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PRONIOSOMES AS A DRUG CARRIER FOR TRANSDERMAL DELIVERY OF MELOXICAM

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تهدف هذة الدراسة الى تقييم البرونيوزومات باعتبارها الناقل عبر الجلد لعقار الميلوكسيكام وهو أحد مضادات الالتهاب غير السترويدية. وقد تم تحضير البرونيوزومات والمحتوية على العقار بنجاح. كما تم قياس مدى كفاءة احتواء العقار داخل البرونيوزومات وكذلك دراسة الشكل الخارجى وحجم البرونيوزومات المحضرة. كما تم قياس نفاذ العقار من البرونيوزومات المحضرة والمصاغة فى صورة هلام عبر غشاء السيليولوز وكذلك عبر جلد الفئران باستخدام خلايا فرانز. كما تم دراسة تقييم حجم الورم فى رجل الفار بعد دهنة ببعض الهلامات المحضرة. وقد أظهرت البرونيوزومات المحضرة نسبة احتواء عالية للعقار. كما أظهرت البرونيوزومات المحضرة والمصاغة فى صورة هلام تحسنا ملحوظا فى نفاذ عقار الميلوكسيكام خلال جلد الفيئران. وأظهرت نتائج دراسة تقييم حجم الورم فى رجل الفار بعد دهنة ببعض الهلامات المحضرة كفاءة الهلام المحتوى على البرونيوزومات الحاملة للعقار.

The current investigation aims to evaluate the potential of proniosomes as a carrier for transdermal delivery of a potent non-steroidal anti-inflammatory, meloxicam. Meloxicam-loaded proniosomes were prepared and characterized for entrapment efficiency, surface morphology and in-vitro permeation across excised rat skin from various proniosome gel formulations using Franz diffusion cells. Various non-ionic surfactants were used to achieve optimum encapsulation efficiency. Niosomes formed from using Spans and Tweens exhibited high encapsulation efficiency. The prepared proniosomes significantly improved drug permeation and reduced the lag time ($p< 0.05$). Proniosomes prepared with Span 60 provided a higher meloxicam flux across the rat skin than did those prepared with Tween 80.

Testing of the anti-inflammatory effect of meloxicam proniosomal gel showed better pharmacological activity when compared with the standard meloxicam gel. The results suggest that proniosomes can act as promising carriers offer an alternative approach for transdermal delivery of meloxicam.

INTRODUCTION

Transdermal delivery systems, when compared with conventional formulations, generally show a better control of blood levels, a reduced incidence of systemic toxicity, no hepatic first-pass metabolism and a higher compliance[1]. A continuous interest toward the dermal and transdermal products can be seen, offering several advantages[2].

Liposomes have attracted a great deal of attention in the delivery drugs because of many advantages: they are biodegradable, non-toxic, amphiphilic in nature, penetration enhancers and effective in the modulation of drug release properties[3]. Although the application of liposomes for improved drug delivery is encouraging, liposomes exhibits some difficulties, including the instability of aqueous dispersions on storage and the leakage of the encapsulated drugs[4]. Moreover, the high cost of synthetic phospholipids and variable purity of natural phospholipids have raised concerns over the adoption of liposomal drug delivery systems[5]. An alternative approach that overcomes several of these problems associated with liposomes involves formation of liposome-like vesicles (niosomal dispersions) from non-ionic surfactants, commonly referred as niosomes[6&7]. Niosomes are capable of entrapping hydrophilic and hydrophobic solutes[8&9]. The greater stability, lesser storage problems and lack of many disadvantages associated with liposomes have prompted the

