Research Article

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Therapeutic Delivery

Gastric ulcer healing by chebulinic acid solid dispersion-loaded gastroretentive raft systems: preclinical evidence

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Background: Chebulinic acid (CA), a component in *Terminalia chebula*, exhibits antiulcer activity, but has poor aqueous solubility. Raft-forming systems incorporating solid dispersions (SDs) of CA, were developed to overcome its poor biopharmaceutical properties and to prolong the gastric residence time for maximum activity. **Methods:** SDs were formulated by a solvent evaporation method using Eudragit EPO. Raft formulations consisted of sodium alginate as a polymer. **Results:** Release of CA in the dissolution medium was 40%, whereas SDs showed 95.45% release. The CA raft system (20 mg/kg) showed curative efficacy in an alcohol-induced gastric ulcer model and increased protection when compared with omeprazole (10 mg/kg) and CA suspension (20 mg/kg). **Conclusion:** These studies demonstrated SD raft systems to be a promising approach for antiulcer therapy by CA.

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Keywords: antiulcer • chebulinic acid • Eudragit EPO • raft system • solid dispersion • *Terminalia chebula*

Gastric ulcers (GUs) present on the inside of the mucosal lining of the stomach, are open sores and are one of the most common gastrointestinal (GI) problems today, afflicting approximately 10% of the population of the world [1]. It is a multifactor and complicated disorder due to the breaking of the equilibrium between the inherent defense mechanism of gastric mucosa, aggressive factors (e.g., secretion of gastric acid and pepsin, oxidants, free radicals, leukotrienes and endothelin) and exogenous factors (e.g., *Helicobacter pylori* infection, alcohol use, nonsteroidal anti-inflammatory drugs) [2,3]. Currently, management and prevention of GUs is done by H_2 antihistamines, proto-pump inhibitors, ulcer protectives and antiulcer drugs [4]. Prolonged use of these chemical substances is often related to challenges, including enterochromaffin-like (ECL) cell hyperplasia, anemia, diarrhea and constipation [5,6].

Terminalia chebula, indigenous to India (vernacular name: *haritaki*, family Combretaceae), is a common medicinal plant used in traditional medicine such as Unani, Ayurveda and Homeopathy [7]. *T. chebula* is also employed as a major ingredient in Ayurvedic formulations, namely *Triphala* and *Abhayaristha,* as a detoxifying agent in GI disorders, purgative in long-standing constipation and as an aid in digestion. It is also reported as an antimicrobial and antiulcer agent [8,9]. A chemical analysis of these formulations (*Triphala* and *Abhayarista*) has revealed a number

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of pharmacologically active compounds arousing interest, like ellagic acid, chebulinic acid, gallic acid, ethyl gallate and tannic acid. Monitoring of the processing method of *Abhayarista* has revealed a breakdown of chebulinic acid during fermentation and an increase in monomeric counterparts like gallic acid, ellagic acid and chebulic acid in the final processed formulation [10,11]. Chebulinic acid has exhibited antiulcer activity since it possesses antisecretory, antioxidative and cytoprotective activities [12]. Chebulinic acid can also act as a prodrug owing to its prolonged retention in the stomach and interaction with gastric fluid, breaking into bioactive compounds, which can also be active for antiulcer activity.

In spite of the immense potential of chebulinic acid in GI disorders, its use in the pharmaceutical field is limited because of its high hydrophobicity and poor aqueous solubility. Therefore, a novel formulation with enhanced aqueous solubility and prolonged gastric residence time for maximizing the action of chebulinic acid against gastric problems is highly desired. In the present work, to overcome the barrier of poor aqueous solubility for therapeutic use, a solid dispersion with a hydrophilic polymer was designed. Additionally, to retain the chebulinic acid solid dispersion in the stomach, it was further embedded in a raft formulation. This approach will not only help to improve the poor solubility of chebulinic acid in the stomach but would also retain the drug at the affected site for an optimum therapeutic period, thus augmenting its activity. The novelty of the work lies in formulating a delivery system containing a chebulinic acid solid dispersion incorporated in a raft for gastric delivery, which has not previously been reported.

Solid dispersion refers to the dispersion of one or more active ingredients in a hydrophilic inert matrix that acts as the carrier to augment solubility and oral bioavailability of poorly aqueous soluble drugs. The technique used here for the preparation of the solid dispersion was the solvent evaporation technique. However, the process was individualized and relies on drug–carrier interactions [13]. A key underlying principle of solid dispersion formulation is the attainment of an amorphous state, which is regarded to be better than the crystalline state. The amorphous state in solid dispersions has its advantages and disadvantages. The major advantage of the amorphous state is evident in the fact that there is no or very little requirement of energy to break the crystal lattice present in the crystalline phase [13]. A cationic polymer, Eudragit, is a copolymer evolved from dimethyl aminoethyl methacrylate, butyl methacrylate and methyl methacrylate. Solid dispersions making use of Eudragit have been prepared for various drugs to enhance their solubility and bioavailability [14]. Eudragit is a polymeric carrier suitable for distinct delivery to the stomach because of its rapid dissolution in the gastric fluid up to a pH of 5.0.

