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Research Article

Theme: Lipid-Based Drug Delivery Strategies for Oral Drug Delivery
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Lipid Nanoemulsions of Rebamipide: Formulation, Characterization, and *In Vivo* Evaluation of Pharmacokinetic and Pharmacodynamic Effects

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Abstract. Rebamipide has low oral bioavailability (10%) due to its low solubility and permeability. Lipid nanoemulsions (LNEs) were prepared in order to improve its oral bioavailability. Rebamipide-loaded lipid nanoemulsions were formulated by hot homogenization and ultrasonication method. Olive oil and egg lecithin in various concentrations as emulsifier were used in the preparation of LNEs. The lipid nanoemulsions were evaluated for various parameters. The globule size, polydispersity index (PDI), and zeta potential (ZP) of the formulations ranged from 230.3 ± 3.88 to 279.8 ± 5.76 nm, 0.204 ± 0.008 to 0.246 ± 0.029, and − 27.7 ± 2.05 to − 31.0 ± 1.87 mV, respectively. Entrapment efficiency and assay values ranged from 99.90 ± 0.006 to 99.92 ± 0.002% and 99.3 ± 0.808 to 99.6 ± 0.360, respectively. Physical stability test results revealed that the optimized LNEs were stable for 2 months at both room (25°C) and refrigerated temperature (4°C). The optimized LNE showed 4.32-fold improvement in the oral bioavailability in comparison to a marketed tablet suspension. *In vivo* anti ulcer activity of rebamipide LNE was studied by testing the prophylactic effect in preventing the mucosal damage in stomach region. The mucosa of stomach in animals was damaged by per oral administration of 80% alcohol. Maximum prophylactic antiulcer activity was observed by per oral delivery of rebamipide as LNE. Our results indicated that LNEs were a promising approach for the oral delivery of rebamipide for systemic effects along with local effects in protecting gastric region, which gets damaged during peptic ulcers.

KEY WORDS: rebamipide; bioavailability; lipid nanoemulsions; antiulcer activity; prophylactic.

INTRODUCTION

Rebamipide is an anti-ulcer agent that acts by stimulating prostaglandin production in the gastric mucosa and inhibiting several inflammatory mechanisms including neutrophil activation in the blood capillary. Therefore, its systemic absorption into the blood circulation is important for its therapeutic action after oral administration (1,2). It is available as 100-mg tablets under various trade names, in Japan (Mucosta), South Korea, China and India (Rebagen), and Russia (Rebagit).

Rebamipide is a Biopharmaceutics Classification System (BCS) class IV drug and has low systemic availability (10%), which is due to its low solubility and low permeability (3). Thus, many attempts were made to improve its bioavailability, such as preparation of microemulsions, solid dispersions, and nano-crystal tablets (3–5). However, there is a necessity to develop more efficient oral formulations for rebamipide. Further, lipid nanoemulsions (LNEs) were not reported for oral delivery of rebamipide.

LNEs are promising delivery systems composed of oil as internal phase and egg lecithin as emulsifier. Drug candidates having limited solubility can be dissolved in the internal oil phase. LNEs were primarily used in the form of parenteral fat emulsions for energy requirements (6). LNEs are also advantageous because of their properties of prolonged release, delivery to target tissues, less irritation, and low toxicity (7–11).

The bioavailabilities of many drugs, such as danazol, cefuroxime axetil, and baicalin were enhanced by formulating them into LNEs (12–14). Further through LNEs, drug targeting was achieved. Here, some studies were included such as diclofenac to inflammatory sites, indinavir to brain, and docetaxel and diferuloylmethane to tumors (15–23).

BCS class II drugs are commonly prepared as LNEs, but class IV drugs are rarely reported through the LNEs. Rebamipide is a BCS class IV drug. The enhancement in bioavailability of rebamipide is only possible when both

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solubility and permeability of the drug are increased. We believe that LNE is a suitable novel delivery system for this drug.

In this work, an effort was made to prepare LNE formulation to enhance the oral performance of rebamipide. The LNEs were formulated using edible oils and excipients that belonged to the GRAS category. The prepared LNEs were characterized and *in vivo* studies were conducted, *i.e.*, anti-ulcer activity and pharmacokinetic effects. Optimized LNE was compared with a marketed rebamipide tablet suspension for *in vivo* performance. We observed enhanced bioavailability coupled with superior prophylactic anti-ulcer activity in our study.