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exploitation of niosomes as alternative promising drug carriers to phospholipids vesicles for industrial production of both pharmaceutical and cosmetic products[10-13]. Niosomes are quite stable, and require no special conditions, such as low temperature or inert atmosphere for production or storage[8]. Niosomes and proniosomes, hydrated by agitation in hot water for a short period of time, have been proposed for a number of potential therapeutic applications, e.g. as carriers of anti-inflammatory drugs[14]. Meloxicam (MLX) is a nonselective, nonsteroidal, antiinflammatory drug (NSAID) with preferential inhibition of cyclo-oxygenase-2 (COX-2) over COX-1. MLX does not have documented cardio-vascular toxicity at doses of less or equal to 15 mg/day which are recommended for the treatment of rheumatoid arthritis and osteo-arthritis[15]. However, when orally administered, nonselective NSAIDs may adversely affect the gastrointestinal tract and can even reduce the life expectancy of patients with rheumatoid arthritis[16]. Transdermal delivery of MLX would avoid major gastrointestinal side effects and provide steady plasma levels from a single dose[17]. In addition, it has been demonstrated that NSAIDs promote local analgesia when administered locally through the skin[18]. Therefore, an alternative non-invasive mode of delivery of the drug is needed. Transdermal administration of MLX can overcome these side effects and higher local concentration can be maintained at the target site, which is desirable for anti-inflammatory agents.

The objectives of this study were to develop a transdermal delivery system of meloxicam using proniosomes and to study their *in-vitro* and *in-vivo* permeation characteristics, as well as the anti-inflammatory efficacy.

MATERIALS AND METHODS

Materials

Span 20, 60 and 80 were supplied from Koch-light laboratories Ltd. (Colebrook Bucks, UK). Tween 20, 60 and 80, acetonitrile HPLC grade, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from BDH Laboratory Supplies (BDH Chemicals Ltd., Poole, UK). Lecithin from eggs (extra pure) was obtained from Merck Company (Darmstadt, Germany). Cholesterol, acetic acid and ethanol were supplied from Riedel Dehae¨n (Darmstadt, Germany). Hydroxpropyl methylcellulose (HPMC K100 Premium CR grade) was obtained from Dow Chemical Company (Midland, MI). Meloxicam (MLX) was purchased from Sigma Chemicals Company (St. Louis, MO).

Methods

Determination of meloxicam (MLX) solubility

An excess amounts of MLX were added to ten ml of water or phosphate buffer pH 7.4, sonicated for one hour, and were shaken in a thermostatically controlled shaker maintained at 37.0±1.0°C for 72 hrs. The suspension was filtered (0.45 µm filter), diluted with 0.1 N ammonia solution and assayed spectrophoto-metrically at 353 nm.

Preparation of proniosomes

The method reported by Fang *et al.*[19] was followed with slight modifications. The method briefly as follows: The surfactant, lecithin and cholesterol were taken in a clean and dry, wide mouth small glass tube and mixed with 2.5 ml of absolute ethanol contains the drug (100 mg of MLX, few drops of ammonia were added to ensure dissolving of meloxicam). After mixing all the ingredients, the open-end of the glass tube was covered with a lid to prevent loss of solvent from it and warmed on a water bath at 65±3°C for 5 min. until the surfactants were dissolved completely. Then 2.0 ml of pH 7.4 phosphate buffer was added and the mixture was further warmed in the water bath for 3 min. so that a clear solution was obtained. The mixture was then left to stand at room temperature until the dispersion was converted to proniosomal gel. The proniosomal gel was then mixed with 2% w/w of polymeric gel (HPMC) to give a final concentration of 0.5% w/w MLX. The pH of the prepared proniosomal gels were 7.4±0.2. A standard MLX gel was prepared, for comparison, as follows: To 5.0 ml of absolute ethanol contains 100mg of MLX, drops of ammonia were added to ensure dissolving of meloxicam, then mixed with aqueous polymeric gel (HPMC) to give a final concentration of 0.5% MLX in 2% w/w of HPMC gel. The prepared standard gel is clear with pH of 9.0. The composition of different proniosomal formulations is shown in table 1.

Table 1: Compositions of various Meloxicam proniosomal formulations (mg).

