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Development and Evaluation of Myricetin Nanoemulsion for Liver Cancer Therapy: In-vitro and cell line study

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Abstract

Objective: -Development and evaluation of Myricetin Nanoemulsion for liver cancer therapy.
Materials and methods: Nanoemulsions were made by aqueous titration method. Sefsol 218, Tween 20 and polyethylene glycol 400 (Smix; 4:1) were used as oil phase, surfactant and co-surfactant while distilled water acted as an aqueous phase. Nanoemulsion was characterized on the basis of physical stability testing, globule size, viscosity, electrical conductivity, Transmittance& refractive index. Further, In vitro release, TEM Analysis & cancer cell line studies were also performed.
Results and discussion: According to physical stability testing F4 formulation is selected. The optimized formulation (F4) with Globule size of 392.8 nm showed and a viscosity of 24.21 cps, maximum drug release (%) in 88.01% in 24 h compared with conventional and standard suspensions of myricetin. The cancer cell line studies on HepG2 of MTT Assay also confirmed that myricetin nanoemulsion reduced the cell viability at different concentration.
Conclusion: Our results concluded that nanoemulsion may be an efficient carrier for oral delivery of myricetin against liver cancer without damaging normal cells.

Keywords: Liver cancer, Nanoemulsion, Myricetin, Cell line, HepG2

1. INTRODUCTION

Cancer is the 2nd main cause of demise in the world after cardiovascular diseases. Half of the men & one-third of women in the (US) United States will grow cancer during their lives. The word cancer is a Greek word that means Karakinos to relate to carcinoma tumors by physician Hippocrates (460–370 B.C), but he was not the first person to find this disease (Akulapalli, 2009). In all types of cancer, the body's cells start to divide without ceasing & extend near tissues. It starts at any place in the human body, it is made up of trillions of cells (Bray et al, 2018). The current 5-year, 10-year, 15-year & 20-year periods approximate all parallel durability rates for interfering cancers together (all races, both sexes) will be about 1%, 7%, 11% & 11%, similarly more than evaluate by cohort-based analysis (Hermann Brenner et al, 2002). It is a group of diseases having a main general health issue worldwide &the 2nd leading cause of mortality in the US. The evaluation of numbers of incidence mortality in 2019 in the US & for all states, an exhausting overview of cancer presence based on the most current population-based data for cancer incidence through 2015 and for mortality through 2016(KD Miller et al, 2020). HCCs develop mainly as slow-growing nodules which may be asymptomatic for many years (HASHEM B et al, 2007). Hepatocarcinoma is made by toxic chemicals, a large amount of consumption of alcohol, infections & autoimmune disorders (Afreen U et al, 2016). Many cases of cancer occur in the liver but do not begin. They metastasized from anywhere in the body; this type of liver cancer is known as secondary liver cancer. In Europe & United States secondary liver cancers are more frequent than primary liver cancer, but in Asia & Africa, primary liver cancer is more common (Afreen U et al, 2017). 10% of liver diseases develop in non-cirrhotic livers (Theise ND et al, 2014). 90% or more of liver disease cases enhance in a chronically inflamed liver, aconsequence of chronic alcohol addiction viral hepatitis, and introduction to digestible carcinogens like aflatoxin B1 & nitrosamines (Peter F et al, 2010). HCC is one of the major causes of cancer deaths in Asia & Africa (Hideaki Tsukuma et al, 2005). Alcoholic liver disease, which is known as fatty liver, hepatitis, fibrosis, cirrhosis & hepatocellular carcinoma is a major cause of illness & death in the United States & worldwide. Rising confirmation supports the advice that strain takes part in a pivotal part in the etiology of alcoholic liver disease (Vishnudutt Purohit et al, 2003).

Myricetin (MYR) will 1st separate in the late 18 century from the bark of *Myrica nagi* Thunb (Myricaceae), it is gathered in India, as light yellow-colored crystals. It is poorly soluble in water. The nutraceuticals & anti-oxidant properties of myricetin are highly valuable. Myricetin is structurally related to several well-known phenolic compounds such as quercetin, Morin, kaempferol & fisetin (Deepak Kumar et al, 2016). It distinguishes by the pyrogallol B-ring & it is a much-hydroxylated structure it is known to be managing for improved biological properties in contrast with other flavonols. MYR is highlighted because of its numerous biological activities like antioxidant, anticancer, anti-inflammatory, anti-amyloidogenic, antibacterial, antiviral, and antidiabetic effects (Kwang-S et al, 2016). In studies of various varieties of carcinoma cells, it is shown to suppress cancer cell invasion & metastasis, and to induce cell cycle arrest & apoptosis of cancer cells. It inhibits their proliferation. These data increased curiosity about MYR as a likely tumor obstacle in human patients (Min Jiang et al, 2019). The low bioavailability of MYR (9.62 and 9.74 % at oral doses of 50 and 100 mg/kg) was also attributed to its poor aqueous solubility (2µg/mL) (Chao H et al, 2014). Recently to enhance the solubility & stability of

MYR involves the formulation of chitosan-based Nanocarriers (Noha N et al, 2020). It is a natural flavonoid and has great interest due to its antioxidant & free-radical scavenging potential. The physicochemical instability critically impairs its design of dosage form, evaluation & administration (Dina M.G et al, 2017).

Nanoemulsion (NE) comprises two words Nano and Emulsion, meaning emulsion whose particle size is in nanometre range i. e.10− 9 m. Miniemulsion was a similar term thathat wased for Nanoemulsion. The components of Nanoemulsion are oil, surfactant, co-surfactant & aqueous phase that show in the isotropic, thermodynamically stable, transparent, or translucent systems (Asad Ali et al, 2017).

NE is used in pharmaceutical industries & the reason that it increases the solubility of lipophilic drugs that show in bioavailability enhancement of these substances by particle size reduction of powdered drugs and Nano-sized droplet formation with range (10-100 nm). Nanoemulsion does not require energy input (Zainab et al, 2020). Nanoemulsion is attractive because they are relatively least sensitive to physical and chemical changes. The main method of preparation of Nanoemulsion is high and low energy (Ankur et al, 2016). They are biphasic dispersion in nature of two immiscible liquids: water in oil (W/O) / oil in water (O/W) droplets maintained by an amphiphilic surfactant (Yuvraj Singh et al, 2017). The investigation was aimed at the development and evaluation of myricetin nanoemulsion against liver cancer. This study's main aim is to improve bioavailability and solubility & miscibility and check evaluation parameters & show effects on liver cancer check its stability assessment and finally work on a cell line study.

