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Original Article

Lornoxicam loaded solid lipid nanoparticles for topical delivery: *ex vivo* assessment and pharmacodynamics activity

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ABSTRACT

The gastric irritant effects of peroral lornoxicam can be attenuated using skin as the route of administration. The present work was focused on the development, characterization, *ex vivo* skin permeation and skin targeting behaviors of lornoxicam-loaded solid lipid nanoparticles (SLN). Lornoxicam loaded SLN was prepared by emulsification solvent evaporation technique. The particle size and polydispersity index were measured by dynamic light scattering technique and was found to be at 180.7±4.4 nm, 0.223±0.006, respectively. The shape and surface topography of SLN were observed by transmission electron microscopy (TEM). Lornoxicam loaded SLN gel and lornoxicam gels were prepared and the gels were evaluated with respect to *in vitro* occlusivity, skin irritation and *ex vivo* skin permeation studies. Lesser skin irritancy and good oclusivity was observed with SLN gel as compared to the lornoxicam gel formulation. The *ex vivo* permeation data showed that SLN gel could significantly increase the extent of lornoxicam in skin and it showed skin targeting effect significantly. The anti-inflammatory activity of lornoxicam loaded SLN gel was stronger than that of lornoxicam gel and marketed formulation in carrageenan induced rat paw edema. These results suggest the SLN gel as the promising carrier for topical delivery of lornoxicam with skin targeting potential.

Kew words: Lornoxicam, SLNs, Topical delivery, Anti-inflammatory, Skin targeting.

INTRODUCTION

Recently, the topical therapy is considered the most innovative research area in drug delivery. Topical delivery associates various advantages over the oral delivery, such as prevention of first-pass metabolism, possible controlled release of drugs, self-administration with ease, convenience, and generally good acceptance by patients, fewer safety concerns because serum absorption tends to be minimal [1-3]. Solid lipid nanoparticles (SLN) were developed as an alternative delivery system because of their several advantages, such as the possibility of incorporating lipophilic and hydrophilic drugs, negligible skin irritation, better physical stability, and lower costs compared to liposomes [4]. Solid lipid nanoparticles is shown an occlusive properties by forming an intact film on the skin surface upon drying, which prevent the trans epidermal water loss and supports the epidermal drug targeting [5-6]. Due to highly specific surface area of SLN, it increases the contact of encapsulated drug with stratum corneum thus it helps to release the drug over prolonged period of time [7-8]. SLN has been used to improve the skin targeting of several drugs such as triptolide [9], podophyllotoxin [10], penciclovir [11], which support that SLN can be used for the topical delivery of lornoxicam. The present work was aimed on the preparation, characterization, *ex vivo* skin permeation and skin targeting behaviors of lornoxicam-loaded SLN. Lornoxicam, a nonsteroidal anti-inflammatory drug (NSAID) of the oxicam class and it is available in oral and parenteral formulations. It is used in the treatment of osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis. The oral therapy with lornoxicam is associated with severe or irreversible adverse effects, which include anemia, thrombocytopenia, leucopenia, hypertension, heart palpitation, hypersensitivity and gastrointestinal hemorrhage [12-13]. Due to its short plasma half-life (3–4 hours), poor solubility in water and side effects in the gastrointestinal tract make lornoxicam a promising candidate for topical delivery [14]. Lornoxicam-loaded SLN were prepared using a lipid PrecirolATO5 (PRE) by emulsification solvent evaporation technique. The

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particle size, polydispersity index and entrapment efficiency of the solid lipid nanoparticles were investigated. The lornoxicam loaded SLN system was finally formulated into hydroxyl propyl methyl cellulose (HPMC) gel for topical delivery and assessed for *ex vivo* skin permeation properties. The anti-inflammatory activity, skin irritation studies of lornoxicam loaded SLN based gel were performed by the topical application on rats' hind paw and rabbits' skin, respectively.

MATERIALS AND METHODS

Materials: The lornoxicam was a gift from Glenmark Pharmaceuticals (Nasik, India), precirol ATO5 was gift from Gattefosse (Saint Priest, France). Lecithin was procured from Acros Organics (New York), cholic acid sodium was procured from Himedia (Mumbai, India), polaxomer188 was a gift from BASF (Germany), HPMC was procured from Acros Organics (New York), and carrageenan was procured from Himedia (Mumbai, India). All other chemicals used in this research were of analytical grade.