Several endeavors are being carried out to design different modified-release gastroretentive drug delivery systems, like high density (sinking) systems that are withheld at the lowest part of the stomach, low density (floating) systems that are buoyant in the fluid of the stomach, systems that result in adhesion to the mucosa of the stomach, unfoldable, extendible or swellable systems which limit evacuation of the delivery system from the pyloric sphincter of stomach, superporous hydrogel systems and magnetic systems [15]. Such delivery systems offer the benefits of localized drug delivery, improved drug absorption and efficacy and reduced dosing frequency due to controlled release [16]. Amidst all these systems, the raft-forming systems are low-density (floating) systems and have been widely used, as they are easily realizable and offer a facile approach for obtaining a predictable and extended delivery of the drug in the GI tract. They comprise effervescence-producing excipients along with gelling polymers that form a floating, cohesive gel possessing the potential to release the incorporated drug in a consistent manner, offering fairly constant drug plasma profiles [17]. The hydrogels form a gel upon coming in contact with the fluids of the body, or with alterations in pH, but are liquid at room temperature. The main objectives for developing this system are a reduction in the frequency of dosing, enhancement in response of the drug by restriction at the action site, diminution of the required dose or allowing consistent drug release. A few raft-forming systems for localizing drug delivery are commercially available, like Topalkan and Almagate FlatCoat for sustained antacid effect [18]. The delivery system-forming raft also has other merits, like a trouble-free manufacturing process, improved patient acceptance and effortless administration [19]. Thus, to enhance the solubility of chebulinic acid in the stomach, solid dispersions were formulated, and to prolong the residence time of the drug in the stomach for maximum ulcer healing effect, floating raft systems were developed. The solid dispersions of chebulinic acid were prepared by varying the ratio of drug and polymer to achieve maximum solubility and dissolution, and then they were characterized employing Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD) and scanning electron microscopy (SEM). Thereafter, the best ratio was selected for formulating the raft system employing sodium alginate and evaluated for floating characteristics and dissolution kinetics. Finally, *in vivo* efficacy was established utilizing an alcohol-induced GU model in rats.

Materials & methods

Materials

The drug, chebulinic acid was isolated and characterized by the previously reported method [20,21]. The solvents (acetone, hydrochloric acid, ethyl alcohol) and excipients employed for making raft system (sodium alginate, sodium bicarbonate, calcium carbonate) were procured from LobaChemie Pvt. Ltd. Mumbai, India. The polymer employed for making the solid dispersion was Eudragit EPO, provided by Evoniks Rohm Gmbh, Germany. Water for HPLC was prepared by Micropore™ assembly installed at Shoolini University, Solan. All other chemicals were of analytical grade and all glassware used was the borosilicate type

Male Sprague-Dawley rats were obtained from the Animal House facility, Shoolini University, Solan, HP, India and habituated to laboratory conditions for 7–10 days before the start of the experiment. Animals were housed in individual cages maintained at a temperature of 23 ± 2°C, in controlled relative humidity conditions (50 ± 5%), and a 12:12 h light and dark cycle. Animal studies were carried out with prior approval of the Institutional Animal Ethical Committee, Shoolini University (IAEC/SU/09/18).

Methods

Determination of the solubility of chebulinic acid

The solubility of chebulinic acid was determined in 0.1N hydrochloric acid employing the equilibrium solubility method. An excess amount of chebulinic acid was added to 0.1N HCl in a volumetric flask. The flasks were placed in an incubator shaker (Orbitek, ScigenicBioteck Pvt. Ltd., Chennai) maintained at 37°C, and shaken for 72 h at 300 r.p.m. The sample was then filtered through Whatman filter paper and the filtrate was diluted and analyzed using UV-VIS double-beam spectrophotometer at λmax 282 nm. The amount of pure chebulinic acid dissolved in hydrochloric acid was obtained using the equation of pure chebulinic acid calibration curve in hydrochloric acid [22].

Preparation & characterization of chebulinic acid solid dispersions

Being a highly hydrophobic drug, the solubility of chebulinic acid is low in water (as can be seen from the results of the solubility study), which ultimately leads to low oral bioavailability [22]. Therefore, there is a need to develop a novel polymer-based formulation for the effective delivery of chebulinic acid to overcome its poor biopharmaceutical properties. Chebulinic acid solid dispersions (CASDs) were formulated using the solvent evaporation method as reported by Kaewnopparat *et al.* [23]. The method has been elaborated here as given in the original publication to maintain transparency. Chebulinic acid and Eudragit EPO were weighed in the ratio of 1:3, 1:5, 1:7.5 and 1:10, respectively. Acetone (50 ml) was used to dissolve chebulinic acid (500 mg) and a weighed amount of Eudragit EPO was added. The solvent was drawn out using a rotary evaporator at 40°C and the resulting viscous solid residue was dried in a vacuum oven at room temperature. Pulverization of the dried samples was done using a mortar and pestle. The CASDs were stored in airtight containers at 25°C, protected from light until further use. Chebulinic acid and Eudragit EPO were mixed in a mortar in the same weight ratios as the solid dispersions to obtain the physical mixtures (PMs). The resulting mixtures were stored in airtight containers, protected from light, after sieving. The solubility determination of the prepared solid dispersions and corresponding PMs was done using the same method as was used for chebulinic acid and the analysis of the resulting final solutions was done spectrophotometrically at λmax 282 nm [24].

Fourier transform infrared spectroscopy

FTIR analysis of chebulinic acid, Eudragit EPO, solid dispersions and PMs was done using FTIR (Agilent technology, Danbury, USA; Cary 630). This was done to check drug–excipient interactions. IR spectra were recorded in absorbance mode in the spectral region of 4000 to 500 cm^{-1} at a resolution of 1 cm^{-1} [25].

Powder x-ray diffraction studies

The XRD studies of chebulinic acid, Eudragit EPO, solid dispersions and PMs were conducted using x-ray diffractometer. The tube voltage was adjusted to 45 kV and the current was 40 mA. The samples were packed in an aluminum sample container. The samples were scanned in a 2Θ range of 5–40° with a step size of 0. 0170. The study was carried out at room temperature [26].