Materials

Rebamipide (gift sample from Daewoong Pharma Pvt. Ltd., Hyderabad, India); egg phosphatidylcholine (EPC80); olive oil (Lipoid, Ludwigshafen, Germany); oleic and acetic acid (SD Fine Chemicals, Mumbai, India); cholesterol and glycerol (Merck, Mumbai, India); centrisart tubes (Sartorius, Goettingen, Germany), dialysis membrane (HiMedia, Mumbai, India), methanol and acetonitrile (Merck) and other chemicals used in the study were of AR grade.

METHODOLOGY

Characterization of Drug by DSC

DSC thermogram of the drug was obtained by using DSC 4000 model, PerkinElmer, Waltham, MA, USA. About 10 mg of drug sample was crimped into DSC crucible. The sample was scanned in the range of 50–350°C and the temperature increment rate was 10°C per minute.

Oil Solubility Studies

Drug solubility in oil was determined after adding excess amount of drug to oil. The contents were shaken for 48 h at 180 rpm on a gyratory shaker. The supernatant obtained after centrifugation was filtered by a membrane filter (0.22 μ). The filtrate was assayed by HPLC after suitable dilution with mobile phase.

Preparation of Rebamipide-Loaded LNEs

The LNE formulations were formulated by hot homogenization and ultrasonication method (16). Oil-soluble components were dissolved in the oily phase and water-soluble components were dissolved in the aqueous phase (Table I) and the two phases were separately heated to 70°C. Then, aqueous phase was added slowly to oily phase with rapid stirring. The mixture was homogenized (DIAX 900 homogenizer, Heidolph, Germany) at 15,000 rpm for about 5 min. The coarse emulsion formed was subjected to ultrasonication using 12 T probe for 20 min at 50% amplitude by ultrasonicator (Vibracell, Sonics Material, Inc., CT, USA).

Table I. Saturation solubility data of rebamipide

Vehicles	Solubility (mg/mL) Average ± SD
Soy bean oil (1 mL)	15.87 ± 0.197
Olive oil (1 mL)	18.98 ± 0.122
Arachis oil (1 mL)	16.13 ± 0.212
Soy bean oil (1 mL) + egg lecithin (100 mg)	22.04 ± 0.528
Olive oil (1 mL) + egg lecithin (100 mg)	24.76 ± 0.522
Arachis oil (1 mL) + egg lecithin (100 mg)	24.71 ± 0.248

Determination of Globule Size, PDI, and ZP

With double-distilled water, LNE was diluted 1:100 times (100–300 kilo counts per second (kcps)) and used in the study. Zeta sizer (Malvern Nano ZS90, Worcestershire, UK) was used for determination of globule size, polydispersity index (PDI), and zeta potential (ZP) (17).

Estimation of Entrapment Efficiency

The entrapment efficiency was calculated by estimating the unentrapped drug in the aqueous phase, being separated by ultrafiltration using centricon apparatus having mol. wt cutoff of 20,000 (Sartorius, USA) (16,18). The sample was spun at 4000 rpm for a period of 15 min. Clear aqueous phase was separated during this technique. The concentration of unentrapped rebamipide in aqueous phase was determined by HPLC.

Determination of Rebamipide Content in Formulations

Serial dilutions of the LNE sample were done with methanol in the ratio of 1:10 (19,20). The diluted samples were assayed by UV method at λ_{max} 237 nm for rebamipide present.

In vitro Drug Release Studies

The rebamipide release was determined by dialysis method using open tube with a dialysis membrane (MWCO, 12,000–14,000 Da, HiMedia, Mumbai) which was previously soaked overnight in double distilled water (21). One milliliter of the emulsion was taken in an open tube for the study and the drug release was conducted for 24 h. The release medium was 0.1 N HCl for first 2 h and 6.8 pH phosphate buffer for the next 22 h. The volume of the release medium taken for the study was 100 mL. The release was conducted on a magnetic stirrer at 100 rpm and room temperature (Remi Equipments, Mumbai, India). One milliliter of sample was collected at 0.25-, 0.5-, 1-, 2-, 3-, 4-, 6-, 8-, 10-, 12-, and 24-h time points for estimation of drug content. Equal volume of fresh medium was added at every time point. The drug concentration in the samples was determined by UV method using a spectrophotometer, SL 159, ELICO, Hyderabad, India, at λ_{max} 237 nm.

Physical Stability of LNE: Effect of Centrifugation, Autoclaving, Dilution Stress, and Storage

The LNE formulations were observed for the changes after subjecting to centrifugation, autoclaving, dilution, and under normal storage conditions (22–26).

Centrifugal Stress Effect

About 1 ml of LNE formulation was taken in Eppendorf tubes and centrifuged in a centrifuge (Heraeus Biofuge, Germany) at a speed of 13,000 rpm for a time period of 10 min and percentage creaming volume for each formulation was determined.