Preparation of SLN: Solid lipid nanoparticles were prepared by emulsification solvent evaporation technique [15] with slight modification. Dichloromethane and water were saturated to ensure thermodynamic equilibrium of both the liquids. Lornoxicam (7.5mg) was dissolved in 3 ml of saturated dichloromethane in which Precirol ATO 5 (300mg) and lecithin (225mg) were previously dissolved. The inner oil phase was emulsified at 15000rpm (Ultra Turrax T-25 IKA Labortechnik) for 7 minutes with 15 ml of solvent-saturated outer aqueous phase containing poloxamer188(150 mg) and cholic acid sodium (75 mg) to prepare O/W emulsion. The emulsion was stirred at 700rpm for 3.5 hours using mechanical stirrer for complete evaporation of organic solvent. The lipid was precipitated out in the aqueous medium and formed solid lipid nanoparticles dispersion.

Purification of SLN dispersions: Purification of lornoxicam loaded SLN was carried out by dialysis bag method. The SLN dispersion was taken in the dialysis bag and tied at both ends. The dialysis bag was kept into 50 ml of double distilled water containing 0.2% w/v sodium lauryl sulphate and stirred at 100 rpm for 20 min. Five milliliter of sample was withdrawn at 5 minutes time intervals for 20 min. The samples were diluted appropriately and quantified the amount of drug by UV-VIS spectrophotometer (Spectrscan UV 2600, Thermo Fisher Scientific Inc, USA).

Characterization of SLN

Particle size: The average particle size and polydispersity index (PDI) of the SLN dispersions were determined by dynamic laser light scattering technique (90Plus Particle size analyzer, Brookhaven, New York, USA). The dispersions were diluted 100 times with deionized water and measurements were obtained at an angle of 90°. The pH of the samples ranged from 6.4 to 6.8. The measurements of particle size were made in triplicate.

Entrapment Efficiency (EE): The lornoxicam SLN suspension (1 ml) was aggregated by acidifying to pH 1.2 with 0.1M HCl and the admixture solution was immediately separated by using ultracentrifugation (Remi, Mumbai) at 11000 rpm for 60 min, at 10°C. The clear supernatant was collected and the lornoxicam content in lornoxicam loaded SLN was measured after dilution with methanol. The percentage EE was calculated by the following equation [16]:

$$\%EE=\left[Wa-\frac{Ws+Wp}{Wa}\right]\times 100$$

Where Wa is the quantity of drug presented in system, Ws is the quantity of drug presented in supernatant after the centrifugation, and Wp is the quantity of drug presented in the purification medium.

Morphology: The morphology of lornoxicam-loaded SLNs dispersion was examined by transmission electron microscope (TEM, JEM-1200EX, JEOL, Tokyo, Japan). One drop of diluted lornoxicam-loaded SLN dispersion was placed on a copper grid and for contrast enhancement; one drop of 2% w/v aqueous solution of phosphotungstate acid (PTA) was used. The sample was dried and examined under the TEM.

Preparation of SLN based Gel: The HPMC powder (2.5%w/w) was added to 40% v/v ethanol-water solvent system and stirred by using a magnetic stirrer at 1500 rpm and the resulting mixture was stirred continuously at 60°C until the gel was formed. The pure lornoxicam (0.25% w/w) for lornoxicam gel or the SLN equivalent to 0.25% w/w lornoxicam for SLN gel were added to HPMC gel and mixed well to make a uniform dispersion. The propylene glycol at 20% w/w level was added to prevent drying out of the gel on storage. The gel formulations were kept overnight at room temperature.

Evaluation of lornoxicam loaded SLN based gel

Determination of drug content, pH and Spreadability: For the determination of drug content, about 1 g of the gel was weighed and placed in 50 ml beaker and dissolved in sufficient quantity of methanol. The sample was filtered and diluted appropriately and quantified for the amount of drug by UV-VIS spectrophotometer. For the determination of spreadability, about 0.5 g test formulation was placed within one cm diameter pre-marked circle on a glass plate. A second glass plate was kept over the first glass plate and 500 g weight was allowed on the upper glass plate for 5min. The increase in the diameter of the test formulation was noted [8]. The pH of test samples was determined using Digital pH meter Model 707 (Digisum Electronics, India) and calibration of pH meter was made by using pH 4.0 and 7.0 standard buffers before use.

Viscosity studies: The viscosity of the different formulations was carried out using a Brookfield Viscometer (Model DV-E, U.S.A) with helipath stand. About 50 g of each formulation was transferred in a beaker. Viscosity of the different formulations was studied at a rotational speed at 10 rpm using a S-64 spindle in triplicate.

Light microscopy: A thin layer of lornoxicam gel and lornoxicam loaded SLN gel was spread on a glass slide and covered with a glass cover slip. Slide was observed under a phase contrast microscope equipped with a camera (Leica DMLP, Germany) to observe the topography of topical formulations. Photomicrographs were taken at suitable magnification.

