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Elastic Liposomal Formulation for Sustained Delivery of Antimigraine Drug: In Vitro Characterization and Biological Evaluation

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The aim of this study was to prepare and characterize a topical formulation for sustained delivery of rizatriptan. Elastic liposomal formulation of rizatriptan was prepared and characterized for different characteristics by evaluating in vitro and in vivo parameters. The in vivo performance of optimized formulation was evaluated for antimigraine activity in mice using morphine withdrawal-induced hyperalgesia. The in vitro skin permeation study across rat skin suggested carrier-mediated transdermal permeation for different elastic liposomal formulation to range between 18.1 ± 0.6 and 42.7 ± 2.3 μg/h/cm^2, which was approximately 8–19 times higher than that obtained using drug solution. The amount of drug deposited was 10-fold higher for elastic liposome (39.9 ± 3.2%) than using drug solution (3.8 ± 1%); similarly the biological activity of optimized elastic liposome formulation was found to be threefold higher than the drug solution. On the basis of the results, it can be concluded that the elastic liposomal formulation provided sustained action of rizatriptan due to depot formation in the deeper layer of skin.

Keywords antimigraine; sustained; topical application; elastic liposomes; scanning electron microscopy; biological evaluation

INTRODUCTION

Migraine is a neurological disorder characterized by recurrent attacks of headache, which typically exist for 4–72 h. It is frequently accompanied by nausea, vomiting and other gastrointestinal disturbances, photophobia, and phonophobia (Villalon, Centurión, Valdivia, Vriesl, & Saxena, 2003). Rizatriptan is a 5-HT$_{1B}$ receptor agonist for treating acute attacks of migraine (Lipton, Pascual, & Goadsby, 2001). Although, it is available in tablet and wafer dosage forms, it exhibits low oral availability of only 30–40% and a short biological half-life of 2–3 h (Bigal, Bordini, Antoniazzi, & Speciali, 2003). According to a recent theory, migraine is thought to be a neurovascular pain syndrome. It is associated with altered central neuronal processing (activation of brain stem nuclei, cortical hyperexcitability, and spreading cortical depression) and involves the trigeminovascular system (triggering neuropeptide release), which produces painful inflammation in cranial vessels and the dura mater (Villalon et al., 2003). In the most recent revision of the headache classification system of the International Headache Society (IHS), migraine has been for the first time divided into an episodic and chronic form with episodic migraine being considered a precursor to chronic migraine (Cady, 2007), implying that migraine can be progressive. These theories showed the need for developing sustained-release formulation for the delivery of antimigraine drug.

Presently, antimigraine drugs are available in different strengths and formulation including oral tablets, orally disintegrating tablets, nasal sprays, and subcutaneous injection. In Europe, sumatriptan is also available as a suppository. Although, oral route is most conventional and convenient route of drug administration, it becomes difficult to administer tablets during migraine attack due to the associated nausea and vomiting (Ronald, 2004). Also, the dose has to be repeated if the first dose does not relieve the headache (Bigal et al., 2003). Therefore, it is important to develop alternate dosage forms that can selectively deliver antimigraine drugs to cranial nerves in the brain and ear (Limmroth, Dowson, Diener, & Dahlof, 2004; Ronald, 2004). In the literature, topical/transdermal delivery of antimigraine drug has been suggested to be beneficial by different authors (Femenía-Font, Balaguer-Fernández, Merino, Rodilla, & López-Castellano, 2005; Femenía-Font et al., 2006; Ronald, 2004). The approaches used in these studies involved use of Transcutol, 2-pyrrolidone, and ethanol as penetration enhancer. However, no attempt has been made using vesicular delivery systems for sustained delivery of antimigraine drugs.

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One of the major advances in vesicle research was the finding that some modified vesicles, for example, elastic liposomes and ethosomes possessed properties that allowed them to successfully deliver drugs in the deeper layers of skin (Cevc, Blume, & Schatzlein, 1997; Touitou, Dayan, Bergelson, Godin, & Eliaz, 2000; Trotta, Peira, Carlotti, & Gallarate, 2004; Trotta, Peira, Debernardi, & Gallarate, 2002). Although the exact mechanism for this has not been conclusively established, their deformable character is suggested to be the main contributing factor (Cevc & Blume, 1992). Fluorescence microscopy has also confirmed their penetration into the deeper skin layers (Jain, Jain, Umamaheshwari, & Jain, 2003). In this study, rizatriptan was encapsulated in vesicular carrier system (elastic liposome). Elastic liposomes are reported to possess better skin permeation ability, and earlier findings have demonstrated that upon nonocclusive application they possess the ability to form a depot in the deeper layers of skin (Jain, Jain, Bhadra, Tiwary, & Jain, 2005; Trotta et al., 2002). Hence, it was hypothesized that elastic liposomal formulation upon topical administration to cranial skin and ears would deliver the drug in the deeper layer of skin. From here the drug would act on cranial nerves resulting in site-specific delivery. In addition, these formulations could be expected to act as depot in the deeper layer of skin and sustain the drug release.

MATERIALS AND METHODS

Materials

Rizatriptan was received as a gift sample from Cipla Ltd. (Mumbai, India). Soya phosphatidyl choline (PC), cholesterol, Sephadex-G-50, ruthenium tetroxide, osmium tetroxide, 6-carboxyfluorescein (6-CF), and Triton-X 100 were purchased from Sigma Chemicals (St Louis, MO, USA). Ethanol, acetonitrile, acetic acid, methanol, xylene, acetic acid, propylene glycol, isopropanol, diethyl ether, chloroform, and Span 80 were procured from E. Merck (Mumbai, India). All other reagents used in the study were of analytical grade.

Preparation of Formulations

The elastic liposomal formulations were prepared by conventional rotary evaporation sonication method described by Cevc et al. (1997). Briefly, phospholipid and surfactant were taken in a clean, dry round bottom flask. This lipid mixture was dissolved in chloroform/methanol (2:1 mixture). Organic solvent was removed by rotary evaporation above the lipid transition temperature (Rotary Evaporator, Superfit, Ambala, India). Final traces of solvent were removed under vacuum overnight. The deposited lipid film was hydrated for 1 h with the drug or fluorescence marker solution in ethanol (7%, vol/vol) by rotating at 60 rpm at 40 ± 1.0°C. The resulting vesicles were swollen for 2 h at room temperature to get large multilamellar vesicles (LMLVs). To prepare smaller vesicles, LMLVs were probe sonicated at 4°C for 20 min at 40 W (Probe Ultrasonicator, Imeco Ultrasonics, Mumbai, India). The sonicated vesicles were extruded through a sandwich of 100 and 200 nm polycarbonate membranes (Millipore, Texas, USA). The final lipid and drug concentration in the vesicular formulations was 5% (wt/vol) and 0.75% (wt/vol), respectively.

