP (2-amino-2-methyl-1-propanol) method. To prepare the working reagent, 4 parts of R1 were mixed with 4 parts of R2. To 20 µl of serum sample, 1000 µl of working reagent was added, mixed well, and incubated for 1 min. After 1, 2, and 3 minutes, absorbance was measured at 405 nm and 37 ºC.

ALP activity (U/L) = ΔA/min × F, where F = 2750.

Estimation of Total Bilirubin

In vitro quantitative determination of total bilirubin in rat serum was performed using the Malloy–Evelyn modified method.

Blank (A1): 750 µl of R1 + 50 µl of serum sample → mix well.

Sample (A2): 750 µl of R1 + 25 µl of R3 + 50 µl of serum sample → mix well.

Both blank and sample solutions were incubated for 5 minutes at 37 ºC, and absorbance was measured at 546 nm.

Bilirubin content (mg/dl) = (A2 – A1) × F, where **F = 16.7**.

Estimation of Antioxidant Marker Enzymes

The antioxidant potential of pure rutin and optimized rutin phytosomes was evaluated by quantifying serum antioxidant marker enzymes. On day 8, after blood collection for LFT, animals were sacrificed under light ether anesthesia. Livers were dissected, washed with ice-cold saline, and homogenized in 0.1 M Tris-HCl buffer (pH 7.4). Homogenates were centrifuged, and supernatants were used for estimation of Reduced glutathione (GSH), Glutathione peroxidase (GPx), Glutathione S-transferase (GST), Glutathione reductase (GRD), Superoxide dismutase (SOD), Catalase (CAT) and TBARS (thiobarbituric acid reactive substances)

Estimation of Reduced Glutathione (GSH): Reduced glutathione reacts with DTNB (Ellman's reagent) to produce a yellow-colored compound measured at 420 nm.[17-20] 0.5 ml liver homogenate + 2 ml of 5% TCA → centrifuge at 3000 rpm for 10 min. To 1 ml supernatant: add 0.3 ml phosphate buffer + 0.5 ml Ellman's reagent. Incubate 15 min at room temperature. Measure absorbance at 420 nm. GSH content expressed as **nmol/mg protein**.

Estimation of Glutathione Peroxidase (GPx): GPx activity is measured by decrease in GSH after reaction with H_2O_2 and detection via DTNB at **420 nm**.[21] Add 0.5 ml liver homogenate + Tris-HCl (0.2 ml) + EDTA (0.2 ml) + NaN_3 (0.1 ml) Add 0.2 ml GSH + 0.1 ml H_2O_2. Incubate 10 min at 37 ºC. Stop reaction with 0.5 ml of 10% TCA and centrifuge. To 1 ml supernatant: add 0.5 ml Ellman's reagent + 3 ml phosphate buffer. Measure absorbance at 420 nm. GPx expressed as nmol of NADPH oxidized/min/mg protein.

Estimation of Glutathione S-Transferase (GST): GST catalyzes conjugation of CDNB and GSH, increasing absorbance at 340 nm [169]. Mix 200 µl phosphate buffer + 20 µl CDNB + 50 µl homogenate. Make volume to 1 ml; pre-incubate 10 min at 37 ºC. Add 50 µl GSH. Measure absorbance at 340 nm every 30 s for 3 min. GST activity expressed as nmol CDNB conjugate/min/mg protein.

Estimation of Glutathione Reductase (GRD): GRD converts GSSG to GSH, consuming NADPH. NADPH reduction is measured at 340 nm.[22,23] Mix 0.5 ml phosphate buffer + 50 µl NADPH + 50 µl GSSG + distilled water. Add 10 µl liver homogenate to initiate reaction. Measure absorbance decrease at 340 nm for 2 min. GRD activity = nmol GSSG utilized/min/mg protein.

Estimation of Superoxide Dismutase (SOD): SOD inhibits auto-oxidation of epinephrine to adrenochrome measured at **450 nm**.[24] Mix 0.1 ml homogenate + 0.75 ml ethanol + 0.15 ml cold chloroform. Centrifuge 3000 rpm, 10 min. Supernatant (0.5 ml) + EDTA (0.5 ml) + carbonate buffer (1 ml). Add epinephrine (0.5 ml). Measure absorbance at 450 nm at 0, 1, and 2 min. SOD expressed as U/mg protein (50% inhibition).