Proniosome code (Formula)	Meloxicam	Span	Tween	Lecithin	Cholesterol
Span 20 (S20)	100	1800	-	1800	200
Span 60 (S60)	100	1800	-	1800	200
Span 80 (S80)	100	1800	-	1800	200
Tween 20 (T20)	100	-	1800	1800	200
Tween 60 (T60)	100	-	1800	1800	200
Tween 80 (T80)	100	-	1800	1800	200

Standard gel formula contains 0.5% MLX in 2% HPMC gel.
Control gel is a plain 2% HPMC gel.

Meloxicam encapsulation efficiency

The concentration of drug entrapped was determined by taking 0.2 g of proniosomal gel, weighed in a glass tube and added to 10 ml of pH 7.4 phosphate buffer. The aqueous suspension was sonicated in a sonicator bath (Transonic T460/H, Elma, Germany). The MLX-containing niosomes were separated from untrapped drug by centrifugation at 25000 rpm (32000×g) at 20°C for 30 min. The supernatant was recovered and assayed by an HPLC method for MLX content. The percentage of drug encapsulation (EP (%)) was calculated by the following equation:

$$EP (\%) = [(C_t - C_f) / C_t] \times 100\% \quad \text{........ Eq (1)}$$

where C_t is the concentration of total MLX and C_f is the concentration of free MLX.

Vesicular shape and surface morphology

Scanning electron microscopy (SEM) was conducted to characterize the surface morphology of the niosomes. One drop of niosomal suspension was mounted on clear glass slab, air dried and sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd., Tokyo, Japan) equipped with a digital camera, at 15 or 20 kV accelerating voltage.

***In-vitro* release study**

The *in-vitro* release meloxicam from proniosomal gel formulations was determined by using Franz diffusion cell. (1.70 cm^2 exposed surface area). Cellophane membrane (after soaking in distilled water for 2 hrs) was mounted on the receptor compartment. The receptor compartment was filled with 12.0 ml of pH 7.4 phosphate buffer maintained at 37±1°C and stirred by a magnetic bar at 600 rpm. One gram of gel formulation was placed on the cellophane membrane and the top of the diffusion cell was covered with paraffin paper. At appropriate time intervals (30, 60, 90, 120, 180, 240, and 300 min), 3 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution to maintain sink conditions. The samples were analyzed by a validated spectrophotometric method at 353 nm. The readings of the calibration curve showed a linear relationship between the absorbance and the concentration of MLX in phosphate buffer pH 7.4 at 353 nm, in concentration range of 2-20 ug/ml. The regression equation is y = 0.058 x +0. 005 and r value is 0.999.

***In-vitro* permeation study**

The permeation of MLX from proniosomal gel formulations was investigated by using an *in-vitro* Franz diffusion cell. The temperature was maintained at 37±1°C. Dorsal full thickness skin of albino rat was used. The fat was removed with the aid of scissor and the skin was washed and soaked in isotonic phosphate buffer (pH 7.4) for 3 hrs. The shaved dorsal skin of albino rat (0.4±0.1 mm thickness and 1.70 cm^2 exposed surface area) was mounted on the receptor compartment with the stratum corneum side facing upwards towards the donor compartment. The receptor compartment was filled with 12.0 ml of pH 7.4 phosphate buffer maintained at 37°C and was constantly stirred by a magnetic stirrer at 600

rpm. Proniosomal gel formulation (1 g) was placed on the skin and the top of the diffusion cell was covered with paraffin paper. Samples (1 ml) were withdrawn through the sampling port of the diffusion cell at pre-determined time intervals (1, 2, 4, 6, 8, 12, 16, 20 and 24 hrs) and analyzed for drug content by HPLC. The receptor medium was immediately replenished with equal volume of fresh diffusion solution. Sink conditions were maintained throughout all the experiment. Triplicate experiments were conducted for each study.