Autoclaving Effect

The LNE formulations were autoclaved at 121°C and pressure of 15 lb./in.^{2} for about 15 min. The effect of autoclaving on globule size, PDI, and ZP were observed.

Dilution Effect

Dilution of LNEs was done with double distilled water in various ratios (1:50, 1:100, 1:500, and 1:1000). The influence of desorption stress on the LNE formulation was studied by using zetasizer.

Physical Stability of LNE during Storage

Rebamipide LNEs were stored at refrigerated temperature (4°C) and room temperature (25°C) for 2 months. The average globule size, ZP, and PDI were determined.

Pharmacokinetic Study

The oral bioavailabilities of the optimized LNE formulation (F4) and rebamipide tablet suspension were estimated by conducting pharmacokinetic studies in male Wistar rats with an single oral dose (10 mg/kg body weight). The study was approved by IAEC of University College of Pharmaceutical Sciences (UCPSc), Kakatiya University (KU) (Warangal, India). Wistar rats of 180–220 g weight were taken in the study (each group $n = 6$). The formulation was administered by using oral feeding tube. Blood was taken from retro-orbital vein at time intervals of 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h after dose. About 0.5 mL of blood was collected, centrifuged at a speed of 3000 rpm for a period of 30 min for separation of plasma. The plasma samples were kept at −20°C until analysis.

Extraction Procedure

To 100 μL of serum, 0.1 mL of losartan 4 μg/mL (internal standard) was added. Methanol (200 μL) was added as protein precipitant and vortexed for 2 min. After centrifugation (at 12,000 rpm for 10 min), the supernatant obtained was evaporated under vacuum to form a residue, further reconstituted in mobile phase (100 μL). The reconstituted samples were analyzed by using HPLC (4).

Chromatographic Conditions

$$\text{Mobile phase} = \text{Methanol/water}\left(\begin{matrix}70:30, v/v, \text{pH adjusted to}\\ 2.6 \text{ with acetic acid}\end{matrix}\right).$$

$$\text{Flow rate} = 1 \text{ mL/min}.$$

$$\text{Column} = \text{Inertsil ODS}-3\left(\begin{matrix}25\text{cm} \times 0.46\text{cm i.d.}, 0.5-\mu\text{m}\\ \text{particle size}\end{matrix}\right).$$

$$\text{Injection volume} = 20\ \mu\text{L}.$$

$$\text{UV detection} = 237\ \text{nm}.$$

Estimation of PK Parameters

Rebamipide present in rat serum was estimated from the HPLC standard graph. Pharmacokinetic (PK) parameters such as C_{max}, $t_{1/2}$, t_{max}, mean residence time (MRT), and AUC_{0-24}, were calculated by Kinetica 2000 software (version 5.0, Innaphase Corporation, Philadelphia). Relative bioavailability of rebamipide LNEs in comparison to rebamipide tablet suspension was estimated using following equation:

$$\%\text{Relative bioavailability} = \left(\begin{matrix}\text{dose}_{\text{tablet suspension}} \times \text{AUC}_{\text{LNE}}/\text{dose}_{\text{LNE}}\\ \times \text{AUC}_{\text{tablet suspension}}\end{matrix}\right) \times 100$$

PK data of optimized LNE was statistically compared with marketed tablet suspension using Student unpaired t test at p value < 0.05. The software used was Graph pad prism, version 5.02.2013 (Graph Pad Prism, San Diego, CA).

In vivo Antiulcer Activity Evaluation

The animals were differentiated into five groups each containing six rats, which were administered with optimized LNE formulation (F4), rebamipide tablet suspension, blank LNE (10 mg/kg body weight). Ethanol (80%) at a dose of 4 mL/kg was given orally for inducing ulcers to all rats except normal control rats (27).

- Group 1 Normal healthy rats were kept as control without any alcohol treatment.
- Group 2 Treated control with ethanol (80%) (4 mL/kg) for inducing ulcers.
- Group 3 Pretreatment with rebamipide tablet suspension (10 mg/kg) and then inducing ulcers with ethanol (80%) treatment.
- Group 4 Pretreatment with rebamipide lipid nanoemulsion (F4) (10 mg/kg) and then inducing ulcers with ethanol (80%) treatment.
- Group 5 Pretreatment with blank submicron lipid emulsion and then inducing ulcers with ethanol (80%) treatment.

Rebamipide tablet suspension, rebamipide LNE, and blank LNE (placebo) were administered to the animals in groups 3–5, respectively. After 3 h, ethanol (80%) was administered (4 mL/kg) to the 2nd–5th groups. Then, the animals were sacrificed after 3 h of alcohol treatment.