2. MATERIALS

Myricetin with a purity greater than 98% was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China) Sefsol 218, and Sefsol228 were obtained as a gift sample from Nikko Chemicals (Tokyo, Japan) Capryol 90, Labrafil m 1944 cs, cremophor EL, cremophor RH- 40, Transcutol-P were procured from Gattefos Saint-Priestiest, Cedex, France). , tween 80, methanol were procured from Merck (Schuchardh, Hokenbrunn, Germany) propylene glycol 400 was purchased from SD Fine Chem. (Mumbai, India) phosphate dihydrogen phosphate, sodium hydroxide, potassium chloride, hydrochloric acid,

3. METHODS-

Selection Of Oil, Surfactant, Co-Surfactant-

The selection was done based on solubility & miscibility studies. The solubility of myricetin was measured by adding an excess amount of the drug into 2 mL of Eppendorf tube by vortex mixing for 5 min (vortex mixer XW-80A, Shanghai, China). Mixtures of oils (Sefsol 228, Sefsol 218, Capryol 90, Labrafil M 1944 CS, Castor oil), surfactants (Labrasol, Cremophor EL, Cremophor ELP, Kolliphor HS 15, Tween 80, Tween 20, Span 80, or Span 85) or cosurfactants (ethanol, Transcutol HP, PEG 400, 1,2-propanediol) with Myr (Jin Qian et al,2017). The equilibrated samples were removed from the shaker and centrifuged at 10,000 rpm for 0.25 h using a high- speed centrifuge (Sigma3K30, Sigma Laboratory Centrifuges, Osterode am Harz, Germany). The supernatant was separated & dissolved in methanol and filtered through 0.2 μM membrane filters (Hi Media, India). Drug concentration was evaluated by using a UV spectrophotometer (Shimadzu Corp, Kyoto, Japan). The solubility studies were done in triplicate & outcomes were announced as ±SD (BrijeshM et al, 2013) For miscibility studies uniform quantity (1:1 ratio) of selected oil attached to surfactant/co-surfactant & mixed by using a vortex shaker (Nirmal International, Delhi, India) up to 0.25 h & the mixtures were permitted to stand for 24 h at room temperature & detect of any sign of turbidity, phase separation/color our change. Those mixtures which appear with great miscibility with no hint of turbidity& phase separation & seem clear were appraised for the expansion of nanoemulsion.

The oil, surfactant & co-surfactant which appear most solubilities of myricetin were taken for other studies (MD Faheem et al, 2018).

Construction of pseudo-ternary phase diagrams-

Construction of pseudo ternary phase diagrams was done by (aqueous titration method) using three components i.e., oil, Smix (surfactant-co-surfactant mixture) & distilled water (T.R. Kommuru et al, 2001). Based on solubility studies, capryol 90 is an oil phase for the expansion of nanoemulsion (M. Srivastava et al, 2014) whereas tween 80 & Transcutol P is used are surfactants and co-surfactant. Surfactant & co- surfactant (Smix) were mixed in different volume ratios (1:1, 1:2, 2:1, 3:1, 4:1 and 5:1) to obtain different pseudo-ternary phase diagrams. For each phase diagram, oil & Smix were mixed & vortexed thoroughly at different volume ratios starting from 1:9to 9:1 in different glass vials [12 &14]. 16 different combinations of oil & Smix, 1:9, 1:8, 1:7, 1:6, 1:5,2:8 (1:4), 1:3.5 (2:7), 1:3 (2:6), 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5(1:1),6:4 (1:0.7), 7:3 (1:0.43), 8:2 (1:0.25), 9:1 (1:0.1) were made so that maximum ratios were covered to form a clear & homogenous system. Slow titration with aqueous phase done to various mixtures of oil & Smix, using micropipette under continuous stirring by vortex mixing until formation of transparent oil in water (O/W) Nanoemulsion take place. Phase diagrams were planned using CHEMIX School software version 4.0 (Arne Standnes, USA). Nanoemulsion about to marked on a pseudo ternary phase diagram with 1 axis act on the aqueous phase, the 2 one act on oil & the third act on Smix (a mixture of surfactant & co-surfactant) at a secure mass ratio (Charles L et al, 2011).

Physical stability testing of nanoemulsion-

Heating-cooling cycle- 6 cycles between refrigerator temperatures (4°C) and 45°C, with storage at each temperature for not less than 48 hours were studied. These formulations that are stable at these temperatures were subjected to a centrifugation test (Javed A et al, 2011).

Centrifugation study- in this study myricetin-loaded NE was centrifuged at 3500 rpm for 30 min to see any phase separation (Javed A et al,2014).

Freeze-thaw cycle- In this study, myricetin-loaded NE was subjected to three freeze-thaw cycles done for the formulations between –21 and +25 °C with formulation performed and formulations are stored at each temperature for not less than 48h (SANJULA B et al,2007 & Asad A et al,2017).

Preparation of myricetin loaded NE-

The aqueous titration method was used for preparing the drug-loaded NE. In this method, a fixed amount of MYR was liquefied in the oil phase (Sefsol 218) using a vortex mixer (Nirmal International, Delhi, India). A mixture of a fixed amount of Smix (Tween 20: PEG-400) was added & mixed continuously on a magnetic stirrer (Remi Instrument Ltd., Mumbai, India). Then the specified amount of distilled water was added drop by drop to this mixture & mixed continuously until transparent and homogeneous NE is produced.

Dispersibility test-

The planning of self-emulsification of NE was appraised using a standard USP XXII dissolution apparatus 2 (Colin W et al, 1997 & Shui-Mei K et al, 1998) .1 ml of every formulation was attached to 500 mL of Distilled water at 37 ± 0.5 °C. The stainless- steel dissolution paddle rotates at 50 rpm to give lenient distress. The In-vitro results of the formulations were visually appeared by the grading system:

Grade A: Fast forming nanoemulsion (within 1 min), having a clear or blue color appear.

Grade B: Fast forming, a bit less clear emulsion, having a blue-white in appearance.Grade C: Milky emulsion forms within 2 min.

Grade D: Dull, greyish white emulsion having less oily in appearance which results in less emulsification (longer than 2 min).

Grade E: Formulation reveals that is bad or less emulsification with large oil globules present on the surface (Sheikh S et al, 2007).

Transmittance (*%T*)

The percentage transmittance (%T) of made nanoemulsion was measured using a UV spectrophotometer (Shimadzu Corp, Kyoto, Japan) at 638.2 nm against distilled wateras a blank (Mukesh K et al,2009).

Viscosity determination-

It is determined by using a Brookfield-type rotary viscometer at different shear rates at many temperatures (Manjit J et al, 2015).

Electro conductivity studies-

To measure of electric conductivity of t nanoemulsion sample is a conductivity meter. The conductivity meter has a cell continual of 0.11cm-1 at a frequency of 94Hz. At a temperature of 25±1 °C, the procedure was performed in a triplicate manner (Halnor V et al, 2018).

Refractive index-

The refractive index n of a method is explained as the ratio (f) to the speed (c) of a wave-like as light or sound case of medium to the phase speed (vp) of the wave in the medium (n=c/vp). It is determined by using an Abbes kind refractometer (Nirmal International) at 25±0.5°C (Ronak P et al, 2012).

Globule size, PDI, and Zeta potential measurements-

These are measured by using a Zetasizer (Nano-ZS90, Malvern Instruments, Worcestershire, UK) after appropriate dilution with double distilled water and filtration through a 0.45 mm membrane filter (Saba k et al,2015). Samples of 1 ml of NE were taken individually into clear polystyrene cuvettes for globule size, and polydispersity index, and into the disposable folded capillary cell for zeta potential respectively (Hamza B et al, 2014 & Kunal J et al, 2013).

