Research Article

Rutin trihydrate loaded liposomal gel formulation and characterization

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ABSTRACT

In this research paper, liposomes were prepared as drug carriers to raising the delivery of an antioxidant Rutin trihydrate for dermal administration. Prepared liposomes were done using 3^2 factorial designs by thin film hydration method and then were characterized for their physiochemical properties (particle size, polydispersity index, zeta potential and morphology).Optimization was accomplished by altering the lipid to cholesterol ratio and keeping the definite amount of drug constant. The formulation and process parameters were optimized to attain multilamellar liposomes with similar size and accurate entrapment. Entrapment efficiency for the optimized batch gave 90%. Liposomal Gels were prepared by using Carbopol 940 as the gelling agent. Prepared liposomal gels release profile for in-vitro and in-vivo compared with conventional gel formulations. The liposomal gel present prolonged drug release up to 11h. The safety of liposomal gels was ensured by conducting skin irritation studies on albino Wistar rats.

Keywords: Gels; Liposomes; Rutin trihydrate.

INTRODUCTION

Liposomes, spherical vesicles consisting of one or more phospholipids bilayers, were first described in mid 60s by Banghum and coworkers. Since then, liposomes have made their way to the market. Liposomes are highly versatile structures for researcher's therapeutic and analytical applications(Shinkar et al., 2015). The liposome drug delivery system has played an important role in the formation of powerful drugs to improve treatment. Recently, the symptoms of liposome formulations decrease and increase conservation at the target site. Liposomes are also used in cosmetic formulations. This review will help researchers working in the field of lymphocytosis. Distribution this article provides an overview of the drug delivery system in the form of liposomes(Nikam et al., 2020). Applications of liposomes in food, cosmetics, gen genetic engineering, immunology, cancer therapy, infection, and also the diagnosis(Mulla et al., 2019).

Nanomedicine or Nanobiomedicine" could impact diagnosis, monitoring and treatment of diseases as well as control and understanding of biological system(Khare et al., 2014). Nanogels are one of the techniques in nanotechnology which has been most popular in effective drug delivery inside the body as well as topical treatment. Certain properties of nanogels make them suitable to carry different types of molecules like DNA, proteins, oligonucleotides, RNA, dyes, quantum dots and certain chemical agents like diclofenac to the target site. Its nano-sized structure has showed the reduced effect of toxicity of drug molecule as well as it provides controlled release of drug at the target site, increased the bioavailability of the drug(Tiwari et al., 2015).

Ketorolac loaded conventional liposomes containing natural phospholipid, So phosphatidyl choline by optimizing various process and formulation related variables such as drug - lipid ratio, cholesterol content, vacuum, speed of rotation, hydration medium and hydration time. Effects of charges over the vesicles were studied by incorporating dicetylphosphate and stearylamine(Begum et al., 2011). In recent times, the utilization of herbal bioactives is increased worldwide because of their incredible therapeutic effects and negligible side effects as compared to the synthetic medicines(Singh et al., 2013). Herbal Therapy is the result of the growing interest of scientists in medicinal plants. In this therapy, instead of a single photochemical, extracts of plant parts such as roots or leaves are use(Sanghi & Tiwle, 2013).

Rutin is 5, 7, 3, 4, tetrahydroxy flavonol -3-rhamanoglucoside and widely used in medicine

for maintenance of capillary integrity. Both possess antioxidant activity and reduce low density lipoproteins [LDL] oxidation. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques. The literature revealed that no UV-spectrophotometric method is not yet reported for the estimation of rutin and gallic acid in Triphala churna(Pawar & Salunkhe, 2013). Methods are establishing and validating a simple, accurate, reliable and economical UV spectrophotometric method for the simultaneous determination of Rutin trihydrate (RUT) and Berberine Chloride (BER) in synthetic mixture and in antidiabetic polyherbal formulation(Chaudhary & Patel, 2019).High performance liquid chromatographic method and Spectrophotometric method by Simultaneous equation for determination of three herbal markers-Rutin Trihydrate (RUT), Berberine Chloride (BER) and Trigonelline Hydrochloride (TRG) in their combined dosage form(Chaudhary & Patel, 2020).

