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RUTIN PHYTOSOMES AS A NOVEL DRUG-DELIVERY SYSTEM: FORMULATION, CHARACTERIZATION, AND ANTI-INFLAMMATORY EVALUATION

Article History:

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Abstract: Background: Phytosomes are novel delivery systems known to enhance the pharmacological performance of phytoconstituents. The present study evaluates the pharmacological activity of rutin phytosomes with particular emphasis on their anti-inflammatory potential. Rutin, a flavonoid possessing antioxidant and anti-inflammatory properties, exhibits limited therapeutic effectiveness due to poor bioavailability. The anti-inflammatory activity of rutin phytosomes was assessed using the carrageenan-induced paw edema model, where a significant reduction in edema was observed compared to free rutin. Additional in vivo studies demonstrated marked antioxidant activity and hepatoprotective effects against paracetamol-induced liver injury, supporting the overall pharmacological efficacy of the phytosomal system. Pharmacokinetic evaluation revealed improved oral bioavailability, contributing to enhanced therapeutic outcomes. The findings of this study indicate that rutin phytosomes exhibit significant anti-inflammatory activity compared with control, along with improved pharmacokinetic performance, highlighting their potential as an effective delivery system for rutin. and improved pharmacological performance, highlighting their potential as an effective therapeutic approach for inflammatory conditions.

Keywords: Phytosomes, Rutin, Bioavailability, Antioxidant, Hepatoprotective, Drug Delivery System, Nanocarriers, Pharmacokinetics, Anti-inflammatory.

INTRODUCTION

The pharmacological activity assessment of rutin phytosomes, particularly for anti-inflammatory action, involves a multi-faceted approach. Preclinical studies have demonstrated that rutin exerts its anti-inflammatory effects through several mechanisms, including inhibition of pro-inflammatory cytokines, reduction of oxidative stress, and modulation of the NF-κB signaling pathway (Singh et al., 2020). Encapsulation into phytosomes enhances these effects by ensuring a sustained release profile and greater systemic availability. Additionally, phytosomal formulation has shown potential to protect rutin from enzymatic degradation, further enhancing its therapeutic efficacy (Roy et al., 2023).

The anti-inflammatory potential of rutin phytosomes can be effectively evaluated using both in vitro and in vivo models. In vitro studies, such as inhibition of nitric oxide production and cyclooxygenase (COX) enzyme assays, provide an initial indication of anti-inflammatory activity. In vivo models, such as carrageenan-induced paw edema and cotton pellet granuloma, offer insights into the systemic efficacy of rutin phytosomes. Studies have consistently shown that rutin phytosomes exhibit significant inhibition of inflammatory markers, often comparable to standard NSAIDs (Verma et al., 2023).

Moreover, the pharmacokinetic profile of rutin phytosomes has been extensively studied, revealing enhanced oral bioavailability, prolonged plasma retention, and improved pharmacodynamic responses compared to unformulated rutin. These advantages highlight the potential of phytosomes as a superior delivery platform for natural anti-inflammatory agents (Kumar et al., 2022).

MATERIALS AND METHODS

Animals were randomly allocated to experimental groups using a simple randomization procedure. Investigators responsible for outcome assessment were blinded to treatment allocation during data collection and analysis. No animals were excluded from analysis, and no formal outlier removal was performed.

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), ITM University, Gwalior, Madhya Pradesh, in accordance with CPCSEA guidelines, Government of India (IAEC Approval No. ITM/IAEC/2023/031, dated 13/04/2023; CPCSEA Registration No. 1894/PO/Re/S/16/CPCSEA**).**

Invitroantioxidant activity

The free radical scavenging activity of pure rutin, Soybean Phosphatidylcholine, and optimized rutin phytosomes was measured, and compared with the activity of standard (Ascorbic acid) using a stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) as per the method described by Jamuna et al., with slight modifications. The 0.1 mM solution of DPPH in methanol was prepared, and from this, 1.55 ml was added to 3.5 ml methanolic solution of pure rutin, SPC, and optimized rutin phytosomes of different concentrations ranged from 10-50 µg/ml. The absorbance of the standard and the samples was measured at 517 nm using Elisa reader.(Kumbhar, S et.al. 2024) All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC), ITM University, Gwalior M.P., in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. IAEC approval number: ITM/IAEC/2023/031, dated 13/04/2023. CPCSEA registration number: 1894/PO/Re/S/16/CCSEA

Formulation and Characterization of Rutin Phytosomes

Materials

Rutin (≥98% purity) and soybean phosphatidylcholine (SPC) were used for phytosome preparation. Ethanol and methanol of analytical grade were employed as solvents. All other chemicals were of analytical grade and used as received.

Preparation of Rutin Phytosomes

Rutin phytosomes were prepared by the solvent evaporation method. Accurately weighed rutin and SPC were taken in molar ratios of 1:1, 1:2, and 1:3 and dissolved in ethanol to obtain a clear solution. The solution was refluxed at 40 ± 2 °C for 2 h to allow complex formation. The solvent was removed under reduced pressure using a rotary evaporator, and the resulting thin film was dried overnight under vacuum. The dried mass was pulverized, sieved (100 mesh), and stored in a desiccator until further use. (Barani, M, et.al., 2021)

Optimization of Formulation

Optimization was carried out based on encapsulation efficiency, particle size, and in vitro drug release profile. Among the tested formulations, the 1:2 rutin:SPC ratio exhibited higher encapsulation efficiency, suitable particle size, and sustained drug release, and was therefore selected as the optimized formulation.

Particle Size and Polydispersity Index

The mean particle size, polydispersity index (PDI), and zeta potential of the optimized rutin phytosomes were determined using dynamic light scattering. Measurements were performed in triplicate (n = 3) for two independently prepared batches. Results are expressed as mean ± standard deviation (SD). The optimized formulation exhibited a nanometric particle size with low PDI (< 0.3), indicating uniform size distribution and good physical stability.(Khanh NV et al.2018)

Zeta Potential

Zeta potential was measured to assess the physical stability of the formulation. The optimized rutin phytosomes exhibited a negative zeta potential, suggesting good stability due to electrostatic repulsion between particles.

Morphological Analysis

The surface morphology of optimized rutin

phytosomes was examined using electron microscopy (TEM/SEM). Representative micrographs with appropriate scale bars revealed discrete, spherical vesicular structures with smooth surfaces and no visible aggregation, confirming successful phytosome formation.

Encapsulation Efficiency and Drug Loading

Encapsulation efficiency (EE%) was determined by separating unentrapped rutin and quantifying the drug spectrophotometrically. Encapsulation efficiency (EE%) and drug loading (%) were determined in triplicate (n = 3) for the optimized formulation and for one independently prepared batch. The optimized rutin phytosomes demonstrated high encapsulation efficiency (>85%) and consistent drug loading, confirming effective complexation and formulation reproducibility.

