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Research Article

Rutin loaded nanoemulsion formulation for brain tumor targeting: *In vitro*, *ex vivo* permeation and *in vitro* cytotoxicity assay

HITENDRA MAHAJAN*, SHAMSHADBELDAR

Department of Pharmaceutics, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, MS, India

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ABSTRACT

Rutin-flavonoid-polyphenolic has gained attention in prevention of brain cancer. The low permeability of Rutin (RU) across the blood-brain-barrier (BBB) leads to its insufficient delivery which in turns result in low therapeutic index. Therefore, developing a novel approaches enhancing the CNS delivery of RU are required for the treatment of Cancer. The aim of this research work was to develop in Nanoemulsion (NE) loaded with RU, for CNS targeting. Rutin is a poorly water soluble anticancer drug, with oral bioavailability is about 4%. Nanoemulsion were fabricated by Spontaneous Emulsification technique. Ethyl oleate was used as oil. Tween 80 was employed as surfactant and Polyethylene glycol 400 was employed as co-surfactant. Optimised RU-NEs (F7) had particle size of 228.60 nm and % drug content of 94.77 %. Finally characterized for different parameters. Differential Scanning Calorimetry and Transmission Electron Microscopy (TEM) study revealed that RU was completely homogenies in Nanoemulsion. Then the formulation were subjected to *in vitro, ex vivo* and *in vitro* cytotoxicity using (U373MG) cell line and Histopathological studies. IC_{50} value was obtained 9.8 µg/mL of RU-NE and it did not show any toxicity and so were safe for intranasal delivery for brain targeting. *In-vitro* diffusion studies revealed that RU loaded NE had a significantly higher release compared to plain drug suspension (PDS). From stability study, it was found that MDS, PDI, zeta potential and % DR was not significantly changed during the period of three months at 25 ± 2°C and 60 ± 5% RH. So, RU loaded Nanoemulsion for intranasal delivery are considered as promising vehicle for its targeting to CNS to treat the brain cancer. RU-NE was a dramatically enhanced anticancer and antiangiogenic activities.

INTRODUCTION

The uncontrolled growth of cells and tissue can arises cancer, cancer was a one of the most distressing and life threading disease that serves date worldwide. Cancer like Brain tumors were an abnormal and uncontrolled growth of cells in brain [1-3]. Brain tumors include all tumors inside the cranium or in the central spinal canal. They are created by an abnormal and uncontrolled cell division, usually in the brain itself, but also in lymphatic tissue, in blood vessels, in the cranial nerves, in the brain envelopes (meninges), skull, pituitary gland, or pineal gland[4]. Within the brain itself, the involved cells may be neurons or glial cells (which include astrocytes, oligo-dendrocytes, and ependymal cells). Diagnosis and treatment of brain tumor was difficult challenge in oncology. Malignant brain tumor are responsible for the brain cancer. The patient survival for malignant tumor was only 48 weeks from diagnosis. Conventional chemotherapy of drug was not more effective against tumor cells, having some disadvantage including several toxic, unwanted effect on healthy tissue, limited delivery of lipophilic drugs to tumor cells. Inability to reach drug molecule intravenously to reach brain tissue for inability to crossing the physiological barrio such as blood brain barrier (BBB) [5, 6]. Higher concentration of drug in tumor site was not effective, this was a one of obstacle of chemotherapy. Modern colloidal nanoparticulate system was novel approach to overcome the problems of chemotherapy. Modern colloidal nanoparticulate system like nanoemulsion was most advanced and novel approach to deliver