4. CHARACTERIZATION OF OPTIMIZED NANOEMULSION-

Transmission electron microscopy (TEM)-

The morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM) (Sheikh S et al, 2007). The negative staining survey with or without dilution always works on nanoemulsion to get basic information (Victoria K et al, 2012). The sample was made by placing a drop of NLC that was previously diluted 50-fold with double-distilled water onto a 400-mesh copper grid coated with carbon film followed by negative staining with 1% phosphotungstic acid. The dried sample was put in the air before the TEM survey. The bright field imaging at an increasing magnification coupled with diffraction modes was used to explore the formand size of the NE (Saba k et al, 2015).

In-vitro release study-

The diffusion of sample preparation toward cellulose acetate membrane (molecular weight cut-off / 12,000 & 14,000 Da) and method is dialysis bag diffusion technique. Cellulose membranes were put all night in the release medium. Three milliliters of the sample were placed into the cellulose membrane and both ends of the bags were tied. Later, dialysis bags were carefully immersed in beakers containing a mixture of phosphate buffer solution and ethanol (ratio 7:3 v/v, pH: 7) (Noor HA et al, 2019). To perform these tests, 2 ml of the formulation & drug solution (containing equivalent to 0.5 mg of drug) were put in a discrete dialysis bag & dipped in 100 ml simulated maintained over a magnetic stirrer (Remi Instrument Ltd., Mumbai, India) (Tausif A et al,2014). 3 milliliters of samples were reserved at regular time intervals of 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 18 & 24 h and the equal quantity of drug-free fresh buffer 7 (pH 7) was replaced every time (Mashooq AB et al,2015). The procedure is performed in 3 times at 264 nm by UV spectrophotometer (Shimadzu Corp, Kyoto, Japan) after suitable dilution.

Cell lines study –

HepG2 cells were obtained from American Type Culture Collection (Manassas, VA). Huh-7 cells were acquired from the Japanese Collection of Research Bioresources (JCRB), Tokyo, Japan (Chiara V et al, 2010). HepG2 and Huh-7 cells were continued in Dulbecco's modification of Eagle's medium (DMEM; Cellgro, Manassas, VA) containing 10% fetal bovine serum, 100 units/ml penicillin, 1% L-glutamine, 100 g/ml streptomycin in 5% C O2 at 37°C. This medium was changed every three days (Steven J et al, 2011).

Statistical analysis-
The results were expressed as mean values ± SD The analysis of variance (ANOVA) was applied to examine the significance of differences in MYR-NE properties (such as droplet size, polydispersity index, percent transmittance, refractive index, viscosity, conductivity, and drug content). In all cases, $p < 0.05$ was considered to be significant.

5. Results And Discussion –

Selection of oil, surfactant, and cosurfactant
Oil is the solubilizing agent for lipophilic drugs in the main component in formulating the NE. The high solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in the solubilized form. The solubility of MYR was determined in oils, surfactants, and co-surfactants.
The results are shown in Fig. 1. Among selected oils, MYR had the highest solubility in Sefsol 218 (35 ± mg/ml) other are Sefsol 228 (30 ± mg/ml). Therefore Sefsol 218 was chosen as the oil phase. Among surfactants, MYR showed the highest solubilityin Tween 20 (27 ± mg/ml) and then Tween 80 (23 ± mg/ml). That's why tween 20 was chosen as the surfactants. Tween 80 belongs to the class of non-ionic surfactants and is widely used since it is less toxic compared to ionic surfactants and is less affected by pH and ionic strength. For o/w emulsion, the surfactant should have HLB N 10. Tween 80 has a hydrophilic-lipophilic balance (HLB) value is 15. Among co- surfactants, PEG- 400 showed the highest solubility of 50 ± mg/ml. That's why PEG- 400 was selected as the co-surfactant. PEG-400 having an HLB value of 9.7 can form transparent and stable NE. Co-surfactant interposes between surfactant molecules which lower the interactions between the polar head group at the interfacial layer, rises flexibility of interfacial film around NE droplets, and also increases the fluidity of the interfacial film by penetrating into the surface monolayer. Fig. 1 represents solubility data for MYR in different oils, surfactants, and co-surfactants. All chemicals used were non-irritant and not sensitizing to the skin, pharmaceutically acceptable, and fall under GRAS (generally regarded as safe) category.
Construction of pseudo-ternary phase diagrams-
Pseudo-ternary phase diagrams were c separately for each Smix ratio and the results are presented in Fig. 2. Ternary plots were constructed using Sefsol 218 as oil phase, Tween 20 as a surfactant, PEG-400 as co-surfactant, and distilled water as the aqueous phase. Six phase diagrams of Smix ratios of 1:1, 1:2, 2:1, 3:1, 4:1, and 5:1 with the shaded region showing the formation of the NE system are presented in Fig.
2. From the figure, it was noticed that a Smix ratio of 4:1 showed maximum NE region when compared to 1:1, 1:2, 2:1, 3:1 and 5:1. By using these three phases along with distilled water as aqueous phase, pseudo-ternary phase diagrams were erect to find out the region into which maximum amount of NE formation took place and the results are presented below. The dotted area shows the O/W NE region in different ratios of surfactant to cosurfactant. (a) Smix (1:1); (b) Smix (1:2); (c) Smix (2:1); (d) Smix (3:1); (e) Smix (4:1); (f) Smix (5:1). The area of NE was found in order of Smix 4:1>3:1>2:1>1:1>1:0. From the figure it was noticed that less NE area is shown in the Smix ratio 1:1 followed by 1:2. As compared to the Smix ratios 2:1 and 3:1, 4:1 showed the maximum NE region based on its ability to solubilize the oil phase while decreasing the system's free energy indicating the increasing emulsification with increasing concentration of surfactant. On further increasing the Smix ratio to 5:1 it was noticed that area was slightly decreased signifying that the further addition of the surfactant does not contribute to the emulsification process. Hence, 4:1 of the Smix was approved for the NE region.
Physical Stability Testing of Nanoemulsion-
As mentioned earlier the chosen formulations were passed through different stress conditions, namely, heating cooling cycle, freeze-thaw cycle, and centrifugation (Table 1). Results demonstrated that the formulations prepared from the Smix ratio of 1:2 and 1:3 were not able to pass the stress test performed to assess the physical stability owing to the formulation's insufficient emulsification. Some formulations turn turbid. Physical stability testing of optimized nanoemulsion was done to check any sign of reliability such as precipitation, phase separation, creaming, cracking, and coalescence under different stress conditions and there was no sign of reliability observed during these stress tests.
The physical stability of the NE was affected by zeta potential. The negative sign is an indication of strong repulsion which ultimately prevents particle aggregation and enhances the physical stability of NE formulation. In the present study it was found that the concentration of oil is proportionate to the negative value of zeta potential, i.e., the higher the oil concentration more negative will be the value of zeta potential.
Preparation loaded myricetin NE-
Table 2 for the preparation of the drug-loaded formulation, MYR was dissolved in the oil phase. This was followed by the addition of the required quantity of Smix and the drop wise addition of distilled water until a clear and transparent liquid was attained.
Transmittance, Globule size, Polydispersity index, and Zeta potential measurements Table 3 shows the Globule size, Polydispersity index, and Zeta potential of & transmittance values of MYR nanoemulsion.
In general, the emulsification capacity was considered the main criterion for evaluating surfactants in NE formulations and was characterized by transmittance and appearance. As shown in Table 3 the transmittance values above 96% with clear & transparent were detected in the systems containing Sefsol 218, PEG-400, and Tween 20, which show excellent emulsification capability.
From the studies it was concluded that an increase in the Smix ratio up to a certain level will result in the small globule size of the NE; however after which the globule size will increase with increasing Smix. This may be due to several factors like the increasing Smix concentration up to a certain level or maybe because of the decline in interfacial tension.
Studies demonstrated that increasing the Smix

concentration up to a certain level will result in the decreased PDI of NE, however on further increase in the Smix concentration will result in increased PDI. Additionally, it may be explained that the concentration of the emulsifies opposite site to the flow resistance ultimately results in the NE of higher viscosity. As a result, a higher coalescence rate results in larger droplet sizes as shown in figure 7a.