The optimized formulation showed high encapsulation efficiency (>85%), indicating effective complexation of rutin with phospholipid. Drug loading was found to be consistent with the composition of the phytosomal system

FTIR Analysis

FTIR spectra of pure rutin, soybean phosphatidylcholine, and optimized rutin phytosomes were recorded and overlaid to evaluate drug–excipient interactions. Characteristic peaks of rutin were retained in the phytosomal formulation with minor shifts and peak broadening, indicating hydrogen bonding and physical complex formation rather than chemical incompatibility. These spectral changes confirm successful rutin–phospholipid complexation.

In Vitro Drug Release Study

The in vitro drug release of optimized rutin phytosomes was evaluated using a dialysis membrane diffusion method. A pre-soaked dialysis membrane (molecular weight cut-off 12,000–14,000 Da) was used as the diffusion barrier. An accurately weighed quantity of optimized rutin phytosomes equivalent to 100 mg of rutin was dispersed in 5 mL of release medium and placed inside the dialysis membrane, which was securely tied at both ends.

The dialysis bag was immersed in 900 mL of phosphate buffer (pH 6.8) containing 0.5% w/v Tween 80 as the dissolution medium to maintain sink conditions. The study was carried out using a USP dissolution apparatus II (paddle method) operated at 50 rpm and 37 ± 0.5 °C.

At predetermined time intervals (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h), 5 mL samples were withdrawn and replaced with an equal volume of fresh medium maintained at the same temperature. The samples were filtered and analyzed spectrophotometrically at the predetermined λmax of rutin. The cumulative percentage drug release was calculated and plotted as a function of time.

All experiments were performed in triplicate (n = 3), and results are expressed as mean ± SD. Sink conditions were maintained by the use of phosphate buffer (pH 6.8) containing 0.5% w/v Tween 80 and a dissolution volume of 900 mL, ensuring that the concentration of released drug remained below its saturation solubility throughout the study.

In vivo Evaluation of Optimized Rutin Phytosomes

A. In vivo hepato protective (Liver function test)and antioxidant activity studies Dosing

The adult male Wistar rats were divided into 4 groups of 6 animals in each. Group I (Normal), animals were treated with distilled water with1%v/v Tween 20 per os for 7days. Group II Paracetamol-induced hepatotoxicity model (CCl4 intoxicated), animals were treated with distilled water with1 %v/v Tween 20 p.o. for 7 days, and an paracetamol dosing equal mixture of CCl4, and olive oil (50 %v/v, 5 ml/kg) as a single dose i.p. on 7thday. Group III (Rutin treated), animals were treated with rutin suspension in distilled water (100 mg/kg) with 1 %v/v Tween 20 per day p.o. for seven days, and on 7th day, an equal mixture of CCl4, and olive oil paracetamol dosing (50 %v/v, 5 ml/kg) as a single dose i.p. Group IV(Optimized rutin phytosomes treated) animals were treated with optimized rutin phytosomes in distilled water (~100 mg/kg rutin) with 1 %v/v Tween 20 per day p.o. for seven days, and on 7th day, an paracetamol dosing equal mixture of CCl4, and olive oil(50 % v/v, 5 ml/kg) as a single dose i.p.

Estimation of liver marker enzymes

To evaluate the effect of pure rutin and optimized rutin phytosomes on rat liver function, liver function test (LFT) was carried out by quantitative determination of liver marker enzymes. The LFT was performed by quantitative determination of serum glutamate pyruvate transaminase (SGPT) also known as Alanine transaminase (ALT), serum glutamate oxaloacetate transaminase (SGOT) and total bilirubin.

1. EstimationofALT (SGPT)

In vitro quantitative determination of ALT in rat serum was carried out by modified 'International Federation of Clinical Chemistry and Laboratory Medicine' (IFCC) method.

Reagent composition

The ALT kitcontains2reagents, R1andR2.

R1: Tris buffer pH 7.5 (100 mmol/l), L-alanine (500 mmol/l), and lactate dehydrogenase (LDH) (≥1200 U/l).

R2:α-Ketoglutarate(16mmol/l)and nicotinamide adenine di nucleotide-reduced(NADH) (0.18 mmol/l).

To prepare working reagent, 4 parts of R1 was mixed with 1 part of R2. Then, to 100 µl of serum sample,

1000 µl of working reagent was added and mixed well. After 1, 2, and 3 min, absorbance was measured at 340 nm and 37 ºC using a semiautomatic analyzer. The ALT activity was calculated as per the following formula, ALT activity (U/L) =ΔA/min X F. Where, F is 1746.

2. Estimation of AST(SGOT)

In vitro quantitative determination of AST in rat serum was carried out by modified IFCC method

Reagent composition

The AST kit contains 2 reagents, R1 and R2.
R1: Tris buffer pH 7.8 (80 mmol/l), L-aspartate (240 mmol/l), malate dehydrogenase (MDH) (≥600 U/l) and LDH (≥600 U/l).
R2:α-Ketoglutarate(12mmol/l)
andNADH(0.24mmol/l).
To prepare working reagent, 4 parts of R1 was mixed with 1 part of R2. Then, to 100 µl of serum sample, 1000 µl of working reagent was added and mixed well. After 1, 2, and 3 min, absorbance was measured at 340 nm and 37 ºC using a semiautomatic analyzer. The AST activity was calculated as per the following formula, AST activity (U/L) = ΔA/minX F. Where, F is 1746.

3. Estimation of total bilirubin

In vitro quantitative determination of total bilirubin in rat serum was carried out by Malloy Evelyn modified method.

Reagentcomposition

The bilirubin kit contains3reagentsR1,R2,andR3.
R1: DMSO (>4 mol/l)
R2:Sulfanilicacid(28.9mmol/l)
R3: Sodium nitrite (43 mmol/l)
For total bilirubin,
Blank (A1):750 µl of R1 was added to 50 µl of serum sample and mixed well.
Sample (A2): 750 µl of R1 and 25 µl R3 were added to 50µl of serum sample and mixed. After mixing well, the bland and sample solutions were incubated for 5 min at 37 ºC, and absorbance was taken at 546 nm and37ºC using a semi automatic analyzer.The bilirubin content was calculated as per the following formula, Bilirubin content (mg/dl) =(A2–A1) X F. Where, F is 16.7.

Estimation of antioxidant marker enzymes

The antioxidant potential of pure rutin and optimized rutin phytosomes was assessed by the quantitative estimation of serum antioxidant marker enzymes. Liver homogenate supernatant was then assayed for reduced glutathione (GSH),superoxide dismutase (SOD), catalase (CAT), and thiobarbituric acid reactive substances (TBARS) by standard procedures.

