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ORIGINAL ARTICLE

Topical liquid crystalline gel containing lornoxicam/cyclodextrin complex

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Abstract Lornoxicam is a potent analgesic non-steroidal anti-inflammatory drug that can be used topically to relieve pain and to reduce inflammation. The objectives of this study were to improve the therapeutic efficacy of lornoxicam by complexation with cyclodextrins and to formulate it in liquid crystalline gel. Lornoxicam and β-cyclodextrin (βCD) or hydroxypropyl-β-cyclodextrin (HPβCD) complexes were prepared using the kneaded method in 1:1, 1:2, 1:3 and 1:4 drug:CD molar ratios. Inclusion complexation in aqueous solution and solid state was evaluated by the ultraviolet, phase solubility diagram, differential scanning calorimetry, X-ray diffractometry and Fourier-transform infrared spectroscopy. The stoichiometry for the inclusion complex was found to be 1:2 drug:CD molar ratio as determined from Job's plot. This result was confirmed by the in vitro dissolution studies for the prepared complexes. Among all the prepared complexes, the complex prepared with βCD in 1:2 drug:CD molar ratio showed highest improvement in drug dissolution and was chosen to be formulated in a topical preparation. For developing liquid crystalline gel, different ratios of Brij 97, glycerol and oils (liquid paraffin, isopropyl myristate and Miglyol® 812) were prepared. The formula composed of Brij 97 and glycerol in 3:1 weight ratio, 10% Miglyol® 812 and 40% water showed higher drug release compared to the other prepared gels. Moreover, this formula showed low ex vivo permeation on excised pigskin thus it could offer high topical effect with low systematic side effects. This formula showed superior anti-inflammatory activity when applied topically on rats' skin after induction of burn compared to that of Feldene® gel.

Keywords Lornoxicam · Cyclodextrin · Complexation · Physiochemical characterization · Dissolution · Liquid crystalline gel · Topical · Burn

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Introduction

Inflammation is a general, non-specific reaction to foreign particles and other noxious stimuli such as toxins and pathogens [1]. Characteristics of the inflammatory response include redness, swelling, pain and heat which are localized at the site of infection [2]. Inflammation may occur due to burns, chemical irritants, infection by pathogens, physical injury, immune reactions due to hypersensitivity, ionizing radiation and foreign bodies [3].

Sunlight is composed of a continuous spectrum of electromagnetic radiation that is divided into three main regions of wavelengths: ultraviolet (200–400 nm), visible (400–700 nm) and infrared (more than 700 nm). Ultraviolet (UV) radiation is divided into three sections, each of which has distinct biological effects: UVA (320–400 nm), UVB (280–320 nm) and UVC (200–280 nm). UVA and UVB radiation both reach the Earth's surface in amounts

sufficient to have important biological consequences to the skin and eyes. Wavelengths in the UVB region of the solar spectrum are absorbed into the skin, producing erythema, burns and eventually skin cancer [4, 5].

Management of inflammatory disorders involves a stepwise approach to the use of therapeutic agents. Relieving of pain and reduction of inflammation are urgent goals to reduce the severity of symptoms [6]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used therapeutics, primarily for the treatment of pain and inflammation. NSAIDs inhibit cyclooxygenase and hence decreases prostaglandin synthesis, reduces UVB-induced erythema when given orally or topically. NSAIDs are therefore used therapeutically for relief of sunburn symptoms [7, 8].

Lornoxicam ($C_{13}H_{10}ClN_3O_4S_2$; 6-chloro-4-hydroxy-2-methyl-*N*-2-pyridyl-2H thieno [2, 3-*e*]-1, 2-thiazine-3-carbox- amide-1,2-dioxide) is a potent analgesic drug with excellent anti-inflammatory properties in range of painful and/or inflammatory conditions. The analgesic effect of 16 mg lornoxicam (IM) is comparable with that of 20 mg morphine (IM) or 50 mg tramadol (IV) [9, 10].

The limited aqueous solubility of lornoxicam causes large variations in its bioavailability. The approach of complexation has been frequently used to increase the aqueous solubility and dissolution rate of water insoluble and slightly soluble drugs in an effort to increase their bioavailability [11]. Generally speaking, cyclodextrins (CDs) are potential carriers for achieving such objectives.

CDs are cyclic oligosaccharides consist of (α-1,4)-linked α-D-glucopyranose units and contain a lipophilic central cavity and a hydrophilic outer surface. The natural *β*-cyclodextrin (*β*CD) consists of seven glucopyranose units [12] and it is the most accessible, the lowest-priced and, generally, the most useful CD [13]. *β*CD molecules form intramolecular hydrogen bonds that diminish their ability to form hydrogen bonds with the surrounding water molecules [14]. Therefore, a much more water-soluble derivative as 2-hydroxypropyl derivative was introduced. The main reason for the solubility enhancement in the alkyl derivatives is that chemical manipulation transforms the crystalline *β*CD into amorphous mixtures of isomeric derivatives [15, 16]. The hydroxypropyl-*β*CD (HP*β*CD) is considered non-toxic at low to moderate oral and intravenous doses [17, 18] and more toxicologically benign than the natural *β*CD [19, 20]. CDs are able to form noncovalent inclusion complexes with many drugs by taking up a drug molecule, or more frequently some lipophilic moiety of the molecule, into the central cavity. The complexation can be used, for example, to increase aqueous solubility, dissolution rate, and bioavailability, and to decrease local irritation and increase the stability of drugs [16, 17, 21].

Lornoxicam shows a distinct pH-dependent solubility characterized by very poor solubility in the acidic conditions of the stomach [22]. Thus, it remains in contact with the stomach wall for a long period which might lead to local irritation and ulceration [23]. Therefore, there is a great interest to develop it in a topical dosage form to avoid the oral side effects of lornoxicam and to provide relatively high drug level at the application site.

Consequently, the rationale of this study was to improve the biological performance of lornoxicam utilizing the approach of inclusion complexation of the drug in cyclodextrins to enhance its solubility and dissolution and to design a new topical formulation for lornoxicam characterized by high therapeutic efficacy. This would be achieved by utilizing liquid crystalline gels as new lornoxicam topical delivery system.

Experimental

Materials

Lornoxicam was kindly donated by Chemipharm Company, Sixth Industrial Area, 6th of October City, Egypt. *β*-cyclodextrin (*β*CD, MW 1135), hydroxypropyl-*β*-cyclodextrin (HP*β*CD, MW 1380), isopropyl myristate (IPM) and Brij®97 were obtained from Sigma-Aldrich Chemical Company, St. Louis, USA. Glycerin 99.5% was obtained from TVV, Cairo, Egypt. Liquid paraffin and absolute ethanol were purchased from El-Nasr Pharmaceutical Co. for Chemicals, Cairo, Egypt. Miglyol® 812 (caprylic/capric triglyceride) was kindly provided by Sasol Germany GmbH, Witten, Germany. Feldene® gel was purchased from Pfizer International Pharmaceutical Industries Co., Cairo, Egypt.