Author for Correspondence:
Email: hsmahajan@rediffmail.com

lipophilic drug to tumor site having greater penetration ability by passing the BBB [7]. Targeted approach of drug reaches maximum concentration to cancer tissue. Intranasal drug delivery was the promising strategies for direct deliver neurotherapeutic agent to nose to brain by passing the BBB via olfactory and trigeminal nerve pathways. The size of nanoemulsion system was a one of the fundamental property for passive targeting of drug molecule and biotransformation within brain tumor. The nanoemulsion size range was 100-500 nm. Rutin (polyphenolic compound) has potent antimetastatic and antiproliferative activity against brain tumors. It was important to suppression of nuclear factor-kB is responsible for tumor proliferation. Rutin having a promising ability to inhibit angiogenesis, it is process for formation of new blood cell in blood vessel for tumor growth, rutin having ability to stop the new blood cell formation in blood vessels responsible for tumor growth and shows antiangiogenic activity [8, 9]. The goal of this study was to design and produce nanoemulsion (NE) containing rutin for intranasal (nose to brain) delivery to central nervous system (CNS). In the present investigation, (RU-NE) were prepared to enhancing CNS delivery for the treatment of brain tumor. RU is highly lipophilic drug having a low oral bioavailability of 4%. RU-NE were prepared by Spontaneous emulsification method with Ethyl Oleate, Tween 80 and Polyethylene glycol 400 as an Oil, surfactant and co-surfactant respectively[10]. The objectives include identification of formulation variables affecting NE formulation and its characteristics like droplet size and zeta potential (ZP). Further objective was to carry out *in vitro, ex vivo* and *in vitro* cyto-toxicity studies of the optimized or developed formulations. Accelerated stability studies showed that there was no significant change in the mean droplet size and % drug content after storage at 25 ± 2ºC/60 ± 5% RH for the period of three months, thus demonstrating a promising perspective for nose to brain delivery of poorly water-soluble RU.

Materials and methods

Materials

Rutin-polyphenolic (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-ramnopyranosyl-(1-6)-β-D-glucopyranosyloxy]-4H-Chromen-4-one) was a gift from Loba Chemie Ltd. (Mumbai, India). Following excipients from respective sources were used as received. Ethyl oleate, Tween 80 (polysorbate 80) and polyethylene glycol 400 (PEG 400) were purchased from Loba Chemie Ltd. (Mumbai, India). Acetonitrile (HPLC grade) and Methanol (HPLC Graded) were purchased from Merck Ltd. (Mumbai, India).Freshly prepared double distilled water (HPLC graded) and buffers solutions were filtered through 0.22 µm membrane filter.

Methods

Formulation of Nanoemulsion (NE)

Selection of Excipients for formulation

The solubility of rutin in various oils (castor oil, ethyl oleate, soya oil, coconut oil, oleic acid and clove oil), surfactants (Tween 20 and Tween 80) and co-surfactant (polyethylene glycol 400 and propylene glycol) was determined by using Screeningtechnique [11, 12-14].

Preparation of Nanoemulsion (NE)

Rutin containing nanoemulsion were prepared by Simultaneous Emulsification technique in which oil phase containing methanol (Merck Pvt Ltd., Mumbai, India), rutin (Loba Chemie Pvt Ltd., Mumbai, India) and ethyl oleate (Loba Chemie Pvt Ltd., Mumbai, India), while water phase contains ratio of tween 80 as a surfactant (Loba Chemie Pvt Ltd., Mumbai, India) and Polyethylene Glycol 400 (Loba Chemie Pvt Ltd., Mumbai, India) as a co-surfactant by using overtaxing them properly, oil phase were slowly added in to water phase drop by drop to achieve desired droplet size [15-17].

Optimization of Formulation

The Nanoemulsion was optimized with respect to oil, ratio of surfactant & co-surfactant. The oil was chosen based on the maximum solubility of drug. The surfactant of no or less skin irritation, hydrophilic lipophilic balance (HLB) > 10 was selected [15-17].

Physicochemical characterization of rutin loaded nanoemulsion (RU-NE)

Photon correlation spectroscopy

The mean droplet size (MDS) were determined by photon correlation spectroscopy (PCS) using a Malvern Zetasizer (Nano ZS 90, Malvern Ltd., Malvern, UK). The measurement using PCS is based on the light-scattering phenomena in which the statistical intensity fluctuations of the scattered light from the particles in the measuring cells are measured [18]. Prior to the measurements, all samples were diluted with double-distilled water to produce a suitable scattering intensity the light scattering was monitored at 25˚c at a 90˚angle[19].

Table 1: Compositions of RU-NE formulations

Formulation batches	Con. of Oil (Ethyl oleate) (µl)	Con. of Surfactant (Tween 80) (ml)	Con. of Co-surfactant (Polyethylene glycol 400) (ml)	Drug (Rutin) (mg)
F1	200	1.00	7	20
F2	200	1.00	9	20
F3	200	0.50	7	20
F4	200	0.50	9	20
F5	180	1.00	9	20
F6	180	1.00	7	20
F7	180	0.50	7	20
F8	108	0.50	9	20

Zeta Potential

The ZP, reflecting the electric charge on the droplet surface and indicating the physical stability of colloidal systems, was measured by determining the electrophoretic mobility using the Malvern Zetasizer (Nano ZS 90, Malvern Ltd., and Malvern, UK). The measurements were performed following dilution in double-distilled water [20, 21]. It was measured using the Dip cell by applying a field strength of 20 V/cm and the average of the ZP was given from 30 runs.