The same method was used for preparing conventional liposomal formulation (phosphatidylcholine/cholesterol, 7:3) that was used for comparison purpose. The final lipid and drug concentration in the liposomal formulation was 5% (wt/vol) and 0.75% (wt/vol), respectively.

Characterization of Elastic Liposomal Formulations

The vesicle size and distribution were determined by dynamic light scattering method (1064L, CILAS, Cedex, France). For morphological characterization transmission electron microscopic (TEM) studies using phosphotungstic acid (PTA) as a negative stain were performed (Morgagni 268D FEI, Philips, Eindhoven, Netherlands). A drop of the sample was placed on a carbon-coated copper grid to leave a thin film on the grid. Before the film dried on the grid, it was negatively stained with 1% PTA. A drop of the staining solution was added to the film and the excess of the solution was drained off with a filter paper. The grid was allowed to thoroughly dry in air and samples were viewed under a TEM. Vesicles without sonication were also visualized using optical microscope (DX31, Olympus, Tokyo, Japan).

The entrapment efficiency was determined after separating unentrapped drug using Sephadex G-50 column. The eluted vesicles were lysed using Triton-X 100 (0.1%, vol/vol) and analyzed for drug content. The turbidity of different formulations was determined by dilution of formulation with distilled water to give a total lipid concentration of 0.312 mM. The formulation was mixed by sonication for 5 min and the turbidity was measured specrtophotometrically at 400 nm (Beckman Du 640B, Fullerton, CA, USA) (Fang, Hong, Chiu, & Wang, 2001).

Elasticity Measurement

For measurement of elasticity of the vesicular membrane, elastic liposomal formulations were extruded at 2.5 bar through the filter membrane having a pore diameter of 60–200 nm using a stainless-steel pressure holder for 25 mm diameter filters with 200 mL capacity barrel. The amount of vesicle suspension, which was extruded during 5 min, was measured and the vesicle shape (by TEM) and size by dynamic light scattering [DLS] was monitored before and after filtration. The elasticity of vesicle membrane was calculated by using the following formula (vanden Bergh, Wertz, Junginger, & Bouwstra, 2001):

$$E = J(r_v/r_p)^2,$$

where E is the elasticity of vesicle membrane, J the amount of suspension, which was extruded during 10 min, r_v the vesicle size (after extrusion), and r_p the pore size of the barrier.

Skin Permeation Study

The in vitro skin permeation of rizatriptan from different formulations was studied using Franz glass diffusion cell maintained at 32 ± 1°C. The effective permeation area of the diffusion cell was 2.303 cm^2. The receptor compartment contained 22.5 mL phosphate buffer saline (pH 6.4) and was constantly stirred at 100 rpm. Excised albino abdomen rat skin was mounted between the donor and the receptor compartments. Elastic liposomal formulation (2.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20-, and 24-h time intervals and analyzed by high-performance liquid chromatography (HPLC). An equal volume of fresh phosphate buffer maintained at 32 ± 1°C was replaced into the receptor compartment after each sampling.

At the end of the permeation experiments (24 h), the surface of the skin was washed five times with 50% ethanol, and then with water to remove excess drug on the surface. The washing protocol was verified and was found to remove more than 95% of the applied dose at zero time. The skin was then cut into small pieces. The tissue was further homogenized with 50% ethanol (10 mL) and left for 24 h at room temperature. After shaking for 5 min and centrifugation for 5 min at 3000 rpm, the rizatriptan content in the upper phase was determined by HPLC assay. In vitro drug released from different formulations was also analyzed using same method with dialysis membrane (MWCO 12,000–14,000, HIMEDIA, Mumbai, India).

Scanning Electron Microscopy

Wistar rats, 4–6 months old, weighing 100–150 g were divided into three groups, each comprising of three animals each. The first group served as control and received topical application of drug solution (1.0 mg/mL) prepared in PBS (pH 6.4). The second and third groups received, respectively, application of 1.0 mL of optimized elastic liposomal formulation (EL-SP3) and conventional liposomal formulation. The formulations were applied nonocclusively to the abdomen side of the rat over an area of 1 cm^2. The treated rats were caged and killed after 6 h of treatment. The skin was removed immediately and fixed at 4°C in Karnvosky's fixative overnight followed by 1% (wt/vol) osmium tetroxide for 2 h and finally in ruthenium tetroxide 0.2% (wt/vol) and $K_3Fe(CN)_6$ 0.25% (wt/vol) for 1 h. Following fixation, the samples were dehydrated in a range of ethanolic solutions 70, 90, 95, and 100% (vol/vol) and coated with gold coater. The coated samples were visualized under scanning electron microscope (SEM, LEO43 SVP, Cambridge). All investigations were performed after approval of the Institutional Ethical Committee of the Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, and in accordance with the disciplinary principles and guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA).

Confocal Laser Scanning Microscopy

Wistar rats, 4–6 months old, weighing 100–150 g were divided into four groups each consisting of three animals. The first group served as control and received topical application of PBS (pH 6.4). The second group received topical application of 1.0 mL of 0.16% (wt/vol) solution of marker 6-CF in PBS (pH 6.4). The third and fourth groups received application of 1.0 mL of conventional liposomes and elastic liposomes loaded with 6-CF (0.16%, wt/vol) as the fluorescence marker, respectively. Fluorescence marker-loaded formulations were applied topically to the abdomen side of the rat at a marked area of approximately 1 cm^2. After application of formulation the animals were caged individually. The animals were killed after 6 h of application, the skin was removed immediately, cut into pieces, and washed with PBS. The skin was blotted and wiped with tissue paper. The wiped tissue was fixed in Carny's fluid (absolute alcohol/chloroform, 3:1) for 3 h. The skin was sectioned into the pieces of 1 mm^2 size and evaluated for depth of penetration of 6-CF. The full skin thickness was optically scanned at different increments through the *z*-axis of a CLS microscope (DMIRE2, Leica, Bensheim, Germany). Optical excitations were carried out with a 489 nm argon laser beam and fluorescence emission was detected above 515 nm for 6-CF.