1. Estimation of reduced glutathione(GSH)

The liver tissue homogenate (0.5 ml) was precipitated with 2 ml of 5 % TCA and centrifuged at 3000 rpm for 10 min using a compact cooling centrifuge. To 1 ml of the supernatant, 0.3 ml phosphate buffer and 0.5 ml Ellman's reagent were added and incubated for 15 min at room temperature. After incubation,the absorbance of the product was measured at λ max 420 nm using a UV-VIS spectrophotometer. A series of standards (10-50 µg/mL) were treated similarly along with the blank containing 1 ml distilled water. The GSH content was expressed as nmol/mg protein.

2. Estimation of super oxided isomutase (SOD)

Liver tissue homogenate (0.1 ml), ethanol (0.75 ml), chloroform chilled in ice (0.15 ml) were taken in a test tube and centrifuged at 3000rpmfor 10 min using a compact cooling centrifuge. To the 0.5 ml of supernatant, EDTA solution (0.5 ml), and carbonate-bicarbonate buffer(1ml)were added and mixed well.The reaction was initiated by the addition of freshly prepared epinephrine solution(0.5 ml)and the change in absorbance at λmax 450 nm was measured using a UV-VIS spectrophotometer at 0, 1, and 2 min. Blank was prepared by replacing the liver tissue homogenate with 0.1 ml of distilled water. The SOD activity was expressed as U/mg protein, i.e. one unit of SOD activity is the amount of protein that is required to give 50 % inhibition of epinephrine auto-oxidation.

3. Estimationofcatalase(CAT)

Liver tissue homogenate (25 µl) was added to the phosphate buffer (1 ml) and mixed well. The enzyme reaction was initiated by the addition of H2O2 solution (250 µl). The decrease in the absorbance was measured at λmax 240 nm using UV-VIS spectrophotometer for 3 min with 30 sec time intervals. Blank was prepared by replacing the H2O2 solution with 250 µl of distilled water. The CAT activity was expressed as U/mg protein, i.e. nmols of H2O2 decomposed/min/mg protein.

Histopatho logical studies

Immediately after scarifying, livers from the animals were dissected and preserved in neutral buffered formalin (10%). Livers were sectioned and stained with haematoxylin and eosin and examined under the digital microscope.

Oral bioavailability studies

Oral bioavailability study of optimized rutin phytosomes was conducted in12 male albino Wistar rats weighing 180-200 g divided into two groups (n=6). Both the group animals were fasted for 12 h with free access to water. Group I and Group II animals received a single dose of pure rutin (100 mg/kg rutin) and optimized rutin phytosomes (~100 mg/kg rutin), respectively. At predetermined time intervals, the blood was collected from the retro-

orbital plexus of animals under light ether anaesthesia into serum separator tubes (5 ml, Red)and centrifuged at 3000rpmfor10 min using a compact cooling centrifuge. The serum was stored at -20 ºC in a deep freezer prior to quantification of rutin in serum by HPLC method.

Quantification of rutin in rat serum by HPLC method

The quantification of rutin in rat serum was performed using an HPLC method developed and validated by Maiti et al., with slight modifications. Briefly, frozen serum samples were thawed at room temperature, and 1 mL of serum sample was added to 5 mL of methanol in a 10 mL volumetric flask. The contents were vortexed and heated at 75 °C for 0.5 h. Methanol was added to make up the volume to 10 mL, followed by centrifugation at 4000 rpm for 15 min using a compact cooling centrifuge. The supernatant was filtered through a 0.2 µm syringe filter, and 20 µL of the filtered sample was subjected to HPLC analysis using a C-18 column (column temperature: 37 °C) and a UV-PDA detector (SPD-M20A) set at λmax 360 nm. The mobile phase consisted of acetonitrile and orthophosphoric acid (45:55, v/v) and was run at a flow rate of 1 mL/min. The concentration of rutin was determined from a standard calibration curve by plotting peak area against concentration. The regression equation was found to be Y = 39267X − 8602 over a linearity range of 1–10 µg/mL. The retention time of rutin was 4.5 ± 0.03 min, and the correlation coefficient (r^2) was 0.999.

Pharmacokineticparameters

The pharmacokinetic parameters such as maximum plasma concentration (C_{max}) and the time required to reach maximum concentration (T_{max}) of optimized rutin phytosomes were determined based on the plasma concentration-time curve. Other parameters such as area under plasma concentration-time curve from zero to time of the final measured sample (AUC_{0-t}) and area under plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$), elimination half-life ($t_{1/2el}$), elimination rate constant (K_{el}), clearance (cl), and volume of distribution (V_d) were calculated using Microsoft Excel Add-In software PK Solver® and compared to the parameters obtained for free rutin. Relative bioavailability (F) is the ratio of the total amount of drug absorbed from optimized rutin phytosomes to the total amount of drug absorbed from pure rutin. Amount of drug absorbed (A_{max}) from a dosage form is a function of V_d, K_{el}, and $AUC_{0-\infty}$. Therefore, the relative bioavailability (F) was calculated by using the following formula,

$$F = \frac{(VdXKelXAUC0\text{-}\omega)\ optimizedrutinphytosomes}{(VdXKelXAUC0\text{-}\omega)\ purerutin} x\ 100$$

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Stability Study

Stability study was carried out on the optimized rutin phytosomes as per ICH guidelines. The dry optimized rutin phytosomes were packed in aluminium foil and subjected to stability studyat 25±2 ºC/ 60±5 % RH and accelerated condition at 40±2 ºC/ 75±5 % RH as per new ICH guidelines. Optimized rutin phytosomes were evaluated for drug content and *in vitro* drug release on 1st, 30th, 60th, and 90th day.

Stability studies of optimized rutin phytosomes were conducted in accordance with International Council for Harmonisation (ICH) Q1A(R2) guidelines. The dried phytosomal formulation was packed in aluminium foil-lined, airtight containers to protect the formulation from moisture, light, and oxidative degradation.

The samples were stored under:

Long-term conditions: 25 ± 2 °C / 60 ± 5% RH

Accelerated conditions: 40 ± 2 °C / 75 ± 5% RH

Samples were withdrawn at 1, 30, 60, and 90 days and evaluated for drug content and in vitro drug release profile using the methods described in this section.

The selected ICH conditions and packaging were justified to simulate normal and stressed storage environments and to ensure the physical and chemical stability of the phospholipid-based phytosomal system

Anti-Inflammatory Activity of Rutin Loaded Phytosomes

Animals

Swiss albino mice weighing 20-25 gm, wistar rats weighing 150- 200 gm were used for this study. The animals were obtained from animal house. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°c and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow. All the experimental procedures are protocols used in this study were reviewed by Institutional Animal Ethics Committee.

Acute Toxicity Studies

Acute toxicity was performed according to OECD 423 guidelines. Selected albino rats were used for toxicity studies. The animals were divided into five groups of three in each. The animals were fasting overnight before the acute experimental procedure. The Rutin Loaded Phytosomes was administered orally to rats in doses graduated as 5, 50, 100, 1000 and 2000 mg / kg of body weight. Immediately after dosing, the animals were observed continuously for the first four hours and close behavioural changes were observed to detect hyperactivity, ataxia, convulsions, salivation, tremors, diarrhoea, lethargy

and sleep. They were then observed for up to 14 days after drug administration to determine mortality, if present. One tenth and one fifth of the maximum tolerated dose (200 and 400 mg / kg, body weight) of the Rutin Loaded Phytosomes selected to evaluate studies of anti-inflammatory activity in rats.