Ex vivo skin permeation study: The permeability behavior of prepared gel was evaluated across the pig ear skin in modified Franz diffusion cell. The pig skin was obtained from local slaughter house and processed as reported elsewhere [17]. Pig skin samples were placed on Franz diffusion cells with the stratum corneum side up [18] and an effective diffusion area of 3.14cm^2. The receiver compartment was filled with 22 ml of 40%V/V methanol in phosphate buffer pH 7.4 .The diffusion cells were maintained at (37±0.5) ^{0}C with stirring at 100 rpm throughout the experiment. The skin samples were kept to equilibrate for 30min before the formulations were placed on the donor compartment [11]. 1 gm of lornoxicam SLN gel and lornoxicam gel containing the 5 mg of lornoxicam were applied on the skin surface. 1ml sample was withdrawn from receiver compartment at predetermined time intervals (0.5, 1, 2, 4, 5, 6, 7, 8 h) and diluted with fresh dissolution medium. All samples were filtered through 0.45µm pore size cellulose membrane filter and concentration of the drug was determined by UV-VIS spectrophotometer at 378 nm. The experiments were performed in triplicate.

The amount of formulation remaining on the skin (donor compartment) was diluted suitably with methanol and filtered through an aqueous 0.45µm pore size cellulose membrane filter. The amount of lornoxicam was determined by UV-VIS spectrophotometer at 378nm. For the determination of lornoxicam present in the skin, the remaining portion of the formulation on the skin was cleaned. The skin was minced and transferred into a test tube containing 10 ml of methanol. The sample was agitated for 15 minutes and the resulting solution was filtered through 0.45 µm pore size cellulose membrane filter. The amount of drug deposited in the skin was analyzed by UV-VIS spectrophotometer at 378nm.

In vitro occlusivity test: It test was performed with the lornoxicam loaded SLN gel and lornoxicam gel formulation [8, 17]. 25 g of distilled water was placed in each beaker and the open end of each beaker was closed with whatman filter paper(9.0cm).200 mg of the test formulation was evenly distributed over the whatman filter paper . These beakers were kept at 30±2^{0}C/ 60±5% RH for 48 h. All formulations were tested in triplicate keeping all the condition constant. The occlusion factor "F" of the formulations was calculated by using following equation:

$$F = 100\left[\frac{A-B}{A}\right]$$

Where, A is the water flux through the blank filter paper and B is the water flux through the filter paper with formulation.

Primary skin irritation test: The evaluation of skin irritation potential of lornoxicam loaded SLN based gel was carried out using hair free skin of rabbits [20-21]. The experimental protocol was approved by the Institutional Animal Ethical Committee of Dibrugarh University, Dibrugarh (Approval No. IAEC/DU/18 dated 17/02/2012, registration no. 1576/GO/A/11/CPCSEA).

500mg of the selected formulations were spreaded uniformly within the area of 4 cm^2 on the hair free skin of rabbits (1.5±0.5 kg). The skin was watched carefully for any visible change such as erythema (redness) or edema (swelling) after 24 h. For the study of the cumulative effect, the test sample was applied once daily on the same site of hair free skin for 7 days. Evaluation of skin irritation potential was carried out on the basis of the following scoring method [20]: 0: No erythema development;

2: scarcely perceive blood vessels and light erythema development; 4: perceive main blood vessels and slight erythema development; 6: main blood vessels more obvious and slight erythema development. Irritation potential was calculated using the following equation [20]:

$$\text{Irritation potential} = \frac{A.B}{\text{number of observation days}'}$$

Where A and B represent erythema value and corresponding day, respectively.

Pharmacodynamics activity

Carrageenan-induced inflammation in rats: Male Wister rats (120-150 gm) of either sex of six per group received topical treatment. The standard group was treated with marketed diclofenac gel (Thermogel). The two experimental groups received lornoxicam loaded SLN gel and lornoxicam gel whereas the control group was received placebo gel. Edema was induced by sub planter injection of 0.05 ml of 1% carrageenan into the left hind paw of each rat after thirty minutes of topical treatment. The left hind paw volume of each rat was measured by using a digital plethysmometer (orchid scientific, PLM 01, India) immediately before and 1 h, 2 h, 3 h, 4 h, 5 h and 6 h after the injection of carrageenan [22]. The edema rate and inhibition rate of each group were calculated by using following equations [23]:

$$\% \text{ Edema rate (E \%)} = \frac{Vt - V0}{V0} \times 100$$

$$\% \text{ Inhibition rate (I \%)} = \frac{Ec - Et}{Ec} \times 100$$

Where Vo is the mean paw volume before carrageenan injection (ml), Vt is the mean paw volume after carrageenan injection (ml), Ec is the edema rate of control group, and Et is the edema rate of the treated group.