Estimation of Catalase (CAT): CAT decomposes H_2O_2; decrease in absorbance is measured at 240 nm.[25] 25 µl homogenate + 1 ml phosphate buffer. Add 250 µl H_2O_2 to initiate reaction. Measure absorbance decrease at 240 nm for 3 min. CAT activity = nmol H_2O_2 decomposed/min/mg protein.

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Estimation of TBARS: MDA reacts with TBA to form a pink product measured at 535 nm.[26] 1 ml homogenate + 2 ml TCA-TBA-HCl reagent. Heat 15 min on boiling water bath. Centrifuge 3000 rpm, 10 min. Read absorbance at 535 nm. TBARS expressed as nmol MDA/mg protein.

Histopathological Studies: Livers were preserved in 10% neutral buffered formalin, sectioned, stained with hematoxylin and eosin (H&E), and examined under a digital microscope.

Statistical Analysis: For in vivo studies (LFT, antioxidant activity, and pharmacokinetics), one-way ANOVA, followed by Tukey's test and Student's t-test, was used ($p \leq 0.05$). In vitro study results were reported as mean ± standard deviation (SD).

Results and discussion

Preparation of rutin phytosomes: The rutin phytosomes prepared by solvent evaporation method, salting out method, and lyophilization method during the preliminary studies. The phytosomal product obtainedby the lyophilization method was found to be dry compared to the products obtained by the solvent evaporation method and the salting out method (Fig. 1). Further, theprepared phytosomes were characterised by DSC and FTIR for the initial confirmation of the complexation between rutin and SPC.

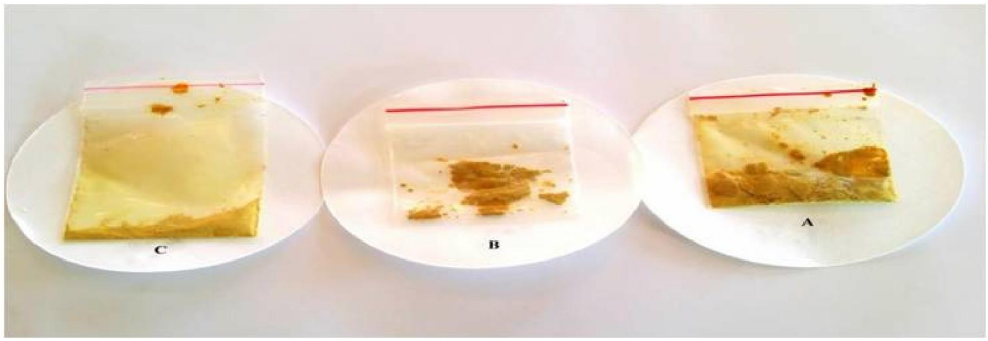

Fig.1. Photograph of phytosomal products obtained by solvent evaporation method (A), salting out method (B) and lyophilization method (C).

Differential scanning calorimetry (DSC): DSC thermograms of pure rutin, SPC, physical mixture of rutin:SPC (1:2), phytosomes prepared by solvent evaporation method, salting out method, and lyophilization method are given in Fig. 3.4A-F. The thermogram of pure rutin (Fig. 3.4A) showed a sharp endothermal peak at 242 ºC, and two endothermal peaks were observed at 68 ºC and 165 ºC in the thermogram of SPC (Fig. 3.4B). In the thermogram of physical mixture of rutin:SPC(1:2)(Fig.)theendothermalpeaksofboth rutin andSPCwereobserved.but shifted towards the lower temperatures (233 ºC and 138 ºC, respectively). Thermograms of phytosomes prepared by solvent evaporation method and salting out method (Fig.) were found to be similar to the thermogram of physical mixture of rutin:SPC (1:2). The thermogram of the phytosomes prepared by lyophilization method showed a broad endothermal peak at 158 ºC.