In-vitro release study-

The drug release studies of MYR from NE and drug solution were performed in liver pH 7. The concentration was calculated by extrapolation of the calibration curve and a graph was plotted between time and percent cumulative release (Figure 5). The cumulative percentage release of MYR from NE was found to be faster i.e., 88.01 % throughout 24 h in comparison to the release from MYR suspension having only 29.11 % drug release after 24 h. In vitro release studies throughout 24 h confirmed an initial burst release and sustained releases go after. The appearance of nanodroplets on NEs surface caused the formulation to release in the majority initially & after that sustained release occur because of the drug's release at the oil-water interface from the oily core which was obstructed by the dialysis bag and aqueous medium. The results of in vitro kinetic release demonstrated that drug release from NE follows a zero-order model owing to the highest value of the coefficient of correlation (R2), i.e., 0.9472 which is nearer to unity.

TEM Analysis-

The morphology of the optimized NE was done by TEM. TEM has indicated that most of the oil globules were of uniform shape (spherical) shape as shown in figure 6.

Cell line-

Cytotoxicity assays the toxicity of the nanoemulsion & the complexes were evaluatedin human hepatoma HepG2 cells through MTT Assay. As shown in figure 7 (A & B) the cell viability to approximately 99.94% as compared with control. The cell viability was reduced to 94.59% and 91.97% at 10 and 100 µg/ml of MYR NE, respectively. Further, MYR at a concentration 250, 500, 1000, 5000 & 10000 µg/ml reduced the viability of cells to 90.07%, 84.07%, 80.80, 77.71%, 43.43% respectively. Although the effects of MYR alone were given on HepG2 cells before but the present study showed better effects as MYR NE reduces the cell viability of cancer cell line in a dose-related without harming the surrounding Chang liver cells (normal cells). The cells used with rising concentrations of NE increased the chromatin condensation as compared with control cells. The optimized formulations also showed in figure 7B reduction in the cell viability and concentration increased MTT Assay of HepG2 against human liver carcinoma cells without harming normal cells. The nanosized particles of MYR followed by a higher surface area may permit a quicker rate of drug release and improved absorption pursued to enhanced bioactivity in a lesser dose ofthe drug.

Table 1 NE formulation composition and physical screening studies

Formulation Code	Surfactant Co-Surfactant (Smix ratio)%	Oil %	Water %	Physical Stability Test			Results
				Centrifugation Test	Heating- cooling Test	Freeze-Thaw	
F1	36.0	4.00	60	PASS	PASS	PASS	PASSED
F2	20	5.00	75	PASS	PASS	PASS	PASSED
F3	42.42	18.18	54.55	PASS	PASS	PASS	PASSED
F4	21.05	14.04	64.91	PASS	PASS	PASS	PASSED
F5	17.91	11.94	70.15	PASS	FAIL	PASS	FAIL
F6	12.50	14.81	55.56	PASS	FAIL	PASS	FAIL
F7	4.62	11.63	65.12	FAIL	FAIL	PASS	FAIL
F8	5.97	23.33	70.15	PASS	PASS	FAIL	FAIL
F9	2	18	80	PASS	PASS	PASS	PASSED
F10	20	10	70	FAIL	PASS	FAIL	FAIL
F11	26.20	8.73	65.07	FAIL	FAIL	PASS	FAIL
F12	20	10	55	PASS	PASS	PASS	PASSED
F13	25	5	65	PASS	FAIL	FAIL	FAIL
F14	47.46	6.78	45.76	PASS	FAIL	FAIL	FAIL
F15	30	5	65.22	PASS	FAIL	FAIL	FAIL
F16	25	5	70	PASS	FAIL	FAIL	FAIL

Table 2: Drug-loaded NE formulation composition and physical screening studies.

FORMULATION CODE	PARTICLE SIZE (d.nm)	ZETA POTENTIAL (mV)	PDI	ELECTRO CONDUCTIVITY (µS/cm)	REFRACTIVE INDEX	TRANSMITTANCE (%)	VISCOSITY (mPa.s)
F1	386.8	-36	0.20	126.6	1.001	96.9%T	18.91
F4	392.8	-30	0.27	127.5	1.356	98.6%T	24.21
F9	300.0	-38	0.19	124.3	1.192	87.1%T	10.32
F12	298.0	-32	0.18	126.9	1.255	84.0%T	9.84

Table 3: Data of particle size, zeta potential, refractive index, transmittance, viscosity, PDI

Formulation Code	Surfactant andco-surfactant Smix ratio%	Oil %	Water %	Physical Stability Test			Results
				Centrifugation Test	Heating-Cooling Test	Freeze-Thaw	
F1	36	4.00	60	PASS	PASS	FAIL	FAIL
F2	20	5.00	75	FAIL	PASS	FAIL	FAIL
F3	42.42	18.18	54.55	FAIL	FAIL	FAIL	FAIL
F4	21.05	14.04	64.91	PASS	PASS	PASS	PASSED
F9	2	18	80	FAIL	PASS	FAIL	FAIL
F12	20	10	55	FAIL	PASS	PASS	FAIL