Drug Content

The drug content of formulation was determinedby UV spectrophotometric method. Rutin from NE formulations was extracted by dissolving 1 ml of NE in methanol [22]. Rutin contentin the Methanolic extract was analyzed spectrophotometrically (UV 1700, Shimadzu, Japan) at257 nm, against the standard Methanolic solution of Rutin [23,24].

Refractive Index, pH Determination, Viscosity

Refractive index of selected formulations was determined in triplicate using an Abbe type Refractometer. The apparent pH of the formulations was measured by a pH meter (Systronics 362 µ pH system, India) in triplicate at 25°C. The viscosity of the CRM-GFT-NE was measured using the small sample UL adapter of a Brookfield Rheometer equipped with spindle no. 00 (Model DV-E Brookfield Engineering Labs., Inc., Stoughton, MA, USA) [22-24].

Morphological examination by using TEM

The structure and morphology of the Nanoemulsion droplet were studied using transmission electron microscopy (TEM) [25]. A combination of bright field imaging at enhancing magnification and diffraction modes was used, to reveal the form and size of oil droplet of the Nanoemulsions [26, 27]. A drop of the diluted nanoemulsions was directly deposited on the holey film grid and studied after drying and the size of the emulsion droplet was determined [28].

Differential scanning calorimetry

Thermal analysis was performed using a differential scanning calorimetry (DSC) (Mettler-Toledo, Greifensee, Switzerland). The instrument was calibrated with indium. DSC Thermogram were recorded for pure RU, lipid-RU physical mixture, and RU-loaded NEs. The samples, weighing 2 mg, were analyzed in sealed and pin-holed standard 40 µl aluminum pan, with a heating rate of 10˚C/min from 30˚C to 400˚C and, during the measurement, the sample cell was continuously purged with nitrogen at a flow rate of 10 ml/min [29, 30].

***In vitro* Drug permeation studies**

In vitro diffusion study of optimized nanoemulsion was carried out by Franz diffusion cell having 2.0 cm diameter and 25 ml capacity. Dialysis membrane (Himedia) having molecular weight cut off range 12000 – 14000 kDa was used as diffusion membrane [31]. Pieces of dialysis membrane were soaked in phosphate buffer saline (PBS) pH 6.4 for 24 h prior to experiment. Diffusion cell was filled with PBS pH 6.4 and dialysis membrane was mounted on cell. The temperature was maintained at 37°C [32]. After a pre-incubation time of 20 minutes, the nanoemulsion equivalent to 20µg of Rutin was placed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for 4 hours and replaced with the sameamount of fresh PBS, and assayed by a UV spectrophotometer at 257 nm.

Ex-vivo Permeation Studies

Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from the local slaughterhouse. Tissue samples were inserted in Franz diffusion cells displaying a permeation area of 0.785 cm. Phosphate buffer saline (PBS) pH 6.4 (25 ml) at 37 °C was added to the acceptor chamber. The temperature within the chambers was maintained at 37°c. After a pre-incubation time of 20 minutes, pure drug solution and formulation equivalent to 20 µg of Rutin was placed in the donor chamber [33]. At predetermined time points, 2 ml samples were withdrawn from the acceptor compartment, replacing the sampled volume with PBS pH 6.4 after each sampling, for a period of 4 hours. The samples withdrawn were filtered and used for analysis. Blank samples (without drug) were run simultaneously throughout the experiment to check for any interference [34]. The amount of permeated drug was determined using a UV Visible spectrophotometer at 257 nm.

The permeation coefficient was calculated by using the following formula:

$$J_{ss} = \frac{\frac{dc}{dt}}{C_0 \times A}$$

Where, Jss is the permeability coefficient, Co is the initial concentration in the donor compartment, A is the area of mucosal surface, dc/dt is the rate of permeability.

Histopathological Studies

Histopathological studies were carried out using isolated sheep nasal mucosa. Three sheep nasal mucosa pieces (M1, M2 and M3) with uniform thickness were selected and mounted on Franz diffusion cells. M1 was treated with PBS (pH 6.4, Negative control, M2 with isopropyl alcohol (Positive control), and M3 was treated with drug loaded nanoemulsion respectively [35]. After treatment for 2 h, all the samples were washed properly with double distilled water, sectioned and stained with hematoxylin and eosin. The mucosa was dissected out, then subjected to histological studies to evaluate the toxicities of NE and photographed by optical microscope [36, 37].