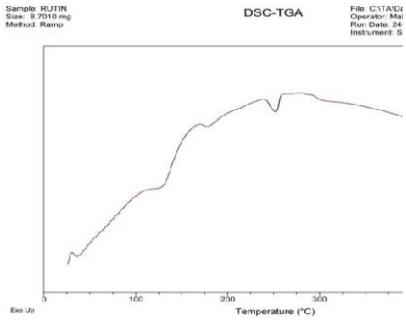

Fig 2: DSC thermogram of pure rutin.

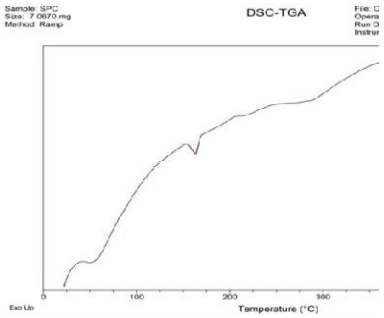

Fig 3: DSC thermogram of SPC.

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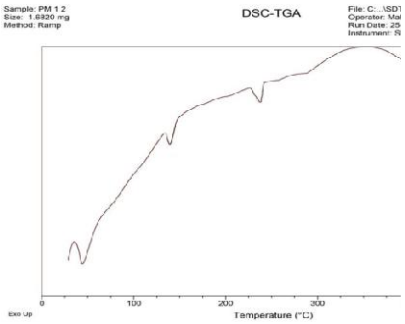

Fig 4:DSC thermogram of rutin:SPC (1:2).

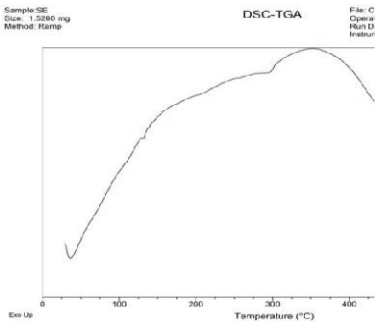

Fig 5:DSC thermogram of rutin phytosomes prepared by solvent evaporation method.

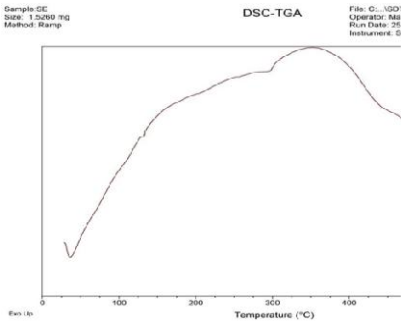

Fig 6 DSC thermogram of Rutin phytosomes prepared by saltingout method.

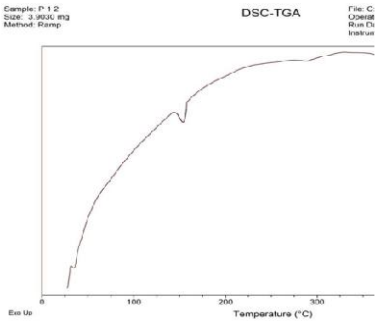

Fig 7: DSC thermogram of rutin phytosomes prepared by lyophilization method.

Fourier transform infrared spectroscopy (FTIR): The IR spectra of the pure rutin, SPC, physical mixture of rutin:SPC (1:2), and rutin phytosomes prepared bythe lyophilization method (Fig. 3.5A-D) were compared, and the characteristic peaks associated with specific functional groups and bonds of the molecule and their presence/ absence were noted in Table 3.3A and B. There was a slight change between the IR spectra of the physical mixture and phytosome in the wavenumber range from 1231 cm^{-1} to 942 cm^{-1} and also broadening of the phenolic (–OH) band of rutin at 3637 $cm^{-1}$was observed in the IR spectrumof rutin phytosomes. The absorption peaks of pure rutin and SPC were present at 1651 cm^{-1} and 2852 cm^{-1}, respectively in the IR spectrum of rutin phytosomes prepared by lyophilization method.