Carrageenan-Induced Paw Edema in Rats

For this experiment, the rats (120-150g) were divided into four groups (n=6). The group I received 0.5% CMC (10ml/kg), while the Group II received Diclofenac Sodium(10mg/kg). The Group III and IV were treated orally with the Rutin Loaded Phytosomes at a dose of 200 mg/kg and 400 mg/kg orally.

GroupI : Normal Control (CMC)
GroupII : Diclofenac Sodium(10 mg/kg)
GroupIII : Test Drug I [Rutin Loaded Phytosomes (200 mg/kg)]
GroupIV : Test Drug II [Rutin Loaded Phytosomes (400 mg/kg)]

Acute inflammation was produced by injecting 0.1 ml of 1% (w/v) Carrageenan suspension into the sub planter region of the right hind paw of the rats. The animals were pretreated with the drug 1hour before the administration of carrageenan. The paw thickness was measured at 1, 2, 3 and 4 h after carrageenan injection by using digital vernier calipers.

RESULTS AND DISCUSSION

In vivo Evaluation of Optimized Rutin Phytosomes

In Vitro Antioxidant Activity (DPPH Assay)

The free radical scavenging activity of pure rutin, soybean phosphatidylcholine (SPC), and optimized rutin phytosomes was evaluated using the DPPH assay and compared with ascorbic acid as a standard. Optimized rutin phytosomes exhibited significantly higher DPPH radical scavenging activity compared with pure rutin at equivalent concentrations (10–50 µg/mL).

SPC alone showed negligible scavenging activity, confirming that the observed antioxidant effect was attributable to rutin. The enhanced antioxidant activity of rutin phytosomes may be attributed to improved solubilization and availability of rutin in the phytosomal system. Statistical analysis demonstrated a significant difference ($P < 0.05$) between pure rutin and optimized rutin phytosomes.

Table 1: In vitro DPPH radical scavenging activity of rutin formulations

Treatment	Concentration (µg/mL)	% DPPH scavenging (Mean ± SEM)
Ascorbic acid (Standard)	10	41.26 ± 1.18
	25	63.84 ± 1.42
	50	82.17 ± 1.36
Pure rutin	10	28.43 ± 0.97
	25	44.62 ± 1.21
	50	61.35 ± 1.09
Optimized rutin phytosomes	10	36.91 ± 1.05*
	25	57.48 ± 1.33*
	50	75.26 ± 1.27*
Soybean phosphatidyl choline (SPC)	50	6.82 ± 0.64

Values are expressed as mean ± SEM (n = 3). $P < 0.05$ compared with pure rutin (one-way ANOVA followed by Dunnett's test).

In vivo Hepatoprotective (Liver Function Test) and Antioxidant Activity Studies

Estimation of Liver Marker Enzymes

In the paracetamol-intoxicated group, the SGPT level increased to 120.73 ± 1.59 U/L, the SGOT level increased to 94.26 ± 1.33 U/L, and the total bilirubin level increased to 1.24 ± 0.01 mg/dL, compared with the normal group in which these enzyme levels were 40.98 ± 0.71 U/L, 36.18 ± 1.03 U/L, and 0.58 ± 0.01 mg/dL, respectively.

Further, in the optimized rutin phytosome-treated group, these enzyme levels were restored more effectively and were found to be 81.75 ± 0.53 U/L, 70.64 ± 0.53 U/L, and 0.87 ± 0.01 mg/dL, respectively.

Table 2: Liver function test after Paracetamol induced hepato toxicity in rats on 8th day(n=6).

Parameters	Group–I (Normal)	Group –II (CCl_4 intoxicated) Paracetamol-intoxicated group	Group – III(Rutin treated)(100 mg/kg)	Group–IV(Optimized rutinphytosomes treated)(~100mg/kgrutin)
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**
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 Paracetamol treated group).

Estimation of Antioxidant Marker Enzymes

The results of the assay of antioxidant marker enzymes in normal, paracetamol-intoxicated, rutin-treated, and optimized rutin phytosome-treated rats are presented. In the paracetamol-intoxicated group, the levels of GSH, SOD, and catalase were 20.18 ± 0.74 nmol/mg protein, 4.37 ± 0.01 U/mg protein, and 96.57 ± 0.74 U/mg protein, respectively. These levels were significantly decreased compared with the normal group, which exhibited enzyme levels of 46.23 ± 1.29 nmol/mg protein, 7.28 ± 0.01 U/mg protein, and 204.76 ± 0.99 U/mg protein, respectively. In the optimized rutin phytosome-treated group, these enzyme levels were better restored toward normal and were found to be 36.57 ± 0.85 nmol/mg protein, 6.24 ± 0.02 U/mg protein, and 164.49 ± 1.01 U/mg protein, respectively. Further, the TBARS level was increased in the paracetamol-intoxicated group (12.89 ± 0.13 nmol of MDA/mg protein) compared with the normal group (4.98 ± 0.01 nmol of MDA/mg protein). In the optimized rutin phytosome-treated group, the TBARS level was better restored toward normal (9.18 ± 0.10 nmol of MDA/mg protein).

Table 3: Effect of optimized rutin phytosomes on the levels of GSH, SOD and CAT in Paracetamol in toxicated rats on day8(n=6)

Parameters	Group–I (Normal)	Group– II (Paracetamol intoxicated)	Group–III (Rutin treated) (100mg/kg)	Group –IV (Optimized rutin phytosomes treated) (~100mg/kgrutin)
GSH (nmol/mg protein)	46.23±1.29**	20.18±0.74	31.29±0.63*	36.57±0.85**
SOD (U/mg protein)	7.28±0.01**	4.37±0.01	4.93±0.04	6.24±0.02**
CAT (U/mg protein)	204.76±0.99**	96.57±0.74	136.64±1.42*	164.49±1.01**

Valuesaremean±Std.EM.*P<0.05,
**P<0.01(significant with respect to Paracetamol-intoxicated group CCl_4 treated group)

