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Preparation, Characterization and In-vivo Evaluation of Span 60-Lornoxicam Niosomes.

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ABSTRACT

The main aim of this study was to prepare and evaluate Lornoxicam loaded niosomes. Lornoxicam niosomes were prepared by thin film hydration method using non-ionic surface active agent, cholesterol and charge inducing agent. Entrapment efficiency of lornoxicam in niosomes was deduced spectrophotometrically. LX niosomes was characterized using several methods. In-vitro release studies were performed using dialysis bag method. Entrapment efficiency percentage of lornoxicam inside niosomes ranged from 47.73 ± 1.07 % to 76.63 ± 0.78 %. Particle size of LX niosomes ranged from 147 to 1606 nm, while zeta potential results revealed stable niosomal formulations. LX release from niosomes was biphasic and the release pattern followed Higuchi's model. The optimized LX niosomal gels exerted a two fold increase in the amount of LX, permeated through rat skin, compared to free LX. Skin irritation study revealed the non-irritancy of Span60 LX niosomes. Percentage edema of LX niosomal gel was lower than free drug showing enhanced anti-inflammatory effect of Span 60 LX niosomes. These results reveal that niosomes could be a promising drug delivery system for encapsulating the anti-inflammatory drug Lornoxicam.

Keywords: Lornoxicam, niosomes, anti-inflammatory, permeation.

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INTRODUCTION

Lornoxicam (LX), an oxicam derivative, is a non-steroidal anti-inflammatory derivative. It is used in muscular, skeletal and joint disorders such as osteoarthritis and rheumatoid arthritis. It is also used in the treatment of other painful conditions including postoperative pain. LX has demonstrated clinical efficacy in relieving chronic pain associated with osteoarthritis [1], rheumatoid arthritis, and ankylosing spondylitis . In the treatment of postoperative pain, LX has been shown to be as effective as morphine [2].

Several side effects in the gastrointestinal tract have been reported upon oral administration of LX. Although it has much more tolerability compared to other NSAIDs, however its renal and hematological adverse effects may be noticed. Topical application of NSAIDs can be an alternate way to overcome the gastro intestinal side effects of NSAIDs, comprising LX [3, 4].

The main purpose of novel drug delivery systems is to maintain constant and effective drug level in the body with simultaneous minimization of side effects [5]. Novel drug delivery systems also localizes the drug action by placing control release system adjacent to diseased tissue or organ; or target the drug delivery by using drug carriers [6]. Such vesicular drug carrier systems alter the plasma clearance kinetics, tissue distribution, metabolism and cellular interaction of the drug. They can be expected to target the drug to its desired site of action and/or to control its release [7, 8].

Niosomes are vesicles prepared using surfactants as the main component. They are similar, to liposomes, in terms of physical properties and structure [9, 10]. They can be prepared, as unilamellar or multilamellar vesicles, applying the same procedures and following the same variety of conditions [11, 12]. The research interest in niosomal formulations is recently widening because niosomes are able to overcome some disadvantages associated with liposomes, as surfactants are easily derivatized and give a higher versatility to the vesicular structure and moreover they have lower costs than phospholipids [9, 11, 13-15].

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The objective of the present study is to prepare and investigate Span60 LX niosomes, through in-vitro and in-vivo evaluations, aiming at enhancing the permeation and antinflamatory activity of LX, and decreasing its adverse effects as well.

MATERIALS AND METHODS

MATERIALS:

LX was a kind gift sample from Eva pharma Co., Cairo, Egypt. Sorbitan monostearate (Span 60) was purchased from Merck Schuchardt OHG, Germany. Dihexadecylhydrogen-Phosphate (Dicetyl Phosphate) (DCP) and Cholesterol (Chol) were obtained from Sigma-Aldrich Chemie GmbH, Germany. Octadecylamine (Stearylamine) (SA) was purchased from Sigma-Aldrich Co., St. Louis, Mo., USA. Chloroform was obtained from Panreac Quimica SA, Barcelona, Spain. All other chemicals were of analytical grade.

METHODS:

Preparation of Span 60-LX niosomes:

LX niosomes were prepared by the thin film hydration method [16, 17]. In a 100 ml pear-shaped flask of the rotary evaporator, 100 mg of (Span 60, Chol and DCP/SA) together with 10 mg LX were dissolved in 10 ml chloroform and rotated at 56°C for 10 minutes. The chloroform was then evaporated under reduced pressure in order to form a thin film of lipids on the wall of the flask. The thin film was hydrated with 10 ml of phosphate buffered saline (pH 7.4) for 30 minutes under rotation at 56°C.

Separation and determination of entrapment efficiency (EE %):

To determine the amount of drug entrapped in niosomes, the unentrapped lornoxicam was separated from the niosomal drug by cooling centrifugation at 5200 xg, at 4 °C, for 30 minutes using the refrigerated centrifuge (Union 32R, Korea). The pellets were resuspended in 10 ml PBS and recentrifuged for another 30

minutes at the same conditions. The supernatant, containing free drug, was filtered through 0.22 µm Millipore filter, and was further diluted. Then the free amount of LX was determined spectrophotometrically at λ_{max} 375 nm [18]. The amount of LX entrapped was then calculated by subtracting the free amount of lornoxicam from the amount added at the start of the preparation (10mg).

$$\text{Entrapment efficiency \%} = [Q_t - Q_s]/Q_t * 100 \quad \text{(Eq. 1)}$$

where Q_t is amount of drug in suspension added, and Q_s is amount of drug detected only in the supernatant [19].