Methods

Investigation of the potentiality of complex formation between lornoxicam and cyclodextrins

Differential spectra of aqueous solutions of 0.1 mM/L lornoxicam in presence of different concentrations of cyclodextrins (5–20 mM/L) were determined at wavelength range from 200 to 700 nm using Shimadzu UV spectrophotometer (2401/PC, Japan). The investigated cyclodextrins were *β*CD and HP*β*CD.

Elucidation of the stoichiometric ratio of lornoxicam–cyclodextrin complexes using the continuous variation technique (Job's method)

The continuous variation method described by Job [24] was utilized to determine the stoichiometric ratio of lornoxicam–cyclodextrin complexes through spectrophotometric measurements.

From two equimolar solutions of lornoxicam and each cyclodextrin, the following series of dilutions were prepared and their absorbance was measured at 378.5 nm:

a- Mixtures of the drug and cyclodextrins of different molar ratios and of the same total molar concentration (0.072 mM).
b- Solutions of lornoxicam, corresponding to those in (a), but without the addition of cyclodextrin and completed to the same final volume.
c- Solutions of cyclodextrin, corresponding to those in (a), but without the addition of the drug and completed to the same final volume.

Effect of cyclodextrins on the solubility of lornoxicam

The effect of βCD and HPβCD on the solubility of lornoxicam was investigated according to the phase solubility technique established by Higuchi and Connors [25]. Excess amounts of lornoxicam were added to 5 mL of aqueous solutions containing increasing concentrations of the previously mentioned CDs in glass vials. The concentrations of CDs in solution ranged from 0 to 20 mM/L. The obtained suspensions were shaken at 25 ± 0.5 °C for 3 days to attain equilibrium. Aliquots were then withdrawn and filtered through a cellulose filter (Millipore® filter 0.45 μm) and their lornoxicam content was assayed spectrophotometrically after appropriate dilution against blank solutions containing the same concentration of CDs at 378.5 nm. Each experiment was carried out in triplicate.

Preparation of lornoxicam–cyclodextrin systems

Drug–CD physical mixtures Physical systems were prepared by homogenous blending of lornoxicam and cyclodextrin in 1:1, 1:2, 1:3 and 1:4 drug: CD molar ratio.

Drug–CD complexes Inclusion complexes of lornoxicam in cyclodextrins under investigation (βCD and HPβCD) were prepared by the kneading method [26], whereby lornoxicam was added to cyclodextrin in 1:1, 1:2, 1:3 and 1:4 drug:CD molar ratio, kneaded thoroughly with least amount of water to obtain a paste which was then dried under vacuum at room temperature in presence of phosphorous pentoxide as a drying agent.

Characterization of lornoxicam–cyclodextrin complexes

Different techniques were used to characterize the prepared complexes and to trace the changes in the physicochemical properties of the drug as crystallinity and chemical interactions. These comprise differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffractometry (XRD).

Differential scanning calorimetry (DSC) The thermal behaviour of lornoxicam, cyclodextrins and lornoxicam–cyclodextrin physical mixtures and complexes were traced using a Shimadzu differential scanning calorimeter (DSC-50, Shimadzu, Japan). The thermograms were obtained by heating the sample in an atmosphere of nitrogen in a temperature range of 20–400 °C at constant heating rate of 10 °C/min.

Fourier-transform infrared spectroscopy (FT-IR) Samples of lornoxicam, cyclodextrins and lornoxicam–cyclodextrin physical mixtures and complexes were mixed with potassium bromide powder and then compressed into discs. The samples were monitored as KBr discs in the range of 4000–500 cm^{-1} at room temperature using a Shimadzu 435 U-O4 IR spectrophotometer, Japan.

X-ray diffractometry (XRD) X-ray diffraction patterns of lornoxicam, cyclodextrins and lornoxicam–cyclodextrin physical mixtures and complexes were obtained using a Diano X-ray diffractometer equipped with Cu Kα. The tube was operated at 45 kV, 9 mA. The scanning rate employed was 2°/min over diffraction angle (2θ) range of 3–70°.

In vitro dissolution studies

Dissolution of lornoxicam or lornoxicam-CD systems was assessed at 32 ± 0.5 °C by the USP Dissolution Tester Type II (SR8 PLUS, Hanson dissolution tester, USA) using 900 mL of phosphate buffer solution (pH = 5.5) as the dissolution medium and at a rotation rate 50 rpm. Aliquots, each of 5 mL from the dissolution medium were withdrawn at different time intervals and replenished by an equal volume of fresh dissolution medium. The samples were withdrawn through sintered glass filter and analyzed for lornoxicam content by measuring its absorbance at 376.5 nm using phosphate buffer solution (pH = 5.5) as a blank. Triplicate experiments were carried out for each dissolution study. Dissolution efficiency (DE) was computed by calculating the AUC values using the trapezoidal method. It is expressed as a percentage of the area of the rectangle corresponding to 100% release for the same total time (6 h) [27].

Preparation of liquid crystalline gel (LCG)

The LCGs were produced by heating the mixture of the oil, glycerol and Brij® 97 at 80 °C. Distilled water was heated up to the same temperature and was added with constant

stirring at 500 rpm. Stirring was continued until the mixture cooled down to room temperature [28, 29].

Characterization of LCGs

Physical properties The appearance and other physical properties, including clarity and precipitation of the prepared LCGs were inspected.

Light microscopy The crystal shape in the LCGs was examined using Olympus BX 50 light microscope (Japan).

pH – value The pH – value of the prepared LCGs bases was measured at 25 °C using Digital pH meter, JENWAY 350, UK.

In vitro release studies

The prepared gels were medicated with lornoxicam–CD complexes, where the amount of complex was adjusted in order to maintain the drug concentration in the gel at 5%. The medicated gel was spread on the surface of a watch glass of 10 cm^2 surface area. The release studies were carried out according to the USP paddle method [30], using 900 mL phosphate buffer (pH 5.5) at 50 rpm and a temperature of 32 °C (SR8 PLUS, Hanson dissolution tester, USA). Aliquots of 5 mL were withdrawn from the release medium at predetermined time interval and replaced by equivalent amounts of the buffer solution. Each experiment was carried out in triplicate. The amount of drug released from the bases was determined spectrophotometrically at 376.5 nm.

To elucidate the mechanism by which drug is released from the prepared bases, the data were analyzed to investigate the best fit to distinct kinetic model (zero, first, and Higuchi order). This was done graphically by plotting mean cumulative amount of drug released versus time, log mean cumulative amount of drug released versus time and mean cumulative amount of drug released versus square root time, respectively and determining the linearity coefficient (R^2) in each case. Release efficiency (RE) was computed by calculating the AUC values using the trapezoidal method. It is expressed as a percentage of the area of the rectangle corresponding to 100% release for the same total time (6 h) [27].