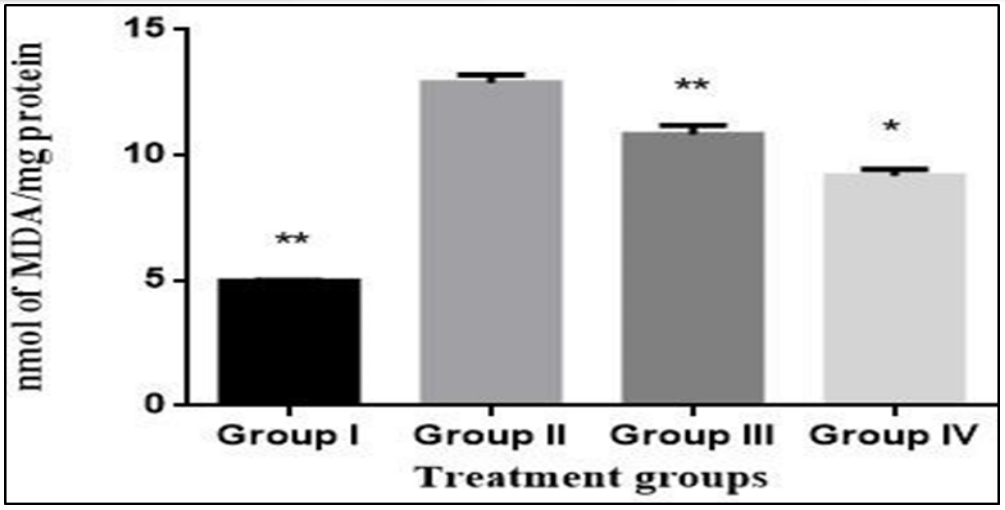

Figure 1: Effect of optimized rutin phytosomes on TBARS

Group I: Normal
Group II: Paracetamol-intoxicated
Group III: Rutin-treated (100 mg/kg)
Group IV: Optimized rutin phytosome-treated (~100 mg/kg rutin)
Values are expressed as mean ± SEM (n = 6).
*P < 0.05, **P < 0.01 (significant with respect to the paracetamol-treated group).

Histopathological Studies

Photomicrographs of rat liver sections from all groups were examined. The normal group liver section showed a clear nucleus, nucleolus, veins, and cytoplasm (Fig. 2A). In contrast, the liver tissues of the CCl_4-intoxicated group Paracetamol-intoxicated group showed visible damage and degeneration of parenchymal cells, degeneration of fatty liver tissue, and damage to the central lobular region.

Pre-treatment with optimized rutin phytosomes (~100 mg/kg rutin) better restored the altered histological changes induced by paracetamol (Fig. 2) compared with pre-treatment using the same dose of pure rutin.

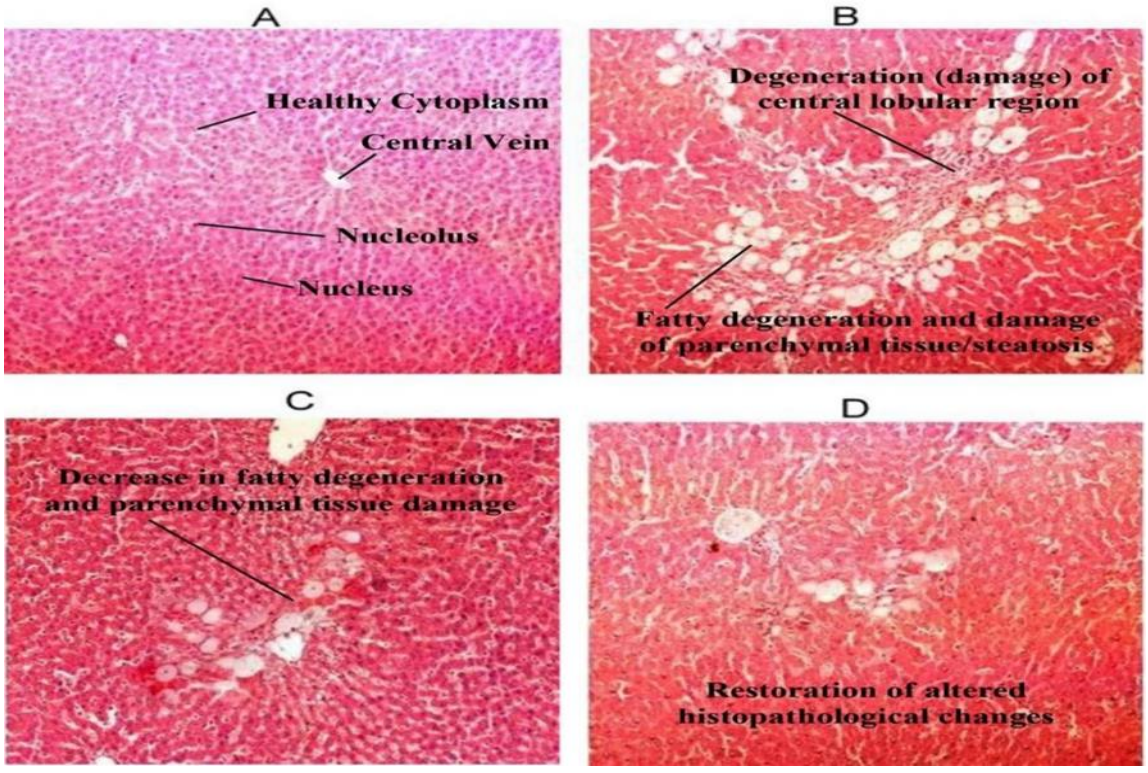

Figure 2: Photo micrographs of normal group rat liver section (A) Paracetamol in toxicated group rat liver section (B) rutin treated group (100 mg/kg) rat liver section (C) and optimized phytosomes treated group(~100mg/kg rutin) rat liver section(D)

Oral Bioavailability Studies

Quantification of Rutin in Rat Serum by HPLC Method

The mean plasma concentration–time curves of pure rutin and optimized rutin phytosomes were obtained by quantification of rutin in the serum of rats treated orally with pure rutin (100 mg/kg, p.o.) and optimized rutin phytosomes (~100 mg/kg rutin). The chromatograms of pure rutin and optimized rutin phytosomes were collected from serum samples at 4 h and 6 h, respectively.

Pure rutin attained a maximum serum concentration (C_{max}) of 6.47 ± 0.12 µg/mL. at 4.0 h, whereas optimized rutin phytosomes showed a higher maximum serum concentration of 9.95 ± 0.57 µg/mL at 6.0 h.