Their stomach regions were isolated and washed with saline. The tissues were stored in formalin (10%) until histopathological examination. The tissue sections were stained using hematoxylin and eosin dyes. The gastro protective effects of the optimized formulation on the gastric damage caused by ethanol (80%) were determined microscopically.

RESULTS AND DISCUSSION

DSC Study

The DSC study was conducted to understand the thermal behavior of the drug. The reported melting point is in the range of 308–310°C. A sharp endothermic peak of rebamipide was observed at 310°C in the developed DSC thermogram, indicating the purity of drug (Fig. 1).

Oil Solubility Studies

Saturation solubility studies of rebamipide were done in various oils and their mixtures with egg lecithin. The solubility of the drug ranged from 16.13 ± 0.212 to 24.76 ± 0.522 mg/mL and showed in Table I. The maximum solubility of 24.76 ± 0.522 mg/mL was observed in a mixture of olive oil and egg lecithin. This combination was used in the formulation of rebamipide LNE.

Formulation of Rebamipide LNE

The formulation composition is given in Table II. The LNEs were prepared by homogenization in hot condition and next subjected to ultrasonication.

Characterization of LNEs

The LNEs were characterized for size, PDI, and ZP, and results are shown in Table III. The particle size, PDI, and ZP of LNEs were in the range of 230.3 ± 3.88 to 279.8 ± 5.76 nm, 0.204 ± 0.008 to 0.246 ± 0.029 and − 27.7 ± 2.05 to − 31.0 ± 1.87 mV, respectively.

Entrapment Efficiency

Entrapment efficiency was determined by HPLC, and results are given in Table III. The entrapment efficiency of LNEs ranged from 99.90 ± 0.006 to 99.92 ± 0.002%.

Assay

Drug content of all the formulations were determined by UV spectrophotometer and results are given in Table III. Assay of LNE formulations ranged from 99.3 ± 0.808 to 99.6 ± 0.360%.

Drug Release from LNEs

The cumulative percent release of drug from the formulations F1–F4 were 57.0 ± 1.72, 60.24 ± 0.89, 66.83 ± 1.59, and 71.89 ± 1.80, respectively in 24 h. In general, the LNEs released slowly in 0.1 N HCl when compared to that in pH 6.8 phosphate buffer, which could be due to the solubility difference of drug in these media. The release profiles of LNE formulations exhibited a typical biphasic pattern with an initial slow phase followed by a rapid phase in phosphate buffer (Fig. 2).

Stability Studies

It was reported earlier that emulsions with higher creaming values showed better stability in centrifugal stress testing (24). In this study, all the formulations showed higher creaming values indicating the stability of LNEs (Table IV) and there was no change in particle size, PDI, and ZP of the formulations upon autoclaving (thermal stress) (Table V). In dilution stress, dilution of emulsion with water disturbs the rigidity of the surfactant layers at the interface leading to changes in ZP (28). In this dilution studies, no significant differences in particle size, PDI, and ZP of the tested formulations were noticed, indicating that all the emulsions were stable (Table VI). In terms of physical stability of LNEs during storage, there were no appreciable changes in particle size, ZP, PDI, or assay indicating that the LNEs were stable on storage at 4°C and room temperature for up to 2 months (Table VII).

Oral Bioavailability Study

The PK parameters of rebamipide in individual rats for optimized LNE and marketed tablet suspension were calculated by using Kinetica software. The PK parameters C_{max}, t_{max}, $AUC_{0–24}$, MRT, and $t_{1/2}$ values were calculated for optimized LNE formulation and compared with that of marketed product as a control. The statistical comparison of data was done by Student unpaired t test at a significance level of p value < 0.05 using GraphPad prism software version 5.02.2013 (GraphPad Software, San Diego, CA). The mean plasma concentration–time profiles of rebamipide for the optimized LNE formulation and marketed tablet suspension are shown in Fig. 3.