Ex vivo permeation studies

The ex vivo permeation studies were conducted across hairless abdominal pigskin. The abdominal hair of male pig was removed carefully using electric razors. After the animals were sacrificed, the abdominal skin was excised and the adhering fat eliminated. The whole skin was equilibrated in ethanol-phosphate buffer solution (pH 7.4, the human blood pH) for 1 h before the experiment. This membrane was mounted on a vertical Franz diffusion cell with the dermis facing the receptor compartment. The donor side was charged with 0.5 gm of the investigated preparation containing 5% lornoxicam. The membrane surface area available for diffusion was 3.14 cm^2. The receptor compartment was filled with 30% ethanol-phosphate buffer to maintain the sink conditions [31]. Temperature was maintained at 37 ± 0.5 °C to simulate human blood temperature. The receptor compartment was constantly stirred at 300 rpm [32].

Samples of the receptor fluid (2 mL) were withdrawn at various time intervals up to 24 h and replaced immediately by fresh buffer solution to maintain the "sink" conditions and a constant volume as well. The samples were then assayed spectrophotometrically at 378.5 nm.

Assessment of anti-inflammatory effect

Exposure to ultraviolet radiation Seventy-two male albino rats aged 12 weeks and weighing between 200 and 240 g were randomly selected and divided into four groups. Group I was used as a negative control, which did not receive any topical medication but it was exposed to UV radiations. Group II was exposed to UV radiations and received topical market product (Feldene® gel). Group III and IV were exposed to UV radiations and received topical placebo of LCG (positive control) and topical lornoxicam LCG, respectively.

First, a skin area on the backs of the rats 3 × 2 cm in size was shaved gently with a razor blade. Twenty-four hours later, the rats were anaesthetized with ketamine 5 mg/kg and xylazine 2 mg/kg via intraperitoneal route. The minimal erythemal dose (MED) of UV radiation for the hairless rat was approximately 30 J/cm^2 [33]; rats were exposed to 60 J/cm^2 (equivalent to 2.0 MED). One MED is defined as the amount of UV radiation necessary to cause a slight reddening of the skin 24 h after exposure.

UV radiation exposure was performed by placing the rats on the floor of the UV radiation cabinet. Food and water were removed during the UV exposure. Rats were irradiated with UVB cabinet (UV lamp, 285–320 nm, Phillips 8 V, France). The lamp was calibrated against Eldonet dosimeter 081 (Germany). The intensity used in the experiments was 1450 μw/cm^2**.** The source to skin distance was 10 cm and the irradiation times were calculated accordingly. The formulations (5 mg/cm^2) were applied to each patch of back skin at 0.5, 24, 48 and 72 h after the UV exposure.

Histological study of sunburned skin Standard 6-mm punch biopsy specimens were obtained from the backs of

six rats after 24, 48 and 72 h of exposure to UV radiations. The specimens were fixed with 10% formalin and embedded in paraffin. For histological examinations, 10 μm thin sections were stained with haematoxylin and eosin. Ten sections were graded for each rat. Slides were examined under Olympus BX 50 light microscope (Japan).

The severity of skin inflammation after UV exposure observed in the histopathological examination was assigned using scores on a scale of none (0), mild (1), moderate (2), and severe (3) inflammation, depending on the number of the inflammatory cells localized in the upper and lower epidermis layer and on the density of leucocyte infiltration localized in both the dermis and epidermis.

Statistical analysis

Data were collected and coded prior to analysis. All data were expressed as mean ± SD. Unpaired t-test or one way analysis of variance test (ANOVA) followed by the least significant difference test (LSD) were performed to compare two or more groups. Statistical analysis was performed using SPSS® software, USA.

Results and Discussion

Investigation of the potentiality of complex formation between lornoxicam and cyclodextrins

Differential scanning ultraviolet spectrophotometry was utilized to investigate the possibility of molecular interaction between lornoxicam and CDs. Figures 1 and 2 show that the UV absorbance of lornoxicam was remarkably altered by increasing βCD and HPβCD concentration, showing hypochromic shift. These data might suggest the possibility of an interaction between lornoxicam and the investigated CDs. The changes in the extinction coefficient of the drug in presence of the investigated cyclodextrins may be attributed to depletion of the guest molecule and formation of drug-cyclodextrin complexes [34].

Elucidation of the stoichiometric ratio of lornoxicam–cyclodextrin complexes using continuous variation technique (Job's method)

The absorbance values for solutions of fixed total concentration (0.072 mM/L) containing different mole fractions of lornoxicam and βCD or HPβCD were measured at 378.5 nm. The measured values were found to be dissimilar to the calculated ones. This can be considered as a further evidence for complex formation between lornoxicam and these cyclodextrins. Plotting the differences between the calculated and the measured absorbance values versus mole fraction (Job's plot) reveals molar ratio at which complexation takes place from the point of inflection of the produced curve [24]. Figure 3 shows a break or change in slope at drug mole fraction of 0.33 indicating formation of 1:2 drug:CD complex for both βCD and HPβCD.

Effect of cyclodextrins on the solubility of lornoxicam

The phase solubility diagrams of lornoxicam with βCD and HPβCD at 25 °C are graphically illustrated in Fig. 4. It is evident that the solubility of lornoxicam increased linearly as a function of increasing CD concentration (within the studied concentration range) for the investigated CDs. The solubilizing power of the investigated cyclodextrins towards the drug calculated as mole lornoxicam solubilized per 1 mol of cyclodextrin was 0.00086 and 0.00229 for βCD and HPβCD, respectively. This result revealed that the solubilizing power of HPβCD is higher than that of βCD.

The values of the stability constants ($K_{1:1}$) calculated from the equation of Higuchi and Connors [25] were found to be 7.023×10^{-6} and 1.755×10^{-5} M^{-1} for βCD and HPβCD, respectively. But the use of phase-solubility is not a reliable method for determination of stability constant ($K_{1:1}$) and error increases with decreasing drug solubility, especially for drugs with solubility <1 mg/mL [35]. Therefore, complexation efficiency (CE) for the investigated CDs was also computed [36] and found to be 8.006×10^{-7} and 2.001×10^{-6} for βCD and HPβCD, respectively. CE of HPβCD is higher than that of βCD indicating that the cavity of HPβCD fits the lornoxicam molecule more comfortably [37].

The calculated higher values of solubilizing power, $K_{1:1}$ and CE obtained for HPβCD compared to those of βCD indicate that lornoxicam interacts more strongly with HPβCD. This might be attributed to that one of the key factors that influence the ability of a cyclodextrin to form an inclusion complex with a guest molecule is steric and it depends on the relative size of the cyclodextrin cavity to the size of the guest molecule [38]. In case of HPβCD, the cavity of βCD is extended by the HP substitution [39], which enhanced the complex forming ability of the drug into the CD cavity.

As phase-solubility studies are performed in aqueous solutions saturated with the drug, it is important to remember that this technique does not indicate whether a given drug forms inclusion complex with cyclodextrin, but only how the cyclodextrin influences the drug solubility [40]. Thus, although correlation is often found between phase-solubility diagrams and the stoichiometry of drug/cyclodextrin complexes determined by other means such as NMR, Job's plots, and molecular modeling, some discrepancies can be found in the literature [37, 40–42].

