

Nanoemulsion-Based Delivery of Ricinoleic Acid Derived from Terminalia chebula: A Novel Strategy for Enhanced Bioavailability and Preclinical Constipation Management

Sharmila Dusi¹

Delhi Pharmaceutical Sciences and Research University

J. Saminathan

swamilingam@gmail.com

Delhi Pharmaceutical Sciences and Research University

S Sivakalai

Delhi Pharmaceutical Sciences and Research University

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Abstract

Constipation is a prevalent gastrointestinal disorder that significantly impacts quality of life. Ricinoleic acid, a potent stimulant laxative isolated from the dried fruit of *Terminalia chebula*, demonstrates significant pharmacological activity, although its therapeutic potential is limited due to poor aqueous solubility and low oral bioavailability. In this study, a nanoemulsion-based drug delivery system was developed and optimized to enhance the solubility, physicochemical stability, and therapeutic efficacy of ricinoleic acid. Formulation parameters including surfactant blend concentration, homogenization pressure, and number of homogenization cycles were optimized using a Box-Behnken experimental design. The optimized nanoemulsion exhibited a mean droplet size of 263.8 nm, a zeta potential of - 0.35 mV, and an entrapment efficiency of 81.5%. In vitro dissolution studies confirmed improved drug release following first-order kinetics. The in vivo laxative efficacy was evaluated using a loperamide-induced constipation model in Wistar rats. Animals treated with the nanoemulsion (50 mg/kg) showed a fecal water content of 55%, which was significantly higher than the disease control group (43%) and comparable to the standard laxative bisacodyl (65%). These findings support the potential of nanoemulsion-mediated delivery as a viable preclinical approach for enhancing the oral bioavailability and therapeutic outcome of ricinoleic acid in the management of constipation. Further preclinical and clinical investigations are warranted to validate its translational applicability.

1. Introduction

Constipation is a prevalent gastrointestinal disorder affecting approximately 14% of the global population, with a higher incidence among the elderly and sedentary individuals [1]. It is clinically characterized by infrequent, difficult, or incomplete defecation, often accompanied by bloating, abdominal discomfort, and reduced quality of life [2]. Chronic idiopathic constipation (CIC), a functional disorder without an identifiable organic cause, necessitates long-term therapeutic management [3]. While conventional laxatives including bulk-forming, osmotic, and stimulant agents provide symptomatic relief, their long-term use may lead to dependency, electrolyte imbalances, and reduced efficacy, necessitating alternative therapeutic approaches [4-5]. Given these limitations, herbal-based therapeutics have gained attention as safer and more effective alternatives for treating gastrointestinal disorders [6-7].

1.1 The Pharmacological Potential of *Terminalia chebula*

Terminalia chebula (Haritaki) has been widely utilized in Ayurvedic, Unani, and Traditional Chinese Medicine (TCM) due to its potent gastrointestinal and hepatoprotective benefits [8]. Its fruit contains a rich phytochemical matrix, including chebulagic acid, chebulinic acid, tannins, flavonoids, and polyphenolic compounds, which exhibit antioxidant, antimicrobial, anti-inflammatory, and gastroprotective properties [9-10]. These bioactives contribute to modulating colonic motility, enhancing stool hydration, and reducing oxidative stress, making *T. chebula* an effective phytopharmaceutical candidate for constipation management [11-12].

However, despite its pharmacological potential, *T. chebula* exhibits poor aqueous solubility, low gastrointestinal permeability, and rapid enzymatic degradation, significantly limiting its systemic absorption and therapeutic efficacy [13-14]. These physicochemical barriers necessitate higher oral doses, which reduce patient compliance and therapeutic effectiveness. To address these challenges, advanced drug delivery strategies, particularly nanoemulsion-based formulations, have been proposed to enhance the solubility, stability, and pharmacokinetics of *T. chebula* [15-16].

1.2 Nanoemulsions as an Advanced Drug Delivery System for Herbal Bioactives

Nanoemulsions are thermodynamically stable, biphasic dispersions consisting of oil-in-water (O/W) or water-in-oil (W/O) droplets, stabilized by surfactants and co-surfactants [17-18]. These Nano dispersions have gained significant attention due to their high solubilization capacity, small droplet size (20–200 nm), and enhanced bioavailability for poorly water-soluble drugs [19].

Unlike conventional emulsions, nanoemulsions prevent coalescence, gravitational separation, and flocculation, ensuring greater stability and controlled drug release [20]. Their small droplet size increases the surface area-to-volume ratio, leading to faster dissolution rates and efficient absorption across biological membranes [8-9]. This unique structural advantage facilitates rapid drug transport across the gastrointestinal epithelium, thereby improving therapeutic outcomes for bioactive compounds.

1.3 Impact of Nanoemulsion Droplet Size on Drug Bioavailability

Research has demonstrated that reducing droplet size in nanoemulsions significantly enhances intestinal permeability and systemic circulation, making them ideal carriers for lipophilic bioactives [21-25]. Smaller droplet sizes increase molecular dispersion within the digestive tract, improving drug transport via passive diffusion and lymphatic uptake [17]. Furthermore, the lipid-based composition of nanoemulsions facilitates micellar solubilization, thereby bypassing hepatic first-pass metabolism and further improving drug bioavailability [11, 23-25].

1.4 Justification for Using Nanoemulsions for *Terminalia chebula*

The poor aqueous solubility and gastrointestinal degradation of *T. chebula* necessitate advanced formulation strategies to enhance its pharmacokinetic profile [13-14]. Nanoemulsions provide a protective environment for bioactives, preventing enzymatic degradation, while

facilitating controlled release and prolonged therapeutic action [10-11]. Compared to traditional formulations, nanoemulsions improve solubility, permeability, and overall pharmacodynamic response [21]. This makes them a promising alternative for delivering *T. chebula* extracts in a bioavailable form, optimizing its therapeutic efficacy for constipation management [17-18].

1.5 Study Objective: Development and Evaluation of a *Terminalia chebula* Nanoemulsion

Given the well-established gastrointestinal benefits of *T. chebula*, encapsulating its bioactives in a nanoemulsion system presents a novel, natural alternative for constipation management with enhanced efficacy. This study aims to develop and optimize a nanoemulsion-based formulation of *T. chebula* using Quality by Design (QbD) principles, ensuring optimal droplet size, stability, and therapeutic performance [10]. The formulated nanoemulsion will undergo comprehensive in vitro characterization, including: Particle size analysis, Zeta potential measurements, Entrapment efficiency and Dissolution testing. Additionally, in vivo laxative efficacy will be evaluated using a loperamide-induced constipation model in Albino Wistar rats to compare the therapeutic effects of nanoemulsions against conventional herbal extracts and marketed formulations. This approach integrates modern nanotechnology with traditional herbal medicine, offering a scientifically validated alternative to synthetic laxatives with improved patient compliance and minimal side effects. The study will further provide insights into formulation optimization, stability studies, and future translational research for clinical applications.