Formaldehyde induced Arthritis in rats: The arthritis was **induced** in rats by using formaldehyde-induced arthritis method [24]. Male Wister rats (120-150 gm) of either sex of six per group received topical treatment. The standard group was treated with marketed diclofenac gel (Thermogel). The two experimental groups received lornoxicam loaded SLN gel and lornoxicam gel whereas the control group was received placebo gel. Arthritis was induced by sub plantar injection of 0.1 ml of 2.5% formaldehyde solution left hind paw of each rat after thirty minutes of topical treatment and same dose of formaldehyde was injected on day 3. Arthritis was estimated by measuring the volume of distilled water displaced by the paw before induction of arthritis and once in a day for 15 days, after induction of arthritis. Application of topical formulation on hind paw was continued onceeveryday for 15 days. The edema rate and inhibition rate of each group were calculated using Eq (4) and (5), respectively.

Statistical analysis: For the determination of statistical analysis, SPSS 14.0 software (USA) was used. All results were expressed as the mean value ±S.D. Statistical data were analyzed, one-way analysis of variance followed by Dunnett's t-test was performed to compare two or more groups and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Characterization of SLN dispersions

Mean particle size and polydispersity index: The mean particle size and Polydispersity index of SLN dispersions are shown in table 1. The mean particle size of lornoxicam free SLN dispersion and lornoxicam loaded SLN dispersion was found to be 176.4±3.8 nm and 180.7±4.4 nm, respectively. The results indicate the mean particle size of the lornoxicam-loaded SLN dispersions was slightly increased over the lornoxicam-free SLN dispersions.

Drug entrapment efficiency: The entrapment efficiency of lornoxicam in the SLN dispersions was found to be 91.34±1.98%.

Particle morphology: The TEM image of lornoxicam loaded SLN is shown in Fig.1. The TEM image showed the particle was nanosize range with spherical in shape.

Evaluation of lornoxicam loaded SLN based gels

Determination of drug content, pH and Spreadability: The amount of lornoxicam from lornoxicam loaded SLN gel and lornoxicam gel was found 97.9±3.5%and 98.60±3.2% respectively. The pH of the topical formulations was found to be less than 7 and it was in tolerable limits. Spreadability is an important characteristic of gel formulation. The results of spreadability studies indicate lornoxicam loaded SLN gel showed better spreadability than the lornoxicam gel (Table 2).

Light microscopy: The photomicrographs of the lornoxicam gel and lornoxicam loaded SLN gel are shown in Fig.2. The microscopic examination of the lornoxicam loaded SLN based gel formulation revealed that the drug loaded SLN were distributed uniformly throughout the gel. However smooth surface was found in plain drug gel.

In vitro occlusivity test: The results of occlusivity are shown in Fig. 3. From the results of occlusivity

test it revealed that the lornoxicam-loaded SLN gel showed significantly higher ($p < 0.05$) prevention of water loss than that of the lornoxicam gel. The solid nature of lipid component in SLN gel and blocking the micropores of filters to prevent the water evaporation hence SLN gel showed better occlusivity [8]. The greater skin occlusivity increases skin hydration properties. Therefore SLN gel increases skin hydration properties which could be helpful for permeation of lornoxicam through the epidermis of skin. Due to the small particle size, lornoxicam loaded SLN gel might have the more suitable occlusive effect than lornoxicam gel. So the high accumulation of lornoxicam loaded SLN gel in skin was obtained.

Ex vivo skin permeation studies: The percentage amount of lornoxicam permeated, retained in skin and presented in donor compartment after 8 h was determined from the lornoxicam loaded SLN gel and lornoxicam gel and the results are shown in Fig. 4. The results reveal that lornoxicam was less permeated in SLN gel as compared to that of lornoxicam gel**.** However, the flux value of lornoxicam loaded SLN gel and SLN gel was calculated and found 7.22±1.07 µg/cm^2.h and 15.8±1.69 µg/cm^2.h respectively. The flux value obtained with the lornoxicam loaded SLN gel was significantly lower ($p < 0.01$) than the lornoxicam gel. The lornoxicam was encapsulated in lipid core hence the slow permeation of lornoxicam from the gel through the skin [25].The extent of drug in epidermis was higher in lornoxicam loaded SLN based gel when compared with plain drug gel (Fig.4). Therefore lornoxicam loaded SLN based gel can improve the skin targeting of lornoxicam and can minimize the systemic release when compared with lornoxicam gel. The similar result has been found by other researchers that SLN can increase the extent of drug in skin [8, 26]. The nanometric size of SLN helps to improve the lipid nanoparticles penetration into skin and accumulation within the skin due to their lipophilic nature. A long term controlled release of lornoxicam may occur from the accumulated SLN and thereby sustained anti-inflammatory effect [27].

Skin irritancy test: The irritation potential of lornoxicam loaded SLN gel was found to be 0.28. The result indicates the lornoxicam loaded SLN gel is supposed to be non-irritant [20].After one week of application of the lornoxicam loaded SLN gel, there was no distinct erythema, and edema on rabbits' skin.