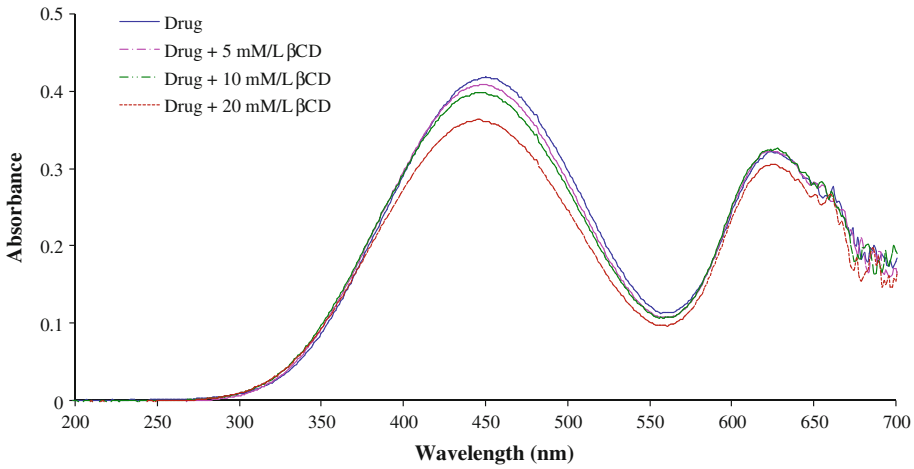

Fig. 1 Differential ultraviolet absorption spectrum of lornoxicam in presence of βCD

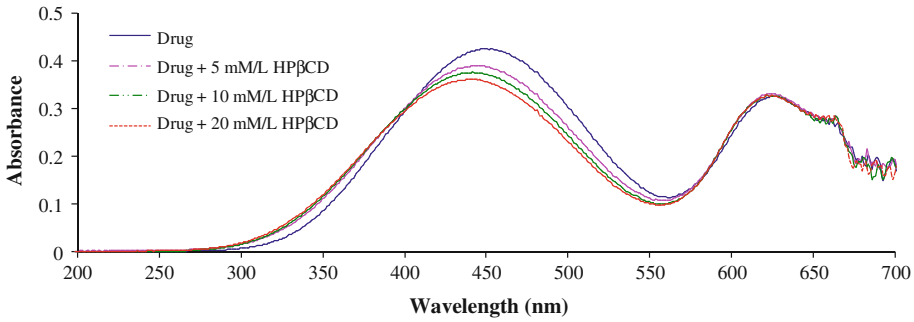

Fig. 2 Differential ultraviolet absorption spectrum of lornoxicam in presence of HPβCD

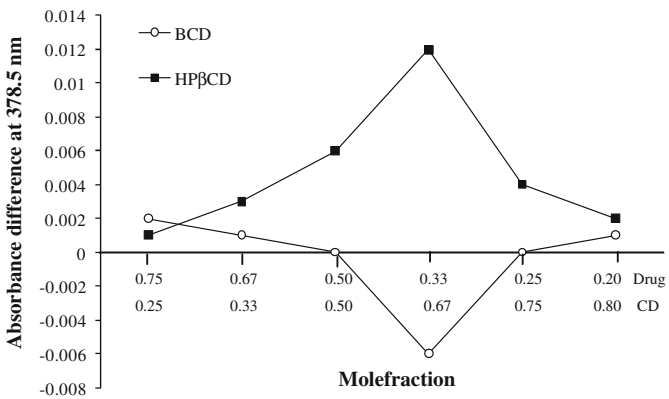

Fig. 3 Elucidation of the stoichiometric ratio of lornoxicam–CDs complexs spectrophotometrically

Characterization of lornoxicam–cyclodextrin complexes

Complexes of lornoxicam with cyclodextrins were examined by differential scanning calorimetry (DSC), Fourier-transform infrared spectroscopy (FT-IR) and X-ray diffractometry (XRD).

Differential scanning calorimetry (DSC)

The DSC thermograms of pure lornoxicam powder, pure CDs powder and their physical mixtures and complexes (Figs not shown) revealed that the DSC thermogram of pure lornoxicam was typical of a crystalline substance, exhibiting a sharp exothermic peak at 234.92 °C corresponding to its melting and decomposition.

In thermogram of βCD, two endothermic peaks were observed. The first peak was observed at 108.08 °C due to loss of water, while the second peak was found at 317 °C due to fusion and decomposition. HPβCD was characterized by the presence of an endothermic peak due to dehydration at 63.54 °C and a second one due to fusion and decomposition at 326.97 °C.

The thermograms of the physical mixtures of lornoxicam with either βCD or HPβCD showed the existence of the drug exothermic peak which might indicate the absence

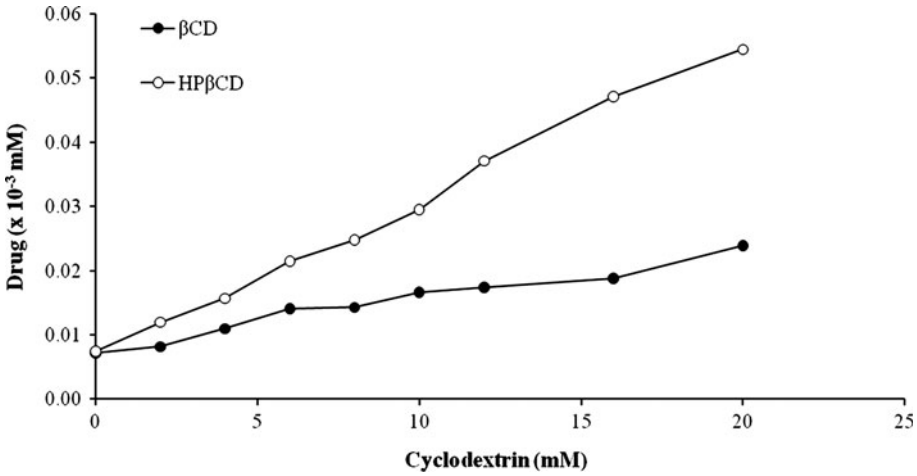

Fig. 4 Effect of cyclodextrins on the solubility of lornoxicam in water at 25 °C

of interaction between lornoxicam and CDs. However, a marked reduction in lornoxicam peak intensity was observed in the aforementioned systems and could be attributed to the low drug to CD molar ratio [43]. On the other hand, the drug-melting exothermic peak was recorded in the complexes prepared using either βCD or HPβCD, but with noticeable broadening and reduction in intensity which could be attributed to increase in the drug–CD interaction as a consequence of the more drastic mechanical treatment during kneading compared to physical mixing [44].

DSC behaviour can be interpreted assuming that mechanical treatment led to a highly dispersed, high-energy state of microcrystalline lornoxicam, which made it prone to interact with the cyclodextrin carrier by the supply of thermal energy during the DSC scan of kneading complexes [45]. Therefore, XRD and FT-IR were considered in conjunction with DSC analysis to reach a definite conclusion.