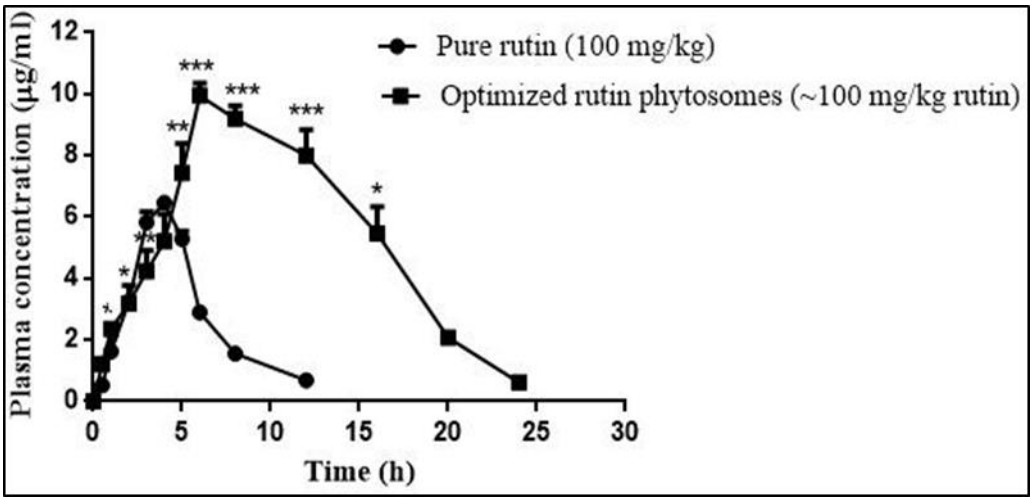

Figure 3: Mean plasma concentration-time profile of rutin (100 mg/kg, p.o.) andoptimized rutin phytosomes (~100 mg/kg of rutin, p.o.).

Values are mean ± Std. EM(n=6). *P<0.05, **P<0.001 and ***P<0.0001(significant with respect to pure rutin treatedgroup)

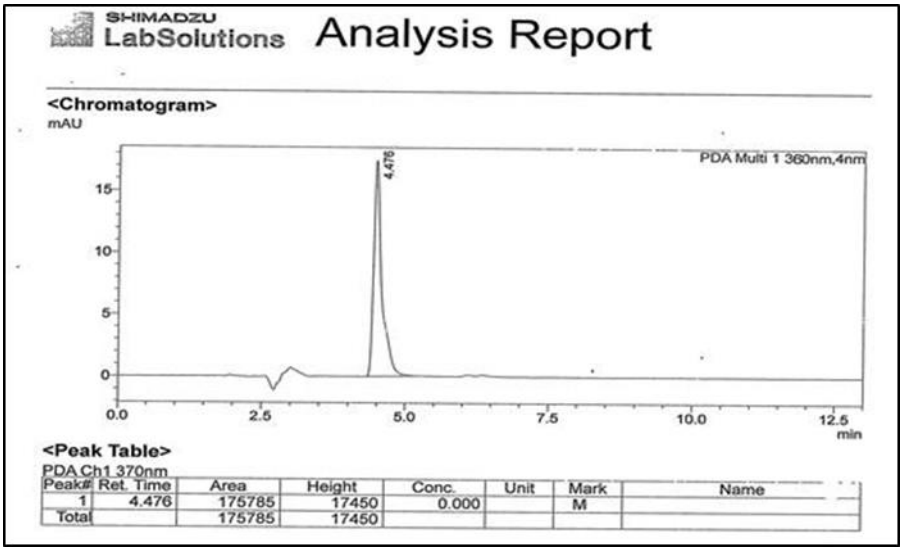

Figure 4:The chromatogram of rutin from pure rutin treated rat serum collected at 4h

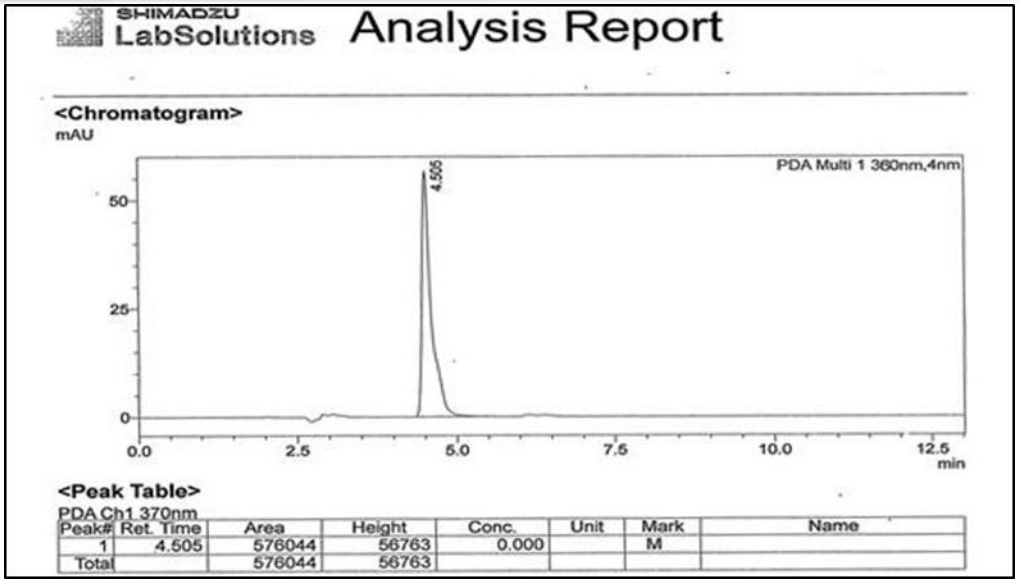

Figure 5: The chromatogram of rutin from optimized rutin phytosomes treated rat serum collected at 6h

Pharmacokinetic parameters

The pharmacokinetic parameters were calculated from the plasma concentration–time curves using the computer software PK Solver®. The C_{max}, T_{max}, and elimination half-life values were increased to 9.95 ± 0.57 µg/mL, 6.0 h, and 115.61 ± 4.69 µg·mL^{-1}·h, respectively, in the serum of animals treated with optimized rutin phytosomes, compared with animals treated with pure rutin, in which these values were 6.47 ± 0.12 µg/mL, 4.0 h, and 39.51 ± 1.16 µg·mL^{-1}·h, respectively.

The elimination rate constant, clearance, and volume of distribution values were decreased in the optimized rutin phytosome-treated rat serum and were found to be 0.17 ± 0.001 h^{-1}, 0.14 ± 0.002 L/h, and 0.98 ± 0.01 L, respectively, in comparison with animals treated with pure rutin, which showed values of 0.41 ± 0.004 h^{-1}, 0.56 ± 0.002 L/h, and 1.32 ± 0.26 L, respectively.

The optimized rutin phytosomes remained in the body for an extended period and exhibited a high relative bioavailability (F) of 86.23 ± 0.46%.Blood samples were collected from the retro-orbital plexus at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, and 24 h following oral administration of pure rutin (100 mg/kg) and optimized rutin phytosomes (equivalent to 100 mg/kg rutin).

Pharmacokinetic analysis was performed using noncompartmental analysis (NCA) with PKSolver® (Microsoft Excel add-in). The parameters Cmax and Tmax were obtained directly from experimental data. The area under the plasma concentration–time curve from 0 to last measurable time ($AUC_{0–t}$) was calculated using the linear trapezoidal method, and $AUC_{0–\infty}$ was calculated as $AUC_{0–t}$ + (Ct/Kel). The elimination rate constant (Kel) was obtained from the terminal log-linear phase, and the elimination half-life (t½) was calculated as 0.693/Kel. Plasma concentrations below the limit of quantification (BLQ) were considered zero for pre-dose samples and were excluded from terminal phase calculations.