In Vivo Evaluation

Antimigraine activity of rizatriptan was investigated using morphine withdrawal-induced hyperalgesia on the hot plate test apparatus and 0.3% acetic acid-induced abdominal constrictions in adult male mice (Arulmozhi, Veeranjaneyulu, Bodhankar, & Arora, 2005).

Determination of Licking Latency Using Hot Plate Method

Morphine withdrawal hyperalgesic activity was determined using the method described by Galeotti, Ghelardini, Grazioli, and Uslenghi (2002). Albino mice weighing 30–40 g, 12–14 weeks old were divided into four groups each consisting of three animals. The first group served as control and received tap water. The second, third, and fourth groups received morphine treatment. Morphine was dissolved in 5% saccharose solution and administered orally with a dose of 0.1 mg/mL at days 1 and 2, 0.2 mg/mL at days 3 and 4, 0.3 mg/mL at days 5 and 6, and 0.4 mg/mL at days 7–15. On day 15, the morphine solution was replaced with tap water. Six hours after replacement with water, the licking latency of each group was determined using the hot plate method. Different rizatriptan formulations were applied topically 45 min before starting the hot plate experiment. The dose selected of rizatriptan for animal study was 3.0 mg/kg as reported by Cumberbatch, Hill, and Hargreaves (1997). In this experiment, the first group received topical treatment with PBS and acted as control. The second, third, and fourth groups received application of rizatriptan solution in PBS (pH 6.4), optimized elastic liposomal formulation (EL-SP3), and conventional liposomal formulation

containing rizatriptan, respectively, to the abdominal skin. At the predetermined time (1, 6, 10, and 24 h) interval, licking latency was determined using hot plate method.

Animals were placed on hot plate maintained at 55 ± 1°C and the time between placement of the animal on the hot plate and the occurrence of either licking of the fore or hind paws was recorded as response latency. Reaction times (s) were measured before (pretest) and after treatment. The end point was the licking of the fore or hind paws. Results of tail flick latencies were expressed in terms of reaction time in seconds.

Measurement of Acetic Acid-Induced Abdominal Constrictions

Albino mice weighing 30–40 g, 12–14 weeks old were divided into four groups each comprising of three animals. The first group received topical treatment with PBS and served as control. The animals in the second, third, and fourth groups received topical application of rizatriptan solution in PBS, optimized elastic liposomal formulation (EL-SP3), and conventional liposomal formulation of rizatriptan, respectively. These formulations were applied topically 45 min before the i.p. injection of 0.3% (vol/vol) acetic acid. The number of abdominal constrictions for 15 min after administration of i.p. injection of 0.3% acetic acid was counted at 1-, 6-, 10-, and 24-h time intervals for each group. The abdominal constrictions consist of constriction of abdominal muscles together with the stretching of hind limbs. Results were expressed as percentage inhibition of abdominal constrictions with respect to control.

Pharmacokinetic Study

Wistar rats, 4–6 weeks old, weighing 100–150 g were divided into three groups each comprising of three animals. The first group received oral administration of rizatriptan solution (1.0 mg/mL) in PBS. The second and third groups received topical application of rizatriptan solution in PBS (1.0 mg/mL) and optimized 1.0 ml of elastic liposomal formulation (EL-SP3) of rizatriptan, respectively. At predetermined time intervals 3-, 6-, 9-, 12-, 18-, and 24-h blood samples were collected. Each blood sample was centrifuged at 2000 rpm for 10 min and the drug concentration after deproteinization with acetonitrile was determined by HPLC.

HPLC Assay

The quantitative determination of rizatriptan was performed using HPLC method as reported by Chen et al. (2004). A mixture of 0.05% triethylamine (adjusted to pH 2.75 with 85% phosphoric acid) in water and acetonitrile (92:8) was used as mobile phase. The injected fluid (20 μL) was eluted in C_8 column at room temperature and rizatriptan was monitored at 225 nm using VP diode array UV detector (Waters, IL, USA). The coefficient of variance (v) for the standard curve ranged from 1.0 to 5.0% and R^2 = .9993.

Statistical Analysis

Data are expressed as the mean ± standard deviation (*SD*). ANOVA followed by Student's *t*-test was used for calculating the significance of results (Graphpad, version 2.01, San Diego, CA, USA). A value of $p < .05$ was considered statistically significant.

RESULTS AND DISCUSSION

Preparation and Characterization

Elastic liposomes are vesicular carriers possessing elastic membrane, which can be used to transport bioactives across biological membrane such as skin. Elasticity of elastic liposomes was achieved by using optimum surface-active agent. Span 80 was selected as an edge activator because it is biocompatible, pharmaceutically acceptable (Liu, Guo, Hua, & Qiu, 2007) and provided optimum elasticity to vesicle membrane as optimized in our previous study (Jain et al., 2005). PC was used as bilayer forming agent. Different batches of elastic liposomes were prepared with Span 80 and PC using conventional rotary evaporation sonication method (Cevc et al., 1997; El Maghraby, Williams, & Barry, 2001).

The compositions of different elastic liposomal formulation are summarized in Table 1. These elastic liposomal formulations were colloidal dispersions having average diameter ranging from 100 to 200 nm. TEM visualization indicated that elastic liposomes were unilamellar vesicles with spherical shape (Figure 1). Elastic liposomal formulation without sonication (particle size 2.35 ± 2.3 μm) when visualized under optical microscope also showed the existence of spherical vesicular structure.

The entrapment efficiency of rizatriptan in elastic liposomes was calculated as percentage of total drug entrapped into the vesicular formulation and determined using Sephadex G-50 minicolumn centrifugation method. The maximum entrapment efficiency obtained was 88.6 ± 3.8 and 62.2 ± 2.8% for elastic liposomes (EL-SP3) and conventional liposomal formulation, respectively (Table 1). The higher entrapment efficiency in elastic liposomes can be attributed to the presence of ethanol in these vesicles. Ethanol is reported to increase the fluidity and intralamellar distance of vesicular membrane (Jain et al., 2005; Touitou et al., 2000), which in turn contributes to enhanced drug entrapment by distributing the hydrophilic rizatriptan both core as well as membrane of the elastic liposomes as opposed to only core in the liposomes (Dayan & Touitou, 2000). Furthermore, the entrapment efficiency of elastic liposomes was found to depend on the surfactant concentration in the bilayer. Initially, with increasing surfactant concentration, there was an increase in entrapment efficiency. However, after a threshold level (above 15%, wt/wt), a further increase in surfactant concentration led to a decrease in entrapment efficiency (Table 1). This is possibly because at lower concentration (<15%, wt/wt) surfactant molecules get associated with the phospholipid bilayer resulting in better partitioning of drug.