Niosomes characterization

Transmission electron microscopy (TEM)

Transmission electron microscope is often used to determine the size, diameter and morphological characteristics of niosomes using negative stain (phosphotungestic acid) [20, 21]. The samples were examined by the TEM (JEOL, JEM-1230, Tokyo, Japan). The experiment was performed at room temperature and transmission electron micrographs were taken at appropriate magnifications.

Differential Scanning Calorimetry (DSC)

The thermal characteristics of drug niosomal formulations as well as drug-free niosomes were analyzed to study the possible interactions between LX and niosomes components using DSC calibrated with indium. Samples were analyzed using Shimadzu TA-60 software. Each niosomal formulation was separated, washed and the dehydrated pellets of niosomal formulations were lyophilized. The individual components of niosomes, namely; Span 60, Chol, SA, and LX were also investigated. The peak transition temperature (T_c) and enthalpy of transition (ΔH) were determined for each peak.

Vesicular size analysis

Particle size and distribution of the prepared niosomal formulations were measured with a dynamic light scattering (DLS) method using Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, UK)[22]. The analyses were performed with a He-Ne laser at 633 nm at a scattering angle of 90.0°. The niosomal preparation was diluted with bidistilled water (1:100 vol/ vol). After dilution; the sample was transferred to a quartz cuvette and measured at room temperature. The polydispersity index (P.I.) was detected as a measure of homogeneity.

Zeta potential determination

The zeta potential of noisome surfaces formulations was measured with a dynamic light scattering (DLS) method using Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, UK)[22]. The niosomes were prepared as usual, separated, washed by cooling centrifuge and diluted with bidistilled water (1:10 vol/vol). After dilution, the samples were transferred to a quartz cuvette and measured at room temperature.

In-vitro release study

After LX niosomes preparation, they were separated from the free drug and the amount of drug entrapped inside the niosomes was determined. The amount of drug entrapped in the niosomes was considered as the total amount of the drug (100%).In-vitro release study of LX from neutral and negatively charged LX niosomal formulations, namely; F1, F2, F4 and F5 LX niosomes, was determined using dialysis bag diffusion technique. Positively charged LX niosomes, namely; F3, and F6 were omitted from this experiment due to their reported aggregation [23] and lower stability previously reported [24].

The dialysis bag, having molecular weight cut off 12000–14000, was soaked in PBS (pH 7.4) for 12h before use. Cellophane bag, filled with niosomal formulation, equivalent to 2 mg drug, was immersed in a beaker containing100 ml PBS (pH 7.4) [25]. The beaker was then placed in a water bath shaker rotating at a

speed 100 rpm and temperature 32°C.Samples were withdrawn at predetermined intervals (1, 2, 3, 4, 5, 6, 7, 8 and 24 hrs) for analysis. A replacement with equal volume of freshly prepared PBS (pH 7.4) was done to maintain a constant volume. The absorbance at each interval was measured spectrophotometrically.

LX niosomal was prepared by dissolving 200 mg of Carbopol 934 in 10 ml niosomal suspension diluted with distilled water. Triethanolamine was slowly added drop by drop (about 7-10 drops) to reach the desired skin pH 7 and to obtain the gel at required consistency [26]. This was accompanied with continuous stirring until homogenous niosomal gel was obtained. Same in-vitro release experiment was performed on selected LX niosomal formulation (F1) after being incorporated in gel, as well as 1% w/w LX standard gel, which was prepared by mixing LX equivalent to 1% w/w with same ingredients.

Kinetic study of release profiles

In vitro release profiles treated with different mathematical models, i.e. zero order kinetics (cumulative percentage drug released vs. time), first order kinetics (log percentage drug retained vs. time) and Higuchi model (cumulative percentage drug released vs. square root of time)[27].The correlation coefficients (R^2) and the release rate constant (K) values were calculated for the linear curve obtained by regression analysis of the above plots. Korsmeyer-Peppas model (log cumulative percentage drug release vs. log time) [28] was also taken in consideration, in order to determine the mechanism of LX release from niosomal formulations. Data obtained were plotted as log cumulative percentage drug release vs. log time. In this model, the release exponent n is ≤ 0.45 for Fickian diffusion release and 0.45 < n < 0.89 for non-Fickian release[29].

Ex-vivo skin permeation studies

Ex-vivo skin permeation experiment

Ex-vivo skin permeation of LX from LX niosomal gel and plain LX gel was evaluated on hairless abdominal Wistar rat skin (120 – 160 gm) using Franz diffusion cell [30]. The surface area of the release membrane was 3.14 cm^2 and receptor volume was 63 ml (phosphate buffer saline pH 7.4) [4, 31, 32]. The epidermal layer of the rat skin was separated carefully. It was fixed on the donor compartment of the diffusion cell. The skin was firmly fastened with a rigid clamp.