Table 1. Composition of various floating raft-forming formulations.

Formulation	Sodium alginate (%w/v)	Calcium carbonate (%w/v)	Chebulinic acid-Eudragit solid dispersion (%w/v)
F1	1	0.5	0.3
F2	2	0.5	0.3
F3	3	0.5	0.3
F4	1	1	0.3
F5	1	3	0.3
F6	1	0.5	0.6
F7	1	0.5	0.9
F8	1	1	0.9
F9	1	1	0.6

Scanning electron microscope study

SEM of the optimized chebulinic acid solid dispersion was performed to check its surface morphology at different magnification powers using SEM (Hitachi s-4800, Japan). The samples for SEM were made on carbon conductive tape and kept inside t\a vacuum chamber [25]. Moving electrons from tungsten filament were made to fall on the sample to reveal the surface morphology.

Characterization of chebulinic acid solid dispersions incorporated into gastroretentive raft systems

Nine raft-forming formulations incorporating the chebulinic acid-Eudragit EPO solid dispersions were formulated. The composition of various floating raft-forming formulations is given in Table 1. The method used for the preparation of the raft-forming formulations was largely adapted from the work of Kerdsakundee *et al.* [2] and Wannasarit [27] and has been briefly described below to maintain transparency. The method was employed since it gives rafts with desired characteristics resulting in successful formulations. A deionized water solution of 1% w/v sodium bicarbonate was used to dissolve sodium alginate (1–3%). Calcium carbonate (0.5–3%) and chebulinic acid-Eudragit solid dispersions (0.3–0.9%) were then added at various levels and thoroughly dispersed by stirring. Deionized water was used to make the volume 100 mL. The chebulinic acid raft-forming formulations were stored protected from light until used [27].

Determination of viscosity

The viscosity of the prepared raft-forming systems was measured using a Brookfield viscometer (INCO, Ambala, India), making use of spindle number 64 at 25 ± 1°C. The measurement of the viscosity of each sample was done in triplicate and each measurement took approximately 30 s [25].

Density measurement

The raft density of all formulations was measured by taking 10 mL of the raft-forming liquid in a measuring cylinder containing 75 mL of 0.1N hydrochloric acid, pH 1.2. The volume and weight of the gel were determined to calculate the density [2].

In vitro floating studies of raft systems

A total of 150 mL of 0.1N hydrochloric acid (pH 1.2) was used as the medium and was introduced into a 250-mL beaker containing the raft-forming liquid at a temperature of 37 ± 2°C. The time required by the formulation to come to the surface of the medium (floating lag time) and the time for which the formulation consistently floated on the medium surface (duration of floating time) were observed and recorded.

In vitro dissolution profile of different raft formulations

The rate of release of chebulinic acid from the raft-forming systems was ascertained at an agitation speed of 50 r.p.m. using a USP 30 rotating paddle apparatus at 37 ± 0.5°C. This speed was appropriate to maintain the physical integrity of the gelled raft as well as to maintain mild agitation, mimicking *in vivo* conditions. The dissolution medium was 200 mL of 0.1N hydrochloric acid (pH 1.2). A disposable syringe was used to inject 10 mL of the formulation into the dissolution medium. Samples (5 mL) were withdrawn and the dissolution flask was replenished with fresh medium at 30, 60, 120, 180, 240, 300, 360, 420 and 480 min. The amount of chebulinic

acid in the withdrawn samples was determined by a UV spectrophotometer, at a wavelength of 282 nm. A plot of the % cumulative release of the chebulinic acid against time was constructed to depict the drug release profiles.

In vivo pharmacodynamic study

Alcohol-induced gastric ulcer model

Ethanol exposure to the GI tract can alter the motility of the esophagus and stomach by causing damage to the gastric mucosa through the exfoliation of cells, leading to increased mucosal permeability and provoking bleeding [28]. Male adult Sprague-Dawley rats weighing 180–250 g were housed in environmentally controlled rooms (25 ± 2°C, 12 h light and dark cycle). Food was deprived for 18 h before exposure to various treatments. The animals were divided into six groups, each group comprising six animals (n = 6). Group I received a vehicle that served as normal control (1% CMC treated), Group II was alcohol-treated (96% absolute alcohol; 1 ml/200 g), Group III was treated with chebulinic acid suspension (20 mg/kg), Group IV was treated with a chebulinic acid blank raft-forming system (20 mg/kg), Group V was treated with a chebulinic acid raft-forming system (20 mg/kg) and Group VI received omeprazole (10 mg/kg) p.o., as standard. All treatments were given 45 min prior to the induction of GUs. On completion of 1 h of alcohol treatment, the animals were sacrificed and the stomach was dissected and opened along the greater curvature and photographs were taken of the stomach. The lengths of the lesions were measured macroscopically and summated to find the ulcer index [29]:

$$\text{Ulcer index}(mm^2) = \text{length}(mm) \times \text{the width of the ulcer}(mm)$$

,

The percent protection was measured using the following formula [30]:

$$\frac{(\text{Control mean ulcer} - \text{test mean ulcer})}{(\text{Control mean ulcer})} \times 100$$

Statistical analysis

Results are presented as mean ± SD and were analyzed using one-way ANOVA followed by Bonferroni's multiple comparison tests as *post hoc* tests. p-values < 0.05 were considered statistically significant.

Results & discussion

Solubility of chebulinic acid

The solubility of chebulinic acid was found to be 30.774 mg/mL in 0.1N HCl.