HPLC analytical method

To each 0.5 ml sample, 0.5 ml of 2% zinc sulphate solution, a protein precipitant, was added and the mixture vortexed (Scientific Industries, Inc., NY) for 1 min and then centrifuged at 13000 rpm for 10 min. The supernatant was directly injected into the HPLC system. The MLX content of these various samples was analyzed by a modified literature HPLC method[20]. The HPLC system consisted of a Waters pump Model 501, Waters, U6k injector (Waters Inc., Bedford, MA), a Waters model 481 variable UV detector. The detector wavelength was set at 354 nm. Separation was achieved by isocratic elution with a mobile phase consisting of 60:40 v/v water/acetonitrile with the pH adjusted to pH 4.5 using glacial acetic acid, delivered at a flow rate of 1 ml/ min at ambient temperature through a μ-Bondapack C18 analytical column, 150x4.6 mm ID, 5 μm particle size (Waters Inc., Bedford, MA). The peak heights were recorded using data module model 746 (Waters Inc., Bedford, MA). The mobile phase used for the current assay provided good separation of MLX, with retention time of 6.8 min. The quantitation of the chromatograms was performed by injecting various concentrations of MLX (1.0-10.0 ug/ml) samples and the obtained peak heights were plotted against MLX concentrations. Each point on the standard curve was based on 3 determinations. Regression analysis of the mean values resulted in the following equation: $Y= 1.23 x - 0.1$ and $r = 0.998$.

By analysis of samples consisting the calibration curve on 3 separate days, the overall intraday and inter-day percent coefficient of variation (%CV) was 1.4 and 1.8 respectively. No interference was observed in samples of the permeability study.

Investigation of the anti-inflammatory efficacy of selected formulation of the prepared gels

The anti-inflammatory efficacy of formulation S60,and the standared gel was evaluated. Male rats (5 per group) weighing 200±20 g were used for such evaluation. The rats in each group were selected so that the average body weight among groups was as close as possible. The rats were anaesthetized with urethane (intrapretonial). Oedema was induced by subcutaneous injection of 0.1 ml carrageenan suspension (1% w/v in pyrogen free saline solution) into the right subplanatar region[21]. One hour after induction of oedema, the first group of rats were treated with the proniosomal gel (1.0 g). The second group received 1.0 g of standard gel formula, the third group received 1g of HPMC plain gel (control). All gels were applied to the induced oedema area and rubbed for 20 seconds. The thickness of rat hind paw was measured by vernier caliper (SMEC, China) before carrageenan injection and after applying the gel at 0 (1 hr after carrageenan injection), and then after 1/2, 1,2,3, and 4 hrs. The percent swelling of the paw was calculated using the following equation[22]:

$$\% \text{ swelling} = (V-Vi \div Vi) \times 100 \quad \text{……... Eq. (2)}$$

Where:

V= The paw thickness at each time interval

Vi= The initial paw thickness (before carrageenan injection).

The average paw swelling in the treated rats was compared with that of control rats and the percent inhibition of oedema was calculated using the following equation.

$$\% \text{ inhibition} = 1 - \frac{\% \text{ Swelling of drug treated group}}{\% \text{ Swelling of control group}} \times 100 \quad \text{………… Eq (3)}$$

Statistical analysis

Data were expressed as the mean of three experiments ± the standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA), followed by Scheffe post-hoc tests using SPSS version 12. The $p< 0.05$ denoted statistical significance.

RESULTS AND DISCUSSION

The determined solubility of MLX in water is 0.012 mg/ml, indicating very low water solubility. As MLX has acidic nature, it is expected that the solubility increased in phosphate buffer pH 7.4. The obtained value is 0.66 mg/ml. To solve the solubility problem of MLX, sufficient amount of conc. ammonia was added to achieve the required solubility (5 mg/ml) during the preparation of standard or proniosomal gel.