The donor side was charged with 2 gm niosomal gel of the investigated formulations containing 2 mg LX. The solution in the receptor side was stirred with magnetic stirrer bar adjusted at 500 rpm [33]. During the experiments the receptor phase was maintained at 37° C ± 0.5. Aliquots (2 ml) were withdrawn at different time intervals (1, 2, 4, 6 and 24 h) and replaced by fresh medium to maintain sink condition [32]. The drug concentration was analyzed using a validated HPLC method. The samples were separated on a C18 column (Zorbax eclipse plus, 4.6 x 250 mm, Germany) with 0.1 M sodium dihydrogenphosphate buffer (pH 6)/ methanol (50:50, v/v) at a flow-rate of 1 ml/min. The detection absorption was at 372 nm [34].

Permeation Data Analysis

The permeation profiles were constructed by plotting the cumulative amount of LX permeated per unit dialysis membrane area (µg/cm2) versus time. Steady state flux (Jss, µg/cm^2/hr) of LX was calculated using linear regression analysis by using the slope of the plot. The permeability co-efficient (Kp) of the drug through the stratum corneum was calculated using the following equation [35].

$$Kp = Jss / C \qquad \text{(Eq. 2)}$$

Where, C is the initial concentration of the drug in the donor compartment. The penetration enhancing effect was calculated in terms of enhancement ratio (ER) by using the equation:

$$ER = Jss \text{ of formulation} / Jss \text{ of control} \qquad \text{(Eq. 3)}$$

Physical stability study of LX niosomes

F1 niosomal formulation was selected for physical stability study for its higher E.E.% and suitable release rate. After niosomal formulations were prepared, the amount of drug entrapped was determined. Following this, ten ml from each batch were sealed in 20 ml glass vials, and stored at refrigerator temperature (2 – 8 ºC) for 3 months [36, 37]. Samples from each batch were withdrawn at specified time intervals after 1, 2 and 3 months of storage to determine the amount of drug retained in the vesicles. Particle size of the selected formulations was also determined at the same time intervals.

In-vivo evaluation of LX niosomes

Animals

Albino Wistar rats of either sex were housed in polypropylene cages. They got free access to standard diet and water. They were kept at 25±1ºC and 45-55% relative humidity with a12 hr. light /dark cycle.

Skin irritation test

The skin irritation of the selected LX niosomal gel formulation was studied on white albino wistar rats, according to the method described by Draize et al.[38]. The rats were anesthetized with thiopental (60 mg/kg) injection (i.p). The animals were divided into 5 groups each group consists of 3 rats: Group 1 served as negative control, group 2 acted as positive control and received 0.8% (v/v) aqueous formalin solution as a standard irritant [39]. Group 3 received blank niosomal gel free from LX while Group 4 received LX niosomes F1 gel for 3 day respectively. Group 5 received free LX in gel. The application sites were examined for edema and erythema after 24 hours, then graded (0- 4) according to a visual scoring scale, performed by the same investigator. The primary irritancy index (PII) was calculated for each group according to the following equation[38]:

Primary irritancy index (PII) = Mean of erythema + Mean of edema…… (Eq. 4)

Thus the formulations were classified as non-irritant if (PII < 2), irritant if (PII =2-5) and highly irritant if (PII=5-8).

In-vivo anti-inflammatory activity

The experiment was performed in accordance with ethical procedures and policies approved by the Medical Research Ethical Committee of the National Research Centre, Cairo, Egypt. Anti-inflammatory activity of LX niosomal gel was studied and evaluated using carrageenan induced rat paw edema model. Wistar rats, of either sex, weighting 200–250 g were used for the study. Animals were divided into five groups; each composed of three rats. Group I served as control, while group II received blank niosomes in gel. Group III received 1g of the selected LX niosomal gel; F1. Group IV received market product Feldene and group V received free LX in gel.The formulae were applied on the shaved dorsal region of all animals half an hour before injection of carrageenan. The right paw was marked with ink at the level of tibiotarsic articulation and basal paw volume was measured, using carrageenan induced inflammation edema model, discussed previously by Swingle et.al [40]. The method involves immersing the paw till the level of tibiotarsic articulation into the container of the plethysmometer and the displacement volume (in ml) was measured by two platinum electrodes introduced into the container. Carrageenan suspension (0.1 ml of 1% w/v, in saline) was injected into the sub-plantar surface of the right hind paw and the paw volume was measured again at 1, 2, 3, 4, 5, 6 and 24 hrs after carrageenan injection. The left paw served as a reference which did not receive any formulation.The percentage difference between right and left paw volumes was recorded as percent edema.

