

Oil of *Mesua ferrea* L. Seed as a Promising Pharmaceutical Excipient in Lipid Based Nanoformulation

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ABSTRACT

This paper reports the studies on the application of *Mesua ferrea* L. (nahor) seed oil as a safe pharmaceutical excipient. The nahor seed oil was extracted by hot continuous percolation process using a soxhelt apparatus and analyzed by TLC and LC-MS. The safety profile of the oil was studied by acute toxicity study in a rat model and MTT assay with mouse brain endothelial cells (bEnd.3). The compatibility of the oil with other excipients was studied by DSC and FT-IR analysis. TLC and LC-MS study suggest the oil is composed of fatty acids similar to other USP grade oils. The acute toxicity study and MTT assay suggest the safe use of the nahor oil at the cellular level in the living system. DSC and FT-IR study confirm the compatibility of the oil with the general components of the lipid nanoparticles. This work provides a new dimension to *M. ferrea* L. seed oil for its safe use in the pharmaceutical formulation.

INTRODUCTION

Pharmaceutical excipients play the greatest role in modern pharmaceutical dosage forms. The importance of excipients has been neglected by the pharmaceutical companies, as we can see almost no or very little money and men power has been utilized to discover new excipients or to determine the safety profile of the existing excipients (Baldrick, 2000). Development of new excipients or finding one in nature gets less importance and funding than an active pharmaceutical ingredient (API) (Bahadur *et al.*, 2014). The formulation development strategies reveal that the same API can act better in the formulations developed with different excipients. Hence, finding a new excipient can lead to the development of stable and efficient pharmaceutical dosage format low cost (Chaudhari and Patil, 2012). Pharmaceutical excipients act as a vehicle to carry the API to the desired site of drug action in the desired dosage form. These vehicles can be either solid or different types of liquids. Fixed oils

are the most important group of non-aqueous pharmaceutical excipients. Detailed specifications for fixed oils have been provided in the USP. These specifications clearly indicate that the fixed oils from vegetable origin are liquid at room temperature, easily metabolized, free from rancidification and stable. The USP has specified standard iodine value, saponification value and free fatty acid content of these oils. A chart of these limits of different physicochemical properties of different USP specified oils has been given in Table 1. These oils are used particularly as vehicles for certain hormone and vitamin preparations. The labels of these formulations must state the name of the oils used in it, so that patients can avoid these formulations if he or she has sensitivity to these oils (Virendrakumar *et al.*, 2015). The vegetable oils used in pharmaceutical formulations have a similar type of composition except for castor oil, which contains a unique fatty acid, the Ricinoleic acid in higher quantities (about 86-90%). Other oils generally contain Oleic acid, Linolenic acid, Linoleic acid, Myristic acid, Palmitic acid, and Stearic acid, in different quantities (Rabasco Alvarez and González Rodríguez 2000; Tunaru *et al.*, 2012). These components have been separated successfully and are used in pharmaceutical formulations either in crude form or after chemical modification along with the oil itself.

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Table 1: Limits of different physicochemical properties of some USP specified oils (NA= Not available).

| Properties | Sesame Oil | Corn Oil | Cottonseed Oil | Peanut Oil | Soybean Oil | Castor oil |
|-----------------------|--------------|-------------|----------------|-------------|-------------|-------------|
| Specific gravity | 0.916- 0.921 | 0.914-0.921 | 0.915-0.921 | 0.912-0.920 | 0.916-0.922 | 0.957-0.961 |
| Iodine value | 103-116 | 102-130 | 109-120 | 84-100 | 120-141 | 83-88 |
| Saponification value | 188-195 | 187-193 | NA | 185-195 | 180-200 | 176-182 |
| Unsaponifiable matter | Not >1.5%. | Not >1.5%. | NA | Not >1.5%. | Not >1.0%. | NA |
| Heavy metals | 0.001%. | 0.001%. | 0.001%. | 0.001%. | 0.001%. | NA |

Table 2: A comparative list of components of different oils used in pharmaceutical formulations (from literature; CA= Component absent) (Lakshminarayana *et al.*, 1984; Were *et al.*, 2006; Labuschagne and Hugo 2010; Shahzad *et al.*, 2011).

| | Fatty acids (in %) | | | | | | | |
|---------------------------|--------------------|---------------|--------------|------------|----------------|---------------|----------------|---------------------------------|
| | Myristic acid | Palmitic acid | Stearic acid | Oleic acid | Linolenic acid | Linoleic acid | Arachidic acid | Special fatty acid |
| Castor Seed Oil | CA | 2.0 | 1.0 | 7.0 | CA | 5.0 | CA | Ricinoleic (86-90) |
| Cotton Seed Oil | 0.4 | 20 | 2.0 | 35 | CA | 42 | CA | CA |
| Groundnut Oil (Penut Oil) | CA | 6.0-9.0 | 3.0-6.0 | 52.0-60.0 | CA | 13.0-27.0 | 2.0-4.0 | Lignoceric 1-3 Behenic acid 1-3 |
| Sesame Oil (Til Oil) | CA | 7.0-9.0 | 4.0-5.0 | 40.0-50.0 | CA | 35.0-45.0 | 0.4-1.0 | CA |

A comparative list of the composition based on the literature of those USP specified oils has been given in Table 2.