2. Materials and Methods

2.1 Materials

Ricinoleic acid ($\geq 98\%$) was generously provided by Acme Synthetic Chemicals, India. Sunflower oil, a commonly used lipid excipient in nanoemulsion formulations, was procured from Merck (Schuchardt, Hokenbrunn, Germany). Transcutol HP (Diethylene glycol monoethyl ether), widely recognized for its solubilizing and permeation-enhancing properties, was obtained from Merck KGaA, Darmstadt, Germany. Tween 20 (Polyoxyethylene sorbitan monolaurate), a non-ionic surfactant essential for nanoemulsion stabilization, was sourced from Merck, Germany.

Ultrapure Milli-Q water was generated using a Millipore water purification system (Massachusetts, USA) to ensure the removal of impurities that might affect the stability and characterization of the nanoemulsion. All additional chemicals and analytical reagents used in this study were of high-performance liquid chromatography (HPLC) or analytical grade, obtained from Merck (Mumbai, India) and S.D. Fine Chem Ltd. (Mumbai, India).

These high-purity reagents were utilized for the physicochemical characterization of the nanoemulsion, including turbidity assessment, viscosity measurement, cloud point temperature determination, refractive index evaluation, particle size and polydispersity index (PDI) analysis, zeta potential measurement, and entrapment efficiency (EE) estimation. Furthermore, these materials played a critical role in in vitro dissolution studies, scanning electron microscopy (SEM) imaging, and in vivo laxative activity assessment to comprehensively evaluate the nanoemulsion's stability, drug release, and therapeutic efficacy.

2.2 Methods

2.3 Preparation of Ricinoleic Acid-Loaded Nanoemulsion

Based on preliminary studies, Tween 80 and Transcutol HP were selected as the surfactant and co-surfactant, respectively. The ricinoleic acid-loaded nanoemulsion was prepared using the spontaneous emulsification method with necessary modifications [26-29].

In brief, ricinoleic acid was dissolved in sunflower oil (5%, v/v) along with Tween 80 (surfactant) and Transcutol HP (co-surfactant) in varying concentrations, forming the oil phase. Milli-Q water was used as the aqueous phase. The oil phase was added dropwise into the aqueous phase while continuously stirring on a magnetic stirrer (600 rpm) for 15 minutes.

Subsequently, the formed mixture was subjected to high-pressure homogenization [30] (Stansted High-Pressure Homogenizer, Essex, UK) at pressures ranging from 20 to 80 MPa, based on formulation requirements. The homogenized nanoemulsion was further processed using bath sonication (Elmasonic S 40 H, Elma Schmidbauer GmbH, Germany) for 15 minutes to ensure uniform droplet size distribution.

The resulting nanoemulsion was stored under refrigerated conditions for further characterization and analysis. The composition of the developed ricinoleic acid-loaded nanoemulsion formulations is detailed in Table 1.

2.4 Characterization Studies

The optimized nanoemulsion formulation was subjected to a series of physicochemical characterizations to assess its stability, homogeneity, and suitability for drug delivery.

2.4.1 Turbidity Measurement

The turbidity of the nanoemulsion was determined using a turbidimeter (Elico D-10, Model 331, India) and expressed in Nephelometric Turbidity Units (NTU) [31]. A 0.5 mL sample was diluted with 250 mL of distilled water, followed by continuous stirring on a magnetic stirrer at ambient temperature before measurement.

2.4.2 Viscosity

The viscosity of the nanoemulsion was analyzed using a Brookfield viscometer (Model DV2T, Brookfield AMETEK, USA) equipped with a C16-1 spindle. A 10 mL sample was placed in the measurement chamber and analyzed at room temperature under a shear rate of 50 rpm to assess flow properties and stability [32-33].

2.4.3 Cloud Point Temperature

The cloud point temperature (T_c) was evaluated to determine the thermal stability of the nanoemulsion. A 0.5 mL sample was diluted in 50 mL of distilled water and subjected to a gradual temperature increase at 0.5°C/min. The temperature at which the formulation turned turbid was recorded as T_c. The process was repeated to ensure reproducibility [34].

2.4.4 Refractive Index

The refractive index of the nanoemulsion was measured using an Abbe refractometer (Atago, Japan) at 25°C. The sample was diluted 100-fold with distilled water before measurement to compare its optical properties with that of pure water [32, 35, 37].

2.4.5 Particle Size and Polydispersity Index (PDI)

The globule size and PDI were determined using a Zetasizer (Malvern Nano ZS-90, UK). A 0.5 mL sample was diluted with distilled water and loaded into a cuvette placed in a temperature-controlled chamber (25°C). The measurements were conducted at a 90° scattering angle, and PDI values were recorded to assess particle homogeneity [38-39].

2.4.6 Zeta Potential

Zeta potential, which indicates the stability and electrokinetic potential of the nanoemulsion, was measured using a Zetasizer (Malvern Nano ZS-90, UK). The sample was appropriately diluted before measurement. Formulations with a zeta potential $\geq \pm 30$ mV were considered electrostatically stable due to strong repulsive forces preventing droplet aggregation [40].

2.4.7 Entrapment Efficiency (EE)

Entrapment efficiency was determined using UV-visible spectrophotometry (Shimadzu 1800, Tokyo, Japan). A 1 mL nanoemulsion sample was centrifuged at 10,000 rpm for 10 minutes, and the supernatant was discarded. The sediment was redispersed in 1 mL of distilled water and subjected to rotary evaporation (Buchi R-210 Advanced, Switzerland) at 35°C under reduced pressure. The residue was dissolved in 10 mL of methanol, incubated at 37°C for 24 hours, and centrifuged at 10,000 rpm for 10 minutes. The absorbance was measured at 270 nm [41-44]. EE was calculated as:

$$\%EE = \frac{\text{Total Ricinoleic acid} - \text{Free Ricinoleic acid in supernatant}}{\text{Total Ricinoleic acid}} \times 100$$

2.4.8 In Vitro Dissolution Study

The dissolution profile of Ricinoleic Acid Nanoemulsion was assessed using a USP Type II dissolution apparatus (Electrolab TDT-08L, India) with simulated gastric fluid (pH 1.2) as the medium [45-46]. The paddle speed was maintained at 50 rpm, and the temperature was controlled at $37 \pm 0.5^\circ\text{C}$. Samples were withdrawn at 5, 10, 20, 30, 45, and 60 minutes, and an equal volume of fresh medium was replaced to maintain sink conditions. The collected samples were filtered (0.45 μm membrane filter) and analyzed using UV spectrophotometry (Shimadzu 1800, Tokyo, Japan) at λ_{max} 270 nm [47].

2.4.9 Drug Release Kinetics

The obtained dissolution data were fitted into zero-order and first-order kinetic models to determine the release kinetics of Ricinoleic Acid nanoemulsions. The mathematical models used for kinetic analysis are as follows [48-49]:

$$\text{Zero-Order Kinetics:} \quad Q = K_0 t \quad (1)$$

Where,

Q = Amount of drug released at time t,

K₀ = Zero-order rate constant,

t = Time (min)

First-Order Kinetics:
$$\log Q_t = \log Q_0 + \frac{K}{2.303} t \quad (2)$$

Where,

Q_t = Amount of drug remaining at time t

Q_0 = Initial drug concentration

K = First-order release rate constant

t = Time (min)

2.4.10 Scanning Electron Microscopy (SEM)

The morphology and surface characteristics of the nanoemulsion were analyzed using Scanning Electron Microscopy (SEM, JEOL JSM-7610F, Japan). A 1:200 v/v diluted sample was deposited onto carbon tape fixed to an aluminum stub and exposed to 2% osmium tetroxide (OsO_4) vapor for 20 minutes to enhance contrast. The sample was air-dried for 24 hours and coated with gold using a Leica EM SCD 500 sputter coater (Austria) [50].