Pharmacodynamics activity

Carrageenan-induced inflammation in rats: The edema was induced in rat's hind paw by using carrageen. The results of lornoxicam gel, lornoxicam loaded SLN gel, and the reference Diclofenac gel (Thermogel) in the carrageenan induced edema at specific time intervals are shown in Table 3. The lornoxicam loaded SLN gel with the smallest particle size showed the strongest anti-inflammatory activity, nearly one and an half fold higher than that of lornoxicam gel. The results revealed that the lornoxicam loaded SLN gel significantly ($P < 0.05$) inhibited the edematous response as compared to lornoxicam gel. The anti-inflammatory effect of lornoxicam loaded SLN gel was found 64.97% of inhibition which was greater than that of the lornoxicam gel 42.65% of inhibition (Table 3).In the present study, it was found that the lornoxicam loaded SLN gel was significantly ($P < 0.05$) inhibited rat paw edema induced by carrageenan as compared to lornoxicam gel and reference diclofenac gel (Thermogel), it may be due to the highly accumulation of lornoxicam in inflammation site. Carrageenan injection helps the secretion of prostaglandin in their inflammatory area and it injured the dermal tissues surround macrophages release Interleukin-1 (IL-1) leads to accumulate polymorphic nuclear cells (PMNs) into the inflammatory area. Polymorphic nuclear cells (PMNs)release the lysosomal enzymes responsible for damage connective tissues and induce paw swelling [28].Lornoxicam could inhibit the polymorpho nuclear leucocyte migration hence it shows anti-inflammatory effects [29].The carrageenan induced rat paw edema could be significantly suppressed by lornoxicam loaded SLN gel.

Formaldehyde induced Arthritis in rats: Formaldehyde was used to persuade arthritis in hind paw of rat. The edema rate of the injected hind paw was increased during the first 6 days. Thereafter, the swelling of hind paw slowly decreased. The effect of the topical formulations on the rapid phase of inflammation revealed that the lornoxicam loaded SLN gel significantly ($P < 0.05$) inhibited the paw edema volume to formaldehyde-induced arthritis as compared to lornoxicam gel. The inhibitory effect of lornoxicam loaded SLN based gel was found 55.72% of inhibition which was greater than that of the lornoxicam loaded gel 42.29% of inhibition (Table 4). It was shown that the lornoxicam loaded SLN based gel was the stronger anti-arthritic activity, as compared to lornoxicam gel. Lornoxicam inhibits the release of superoxide from polymorphs and stimulates proteoglycan synthesis for strong healing effect in rheumatoid arthritis [30].The leukocyte migration from cells persuades superoxide radicals release which is responsible for tissue inflammation [31]. Due to high accumulation of lornoxicam from lornoxicam loaded SLN gel in inflammation site

thus the lornoxicam could reduce the release of superoxide radicals by inhibiting leukocyte migration. Tissue necrosis is shown in later stage of arthritis induced by formaldehyde. The necrotic tissues can induce inflammation by releasing the mediators from dead passenger leukocytes [32].The lornoxicam loaded topical formulations may promote tissue repair by the alleviating actions of secretions of these cells [33].

CONCLUSION

In this study Lonoxicam loaded solid lipid nanoparticles were prepared by emulsification solvent evaporation technique. The lornoxicam loaded solid lipid nanoparticles were evaluated and showed the satisfactory results. Further SLNs were introduced in to the gel for topical delivery of lornoxicam. The *ex vivo* permeation studies showed the less systemic uptake of lornoxicam from lornoxicam loaded SLN gel when compared with the lornoxicam gel. Lesser skin irritancy and good occlusivity was observed with the lornoxicam loaded SLN based gel as compared to gel formulation. The anti-inflammatory activity of lornoxicam loaded SLN gel was stronger than lornoxicam gel and marketed formulation in carrageenan induced rat paw edema. However, the anti-arthritic activity of lornoxicam loaded SLN based gel was less affective when compared to the marketed formulation in formaldehyde induced rat paw edema. Formulation of lornoxicam loaded SLN based gel with skin targeting and successively anti-inflammatory affects could be successful for topical delivery of lornoxicam.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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Table 1: Mean particle size and Polydispersity index of SLN dispersion

Formulations	Mean particle size	Polydispersity index
Lornoxicam free SLN Dispersion	176.4±3.8	0.218±.013
Lornoxicam loaded SLN dispersion	180.7±4.4	0.223±.004

Data presented as mean ± S.D (n = 3).

Table 2: Evaluation of different parameters of various topical formulations

Parameters	Gel	SLN GEL
pH	6.5 ± 0.20	6.7±0.15
Spreadability (cm)	5.96±0.18	6.82±0.36
Spredability coefficient (cm^2/g)	0.055±0.003	0.073±0.007
% drug content	98.60±3.2	97.9±3.5
Viscosity at 10 rpm (Pa.S)	10.14±1.2	11.38±1.6

Data presented as mean ± S.D (n = 3).