The plant *Mesua ferrea* L. (Family: Clusiaceae) is commonly known as Nahorin North-East India. It is readily available in wild in almost all the districts of north and north-eastern states of India and Bangladesh (Arunachalam *et al.*, 2003). The aerial parts of the plant are used in traditional medicine for several indications such as rheumatism (Jalalpure *et al.*, 2011), cough, sore throat, fever, vomiting, dysentery, itch from the ancient times. Flower extracts are proven to show the abortifacient property. The uses of *M. ferrea* L. seed oil is of nonedible type and are used for poulticing wounds, treating itch, skin eruptions and dandruff (Chanda *et al.*, 2013). In Java, a decoction of the flowers is drunk by women after childbirth. The stamens of *M. ferrea* L. is used with other herbal ingredients like seeds of *Wrightia tinctoria*, and the shells of pearl oysters (*Pinctada margaritifera*) to formulate Qurs-e-Habis, which is a traditional medicine used as a hemostatic, antidysenteric and antidiarrhoeal (Rasheed and Gupta 2010).

The acute toxicity of the methanolic extract of the leaf of *M. ferrea* L. was determined by Udayabhanu *et al.*, in Swiss albino mice model. They concluded that the oral administration of the methanolic leaf extract to mice does not cause any acute adverse effect or death to the animal model (Udayabhanu *et al.*, 2014).

The oils mostly used for pharmaceutical formulation purposes are edible and have an ever increasing demand with the growing world population and hence has a higher price tag. Peanut oil, corn oil, cottonseed oil, and sesame oil are some of the USP prescribed oils. Hence, finding some other oil from nature, which can be used as a pharmaceutical excipient having similar properties and safety profile with that of the edible oils, is very much important. It may reduce the use of edible oils in pharmaceuticals and also reduce the formulation cost. The present study evaluates the oil of *M. ferrea* L. seed for its potential application in pharmaceutical nanoformulation. The acute toxicity profile of the oil was determined in a rat model as per OECD guidelines. The chemical and biological compatibility of the oil

were also studied in the form of nanostructures lipid carriers to establish the oil as a valuable and safe pharmaceutical excipient.

MATERIALS AND METHODS

Materials

Petroleum ether (B.P.: 60-80 °C), Stearic acid, Palmitic acid, Cholesterol, Silica gel G were purchased from HiMedia, Mumbai, India. Ethanol was purchased from Merck Millipore, Mumbai, India. Vitamin E and Phosphoric acid were purchased from Sigma Aldrich, Bengaluru, India. All the other chemicals and reagents used were of analytical grade. The nahor fruits were collected from *M. ferrea* L. plants in the Dibrugarh University campus. The herbal sample was authenticated by Botanical Survey of India (BSI), Eastern Regional Centre, Shillong, Meghalaya, India.

Methods

Extraction of *M. ferrea* L. seed oil

The collected nahor fruits were shade dried for 7 days. Seed kernels were removed from the dried fruit shells. The kernels were sun dried for another 3-4 days. Kernels were then cut into small chips and again sun dried until it becomes non sticky. The dried seed kernels were ground coarsely (# 30) and stored in air tight containers for future use.

The powdered samples were extracted with continuous hot percolation process at 80 °C using petroleum ether (B.P. 60 – 80 °C) in a soxhlet apparatus (Borosil®, Gujarat, India) for 24 hours. After extraction, petroleum ether was removed from the oil by rotary vacuum evaporator (IKA® RV10 digital, IKA, Germany) with 100 m Bar pressures at 80 °C until viscous oil was obtained.

The oil was then placed in a hot air oven at 45 °C until complete disappearance of the smell of petroleum ether. Finally, the oil was stored in amber colored glass bottle at 4-8 °C for further analysis.

Physical and chemical characterization of *M. ferrea* L. seed oil

The yield (%) was calculated from the weight of powder taken for extraction and the weight of the oil obtained (Nkafamiya *et al.*, 2010). Sudan IV test and translucence Lipid Test was performed to confirm that the extracted material was a lipid. The specific gravity of the extracted oil was determined at room temperature with a Pycnometer. The density of the extracted oil was also calculated using the pycnometer data. The moisture content (%) of the oil was determined by drying the oil at 80 °C in a hot air oven until it achieved a constant weight. The acid value, saponification value, un-saponification matter in the oil, peroxide value, iodine value was determined by the standard methods (Hilditch 1946; de Peña *et al.*, 1992; Nzikou *et al.*, 2010; Mahale and Goswami-Giri 2011). The amount of vitamin E and cholesterol was also determined by standard methods (Rutkowski and Grzegorzczak 2007; Daksha *et al.*, 2010). The chemical stability of the oil was checked after 6 months of storage at room temperature.