The method of preparation of proniosomes is based on the simple idea that the mixture of surfactant: alcohol: aqueous phase can be used to form the concentrated proniosomal gel, which can spontaneously be converted to a stable niosomal dispersion by dilution with excess aqueous phase[19]. Meloxicam was successfully encapsulated within the proniosomal gel. HPMC gel was chosen in this study as a suitable polymeric gel vehicle due to the negligible effect on drug release rate from proniosomes when compared to other polymeric gel polymers; sodium carboxy-methylcellulose and carbopol 934, which resulted in an enhancement or retardation of drug release rate[23].

Encapsulation efficiency

As shown in table 2, niosomes formed from using Spans and Tweens exhibited high encapsulation efficiency. This could be explained on the basis that the highly lipophilic portion of the drug is expected to be housed almost completely within the lipid bilayer of the niosomes. Similar observations have been previously reported[24]. The results are also consistent with the high entrapment efficiency of levonorgestrel in proniosomes[12]. Most of the surfactants used to make non-ionic surfactant vesicles have a low aqueous solubility. However, freely soluble non-ionic surfactants such as Tweens can form micelles on hydration in the presence of cholesterol[11]. The Tween formulations in the present study were also able to entrap MLX efficiently. The increased entrapment efficiency of proniosomes prepared using Span 60 may be attributed to the increase in the availability of lipophilic ambience, which can accommodate the drug molecules to a higher extent. A similar observation was also noted with the antipsoriatic drug, dithranol[25].

Table 2: Encapsulation efficiency of Meloxicam in proniosomal formulations after dilution with phosphate buffer pH 7.4.

Formula	Encapsulation efficiency (%)
S20	91.40 ± 4.5
S60	95.20 ± 3.6
S80	92.3 ± 4.8
T20	81.5 ± 5.1
T60	82.4 ± 4.2
T80	87.6 ± 4.3

Each value represents the mean ± SD (n = 3).

Based on the encapsulation efficiency results, Span 60 and Tween 80 (showing higher encapsulation efficiency in both of Span or Tween series used) were selected for further studies.

Morphology and vesicle size of proniosomes

The scanning electron microscopy images of the niosomes prepared from Span 60 and Tween 80 are shown in figure 1.

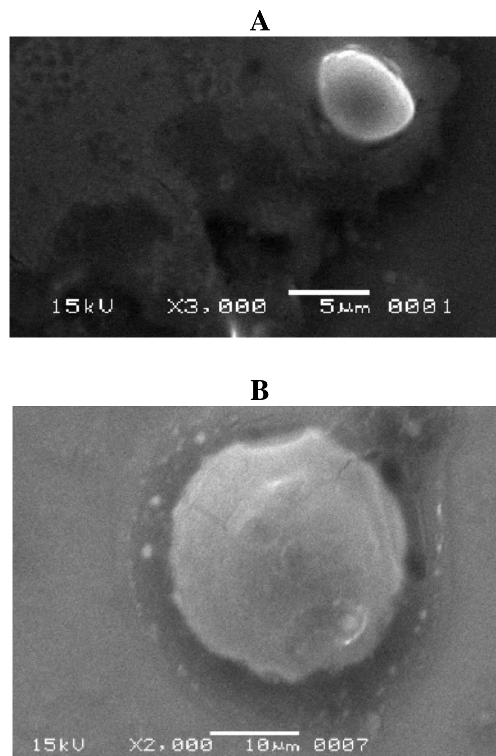

Fig. 1: Scanning electron microscopy (SEM) images of different proniosomal formulations: (A) formula S60, (B) formula T80.

The vesicles are spherical and discrete with sharp boundaries. The mean vesicle sizes of the niosomes formed from MLX proniosomal formulations are presented in table 3. Niosomes prepared with Tween 80 (Fig. 1b) were significantly larger ($p < 0.05$) than those prepared with Span 60 (Fig. 1a). In a previous study[26], the relationship observed between noisome size and Span hydrophobicity has been attributed to the decrease in surface energy, thus increasing hydrophobicity, resulted in smaller vesicles. This would also explain the larger vesicle size of niosomes prepared with Tween 80 which has a much lower hydrophobicity than does Span.