Topical drug delivery has been prolonged search after, as it is joined with well-established benificially like localized drug delivery, to avoide the first pass metabolism, and enhance thecompliance of affected person owing to its non-invasive nature and reduced the systemic aspect outcomes (Shew & Deamer, 1985). Apart of these merits, the brick and mortar structure of stratum corneum i.e. the horny layer serves as themost important barrier to the percutaneous absorption of table (Murry & Blaney, 2000). Owing to these barrier houses of the skin, the shipping of energetic supplies from traditional formulations is typically compromised. Thus, there arises the want for a appropriate provider to decorate drug transport(Padamwar & Pokharkar, 2006).

Topical drugadministriation is a localized drug transport gadget somewhere in the physique via ophthalmic, rectal, vaginal and pores and skin as topical routes. Rutin trihydrate used to be chosen as the powerful molecule so they are effective antioxidant to be reduced the free-radical mediated cytotoxicity and lipid per oxidation. These are partially soluble in water (0.125 g/L) and this limits use in topical delivery. Hence liposomes have been chosen to be drug carriers because they are amphiphilic in nature and hydrophobic molecules get entraped in their concentric bilayers. Liposomes being physiologically resemble to cell membrane are not critical in nature and are effortlessly re-absorbed from the dermis into the deepest layers(Rahimpour & Hamishehkar, 2012).Since first being described by using English hematologist Alec Bangham in 1961(Bangham & Horne, 1964). Artificial As chemical micro reactors and as mannequin biomembrane structures(Lasic et al., 1991). No longer to point out the potential to mimic the biophysical residences of residing cells(Mozafari, 2005). These ''dynamic'' behaviors refer to features such as membrane deformation and appearing polymerization which impart cell-like kinetic conduct to liposomes The fundamental benefits that have made these vesicular structures beneficial for drug transport are their tissue compatibility, biodegradability, security and their capability to entrap nearly any drug in the bilayer core or in the outer domain. Additionally, liposomal drug formulations can be used to overcome a drug's non-ideal residences and destructive pharmacokinetic profiles(Hamilton et al., 1980).

Antioxidant widely used as topical agent as an important method in cosmeceutical enterprise to remove the wrinkling of the pores and skin and protect it from degenerative consequences such as picture aging, sunburn, picture carcinogenesis etc(Alinaghi et al., 2013).

Clinically Available Liposome-Based Products

At present, there are acceptable quantities of liposome-based medications accessible for human use.

Doxil

Doxil liposomes are made up of high stage change temperature (Tm) phosphatidylcholine (HSPC), cholesterol and N-(carbonyl-methoxypolyethylene glycol 2000) - 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE) in a molar proportion of 56:38:5(Shahi & Athawale, 2010). This remote medication stacking approach takes into account methodical aggregation of DOX inside the liposome hydrophilic center (around 15,000 DOX atoms/vesicle), with the vast majority of the medication(Barenholz, 2012).

Depocyt

Depofoam innovation comprises of tiny round particles (3-30 m) and is reasonable for embodying hydrophilic mixes, for example, Ara-C. These lipid froth based particles are included 96% watery froth and 4% biodegradable lipid(Lasic et al., 1991). The Depocyt suspension is kept up at a last pH of 5.5 to 8.5 Because of the higher thickness of Depofoam ™ particles than that of the suspending medium(Murry & Blaney, 2000).

Ambisome

Ambisome is endorsed for the treatment of hazardous parasitic diseases including leishmaniasis, aspergillosis, impact mycosis, coccidioidomycosis in febrile, neutropenic patients and a specific type of meningitis in individuals tainted with HIV(Petre & Dittmer, 2007). Ambisome is additionally endorsed for the treatment of intrusive fundamental contamination brought about by Aspergillums Candida or Cryptococcus in patients those cann't endure traditional AmB treatment or renally disabled patients(Lister, 1996).

Epaxal

Epaxal is profoundly compelling after organization of the main portion, offering defensive invulnerability for a constrained span. It gives invulnerability to as long as 20 years following the subsequent promoter portion(Janoff et al., 1993). The lipid parts of Epaxal virosomes are 1, 2- Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and DOPC present in a molar proportion of 25:75(Clarke et al., 2006).