Composition of *M. ferrea* L. seed oil

The separation and identification of the components of the *M. ferrea* L. Oil was performed by analytical TLC method using some probable standard components along with the test sample (Asha 2015). TLC plates were prepared by pouring thick slurry of silica gel G on a glass plate (15cm×20 cm) followed by activation of the plate by heating at 120°C for 30 minutes. Standard components (oleic acid, linoleic acid, linolenic acid, stearic acid, palmitic acid, myristic acid, cholesterol, vitamin E) and the sample were dissolved in petroleum ether and the spot was applied on the TLC plate with capillary tubes (Sasidharan *et al.*, 2011). The spots were air dried and TLC was run with ternary mixture of n-Hexane, diethyl ether, acetic acid (70:30:1) as the mobile phase. The diluted sulphuric acid (H₂SO₄:H₂O = 1:9) was sprayed as detecting reagent followed by heating the plate at 110°C until the spots were visible (Nour *et al.*, 2010). The composition of the eluent was finalized using different solvent systems in trial and error method. The R_f values of all the spots on the TLC plate were determined and the R_f values of the sample components were compared with those of the standards.

The quantitative fatty acid composition of *M. ferrea* L. seed oil was studied by LC-MS (Waters ZQ-4000, Milford, Massachusetts, USA). The sample was dissolved in acetonitrile and analyzed with a reversed-phase separating column (Brownlee SPP C18 column, 2 mm x 10 cm). Acetonitrile/ethyl acetate was used as mobile phase in a gradient manner at a flow rate of 0.4 mL/min. Components were identified manually by observing the m/z values and the peak heights of the parent peaks in the mass spectrum (Mamoci *et al.*, 2011).

Acute toxicity study of *M. ferrea* L. seed oil

Acute toxicity study was performed as per the Organization for Economic Cooperation and Development (OECD) guidelines 423. The Institutional Animal Ethical Committee (IAEC) of Dibrugarh University approved the

experimental protocol (Approval No. IAEC/DU/120 dated 18/02/2016).

The acute toxicity study was performed using healthy young adult Wistar rats of both sex, nulliparous, non-pregnant and weighing 80-150 g. Rats were randomly divided into five groups each of three rats. Identification of the rats was done by putting ink marks on the tail. The animals were kept in polypropylene cages (55×32.7×19 cm), bottom covered with sawdust in an air and temperature controlled room (23 ± 2 °C). 12h of light and 12h of dark cycle was maintained for each 24h period with artificial lighting. Each animal cage contained an identification card. Number and weight of the animals, test substance code, route of administration, drug dose etc. were written on that cage identification card. Rats were fed with laboratory animal food pellets (Oxbow Regal Rat Food, Rhinelander, Wisconsin, USA) with water *ad libitum*.

Rats were fasted overnight before dosing (only food was withheld but not water). In the morning of the dosing day, animals were weighed and *M. ferrea* L. oil was administered orally at a dose of 5,50, 300 and 2000 mg/kg of body weight using specially designed rat oral needle. The control group received double distilled water at a dose of 2000 mg/kg of body weight. At 3 h post administration the rats were allowed to food. The administration volume was kept at 1ml/kg body weight of the animal. The quantity of the test substance was calculated based on the body weight of the animal on the day of treatment.

Observation of the treated and control group rats started just after dosing was completed. All the animals were observed individually each hour on the first day of dosing, 4 times daily for next 7 days, and twice daily on the remaining 6 days for a total period of 14 days. Any symptoms of ill-health or abnormal behavior (tremors, salivation, diarrhea, sleep, and coma, changes on skin and fur, eyes and mucus membrane, behavior pattern and death) were recorded as per OECD guideline no. 423.

The body weights of an individual animal of each group were recorded at 1, 3,7,10 and 14th day of the experiment and average weight of each group was tabulated. The data were statistically analyzed by one-way analysis of variance (ANOVA, one way) using Graph Pad Prism (version 5.03) software. Significance of difference between control and test data was analyzed by Student's *t*-test. A statistical difference P >0.05 was considered significant.

Development of Nanostructured Lipid Carriers (NLCs) with *M. ferrea* L. seed oil

The NLCs formulation (Table 3) was developed by modified emulsion solvent diffusion method using extracted *M. ferrea* L. seed oil as a liquid lipid along with behenyl alcohol as a solid lipid (Fan *et al.*, 2014). Pluronic F68 was used as the surfactant. Briefly, 10 mg of behenyl alcohol was mixed with 7.5 mg of *M. ferrea* L. seed oil. The lipid mixture was mixed with 2 ml of absolute ethanol and heated to 55°C in a beaker over a hot plate cum magnetic stirrer (IKA® RCT basic, IKA, Germany). The lipid mixture was added gradually into 10 ml of 1% w/v Pluronic

F68 at 55 °C with stirring at 500 rpm for 10 min. This mixture was then ultra-sonicated (power 100 W, frequency 40 Khz) for 20 minutes at 55 °C in a bath sonicator (UCB 30, Spectra Lab, India). The formulation was then stirred on a magnetic stirrer until complete evaporation of ethanol (~2.5-3.0 h). Finally, the formulation was lyophilized (temperature -80 °C, pressure 0.02 m.bar) with 5% w/v sorbitol using a lyophilizer (SS1-LYO, Southern Scientific Lab Instruments, Chennai, India) and stored in refrigerator for further study (Shete and Patravale 2013; Tamjidi *et al.*, 2014).