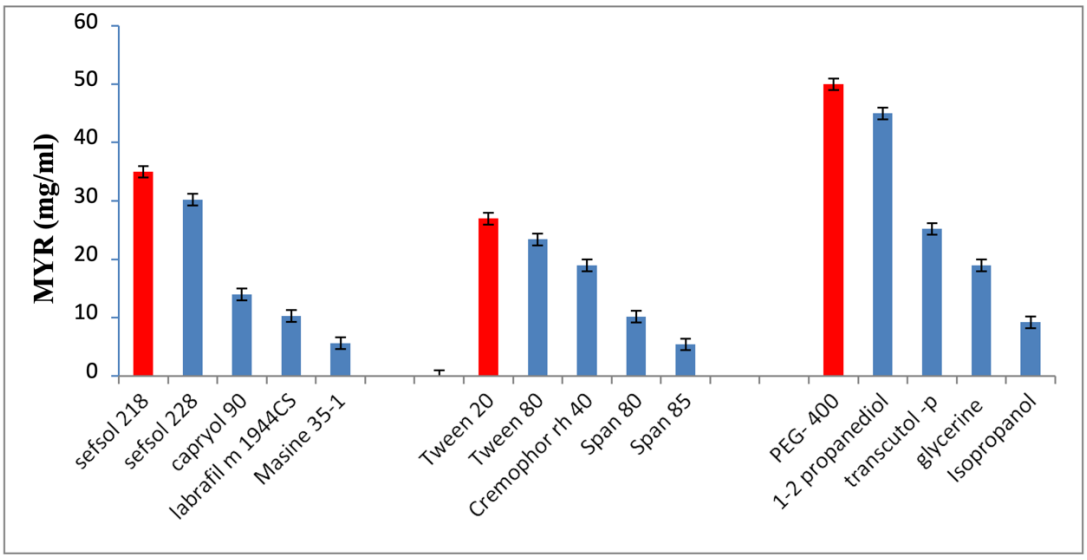


Figure 1. Solubility study data for MYR in oils, surfactants, and co-surfactants

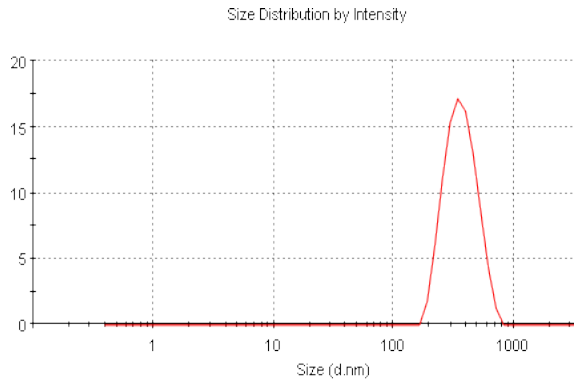
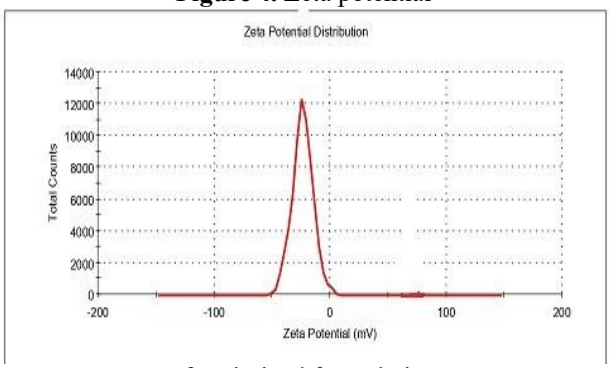


Figure 3. Globule size distribution of optimized formulation

Figure 4. Zeta potential



of optimized formulation

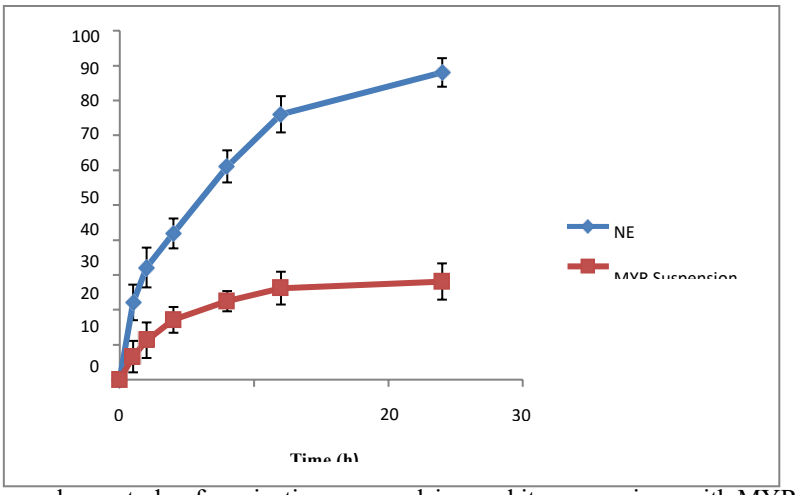


Figure 5. Drug release study of myricetin nanoemulsion and its comparison with MYR suspension