Tail Flick Test

The tail flick test is a test of the pain response in animals. A light beam is focused on the animal's tail and a timer starts. When the animal flicks its tail, the timer stops and the recorded time is a measure of the pain threshold. Tail flick test was conducted to examine the analgesic effect of the prepared LX niosomes,

compared to Feldene. Animals were divided randomly into five groups; each group is composed of 6 rats. Group 1 served as control while group 2 received gel of blank niosomes. Groups 3 served as test group where LX loaded niosomal gels (F1) was applied. Groups 4 received Feldene gel while group 6 received free LX. The animals were placed on the hot plate. Baseline was measured for all rats before start of the experiment. Tail flick test started 30 min after applying 0.1 g of the assigned preparation onto about 5 cm in length of the distal part of the tail of each rat and wiping off excess and the response was measured after 30, 60 and 120 minutes. Changes in tail flick latency were determined in comparison to data obtained from the relevant negative control group [41].

Data analysis and statistics

Data are expressed as mean ± standard deviation (S.D.). Statistical analysis was performed using (SPSS® statistics for windows, version 17.0). Analysis of variance (ANOVA, single factor) followed by Fisher LSD's post-hoc test, was employed in the statistical analysis of the determined parameters. A P-value < 0.05 was considered statistically significant

RESULTS AND DISCUSSION

Entrapment efficiency percentage (E.E. %)

The entrapment efficiencies (EE %) of all niosomal formulations are illustrated in (Table 1), where lornoxicam was successfully entrapped in all these formulations.

Effect of niosomal surface charge on E.E. %

The results reveal that the neutral niosomes of the mola ratio Span 60: Chol: DCP/SA (1:1:0.1) exhibited the highest entrapment efficiency (76.63 ± 0.78 %), that was followed by negatively (61.6 ± 1.07%) and positively (47.73 ± 1.09%) charged niosomal formulations, with statistical significant difference (P > 0.05). On the other hand, for the molar ratio Sp60: Chol: DCP/SA (2:1:0.2), similar behavior was observed where neutral LX niosomes exhibited the highest E.E. % (59.69 ± 1.12 %), which was followed by negatively (57.58 ± 3.87 %) and positively (55.51 ± 0.9 3%) charged niosomes. Neutral, negative and positive niosomes showed statistical insignificant difference (P < 0.05) when compared to each other. Incorporation of Chol in the niosomes bilayer increases the entrapment efficiency of the drug in the vesicles. Cholesterol increases viscosity of niosomal dispersion and imparts rigidity to the flexible bilayer, which results in the formation of highly ordered structures of surfactants with cholesterol embedded in the bilayer. This further facilitates partitioning of the drug in the bilayer and increases the entrapment of the drug in niosomal vesicles [19].

Effect of molar ratio on E.E. %

Table (1) reveals that neutral and negatively charged LX niosomes, F1 and F2 of the molar ratio Span 60: Chol (1:1) and Span 60: Chol: CIA (1:1:0.1), possessed significantly higher entrapment efficiencies (P<0.05) viz., 76.63 ± 0.78 %, and 61.60 ± 1.07 %, respectively, when compared to neutral and negatively charged LX niosomes of the molar ratio Span 60: Ch (2:1) and Span 60: Ch: CIA (2:1:0.2), which possessed entrapment efficiencies of 59.69 ± 1.12% and 57.58 ± 3.87% respectively. While positive niosomal formulation of the molar ratio Span 60: Ch: CIA (1:1:0.1) F3, exhibited lower entrapment efficiency than its alternative of the molar ratio Span 60: Ch: CIA (2:1:0.2) F6, with E.E. % 47.73 ± 1.07 % and 55.51 ± 0.93% respectively. These results are in accordance with what was reported previously [12] that in a series of sorbitan monoesters, there was an almost linear relationship between encapsulation efficiency and Ch content up to 50% .

Niosomes Characterization

Four methods were performed for the characterization of the LX niosomes namely, Transmission Electron Microscopy (TEM), Differential Scanning Calorimeter (DSC), particle size analysis and zeta potential determination.

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Transmission electron microscopy (TEM)

Different vesicle shapes of LX niosomal formulations at different magnification powers are demonstrated in Figure (1). The micrographs show sphere like shaped vesicles having a large internal aqueous space and a smooth vesicle surface. Positively charged niosomes micrographs showed some aggregation. This comes in accordance with previously reported publication [23].