MATERIALS AND METHODS:

Formulation of Rutin Trihydrate Liposomes: Bangham describe the thin film hydration technique for the preparation of multilamellar vesicles. Phosphatidylcholine, Cholesterol and Rutin trihydrate constituted the lipid mixture. The lipid mixture will be dissolved in methanol and chloroform (1:2) in 500ml round bottom flask (RBF), 25g glass beads will be added for homogeneous film formulation. The RBF will be rotated with the help of hand over thermostated water bath at 40°C. The organic solvents will be removed by evaporation leading to lipid film deposition on the flask wall. The film dried for 65 min for complete removal of the solvent andthe dried lipid film hydrated with phosphate buffer (pH: 5.4) at 150 r/min and 45°C that is above the transition temperature of lipid. The suspension will be also outfall for few minutes. Characterization of liposomal suspension will be done by physical appearance, settling time, entrapment efficiency, re-dispersion time and vesicle size by optical microscopy. Then it is filled in amber colored glass bottles to store at 4°Cuntil further use. 10mg drug, 2.3ml Tween 80 and 10ml phosphate buffer pH 6.4 was taken. All were uniform in all batches.

Table1:Factorial design for formula optimization based on Drug:Lipid and SL:CH ratio

Batch No.	Amount of SL (%) (mg)	Amount of CH (mg)	SL:CH (molar ratio)
F1	15	135	1:9
F2	30	120	2:8
F3	45	105	3:7
F4	60	90	4:6
F5	75	75	5:5
F6	90	60	6:4
F7	105	45	7:3
F8	120	30	8:2
F9	135	15	9:1

Characterization of Rutin Trihydrate Liposomes

Characterization of Rutin trihydrate Liposomes were done byFTIR using KBr pellet. The sample pellet was mounted in FTIR spectrophotometer and taken scan at wavelength 4000cm-1 – 400cm-1. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) for optimized drug loaded optimized liposomal suspension was determined. Particle size, Zeta potential and PDI measured in triplicate with the help of Malvern zetasizer.The pH of different formulations of gels was determined on digital pH meter. Entrapment Efficiency was estimated using UV spectroscopy(Upadhyay & Pandit, 2011, 2012).

In vitro drug release studies

In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane (HiMedia molecular weight 5000) was placed between receptor and donor compartments. Rutin trihydrate liposomal gel suspension was placed in the donor compartment and the receptor compartment was filled with phosphate buffer, pH 7.4 (18 ml). The diffusion cells were maintained at 37 ± 0.5°C with stirring at 200rpm throughout the experiment. At fixed time intervals, 1ml of aliquots were withdrawn from receiver compartment through side tube and analyzed by UV-Visible Spectrophotometer at 266nm. Data obtained from in vitro release studies were fitted

to various kinetic equations to find out the mechanism of Rutin trihydrate release from liposomal gel suspension.

Kinetics of drug release

To analyze the Kinetics of drug release from the Liposomal gel, the in vitro dissolution data will be fitted to zero order, first order, Higuchi release model, Korsmeyer and Peppas model. Drug released from the matrix devices by diffusion has been described by Higuchi's classical diffusion equation. The release rates from controlled release polymeric matrices can be described by the equation proposed by korsmeyer et al.

Statistical Analysis

The statistical analysis of the simplex lattice design batches was performed by multiple regression analysis using Microsoft Excel. To evaluate contribution of each factor with different levels on response, two way analysis of variance (ANOVA) ($P < 0.05$) was performed.(Upadhyay et al., 2014).

Animals studies

The male and female Wistar albino rat (150-200g) was procured from animal house facility of IFTM University, Moradabad UP (India). The animals will be housed in standard polypropylene cages and maintained under controlled room temperature ($22 \pm 2°C$) and humidity ($55 \pm 5\%$) with 12 h light and 12 h dark cycle. All the rats in Normal group will be provided with commercially available rodent chow diet) and tap water ad libitum. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India will be followed and prior permission was sought from the Institutional Animal Ethics Committee (2019/837ac/MPH/32), IFTM University, Moradabad (U.P) India for conducting the study.