From above results, it was found that optimized LNE formulation showed high C_{max}, t_{max} $AUC_{0–24}$, MRT, and $t_{1/2}$ values (0.652 ± 0.133 μg/mL, 3 h, 3.747 ± 0.734 μg × h/mL, 9.807 ± 2.11 h and 9.167 ± 2.99 h), respectively, when compared to marketed product, which showed lower C_{max} and $AUC_{0–24}$, MRT, and $t_{1/2}$ values (0.223 ± 0.03 μg/mL, 2 h, 0.866 ± 0.19 μg × h/mL, 4.738 ± 0.58 h and 2.580 ± 0.49 h) (Table VIII) and there is statistically significant difference between C_{max}, t_{max}, $AUC_{0–24}$, MRT, and $t_{1/2}$ values of all the two groups. The drug suspension generally acts a reservoir of insoluble solid particles and being surrounded by saturated solution of drug. As a result, the drug molecules readily get absorbed. Not only this, the dissolution of insoluble particles may also start quickly. Where as in the case of test formulation (LNE), the drug would be slowly released from the oil globules into the aqueous environment and then get absorbed. However, this delivery system is a nanoemulsion, which provided enormous surface area for release of drug into surrounding aqueous environment. Consequently, the drug absorption is also very fast, which is also reflected in our observations. Hence, t_{max} of suspension is less than t_{max} of LNE. Here, a critical examination of plasma drug

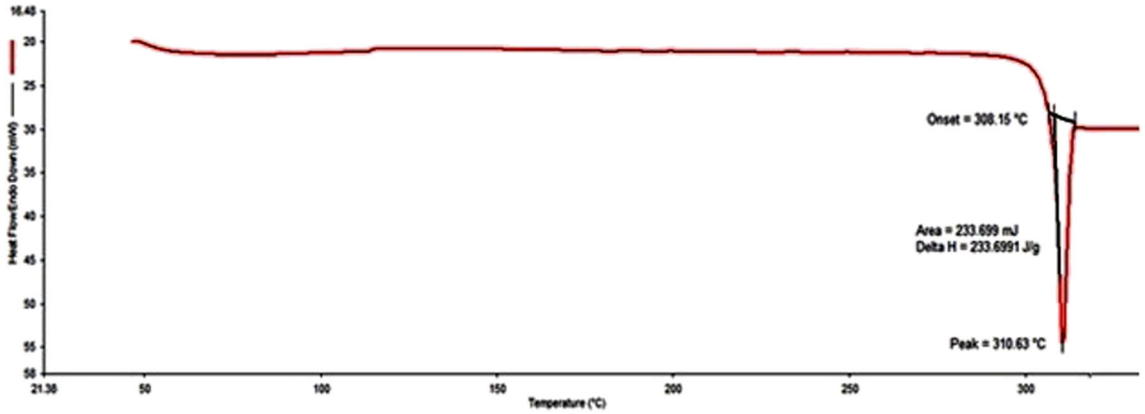

Fig. 1. DSC thermogram of rebamipide (pure drug) showing an endotherm peak at 310°C

Table II. Formulation ingredients of LNEs of rebamipide

Ingredients	F1	F2	F3	F4
Drug (rebamipide) (mg)	100	100	100	100
Olive oil (g)	4	4	4	4
Egg lecithin, EPC-80 (mg)	100	200	300	400
Glycerol (mg)	225	225	225	225
Oleic acid (mg)	25	25	25	25
Cholesterol (mg)	30	30	30	30
Double distilled water (mL)	Up to 10 mL	Up to 10 mL	Up to 10 mL	Up to 10 mL

Table III. Physical parameters of rebamipide LNEs

Formulation	Size (nm)	Polydispersity index	Zeta potential (mV)	Assay (%) ± SD	EE (%) ± SD
F1	279.8 ± 5.76	0.211 ± 0.015	− 27.7 ± 2.05	99.4 ± 0.346	99.90 ± 0.001
F2	258.4 ± 4.29	0.246 ± 0.029	− 29.4 ± 3.32	99.3 ± 0.808	99.92 ± 0.002
F3	248.4 ± 7.35	0.204 ± 0.008	− 30.5 ± 5.92	99.6 ± 0.360	99.91 ± 0.003
F4	230.3 ± 3.88	0.224 ± 0.022	− 31.0 ± 1.87	99.3 ± 0.288	99.90 ± 0.006

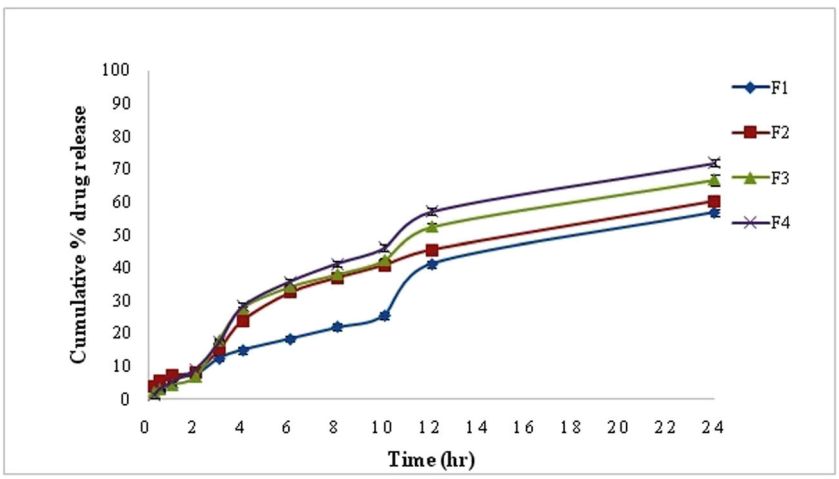

Fig. 2. *In vitro* drug release from LNE formulations

Table IV. Creaming volume percentage of the prepared LNEs, subjected to centrifugal stress