Chebulinic acid solid dispersions

For the preparation of solid dispersion, chebulinic acid and Eudragit EPO were used in the ratio of 1:3, 1:5, 1:7.5 and 1:10 [23]. Solvent evaporation was the method of choice for preparing solid dispersions, as the drug and carrier are not subjected to high temperatures, thus maintaining their chemical integrity. It is also an uncomplicated process that is easily applied.

Characterization of chebulinic acid solid dispersions

Solubility of chebulinic acid solid dispersions

Solubility values obtained for CASDs and chebulinic acid PMs in 0.1N hydrochloric acid are depicted in Figure 1. The solubility of chebulinic acid from all the prepared solid dispersions was much higher vis-á-vis their corresponding PMs. The higher solubility of solid dispersions can be attributed to strong intermolecular interactions. Among the different solid dispersions, the drug:polymer ratio 1:5 exhibited maximum solubility (i.e., 73.44 mg/mL) in comparison to the other ratios (1:3, 1:7.5 and 1:10, respectively). At the ratios 1:7.5 and 1:10 of drug and polymer in the solid dispersion, the solubility of chebulinic acid decreased considerably due to a cohesive interaction between the EudragitEPO particles. There must not be sufficient space or chain flexibility to accommodate a liquid molecule to facilitate solubility. Therefore, solid dispersion with a ratio of drug:polymer of 1:5 were selected for the formulation of the raft system and further studies.

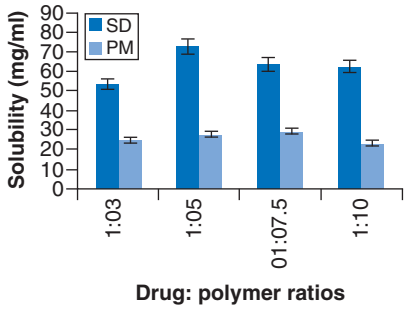

Figure 1. The solubility of chebulinic acid solid dispersions and physical mixtures in 0.1N HCl (pH 1.2).
SD: Solid dispersions; PM: Physical mixture.

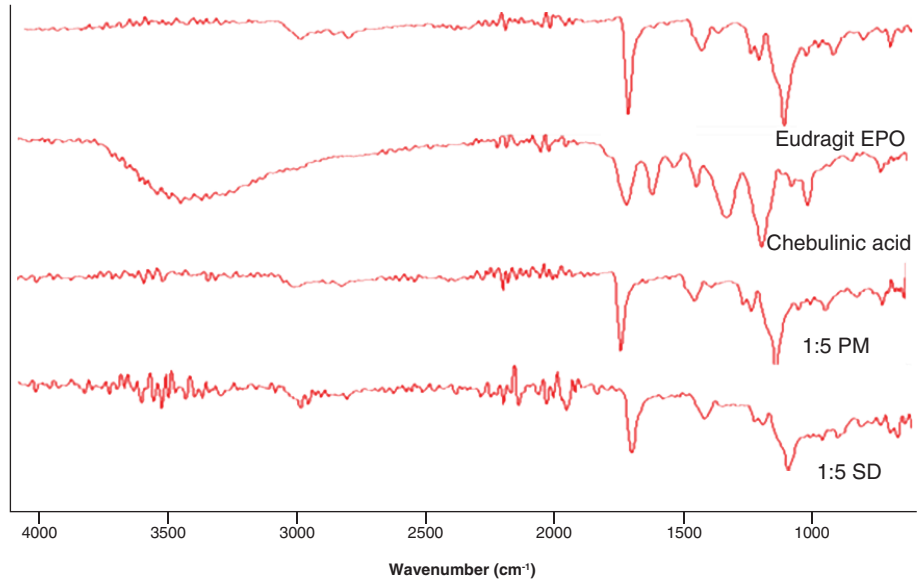

Figure 2. Fourier transform infrared spectroscopy spectra of Eudragit EPO, chebulinic acid, physical mixtures and solid dispersions.
SD: Solid dispersions; PM: Physical mixture.

Fourier transform infrared spectroscopy studies

The FTIR spectra elucidated the interaction between chebulinic acid and Eudragit EPO in the PM and solid dispersion. The FTIR spectra of chebulinic acid showed C-H stretching with vibration at 3372 cm^{-1} and C-OH stretching at 1200 cm^{-1}. Eudragit EPO showed C=O stretching at 1730 cm^{-1}C-OH stretch at 1150 cm^{-1}. In the case of solid dispersions, broadening of all characteristic peaks of chebulinic acid occurred, denoting intermolecular interaction. The supposed intermolecular interaction may be due to hydrogen bonding between the hydroxyl group of chebulinic acid and the Eudragit EPO cationic functional group. The FTIR spectra of the PM revealed that all the peaks were similar to those present in the FTIR spectra of chebulinic acid and Eudragit EPO, which indicates no interaction between chebulinic acid and Eudragit EPO [2]. Although FTIR spectra were obtained for all ratios of PMs and solid dispersions formed, spectra of the PM and solid dispersion 1:5 have been illustrated, as this ratio was chosen for further studies. The results are shown in Figure 2.