Grouping of animals

Then they will be divided into 4 groups of 4 animals each & treated as follows:

S. No.	Group	Dose	Treatment	No. of Rats
1.	G-I	Normal saline (10ml/kg)	Control/Blank	6
2.	G-II	Standard formulation	Standard	6
3.	G-III	Formulation 1	Low dose	6
4.	G-IV	Formulation 2	High dose	6

Anti inflammatory activity by
Xylene-Induced Ear Edema method

This experimental procedure will perform using the method of Rats will be used, and test drugs will be administered orally on a once daily dosage regimen for 5 days, and the control group received vehicle(Hosseinzadeh et al., 2003). The edema will induced in each Rat by applying 50 µL Xylene to the inner surface of the right ear. Ninety minutes after Xylene daubing, the rat will be executed by cervical dislocation, and both ears will be removed and weighed(Chen et al., 1991). The difference between the right and left ears will determined for each group, and % inhibition = (Difference of ear weight in control group - Difference of ear weight in test group) Difference of ear weight in control group ×100.

Carrageenan-Induced Rat Paw Edema

Rats will be used, and test drugs will be administered orally for 5 days. One hour after the last treatment, the edema will induce by injection of 100 µL Carrageenan (prepared by using 1% in normal saline) in the sub plantar tissue of the right hind paw. Paw volume will be measured using the to be volume measure meter before and 1, 2, 3, 4, and 5 h after injection of Carrageenan(Chen et al., 1991).

RESULTS AND DISCUSSION:
Liposomes were prepared using 3^2 factorial design

Multilamellar liposomes were formulated by thin film hydration method.Thin film hydration method was selected because theRutin trihydrate is hydrophobic in nature due to low aqueous solubility, Hencethe capability for loading the higher mass of hydrophobic drug is more to multilamellar vesicles than unilamellar vesicles.

Entrapment of the drug depends on pKa value for a considerable extent. The pKa value of Rutin trihydrate is 6.4 mentioned in the literature. Maximum 90% ionization occur at this pH values..

U.V. Standard Curve of Rutin Trihydrate

The absorption maxima of drug solution (10µg/ml) was determined by UV Spectrophotometry was found to be 290 nm in 0.1 M HCl (pH 1.2) when it was scanned between 400 – 200 nm.

FTIR Characterization of Rutin Trihydrate

The FTIR spectrum of Rutin Trihydrate showed a characteristic secondary amine –NH stretch at 3279 cm-1, a C–H stretch at 2966 cm-1, an aryl C=C stretch at 1564 cm-1, an aryl O–CH2 asymmetric stretch at 1243 cm-1, an aryl O–CH2 symmetric stretch at 1022 cm-1 and a

peak at 797 cm-1 due to alpha-substituted naphthalene.

FTIR Characterization of Rutin Trihydrate Liposomes Gel

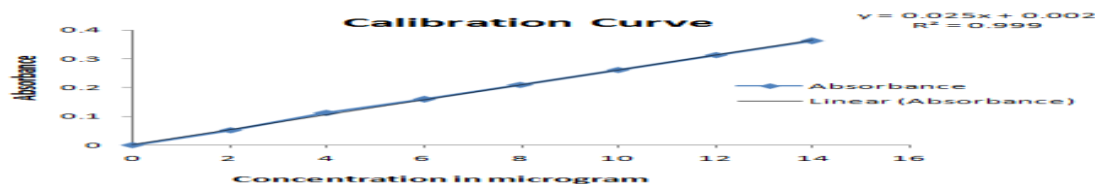The FTIR spectra of Rutin Trihydrate Liposomes Gel (Fig) exhibited absorption band at 2925, 3431and 1306 cm–1 are due to -CH2 bending/wagging, stretching of hydroxyl group and –CH2 stretching vibrations.
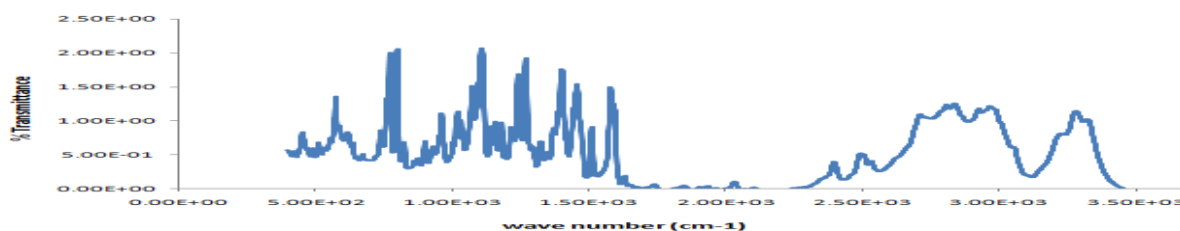
Drug Polymer interaction studies