Formulation code	Creaming volume percentage
F1	98.70 ± 0.08
F2	98.82 ± 0.17
F3	98.79 ± 0.13
F4	98.98 ± 0.32

concentrations at second hour time point for both delivery systems clearly indicated that LNEs were showing superior absorption of drug. The bioavailability of optimized formulation was found to be increased by 4.32 times than that of marketed tablet suspension. Further, the MRT and $t_{1/2}$ values were also increased considerably. This enhancement in bioavailability is due to the lipid digestion of nanoemulsion components and faster release of drug in GIT. The enterocytes are responsible for the absorption of lipid digestion products, drug molecules and genesis of chylomicrons and other lipoproteins. Finally, they get exocytosed into the lymphatic vessels. The lymphatic transport of drug along with lipoproteins and chylomicrons is a known mechanism (29–30).

In vivo Anti-ulcer Activity

Anti-ulcer activities of the formulations were tested by checking the prophylactic effect on the possible damage caused to GI mucosa due to alcohol. The following conclusions were observed from the histopathological studies:

Group 1 All the control animals showed intact mucosal lining in stomach region without any visible damage (Fig. 4a, b).

Group 2 Sections from gastroesophageal junctional tissue revealed ulceration, congestion, and other damages in the stomach area (Fig. 4c, d).

Group 3 Majority (5/6) in this group of animals, being treated by rebamipide tablet suspension, revealed no specific ulceration, but some damage was seen. This indicated protection of gastric mucosal damage to some extent only but not complete (Fig. 4g, h).

Group 4 In this group (5/6), animals revealed no clear cut ulceration and fifth rat showed the areas with ulceration. However, the extent of protection in individual rats is far superior to that of single tablet suspension (Fig. 4i, j).

Group 5 In this group also (5/6), animals revealed no specific ulceration, but three animals

Table V. Effect of autoclaving on LNEs

Formulation code	Before autoclaving			After autoclaving		
	Size (nm)	Polydispersity index	Zeta potential (mV)	Size (nm)	Polydispersity index	Zeta potential (mV)
F1	279.8 ± 5.76	0.211 ± 0.015	−27.7 ± 2.05	278.6 ± 2.44	0.229 ± 0.013	−28.9 ± 3.54
F2	258.4 ± 4.29	0.246 ± 0.029	−29.4 ± 3.32	271.6 ± 6.58	0.244 ± 0.055	−31.7 ± 4.18
F3	248.4 ± 7.35	0.204 ± 0.008	−30.5 ± 5.92	255.6 ± 5.19	0.211 ± 0.021	−29.0 ± 2.12
F4	230.3 ± 3.88	0.224 ± 0.022	−31.0 ± 1.87	228.6 ± 2.35	0.236 ± 0.054	−31.8 ± 4.93

Table VI. Effect of dilution stress on LNEs

Formulation code	Dilution ratio	Size (nm)	Polydispersity index	Zeta potential (mV)
F1	1:50	279.8 ± 5.76	0.211 ± 0.015	−27.7 ± 2.05
	1:100	282.7 ± 6.29	0.213 ± 0.011	−25.3 ± 1.52
	1:500	284.9 ± 8.14	0.221 ± 0.019	−26.3 ± 2.11
	1:1000	298.8 ± 11.58	0.218 ± 0.022	−23.7 ± 3.14
F2	1:50	258.4 ± 4.29	0.246 ± 0.029	−29.4 ± 2.36
	1:100	262.2 ± 5.89	0.251 ± 0.016	−28.5 ± 2.20
	1:500	262.5 ± 6.82	0.230 ± 0.019	−28.4 ± 1.88
	1:1000	263.3 ± 3.98	0.225 ± 0.031	−28.2 ± 3.02
F3	1:50	248.4 ± 7.35	0.204 ± 0.008	−30.5 ± 5.92
	1:100	250.9 ± 9.44	0.203 ± 0.014	−30.1 ± 2.93
	1:500	252.5 ± 12.50	0.194 ± 0.058	−29.8 ± 1.62
	1:1000	255.6 ± 10.66	0.211 ± 0.037	−29.8 ± 2.77
F4	1:50	230.3 ± 3.88	0.224 ± 0.018	−31.0 ± 1.87
	1:100	229.3 ± 2.80	0.294 ± 0.075	−31.7 ± 1.22
	1:500	228.6 ± 3.71	0.266 ± 0.063	−31.8 ± 1.53
	1:1000	247.6 ± 5.94	0.202 ± 0.010	−30.4 ± 1.29