Fourier-transform infrared spectroscopy (FT-IR)

The IR spectra of pure lornoxicam, pure CDs, their physical mixtures and complexes were analysed (Figs not shown). The IR spectrum of lornoxicam showed the presence of the following peaks: at 3098.08 cm^{-1} due to the stretching vibration of the –NH group, at 1644.98 cm^{-1} due to the stretching vibration of the C = O group in the primary amide and at 1593.88 cm^{-1} due to the bending vibration of N–H group in the secondary amide. The stretching vibration of the O = S = O group appeared at 1148.40 cm^{-1}, aromatic CH stretching vibration appeared at 3064.33 cm^{-1} and the –CH aromatic ring bending appeared at 828.277 cm^{-1}.

The IR spectrum of βCD showed intense band due to O–H stretching vibrations at 3394.1 cm^{-1}. The stretching vibrations of –CH and $-CH_2$ groups appeared at 2925.48 cm^{-1}. The IR spectrum of HPβCD showed intense band due to O–H stretching vibrations at 3395.07 cm^{-1}. The stretching vibrations of –CH and $-CH_2$ groups appeared at 2929.34 cm^{-1}. A shorter band appeared in the region 1500–1200 cm^{-1} that could be ascribed to the hydrated bonds with CD molecules. Another large band assigned to the C–O–C stretching vibration occurred between 1200 and 1030 cm^{-1} [43, 46].

The intense peak due to N–H bending of lornoxicam present at 1593.88 cm^{-1} was the main characteristic band used to assess the drug–CDs interactions due to absence of overlapping between this peak and CD peaks.

The FTIR spectra of the investigated drug and βCD physical mixtures showed a shift in the characteristic N–H bending of lornoxicam. Likewise, generally, there was the same extent of shift in this characteristic peak of lornoxicam in the spectrum of their complexes with βCD. However, generally the same peak was shifted in the case of the lornoxicam–HPβCD complexes to higher frequency when compared to that of their corresponding physical mixture.

The FTIR spectra indicate that, although an interaction occurs with both cyclodextrins, the interaction is notably more intense with HPβCD than βCD. This is probably because HPβCD has a greater content of pendant hydroxyl groups, that are more accessible for establishing hydrogen bonds [47]. For βCD, the use of X-ray diffractometry seems to be necessary for such interaction to be detected.

X-ray diffractometry (XRD)

X-ray diffractometry is a useful method for the detection of inclusion complexation in powder. The diffraction pattern of lornoxicam powder revealed several sharp high intensity peaks at 24.744°, 21.575° and 23.013° (2θ), suggesting that it exists as a crystalline material. The XRD of βCD and HPβCD showed that βCD exhibit a crystalline diffractogram, while a diffuse halo pattern was recorded for HPβCD.

Table 1 Values of relative degree of crystallinity (RDC) for lornoxicam–cyclodextrins systems

Drug:CD molar ratio		RDC	
		Physical mixture	Complex
Lornoxicam–βCD	1:1	0.387	0.237
	1:2	0.294	0.233
	1:3	0.301	0.244
	1:4	0.241	0.155
Lornoxicam–HPβCD	1:1	0.393	0.500
	1:2	0.357	0.267
	1:3	0.315	0.282
	1:4	0.260	0.285

Crystallinity can be determined by comparing some representative peak heights in the diffraction patterns of the prepared systems with those of a reference. The relationship used for the calculation of crystallinity was relative degree of crystallinity (RDC). RDC = I_{sam}/I_{ref}, where I_{sam} is the peak height of the sample under investigation and I_{ref} is the peak height at the same angle for the reference with the highest intensity [48]. Pure drug peak at 24.744° (2θ) was used for calculating RDC-values for the systems. The RDC-values calculated for physical mixtures and complexes of lornoxicam-βCD and HPβCD are presented in Table 1.

Reduction in crystallinity was observed in the complexes with βCD compared to those of their physical mixtures showing reduction in RDC-values (Table 1) attributable to a new solid phase with low crystallinity indicating inclusion complex formation with βCD (more water soluble).

The diffractograms of the complexes of HPβCD showed that only the 1:2 drug-cyclodextrin complex shows an evident greater amorphousness compared to its corresponding physical mixture as revealed by its RDC-value (Table 1). The formation of mixed crystalline particles during the desiccation process might be the reason for the high RDC-values for the drug: HPβCD complexes in 1:1 and 1:4 molar ratio compared to their corresponding physical mixtures [49].

Effect of cyclodextrins on the dissolution of lornoxicam

Both the physical mixtures and complexes of lornoxicam with cyclodextrins exhibit higher dissolution efficiency ($P < 0.05$) compared to that of the free drug (Figs. 5, 6). The improvement of lornoxicam dissolution efficiency obtained with the physical mixtures and even more with kneaded products can be attributed to two factors [50, 51]: (1) local solubilizing action of the carrier, operating in the microenvironment on the hydrodynamic layer surrounding the drug particles, which improve lornoxicam wettability and/or solubility; (2) in situ formation of readily soluble complexes in the dissolution medium.

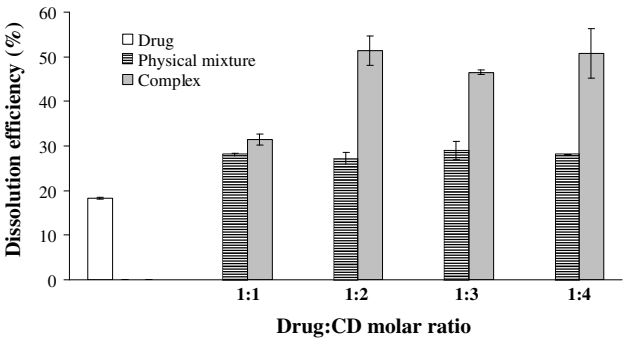

Fig. 5 Effect of βCD on the dissolution efficiency of lornoxicam

The effect of the CD type was also obvious on the dissolution of lornoxicam, where the physical systems prepared using HPβCD showed superior enhancement in lornoxicam dissolution compared with those prepared using the parent βCD. This could be explained on the basis of greater water solubility, better wetting ability and solubilizing power of HPβCD towards the drug in the solid state [52]. These results are in perfect agreement with the calculated values of $K_{1:1}$ and CE obtained for lornoxicam with the investigated CDs.

Lornoxicam–βCD complex showed generally higher dissolution efficiency-values than those obtained by the use of HPβCD as complexing agent. This behaviour may be due to the ability of βCD to reduce the drug crystallinity as shown by the X-ray studies.

All the lornoxicam–βCD complexes showed markedly higher drug dissolution efficiency-values than their corresponding physical mixtures (Fig. 5). This is a consequence of decreasing in drug crystallinity as provided by the X-ray study.