Table 3: Formulation of NLCs.

| Components | Solid lipid (Behenyl alcohol) | Liquid lipid (Nahor seed oil) | Surfactant (Pluronic F68) | Organic Phase (Ethanol) | Aqueous Phase (Water) |
|------------|-------------------------------|-------------------------------|---------------------------|-------------------------|-----------------------|
| Quantity | 10 mg | 7.5 mg | 100 mg | 2 ml | 10 ml |

The average particle size, polydispersity index (PDI) and zeta potential of the NLCs were determined using particle size analyzer (Zetasizer Nano ZS90, Malvern, UK). The particle size of the NLC was measured at a 90° scattering angle using Dynamic Light Scattering. The zeta potential of the NLC formulation was measured by using Laser Doppler Micro-electrophoresis. A 1:100 dilution of the sample was made using double-distilled water before the measurements (Tsai *et al.*, 2012).

Compatibility study of the *M. ferrea* L. oil with other NLC components

To determine any incompatibility between the components of the NLC formulation, Differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FT-IR) study of the individual components, physical mixture of the formulation components and the lyophilized formulation were done (Tita *et al.*, 2014). The sample was simply placed over the sample plate of the FT-IR instrument (Bruker, ALPHA, Billerica, Massachusetts, USA) and the covering probe was placed over the sample. IR spectrum was obtained over a wave number region of 400 to 4000 cm⁻¹ (wavelength of 2.5 to 25 μ).

DSC curves were recorded with a Differential Scanning Calorimeter (PerkinElmer, DSC 4000, Waltham, Massachusetts, USA) in a closed aluminum crucible. 5-10 mg of the sample powder was placed in the alumina crucible and heated between 30 - 445°C with nitrogen flow at 50 mL/min with a heating rate of 10°C/min. The curves were analyzed visually with references from the literatures.

Cytotoxicity study of NLC formulation

Cytotoxicity study of the NLC formulation was done by MTT assay on mouse (*Mus musculus*) brain endothelial cell line (bEnd.3; ATCC, USA). For the MTT assay, 5000 cells/well were seeded in 96 well plates with DMEM mixed with fetal bovine serum (10 % v/v), Glutamax™ (1 %), penicillin G (100 U/ml) and streptomycin (100 mg/ml) and placed inside a CO₂ incubator (5% CO₂) at 37°C. On the confluent growth of the cells, plates were

treated with four concentrations between 1 – 1000 μg/ml of the reconstituted lyophilized NLC formulation in distilled water for 3 time points at 24 h, 48 h and 72 h. The positive and negative control was dimethyl sulphoxide (DMSO, 10 % v/v) and blank media, respectively. After specific time points of the treatment, plates were collected and cells were treated with 120 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, EMD Bioscience Inc, CA, USA) and incubated for 3 h. Living cells convert the MTT dye to its insoluble form, formazan, with the help of NAD(P)H-dependent cellular oxidoreductase enzyme. The higher is the number of living cells, the higher is the formation of formazan. The media were aspirated and 200 μL of DMSO was added to each well, plates were incubated for 10 min with shaking and absorbance was read at 570 nm with a reference at 650 nm using a micro plate reader (FLUOstar Omega, BMG LABTECH, UK). Higher reading gives a direct indication of the presence of higher number of living cells. The data was then analyzed with MS Office Excel by comparing the readings of the test samples with the negative and positive control (van Meerloo *et al.*, 2011; Angius and Floris 2015).

RESULTS AND DISCUSSION

The vegetable oils are mainly used as formulation vehicle in the drug delivery systems. They are inert in nature with some exceptions e. g castor oil is used as laxative. The vegetable oil based formulations are mainly for oral administration, but some oil formulations are injected intramuscularly for giving a controlled release property. Different parts of *M. ferrea* L. plant have been used traditionally in fever, cold, asthma, inflammation, septic conditions. It has been utilized as a diuretic, antipyretic, carminative, expectorant, cardio tonic etc. (Rai *et al.*, 2000). It has been tested for various medicinal benefits for its antioxidant and hepato-protective property (Makchuchi *et al.*, 2010), antiulcer activity (Gopalakrishnan, 1980), sore throat (Bandaranayake *et al.*, 1975), cough and asthma (Bala and Seshadri, 1971). The physicochemical properties and composition of some vegetable oils mentioned in USP are given in Table 1 and Table 2, respectively.

Extraction and physicochemical characterization of extracted oil

The solvent extraction of *M. ferrea* L. seed yielded about 68.98% oil. Identification tests confirm that the extracted material is a lipid. The physical and chemical properties of the extracted oil were evaluated by standard methods (Hilditch 1946; Nzikou *et al.*, 2010; Rutkowski and Grzegorzczak 2007; Daksha *et al.*, 2010) and are presented in the Table 4 and Table 5.

Physicochemical properties of the extracted *M. ferrea* L. oil are very similar to the other vegetable oils mentioned in USP (Table 1). Vegetable oils have a tendency to undergo oxidation on standing by environmental oxygen, metal ions, free radicals etc., which is known as rancidity. This leads to a higher acid value of the oils. But *M. ferrea* L. oil contains a higher amount of

antioxidants Vitamin E, which slows down the oxidation process. Data of the chemical properties from two different time points shows that the oil is stable without a major alteration of its chemical properties. The higher stability of nahor oil is advantageous to use it in the pharmaceutical formulations.