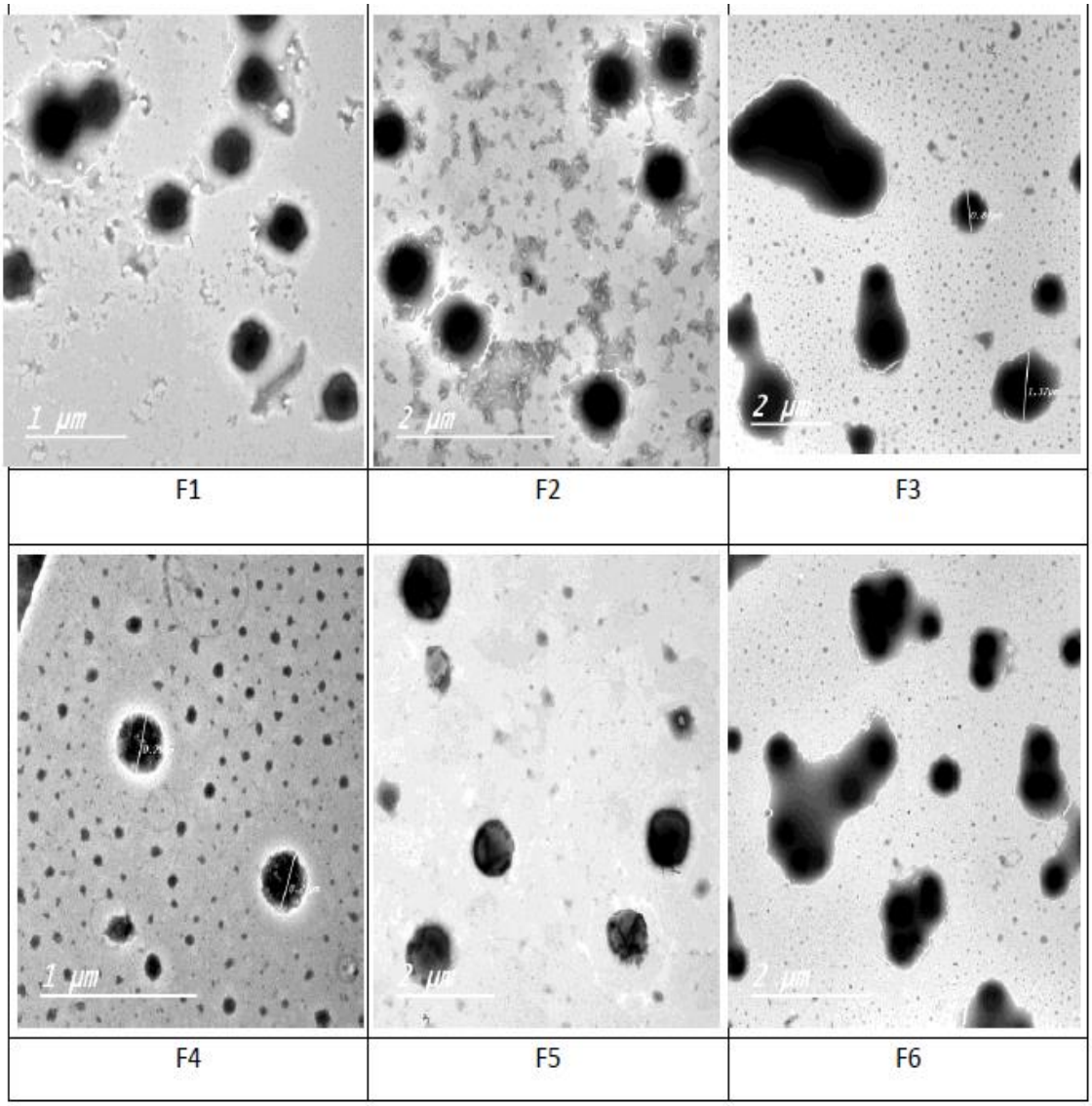

Figure 1. TEM micrographs of Span 60 LX niosomes.

Differential Scanning Calorimetery (DSC)

The thermograms of individual components showed sharp endothermic peaks at each component's transition temperature, namely; 53.92, 50.57, 145.4 and 50.27 °C respectively, indicating their crystallinity. While the thermogram of LX showed sharp exothermic peak at 221.54°C [18, 42], as shown in Figure (2). Same figure reveal the thermodynamic parameters of drug-free and drug loaded niosomal formulations. It is observed in all formulations prepared, that there was a decrease in the endothermic peak of span 60, disappearance of the significant peaks of cholesterol and LX. These findings indicate that there were significant good interactions of all niosomal components, forming the bilayers of niosomes and enhanced entrapment of LX into these formulations.

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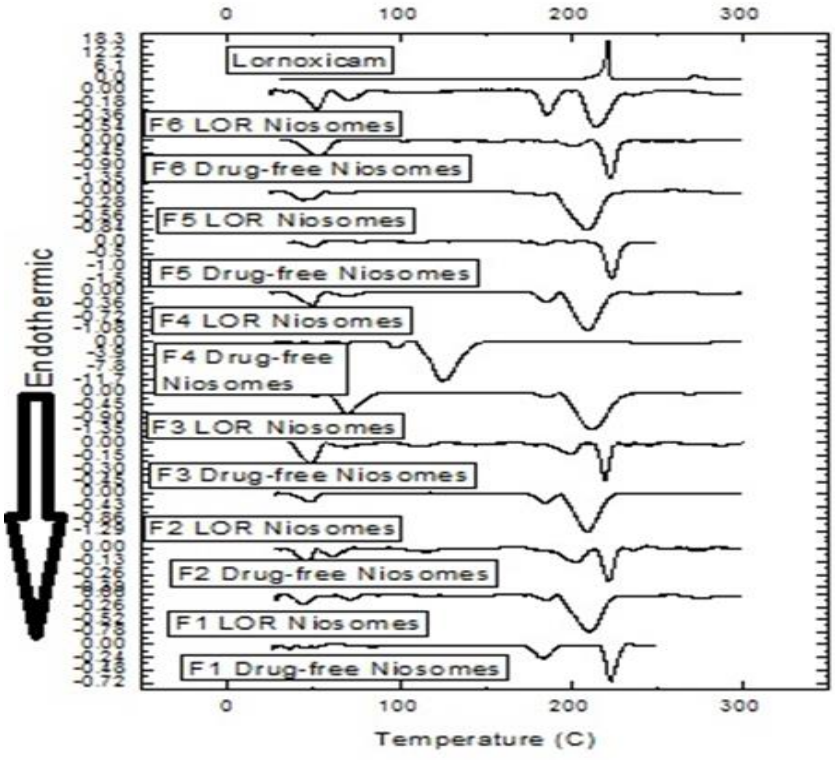

Figure 2. DSC thermograms of LX niosomal formulations, drug free niosomal formulations and free drug LX.