Powder x-ray diffraction studies

The powder x-ray diffractograms of chebulinic acid, Eudragit EPO, the PM (1:5), and solid dispersion (1:5), are depicted in Figure 3. A 1:5 ratio was considered for further studies. The diffraction peaks that appear at 5.1380°, 6.4769°, 8.2921°, 10.0470°, 13.3075°, 18.1294° and 20.329°, were the characteristic peaks of chebulinic acid and suggested that chebulinic acid was present in the crystalline form. The diffractogram of the PM presented the characteristic peaks that were analogous to chebulinic acid and established that the crystallinity of chebulinic acid did not change in the PMs. In contrast, the solid dispersion demonstrated a halo pattern, without any characteristic peaks of chebulinic acid, indicating the drug to be present in its amorphous form [31]. The conversion of the drug

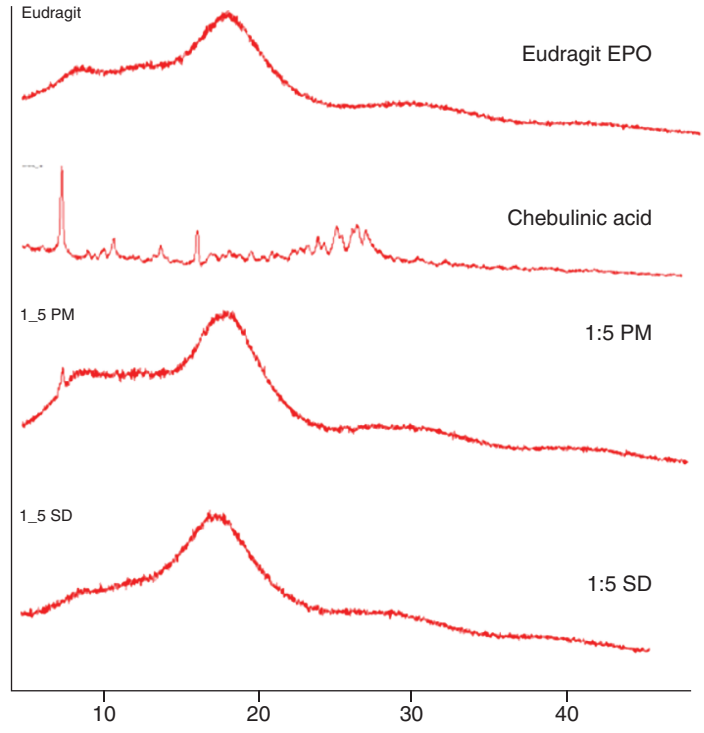

Figure 3. Powder x-ray diffractograms of chebulinic acid, Eudragit EPO, physical mixtures and solid dispersions.
SD: Solid dispersions; PM: Physical mixture.

from crystalline to amorphous form also suggests enhancements in aqueous solubility, as amorphous forms have been reported to possess better aqueous solubility over their crystalline form. These results may have been caused by Eudragit EPO prohibiting the association of chebulinic acid molecules to form crystal nuclei and preventing their growth. Moreover, there may be certain bonding interactions, such as the hydrogen bonding between chebulinic acid and the Eudragit EPO, which further prevented chebulinic acid from crystallizing [25,32].

Scanning electron microscopy

A scanning electron microscope was used to characterize particle state and morphology. SEM photographs of 1:5 CASDs are depicted in Figures 4A & B, as this ratio presented the highest enhancement in solubility of chebulinic acid and was therefore selected for further studies. The pictures revealed a uniform and homogeneously mixed mass of the solid dispersion. These results clearly indicate that chebulinic acid was completely adsorbed onto Eudragit EPO and was homogeneously dispersed in the polymer at the molecular level. Further, only amorphous mass was visible and no drug crystals were observed in the pictures, indicating the complete adsorption of chebulinic acid onto the polymer employed, thus reinforcing the results of XRD.

Characterization of chebulinic acid solid dispersion-incorporated raft systems

All nine raft-forming formulations prepared were characterized by determining their viscosity, density, total *in vitro* floating duration, floating lag time and *in vitro* drug release profile. Based on the results of characterization, an optimized formulation was selected.

Determination of viscosity

The formulation should have good swelling properties achievable by optimum viscosity, which then results in floating due to ionic interaction [33]. The viscosity of the chebulinic acid raft-forming formulations is illustrated in Figure 4C. The viscosity of all the raft gels was in the range of 70–77 cps and that of the raft solutions was in the 1.2–2.6 cps range. Of all the variables studied, sodium alginate concentration influenced the viscosity in a considerable manner. There was a marked rise in viscosity with the increase in the concentration of sodium alginate, owing to its high solubility in water, which might have favored entropic contribution from the free counter ions. Further, the increase in viscosity on increasing the sodium alginate concentration could be due to strong cross-linking in the polymer matrix. However, $CaCO_3$ CASD concentration did not significantly affect the viscosity of the raft formulations.