Figure 6 reveals that the kneaded products for lornoxicam–HPβCD showed approximately the same dissolution behavior of the physical mixtures (except 1:2 drug : HPβCD complex) which is in complete accordance with the X-ray characterization. The limited improvement effect of the kneading method is the direct result of employing a semisolid medium during the preparation, where the interactions between the drug and this cyclodextrin might be limited [44, 53].

It was found that by decreasing drug to cyclodextrin ratio from 1:1 to 1:2 there was a significant increase in the dissolution efficiency ($P < 0.05$) (Figs. 5 and 6). This increase in dissolution might also be due to the availability of more CD molecules in the hydrodynamic layer surrounding the drug to undergo in situ inclusion of drug molecules [43]. On the other hand, further decrease in this ratio to 1:3 or 1:4 did not show further significant increase

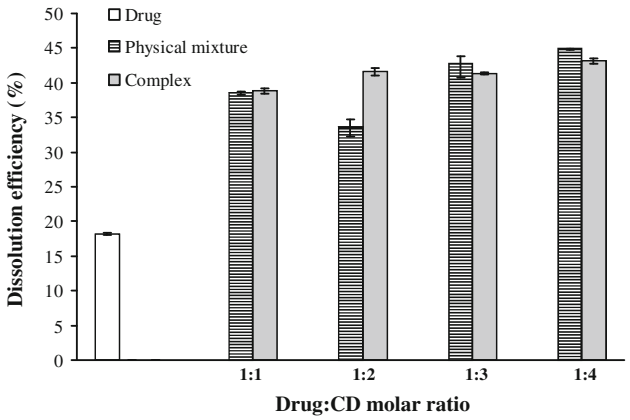

Fig. 6 Effect of HPβCD on the dissolution efficiency of lornoxicam

in the dissolution efficiency values ($P > 0.05$). These results confirm that the complex systems prepared at 1:2 (drug:CD) molar ratio showed the most superior and significant enhancement effect on the dissolution pattern of lornoxicam. These results run parallel with the stoichiometric ratio results obtained with Job's method.

The complex system prepared using βCD at 1:2 drug:CD molar ratio showed the most superior and significant enhancement effect on the dissolution pattern of lornoxicam with the highest release efficiency. Accordingly, lornoxicam–βCD complex at 1:2 drug:CD molar ratio was chosen for further incorporation in topical gel preparations.

Characterization of liquid crystalline gels (LCGs)

The design of new dosage forms that increase the effectiveness of the existing drugs is one of the trends observed in pharmaceutical technology [54]. In this context, liquid crystalline gels have aroused great interest as novel dosage forms due to their considerable solubilizing capacity for both oil and water soluble compounds [55]. It has been reported that the possibilities for the development of dermal systems are inherent in these systems due to their stability and similarity in skin structure [56].

Liquid crystalline gels were prepared using a mixture of lipophilic and hydrophilic vehicles (Table 2). In the presented study, liquid paraffin, isopropyl myristate and Miglyol® 812 were used as oil phase, Brij®97 was used as surfactant and glycerol representing cosurfactant in order to assist in achieving skin compatible composition. Previous studies reported that a more skin compliant composition can be achieved by decreasing the surfactant concentration using glycerol as a cosurfactant [56]. In this respect, different multicomponent LCGs with different surfactant : cosurfactant ratios were prepared characterized and assessed for their effect in in vitro drug release [56]. The nonionic surfactant (Brij®97) was used in order to increase the solubility of lornoxicam by means of solubilization [57–60].

Physical properties

All the prepared liquid crystalline gels showed a homogenous yellow coloured gels.

Light microscope

Light microscope was used to determine morphology of the crystals in the preparations. Figure 7 shows a needle shaped crystals in the LCG field. This shape of crystals was also found in a previous study [48].

pH-value

The pH-values of the prepared liquid crystal gels were within the acceptable range for non-irritant topical preparations [61, 62] (Table 3).

Table 2 Composition of the prepared liquid crystalline gels (%w/w)

Formula	Oil	Brij®97: glycerol	Brij®97	Glycerol	Oil	Distilled water
F1	Liquid paraffin	3:2	36.0	24.0	10	30
F2		2:1	40.0	20.0	10	30
F3		3:1	45.0	15.0	10	30
F4	Isopropyl myristate	3:2	36.0	24.0	10	30
F5		2:1	40.0	20.0	10	30
F6		3:1	45.0	15.0	10	30
F7	Miglyol® 812	3:2	36.0	24.0	10	30
F8		2:1	40.0	20.0	10	30
F9		3:1	45.0	15.0	10	30
F10		3:1	37.5	12.5	10	40
F11		3:1	37.5	12.5	20	30
F12		3:1	30.0	10.0	30	30
F13		3:1	30.0	10.0	10	50

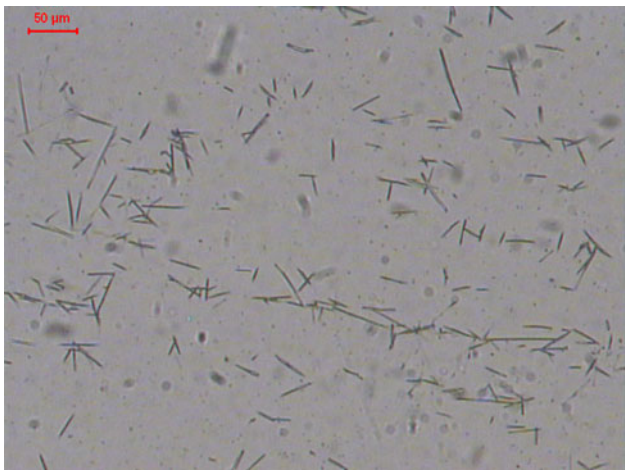

Fig. 7 Representative microscopic picture of LCG

Table 3 pH – values of liquid crystalline gels

Formula	pH
F1	5.34
F2	5.54
F3	5.21
F4	5.18
F5	5.27
F6	5.23
F7	6.11
F8	5.99
F9	6.09
F10	6.14

In vitro release studies

Ten multicomponent lornoxicam LCGs with different surfactant : cosurfactant ratios were prepared, characterized and assessed for their effect on in vitro drug release. The release of LCGs containing lornoxicam–βCD complex in 1:2 drug:CD molar ratio showed that LCGs could be best expressed by Higuchi's equation, where plots showed high linearity (R^2).

The rank order of the release efficiency from different systems was found to be generally in the following order: formulations containing Miglyol® 812 > liquid paraffin > isopropyl myristate (Fig. 8).

In LCGs prepared using liquid paraffin as oil phase, there was no significant increase in the release efficiency of lornoxicam by increasing surfactant concentration from 3:2 to 2:1 or 3:1 ($P > 0.05$) as F1, F2 & F3 showed release efficiency values of 69.3 –70.6%.

In LCGs prepared using IPM or Miglyol® 812 as oil phase, the formula F6 and F9 with high surfactant concentration (45%) showed the higher drug release efficiency.