Vesicular Size Analysis

Physicochemical and biopharmaceutical properties of drug substances and dosage forms can be affected by the particle size, a critical parameter in pharmaceutical production [43]. The results, tabulated in table (1), reveal that LX Span 60 niosomes exhibited vesicular size that ranged from 147 to 1606 nm. Charged span 60 niosomes (F2, F3/ F5, F6) exhibited larger particle diameters than that of their neutral counterparts (F1/F4). Incorporation of a CIA in niosomes results in an enhancement in the spacing between adjacent bilayers[44], leading to an increase in the size of the internal aqueous compartment leading to the formation of larger niosomes. PDI values, shown in Table (1) calculated for LX niosomal formulations are high, indicating a heterogenous population.

Table 1. Entrapment efficiency percentages, vesicular size, polydispersity index and zeta potential of prepared LX niosomes.

Formula	E.E. (%) ± S.D.	Particle size (nm)	PDI	Zeta potential (mV) ± S.D.
F1 Span 60: Ch (1:1)	76.63 ± 0.78	147	1	-41.5 ± 11.5
F2 Span 60: Ch: DCP (1:1:0.1)	61.6 ± 1.07	264.6	1	-50.5 ± 10.3
F3 Span 60: Ch: SA (1:1:0.1)	47.73 ± 1.09	1063	0.84	-23.6 ± 6.4
F4 Span 60: Ch (2:1)	59.69 ± 1.12	299.3	1	-42.9 ± 13.7
F5 Span 60: Ch: DCP (2:1:0.2)	57.58 ± 3.87	838	0.155	-49.9 ± 10.8
F6 Span 60: Ch: SA (2:1:0.2)	55.51 ± 0.93	1606	0.565	-29.5 ± 6.32

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Zeta potential

Table 1 reveal the zeta potential of prepared LX niosomes (F1-F6). Particles with ZP values either more positive than (+30 mV) or more negative than (-30 mV) are normally considered stable [45].The results reveal that all neutral LX niosomes (F1 and F4) gave negative zeta potential values, i.e.-41.5 mv± 11.5, -42.9 ± 13.7 mV, -46.9 ± 9.83 mV and -56.9 ± 11.6 mV, respectively, indicating stable systems. Negative LX niosomes (F2 and F5) gave higher negative values of ZP (-50.5 ± 10.3 mV and -53.6 ± 6.78 mV), respectively, which indicates more of these colloidal systems compared to the other investigated niosomal formulations. On the other side, positively charged LX niosomes (F3 and F6) neutralized the negative charge carried by the system giving lower ZP values (-23.6 ± 6.4 mV and -21.2 ± 5.48 mV) resulting in aggregation of the particles which indicates low stability of these systems.

In-vitro release studies

Figure (3) demonstrate that the release pattern of LX from various niosomal formulations (F1-F6) was biphasic, with was an initial rapid drug release that was followed by a sustained release profile. The rapid release may be due to Lx is incorporated in fatty acid chains of lipid bilayers. This led to rapid release of LX upon dispersing vesicles in buffer until reaching equilibrium [46]. The release pattern of LX niosomal gels formulations showed similar behavior, where it occurred in two phases, a controlled release phase that lasted for 8 hours, followed by a steady phase with a reduced release rate which lasted for 24 hours.

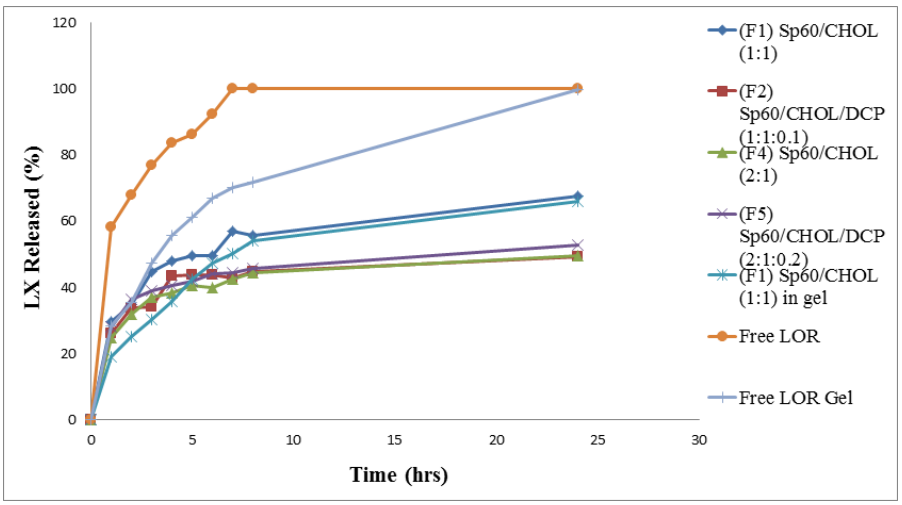

Figure 3. In-vitro release profiles of free drug, different LX niosomal formulations and selected LX niosomes (F1) in gel.