2.4.11 In Vivo Laxative Activity

The laxative activity of the nanoemulsion was evaluated using an Albino Wistar rat model, following ethical approval from the Institutional Animal Ethics Committee (IAEC) as per the CPCSEA guidelines, Government of India. Rats were housed under standard laboratory conditions ($22 \pm 2^\circ\text{C}$, 12 h light/dark cycle) and acclimatized for 7 days before experimentation [51-56].

Experimental Groups:

Group I: Normal control (treated with saline)

Group II: Disease control (treated with Loperamide, 5 mg/kg)

Group III: TC Extract (100 mg/kg)

Group IV: Nanoemulsion (50 mg/kg)

Group V: Standard (Bisacodyl, 0.25 mg/kg)

The formulations were administered orally, and fecal output was recorded at 8 hours and 16 hours post-administration.

The percentage of fecal water content was calculated using:

$$\% \text{ of water content of feces} = \frac{\text{wet weight of the feces} - \text{Dry weight of the feces}}{\text{wet weight of the feces}} \times 100$$

2.4.12 Statistical Analysis

All data were represented as mean $\pm$ standard deviation (SD). The statistical evaluation was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to compare multiple groups. Statistical computations were performed using GraphPad Prism software, version 9.0 (GraphPad Inc., San Diego, USA). A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3. Results

3.1 Preparation of Ricinoleic Acid-Loaded Nanoemulsion

The ricinoleic acid-loaded nanoemulsion was developed using the spontaneous emulsification method with essential modifications to optimize its physicochemical stability and drug delivery efficiency. The formulation process was carefully designed to achieve a stable and homogenous nanoemulsion system. The selection of ingredients, including oils, surfactants, and co-surfactants, was based on their compatibility, solubilizing potential, and ability to enhance the formulation's therapeutic efficacy. The primary oil phase consisted of sunflower oil, which was chosen due to its excellent emulsification properties and compatibility with oral pharmaceutical formulations. Sunflower oil has been widely used as a lipid carrier in nanoemulsion systems due to its ability to improve drug solubility and stability [57]. Ricinoleic acid was dissolved in sunflower oil, followed by the incorporation of the surfactant (Tween 80) and the co-surfactant (Transcutol HP) in varying ratios based on experimental design parameters Table 1. The use of Tween 80 as a surfactant was justified by its well-established emulsification properties and compatibility with lipid-based

systems, whereas Transcutol HP was employed as a co-surfactant to enhance the solubilization capacity and drug loading efficiency of the nanoemulsion [58]. To ensure proper solubilization, the oil phase was subjected to continuous magnetic stirring at 600 rpm for 15 minutes. The aqueous phase (Milli-Q water) was gradually introduced while stirring at 710 rpm, facilitating the formation of a coarse emulsion. Subsequently, this pre-emulsion underwent high-pressure homogenization using a Stansted High-Pressure Homogenizer (Essex, UK) at pressures ranging from 20 to 80 MPa, depending on the formulation requirements. High-pressure homogenization plays a crucial role in achieving nanoscale droplet size, which enhances the stability and bioavailability of the encapsulated drug [59]. A 3² factorial design was employed using Design-Expert® Software (Version 13.0, Stat-Ease Inc., USA) to optimize the nanoemulsion formulation. Two independent variables, X₁ (Smix concentration %) and X₂ (homogenization pressure MPa), were evaluated for their influence on Y₁ (globule size nm) and Y₂ (entrapment efficiency %). A total of seventeen formulations (F1–F17) were developed and assessed as part of the optimization process. The stability of the Nano emulsions was determined after 24 hours of equilibration, during which no visible signs of phase separation, flocculation, or creaming were observed. The stability and homogeneity of the formulation were attributed to the selection of surfactants with an appropriate hydrophilic-lipophilic balance (HLB). The HLB value of Tween 80 (HLB = 15) was suitable for stabilizing the sunflower oil phase, ensuring effective Nano emulsion formation [55]. Previous studies have demonstrated that surfactants with HLB values close to the required HLB of oils result in the formation of more stable emulsions [60–62]. After 24 hours, all formulations were analyzed for critical physicochemical parameters, including globule size, zeta potential, Polydispersity index (PDI), turbidity, viscosity, refractive index, cloud point temperature, and entrapment efficiency. The optimized formulation was selected based on the smallest globule size, high zeta potential to ensure stability, and maximum entrapment efficiency. Further characterization studies, including in vitro dissolution studies, drug release kinetics, and scanning electron microscopy (SEM) analysis, were conducted to confirm the suitability of the nanoemulsion as an efficient nano-drug delivery system for ricinoleic acid. The optimized formulation demonstrated improved colloidal stability and bioavailability, making it a promising candidate for therapeutic applications in constipation management.

Table 1. Formulation Batches of Ricinoleic Acid Nanoemulsions

Ingredients (% w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12	F13	F14	F15	F16	F17
Ricinoleic Acid	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Sunflower Oil	10	12.5	15	17.5	20	22.5	25	27.5	30	10	12.5	15	17.5	20	22.5	25	27.5
Tween 80	30	27.5	25	22.5	20	17.5	15	12.5	10	32.5	30	27.5	25	22.5	20	17.5	15
Transcutol HP	20	22.5	25	27.5	30	32.5	35	37.5	40	20	22.5	25	27.5	30	32.5	35	37.5
Distilled Water (q.s)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Surfactant: Co-surfactant Ratio	2:01	2:01	2:01	2:01	2:01	2:01	2:01	2:01	2:01	3:01	3:01	3:01	3:01	3:01	3:01	3:01	3:01
Oil: Surfactant Ratio	1:09	1:08	1:07	1:06	1:05	1:04	1:03	1:02	1:01	1:09	1:08	1:07	1:06	1:05	1:04	1:03	1:02

3.2 Results of Characterization of Ricinoleic Acid Nanoemulsion

The physicochemical characterization of the ricinoleic acid-loaded nanoemulsion (RA-NE) was systematically conducted to evaluate its stability, uniformity, and suitability for therapeutic applications. The assessment included turbidity, viscosity, cloud point temperature, refractive index, particle size, polydispersity index (PDI), zeta potential, entrapment efficiency (EE), in vitro drug release, and in vivo laxative activity.

3.2.1 Turbidity Analysis

Turbidity is a critical parameter that determines the optical clarity and colloidal stability of a nanoemulsion system. The turbidity values of the RA-NE formulations ranged between 98.5 and 251.2 NTU, indicating adequate dispersion and stability Table 2. Higher turbidity values correlate with larger droplet sizes and increased oil phase content, while lower values suggest efficient emulsification and homogeneous particle distribution [63]. Similar findings were reported in previous studies demonstrating that nanoemulsions with optimized surfactant-to-oil ratios exhibit greater transparency [58].