Table 3: Characterization of Meloxicam proniosomal formulations by vesicle size after dilution with phosphate buffer.

Proniosome code	Vesicle size (μm)
S60	5.82 ± 2.6
T80	28.20 ± 6.8

Each value represents the mean ± SD (n = 25).

In-vitro release study

Figure 2 shows the cumulative release profile of meloxicam from proniosomal gel formulations compared to its release from standard HPMC polymeric gel. It is obvious that there is a significant difference between the drug release rates from HPMC and proniosomal gels formulations in the release rate ($p < 0.05$). This could be explained on the basis of high lipophilicity characteristics of meloxicam which favors higher partition in the proniosmal gels. This concept could also explain the lower release rates observed from Span 60 formulation compared to Tween 80 formulation. This indicates that lipophilicity and hydrophilicity of surfactant has a main role in release rates of meloxicam. Similar results were previously reported[26]. The higher release rate obtained from the standard gel could be attributed to presence of MLX in the ammonium salt form.

In-vitro permeation study

Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the skin. On hydration, the produced nisomal suspension form micelles and can entrap solutes[7&8]. Several mechanisms could explain the ability of niosomes to modulate drug transfer across skin including: (i) adsorption and fusion of niosomes on to the surface of skin would facilitate drug permeation, (ii) the vesicles act as penetration enhancers to reduce the barrier properties of the stratum corneum and (iii) the lipid bilayers of niosomes act as a rate-limiting membrane barrier for drugs[27].

Fig:2. In-vitro release of Meloxicam from gel formulations

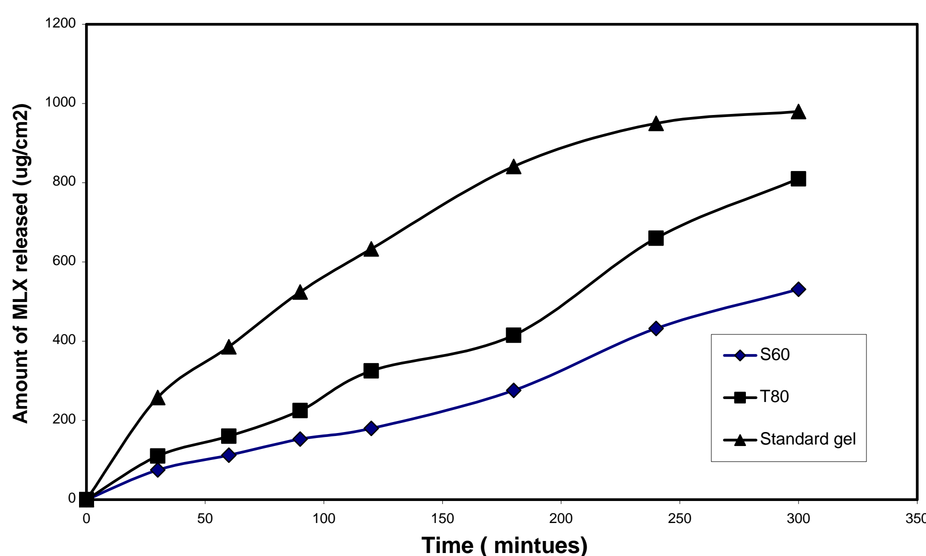

Fig. 2: *In-vitro* release of Meloxicam from gel formulations.