In-vitro cytotoxicity assay of Rutin Nanoemulsion in (U373MG) Cell – line

The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microliter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microliter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microliter wells already containing 90 µl of medium, resulting in the required final drug concentrations i.e.10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1 % acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells * 100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:[Ti/C] x 100 %[38-40].

Accelerated Stability Studies

The stability of optimized RU-NE was assessed at three different temperatures for 3months is refrigerating condition (2-8 ±2 °C/ 75±5 % RH); room temperature (25±2 °C/ 65±5 %RH); elevated temperature (40 ±2 °C/ 75±5 % RH) as

per ICH Guidelines. The nanoemulsion formulation were evaluated at 0, 30, 60 and 90 days by visual inspection (droplet size, Polydispersity index and drug content) [41].

RESULTS AND DISCUSSION

Preparation and characterization of nanoemulsion

The RU-loaded NEs were prepared using spontaneous emulsification technique. For the preparation of Ethyl oleate as a liquid lipid. Tween 80 were selected as a surfactant and Polyethylene glycol 400 as a co-surfactant a stabilizer, respectively. The balance of emulsifiers is required at the oil–water interface for the stability of dispersions. NEs can be prepared by various methods including hot and cold homogenization, Microfluidization, and low energy emulsification method. The spontaneous emulsification method was simple and quick at laboratory scale in the development of formulation [42, 43]. Therefore, for the preparation of RU-NEs, spontaneous emulsification(low energy) technique was selected with ethyl oleate, tween 80 and PEG 400 which provided the highest drug pay-load and there was no problem of drug leakage out.

Photon correlation spectroscopy

The small peak was followed by a broader peak, indicating two populations of particles: the first one can observe a negligible first population of particles having a diameter of 17.90 nm and a second broader peak represented one with a larger diameter of 228.6 nm (F7) with Polydispersity indexes (PDI) of 0.44 (Fig. 1). The

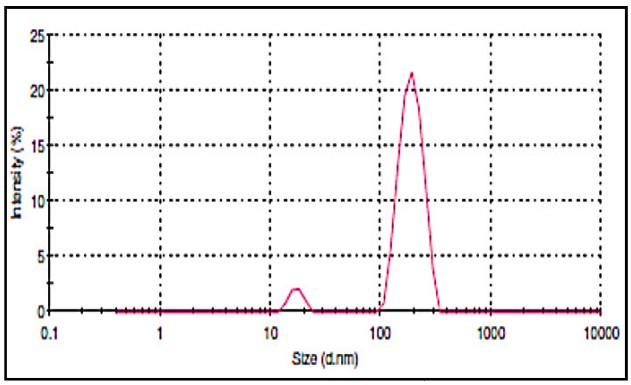


Figure 1: Droplet size analysis of optimized batch

droplet size of the NE is a crucial factor because it determines the rate and extent of drug release as well as drug absorption. The smaller particle size provides a larger interfacial surface area for drug absorption [44]. The globules having average diameter up to 500 nm could be easily transported transcellular via intranasal route. In addition, it was suggested that the smaller globule size permit a faster release rate. Also, it has been reported that the smaller particle size may lead to more rapid absorption and improve the bioavailability [45]. Also, PDI measures the width of particle size distribution. If PDI is lower than 0.5, it might be associated with a high homogeneity in the globule population, whereas high PDI values suggest a broad size distribution.

Zeta Potential

The measurement of the zeta potential (ZP) allows prediction about the stability of colloidal dispersion. ZP is the electric charge on particle surface which creates an electrical barrier, and acts as a 'repulsive factor' in the process of emulsion stabilization. High surface energy plays a major role in the stability of the formulation as like charges at the interface resists coalescence of globules [46]. Zeta potential of optimized nanoemulsion (F7) (Fig. 2) was -0.649 mV. The negative zeta potential indicates that globules of nanoemulsion having no charge that is system is stable Zeta potential was determined by using Zetasizer. There was no charge on globules so no flocculation and creaming was observed which shows that optimized nanoemulsion was found to be stable.