TABLE 1
Composition and Characterization of Rizatriptan-Loaded Formulations

Formulation Code	Composition (PC/S)	Vesicle Size (nm)	Entrapment Efficiency (%)	Shape	Turbidity	Elasticity	% Drug Release at 24 h
EL-SP1[a]	95:5	120 ± 11	58.1 ± 2.4	Vesicular	0.5968 ± 0.2	28.3 ± 1.8	62.1 ± 1.9
EL-SP2	90:10	125 ± 10	61.6 ± 2.7	Vesicular	0.7546 ± 0.2	40.5 ± 2.4	70.7 ± 2.0
EL-SP3	85:15	132 ± 15	88.4 ± 3.8	Vesicular	0.9875 ± 0.8	58.2 ± 3.1	81.1 ± 2.5
EL-SP4	80:20	94 ± 10	72.2 ± 3.4	Vesicular + micelles	0.5149 ± 0.1	25.3 ± 1.5	66.7 ± 1.8
EL-SP5	75:25	99 ± 8.0	68.4 ± 3.1	Vesicular + micelles	0.2456 ± 0.1	9.4 ± 1.2	56.4 ± 1.6
Lipo.[b]	–	112 ± 13	62.2 ± 2.8	Vesicular	0.8125 ± 0.2	2.1 ± 0.6	60.1 ± 3.2

Values are represented as mean ± *SD* (*n* = 3).
[a]EL-SP: elastic liposomal formulation containing different concentrations of Span 80.
[b]Lipo.: liposomes used for comparison containing phosphatidylcholine:cholesterol (70:30).
PC, phosphatidylcholine; S, Span 80.

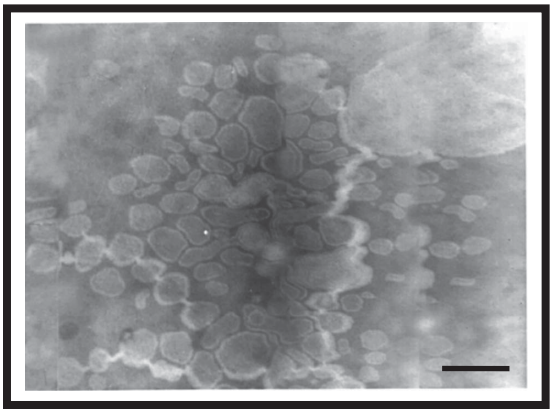

FIGURE 1. Visualization of optimized elastic liposomal formulation (EL-SP3) by transmission electron microscopy. Scale bar = 500 nm. Magnification, × 15,000.

However, above this optimum concentration (>15%, wt/wt), surfactant molecules start forming micelles in bilayer, resulting in pore formation in the vesicle membrane, which decreased the entrapment efficiency (Jain, Tiwary, & Jain, 2006). Another fact associated with the increase in surfactant concentration is the induction of the conversion of vesicle membranes into mixed micelles. These mixed micelles are reported to possess lower drug carrying capacity and poor skin permeation due to their structural features (Cevc, Blume, Schatzlein, Gebauer, & Paul, 1996; Lichtenberg, Robson, & Dennis, 1983). Optical microscopy photomicrograph of elastic liposomal formulation (without sonication) at different surfactant concentrations as shown in Figure 2A–C reveals significant decrease in vesicle population and conversion of elastic liposomes into mixed micelles when concentration of Span 80 was increased from 15 to 25% (wt/wt) of PC. Turbidity measurement also showed significant decrease in turbidity with increase in the surfactant concentration (Table 1). The theory of conversion of elastic liposomes at higher concentration of surfactant is well correlated with existing literature reports (Honeywell-Nguyen, Frederik, Bomans, Junginger, & Bouwstra, 2002; Jain et al., 2006; Lichtenberg et al., 1983). It has been suggested that at an optimum concentration, surfactants provide maximum elasticity to vesicle membranes that results in their better skin permeation (Jain et al., 2006). Increasing the concentration of membrane-softening component beyond an optimum level or even to the point of bilayer solubilization does not contribute to skin permeation efficiency (Hofer et al., 2000). Entrapment efficiency, deformability measurement, and skin permeation data supported this hypothesis.

Skin Permeation and Deposition Study

The permeation parameters for in vitro permeation of rizatriptan across excised rat abdominal skin are summarized in Table 2. The value of transdermal flux for different elastic liposomal formulations was observed to range between 18.1 ± 0.6 and 42.7 ± 2.3 μg/h/cm^2. This was about 20-fold higher than that obtained after application of rizatriptan solution and fivefold higher than its conventional liposomal formulation. Permeation of rizatriptan from elastic liposomes was found to depend on the concentration of Span 80. It increased till the concentration increased to (15%, wt/wt, of PC) and then decreased with further increase in surfactant concentration. This is in accordance with the entrapment efficiency, elasticity, and turbidity measurement data (Table 1). The permeation of rizatriptan from elastic liposomal formulation (EL-SP3) was found to be highest and this formulation also exhibited the highest entrapment efficiency (88.6 ± 3.8) and elasticity (58.2 ± 3.1).

Skin retention studies were carried out with the objective of determining the depot effect of elastic liposomes in the deeper layers of skin. For effective management of migraine, the dosage form should deliver the drug to anastomoses located mainly in the cranial skin and ears. Here, the drug would close the shunts and restore blood flow to brain thus, relieving migraine (Pathirana, Kariyaswasam, & Tibbotumununuwa,

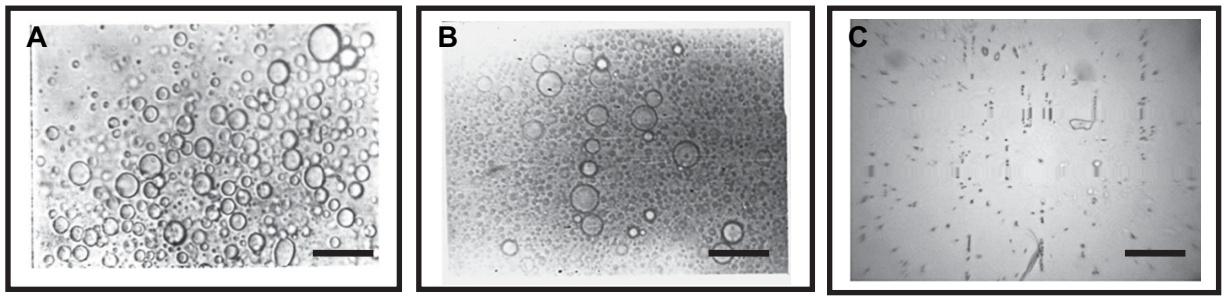

FIGURE 2. Optical microscopy photomicrograph of elastic liposomal formulation (without sonication) prepared with (A) 15% (wt/wt) (EL-SP3, × 450), (B) 20% (wt/wt) (EL-SP4, × 450), and (C) 25% (wt/wt) (EL-SP5, × 450) concentrations of Span 80.