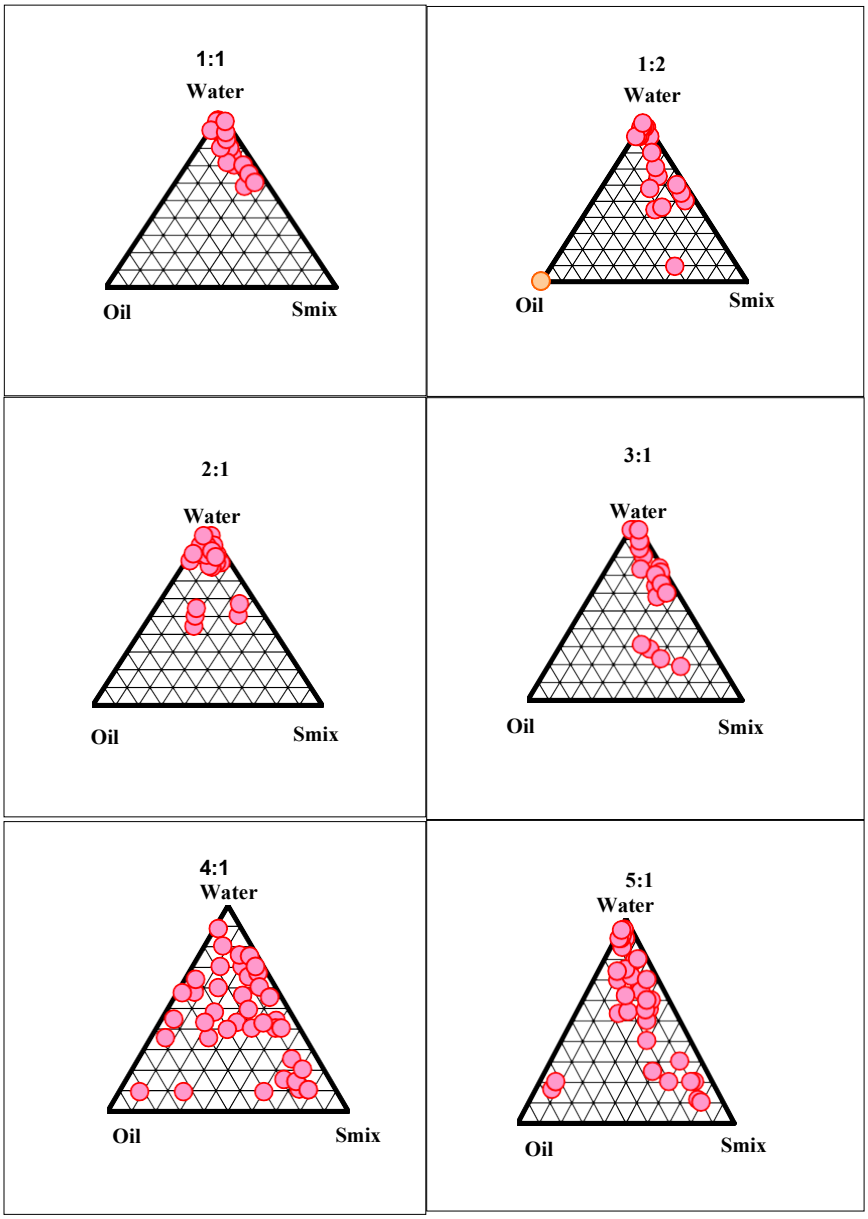
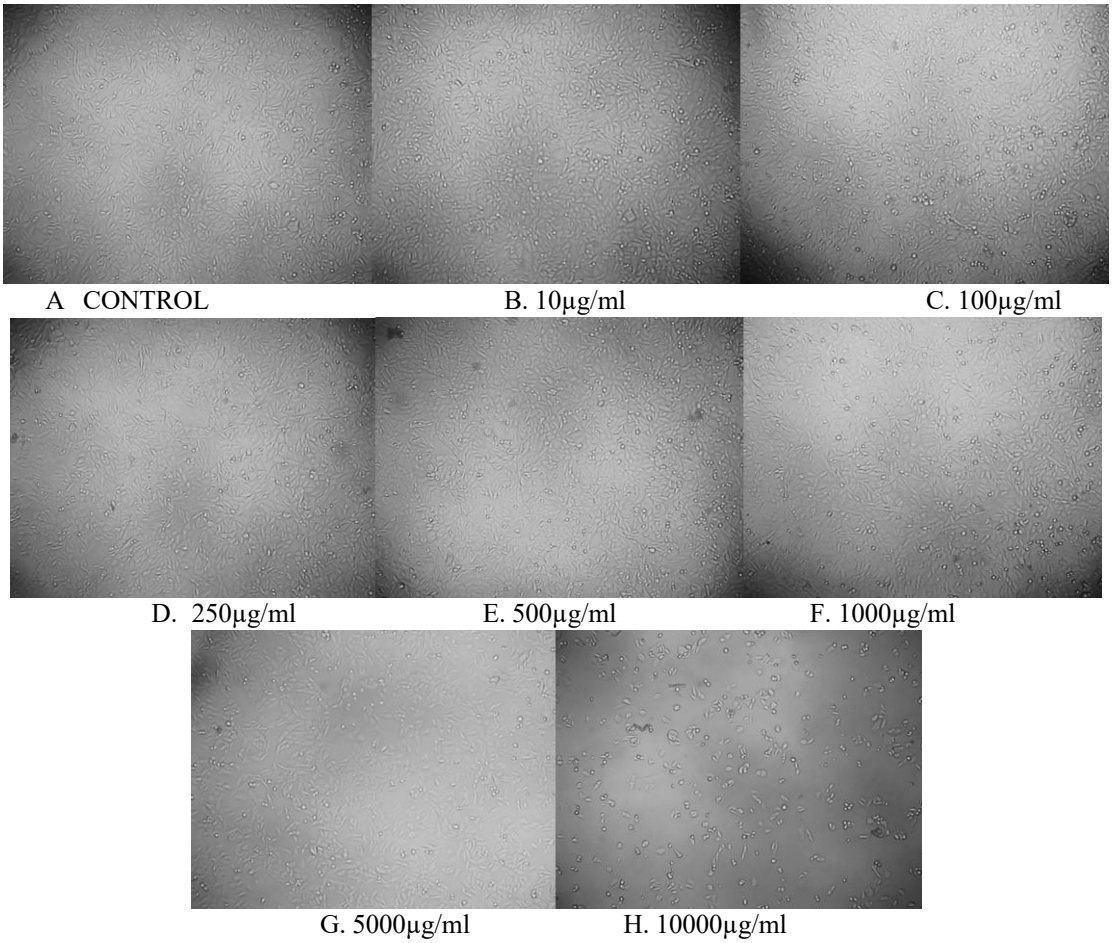


Figure 2. Pseudoternary phase diagrams system containing the following components: Sefsol 218 as oil, Tween 20 as surfactant, and PEG-400 as cosurfactant. The dotted area shows the O/W NE region in different ratios of surfactant to cosurfactant. (a) Smix (1:1); (b)Smix (1:2); (c) Smix (2:1); (d) Smix (3:1); (e) Smix (4:1); (f) Smix (5:1).

(A)



(B)