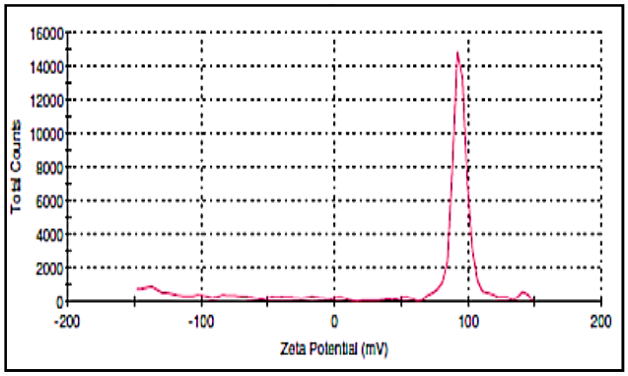


Figure 2: Zeta potential distribution of optimized batch

Drug Content (drug loading)

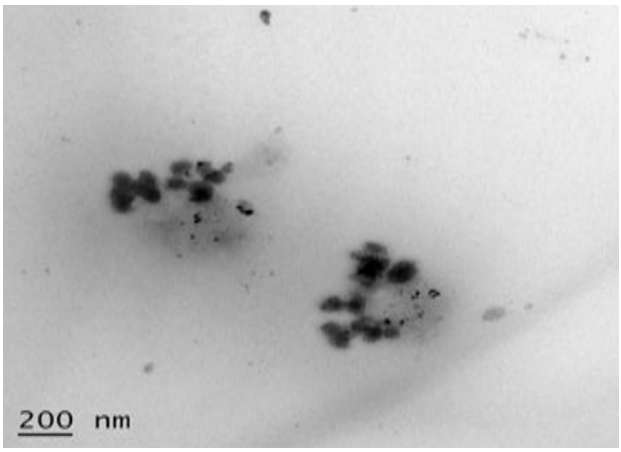
The concentration of oil and surfactant: co-surfactant was important effect on drug content. The oil content (ethyl oleate) was increases, to increase drug content and the surfactant and co surfactant concentration (tween 80 + PEG 400) decreases to increase drug content [47, 48]. Because drug having maximum solubility in oil phase and drug content of optimized formulation was found to be 94.77 %.

Refractive Index, pH Determination, Viscosity

The refractive index value for optimized formulation (F7) was found to be 1.783± 0.12 (mean ± SD, n=3). The pH of optimized nanoemulsion (F7) was found 6.5 ± 0.16 (mean ± SD, n=3), in the range of 4.5 to 6.5 which is required for the nasal drug delivery to minimize nasal mucosal irritation. Viscosity of optimized batch (F7) was found to be 280±2.5.

Morphological examination by using TEM

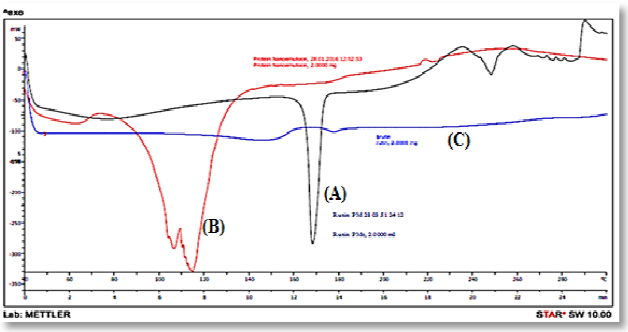
Morphological study of optimum formulation (F7) was done by taking TEM photograph of prepared NE. It was revealed that they were ovoid in shape (Fig. 3). Studies showed that the predicted globule size and measured size with a zeta seizer was comparable with size of globule that were observed by TEM.



Figures 3: TEM image of optimized batch

Differential scanning calorimetry

DSC was a basic method to investigate the crystallization or amorphous state of drug in the compounds and NEs by determining the variation of temperature and energy at phase transition. (Fig.4.) shows the DSC curve of RU, RU + physical mixture, and RU-NEs (F7). The melting peak for the RU was not detected in the thermogram of the RU-NEs. The physical state of a drug in the dosage form affects the *in vitro* and *in vivo* release characteristics of the drug [49]. The drug loaded nanoemulsion did not show any peaks under the studied temperature range indicating that RU in nanoemulsion was present either in a solubilized form or in a disordered crystalline form of a molecular dispersion [50].



Figures 4: DSC thermogram of (A) Pure Drug (RU), (B) Drug+ Physical Mixture, (C) Drug loaded nanoemulsion (RU-NE-F7)

***In vitro* Drug permeation studies**

The release profile of RU from RU-NEs (F7) and plain drug suspension (PDS) through the dialysis membrane in PBS (pH 6.4) is shown in (Fig. 5.) The release pattern of optimized nanoemulsion appears to be fast release with negligible burst effect.