FTIR studies shows that drug and polymer were found to be compatible with each other and there was no interaction found between them.

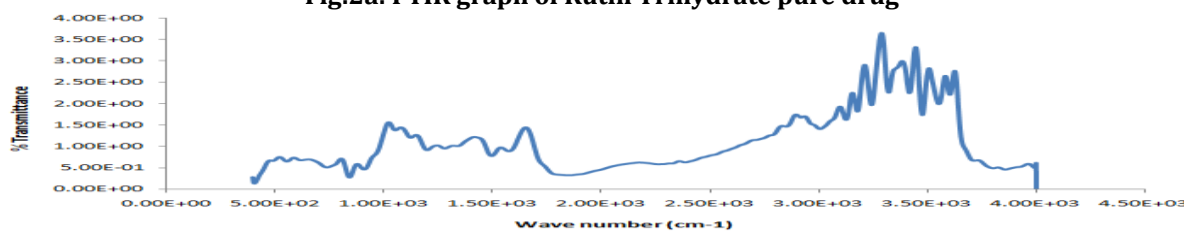**Table 2: Calibration curve data of Rutin Trihydrate**

Concentration(µg/ml)	Absorbance
2	0.052
4	0.112
6	0.159
8	0.210
10	0.262
12	0.313
14	0.362

Fig.1: UV Standard curve of Rutin Trihydrate pure drug

Fig.2a: FTIR graph of Rutin Trihydrate pure drug

Fig.2b:FTIR spectra of Rutin TrihydrateLiposomes Gel

Scanning Electron Microscopy (SEM) :The Morphology and surface appearance of Liposomes were examined by using SEM. The SEM photographs of F2 and F6 formulation showed that the particles have smooth surface. The SEM images were shown in Fig No3.


Fig.3: SEM photography of Liposomal solution formulation.

Transmission Electron Microscopy (TEM): At magnification of 100xs when optical microscope is used the MLV's of size 0.5 to 1 µm were visualized easily. TEM imaging for optimized formulation represent the drug loaded liposomes of spherical shape. Surrounded faint background showed for vesicles inner dark spherical core.

Vesicle size for optimized batch was found to be   approximately600 nm (Figure 4).
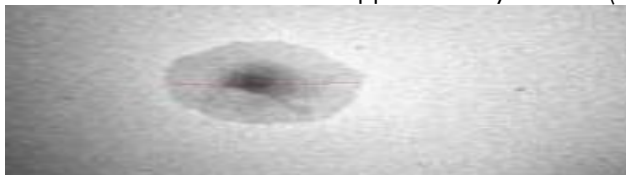
Fig.4: Negative staining TEM imaging of Rutin trihydrate liposomes

Physicochemical characterization,Vesicle Size and Zeta Potential Measurement

Table 3:Zeta potential and Droplet size.

FormulationCode	Zeta Potential(mV)	Droplet Size(nm)	PDI(Poly dispersive index)
F1	-0.112	18.39	0.401
F2	-0.051	250.1	0.963
F3	-0.069	156.1	0.381
F4	**-0.030**	161.4	**0.191**
F5	-0.011	75.53	0.241
F6	0.037	183.2	0.424
F7	0.158	106.2	0.220
F8	0.140	224.4	0.520
F9	0.141	567.2	**1.000**