3.2.2 Viscosity Measurement

The viscosity of the RA-NE formulations varied between 76.9 and 112.3 cps, with the optimized formulation exhibiting a viscosity of 87.2 ± 5.4 cps Table 2. This range indicates moderate viscosity, ensuring ease of administration while preventing phase separation. Higher viscosity formulations

showed better physical stability, consistent with previous reports demonstrating that well-balanced surfactant and co-surfactant compositions enhance nanoemulsion viscosity [60]. The results align with previous studies, which indicated that increased oil phase concentration contributes to higher viscosity [61].

3.2.3 Cloud Point Temperature (Tc) Analysis

The cloud point temperature (Tc), which determines the thermal stability of nanoemulsions, was found to be above 72.8°C for all formulations Table 2. These values confirm that the formulations remain stable at physiological temperatures, reducing the likelihood of phase separation upon storage. Studies have shown that nanoemulsions stabilized with surfactants of appropriate hydrophilic-lipophilic balance (HLB) values exhibit superior cloud point stability [62].

3.2.4 Refractive Index Measurements

The refractive index (RI) is a useful parameter to determine nanoemulsion uniformity and optical clarity. The RA-NE formulations exhibited consistent RI values of approximately 1.40, confirming homogeneous droplet dispersion and optical transparency Table 2. These results are in line with previous studies, demonstrating that nanoemulsions with stable droplet formation typically exhibit RI values within this range [64].

3.2.5 Particle Size and Polydispersity Index (PDI)

Particle size and PDI were analyzed using dynamic light scattering (DLS), and the results are presented in Table 2. Particle size ranged from 92.5 nm to 611.4 nm, indicating the effect of formulation variables on droplet size. The optimized formulation (RCF6) exhibited a particle size of 263.1 ± 12.4 nm, confirming nano-sized dispersion. PDI values varied from 0.146 to 0.418, indicating variations in droplet distribution across formulations. Lower PDI values (<0.3) suggested improved uniformity, confirming the formulation's suitability for nano-drug delivery applications [65]. These findings are consistent with studies showing that higher surfactant levels stabilize emulsions, preventing droplet coalescence and yielding smaller particle sizes [66].

3.2.6 Zeta potential

The zeta potential of the nanoemulsions ranged from −0.35 mV to −13.7 mV, reflecting their surface charge characteristics. The negative values were attributed to nonionic surfactants and ionic interactions in the aqueous phase. While zeta potentials exceeding ±30 mV are typically indicative of electrostatic stability, the optimized formulation (RCF6) demonstrated colloidal stability despite a zeta potential of −0.35 ± 1.23 mV. This can be attributed to steric stabilization conferred by the nonionic surfactant Tween 80, which forms a hydrated, adsorbed layer around the droplets. This layer acts as a steric barrier, preventing droplet coalescence and aggregation. The absence of phase separation or visible instability over 24 hours supports the effectiveness of this mechanism in stabilizing nanoemulsions with low surface charge, ensuring adequate stability in Table 2.

3.2.7 Entrapment Efficiency (EE) Evaluation

Entrapment efficiency (EE) determines the extent of ricinoleic acid encapsulation within the nanoemulsion system. EE values ranged from 72.1% to 91.8%, with the optimized formulation (RCF6) achieving 81.5 ± 2.9% EE in Table 2. The high EE values confirm successful encapsulation, preventing premature drug leakage [67].

Table 2. Characterization of Nanoemulsion Formulations

Formulation code	Turbidity (NTU)	Viscosity (cps)	Cloud point temperature (°C)	Refractive index	Particle size (nm)	PDI	Zeta potential (mV)	EE (%)
RCF1	102 ± 14	86.5 ± 2.2	71.3 ± 2.6	1.43 ± 0.04	198.3 ± 14.2	0.241	- 4.3 ± 1.26	76.4 ± 2.8
RCF2	240 ± 12	84.9 ± 1.6	72.5 ± 1.8	1.46 ± 0.07	327.4 ± 26.5	0.326	- 7.2 ± 2.45	70.9 ± 1.3
RCF3	222 ± 9	81.3 ± 1.8	74.2 ± 1.5	1.45 ± 0.07	539.1 ± 13.2	0.263	- 13.7 ± 3.62	77.2 ± 3.5
RCF4	198 ± 15	82.6 ± 3.1	73.8 ± 2.4	1.42 ± 0.08	106.1 ± 14.2	0.196	- 2.90 ± 1.64	62.4 ± 2.7
RCF5	184 ± 18	79.5 ± 2.7	72.4 ± 1.6	1.44 ± 0.06	127.4 ± 6.8	0.216	- 8.6 ± 2.37	75.3 ± 2.4
RCF6	160 ± 17	78.3 ± 2.4	74.3 ± 3.1	1.45 ± 0.05	263.1 ± 12.4	0.308	- 0.35 ± 1.23	78.1 ± 3.8
RCF7	120 ± 13	83.7 ± 1.5	72.9 ± 2.8	1.43 ± 0.07	92.1 ± 26.2	0.215	- 12.3 ± 1.89	58.0 ± 1.4
RCF8	227 ± 20	81.2 ± 1.9	75.1 ± 2.3	1.47 ± 0.08	334.0 ± 48.2	0.352	- 7.6 ± 2.21	72.5 ± 2.9
RCF9	182 ± 21	88.7 ± 3.5	76.4 ± 2.1	1.46 ± 0.06	467.5 ± 12.4	0.282	- 3.8 ± 1.39	74.8 ± 2.5
RCF10	106 ± 8	85.3 ± 2.2	71.5 ± 1.8	1.47 ± 0.05	628.2 ± 26.3	0.418	- 5.2 ± 2.04	90.0 ± 3.4
RCF11	101 ± 9	84.9 ± 2.6	72.3 ± 1.4	1.43 ± 0.03	175.4 ± 8.7	0.316	- 2.8 ± 3.46	65.9 ± 1.6
RCF12	235 ± 10	82.1 ± 1.7	75.4 ± 1.7	1.46 ± 0.01	340.9 ± 26.3	0.254	- 0.96 ± 1.29	73.4 ± 2.3
RCF13	225 ± 11	80.7 ± 1.4	74.7 ± 2.5	1.45 ± 0.06	321.5 ± 19.4	0.198	- 2.2 ± 2.73	71.6 ± 4.1
RCF14	102 ± 7	78.6 ± 2.1	75.2 ± 1.6	1.47 ± 0.03	428.5 ± 12.8	0.282	- 4.3 ± 2.09	83.4 ± 3.5
RCF15	186 ± 12	81.5 ± 2.6	71.3 ± 2.7	1.45 ± 0.05	580.2 ± 26.7	0.404	- 8.6 ± 1.73	87.7 ± 1.8
RCF16	229 ± 15	80.2 ± 1.3	72.6 ± 3.1	1.47 ± 0.08	343.7 ± 25.4	0.146	- 3.9 ± 2.07	70.2 ± 2.6
RCF17	176 ± 9	73.6 ± 2.8	74.8 ± 2.5	1.46 ± 0.06	223.4 ± 16.3	0.239	- 13.7 ± 2.49	67.9 ± 2.8

3.2.8 In Vitro Dissolution Study

Drug release was evaluated using a USP Type II dissolution apparatus under simulated gastric conditions (pH 1.2, 37 ± 0.5 °C, 50 rpm). Data for RCF1–RCF17 are shown in Tables 3–6. Dissolution kinetics ($T_{90\%}$ = 28.4 min) are summarized in Table 7. Comparative cumulative release for RCF6 and plain ricinoleic acid is given in Table 8 and illustrated in Figure 1. The profiles followed first-order kinetics, indicating concentration-dependent release, consistent with prior studies [68].