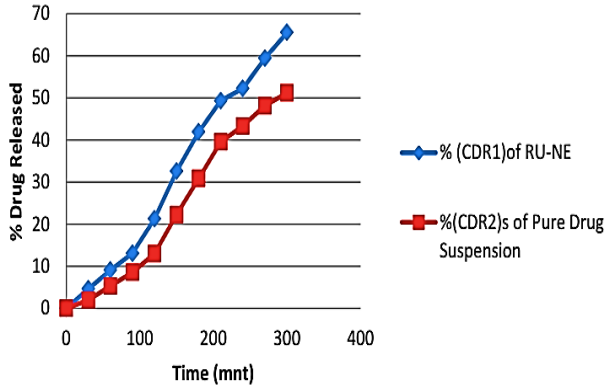
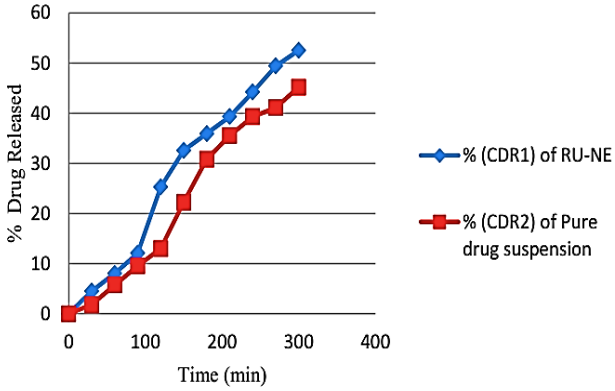


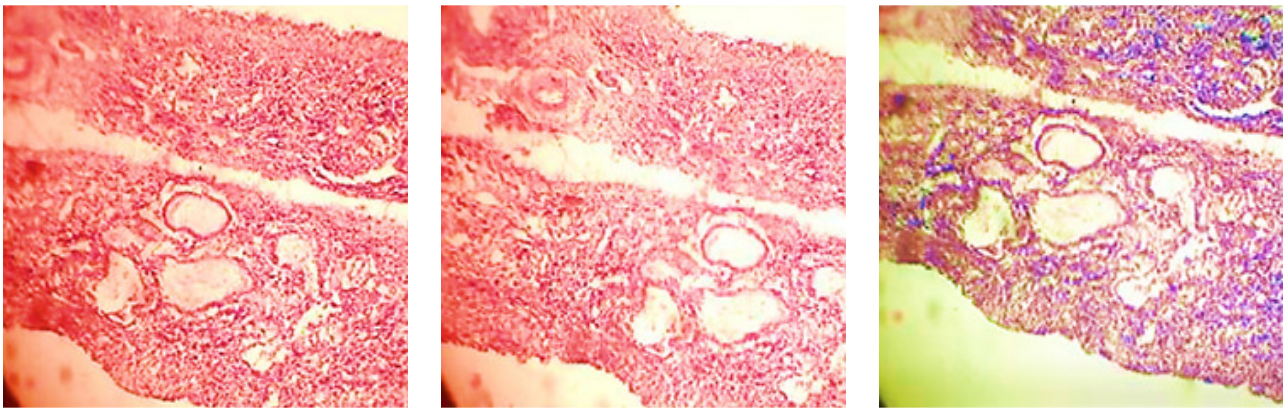
Figure 5: *In vitro* drug release of RU - suspension and optimized RU –NE

***Ex-vivo* Permeation Studies**

NE formulation was subjected to *ex-vivo* permeation studies using the sheep nasal mucosa. Ex vivo nasal mucosa permeability profile was shown in (Fig. 6.). The drug diffused at faster rate and the total percentage diffusion was much higher from the nanoemulsion system[51] than PDS. After 11 hours of diffusion 52.52 % of drug was diffused from drug loaded NE.