Table 4: Physical properties of *M. ferrea* L. oil.

| | |
|-------------------------|--------|
| % Yield | 68.98% |
| Sudan IV test | +ve |
| Translucence Lipid Test | +ve |
| % moisture content | 11.86% |
| Specific gravity | 0.8915 |
| Density (g/ml) | 0.8880 |

Table 5: Chemical properties of *M. ferrea* L. oil.

| | Fresh oil | After 6 months |
|-------------------------------------|-----------|----------------|
| Acid Value (mg KOH/g) | 99.7239 | 100.7847 |
| Saponification Value (mg KOH/g) | 180.9324 | 178.4238 |
| Ester Value (mg KOH/g) | 81.2085 | 88.0639 |
| Un-saponifiable matter (%) | 2.2305 | 2.4728 |
| Iodine value (g of I / 100g of oil) | 16.065 | 15.879 |
| Peroxide Value (meq/kg) | 10.2215 | 11.1234 |
| Vitamin E (mg/gm of lipid) | 14.99 | 13.58 |
| Cholesterol (mg/mg of lipid) | 48.17 | 47.82 |

Composition of extracted oil

The composition of nahor oil was analyzed by TLC method. Fig. 1 represents a total of 8 well separated spots with different R_f values meaning that the sample contains a minimum of 8 different compounds. When the R_f values of the sample spots were compared with the R_f values standard substances, it became clear that the sample contains stearic acid, palmitic acid, oleic acid, linoleic acid, myristic acid, cholesterol and vitamin E, but no linolenic acid. TLC of the sample shows an additional spot at the top of the solvent front, which means the sample may contain some other components which are having different R_f values than the standards used.

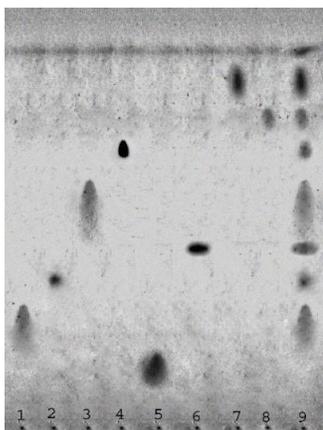


Fig. 1: TLC of *M. ferrea* L. seed oil. 1=stearic acid, 2=palmitic acid, 3=oleic acid, 4=linoleic acid, 5=linolenic acid, 6= Myristic acid, 7=cholesterol, 8= vitamin E, 9= *M. ferrea* L. seed oil.

The quantitative analysis of fatty acid composition in nahor oil was performed using LC-MS and data are presented in Table 6. The content (%) of the various components was calculated by comparing the peak heights of the components. In

Fig. 2, the combined height of the peaks was considered as 100%, and comparing the individual peak heights with the combined value, the quantity of fatty acid components were calculated. The peaks, which are given by the fragments of the fatty acids, were neglected in the calculation process. LC-MS study confirms that the nahor oil is composed of oleic acid (58.12%), linoleic acid (12.64%), stearic acid (13.45), palmitic (9.76%), myristic acid (2.72%) and arachidic acid (3.14%). The content of nahor oil is very similar with the other USP grade oils (Table 2).

Table 6: Fatty acid composition of *M. ferrea* L. seed oil.

| Fatty acids | Relative percentage (%) |
|-------------------------|-------------------------|
| Myristic acid (C14: 0) | 2.72 |
| Palmitic acid (C16: 0) | 9.76 |
| Linoleic acid (C18: 2) | 12.64 |
| Oleic acid (C18: 1) | 58.12 |
| Stearic acid (C18: 0) | 13.45 |
| Arachidic acid (C20: 0) | 3.14 |

Acute toxicity study of extracted oil

Acute toxicity study of nahor seed oil was carried out in rat model with oral administration at 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg body weight. There are no significant changes in the body weights ($P < 0.0001$) among the treated animal groups. Student's *t*-test shows that the body weight changes of the individual test group compared to the control group is insignificant ($P < 0.0001$) (Table 7).

Table 7: Body weight of control and drug treated animals.

| | Control | 5 mg/kg | 50 mg/kg |
|---------|--------------|-----------------|-----------------|
| Initial | 130.33±0.882 | 140.67±1.764*** | 120.67±1.453*** |
| Day 3 | 130.67±0.882 | 141.33±2.028*** | 121.00±1.155*** |
| Day 7 | 131.00±0.577 | 141.67±1.764*** | 121.33±0.882*** |
| Day 10 | 131.33±0.333 | 142.00±1.732*** | 121.67±0.667*** |
| Day 14 | 131.67±0.333 | 141.67±1.202*** | 122.00±0.577*** |

| | 300mg/kg | 2000mg/kg |
|---------|-----------------|-----------------|
| Initial | 119.67±0.882*** | 121.33±1.202*** |
| Day 3 | 120.00±0.577*** | 121.67±0.882*** |
| Day 7 | 120.33±0.667*** | 122.00±1.000*** |
| Day 10 | 120.67±0.333*** | 122.33±0.667*** |
| Day 14 | 121.00±0.577*** | 122.67±0.667*** |

Values are expressed as the mean ± S.E.M. (n=3); Statistical significance (P) calculated by one-way ANOVA. *** $P < 0.0001$, NS – non-significant calculated by comparing treated group with the control group.