Table VII. Stability of optimized LNE formulation (F4) under storage

Time (month)	Room temperature (25°C)				Refrigerated temperature (4°C)			
	Size (nm)	Polydispersity index	Zeta potential (mV)	Assay (%) ± SD	Size (nm)	Polydispersity index	Zeta potential (mV)	Assay (%) ± SD
0	230.3 ± 3.88	0.224 ± 0.018	− 31.0 ± 1.87	98.66 ± 0.35	230.3 ± 3.88	0.224 ± 0.018	− 31.0 ± 1.87	98.66 ± 0.35
1	235.6 ± 2.12	0.226 ± 0.005	− 29.8 ± 5.65	98.46 ± 0.60	237.7 ± 5.11	0.229 ± 0.064	− 31.4 ± 2.34	98.55 ± 0.42
2	239.7 ± 7.35	0.229 ± 0.022	− 31.9 ± 3.88	98.26 ± 0.34	240.8 ± 6.25	0.231 ± 0.072	− 30.0 ± 3.86	98.32 ± 0.22

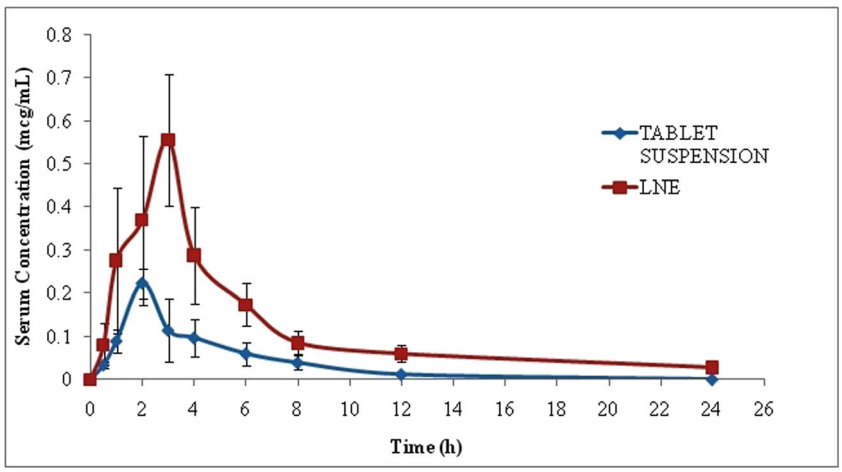

Fig. 3. Comparative pharmacokinetic profiles of rebamipide tablet suspension and LNE formulation

revealed focal areas of superficial ulceration. This study, showed some protection due to emulsion excipients. This is a kind of placebo effect but the protection was not to the same extent as that of LNE with drug (Fig. 4e, f).

The rank order of prophylactic anti-ulcer activity against the damage caused by ethanol (80%) is as follows: F4 (LNE) > tablet suspension > blank LNE (placebo) > control (ethanol treated).

CONCLUSION

In conclusion, LNEs containing rebamipide were prepared. The relative bioavailability of optimized LNE was 4.32 -olds in comparison to marketed tablet suspension. Further, prophylactic effect of the rebamipide LNE was also studied, in comparison with tablet suspension and blank emulsion. The rank order of protective effect against the damage caused by ethanol (80%) was; F4 (LNE) > tablet suspension > blank LNE (placebo) > control (ethanol treated). By administering rebamipide in the form of lipid nanoemulsion maximum

Table VIII. Pharmacokinetic parameters of rebamipide tablet suspension and LNE in Wistar rats ($n = 6$)

Pharmacokinetic parameter	Rebamipide tablet suspension	Rebamipide LNE (F4)
C_{max} (μg/mL)	0.223 ± 0.03	0.652 ± 0.133***
AUC_{tot} (μg h/mL)	0.866 ± 0.19	3.747 ± 0.734***
$t_{1/2}$ (h)	2.580 ± 0.49	9.167 ± 2.99***
T_{max} (h)	2.0	3.0***
MRT (h)	4.738 ± 0.58	9.807 ± 2.11***