Zeta potential (mV) was found to be in the range of -30 to + 30 (mV) for all the formulation F1 to F9. The minimum droplet size of liposome formulation for F1 was found to be 18.39 nm whereas the highest was found to be for F9 formulation that is 567.2nm, followed by F2, F8, F6, F4, F3 and F7 (nm). PDI was found to be minimum for F4 that is 0.191 which shows its stability and highest for F9. The stability of vesicles which are formed by thin film hydration method using zeta potential as an indicator. The zeta potential and PDI of the optimized batch F4 was -0.030 and 0.191 showing sufficient stability.

pH of gels
The pH values of prepared Rutin liposomal gel were found to be in the range of 5.5±0.20 and 5.7±0.20, respectively.
3.8. % Entrapment efficiency:

Table 4: % Entrapment efficiency in liposomal gel

Formulation Code	% Entrapment efficiencyin liposomal gel
F1	70.45±0.005
F2	79.14±0.150
F3	80.10±0.020
F4	86.18±0.042
F5	72.62±0.049
F6	86.82±0.042
F7	76.89±0.140
F8	61.67±0.151
F9	70.69±0.019

The % Entrapment efficiency in liposome gel was found to be highest for F6 and F4 that is 86.82% and 86.18 % and lowest for F8 that is 61. 67%.

in-vitro Dissolution data
The in-vitro dissolution profile of prepared formulations was determined by membrane diffusion method. The dissolution was carried out for a period of 24 hrs at pH 7.4 phosphate buffer. The cumulative percent release of F1 to F9 formulations at various time intervals was calculated and tabulated in Table No:5 The cumulative percent drug release in all formulations was plotted against time in Graph No: 1 and 2. The Maximum percent of drug release was found in F9 formulation which contains maximum drug entrapment.

Table 5: in-vitro cumulative % drug release profile of Rutin trihydrate liposomal formulations.

Time (mins)	Cumulative % drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
50	1.29	1.38	1.65	1.74	2.12	2.15	2.89	3.11	3.98
100	2.36	2.92	3.21	3.37	4.02	4.07	4.98	5.34	6.10
150	4.32	4.41	5.01	5.11	6.12	6.19	7.43	8.21	9.45
200	5.65	5.93	6.56	6.82	8.32	8.43	9.34	10.12	11.56
250	8.53	8.77	9.85	10.5	12.92	13.01	15.11	16.65	17.87
300	11.21	11.65	13.56	13.9	17.53	17.62	18.89	19.23	20.21
350	15.25	15.63	17.04	17.38	21.45	21.62	23.31	24.74	26.14
400	18.65	18.91	20.53	20.86	25.35	25.56	27.67	28.64	30.43
450	25.98	26.73	29.62	29.82	35.95	36.47	40.21	43.89	46.34

Percentage Drug Release ofRutin trihydrate liposomalin Different Formulations
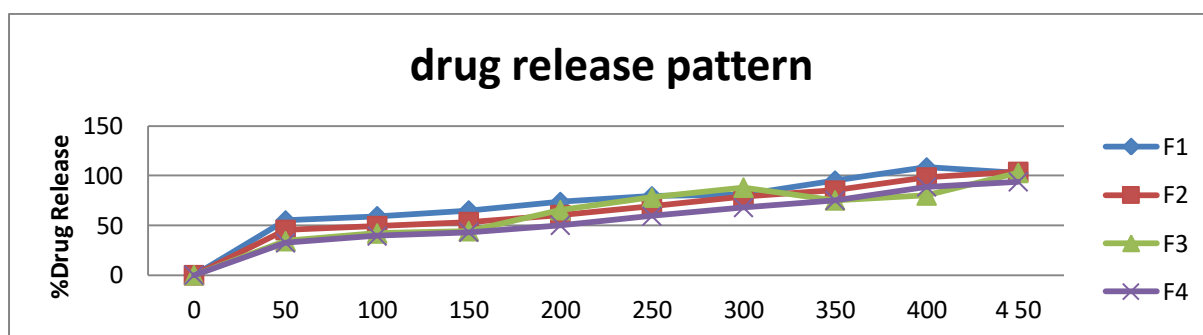
Graph 1: Comparison of in-vitro release studies for optimized formulations
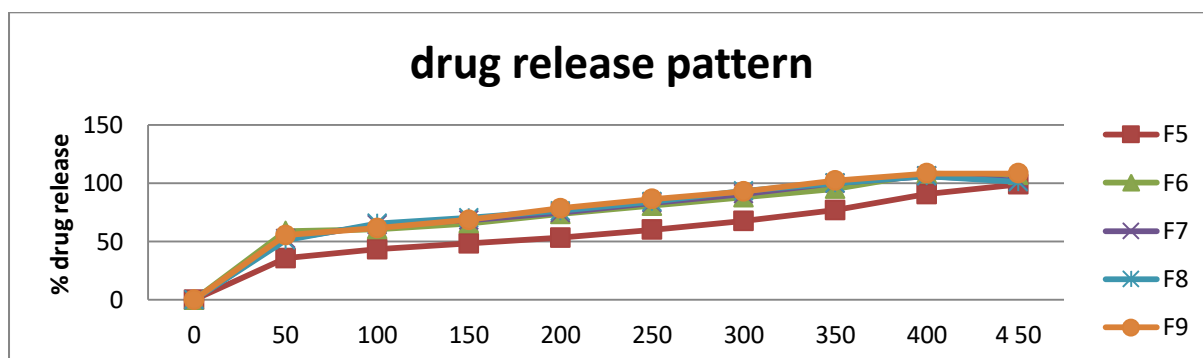
Graph 2: Comparison of in-vitro release studies for optimized formulations