S.NO	Functionalgroup	Reported frequency (cm^{-1})	ObservedFrequency(cm^{-1})		
			SPC	Physical mixture 1:2	Phytosomes1:2 (by lyophilization method)
1	C-H (Alkane, stretch)	3000-2850	2852.81	2852.53	2852.74
2	C=O (Ketone)	1725-1705	1734.73	1736.98	1731.90
3	C=C (Alkene)	1680-1600	1637.71	1651.65	1651.13
4	C-O-C (Ether)	1300-1000	1265.63	1295.22	1296.72
5	P=O (Phosphoricacid)	1320-1140	1231.91	1232.21	1237.95
6	C–C (Alkane)	1450-1400	1431.08	1424.32	1447.36

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Formulation of Rutin Phytosomes and Optimization

Three level factorial design

Std	Run	Factor1 A:RUTIN:SPC (w:w)	Factor2 B:MANNITOL (%w/v)	Response1 ParticleSize(nm)	Response2 DrugContent (% w/w)
4	1	-1	0	455.6	82.46
9	2	1	1	342.5	82.81
7	3	-1	1	488.2	79.93
3	4	1	-1	323.7	85.60
1	5	-1	-1	472.6	80.67
6	6	1	0	301.4	87.83
2	7	0	-1	286.7	90.16
8	8	0	1	298.5	88.37
5	9	0	0	274.2	93.24

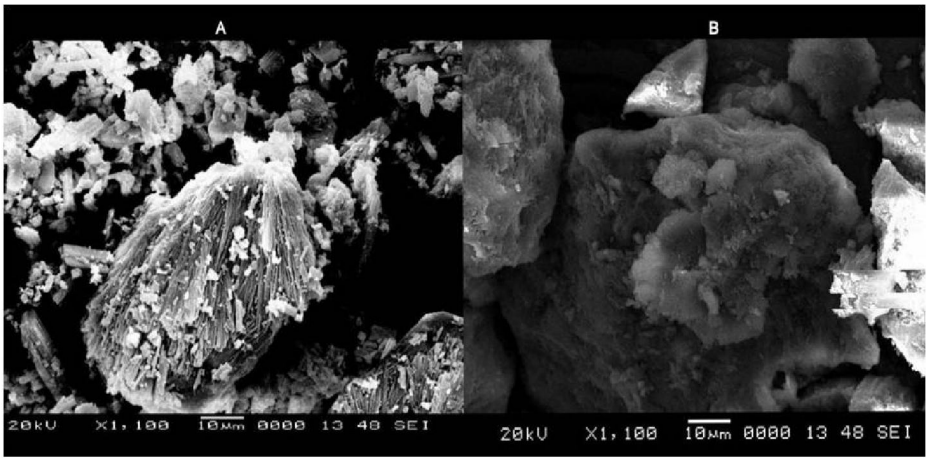
***Invitro* Evaluation of Optimized Rutin Phytosomes**

Average particle size, particle size distribution (PDI) and zeta potential: The PS and ZP obtained for all the rutin phytosome formulations generated by DoE software are given in Table 3.4. The PS, PDI, and ZP ofoptimized rutin phytosomes was foundtobe272.6±2.48nm ,0.376±0.02and-28.2±0.10mV, respectively.

Extent of rutinin corporation in rutin phytosomes (drug content): The drug content of all the rutin phytosome formulations generated by DoE software.The rutin content in the optimized rutin phytosomes was found to be 91.38±0.24%w/w

Surface morphology

Scanning electron microscopy (SEM): The SEM image of the pure rutin revealed the crystalline nature of the drug rutin whereas optimized rutin phytosomes showed the amorphous nature and fluffy, porous and rough surface.



Transmission electron microscopy (TEM): The TEM image of the optimized rutin phytosomes showed well formed, discrete vesicles with no evidence of aggregation or decomposition

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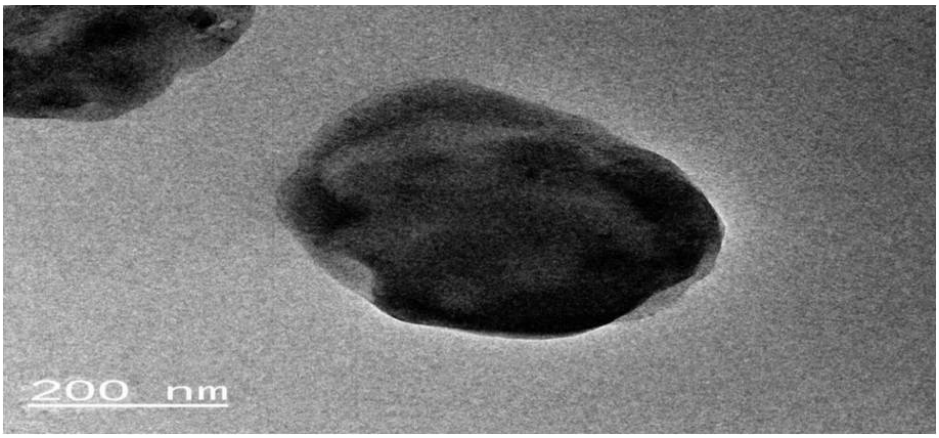

