

## Research Article

# Formulation and Evaluation of Quercetin Nanoemulsions for Treatment of Brain Tumor via Intranasal pathway

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## Abstract

**Objective:** Quercetin-flavonoid-polyphenolic has gained attention in prevention of brain cancer. The low permeability of Quercetin (QUR) 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 QUR are required for the treatment of Cancer. The aim of this research work was to develop in Nanoemulsion (NE) loaded with QUR, for CNS targeting. **Method:** Quercetin is a poorly water soluble anticancer drug, with oral bioavailability is about 4%. Nanoemulsion were fabricated by Spontaneous Emulsification technique. Oleic acid was used as oil. Tween 80 was employed as surfactant and Polyethylene glycol 400 was employed as co-surfactant. **Conclusion:** QUR loaded NE for intranasal delivery are considered as promising vehicle for its targeting to CNS to treat the brain cancer.

**Keywords:** Intranasal delivery, Nanoemulsion, Brain targeting, Spontaneous emulsification technique.

## 1.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. Modern colloidal nanoparticulate system was novel approach to overcome the problems of chemotherapy.

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. Quercetin (QUR) (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. QUR having a promising ability to inhibit angiogenesis, it is process for formation of new blood cell in blood vessel for tumor growth, QUR having ability to stop the new blood cell formation in blood vessels responsible for tumor growth and shows antiangiogenic activity. The goal of this study was formulate nanoemulsion (NE) containing QUR for intranasal (nose to brain) delivery to central nervous system (CNS) for the treatment of brain tumor (Shah et al., 1994; Savale et al., 2015; Savale et al., 2017).

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## Materials and methods

### Materials

Quercetin (QR) was a gift from Loba Chemie Ltd. (Mumbai, India). Oleic acid, Tween 80 and polyethylene glycol 400 (PEG 400) were purchased from Loba Chemie Ltd. (Mumbai, India).

### Methods

#### Formulation of Nanoemulsion (NE)

#### Selection of Excipients for formulation

The solubility of Quercetin (QR) in various oils (castor oil, oleic acid, ethyl oleate, soya oil, coconut oil, oleic acid and clove oil), surfactants (Tween 20 and Tween 80) and co-surfactant

(polyethylene glycol 400, polyethylene glycol 200 and propylene glycol) was determined by using Screening technique (Savale et al., 2017).

#### Preparation of Nanoemulsion (NE)

QR-NE were prepared by spontaneous emulsification technique (Low energy emulsification technique) by slowly pouring the oil, surfactant and co-surfactant mixture using Vortex mixer (Sphinx Ltd, India) into aqueous phase. QR (150 mg/ml) was dissolved in mixture of Oleic acid ( $\mu$ l), Tween 80 (ml) and PEG 400 (ml) was slowly added with stirring at 500 rpm using magnetic stirrer and formulation composition was reported in Table 1.

**Table 1. Compositions of QR-NE formulations**

Formulation batches	Con. of Oil (Oleic acid) ( $\mu$ l)	Con. of Surfactant (Tween 80) (ml)	Con. of Co-surfactant (PEG 400) (ml)	Particle size in (nm)
F1	200	2.00	7	195.12
F2	200	2.00	9	155.95
F3	200	3.50	7	126.84
F4	200	3.50	9	106.29
F5	180	2.00	9	115.36
F6	180	2.00	7	177.25
<b>F7 (op)</b>	<b>170</b>	<b>1.00</b>	<b>5</b>	<b>65.41</b>
F8	108	2.50	9	108.25

### Physicochemical characterization of quercetin loaded nanoemulsion (QR -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. 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 (Yang et al., 2004).

#### 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. 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 (Vanitasagar et al., 2013).

#### Drug Content

The drug content of formulation was determined by UV spectrophotometric method. QR from NE formulations was extracted by dissolving 1 ml of NE in methanol. QR content in the Methanolic

extract was analyzed spectrophotometrically (UV 1700, Shimadzu, Japan) at 257 nm, against the standard Methanolic solution of QUR (Savale et al., 2017).

#### ***In vitro* Drug permeation studies**

*In vitro* diffusion study of optimized NE 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. 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. After a pre-incubation time of 20 minutes, the NE equivalent to 20 µg of QUR was placed in the donor chamber. Samples were periodically withdrawn from the receptor compartment for 4 hours and replaced with the same amount of fresh PBS, and assayed by a UV spectrophotometer at 257 nm (Savale et al., 2017).

#### **Histopathological Studies**

Histopathological studies were carried out using isolated sheep nasal mucosa. Three sheep nasal mucosa pieces (A, B and C) with uniform thickness were selected and mounted on Franz diffusion cells. A was treated with PBS (pH 6.4, Negative control), C with isopropyl alcohol (Positive control), and B was treated with drug (QUR) loaded NE respectively. After treatment for 8 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 (Patel et al., 2005).

#### **Results and discussion**

##### **Preparation and characterization of nanoemulsion**

The QUR-loaded NEs were prepared using spontaneous emulsification technique. For the

preparation of oleic acid as a liquid lipid. Tween 80 were selected as a surfactant and Polyethylene glycol 400 as a co-surfactant a stabilizer, respectively.

##### **Photon correlation spectroscopy and Zeta Potential**

The particle size (nm) and zeta potential (mV) of QUR-loaded NEs (F7) was found to be 65.41 nm and -38.12 mV respectively.