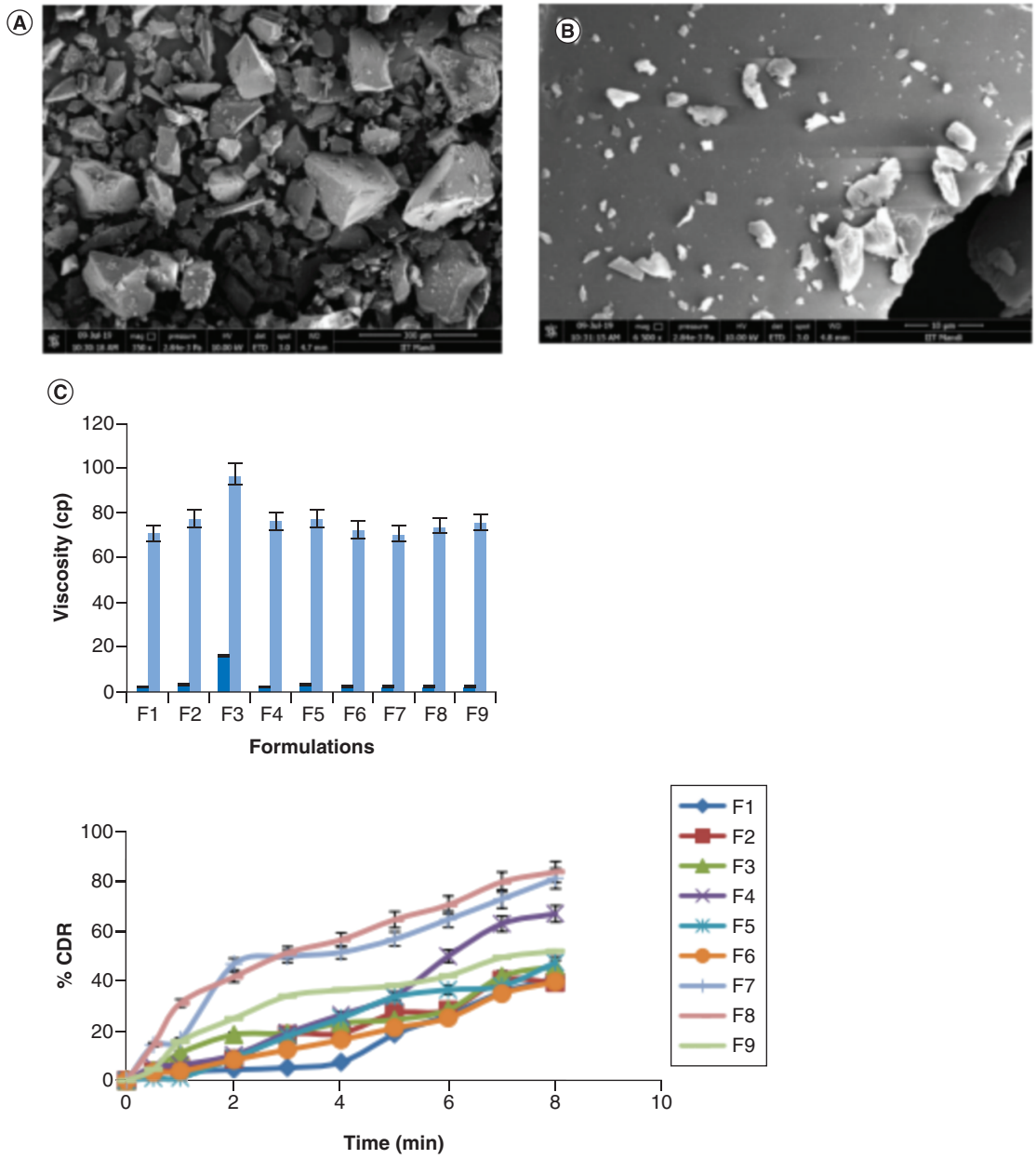

Figure 4. Results of characterization of solid dispersion and raft formulations of chebulinic acid. (A) Scanning electron microscopy pictures of 1:5 solid dispersions at 330×. **(B)** 1:5 solid dispersions at 6500×. **(C)** Viscosity of chebulinic acid solid dispersions incorporated in raft systems. Light blue bars represent viscosity of raft formulations after gelling and dark blue bars represent viscosity before gelling. **(D)** Dissolution profile of chebulinic acid and different raft formulations.
CDR: Cumulative drug release.

Density measurement

The density of different floating raft formulations ranged between 0.982 g/mL and 1.012 g/mL, which was smaller in comparison to the density of the gastric contents (~1.0597 g/mL). These results established the buoyant nature of the raft in the gastric fluid that is expected to have a longer residence time in the stomach, locally facilitating sustained slow drug release. The interaction between alginate and monovalent or divalent cations led to the conversion from liquid to gel. The entrapped carbon dioxide in the gel causes the buoyancy of the raft. In the raft-forming formulation, $CaCO_3$ was present in an insoluble state but it dissolves at the acidic pH of 1.2–3.5 [34]. Calcium carbonate releases calcium ions (Ca^{2+}) and CO_2 under the conditions prevailing in the stomach. These

Table 2. Densities and floating ability of raft formulations.

S. No.	Raft formulation	Floating lag time (s)	Duration of floating	Density (g/ml)
1	F1	8	More than 24 h	1.012 ± 0.001
2	F2	29	More than 24 h	0.984 ± 0.002
3	F3	55	More than 24 h	1.005 ± 0.001
4	F4	6	More than 24 h	0.986 ± 0.002
5	F5	5	More than 24 h	0.998 ± 0.001
6	F6	7	More than 24 h	0.996 ± 0.003
7	F7	8	More than 24 h	1.001 ± 0.002
8	F8	6	More than 24 h	0.985 ± 0.001
9	F9	6	More than 24 h	0.982 ± 0.002

free calcium ions take the place of sodium ions in sodium alginate. Crosslinking occurs between the calcium ions and adjacent alginate strands forming egg-box structures that increase the network strength of the gel [35]. The entrapment of released carbon dioxide in the gel leads to floatation on the surface of the gastric medium. The raft density was affected by different alginate concentrations. The density of the raft is directly proportional to the alginate content present. However, even at high alginate content, the raft-forming formulation still forms a gel and floats in the acidic medium. Analogous to the results obtained in the case of viscosity, an increase in the concentration of sodium alginate also resulted in an increase in density. However, the concentration of calcium carbonate did not influence the density of rafts. The results are shown in Table 2.