Table 3: The anti-inflammatory effect of carrageenan-induced paw edema in rats

	% edema rate					
Group	1h	2h	3h	4h	5h	6h
Control	31.6± 2.3	36.0± 2.2	42.9±3.4	41.6±4.6	38.1±5.1	35.4±4.2
Gel	26.8±1.4 (15.08)	29.1±3.7** (19.16)	36.7±3.2 (14.45)	30.3±2.7** (27.16)	24.5±3.4** (35.69)	20.3±2.9* 42.65)
SLN Gel	20.4±4.1* (35.44)	22.6±3.9* (37.22)	23.4±6.3* (45.45)	20.1±1.8* (51.64)	17.3±3.5* (54.49)	12.4±1.3* (64.97)
Diclofenac Thermogel	21.3±3.6** (32.59)	24.8±5.1* (31.11)	26.6±4.7* (37.99)	22.9±3.1* (44.95)	19.4±1.9* (49.08)	15.1±2.8* (57.34)

Data presented as mean ± S.D of six animals for each group. Each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control: *P<0.01 and **P<0.05 (Dunnett's t-test)

Table 4: The anti-arthritic effect of formaldehyde-induced paw edema in rats

Group	% edema rate					
	1 day	3 days	6 days	9 days	12 days	15 days
Control	41.0±2.7	51.1±4.9	62.1±6.3	56.7±5.2	48.3±3.9	45.4±4.6
Gel	32.4±4.6 (20.97)	40.9±3.6 (19.96)	46.8±5.1** (24.60)	39.8±2.6** (29.80)	32.0±3.5* (33.74)	26.2±2.4* (42.29)
SLN Gel	28.6±3.1* (30.24)	31.2±3.8* (38.94)	36.4±1.9* (41.38)	29.7±4.2* (47.61)	26.4±2.7* (45.34)	20.18±4.3* (55.72)
Diclofenac Thermogel	26.7±2.9* (34.87)	30.3±1.7* (40.70)	34.2±2.1* (44.92)	29.1±3.7* (48.67)	24.8±1.8* (48.65)	17.2±3.8* (62.11)

Data presented as mean ± S.D of six animals for each group. Each value in parenthesis indicates the percentage inhibition rate. Statistically significant from control: *P<0.01 and **P<0.05 (Dunnett's t-test)