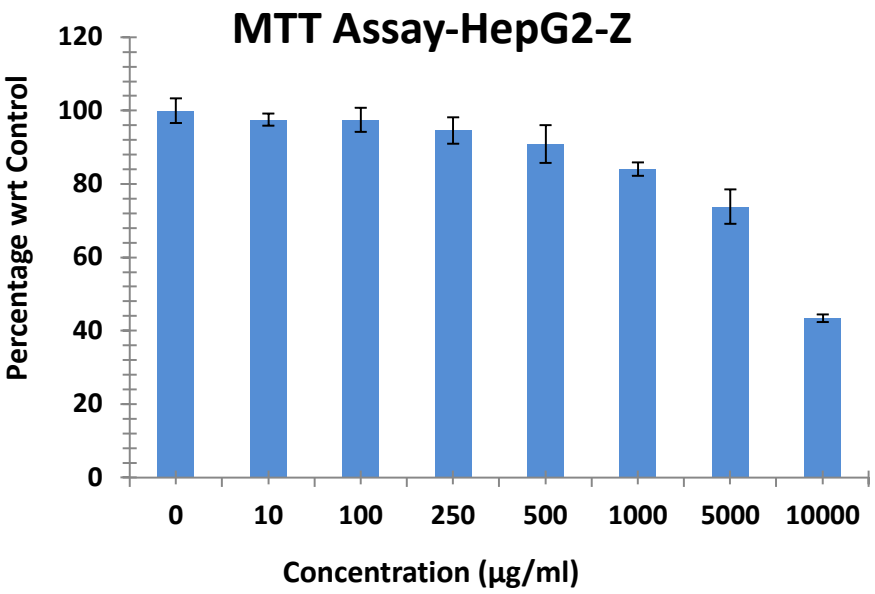


Figure 7. figure A & B represent cytotoxicity test

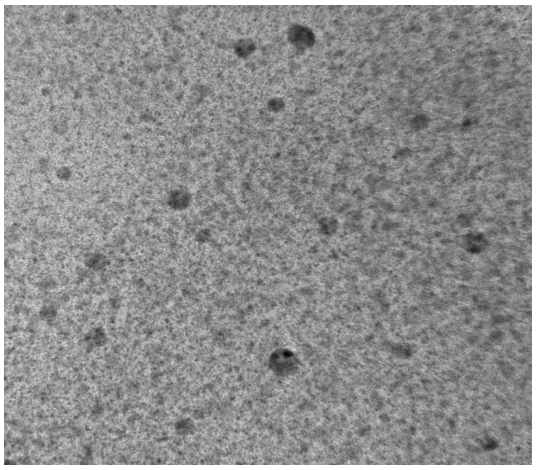


Figure 6. TEM image of optimized formulation

CONCLUSION

In the present study, Myricetin-loaded NE was formulated. Based on solubility, the composition is selected like oil phase (Sefsol 218) 35±1.31, of Smix (Tween 20 and PEG 400)27±1.96 & 50±2.43, as a surfactant and co-surfactant, respectively) and distilled water as an aqueous phase and the construction phase diagram is plotted whereas 4:1 show most NE. The Data obtained from formulations were selected based on the physical stability test & F1, F2, F3, F4, F9, and F12 were selected. Then after adding the drug, the selected formulation should also perform a physical stability test & F4 formulation is selected which does not show any sign of creaming, cracking, or phase separation. Then the F4 formulation was evaluated based on globule size 392. 8dn.m, zeta potential -30mV, PDI 0.27, viscosity 24.21, Transmittance 98.6%T, electro-conductivity 127.5µS/cm & refractive index 1.356. The In- vitro drug release of NE is 88.01% & MYR Suspension 28.11%. The TEM analysis was performed. The cell-line study of HepG2 (MTT Assay) in control 99.98% & with treated decreases at every concentration with cell viability. The cell viability was drastically reduced to 10, 100, 250,500,1000,5000 &10000 µg/ml concentration which show cell viability 94.59%, 91.97%, 90.07%, 84.07%, 80.80, 77.71%, 43.43% respectively of MYR NE. Nanoemulsion may be an efficient carrier for oral delivery of myricetin against liver cancerwithout damaging normal cells.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

1. Sudhakar A. History of cancer, ancient and modern treatment methods. Journal of cancer science & therapy. 2009;1(2):1. doi:10.4172/1948-5956.100000e2.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394-424. https://doi.org/10.3322/caac.21492
3. Brenner H. Long-term survival rates of cancer patients achieved by the end of the 20th century: a period analysis. The Lancet. 2002;360(9340):1131-5. https://doi.org/10.1016/S0140-6736(02)11199-8.
4. Siegel RL, Miller KD, Goding Sauer A, Fedewa SA, Butterly LF, Anderson JC, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2020. CA: a cancer journal for clinicians. 2020;70(3):145-64. https://doi.org/10.3322/caac.21601
5. El–Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology. 2007;132(7):2557-76. https://doi.org/10.1053/j.gastro.2007.04.061.
6. Usmani A, Mujahid MD, Khushtar M, Siddiqui HH, Rahman MA. Hepatoprotective effect of Anacyclus pyrethrum Linn. against antitubercular drug-induced hepatotoxicity in SD rats. Journal of Complementary and Integrative Medicine. 2016;13(3):295-300. https://doi.org/10.1515/jcim-2016-0001.
7. Usmani A, Mishra A. Current updates on risk factors of hepatocellular carcinoma.Res Rev. 2017; 8:23-31.
8. Eun JR, Jung YJ, Zhang Y, Zhang Y, Tschudy-Seney B, Ramsamooj R, Wan YJ, Theise ND, Zern MA, Duan Y. Hepatoma SK Hep-1 cells exhibit characteristics of oncogenic mesenchymal stem cells with highly metastatic capacity. PloS one. 2014;9(10):e110744. https://doi.org/10.1371/journal.pone.0110744.
9. Ferenci P, Fried M, Labrecque D, Bruix J, Sherman M, Omata M, Heathcote J, Piratsivuth T, Kew M, Otegbayo JA, Zheng SS. World gastroenterology organization global guideline. hepatocellular carcinoma (hcc): a global perspective. J gastrointestin liver dis. 2010;19(3):311-7. doi: 10.1097/ MCG.0b013e3181d46ef2.
10. Tsukuma H, Tanaka H, Ajiki W, Oshima A. Liver cancer and its prevention. Asian Pacific Journal of Cancer Prevention. 2005;6(3):244.
11. Purohit V, Russo D, Salin M. Role of iron in alcoholic liver disease: introduction and summary of the symposium. Alcohol. 2003;30(2):93-7. https://doi.org/10.1016/S0741-8329(03)00132-0.
12. Semwal DK, Semwal RB, Combrinck S, Viljoen A. Myricetin: A dietary molecule with diverse biological activities. Nutrients. 2016;8(2):90. https://doi.org/10.3390/nu8020090.
13. Park KS, Chong Y, Kim MK. Myricetin: biological activity related to human health.Applied Biological Chemistry. 2016;59(2):259-69. https://doi.org/10.1007/s13765- 016-0150-2.
14. Jiang M, Zhu M, Wang L, Yu S. Anti-tumor effects and associated molecular mechanisms of myricetin. Biomedicine & Pharmacotherapy. 2019; 120:109506. https://doi.org/10.1016/j.biopha.2019.109506.
15. Yao Y, Xie Y, Hong C, Li G, Shen H, Ji G. Development of a myricetin/hydroxypropyl-β-cyclodextrin inclusion complex: Preparation, characterization, and evaluation. Carbohydrate polymers. 2014; 110:329-37. https://doi.org/10.1016/j.carbpol.2014.04.006.
16. Nafee N, Gaber DM, Elzoghby AO, Helmy MW, Abdallah OY. Promoted antitumoractivity of myricetin against lung carcinoma via nanoencapsulated phospholipid complex in respirable microparticles. Pharmaceutical Research. 2020;37(4):1-24. https://doi.org/10.1007/s11095-020-02794-z.
17. Gaber DM, Nafee N, Abdallah OY. Myricetin solid lipid nanoparticles: Stabilityassurance from system preparation to site of action. European Journal of Pharmaceutical Sciences. 2017; 109:569-80. https://doi.org/10.1016/j.ejps.2017.08.007.
18. Ali A, Ansari VA, Ahmad U, Akhtar J, Jahan A. Nanoemulsion: An advanced vehicle for efficient drug delivery. Drug research. 2017;67(11):617-31. DOI: 10.1055/s-0043-115124.
19. Sadeq ZA. Review on nanoemulsion: Preparation and evaluation. InternationalJournal of Drug Delivery Technology. 2020;10(1):187-9. ; DOI: 10.25258/ijddt.10.1.33.
20. Gupta A, Eral HB, Hatton TA, Doyle PS. Nanoemulsions: formation, properties and applications. Soft matter. 2016;12(11):2826-41. DOI: 10.1039/C5SM02958A.
21. Singh Y, Meher JG, Raval K, Khan FA, Chaurasia M, Jain NK, Chourasia MK. Nanoemulsion: Concepts, development and applications in drug delivery. Journal of controlled release. 2017; 252:28-49. https://doi.org/10.1016/j.jconrel.2017.03.008.
22. Qian J, Meng H, Xin L, Xia M, Shen H, Li G, Xie Y. Self-