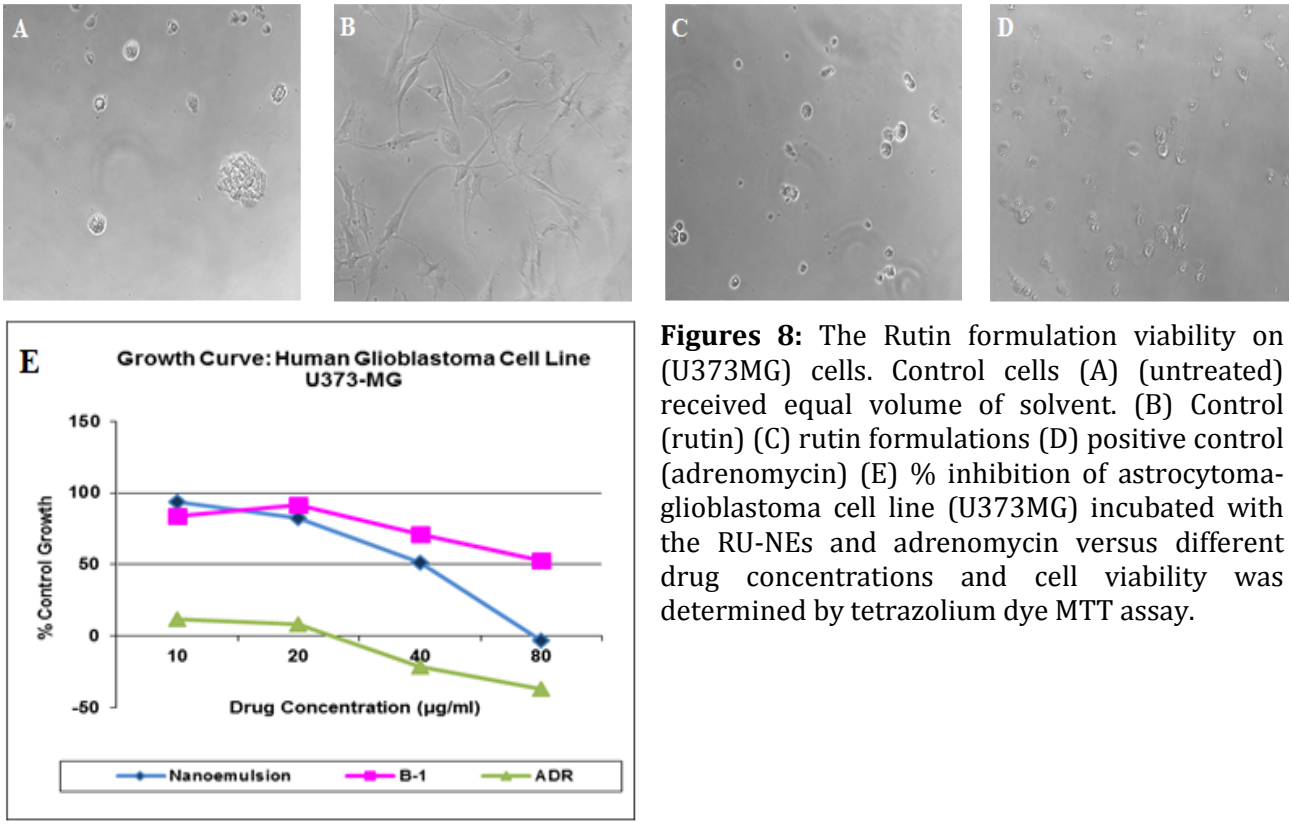


Figures 6: *Ex-vivo* permeation profile of RU-NE and plain drug suspension (PDS) through sheep nasal mucosa



A B C

Figures 7: Microscopic images illustrate the Histopathological condition of nasal mucosa after 2 h exposure of (A, negative control) simulated nasal fluid pH 6.4; (B) RU-loaded NE (C, positive control) IPA



Figures 8: The Rutin formulation viability on (U373MG) cells. Control cells (A) (untreated) received equal volume of solvent. (B) Control (rutin) (C) rutin formulations (D) positive control (adrenomycin) (E) % inhibition of astrocytoma-glioblastoma cell line (U373MG) incubated with the RU-NEs and adrenomycin versus different drug concentrations and cell viability was determined by tetrazolium dye MTT assay.

Histopathological studies

It is necessary to examine histological changes in nasal mucosa caused by formulations, if it is to be considered for practical use [52, 53]. Histological studies show negative control mucosa (normal nasal mucosa) and positive control mucosa stained with hematoxylin-eosin and the effect of formulation on sheep nasal mucosa, 2 hours after applying the formulations (Fig.7.). No change in mucosal structure was seen when treated with drug loaded NE (F7) as compared to the positive control. The section of mucosa treated with formulation RU-NE showed no changes in nasal epithelium [54]. There was no sign of remarkable destructive effect of formulations on the treated nasal mucosa [55].