Kinetics study

Table (2) reveals that the release of LX from either Span 60 niosome or niosomal gel, is best fitted to diffusion controlled mechanism (Higuchi's model), where it showed the highest (R^2) values. The kinetics of drug release was analyzed by applying Korsmeyer-Peppas equation which often used for identifying the release mechanism. Data shown in Table (2) revealed that the drug release from niosomes follows Fickian diffusion release; where n < 0.45.

Table 2. Release rate constants (k) and correlation coefficients (R2) of LX release profiles from different niosomal / niosomal gel formulations.

Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas		
	R^2	K	R^2	K	R^2	K	R^2	K	n
F1	0.654	0.489	0.536	0.003	0.808	5.207	0.903	33.11	0.205
F2	0.583	0.283	0.476	0.002	0.724	3.028	0.832	30.90	0.152
F4	0.563	0.268	0.464	0.002	0.729	2.928	0.858	29.51	0.154
F5	0.569	0.255	0.487	0.002	0.739	2.792	0.879	32.35	0.136
F_1 in gel	0.645	0.628	0.495	0.005	0.802	6.713	0.884	23	0.311

Physical stability study

Visual appearance of the selected niosomal formulation (F1) showed good physical appearance without coarse particles or layer separation. Table (3) represents the percentages of drug retained in niosomes after 30, 60 and 90 days which are 99.41 ± 0.13 %, 99.00 ± 0.45 % and 98.48 ± 0.12 %, respectively, from their initial drug entrapped. The effect of storage on vesicular size was also studied. The data; Table (3), showed a slight increase of vesicle size after 30, 60 and 90 days. This increase in vesicle size after storage may be due to aggregation and fusion of the niosomal particles [47]. It is reported that liposomes up to 600 nm of diameter can penetrate through skin[48]. This reassures that storage of LX niosmes up to 3 months led vesicles of suitable size range that is appropriate for skin penetration.

Table 3. Effect of storage at (2 – 8 ºC) on mean drug retained, mean vesicle size and polydispersity index of LX niosomes F1.

	Drug retained* (% ±S.D.)	Mean vesicle size (nm)	P.I.
Initial	100 ±0.00	147	1
30 days	99.41±0.13	410	0.911
60 days	99±0.45	430	0.911
90 days	98.48±0.12	550	0.361
Initial	100 ±0.00	147	1
30 days	99.41±0.13	410	0.911

*Mean of 4 batches

In-vitro skin permeation

The permeation profiles of LX from plain ge,l as well as from selected LX niosomes (F1) in gel, through rat skin are shown in Figure (4). Results show that permeation of LX from niosomal gel is higher than its permeation from plain LX gel. The steady state flux (Jss) was 6.68 ± 0.12 ug/cm^2/hr and 3.33 ± 0.29 ug/cm^2/hr for F1 niosomal gel and free LX gel, respectively. Statistial analysis reveal a significant difference ($P<0.05$) in the permeation coefficient (Kp) of LX when encapsulated in niosomes ($3.341x10^{-3}$ ± $6.27x10^{-5}$ cm/hr) compared to free LX gel ($1.66x10^{-3}$ ± $14.88x10^{-5}$ cm/hr). There is a two fold increase in deposition of LX when encapsulated into the F1 niosomes-based gel compared to plain LX gel, as revealed by enhancement ratio.

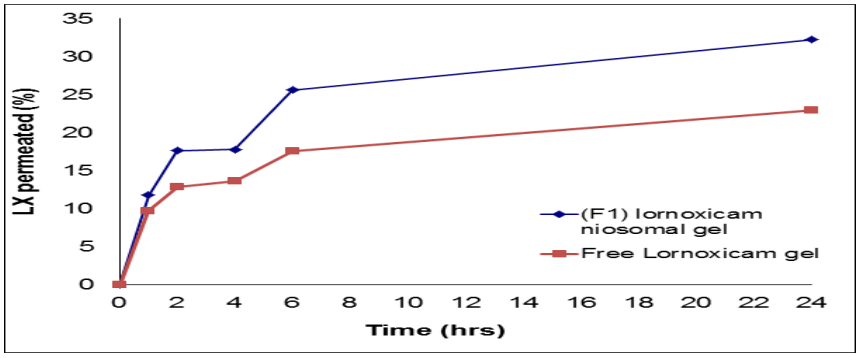

Figure 4. Permeation profiles of plain LX gel and selected LX niosomes (F1) in gel through rat skin.