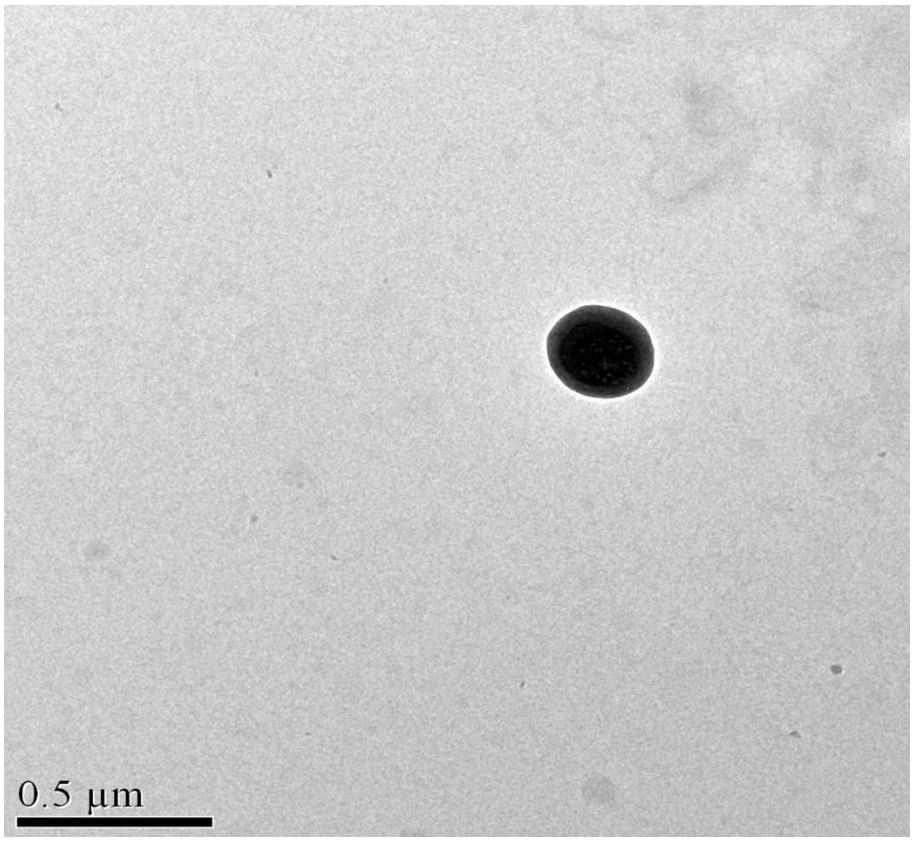

Figure 1 Transmission electron microscopy of lornoxicam loaded SLN

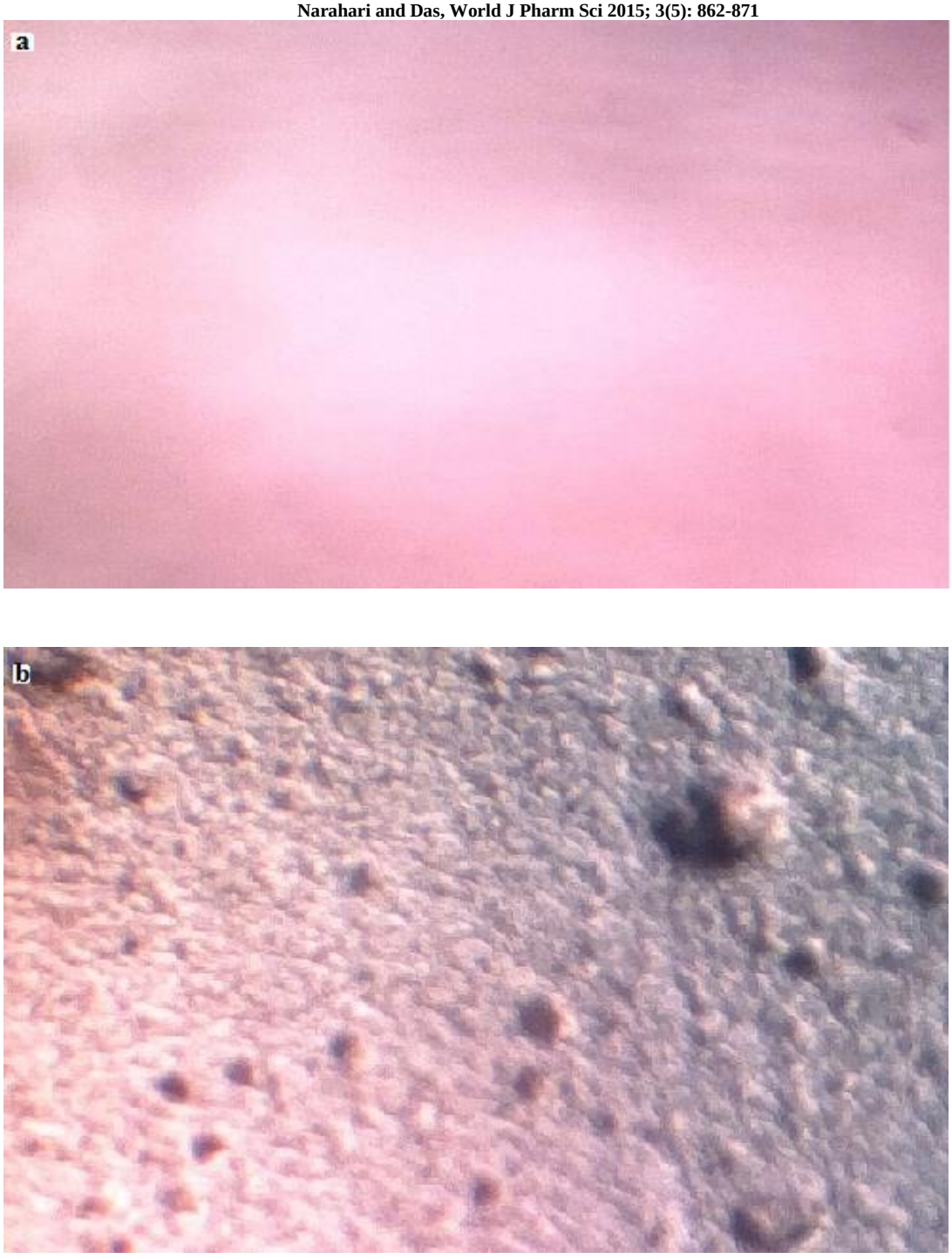

Figure 2 Light Microscopy of (a) conventional lornoxicam loaded gel (b) lornoxicam loaded SLN based gel