TABLE 2
Transdermal Permeation Parameters of Different Rizatriptan Formulations Across Rat Skin

Formulation Code	J_{ss}[a] (μg/cm^2/h)	% Drug Deposited	ER[b]
EL-SP1	22.0 ± 1.0	20.3 ± 1.9	08.1
EL-SP2	38.4 ± 1.9	28.3 ± 2.2	11.7
EL-SP3	42.7 ± 2.3	39.9 ± 3.2	20.5
EL-SP4	31.7 ± 1.2	26.3 ± 2.0	10.6
EL-SP5	18.1 ± 0.6	19.6 ± 1.8	09.1
Liposomes	7.1 ± 0.5	07.3 ± 1.2	05.7
Drug solution	2.2 ± 0.1	03.8 ± 1.0	–

Values are represented as mean ± *SD* (*n* = 3).

[a]J_{ss}: transdermal flux, calculated from the slope of Cartesian plot of cumulative amount of drug present in receptor compartment versus time.

[b]ER: enhancement ratio; it is the ratio of transdermal flux from elastic liposomes to drug solution.

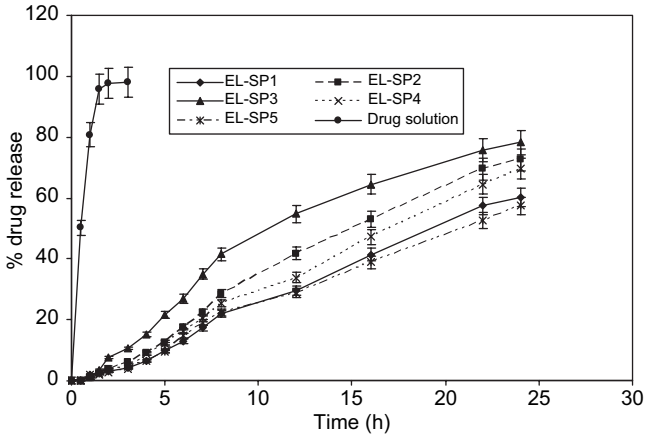

FIGURE 3. Percentage of drug release of rizatriptan across the cellophane membrane from elastic liposomal formulation prepared with different concentration of Span 80 (5–25% [wt/wt] of phosphatidylcholine [PC]) and drug solution in phosphate buffer saline (PBS). All values are shown as mean ± *SD* (*n* = 3).

2006). Table 2 compares the percentage of skin deposition of rizatriptan after topical application of EL-SP3, liposomal formulation, and drug solution in PBS. The amount of rizatriptan deposited in skin was 10-fold higher after application of elastic liposomes (39.9 ± 3.2%) than its solution (3.8 ± 1%) and five-fold higher than its liposomal formulation (7.3 ± 1.2%). This could be attributed to the difference in the mechanism of drug transport across the skin from elastic liposomes and drug solution. In contrast with rizatriptan molecule, elastic liposome is too large to enter into the cutaneous blood circulation directly. Locally, they bypass the cutaneous capillary bed, cross over to the subcutaneous capillary bed, and finally to the subcutaneous tissue where they act as depot and sustain the drug release (Cevc & Blume, 2002; Jain et al., 2005).

Release Rate Kinetics

Figure 3 compares the release rate of rizatriptan from elastic liposomal formulation and its solution form the synthetic cellophane membrane. The release rate of rizatriptan from elastic liposomes was significantly lower ($p < .05$) than its solution form. After 3 h, maximum drug was released from the drug solution (98.1 ± 5.1%). For comparison, 10.6 ± 0.7% of rizatriptan was released from the elastic liposomal formulation (EL-SP3) in 3 h and after 24 h, 78.2 ± 6.1% of drug was released. The release experiments clearly indicated sustained release of rizatriptan from elastic liposomal formulations. For ideal antimigraine therapy, higher skin permeation as well as sustained release of rizatriptan is desirable. In vitro release experiments indicated the ability of elastic liposomal formulation to sustain the release of rizatriptan to 24 h.

Drug release parameters of different formulations are summarized in Table 3. Elastic liposomal formulations exhibited correlation coefficient in the range 0.8909–0.9491. The optimized elastic liposomal formulation exhibited a zero-order release pattern during the permeation studies (R^2 = .8909). Because the concentration of drug is in equilibrium

TABLE 3
Release Kinetics of Rizatriptan-Loaded Elastic Liposomal Formulations[a]

Formulation Code	Log k	n	R^2	Order of Release
EL-SP1	0.6640	0.9137	.9383	Non-Fickian
EL-SP2	0.7780	0.8637	.9470	Non-Fickian
EL-SP3	0.9830	0.7790	.8909	Non-Fickian
EL-SP4	0.6876	0.9089	.9466	Non-Fickian
EL-SP5	0.5687	0.9479	.9491	Non-Fickian
Plain drug	2.2053	10.624	.7583	

[a]Analyzed by the regression coefficient method.

with the inner surface of the elastic liposomal vesicle membrane and diffusion path length was constant, a zero-order permeation profile can be expected with the vesicular system. The log release rate constant (k) for optimized elastic liposomal formulation and drug solution was found to be 0.9830 and 2.2053, respectively. The significant ($p < .05$) lower value of the release rate constant of rizatriptan for optimized elastic liposomal formulation as compared with the solution form indicated its sustained release from elastic liposomes.

Vesicle Skin Interaction Study

Results of skin permeation study showed that elastic liposomal formulation exhibited higher skin permeation (Table 2). The mechanism responsible for better skin permeation ability of elastic liposomes is not yet clear. Different groups have hypothesized different reasons for better skin permeation ability of elastic liposomal formulation. Cevc and Blume (1992) reported for the first time the mechanism of intact vesicle permeation, whereas El. Maghraby et al. (2001) reported molecular mixing of phospholipid with skin lipids to be the main contributors. Jain et al. (2006) reported the combination of both these mechanisms to be responsible for better skin permeation ability of elastic liposomes.