##### **Drug Content**

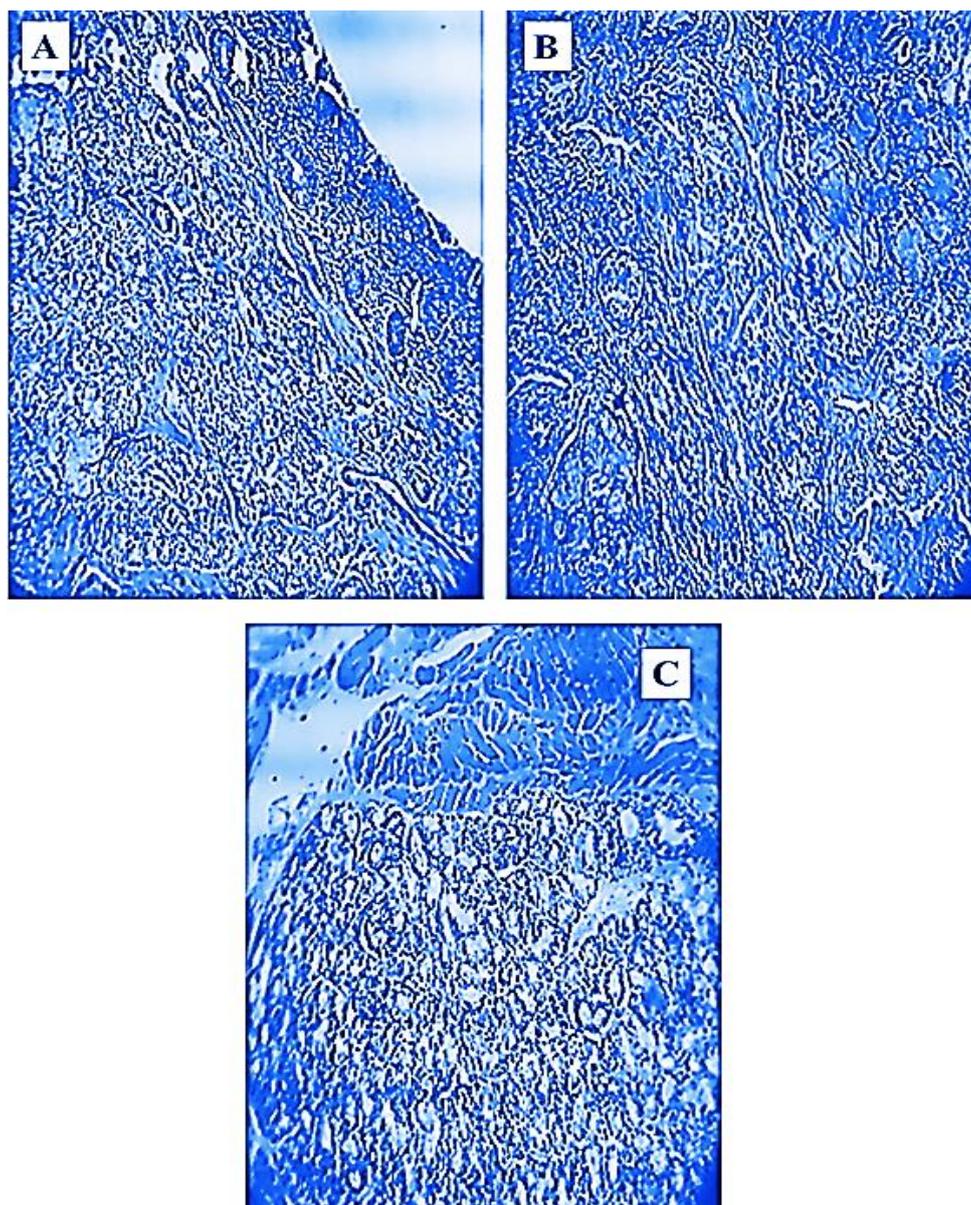
The concentration of oil and surfactant: co-surfactant was important effect on drug content. The oil content (oleic acid) was increases, to increase drug content and the surfactant and co surfactant concentration (tween 80 + PEG 400) decreases to increase drug content. Because drug having maximum solubility in oil phase and drug content of optimized formulation was found to be 99.16 %.

##### ***In vitro* Drug permeation studies**

The release profile of QUR-loaded NE (F7) through the dialysis membrane in PBS (pH 6.4) was found to be 99.55 %. The release pattern of optimized NE appears to be fast release with negligible burst effect.

##### **Histopathological studies**

It is necessary to examine histological changes in nasal mucosa caused by formulations, if it is to be considered for practical use. 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, 8 hours after applying the formulations (Figure 1). 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 QUR-NE showed no changes in nasal epithelium. There was no sign of remarkable destructive effect of formulations on the treated nasal mucosa.



**Figure 1: Microscopic images illustrate the Histopathological condition of nasal mucosa: simulated nasal fluid pH 6.4 (A, negative control); QUR-loaded NE (B); positive control IPA (C).**

### Conclusion

QUR have various activities as it may be anticancer, antioxidant, anti-inflammatory drug lipophilic in nature having low oral Bioavailability (4%) is selected as candidate for the development of NE for its intranasal delivery to target the CNS. The result of present investigation shows that drug loaded QUR-NE for intranasal administration may be very a promising approach for delivering an anticancer agent in order to achieve CNS targeting for the treatment of brain tumor, in particular, for producing the cytotoxic effect. However, clinical benefits to the risk ratio of the formulation

developed in this investigation will decide its values in the clinical practice for the treatment of brain tumor.

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