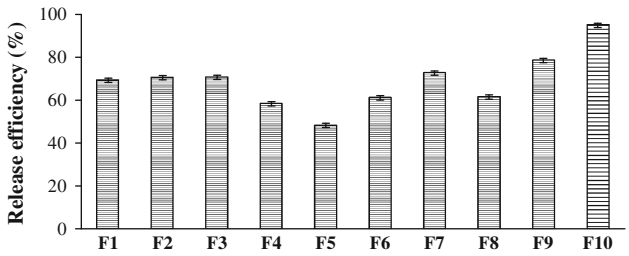

Fig. 8 Release efficiency of lornoxicam–βCD complex (1:2 molar ratio) from different liquid crystalline gels

This is probably due to increasing wettability and solubilization of the drug by Brij® 97. Also being an emulsifying agent, the surfactant acts at the oil–water interface, reducing the interfacial tension which can help the flow out of lornoxicam molecules from the interface to the medium [63, 64].

Comparing the release parameters of lornoxicam from the prepared LCGs, it was found that formula F9 containing drug-βCD complex showed the highest release efficiency. Thus, F9 was chosen for further modifications.

In an attempt to study the effect of water content on the release of lornoxicam from LCGs, it was found that by increasing the water content from 30 (F9) to 40% (F10), the amount of drug released increased significantly ($P < 0.05$). This maybe attributed to the decrease in the solubility of the drug in the vehicle during the water uptake leading to 'supersaturation', which results in an increase in thermodynamic activity of the drug in the oil phase [65]. Further increase in water content (more than 40%), showed phase separation of the prepared formulation (F13).

The oil content in F10 was increased from 10 to 30% (F11 and F12, respectively). The increase of oil content was combined with a decrease in surfactant/cosurfactant system content (Brij®97/glycerol) in F11 and F12 which resulted in decrease in the LCG stability as phase separation was observed.

On the basis of the previous results, F10 showed the higher lornoxicam release efficiency-value, therefore it was chosen for further investigations.

Ex vivo permeation studies

The permeation of lornoxicam from F10 through pigskin did not exceed 20% after 24 h. This low level of drug permeation is important when only a topical effect is required in order to avoid systemic side effects of the drug.

Assessment of anti-inflammatory effect

Exposure to UV radiation induces functional changes in both resident skin cells as well as infiltrating cells of the

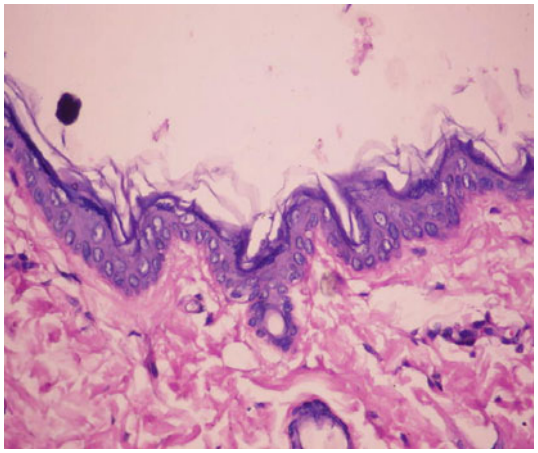

Fig. 9 Normal rat epidermis and dermis (haemotoxylin and eosin × 400)

immune system, which may ultimately contribute to the development of skin cancer [66–69]. Exposure to UVB light is initially associated with an inflammatory response characterized by increased blood flow and vascular permeability that results in edema and erythema, the infiltration of neutrophils into the dermis and the induction of pro-inflammatory cytokines [66, 70–72].

The acute UVB-induced inflammatory response in the skin is characterized by the rapid induction of COX-2 gene expression with the subsequent production of prostaglandins, including PGE2, resulting in dermal edema and the infiltration of activated neutrophils into the dermis [73].

The histopathology of normal skin which did not receive any topical medication or exposed to UV radiation (Fig. 9) showed normal epidermal and dermal layer with no leucocytes infiltration in the epidermis, dermis and in the epidermis and dermis junction.

The histopathological changes after UV exposure for the four groups are shown in Figs. 10, 11, 12 and 13. The Histopathology scores for the four groups after exposure to UV radiation were assessed**.**

The inflammation rating scores observed for group I (negative control) at 24, 48 and 72 h after UV exposure was 100% of severe inflammation (score level of 3). The rat skin showed severe inflammation with suppurative inflammation of superficial layer (epidermis) of the skin at 24 and 48 h after UV exposure. Furthermore, diffused necrosis and suppurative inflammation of the epidermis was observed at 72 h after UV exposure.

The scores of inflammation for group II (receiving market product) showed that 50% of the rat skin had zero score with no histopathological changes and 50% of the rat skin had score level of 1 showing mild inflammation where normal epidermis with few inflammatory leucocytes infiltration (mainly neutrophils) was observed in the dermis at 24 and 48 h after UV exposure. About 67% of the rats showed zero score with no histopathological changes while 33% of the rats showed moderate inflammation with suppurative inflammation of the epidermis at 72 h after UV exposure with inflammation score of 2.

The inflammation rating scores observed for group III (positive control) at 24, 48 and 72 h after UV exposure was 3 as 100% of the rats showed a severe inflammation of the skin at 24 and 48 h after UV exposure showing suppurative inflammation and infiltration of inflammatory cells in the epidermis and dermal junction. At 72 h after UV exposure, there was diffused necrosis and suppurative inflammation of the epidermis. This study demonstrates that placebo formulation (group III) of the liquid crystalline gel did not have any anti-inflammatory effect.

The scores of inflammation for group IV (receiving F10 of lornoxicam LCG) showed that 67% of the rat skin had zero score with no histopathological changes and 33% of the rat skin had score level of 2 with moderate inflammation showing suppurative inflammation of the epidermis at 24 h after UV exposure. On the other hand, 100% of the rat skin showed no histopathological changes at 48 and 72 h after UV exposure with inflammation score of 0.

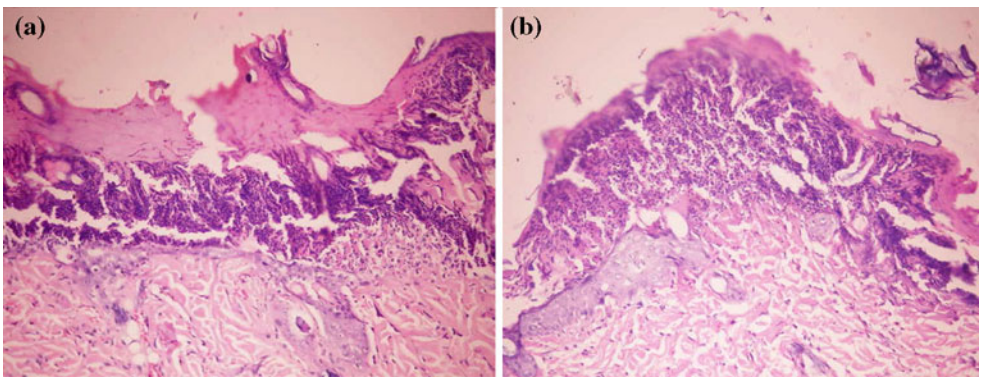

Fig. 10 Group I histopathological pictures; **a** suppurative inflammation of epidermis, **b** diffused necrosis and suppurative inflammation of epidermis (haemotoxylin and eosin × 200)