No mortality of test animals among any of the groups was seen. No sign of toxicity (changes on skin and fur integrity, eye color, mucus membrane, behavior, tremors, salivation, diarrhea, sleep, coma and death) was seen in any rat throughout the study (Halim *et al.*, 2011).

Characterization of the NLC formulation

The average particle size of the NLC formulation was found to be 266.03±6.67 nm and the zeta potential was found to be -34.06±0.473. The polydispersity index (PDI) of the formulation was found as 0.313±0.098. The results show that nano sized NLC formulations were obtained within a very narrow particle size range. Zeta potential suggests that the surface charges of the NLC particles are negative in nature and have good storage stability.

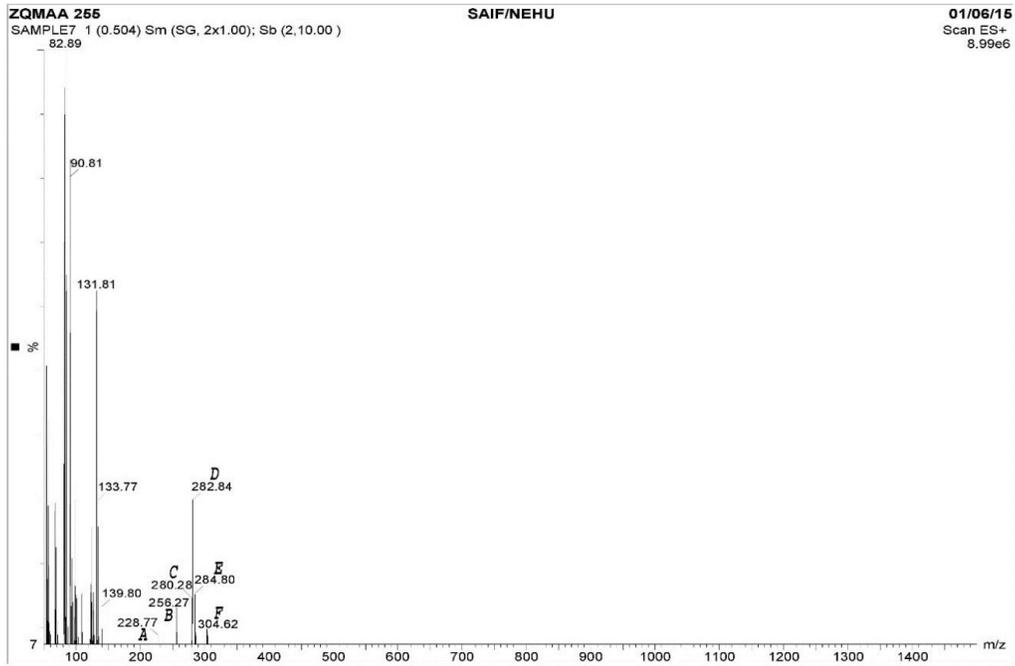


Fig. 2: LC-MS study report of *M. ferrea* L. seed oil. (A= Myristic acid, B= Palmitic acid, C= Linoleic acid, D= Oleic acid, E= Stearic acid, F=Arachidic acid).

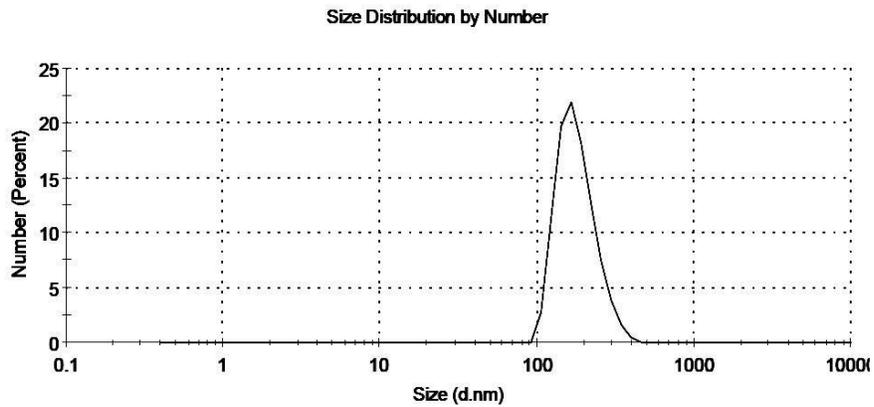


Fig.3: Particle size distribution of the NLC formulation.

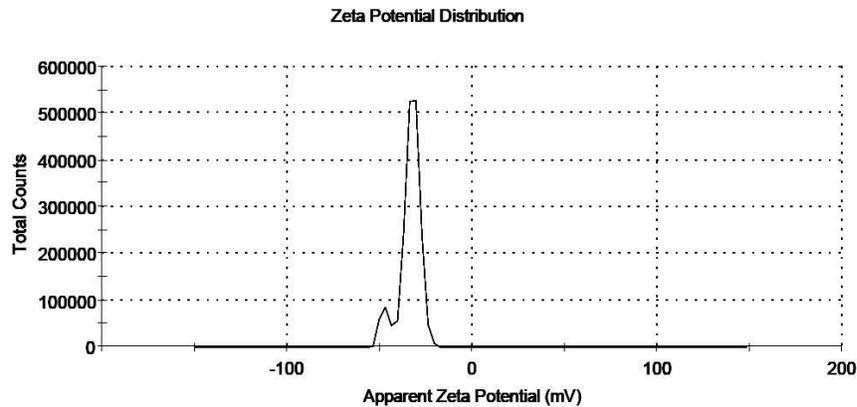


Fig. 4: Zeta potential of the NLC formulation.