Table 3. % dissolved data from the formulations RCF1 – RCF4

Time (min.)	% Drug dissolved			
	RCF1	RCF2	RCF3	RCF4
5	38.91 ± 1.42	28.61 ± 3.23	25.89 ± 3.06	49.56 ± 3.71
10	54.93 ± 2.56	50.23 ± 1.64	41.54 ± 2.38	69.27 ± 2.68
20	79.13 ± 3.84	80.12 ± 2.98	58.65 ± 2.44	88.55 ± 1.54
30	94.07 ± 1.81	85.77 ± 3.22	77.98 ± 1.86	97.41 ± 1.92
45	98.18 ± 2.43	97.23 ± 1.85	88.36 ± 2.34	99.51 ± 0.76
60	99.62 ± 0.57	98.91 ± 1.12	96.58 ± 2.51	--

Table 4. % dissolved data from the formulations RCF5 – RCF8

Time (min.)	% Drug dissolved			
	RCF5	RCF6	RCF7	RCF8
5	36.13 ± 4.15	30.96 ± 2.41	49.32 ± 2.92	24.92 ± 1.78
10	62.51 ± 5.23	58.39 ± 3.66	75.07 ± 4.61	48.28 ± 3.26
20	77.99 ± 2.62	73.98 ± 4.08	92.62 ± 2.54	74.23 ± 5.01
30	97.18 ± 1.37	93.71 ± 1.29	97.13 ± 2.03	86.13 ± 2.87
45	99.65 ± 0.76	97.42 ± 2.13	99.14 ± 1.15	97.09 ± 1.44
60	--	99.51 ± 0.45	--	98.72 ± 0.95

Table 5. % dissolved data from the formulations RCF9 – RCF12

Time (min.)	% Drug dissolved			
	RCF9	RCF10	RCF11	RCF12
5	23.79 ± 1.95	25.34 ± 2.37	40.81 ± 3.17	34.88 ± 2.42
10	40.44 ± 3.07	32.94 ± 1.76	65.32 ± 5.11	60.83 ± 4.16
20	65.01 ± 4.62	50.68 ± 3.14	87.04 ± 2.39	77.82 ± 3.68
30	78.91 ± 1.84	68.47 ± 4.69	92.21 ± 3.02	85.11 ± 1.35
45	91.30 ± 2.44	83.55 ± 1.83	98.75 ± 1.75	96.04 ± 2.08
60	97.23 ± 2.03	93.42 ± 2.12	99.92 ± 0.43	98.63 ± 1.14

Table 6. % dissolved data from the formulations RCF13 – RCF17

Time (min.)	% Drug dissolved				
	RCF13	RCF14	RCF15	RCF16	RCF17
5	26.58 ± 1.49	29.91 ± 3.02	31.73 ± 2.66	35.82 ± 2.37	40.59 ± 3.26
10	53.80 ± 4.27	40.11 ± 1.67	35.62 ± 3.49	46.33 ± 4.06	45.52 ± 1.69
20	74.77 ± 5.11	70.87 ± 3.52	52.46 ± 2.38	68.49 ± 2.74	72.79 ± 3.45
30	86.80 ± 3.42	80.46 ± 2.84	70.04 ± 1.55	88.91 ± 3.91	94.86 ± 2.14
45	97.25 ± 2.19	92.28 ± 2.36	89.54 ± 3.42	94.37 ± 2.33	98.19 ± 1.38
60	98.62 ± 1.34	97.74 ± 1.74	95.71 ± 2.31	98.45 ± 1.02	--

Table 7. Dissolution Kinetics Data

Formulation	Regression (R^2) value		First-order Dissolution rate constant (k , min ⁻¹)	T90% (min.)
	Zero-order	First-order		
RCF1	0.874	0.993	0.089	25.91
RCF2	0.893	0.991	0.076	30.49
RCF3	0.934	0.986	0.052	44.05
RCF4	0.859	0.993	0.118	19.49
RCF5	0.901	0.937	0.105	21.98
RCF6	0.883	0.982	0.083	27.86
RCF7	0.844	0.994	0.123	18.66
RCF8	0.907	0.993	0.073	31.55
RCF9	0.931	0.994	0.057	40.65
RCF10	0.954	0.986	0.042	54.35
RCF11	0.849	0.989	0.095	24.21
RCF12	0.874	0.992	0.071	32.36
RCF13	0.896	0.991	0.073	31.45
RCF14	0.917	0.992	0.060	38.31
RCF15	0.945	0.977	0.049	46.73
RCF16	0.901	0.991	0.068	34.01
RCF17	0.922	0.971	0.088	26.11

Table 8. Cumulative Drug Release Profile of Ricinoleic Acid Nanoemulsion (RCF6) and Plain Ricinoleic Acid (RC) after 60 Minutes in Simulated Gastric Conditions (pH 1.2, 37°C)

Time (minutes)	RCF6 (% Drug Released)	Plain RC (% Drug Released)
5	30.96	12.5
10	58.39	28.8
20	73.98	46.75
30	93.71	64.2
45	97.42	75.95
60	99.51	82.5

This figure compares the enhanced dissolution efficiency of the optimized Nano emulsion formulation against unformulated ricinoleic acid.

3.2.9 Scanning Electron Microscopy (SEM) Analysis

The spherical morphology and uniform droplet distribution of the optimized formulation are shown in Figure 2. SEM imaging provided insights into morphology, dispersion, and surface characteristics of the nanoemulsion. Droplets appeared spherical with smooth surfaces, confirming effective surfactant stabilization [69]. The measured particle size (263.8 nm) in aligned with DLS results in figure 2. The absence of significant aggregation indicated high colloidal stability, supporting prior findings [70].

The figure showcases the surface morphology, particle uniformity, and dispersion characteristics of the optimized formulation.

3.2.10 Statistical Characterization of Ricinoleic Acid Nanoemulsions

a) Model Selection and Statistical Analysis

A Sequential Model Sum of Squares (SMSS) analysis was performed to determine the most suitable regression model for both particle size and entrapment efficiency. Based on statistical analysis, a quadratic model was identified as the best fit for both responses. Analysis of variance (ANOVA) was conducted to assess the significance of the model and its individual factors, ensuring statistical reliability. The adjusted R^2 values for

particle size and entrapment efficiency were 0.9782 and 0.9174, respectively, indicating a strong correlation between observed and predicted values. The predicted R² values of 0.8589 and 0.8983 for particle size and entrapment efficiency, respectively, confirmed the robustness of the model. Predicted vs. actual plot for Response R1. The statistical analyses for particle size and entrapment efficiency, including SMSS and ANOVA results, are summarized in Tables 9-12.

b) Effect of Formulation Variables on Responses

Contour plots and 3D surface plots were generated to visualize the influence of Smix concentration, homogenization cycles, and pressure on particle size and EE.