Release Kinetics:

The release kinetics of F1 to F9 formulations was studied. All formulations follow Zero order release kinetics and follow case II transport when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. F9 formulation has better kinetic results when compared to F2, F6 and F4 formulations. The results are shown in Table N0: 6.

Table 6: Curve fitting data of release rate profile of Formulations F1, F2, F3, F4, F5, F6, F7, F8 & F9.

Type of Formulation	Zero-order	First-order	Higuchi	Korsmeyer –Peppas	
	(R^2)				n value
F1	0.8243	0.9453	0.9569	0.0171	0.0158
F2	0.9052	0.9167	0.9818	0.3337	0.0625
F3	0.8707	0.9377	0.8687	0.5661	0.0267
F4	0.9329	0.9858	0.9867	0.1204	0.0387
F5	0.8329	0.9324	0.9764	0.4931	0.0765

F6	0.8233	**0.9679**	0.9753	0.5691	0.085
F7	0.8144	0.9531	0.9651	0.8646	0.1419
F8	0.7931	0.9290	0.9534	0.2653	0.0961
F9	0.8362	0.8698	**0.9842**	0.7417	0.0477

As per the n value from korsmeyer peppas model, All the formulation F1 to F9 follows the Fickian diffusion or drug release mechanism for Swellable system. As per the r^2 value, F4 formulation follow the Zero order followed by F2 form similarly F4 also followed the first order release Kinetics followed by F6, F7 and F1. Also, F4 follows Higuchi's followed by F9, F2, and F5 Formulations.

in-vivo animal studies effect
Carrageenan- induced rat paw edema
By using the Carrageenan-induced rat paw edema method, the in-vivo performance determined for the selected Rutin trihydrate liposome loaded gels. The results are represented in table 7.

Table7: Percent of Inhibition of Edema

Sr. No	Time (in Hrs)	Percent of inhibition of edema Treated with optimized F4 liposomal gel	Test plane gel	Std. Diclofenac Diethylamine gel
1	0	0	0	0
2	2	25.63±0.0932	30.31±0.1013	18.14±0.0434
3	4	31.74±0.1208	34.12 ±0.0424	25.36±0.1343
4	6	40.28±0.0357	37.46±0.05585	31.61±0.1378
5	8	47.88±0.1572	41.35±0.1364	38.77±0.0497
6	10	56.25±0.1028	46.35±0.0974	45.20±0.1011
7	12	68.04±0.0518	50.55±0.0671	52.21±0.0761
8	24	90.83±0.1648	64.58±0.0738	69.51±0.1545