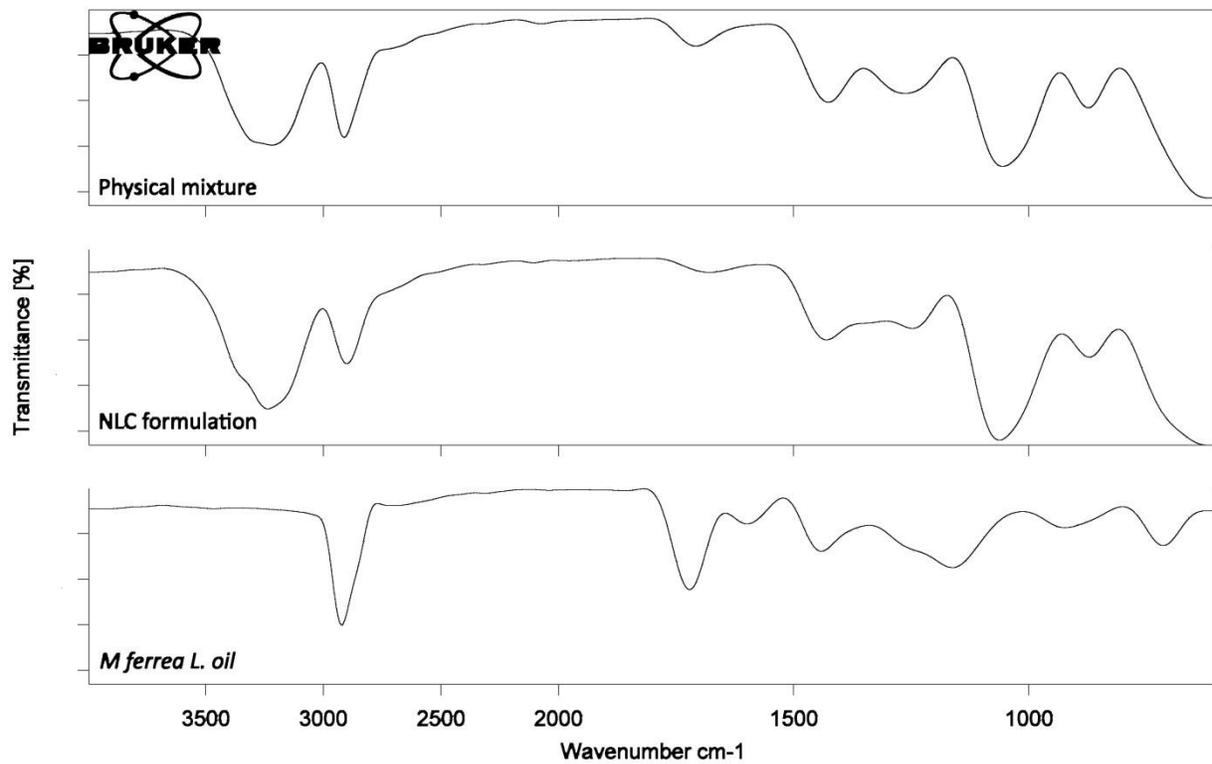


Fig. 5: IR spectrum of the physical mixture of the components and the lyophilized NLC formulation.

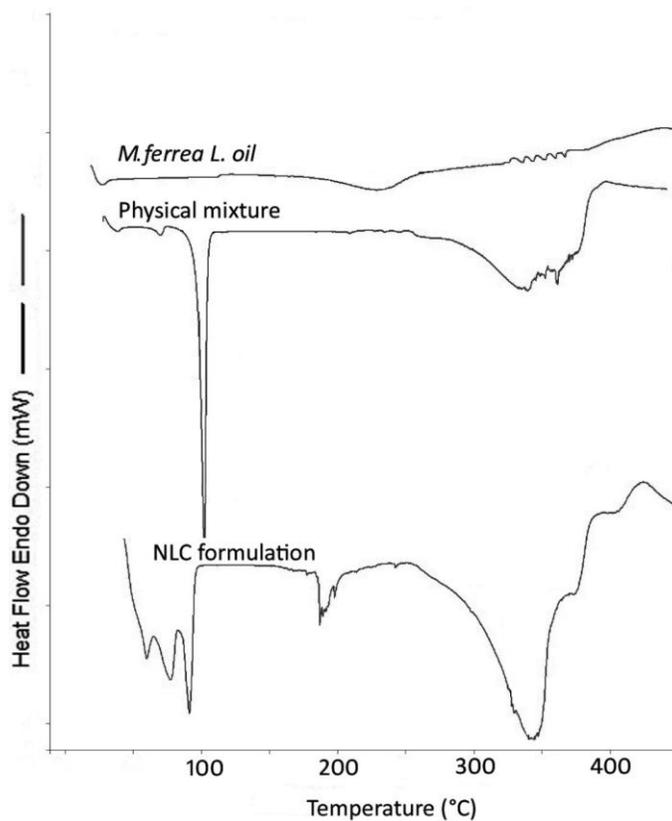


Fig. 6: DSC thermogram of NLC formulation and its physical mixtures.