***In-vitro* drug release:** *In vitro* drug release profile ofpure rutin and optimized rutin phytosomes in acidic buffer pH1.2(upto2h) and phosphate buffer pH 7.4 (2-24h). The pure rutin showed1 0.4±0.09% drug release at the end of 2 h in acidic pH 1.2.and 19.13±0.96% drug release at the end of 24 h after reaching maximum value 34.35±0.86 in 12 h in phosphate buffer pH 7.4. Unlike pure rutin, the optimized rutin phytosomes showed high drug release of 74.4±0.99 % at the end of24 h, but in acidic pH the drug release was less than that of the pure rutin.

In-vitro drug release profile of pure rutin and optimized rutin phytosomes in acidic buffer pH 1.2 (upto 2 h) and phosphate buffer pH 7.4 (2-24 h).

Time(h)	PureRutin	Optimizedrutin phytosomes
0	0.00	0.00
2	10.4±0.09	9.7±0.23
4	18.1±0.73	23.5±1.20
6	24.59±0.54	26.8±1.35
8	29.33±0.87	28.6±1.01
10	33.24±0.21	35.4±1.12
12	34.35±0.86	42.6±1.87
14	33.19±0.38	51.5±1.72
16	30.34±0.98	56.7±1.60
18	28.88±0.61	59.8±1.27
20	26.36±1.18	63.7±2.18
22	23.77±0.78	67.1±1.18
24	19.13±0.96	74.4±0.99

Values are mean ± SD (n=6).

In vitro Antioxidant Activity: The percentage inhibition of DPPH at 50 µg/ml concentration by ascorbic acid, pure rutin, and optimized rutin phytosomes was found to be 91.75 ± 1.17%, 29.30 ± 0.71%, and 30.74 ± 1.09%, respectively. The percentage inhibition of DPPH at 50 µg/ml concentration by SPC was negligible.

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Percentage inhibition of DPPH by standard (ascorbic acid), pure rutin, SPC, and optimized rutin phytosomes

Concentration (µg/ml)	%Inhibition of DPPH			
	Standard (Ascorbic acid)	Purerutin	SPC	Optimized rutin phytosomes
10	87.0±1.74	24.19±0.62	4.31±0.99	25.55±1.84
20	87.63±1.81	24.46±1.72	6.88±0.70	25.28±1.24
30	89.15±1.61	24.55±1.24	5.33±0.26	25.91±1.41
40	89.42±0.87	28.58±1.35	6.61±0.38	29.76±0.99
50	91.75±1.17	29.30±0.71	7.22±0.49	30.74±1.09

Values are mean ± SD (n=6).

Estimation of liver marker enzymes

The results of liver function tests are tabulated in Table 3.24. In CCl_4 intoxicated group, theSGPTlevelwasincreasedto120.73±1.59U/l,SGOTlevelwasincreasedto94.26±1.33 U/l, SALP levelwas increased to 224.79±0.71 U/landthetotalbilirubin level was increased to 1.24±0.01 mg/dl in comparison with normal group in which these enzyme levels were found to be 40.98±0.71 U/l, 36.18±1.03 U/l, 146.81±1.05 U/l, and 0.58±0.01 mg/dl, respectively. Further, in the optimized rutin phytosomes treated group these enzyme levels were restored better which was found to be 81.75±0.53 U/l, 70.64±0.53 U/l, 172.49±0.53 U/l, and 0.87±0.01 mg/dl, respectively.

Liver function test after CCl4 induced hepatotoxicity in rats on 8th day (n=6).