In vivo evaluation of LX niosomes

Skin irritation test

Table (4) reveals the results of the skin irritation test expressed as PII. The results show that the LX niosomal gels were free of any irritation when applied to the skin. There were no signs of erythema and edema .According to Draize et al.[38] all the tested transdermal patches were considered to be negative (non-irritant) [PII< 2]. The irritation indices proved the non-irritancy of the drug or any of the formulation components.
3.9.2. In-vivo anti-inflammatory activity.

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Table 4. Primary irritancy indices (PII) calculated after application of different formulations on shaved rat skin.

Groups	Negative control		Positive control		Niosomal gel Free from LX (Placebo gel)		LX niosomal gel (F_1)		LX gel	
	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema	Erythema	Edema
Score	0.00	0.00	3.5	2	1	0.00	0.00	0.00	0.00	0.00
PII*	0.00		5.5		1		0.00		0.00	

*: mean of 3 values

The results, revealed in Figure (5), show that both control and blank groups showed the highest edema percentages throughout the whole experiment. F1 LX niosomal gel showed significantly lower (P<0.05) edema percentage, compared to free drug group staring from 4 hours and through all intervals studied till reaching 24 hours (27.05±7.25 compared to 62.19±9.83 for free drug group). This reveal enhanced anti-inflammatory activity of LX when incorporated in niosomes.

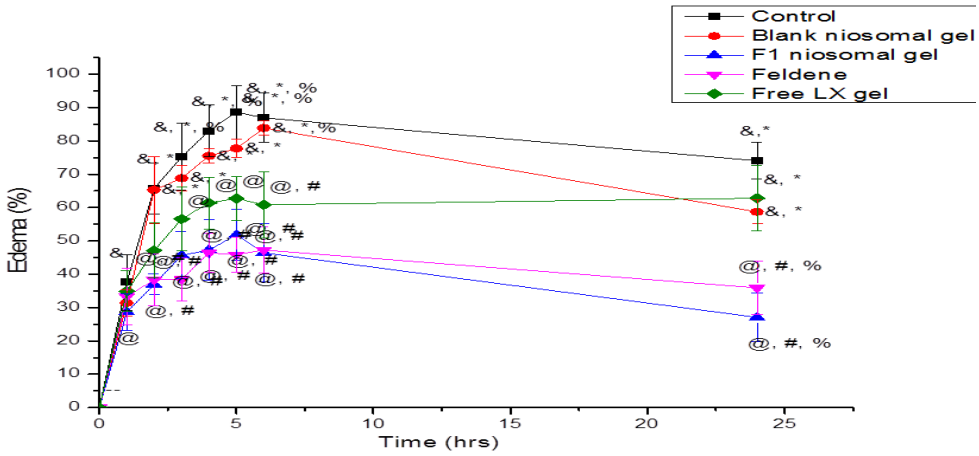

Figure 5. Percentage edema calculated for LX niosomal gel (F1), free drug gel and Feldene.

"@" significantly different (s.d.) from control group, "#" s.d. from blank group, "&" s.d.from F1 group, "*"s.d.from feldene group, "%" s.d. from free drug

Tail Flick Test

Results, shown in Table (5), reveal that inclusion of LX in niosomal gel significantly (P>0.05) prolonged the duration of analgesic effect when compared to free drug group after 120 min (i.e. 8.31±1.95 sec for F1, compared to 5.1±0.66 for free LX group). This prolongation of the analgesic activity can be attributed to the sustained release of LX entrapped in niosomal formulations. Statistical analysis revealed insignificant difference (P>0.05) between the effect of Feldene group and LX niosomal gel F1 at all times investigated.

Table 5. Time recorded to tail flick after topical application of LX niosomeal gel, Feldene and free drug gel.

Groups	Time to tail flick (sec)± S.D.			
	Baseline	30 min	60 min	120 min
Control	3.65±1.17	4.41±0.95 & * %	4.51±1.29& * %	4.25±0.75 & *
Blank niosomes	4.45±1.43	3.98±0.66 & * %	4.55±1.41& * %	4.45±1.21 & *
F1niosomal gel	3.86±0.85	5.86±1.25@#	9.43±2.02@#	8.31±1.95 @# %
Feldene	3.78±1.26	7.15±1.12@#	8.68±2.18@#	7.73±1.27 @# %
Free drug gel	4.35±1.34	6.86±1.27@#	7.11±1.96@#	5.1±0.66 & $ *

"@" significantly different (s.d.) from control group, "#" s.d. from blank group, "&" s.d. from F1 group, "*" s.d. from feldene group, "%" s.d. from free drug

CONCLUSIONS

Span60 LX niosomes were successfully prepared, yielding high entrapment efficiency, using the non-ionic surfactant Span 60, cholesterol and a charge inducing agent. They exhibited spherical shaped vesicles, suitable size, and were generally stable as deduced from zeta potential values. Stability experiments revealed that they were physically stable with insignificant difference in entrapment efficiencies after 3 months of storage. LX niosomes increased the permeation of the drug by 2 folds, compared to a free LX gel. As a result, Span60 LX niosomal gel were concluded to offer benefits for the management of inflammatory and painful conditions via transdermal application, thus they could decrease side effects of the drug that are caused by oral administration.

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