The pharmacokinetic data was statistically compared by Student unpaired t test at p value 0.05
***There was significant difference observed between optimized LNE (F4) when compared to tablet suspension in terms of C_{max}, T_{max}, AUC, $t_{1/2}$, and MRT (at $p < 0.0005$)

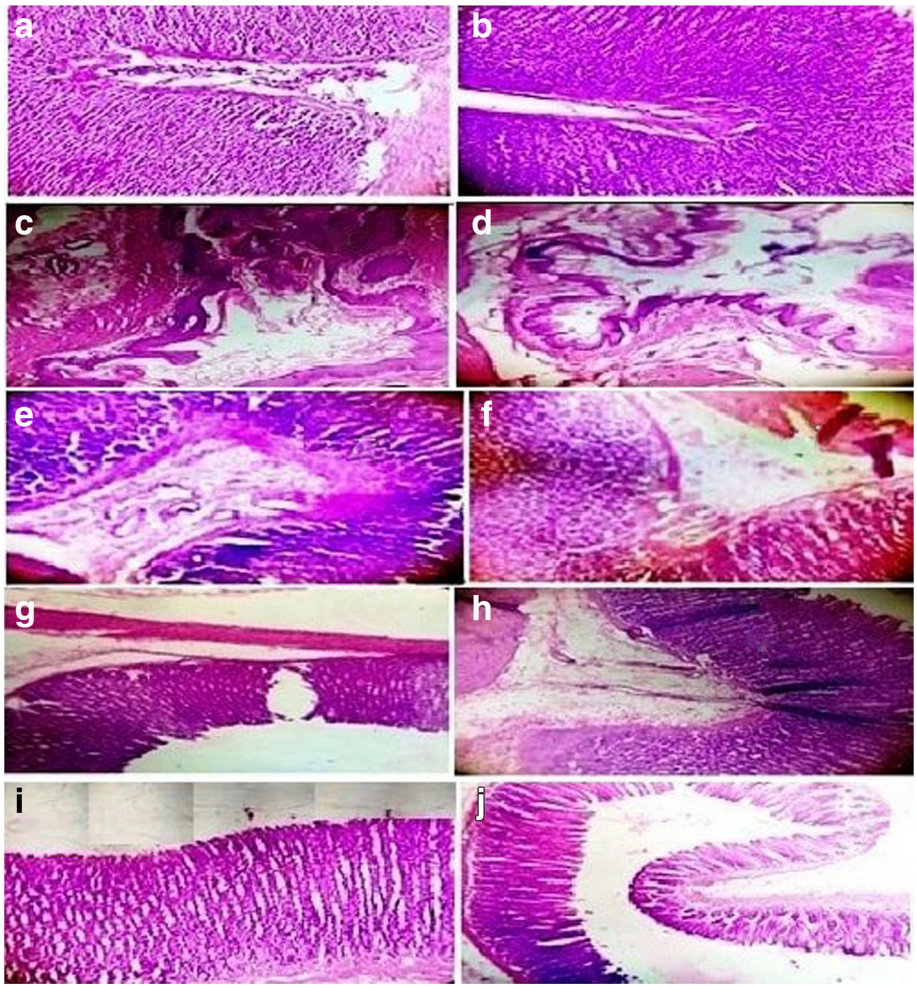

Fig. 4. **a**, **b** Normal (control) rat stomach sections showing intact mucosal lining without any damage at mucosal surface (group 1) (control group). **c**, **d** Stomach sections of rats showing ulcers, being induced by 80% ethanol (group 2) (untreated control group – mucosal surface is damaged). **e**, **f** Stomach sections of rats showing negligible prophylactic effect of blank LNE (group 5) due to excipients after ethanol (80%) treatment (blank LNE-treated group with ulcers). **g**, **h** Stomach sections of rats showing some prophylactic effect of tablet suspension after ethanol (80%) treatment (group 3) (suspension-treated group with ulcers). **i**, **j** Stomach sections of rats showing good prophylactic effect of optimized LNE formulation (F4) after ethanol (80%) treatment (group 4) (LNE-treated group with ulcers). (Magnification × 100)

prophylactic antiulcer activity was observed. Taken together, the developed rebamipide lipid nanoemulsion (F4) containing phospholipids, showed superior performance in terms of pharmacokinetic and pharmacodynamic effects in rats over the tablet suspension.

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COMPLIANCE WITH ETHICAL STANDARDS

Ethical Committee Approval The animal protocol was approved by the IAEC (Protocol no. IAEC/4/UCPSC/KU/2017). The animal treatment in the study was according to CPCSEA guidelines.

Declaration The authors declare that there is no conflict of interest.

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