In vitro floating study

The floating study of the dosage form can assist in developing an optimized drug formulation with faster onset and continuous floatation, thus preventing premature evacuation from the stomach. The time in which the formulation moved from the bottom of the medium to the surface (floating lag time) and the time for which the formulation remained on the medium surface, (duration of floating) are shown in Table 2. All raft formulations took less than 1 min to float on the surface and floated for more than 24 h. By increasing the sodium alginate concentration, the floating lag time increased as can be seen from the results of the F1 to F3 (Table 2) batches in which sodium alginate concentration was increased from 1 to 3%. This could be because increased sodium alginate might have reacted with calcium carbonate to produce a crosslinked 3D gel network and swollen structure that restricted further liberation of carbon dioxide and drug molecules, thus reducing the buoyancy and increasing the lag time.

In vitro dissolution studies of chebulinic acid & raft formulations

The dissolution profiles of all raft-forming formulations are shown in Figure 4D. The *in vitro* drug release can be divided into two phases. The initial phase, or the burst phase, occurred due to no or partial gelation. After adequate formation of the gel raft, the residual drug was released gradually as it was confined to the gel network. This represented the second phase (moderate release phase) of drug release. In the formulations F1, F2 and F3, only the sodium alginate concentration was increased to 3% from 1%, while calcium carbonate ($CaCO_3$) and CASD concentrations were constant. Increasing sodium alginate concentration to 3% from 1% increased the percentage of cumulative drug release (%CDR). This is attributed to the high polymer concentration, which resulted in increased floating lag time (Table 2; i.e., 8 s for F1, 29 s for F2 and 55 s for F3) and decreased density. Less floating lag time of F1 might have resulted in poor wetting, consequently exhibiting slower release of the drug, whereas the other raft formulations, F2 and F3, due to more floating lag time, were better wetted and thus had a higher %CDR vis-à-vis F1. On increasing the concentration of $CaCO_3$ in the raft formulation 0.5% in F1 to 1% in F4, there was an increase in the %CDR. However, a significant difference in the %CDR was observed when $CaCO_3$ was further increased to 3% in F5. Although a stronger raft was formed from calcium ions in F4 and F5, the presence of carbon dioxide (CO_2) in the gel network made it porous that increased the release rate. Elevated concentrations of $CaCO_3$ not only produced more calcium ions but also more CO_2, thus affecting the release rate. Similar results were also seen in raft formulations F7 and F8, wherein the concentration of $CaCO_3$ was increased from 0.5 to 1%. Further, while the %CDR of F8 was more than F9, both having the same $CaCO_3$ concentration, the only difference was in the CASD amount, which was more in F8 (0.9%) than F9 (0.6%). This could be attributed to the high concentration of CASD present in the case of F8 vis-á-vis F9, which helped maintain a

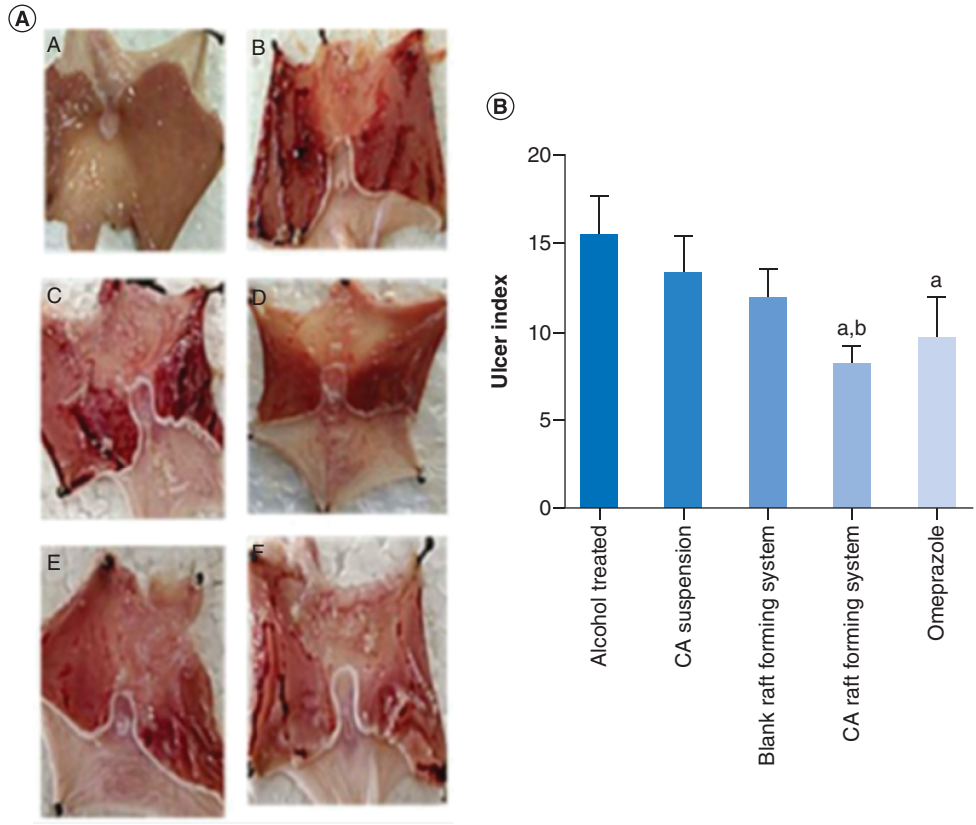

Figure 5. Results of in vivo study for chebulinic acid raft formulation. (A) Gastric ulcers induced by ethanol (1 ml/200 g) and protective effects of chebulinic acid raft-forming system; normal saline; ethanol-treated. **(B)** Ethanol + CA suspension. **(C)** Ethanol + CA raft-forming system (20 mg/kg). **(D)** Ethanol + placebo (20 mg/kg). **(E)** Ethanol + omeprazole (10 mg/kg). **(F)** Ethanol caused extensive hemorrhagic ulcers. **(B)** Curative effect of CA suspension, CA raft-forming system and omeprazole on alcohol-induced ethanol-treated gastric ulcer in Sprague-Dawley rats. Mean ± SD analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test: $p < 0.05$ versus alcohol-treated, $p < 0.05$ compared with the CA suspension-treated rats.
CA: Chebulinic acid.

higher concentration gradient in F8, as required for the dissolution process to occur according to the modified Noyes-Whitney law of dissolution.