Blood samples were collected at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12, and 24 h post-dosing. The HPLC method was validated over a defined linear range (LLOQ–ULOQ). Pre-dose and post-dose concentrations below the limit of quantification (BLQ) were treated as zero and excluded from terminal phase calculations. The terminal elimination phase was identified by log-linear regression of the final quantifiable concentration–time points.

Table 4: Pharmacokinetic parameters of pure rutin (100 mg/kg) and optimized rutin phytosomes(~100 mg/kgrutin)inrats

Pharmacokineticparameters	Purerutin	Optimized rutinphytosomes
C_{max}(µgml^{-1})	6.47±0.12	9.95±0.57
T_{max}(h)	4.0	6.0
AUC_{0-t}(µgml^{-1}h)	39.51±1.16	115.61±4.69
$AUC_{0-\infty}$(ml^{-1} h)	40.44±2.76	152.34±6.48
Elimination half-life($t_{1/2el}$)(h)	1.97±0.10	3.49±0.27
Elimination rate constant(K_{el})(h^{-1})	0.41±0.004	0.17±0.001
Apparent oral clearance (CL/F)	0.56±0.002	0.14±0.002
Apparent volume of distribution (Vd/F)	1.32±0.26	0.98±0.01

Values are mean± Std.EM(n=6).

Relative oral bioavailability (F) of optimized rutin phytosomes was calculated using dose-normalized $AUC_{0-\infty}$ values. The phytosomal formulation showed a significant increase in systemic exposure compared with pure rutin, and relative bioavailability is expressed with 95% confidence intervals.

Stability study

The in vitro drug content and drug release study results of the optimized rutin phytosomes during the stability study under normal conditions are presented. Upon storage under normal conditions, optimized rutin phytosomes showed 89.97 ± 0.20% drug content and 72.53 ± 1.99% drug release at 24 h on day 90, without showing a significant difference compared with the drug content and drug release values measured on day 1 (Tables 45 and 65).

The in vitro drug content and drug release study results of the optimized rutin phytosomes during the accelerated stability study are shown in Tables 67 and 87, respectively. Upon storage under accelerated conditions, optimized rutin phytosomes showed 87.62 ± 0.26% drug content and 73.44 ± 1.71% drug release at 24 h on day 90, without showing a significant difference compared with the drug content and drug release values measured on day 1.

Table 5: Drug content of the optimized rutin phytosomes during the stability study

EvaluationParameter	Time(Days) (Normalconditionsat25±2 ˚C&60±5 %RH)			
	1st day	30thday	60thday	90thday
Drug Content(%w/w)	91.38±0.19	90.66±0.08	90.13±0.31	89.97±0.20

Valuesaremean±SD(n=6).

Table 6: In vitro drug release profile of the optimized rutin phytosomes during the stability study (normal condition)

Time(h)	%CDR (Normal condition at 25±2˚C&60±5%RH)			
	1stday	30thday	60thday	90thday
0	0	0	0	0
2	9.73±0.18	10.24±0.37	8.61±0.53	9.10±0.93
4	23.51±0.08	20.33±1.90	21.75±1.71	22.55±0.80
6	26.88±0.99	24.36±1.15	27.88±1.02	29.34±0.62
8	28.66±1.92	27.44±1.81	28.67±1.61	30.65±1.39
10	35.48±0.95	32.33±1.42	37.56±1.72	36.22±1.84
12	42.63±0.80	43.71±0.78	44.66±1.37	45.98±2.89
14	51.57±1.00	50.41±1.26	55.83±1.00	52.65±2.25
16	56.74±1.86	57.39±0.29	58.64±0.33	56.66±1.26
18	59.81±1.17	60.62±3.02	62.37±1.90	59.69±0.54
20	63.76±1.59	65.30±2.10	67.42±2.24	66.87±1.86
22	67.18±1.84	69.46±2.13	69.13±2.60	67.11±3.25
24	74.45±0.71	73.61±1.52	70.78±2.09	72.53±1.99

Values are mean±SD(n=6).

Table 7: Drug Content of Optimized Rutin Phytosomes (Accelerated Stability Study)

EvaluationParameter	Time(Days) (Acceleratedconditionat40±2 ˚C&75±5%RH)			
	1st day	30thday	60thday	90thday
Drug Content(%w/w)	90.47±0.44	89.54±0.22	88.10±0.17	87.62±0.26

Valuesaremean±SD(n=6).

Table 8: In Vitro Drug Release of Optimized Rutin Phytosomes (Accelerated Stability Study)

Time(h)	%CDR (Accelerated condition at 40±2 ˚C & 75±5%RH)			
	1stday	30thday	60thday	90thday
0	0	0	0	0
2	10.12±0.29	9.66±0.68	10.22±0.94	11.26±0.89
4	22.35±0.94	21.48±0.99	23.33±1.37	24.26±1.57
6	27.43±1.49	26.47±0.93	29.20±1.84	27.38±0.99
8	29.45±0.98	29.35±1.37	32.63±1.95	32.22±0.59
10	32.94±0.97	33.82±1.35	36.42±1.24	38.47±1.58
12	39.63±0.80	45.33±1.10	42.79±1.86	43.83±1.91
14	47.72±0.51	52.54±1.83	50.71±1.71	50.56±1.62
16	55.35±1.12	56.33±1.62	57.66±0.95	55.55±1.95
18	58.39±2.21	61.66±2.26	61.44±1.12	59.87±0.54
20	61.99±1.02	66.20±2.62	66.66±0.62	66.87±2.35
22	66.82±2.62	70.49±1.91	70.38±1.86	70.32±2.25
24	76.55±1.37	75.34±1.02	77.71±1.62	73.44±1.71

Valuesaremean±SD(n=6).

The analysis of the results gave the significant observations which were discussed in the light of current concepts and interrelationships among the other experimental results.