Figure 4A–F depicts the 2D and 3D SEM photomicrographs of rat skin treated with PBS that acted as control or elastic liposomes for 6 h. In comparison with the control skin, on the vesicle-treated skin, few vesicular structures were observed to be present on the surface of skin (Figure 4C). The morphology of cell was slightly altered and partial disappearance of intercellular lipid was observed. An increase in interlamellar distance of stratum corneum (SC) lipids was also observed (Figure 4F). However, there was absence of intercellular vesicular structures in SC incubated with PBS (Figure 4A and D). The vesicular suspension formed networks and stacks of lipid bilayers at the interface of the SC. Intercellular vesicular structures were observed in superficial layers of the SC and their appearance could be attributed to desquamating corneocytes with a leaky membrane, through which elastic liposomes might have penetrated. In comparison, conventional liposomes did not appear to significantly alter the ultrastructure of skin (Figure 4B and E). This supports the mechanism of intercellular penetration of elastic liposomes due to their deformable nature. These results can be correlated with our previous findings, where the TEM of skin treated with elastic liposomes revealed the presence of vesicular stacks in the deeper layers of skin (Jain et al., 2006). Hence, the results of SEM studies suggested that the better skin permeation potential of elastic liposomes to be due to their deformable nature as well as perturbation of intercellular skin lipids.

The superior skin penetration and deposition potential of elastic liposomes (EL-SP3) was further confirmed by confocal laser scanning microscopy (CLSM). The extent of vesicular penetration measured by CLSM after application of elastic liposomes, conventional liposomes, and 6-CF solution in PBS as compared with untreated skin is shown in Figure 5A–D. The untreated rat skin did not show any fluorescence (Figure 5A). The florescent marker 6-CF does not normally get into the deeper layer of skin, when applied in the form of aqueous solution (Jain et al., 2003). However, it was transported extensively and reached 200 μm deep in rat skin when applied in the form of elastic liposome formulation (Figure 5D). In comparison, conventional liposomes and the PBS solution of 6-CF penetrated only 40 and 15 μm deep, respectively, in rat skin after 6 h of topical application. This nearly showed fivefold deeper skin penetration and deposition potential of elastic liposomes as compared with conventional liposomes. For the effective treatment of migraine, higher drug accumulation in the deeper layer of skin is required. The CLSM study indicated that the elastic liposomes were capable of penetrating deeper into the skin and hence, could deliver the drug to the deeper layers of skin in appreciable concentration.

Evaluation of Biological Antimigraine activity

The mouse model is effectively correlated with human migraine pain because morphine withdrawal syndrome is characterized by hyperalgesia very similar to the reduction of pain threshold characteristic of migraineurs (Ghelardini, Galeotti, Donaldson, & Bartolini, 1998). The potential antimigraine agent sumatriptan and ergotamine have been found effective in this model (Galeotti et al., 2002).

Figure 6A compares the licking latency response of morphine-treated mice after the application of elastic liposomes with that obtained after an application of the solution form of rizatriptan. Morphine withdrawal syndrome significantly ($p < .05$) reduced the licking latency at 6 h morphine withdrawal (04 ± 1.0 s) in control group with respect to licking latency observed at 0 h (35 ± 6.0 s). Topical application of solution, optimized elastic liposomes (EL-SP3), and conventional liposomes of rizatriptan increased the licking latency in the hot plate test.

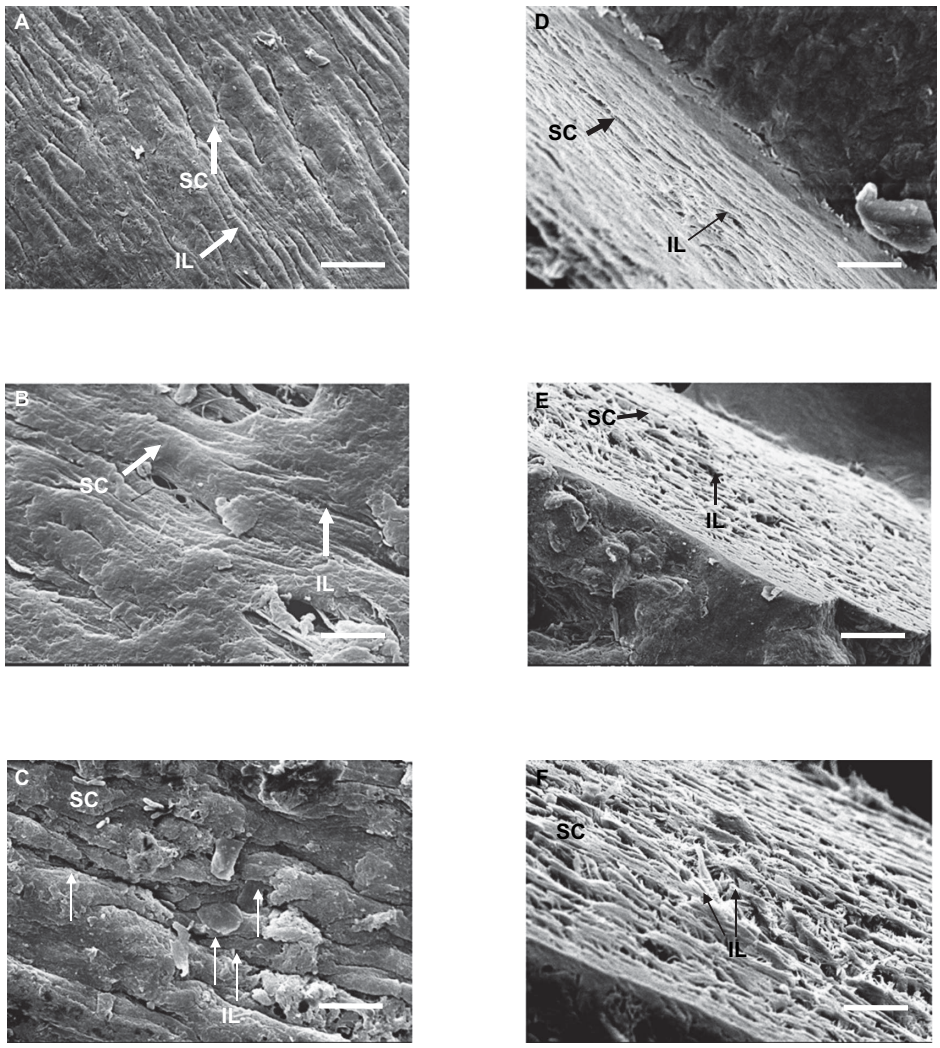

FIGURE 4. 2D and 3D scanning electron microscopy micrograph of viable rat skin treated with phosphate buffer saline as control (A [2D], D [3D]), liposomes (B [2D], E [3D]), and optimized elastic liposomes (EL-SP3) (C [2D] and F [3D] after 6 h of treatment. SC, stratum corneum; IL, interlamellar space. Scale bar = 100 nm. Magnification, × 15,000.