Parameters	Group – I (Normal)	Group – II (CCl_4 intoxicated)	Group – III (Rutin treated) (100 mg/kg)	Group – IV (Optimized rutin phytosomes treated) (~100 mg/kg rutin)
SGPT (U/l)	40.98±0.71**	120.73±1.59	93.40±0.69**	81.75±0.53**
SGOT(U/l)	36.18±1.03**	94.26±1.33	81.25±2.97*	70.64±0.53**
SALP(U/l)	146.81±1.05**	224.79±0.71	196.43±0.61*	172.49±0.53**
Total bilirubin (mg/dl)	0.58±0.01**	1.24±0.01	0.96±0.01**	0.87±0.01**

Values are mean±Std.EM.*P<0.05, **P<0.01 (significant with respect to CCl4 treated group).

Here is the entire passage rewritten with **proper spacing, paragraph structure, and readability**, while keeping all scientific values unchanged:

Estimation of Antioxidant Marker Enzymes

The results of the assay of antioxidant marker enzymes in normal, CCl_4-intoxicated, rutin-treated, and optimized rutin phytosomes–treated rat groups

In the **CCl_4-treated group**, the levels of GSH, GPx, GST, GRD, SOD, and catalase were found to be:

GSH: 20.18 ± 0.74 nmol/mg protein

GPx: 186.32 ± 1.39 nmol of NADPH oxidized/min/mg protein

GST: 156.14 ± 1.93 nmol of CDNB conjugate formed/min/mg protein

GRD: 8.39 ± 1.92 nmol of oxidized glutathione (GSSG) utilized/min/mg protein

SOD: 4.37 ± 0.01 U/mg protein

Catalase: 96.57 ± 0.74 U/mg protein

These enzyme levels were significantly decreased compared with the **normal group**, which showed:

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GSH: 46.23 ± 1.29 nmol/mg protein
GPx: 324.47 ± 1.94 nmol of NADPH oxidized/min/mg protein
GST: 292.71 ± 1.68 nmol of CDNB conjugate formed/min/mg protein
GRD: 20.27 ± 0.82 nmol of GSSG utilized/min/mg protein
SOD: 7.28 ± 0.01 U/mg protein
Catalase: 204.76 ± 0.99 U/mg protein

In the optimized rutin phytosomes–treatedgroup, these enzyme levels were better restored toward normal values and were observed as:

GSH: 36.57 ± 0.85 nmol/mg protein
GPx: 269.38 ± 1.22 nmol of NADPH oxidized/min/mg protein
GST: 234.47 ± 1.45 nmol of CDNB conjugate formed/min/mg protein
GRD: 15.39 ± 0.35 nmol of GSSG utilized/min/mg protein
SOD: 6.24 ± 0.02 U/mg protein
Catalase: 164.49 ± 1.01 U/mg protein

Furthermore, the TBARS level (Fig. 3.37) was significantly increased in the CCl_4-intoxicated group (12.89 ± 0.13 nmol MDA/mg protein) compared with the normal group (4.98 ± 0.01 nmol MDA/mg protein). In the optimized rutin phytosomes–treated group, the TBARS level was substantially restored toward normal (9.18 ± 0.10 nmol MDA/mg protein).

Effect of Optimized Rutin Phytosomes on the Levels of GSH, GPx, GST, and GRD in CCl_4-Intoxicated Rats on Day 8 (n = 6)

Parameters	**Group–I** (Normal)	**Group–II** (CCl_4 intoxicated)	**Group – III** (Rutintreated) (100 mg/kg)	**Group – IV** (Optimizedrutinphytosomes treated) (~100mg/kgrutin)
GSH (nmol/mgprotein)	46.23±1.29**	20.18±0.74	31.29±0.63*	36.57±0.85**
GPx (nmol of NADPH oxidized/min/mgprotein)	324.47±1.94**	186.32±1.39	240.09±1.25**	269.38±1.22**
GST (n mol of CDNB conjugate formed/min/mg protein)	292.71±1.68**	156.14±1.93	210.55±1.23	234.47±1.45*
GRD (n mol of oxidized glutathione [GSSG] utilized/min/mg protein)	20.27±0.82**	8.39±1.92	12.45±0.23	15.39±0.35**
SOD (U/mgprotein)	7.28±0.01**	4.37±0.01	4.93±0.04	6.24±0.02**
CAT (U/mgprotein)	204.76±0.99**	96.57±0.74	136.64±1.42*	164.49±1.01**