nanoemulsifying drug delivery systems of myricetin: Formulation development, characterization, and in vitro and in vivo evaluation. Colloids and Surfaces B: Biointerfaces. 2017; 160:101-9. https://doi.org/10.1016/j.colsurfb.2017.09.020.
23. Haider MF, Khan S, Gaba B, Alam T, Baboota S, Ali J, Ali A. Optimization of rivastigmine nanoemulsion for enhanced brain delivery: In-vivo and toxicity evaluation. Journal of Molecular Liquids. 2018; 255:384-96. https://doi.org/10.1016/j.molliq.2018.01.123.
24. Kommuru T, Gurley B, Khan MA, Reddy IK. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. International journal of pharmaceutics. 2001;212(2):233-46. https://doi.org/10.1016/S0378-5173(00)00614-1.
25. Srivastava M, Kohli K, Ali M. Formulation development of novel in situ nanoemulgel (NEG) of ketoprofen for the treatment of periodontitis. Drug Delivery. 2016;23(1):154-66. https://doi.org/10.3109/10717544.2014.907842
26. Guo RX, Fu X, Chen J, Zhou L, Chen G. Preparation and characterization of microemulsions of myricetin for improving its antiproliferative and antioxidative activities and oral bioavailability. Journal of agricultural and food chemistry. 2016;64(32):6286-94. https://doi.org/10.1021/acs.jafc.6b02184.
27. Lovelyn C, Attama AA. Current state of nanoemulsions in drug delivery. Journal of Biomaterials and Nanobiotechnology. 2011;2(05):626. doi:10.4236/jbnb.2011.225075.
28. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. Alternativemedicine review. 2011;16(3):239.
29. Akhtar J. Novel oral nanoemulsion based drug delivery system of antidiabetic drugs.
30. Faiyaz S, Sanjula B, Alka A, Javed A, Mohammed A, Sheikh S. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. AAPS PharmSciTech. 2007;8(4):1-9.
31. Gaba B, Khan T, Haider MF, Alam T, Baboota S, Parvez S, Ali J. Vitamin E loaded naringenin nanoemulsion via intranasal delivery for the management of oxidative stress in a 6-OHDA Parkinson's disease model. BioMed research international. 2019; 2019:1-20. https://doi.org/10.1155/2019/2382563.
32. Shafiq-un-Nabi S, Shakeel F, Talegaonkar S, Ali J, Baboota S, Ahuja A, Khar RK, Ali M. Formulation development and optimization using nanoemulsion technique: a technical note. AAPS pharmscitech. 2007;8(2):12-7. https://doi.org/10.1208/pt0802028.
33. Kumar M, Pathak K, Misra A. Formulation and characterization of nanoemulsion- based drug delivery system of risperidone. Drug development and industrial pharmacy. 2009;35(4):387-95. https://doi.org/10.1080/03639040802363704
34. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123-7. https://doi.org/10.1007/s13205-014-0214-0.
35. Halnor VV, Pande VV, Borawake DD, Nagare HS. Nanoemulsion: A novel platform for drug delivery system. J Mat Sci Nanotechol. 2018;6(1):104.
36. Patel RP, Joshi JR. An overview on nanoemulsion: a novel approach. International Journal of Pharmaceutical Sciences and Research. 2012;3(12):4640.
37. Bshara H, Osman R, Mansour S, El-Shamy AE. Chitosan and cyclodextrin in intranasal microemulsion for improved brain buspirone hydrochloride pharmacokinetics in rats. Carbohydrate polymers. 2014; 99:297-305. http://dx.doi.org/10.1016/j.carbpol.2013.08.027.
38. Jain K, Kumar RS, Sood S, Gowthamarajan K. Enhanced oral bioavailability of atorvastatin via oil-in-water nanoemulsion using aqueous titration method. Journal of Pharmaceutical Sciences and Research. 2013;5(1):18.
39. Klang V, Matsko NB, Valenta C, Hofer F. Electron microscopy of nanoemulsions: an essential tool for characterisation and stability assessment. Micron. 2012;43(2-3):85- 103. https://doi.org/10.1016/j.micron.2011.07.014.
40. Khan S, Baboota S, Ali J, Narang RS, Narang JK. Chlorogenic acid stabilized nanostructured lipid carriers (NLC) of atorvastatin: formulation, design and in vivo evaluation. Drug development and industrial pharmacy. 2016;42(2):209-20. https://doi.org/10.3109/03639045.2015.1040414.
41. Arbain NH, Salim N, Masoumi HR, Wong TW, Basri M, Abdul Rahman MB. In vitro evaluation of the inhalable quercetin loaded nanoemulsion for pulmonary delivery. Drug delivery and translational research. 2019;9(2):497-507. https://doi.org/10.1007/s13346-018-0509-5.
42. Alam T, Pandit J, Vohora D, Aqil M, Ali A, Sultana Y. Optimization of nanostructured lipid carriers of lamotrigine for brain delivery: in vitro characterization and in vivo efficacy in epilepsy. Expert Opinion on Drug Delivery. 2015;12(2):181- 94. https://doi.org/10.1517/17425247.2014.945416.
43. Bhat MA, Iqbal M, Al-Dhfyan A, Shakeel F. Carvone Schiff base of isoniazid as a novel antitumor agent: nanoemulsion development and pharmacokinetic evaluation.Journal of Molecular Liquids. 2015; 203:111-9. https://doi.org/10.1016/j.molliq.2014.12.037.
44. Dave V, Telange D, Wavare K, Umekar M, Anand S, Patil A. Drug-phospholipid complex-loaded matrix film formulation for the enhanced transdermal delivery of quercetin. Journal of Excipients and Food Chemicals. 2018;9(2):3718.
45. Dave V, Telange D, Denge R, Patil A, Umekar M, Gupta SV. Pentaerythritol as an excipient/solid-dispersion carrier for improved solubility and permeability of ursodeoxycholic acid. Journal of Excipients and Food Chemicals. 2018 ;9(3):4708.
46. Dixit P, Jain DK, Dumbwani J. Standardization of an ex vivo method for determination of intestinal permeability of drugs using everted rat intestine apparatus. Journal of pharmacological and toxicological methods. 2012;65(1):13-7. https://doi.org/10.1016/j.vascn.2011.11.001.
47. Hamilton KL, Butt AG. Glucose transport into everted sacs of the small intestine of mice. Advances in physiology education. 2013;37(4):415-26. doi:10.1152/advan.00017.2013.
48. Kumbhar SA, Kokare CR, Shrivastava B, Gorain B, Choudhury H. Preparation, characterization, and optimization of asenapine maleate mucoadhesive nanoemulsion using Box-Behnken design: In vitro and in vivo studies for brain targeting. International journal of pharmaceutics. 2020;586:119499. https://doi.org/10.1016/j.ijpharm.2020.119499.
49. Vecchi C, Montosi G, Pietrangelo A. Huh-7: A human "hemochromatotic" cell line. Hepatology. 2010;51(2):654-9. https://doi.org/10.1002/hep.23410.
50. Meex SJ, Andreo U, Sparks JD, Fisher EA. Huh-7 or HepG2 cells: which is the better model for studying human apolipoprotein-B100 assembly and secretion?[S]. Journal of lipid research. 2011;52(1):152-8. https://doi.org/10.1194/jlr.D008888.
51. Date AA, Desai N, Dixit R, Nagarsenker M. Self-nanoemulsifying drug delivery systems: formulation insights, applications and advances. Nanomedicine. 2010;5(10):1595-616. https://doi.org/10.2217/nnm.10.126.
52. Fraga M, Laux M, Rejane dos Santos G, Zandona B, Dos Santos Giuberti C, De Oliveira MC, da Silveira Matte U, Ferreira Teixeira H. Evaluation of the toxicity of oligonucleotide/cationic nanoemulsion complexes on Hep G2 cells through MTT assay. Die Pharmazie-An international journal of pharmaceutical sciences. 2008;63(9):667-70. https://doi.org/10.1691/ph.2008.8093.