***In vitro* cytotoxicity assay of CRM-NLCs**

The in vitro cytotoxicity of freeze-dried RU-NEs' as the untreated equal volume of solvent (A) to control (B) with RU-NE formulation to positive control (adrenomycin) (D) and the graph of % inhibition of astrocytoma-glioblastoma cell line (U373MG) incubated with the RU-NEs and

Table 3: Stability study of RU loaded NE (F7) in terms of MDS, Transparency, Zeta potential and %DC (n = 3)

Stability Parameter	Test period			
	0 month	**1 month**	**2 month**	**3 month**
Transparency	Transparent	Transparent	Transparent	Transparent
MDS (nm)	228±0.027	229.6 ± 1.80	229.4± 0.03	230.1 ±0.013
Zeta Potential	-0.0649	-0.0604	-0.0594	0.0521
PDI	0.189 ± 0.89	0.197 ± 1.83	0.195 ± 0.92	0.262 ± 1.045
% DC	94.72 ± 1.14	92.02 ± 0.41	90.98 ± 1.05	90.98 ± 1.05

adrenomycin versus different drug concentrations (E). From the results, we can see that at the same concentration, RU-NEs produced higher inhibition on the cells. Results are shown in Table 2 and Fig.8. Graph of % inhibition of astrocytoma-glioblastomacell line (U373MG) incubated with the RU-NE and adrenomycin Vs. different drug concentrations. From results, we can see that at the same concentration, RU - NE produced cell inhibition on the cells when the drug concentration is 40µg/ml. The pure drug Rutin is only able to cause cell inhibition. Rutin does not show cytotoxicity. To explain the cytotoxic effect of NE, it was hypothesized that NE absorbed to cell surface and released RU close to the membrane, leading to a high local drug concentration gradient or was brought in the cells and then released from the NE. The IC50 values were found tobe 41.8 µg/ml for RU-NEs and < 10 µg/ml for adrenomycin, demonstrating effectiveness of RU-NEs against the gliblastoma cell line. Thus, RU-NEs show higher cellular viability which means that it decreased the cytotoxicity of formulation towards cells. To explain the cytotoxic effect of NEs, it was hypothesized that NEs absorbed to cell surface and released RU close to the membrane, leading to a high local drug concentration gradient or was brought in the cells and then released from the NEs.

Table 2: Drug concentrations (µg/ml) calculated from graph

U373-MG	LC50	TGI	GI50*
Nanoemulsion	NE	77.7	41.8
B-1	NE	>80	84.9
ADR	>80	24.3	<10

Accelerated Stability Study

Formulation showing optimum droplet diameter, PDI and zeta potential was selected for stability studies. According to ICH guidelines, selected formulation (F7) was stored at 40˚C temperature and 75 % relative humidity (RH) for a period of 3 months [56, 57-59]. Formulations were evaluated at periodical intervals of one month for various evaluation parameters which shown that all results are in acceptable limits (Table 3). The optimized batch shows that formulation can be stored well at room temperature (25˚C). From stability studies, it was observed that globule size was slightly increased from 228 ± 0.027 nm to nm and % DC was decreased to 90.98 ± 1.05 % during storage. Additionally, there was not much change in PDI means, initially it was 0.189 ± 0.89 and changed to 0.262 ± 1.045. Minimum loss of % DC indicates that the drug was retained within the homogeneous system during the stability period and minimum loss of drug was occurred. The obtained results revealed that there was no significant change in the MGS, PDI and % DC indicating that they were found to be stable at 25 ± 2°C, 60 ± 5% RH for a total period of 3 months.

CONCLUSION

Rutin Nanoemulsion were fabricated by Spontaneous Emulsification technique using Tween 80, Polyethylene glycol 400 and Ethyl oleate and finally characterized for different parameters like, Differential Scanning Calorimetry (DSC) and transmission electron microscopy (TEM) study revealed that Rutin was completely homogenies in Nanoemulsion. Then the formulations were subjected to *in vitro* cytotoxicity using U373MG cell line and Histopathological studies. IC_{50} value was obtained 9.8 µg/mL of Rutin nanoemulsion and it did not show any toxicity and so were safe for

intranasal delivery for brain targeting. In-vitro diffusion studies revealed that Rutin loaded nanoemulsion (RU-NE) had a significantly higher release. From stability study, it was found that MDS, ZP and % DC was not significantly changed during the period of three months at 25 ± 2°C and 60 ± 5% RH. Hence, in colloidal drug delivery system, Rutin was successfully loaded into Nanoemulsion by Spontaneous method with high Drug content. The results shows that drug loaded nanoemulsion for intranasal administration may be very promising approach for delivering an anticancer agent, at a required site of action for treatment of brain tumor.

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CONFLICT Of INTEREST

The authors report no declarations of interest

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