Selection of optimized formulation

Based on the results of the characterization, formulation F8 was selected as the optimized batch. The F8 formulation exhibited a blend of desirable characteristics like optimum viscosity before and after gelling, total floating time of more than 24 h, small floating lag time and a low density along with a high %CDR as can be seen from Table 2 & Figure 4C & D.

In vivo pharmacodynamic study

Alcohol-induced gastric ulcer model

The gastroprotective effect of pretreatment with chebulinic acid raft systems on ethanol-induced gastric lesions was determined using the F8 formulation, as this was selected as the optimized formulation based on the results of the characterization studies. The results are shown in Figure 5A & B & Table 3. Normal saline, chebulinic acid suspension, blank raft system, chebulinic acid raft-forming system (F8) and omeprazole were given 45 min preceding induction of GU. On completion of 1 h of alcohol treatment, the animals were sacrificed and the stomach was dissected and opened along the greater curvature to take the photographs of the stomach. In the vehicle-treated group, no macroscopic lesions were found (Figure 5A). In the control group (ethanol-treated), severe gastric lesions and linear hemorrhages were seen in the mucosal layer (Figure 5A & B). Pretreatment with

Table 3. Percentage protection effect of chebulinic acid suspension, chebulinic acid raft-forming system and omeprazole on alcohol-induced gastric ulcer in Sprague-Dawley rats.

S. No.	Groups	% Protection
1	Normal control	–
2	Alcohol treated	–
3	Chebulinic acid suspension	14.95
4	Chebulinic acid blank raft-forming system	21.58
5	Chebulinic acid raft-forming system	47.0
6	Omeprazole	37.22

chebulinic acid suspension, chebulinic acid raft system (20 mg/kg), omeprazole (10 mg/kg; Figure 5A, C, D, F) significantly decreased the ulcer index of lesions compared with the control group. A significantly more potent therapeutic efficacy with a decrease in ulcer index was observed in the chebulinic acid raft-forming system given at 20 mg/kg than in the chebulinic acid suspension group (20 mg/kg). This curative effect could be ascribed to the better efficacy of chebulinic acid from the gastroretentive raft system. Owing to the formation of a thick barrier on the top of the gastric fluid by the raft system, which subsequently obstructs the backflow of gastric fluid to the esophagus thereby reducing the symptoms of gastric acid reflux in the esophagus (GERD) and providing protection against GUs. The percent protection effect for different treatments, on alcohol-induced GUs in SD rats, followed the trend of the chebulinic acid suspension (i.e., 14.95), <blank raft system (i.e., 21.58), <chebulinic acid raft-forming system (i.e., 47.0), >and omeprazole (i.e., 37.22). The decrease in the ulcer index of the chebulinic acid raft-forming system also expresses significantly ($p < 0.05$) superior curative effect on the GUs in comparison to the standard drug, omeprazole, and chebulinic acid suspension

Conclusion & future perspective

The present work resulted in the successful formulation and development of raft-forming systems loaded with chebulinic acid Eudragit EPO solid dispersion for improvement in dissolution rate and availability of poorly soluble active constituent chebulinic acid. The optimized formulation, F8, comprised 1 g sodium bicarbonate, 1 g sodium alginate, 1 g calcium carbonate and 0.9 g of solid dispersion, eliciting optimum characteristics for gastric delivery. The raft-forming delivery system exhibited biphasic *in vitro* drug release following zero-order kinetics. The XRD study revealed the amorphous state of the drug in solid dispersion, justifying the enhancement in dissolution rate. Chebulinic acid raft-forming systems given orally in the dose of 20 mg/kg in a rat model showed significantly higher curative efficacy with a decrease in ulcer index and increased percent protection in comparison to chebulinic acid suspensions during pharmacodynamic study. The study gave compelling evidence for use of the CASD-raft-forming system for providing therapeutic benefits in GUs.

Raft systems may prove conducive in enhancing the efficacy of drugs with an absorption window in the stomach and with poor stability at the alkaline pH. It would be a boon for drugs having localized action in the stomach. However, further studies need to assess the impact of fed and fasted states on the residence time of the system and to find ways to alleviate this effect. The simplicity of the modulation of residence time in the stomach of the raft systems would assist in the translation of this approach from research to commercially viable products.

Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Summary points

- Solid dispersion of chebulinic acid with a hydrophilic polymer was designed to overcome the poor biopharmaceutical properties.
- Raft-forming systems incorporating chebulinic acid Eudragit EPO solid dispersion were developed to increase residence time in the stomach.
- Rafts were prepared and optimized with respect to sodium bicarbonate, sodium alginate, calcium carbonate and solid dispersion.
- The release of chebulinic acid in the dissolution medium, was less than 40% within 2 hours, whereas the solid dispersion ratio of 1:5 showed 95.45% drug release. The best floating raft system exhibited 80% drug release in 8 hours with sustained effect.
- The best floating raft system revealed a floating lag time of less than 8 seconds and a density of less than 1 g/mL.
- Chebulinic acid raft-forming systems showed potent therapeutic effectiveness with a decrease in the ulcer index and increase in percent ulcer protection as compared with the standard drug, omeprazole.

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