Prepared Rutin trihydrate liposome loaded gel sustained the magnitude and decreased the inflammation at larger magnitude. In Rutin trihydrate liposome loaded gel formulation the maximum inhibition was observed at 12th hr with higher value 68.04%, and even after 24hr, 90.83% inhibition was observed as compared to plane. Rutin trihydrate gel and standard Diclofenac Diethylamine gel at 12 hr was 50.55%, 52.21 and at 24 hrs 64.58, 69.51 respectively. The possible reason could be the drug concentration in the blood, which was maintained for longer duration in case of formulated Rutin trihydrate liposome loaded gel which compared to the plane Rutin trihydrate gel. In comparison to Rutin trihydrate gel, the prepared Rutin trihydrate liposome loaded gel was applied transdermally gave good results. The anti-inflammatory activity of the prepared Rutin trihydrate Liposome Loaded Gel was maintained for longer period of time due to slow release of

the drug. This was achieved to gel structure and the surface active properties of the gel.
The Rutin trihydrate liposomal gel represents the better pharmacological activity when compare with the plane Rutin trihydrate gel this is due to the penetration capability of liposome into the deeper layers of the skin and presents the better results compare the plane Rutin trihydrate gel.

Effect of Rutin trihydrate on Xylene-Induced Mouse Ear Edema
Topical application of Xylene on the ear of rats in the control group caused a marked increase in the weight of the ears (Table 8). Topical application of Rutin trihydrate liposomal gel(1, 2.5, and 5 mg/kg) simultaneous with xylene, suppressed xylene-induced ear edema in mice ($P < 0.05$ and $P < 0.01$). Indomethacin (0.5 mg/ear) did not exhibit a considerable anti-inflammatory effect in the model of xylene-induced ear edema.

Table 8: Effects of the Rutin trihydrate liposomal gel (1, 2.5, and 5 mg/ear) and Indomethacin (0.5 mg/ear) on Xylene-induced Ear Edema in rats

Group	Dose, mg/ear	Weight of Ear, mg
Control	-	12 ± 0.81
Indomethacin	0.5	6.1 ± 1.50[a]
Rutin trihydrate liposomal	1	4.4 ± 0.85[b]

| gel | 2.5 | 4 ± 0.90^c |
| | 5 | 3 ± 0.70^d |

a Values are expressed as Mean ± S.E.M. (n = 6).
b P < 0.05 statistically significant relative to the control.
c P < 0.01 statistically significant relative to the control.
d P < 0.001 statistically significant relative to the control

CONCLUSION

The main objective of this work was designed to prepare and evaluate the Rutin trihydrate Liposomes. This formulation will target the site of action with effect of various stabilizers on drug entrapment efficiency, and to reduce the side effects by formulating non-PEGylated Liposomes. This liposomal formulation was formulated using the soyabeanlicithin and cholesterol which has lesser toxicity. The prepared Liposomes of F2, F4, F6 and F9 formulations were evaluated for physical and chemical characteristics like average vesicle size, shape and zeta potential. The evaluated batches showed good physicochemical characteristics in F4 formulation when compared to the F2, F4 and F6 formulations. The release kinetics of F2, F4, F6 and F9 Formulations were study. The stability of the Rutin trihydrate Liposomes was evaluated after stored at 4°C and room temperature for 90 days. The assay of the samples was determined as a function of the storage at different time intervals. The Liposomes stored at 4°C were found to be stable for duration of three months.From the results of physical characterization, in-vitro evaluation, release kinetics and stability studies, it was found that Liposomes gel containing rutin trihydrate might be used for the treatment of a anti-inflammation activity when compared to the normal drug and neutral Liposomes. From the executed experimental results, it could be concluded that the stabilizers like Stearylamine and Rutin trihydrate along with Soya lecithin and cholesterol were suitable carrier for the preparation of Rutin trihydrate Liposomes. Though the preliminary data based on in-vitro dissolution profile, release kinetics and stability studies proved that the suitability of such formulations, Still a thorough experiment will be required based on the animal studies. There after we can find the actual mode of action of this kind of dosage form. Therefore, a future work will be carried out as follows.
➢ Long term stability studies
➢ in-vitro Cytotoxicity studies
➢ in-vivo Pharmacological work on animals.
➢ in-vivo pharmacokinetic studies on animals.

Ethical Clearance

The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India will be followed and prior permission was sought from the Institutional Animal Ethics Committee (2019/837ac/MPH/32), IFTM University, Moradabad (U.P) India for conducting the animal study.

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