Anti-Inflammatory Activity

Carrageenan-Induced Paw Edema in Rats

The anti-inflammatory effect of Rutin Loaded phytosomes on carrageenan – induced hind paw edema. The Rutin Loaded phytosomes at doses 200 and 400 mg/kg produced a significant effect against carrageenan induced inflammatory effect. The dose of 400 mg/kg exhibited a significant inhibition of 48 % after 3 h, the effect increased after 3h (52%). Rutin-loaded phytosomes produced a significant reduction in carrageenan-induced paw edema compared with the control group and demonstrated anti-inflammatory activity comparable to the standard drug diclofenac sodium at the tested doses**.**

Table 9: Anti-inflammatory activity of Rutin Loaded phytosomes on Carrageenan induced paw edema method in Wistar rats

Group	Paw thickness in mm					% Inhibition at 3hr
	0 hr	1hr	2hr	3hr	4hr	
Group-I Carrageenan (control)	1.4±0.03	3.4±0.06	4.9±0.06	6.4±0.05	4.8±0.02	------
Group-II Diclofenac Sodium (10mg/kg)	1.4±0.04	2.2±0.03**	2.9±0.04**	3.1±0.02**	2.2±0.04**	52
Group-III (200mg/kg of RLP)	1.2±0.02	3.0±0.04	4.2±0.03	4.7±0.01*	3.5±0.04**	27
Group-IV (400mg/kg of RLP)	1.1±0.01	2.7±0.04**	3.5±0.02*	3.3±0.06**	2.8±0.04**	48

Values were mean ± SEM, (n=6), *P<0.05, **P<0.01 Vs control

RLP= Rutin Loaded phytosomes

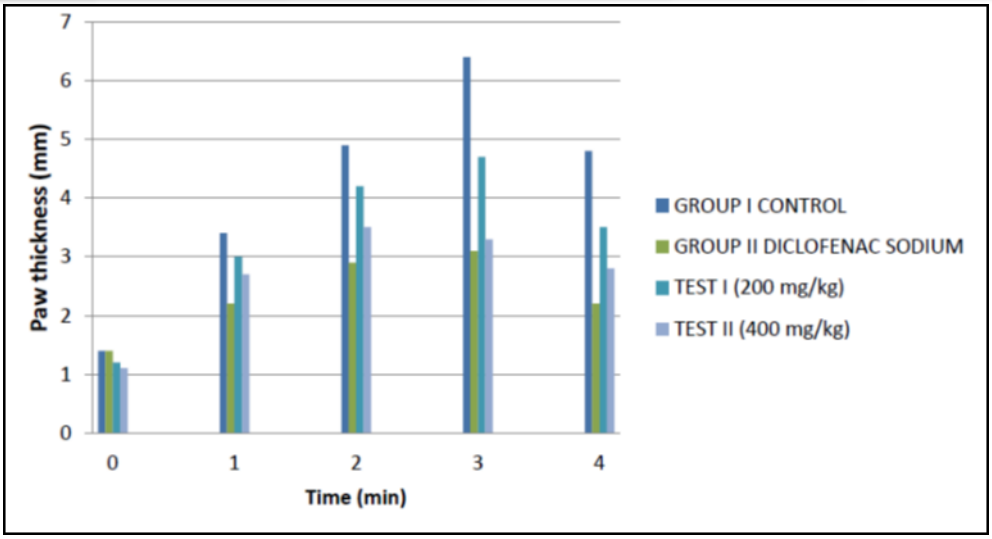

Figure 6: Anti-inflammatory activity of Rutin Loaded phytosomes on Carrageenan induced paw edema method in Wistar rats

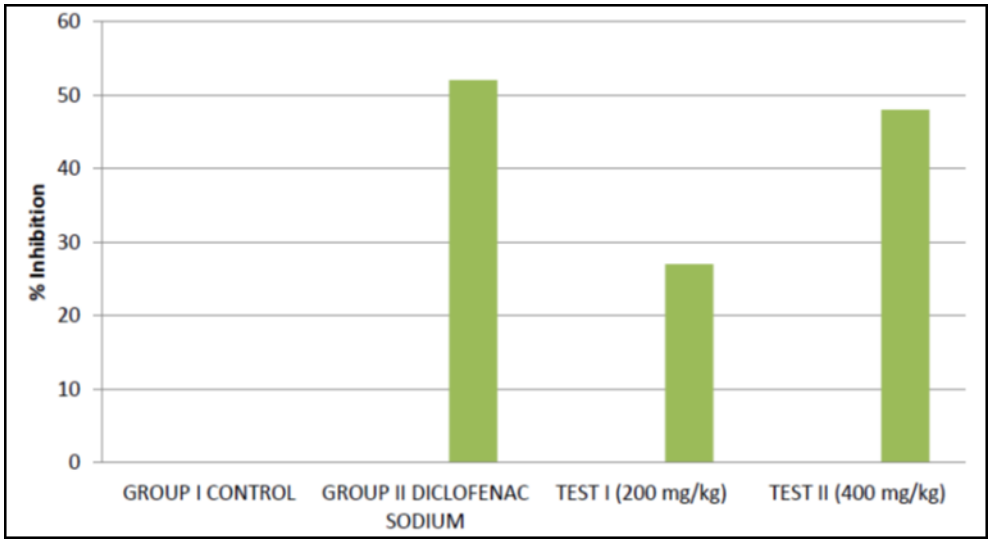

Figure 7: Anti-inflammatory activity of Rutin Loaded phytosomes on carrageenan induced paw edema method in Wistar rats

It should be noted that a free rutin group at an equivalent dose was not included in the present carrageenan-induced paw edema study. Therefore, direct head-to-head comparison and estimation of effect sizes between free rutin and rutin-loaded phytosomes could not be performed. The enhanced anti-inflammatory response observed for the phytosomal formulation may be attributed to improved oral bioavailability and prolonged systemic exposure, as supported by the pharmacokinetic findings. Future investigations incorporating a free rutin comparator are warranted to establish formal superiority with confidence interval–based effect size estimation.

Statistical Analysis

All experimental data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software. Prior to analysis, data distribution was assessed for normality using visual inspection and standard normality assumptions applicable to parametric tests.

Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test for comparison against the control group. Pharmacokinetic parameters between pure rutin and optimized rutin phytosomes were compared using an unpaired Student's t-test.

A P value < 0.05 was considered statistically significant. Sample size ($n = 6$ animals per group) was selected based on previous pharmacological studies using similar in vivo models, which consistently

Results & Discussion

Characterization of an independently prepared batch of the optimized formulation showed comparable particle size,

PDI, zeta potential, encapsulation efficiency, and drug loading values, confirming batch-to-batch reproducibility of the rutin phytosomal system.
The cumulative drug release data of optimized rutin phytosomes were subjected to kinetic modeling using zero-order, first-order, Higuchi, and Korsmeyer–Peppas equations. The release profile showed the highest correlation with the Higuchi/Korsmeyer–Peppas model, indicating diffusion-controlled release from the phytosomal matrix.
Differences are reported as mean differences with 95% confidence intervals and exact P-values.

CONCLUSION

The anti-inflammatory activity of rutin-loaded phytosomes was evaluated using a carrageenan-induced paw edema model in rats. The results demonstrated significant anti-inflammatory effects of rutin-loaded phytosomes compared with control, with efficacy comparable to the standard anti-inflammatory drug diclofenac sodium

Overall, this study contributes valuable insights into the development and evaluation of rutin-loaded phytosomes, highlighting their promising therapeutic applications in various disease conditions. Further research is warranted to explore their clinical efficacy and safety profiles, with potential implications for pharmaceutical and nutraceutical industries.
A free rutin dissolution profile was not included in the present release study; therefore, direct comparison of release behavior could not be performed. Future studies will incorporate a free-drug comparator to further elucidate the release enhancement achieved by phytosomal formulation.

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