Figure 3 shows the permeation profile of MLX across excised rat skin from standard and proniosomal gel formulations. MLX of standard gel passed through the skin is significantly lower than MLX of proniosomal formulations prepared using non-ionic surfactants, suggesting that the lipid bilayers of niosomes act as a permeation enhancer for MLX across rat skin[19]. It is clear from tables 4 and 5 that the release rate of MLX across the cellulose membrane is significantly higher than its flux across the skin ($p < 0.05$), indicating the barrier properties of skin for the drug. One of the possible reasons for niosomes to enhance the permeability of drugs is structure modification of the skin[19]. It has been reported that the intercellular stratum corneum lipid barrier in the stratum corneum would be dramatically looser and more permeable following treatment with liposomes and niosomes[27&28]. Another explanation was that the niosomes vesicles in contact with stratum corneum aggregated and fused at the interface of the stratum corneum and a high local drug concentration in the bilayers generated a high thermodynamic activity of MLX in the upper part of the stratum corneum to the surface of skin results in, as demonstrated in a previous report[19], higher flux of the drug. Proniosomes prepared with Span 60 showed a higher enhancement effect (permeation rate) than those prepared with Tween 80. This was expected due to the smaller size of the vesicles and the higher lipophilic nature of vesicles prepared with Span 60, which makes it easier for the vesicles to penetrate or fuse with the skin. Similar observations were previously reported for piroxicam[26].

Table 4: Release rate of meloxicam through a cellophane membrane from different investigated formulations.

Formula	Release rate of MLX ($\mu g/cm^2/h$)
S60	100.20 ± 2.6
T80	163.5 ± 3.3
Standard gel	190.20 ± 2.9

Each value represents the mean ± SD (n = 3).

Vesicular system mechanisms have been explored using different vesicles models, soybean phosphatidylcholine, liposomes and Span 60 niosomes, to investigate the reasons for permeation enhancement[29]. It was found that permeation across Span 60-treated skin was significantly higher than that across soybean phosphatidylcholine and non-treated skin. Formulations treated with Span 60 were superior facilitating the permeation of enoxacin as well as drug deposition into the skin[29].

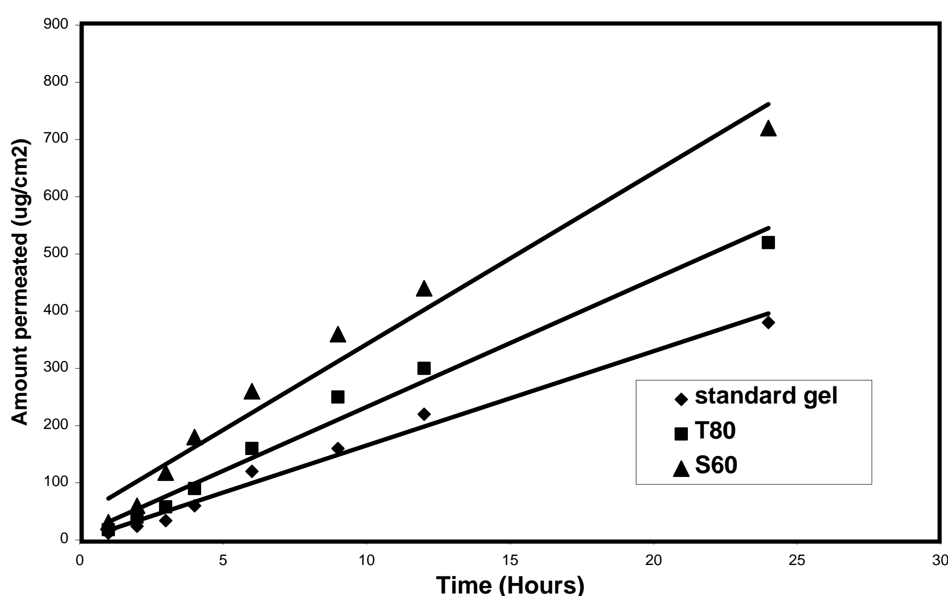

Fig. 3: Meloxicam permeation from gel formulations through rat skin.

Table 5: Permeation parameters for meloxicam from the investigated gel formulations across excised rat skin.