- 1. **Particle Size (R1):** Increasing Smix concentration led to an increase in globule size due to reduced hydrophilic-lipophilic balance (HLB). A higher number of homogenization cycles and increased pressure resulted in smaller globules due to enhanced shearing forces.
- 2. **Entrapment Efficiency (R2):** Higher Smix concentrations improved EE by preventing drug leakage from the oil phase, whereas increased homogenization cycles and pressure reduced EE by increasing surface area exposure to the external aqueous phase.

c) Particle Size Analysis

The quadratic regression equation for particle size (Response 1) based on the identified factors is as follows:

Size=333.50 + 211.39A – 34.47B – 69.96C – 4.95AB – 13.63AC + 29.35BC + 39.31A² – 34.61B² – 26.29 C²

The statistical significance of the model was confirmed through an analysis of variance (ANOVA), with an adjusted R² value of 0.9782 and a predicted R² value of 0.8589, suggesting a good fit between the observed and predicted values. The SMSS and ANOVA findings are summarized in Tables 9 and 10, respectively. The model’s validity was further supported by the Predicted vs. Actual Response Plot. The Contour and 3D surface plots for particle size.

Table 9. SMSS analysis of the Response 1

Source	Sum of Squares (SS)	Df	Mean Sum of Squares (MSS)	F-value	p-value	inference
Mean	1.777x10 ⁶	1	1.777 x10 ⁶			
Linear	4.061 x10 ⁵	3	1.354 x10 ⁵	79.21	< 0.0001	
2FI	4286.26	3	1428.75	0.7968	0.5233	
Quadratic	13847.77	3	4615.92	7.91	0.0119	Suggested
Cubic	3744.01	3	1248.00	14.67	0.0126	Aliased
Residual	340.26	4	85.07			
Total	2.206 x10 ⁶	17	1.297 x10 ⁵			

Table 10. Results of ANOVA test for the quadratic model for the Response 1.

Source	SS	Df	MSS	F-value	p-value	Inference
Model	4.243x10 ⁵	9	47141.96	80.80	< 0.0001	significant
A-Smix	3.575 x10 ⁵	1	3.575 x10 ⁵	612.68	< 0.0001	significant
B-No. of Cycles	9508.20	1	9508.20	16.30	0.0050	significant
C-Pressure	39158.01	1	39158.01	67.11	< 0.0001	significant
AB	98.01	1	98.01	0.1680	0.6942	
AC	742.56	1	742.56	1.27	0.2964	
BC	3445.69	1	3445.69	5.91	0.0454	
A ²	6507.25	1	6507.25	11.15	0.0124	
B ²	5044.32	1	5044.32	8.65	0.0217	
C ²	2909.61	1	2909.61	4.99	0.0607	
Residual	4084.27	7	583.47			
Lack of Fit	3744.01	3	1248.00	14.67	0.0726	Not significant
Pure Error	340.26	4	85.06			

d) Entrapment Efficiency Analysis

The quadratic model was also identified as the best fit for Response 2 (Entrapment Efficiency, EE). The regression equation for EE is:

$$EE = 73.41 + 7.13A - 3.75B - 7.88C$$

The ANOVA results confirmed that the model was statistically significant, with adjusted and predicted R² values of 0.9174 and 0.8983, respectively. The model validation is presented in Table 11 and Table 12, while the Predicted vs. Actual Response Plot. The Contour and 3D surface plots for entrapment efficiency.

Table 11. SMSS analysis of the Response R2

Source	SS	Df	MSS	F-value	p-value	inference
Mean	91617.88	1	91617.88			
Linear	1014.75	3	338.25	48.13	< 0.0001	Suggested
2FI	5.50	3	1.83	0.2135	0.8848	
Quadratic	42.82	3	14.27	2.32	0.1619	
Cubic	36.25	3	12.08	7.11	0.0442	Aliased
Residual	6.80	4	1.70			
Total	92724.00	17	5454.35			

Table 12. Results of ANOVA test for the linear model for the Response R2.

Source	SS	Df	MSS	F-value	p-value	Inference
Model	1014.75	3	338.25	48.13	< 0.0001	significant
A-Smix	406.13	1	406.13	57.78	< 0.0001	significant
B-No. of Cycles	112.50	1	112.50	16.01	0.0015	significant
C-Pressure	496.12	1	496.12	70.59	< 0.0001	significant
Residual	91.37	13	7.03			
Lack of Fit	84.57	9	9.40	5.53	0.0574	not significant
Pure Error	6.80	4	1.70			
Cor Total	1106.12	16				

3.2.11 Statistical Analysis (ANOVA)

a) ANOVA for Particle Size

The statistical analysis results for Response 1 (Particle Size) are summarized in Table 9. The model was found to be highly significant ($p < 0.0001$), with significant individual contributions from Smix concentration (A), the number of homogenization cycles (B), and applied pressure (C).

b) ANOVA for Entrapment Efficiency

The ANOVA results for Response 2 (Entrapment Efficiency) presented in Table 11 demonstrated that Smix concentration (A), number of homogenization cycles (B), and applied pressure (C) were all statistically significant predictors of EE ($p < 0.0001$).

The contour plots and 3D surface plots provide a graphical representation of the effects of the independent variables on the responses.

c) Optimization and Validation of Nano emulsion Formulation

The desirability function approach in Design Expert software was used to optimize the formulation. The optimal formulation was determined as 38.5% Smix concentration, 3 homogenization cycles, and 20 MPa pressure, resulting in graph.

Particle Size: 263.8 nm

Entrapment Efficiency: 81.5%

These values were within the 95% confidence interval of the predicted values, validating the effectiveness of the experimental design. The optimized nanoemulsion was further characterized for turbidity, viscosity, cloud point temperature, refractive index, in vitro drug release, and in vivo laxative activity to confirm its suitability as a stable and effective drug delivery system for ricinoleic acid.

3.2.11 In-Vivo Laxative Activity

The in-vivo laxative efficacy of nanoemulsion and TC extract was evaluated and compared with the standard laxative (Bisacodyl, 0.25 mg/kg). The results demonstrated a significant increase in fecal water content in rats treated with TC extract (100 mg/kg) and nanoemulsion (50 mg/kg), with fecal water content recorded at 52% and 55%, respectively. The highest fecal water content was observed in the standard drug (Bisacodyl)-treated group, reaching 65%. In contrast, the disease control group (Loperamide-treated) exhibited a significantly lower fecal water content, confirming the induction of constipation. The fecal output in nanoemulsion-treated rats was statistically significant ($p < 0.05$) compared to the disease control group, indicating its efficacy in alleviating constipation. The laxative effect parameters for each treatment group, including fecal counts, weights, and water content, are detailed in Table 13. The differences in fecal water content between treatment groups are depicted in Figure 3.