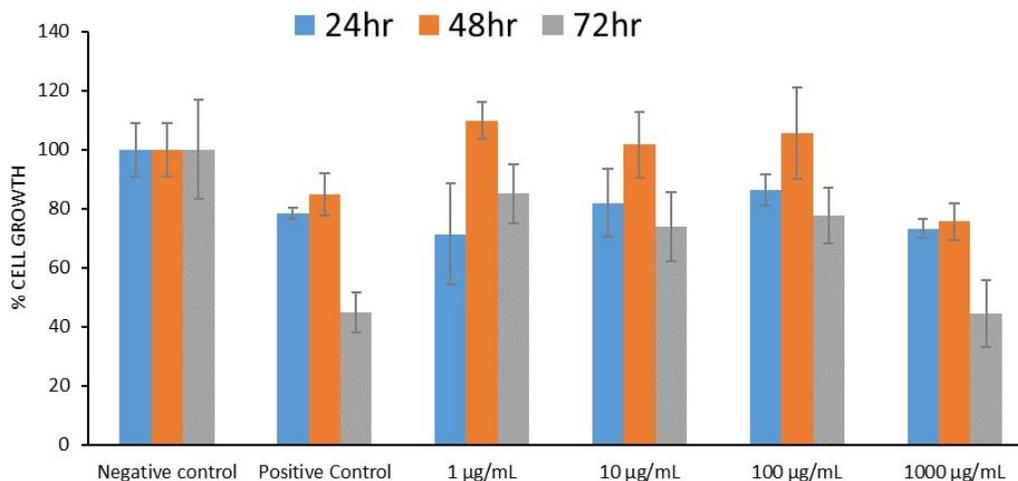


Fig. 7: Graphical presentation of the Cytotoxicity of NLC formulation on bEnd.3 cell.

Compatibility study of the *M. ferrea* L. oil with other NLC components

IR spectrum of behenyl alcohol, *M. ferrea* L. seed oil, pluronic F68, sorbitol, a physical mixture of all the components and the NLC formulation was obtained. In the formulation as well as in the physical mixture, the major peaks of the individual components can be seen (Table 8). There is no evidence of any additional peaks appearing in the IR spectrum of the formulation as well as in the physical mixture. This phenomenon explains that all the components are compatible with one another, as if there was any incompatibility, some additional peaks might have appeared.

Table 8: Interpretation of the FTIR spectrum of physical mixture and NLC formulation.

| | Standard (cm ⁻¹) | Peaks (cm ⁻¹) |
|------------------------------------|------------------------------|---------------------------|
| O-H | 3600 - 3200 | 3230.94 |
| CH ₃ or CH ₂ | 2960 - 2850 | 2891.87 |
| RCOH | 1750-1660 | 1669.37 |
| CH ₃ or CH ₂ | 1470 - 1430 | 1439.19 |
| O-H | 1410 - 1260 | 1268.56 |
| C-OH | 1150 - 1040 | 1076.51 |

On comparing the DSC thermograms of the physical mixture and the lyophilized NLC formulation, it is clear that both contain the same endothermic peaks. In the thermogram of physical mixture, the peaks are more prominent and sharper than that of the formulation thermogram. This is because, in the formulation, some components get covered, entrapped by other components, which is not possible in the case of the physical mixture. There were no additional peaks appearing in the DSC thermogram of the NLC formulation than physical mixtures of all ingredients (Balestrieri *et al.*, 1996). The absence of any exothermic peaks in the physical mixture or in the formulation thermogram means that there is no chemical reaction occurring between the components (Łaszcz *et al.*, 2007). All these study results confirm that there is no incompatibility between the excipients used in the NLC formulation (Mallik *et al.*, 2011).

Cytotoxicity study of NLC formulation

MTT assay results indicate that the NLC formulation is nontoxic up to a dose level of 100µg/ml. The 1000 µg/ml dose shows little toxicity till 48h and then it shows similar toxicity with DMSO solution. It is clear that at 48 h of the study, there is a better growth of the cells at all concentrations of the formulation, which gradually decreases at 72 h. 80% of the cell remains alive at a dose level of 100µg/ml after 72 h. This study indicates that the NLC formulation is nontoxic to bEnd.3 cells at a dose level of 100µg/ml.

CONCLUSION

This study reports the successful extraction and evaluation of *M. ferrea* L. seed oil as pharmaceutical excipient. The TLC and LC-MS study suggest the oil is composed of fatty acids similar to any USP grade oil. The acute toxicity study and MTT assay suggest the safety profile of the navor oil at cellular level in the living system. DSC and FT-IR study confirm no chemical interaction with the general components of the lipid nanoparticles. This work provides a new dimension to *M. ferrea* L. seed oil for its effective use in the pharmaceutical nanoformulation.

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