Histopathological studies

Photomicrographs of the rat liver sections of all the groups are shown in Fig. 3.38. The normal group rat liver section showed the clear nucleus, nucleolus, veins and cytoplasm (Fig. 3.38A), but the rat liver tissues of CCl_4 intoxicated group showed visible damageand degeneration of parenchyma cells, degeneration of liverfatty tissue and the damageof central lobular region (Fig. 3.38B). Optimized rutin phytosomes (~100 mg/kg rutin) pre-treatment better restored the altered histological changes induced by CCl_4 (Fig.3.38D) compared to the same dose of pure rutin pre-treatment.

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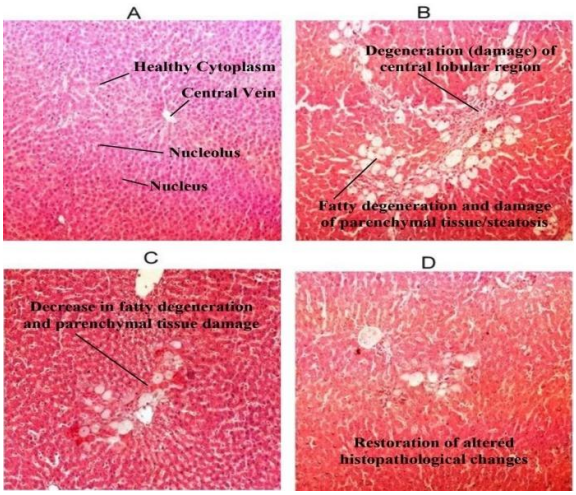

Photomicro graphs of normal group ratliver section (A), CCl_4 intoxicated groupr at liver section(B), rutintreated group (100mg/kg) rat liver section(C) and optimized rutin phytosomes treated group (~100 mg/kg rutin) rat liver section (D).

II. CONCLUSION

Among the three methods evaluated—solvent evaporation, salting out, and lyophilization—only the lyophilization method successfully produced a stable and dry rutin–phospholipid complex. Solvent evaporation failed due to the poor solubility of rutin in the volatile solvent system and the high boiling point of DMSO, while the salting out method yielded only a weak, unstable complex. DSC analysis further confirmed that lyophilization resulted in proper complex formation, making it the most suitable technique for preparing rutin phytosomes. Optimization results showed that a quadratic model best described both particle size and drug content. In both responses, the rutin:SPC ratio (factor A) was the major influencing factor, while mannitol (factor B) showed minimal effect and no significant interaction (AB) was observed. Particle size decreased with increasing rutin:SPC ratio up to an optimum level and increased thereafter. Similarly, drug content increased initially with higher rutin:SPC ratios and then declined. Model fit, ANOVA significance, linear predicted–actual plots, and contour/surface plots all confirmed the reliability of the quadratic model for predicting both responses. The optimized rutin phytosomes exhibited suitable particle size, low PDI, and a stable negative zeta potential, confirming good physical stability for oral delivery. High drug content indicated efficient incorporation of rutin into the phytosomal complex. DSC and FTIR analyses supported successful complex formation through weak interactions between rutin and phospholipids. SEM and TEM images further confirmed structural transformation and formation of uniform, stable vesicular nanostructures. Overall, the characterization results validated the successful development of stable and well-formed rutin phytosomes. The optimized rutin phytosomes retained strong in vitro antioxidant activity, confirming that complexation with SPC did not affect rutin's free radical–scavenging properties. In vivo, CCl_4 caused marked hepatic damage, as shown by elevated liver enzymes and reduced antioxidant markers. Treatment with optimized rutin phytosomes significantly restored liver function markers, antioxidant enzyme levels, and TBARS values toward normal more effectively than pure rutin. Histopathological observations further confirmed superior hepatoprotection, with optimized phytosomes showing near-normal liver architecture. Overall, the optimized rutin phytosomes provided enhanced antioxidant and hepatoprotective effects compared to free rutin.

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