Formula	Permeation parameters				
	Cumulative amount ($\mu g/cm^2$)	Flux ($\mu g/cm^2/h$)	Permeability Coefficient (cm/h)	Lag Time (h)	ER
S60	720 ± 8.2	29.9 ± 2.9	6.0×10^{-3}	0.230	1.82
T80	520 ± 11	22.30 ± 2.6	4.46×10^{-3}	0.311	1.35
Standard gel	380 ± 4.5	16.47 ± 2.30	3.29×10^{-3}	0.580	1

ER = enhancement ratio, each value represents the mean ± SD (n = 3).

Fig: 4. Anti-inflammatory activity of Meloxicam gel formulations (presented as % inhibition of oedema)

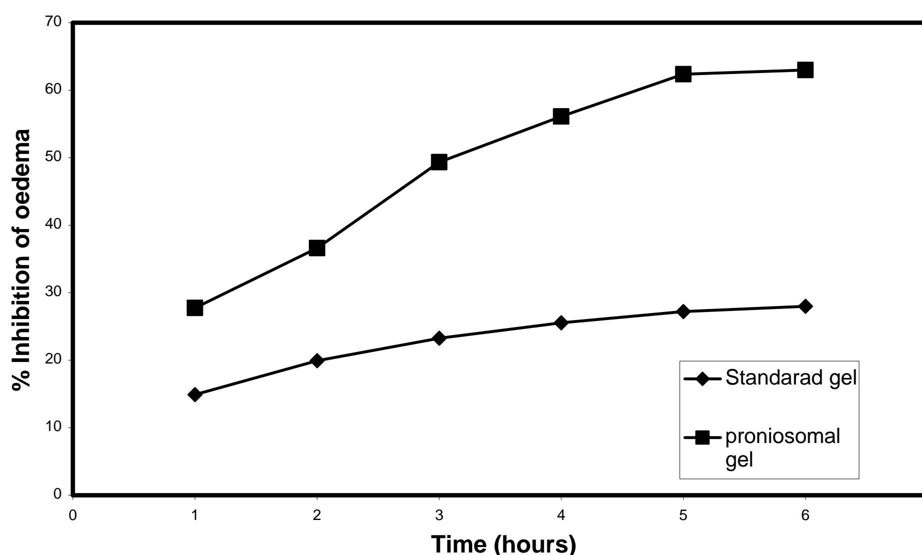

Fig. 4: Anti-inflammatory activity of Meloxicam gel formulations (presented as % inhibition of oedema).

Anti-inflammatory activity study

Based on the results of *in-vitro* permeation study, pronisomal MXL gel prepared with Span 60 which showed higher permeation rate was subjected to the anti-inflammatory activity test in comparison with standard MLX gel. In the anti-inflammatory activity test using carrageenean as phlogistic compound, pronisomal and standard MLX gel exhibited anti-inflammatory activity up to 6 hrs and peak activity was observed between 3-5 hrs for both formulations as shown in figure 4. Pronisomal MXL gel exhibited significantly higher anti-inflammatory activity compared to standard gel ($p< 0.05$). Pronisomal MLX gel application resulted in 25% inhibition at the end of 1 hr after application of the gel, and it further increase to 65% at 6 hrs. Inhibition produced by the application of standard gel was 10 and 29% after 1 and 6 hrs respectively (Fig. 4). The observed activity difference between pronisomal MLX gel and standard gel was around two–folds. The results confirm the fact that a significant amount of MLX was delivered from the gel through rat skin to induce the anti-inflammatory effect.

Conclusions

This paper has shown that meloxicam can be entrapped in niosomes with high efficiency using various compositions and types of non-ionic surfactants. The present study revealed that MLX-loaded proniosomes provided an enhanced transdermal flux, lower lag time and high entrapment efficiency. The meloxicam proniosomal gel showed better pharma-

cological activity when compared with the standard meloxicam gel. Thus leading to the conclusion that MLX-loaded proniosomes offers a suitable approach for transdermal delivery for meloxicam.

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