Table 13. Laxative Effect of *Terminalia chebula* Hydro alcoholic extract and Nanoemulsion in Loperamide-Induced Constipated Albino wistar rats.

Treatment Groups	Dose	No. of Feces in 12hrs	Weight of wet feces	weight of dry feces	% fecal water content
Group 1 (Normal control)	10ml/kg	9.38 ± 1.27 ^{b1}	0.95 ± 0.02 ^{b1}	0.39 ± 0.02	58.94 ± 1.24 ^{b3}
Group 2 (Positive control)	5 mg/kg	3.78 ± 0.78	0.74 ± 0.04	0.42 ± 0.03	43.24 ± 1.47 ^{b1}
Group 3 (Test Drug 1)	100 mg/kg	7.58 ± 0.84	0.82 ± 0.04 ^{b1}	0.39 ± 0.03	52.43 ± 1.94 ^{b2}
Group 4 (Test Drug 2)	50 mg/kg	7.78 ± 1.97	0.98 ± 0.03 ^{b2}	0.44 ± 0.02	55.10 ± 2.05 ^{b2}
Group 5 (Standard control)	0.25 mg/kg	8.98 ± 0.98	1.08±0.09 ^{b3}	0.37 ± 0.02	65.74±2.31 ^{b3c3d2e2}

*Results expressed as mean + SEM (n ¼ 5). 1 P < .05, 2 P < .01, 3 P < .001, a: compared to group 1, b: compared to group 2, c: compared to group 3, d: compared to group 4, e: compared to group 5. Group 1 and group 2 animals are normal and constipated controls, respectively, and treated groups are all constipated

Values are expressed as the mean + standard error of mean (n=6). P < 0.05 compared to control group (One-way analysis of variance followed by Dunnett's multiple comparison test)

The figure 3. compares the therapeutic efficacy of different treatment groups by analyzing fecal water content in a loperamide-induced constipation model.

4. Discussion

Chronic idiopathic constipation (CIC) is a widespread gastrointestinal disorder, affecting a significant portion of the global population, particularly the elderly and those with a sedentary lifestyle [71]. While conventional laxatives, such as osmotic and stimulant agents, provide symptomatic relief, their long-term use often leads to issues like dependency, reduced efficacy, and electrolyte imbalances [72, 73]. This necessitates the exploration of alternative therapeutic approaches, such as the use of natural bioactive compounds and advanced drug delivery systems. In this context, the development of ricinoleic acid-loaded nanoemulsions (RA-NE) presents a promising alternative for the management of CIC. The present study focuses on optimizing the pharmacokinetic and therapeutic properties of ricinoleic acid using nanoemulsion technology, which has shown great potential in enhancing the solubility, stability, and bioavailability of lipophilic compounds [74, 75]. The RA-NE formulation developed in this study demonstrated excellent physicochemical properties, with an optimized droplet size of 263.8 nm. This size is within the ideal range for improving the bioavailability of poorly water-soluble compounds, as smaller droplet sizes increase the surface area-to-volume ratio, facilitating faster dissolution and absorption [76]. These findings are consistent with previous studies, which have reported that nanoemulsion formulations with smaller droplet sizes exhibit enhanced drug absorption and bioavailability compared to larger emulsions [77]. The low polydispersity index (PDI) observed in this study further indicates a uniform and stable particle distribution, which is essential for consistent therapeutic effects [76]. The controlled release profile observed in the RA-NE formulation is another key finding. The optimized formulation exhibited first-order release kinetics, suggesting a concentration-dependent release, which is crucial for chronic conditions such as CIC where sustained therapeutic action is needed [8]. This controlled release property is in line with other nanoemulsion studies, which have shown that lipid-based nanoemulsions can offer controlled drug delivery, enhancing therapeutic outcomes and reducing side effects [75, 79]. The *in vivo* evaluation of RA-NE in the loperamide-induced constipation rat model demonstrated significant laxative efficacy compared to both plain ricinoleic acid and conventional formulations. This result suggests that the nanoemulsion's ability to enhance the solubility and permeability of ricinoleic acid is crucial for improving therapeutic efficacy. The results align with those of previous studies, where nanoemulsion formulations of bioactive compounds have shown enhanced colonic motility modulation and improved therapeutic outcomes for gastrointestinal disorders [78-79]. While these results are promising, the study does have certain limitations. For instance, the long-term stability of the RA-NE formulation under varying environmental conditions, such as temperature and humidity, was not fully explored. Further research is required to evaluate the scalability of this formulation for large-scale production, as well as its ability to maintain stability and efficacy under real-world storage conditions. Moreover, while the *in vivo* studies demonstrated significant therapeutic potential, clinical trials in human subjects are essential to confirm the safety, efficacy, and potential side effects of the RA-NE formulation in a clinical setting. Future research could also explore the combination of ricinoleic acid with other bioactive compounds or excipients to further optimize the therapeutic effects. Additionally, investigating the potential of RA-NE for targeting other gastrointestinal disorders, such as irritable bowel syndrome (IBS) could expand its clinical applications. Furthermore, the development of personalized medicine approaches, where RA-NE formulations are tailored to an individual's specific gastrointestinal needs, could further enhance the clinical applicability and efficacy of this treatment.

5. Conclusion

In this study, we developed and optimized a ricinoleic acid-loaded nanoemulsion (RA-NE) formulation aimed at improving the therapeutic efficacy of ricinoleic acid in managing chronic idiopathic constipation (CIC). The results demonstrated that the nanoemulsion formulation significantly enhanced the solubility, stability, and bioavailability of ricinoleic acid, which in turn improved its laxative effects in an *in vivo* loperamide-induced constipation model. The optimized formulation, with an ideal droplet size of 263.8 nm, exhibited controlled drug release, indicating a sustained therapeutic effect ideal for chronic conditions like CIC. These findings underscore the potential of RA-NE as an effective alternative to conventional laxatives, offering a more targeted and long-lasting therapeutic response. The *in vitro* and *in vivo* studies confirmed that RA-NE enhanced the absorption and therapeutic outcomes of ricinoleic acid compared to hydroalcoholic extract and conventional formulations, suggesting that nanoemulsion technology can overcome the challenges associated with poorly water-soluble bioactive compounds. Additionally, the formulation was stable and demonstrated good physicochemical properties, ensuring consistency in its performance. These findings provide valuable insight into the development of nanoemulsion-based formulations for gastrointestinal disorders, positioning RA-NE as a promising candidate for the management of constipation. While the preclinical results are promising, further clinical studies are needed to evaluate the safety, efficacy, and long-term benefits of RA-NE in human subjects. The successful application of RA-NE in CIC management could pave the way for safer and more effective alternatives to traditional laxatives, minimizing side effects and enhancing patient compliance.

Declarations

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Competing Interests

The authors declare no competing interests.

Ethics Approval and Consent to Participate

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Delhi Pharmaceutical Sciences and Research University with reference number 215/GO/ReBi/2000/CPCSEA. All procedures were conducted in accordance with the CPCSEA guidelines on the protection of animals used for scientific purposes.