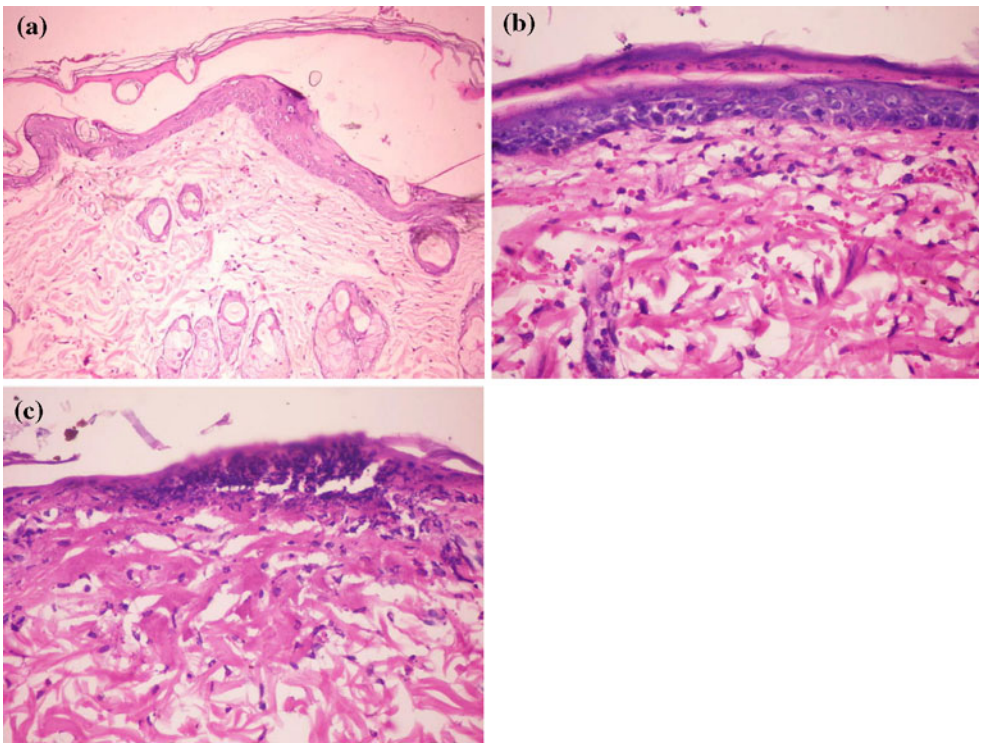

Fig. 11 Group II histopathological pictures; **a** no histopathological changes (haemotoxylin and eosin × 200), **b** normal epidermis and few inflammatory cells infiltration in the dermis (haemotoxylin and eosin × 400), **c** suppurative inflammation of epidermis (haemotoxylin and eosin × 200)

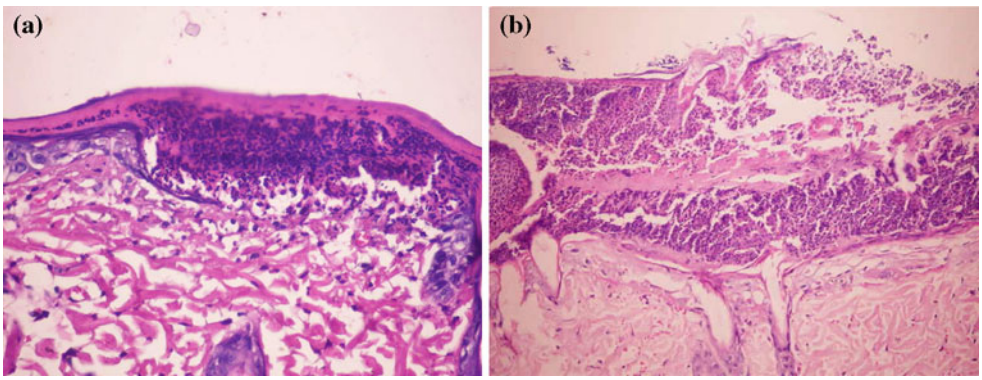

Fig. 12 Group III histopathological pictures; **a** suppurative inflammation of epidermis (haemotoxylin and eosin × 200), **b** diffused necrosis and suppurative inflammation of epidermis (haemotoxylin and eosin × 200)

Infiltration of inflammatory cells in the dermis at 24 h after UV exposure was found to be reduced in the group which received lornoxicam LCG (group IV), when compared to the group receiving the market product (group II). Furthermore, the histological examination, at 48 and 72 h after UV exposure, showed suppurative inflammation of the epidermis in group II which received the market product, while group IV which received lornoxicam LCG showed normal skin structure.

This data suggest that topical treatment with lornoxicam LCG decreased the number of neutrophils infiltrating in the dermis. A number of previous studies have reported that topical application of non selective NSAIDs are effective in inhibiting UVB mediated inflammatory responses [74, 75].

Conclusion

The present study confirms that complex system prepared using βCD at 1:2 lornoxicam:CD molar ratio showed the most superior and significant enhancement effect on the dissolution of lornoxicam. In addition, lornoxicam liquid

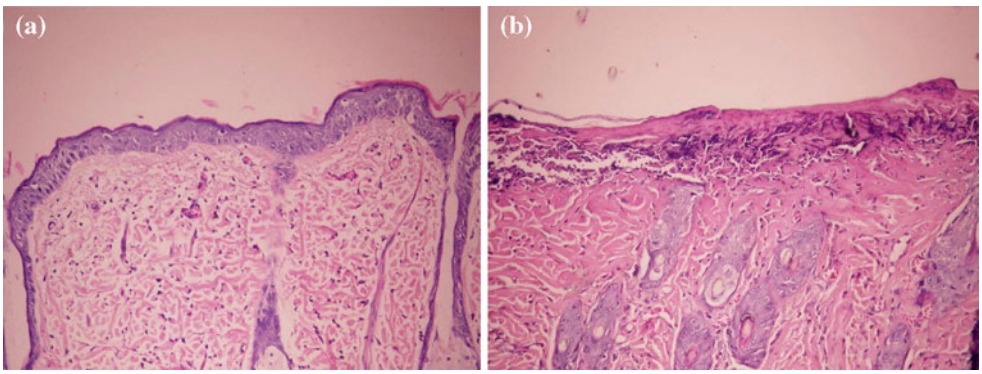

Fig. 13 Group IV histopathological pictures; **a** no histopathological changes, **b** suppurative inflammation of epidermis (haemotoxylin and eosin × 200)

crystalline gel composed of Brij® 97 and glycerol in 3:1 weight ratio, 10% Miglyol® 812 and 40% water showed promising anti-inflammatory activity when applied topically after UVB light exposure and suggested that topical application of NSAIDs may be effective in modulating the deleterious effects caused by acute and potentially long term UVB exposure.

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