The increase in latency response after topical application of rizatriptan solution (8.0 ± 2.0 s) and elastic liposomes (22 ± 4.0 s) was significantly ($p < .05$) higher at 1 h as compared with saline treatment (5 ± 2.0 s). Furthermore, at 24 h postapplication, the increase in licking latency response for drug solution application decreased significantly ($p < .05$), but it was maintained after treatment with elastic liposomes (15 ± 3.0 and 4 ± 1.0 s, respectively, for elastic liposomes and drug solution). This indicated sustained release of rizatriptan from elastic liposomes.

Results of abdominal constriction test are shown in Figure 6B. It is evident that topical application of rizatriptan solution and optimized elastic liposomal preparation elicited a significant ($p < .05$) decrease in the number of abdominal constrictions in the acetic acid test as compared with mice treated with saline. The results indicated that elastic liposomal preparation decreased the number of abdominal constrictions to a greater extent as compared with rizatriptan solution (11.4 ± 1.0 and 57.1 ± 4.5% reduction, for drug solution and EP-SL3, respectively, at 15 min). Furthermore, at 6 h postapplication, the decrease in number of constrictions was significantly higher ($p < .05$) as compared with that after application of drug solution (18.9 ± 2.2 and 75.6 ± 8.0%, for drug solution and elastic liposomal formulation, respectively). The drug solution did not show any activity but elastic liposomal formulation showed 43.7 ± 5.0% reduction in abdominal construction at 24-h posttreatment. These results further strengthen the contention that the elastic liposomes exhibited better skin penetration and deposition in the deeper layers of skin thus, sustaining the release of rizatriptan, which sustained the drug action.

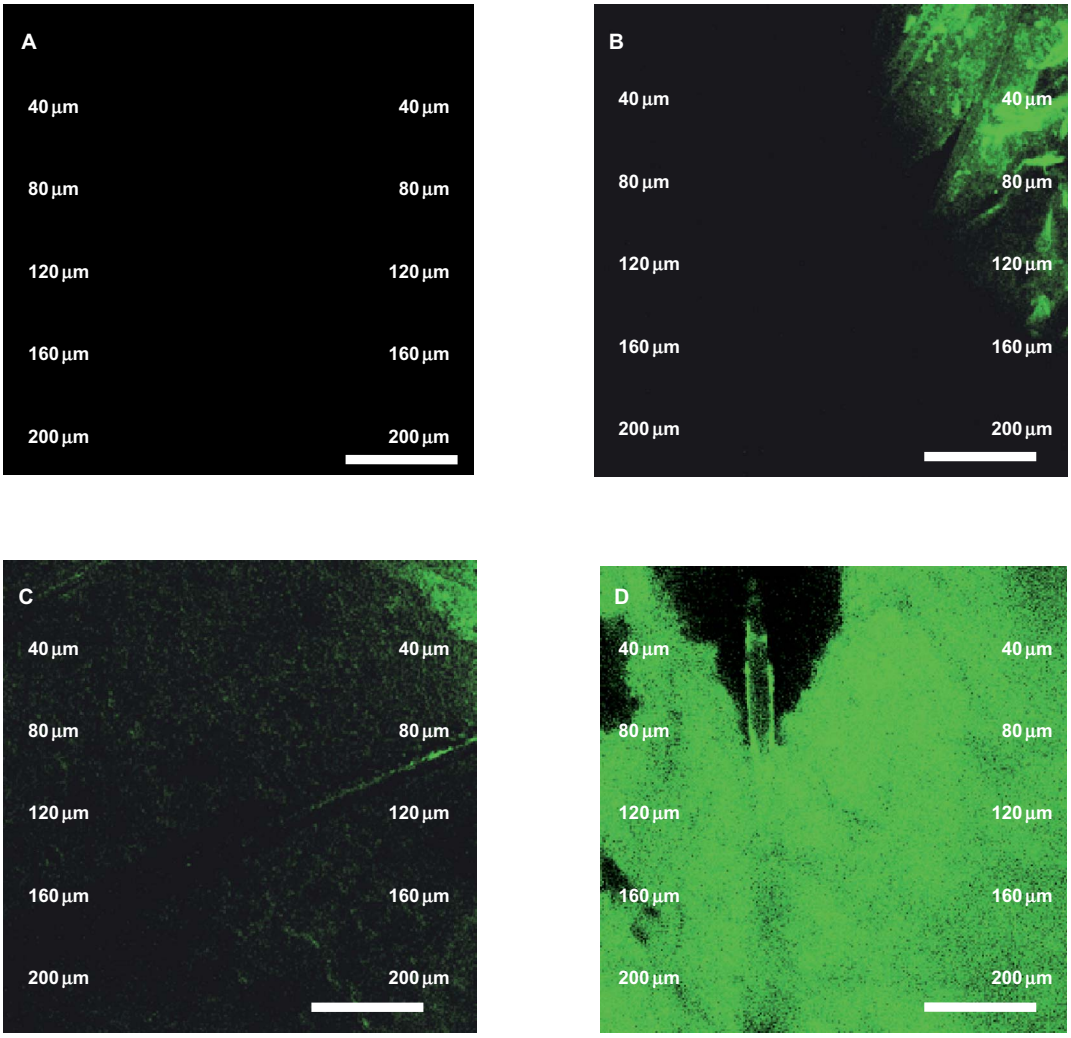

FIGURE 5. Confocal Laser Scanning Microscopy image of rat skin for penetration of 6-CF as fluorescence marker applied as (D) elastic liposomes (EL-SP3) (C) liposomes c. Phosphate buffer saline solution and (A) Control skin after 6 h of treatment. Scale bar = 100 nm. Magnification × 10,000.