Consent for Publication

Not applicable.

Availability of Data and Material

All data generated or analyzed during this study are included in this published article.

Authors' Contributions

The study was conceived and designed by the lead investigator. The initial draft of the manuscript was prepared by two contributing researchers. All authors participated in reviewing the results and approved the final version of the manuscript.

The authors used Grammarly software solely for grammar and language editing. No AI-based writing tools were used in the preparation of this manuscript.

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Figures

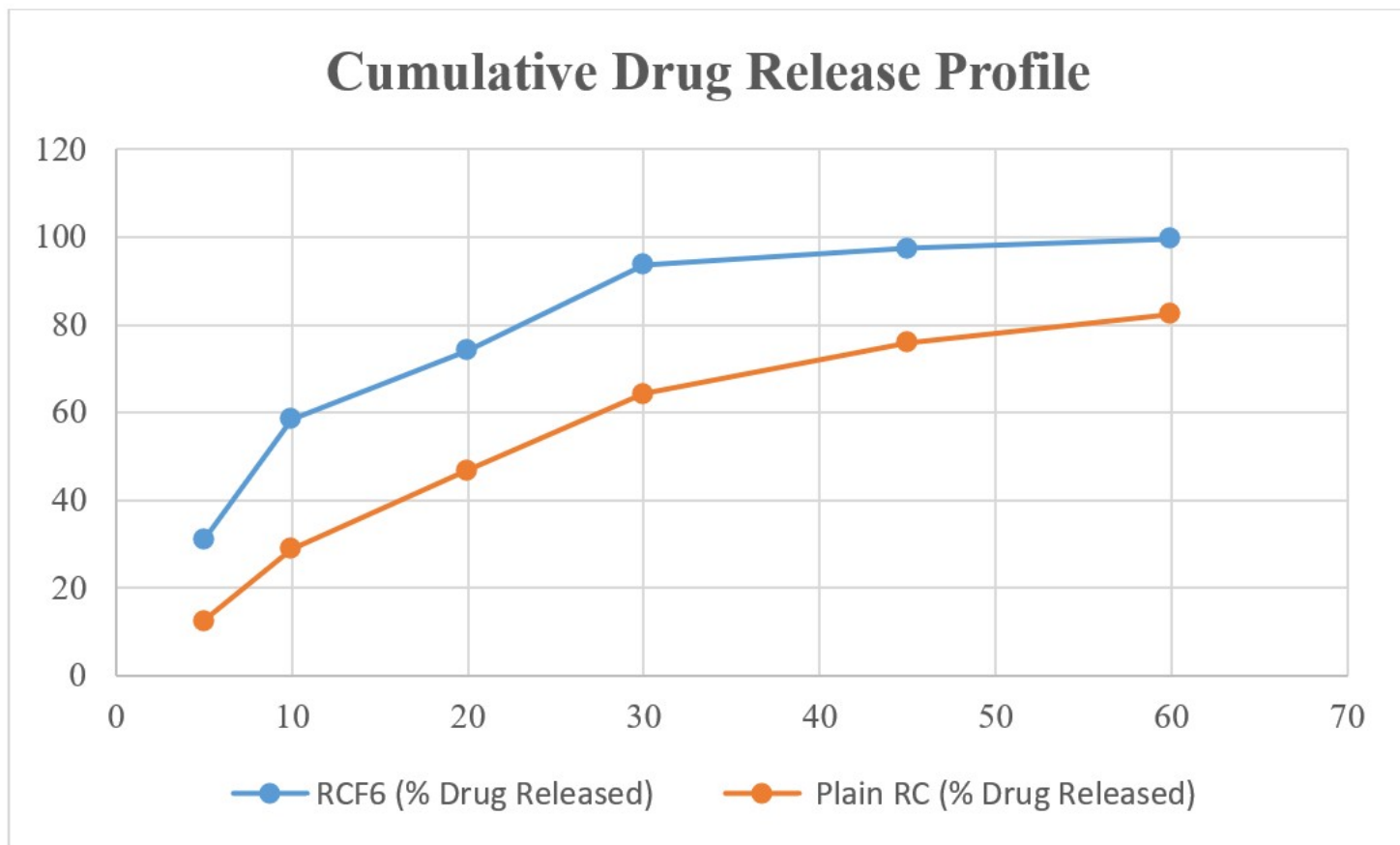


Figure 1

Cumulative drug release profile of ricinoleic acid Nano emulsion (RCF6) versus plain ricinoleic acid under simulated gastric conditions (pH 1.2, 37°C)

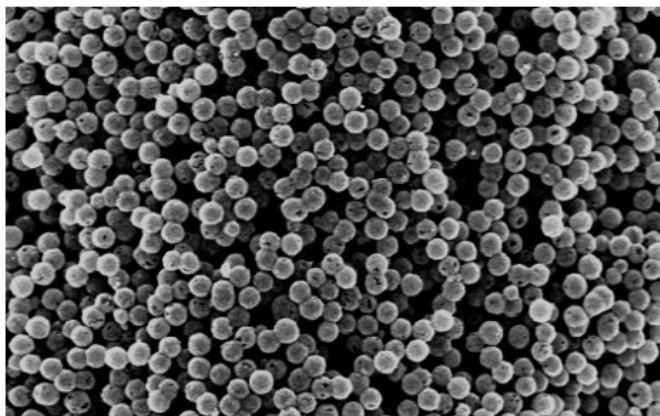


Figure 2

Scanning Electron Microscopy (SEM) image of the optimized ricinoleic acid Nano emulsion at 263.8nm.

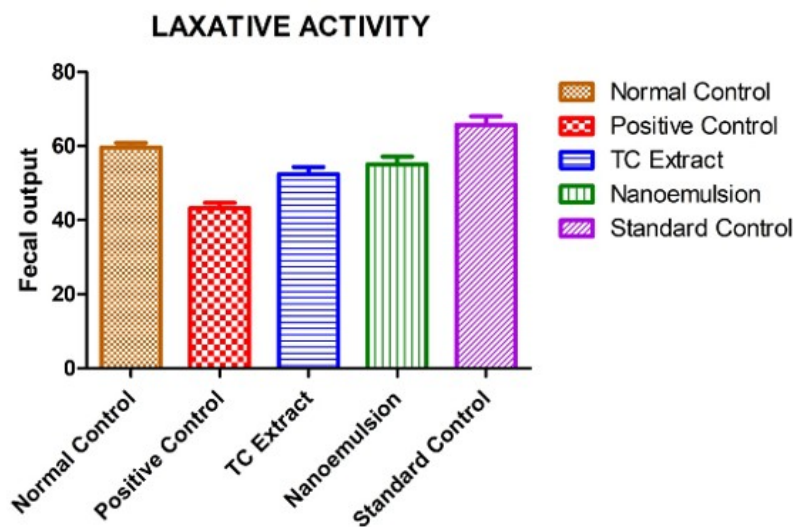


Figure 3

Graphical representation of fecal water content (%) in rats treated with *Terminalia chebula* extract, Nano emulsion, and standard drug.

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