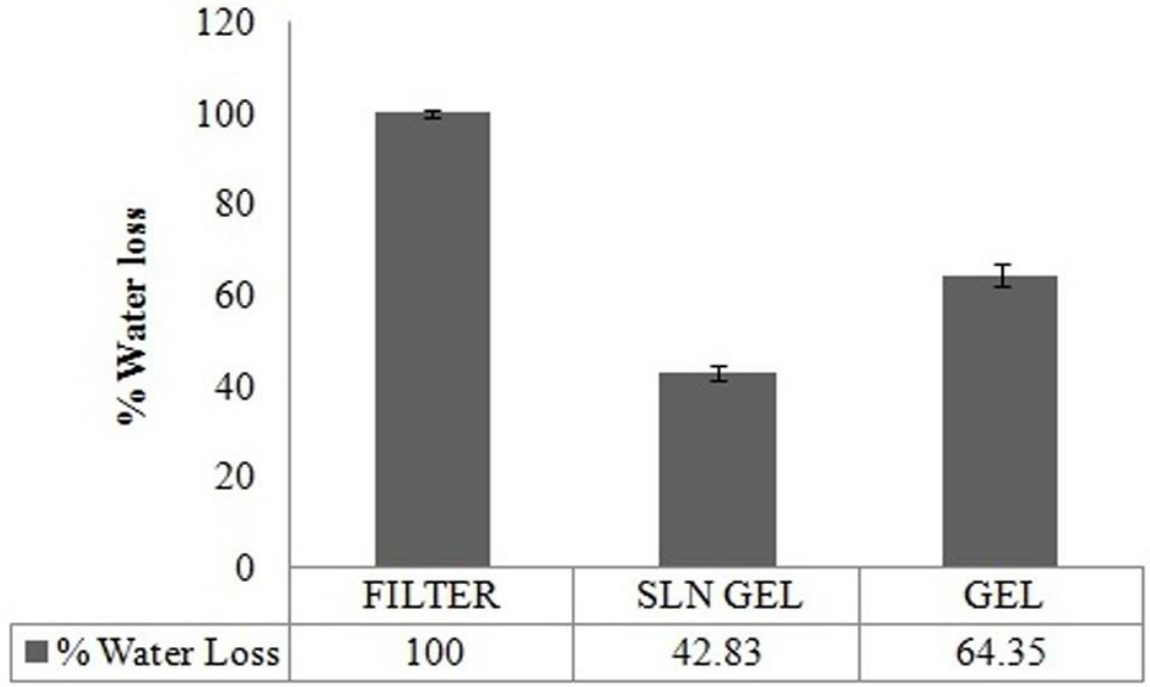

Figure 3 Comparative *in vitro* occlusivity of various topical formulations (n=3).

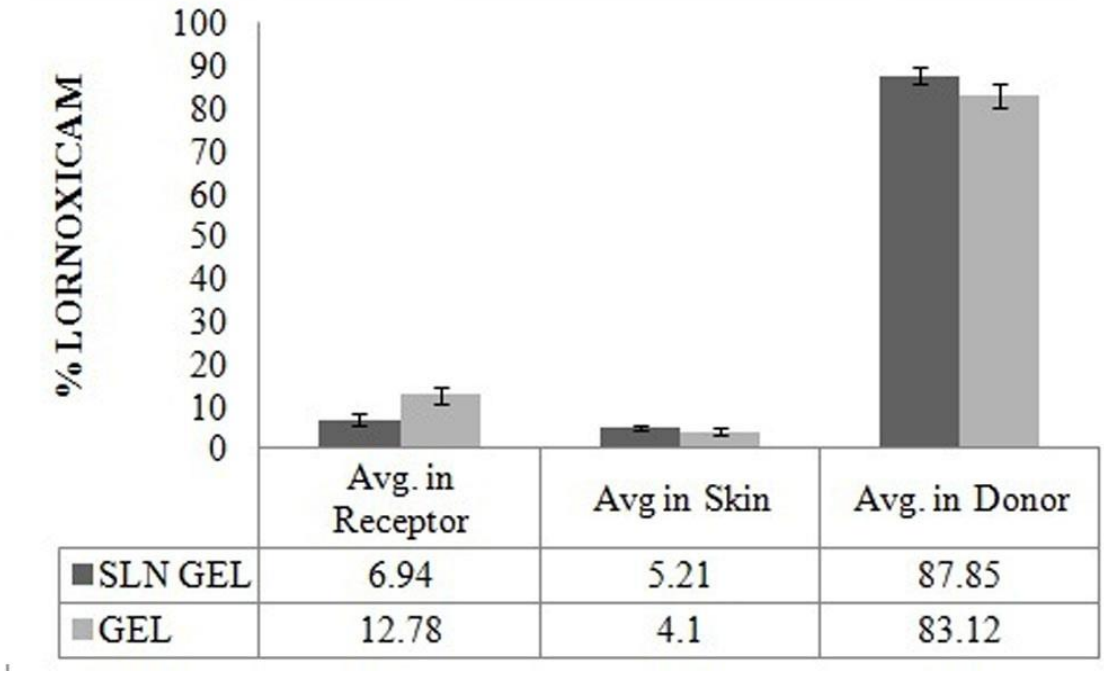

Figure 4 Amount of lornoxicam permeated and deposited through pig skin from various topical formulations at the end of 8 h (n=3).

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