The plasma drug profile of rizatriptan following administration of rizatriptan formulations is shown in Figure 7. The plasma concentration of rizatriptan in the group administered with drug solution topically and orally was maintained only for 6 and 9 h, respectively, whereas plasma level after application of optimized elastic liposomal formulation was maintained for 24 h (0.18 ± 0.1 µg/mL). The area under the curve AUC_{0-24} h after application of the elastic liposomes formulation was nearly sixfold higher (6.09 ± 0.7 µg/h/mL) than that after application of the drug solution topically (1.04 ± 0.3 µg/h/mL) and orally (0.96 ± 0.2 µg h/mL). These results established the sustained delivery of rizatriptan from the elastic liposomes formulation.

CONCLUSION

Patients of migraine are increasing exponentially. Conventional oral tablets of rizatriptan are not effective in patients experiencing nausea and vomiting due to migraine. The elastic liposomes of rizatriptan developed in this investigation seem to be a better option for sustained delivery of rizatriptan. The results of this study demonstrated that encapsulation of rizatriptan in elastic liposomes increased its skin deposition by 10-fold and sustained its release through 24 h as compared with its solution form. The biological activity also increased three- to fourfold by encapsulation in elastic liposomes as compared with the drug solution.

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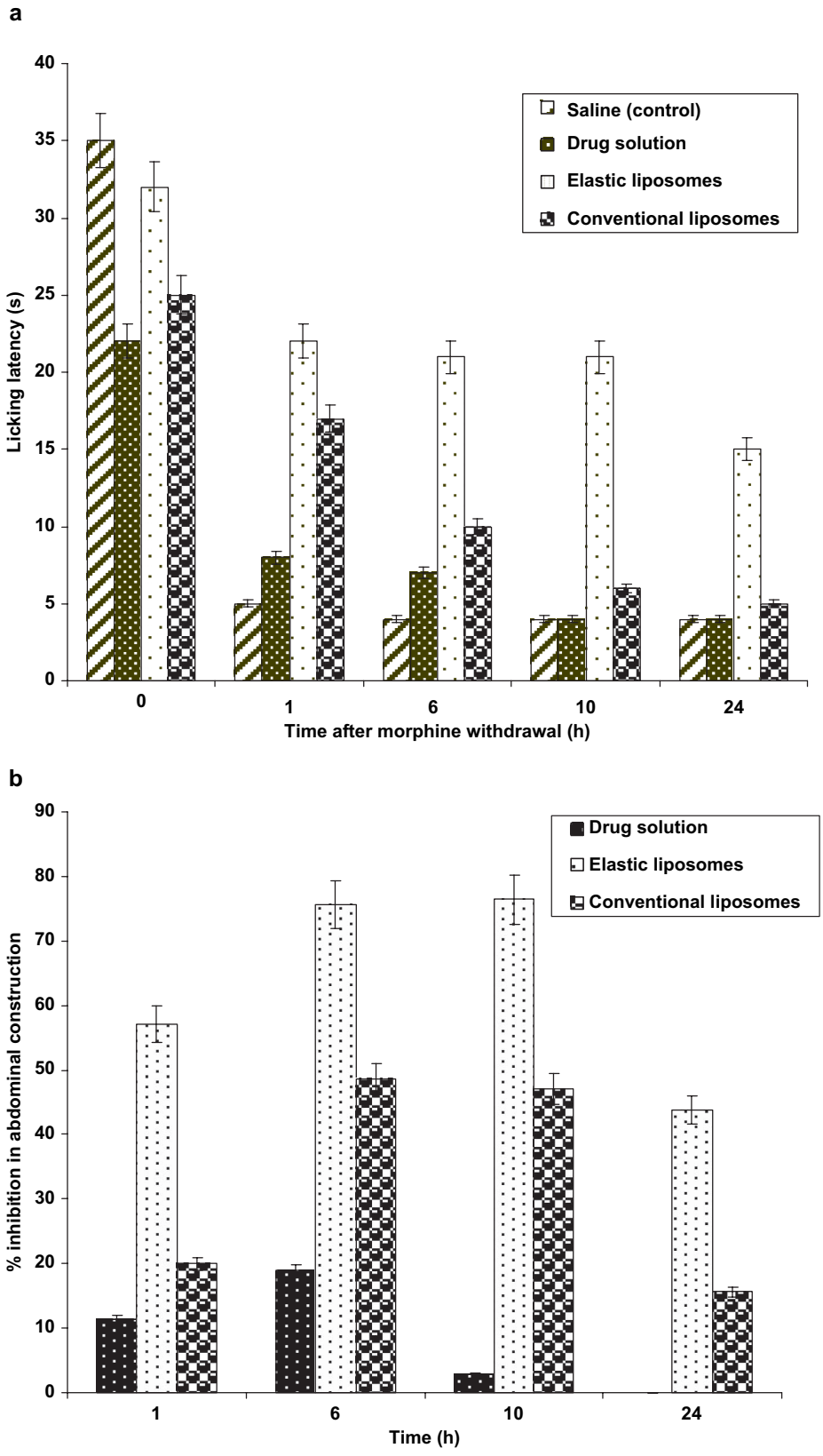

FIGURE 6. Effect of topical treatment of drug solution and elastic liposomal formulation (EL-SP3) of rizatriptan at 3.0 mg/kg on licking latency of morphine withdrawal hyperalgesic mice (A) and acetic acid-induced abdominal constrictions (B) at different time intervals. All values shown as mean ± *SD* (*n* = 3).

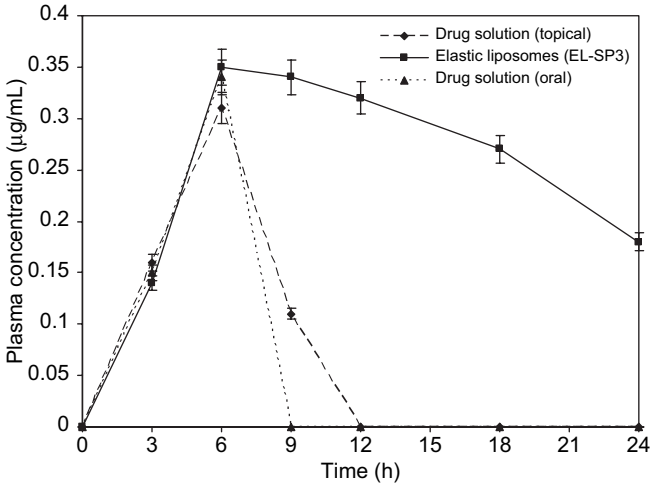

FIGURE 7. Comparison in plasma concentration of rizatriptan after administered in the form of drug solution in phosphate buffer saline (PBS) and elastic liposomal formulation (EL-SP3). All values shown as mean ± *SD* (*n* = 3).

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