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RESEARCH ARTICLE

In vitro and in vivo evaluation of optimized sustained release Ketoprofen niosome for once daily administration

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Abstract

The present work aimed to use niosomes for achieving sustained release of Ketoprofen in tablet form to be administered once daily. Ketoprofen niosomes were prepared by lipid hydration and formed from mixed surfactants (Span 20 and Span 60) and cholesterol in the molar ratio 0.66:1. The prepared niosomes were characterized for entrapment efficiency and particle size. After lyophilization, 185 mg of the dried niosomes (Equivalent to 100 mg Ketoprofen) was mixed with 282.5 mg of Avicel PH 101, 2.5 mg of Cab-o-Sil (0.5%), 25 mg of Starch (5%), and 5 mg of Magnesium stearate (1%). A single dose of the tested Ketoprofen niosomal tablets was orally administered to 12 healthy volunteers and the Ketoprofen plasma levels were compared with those obtained in the same subjects after administration of one dose of the commercial Ketofan® capsules. The results showed that the mean C_{max} values were 4.64 and 5.6 µg/ml and T_{max} values were 2 and 4 hr for the tested tablet formulation and the reference Ketofan® capsule respectively. During a 24 h period after administration, mean Ketoprofen plasma levels never fell to 1 µg/ml with the tested Ketoprofen tablet whereas with Ketofan® capsule they sometimes fell below the therapeutic threshold during the final 4 h. The percentage of relative bioavailability (RB %) for the tested tablet formulation was found to be 123.06 %. In conclusion, the new sustained release 100 mg Ketoprofen niosomal tablet formulation can ensure therapeutic Ketoprofen plasma levels for the entire 24 h period with a constant release of Ketoprofen guaranteeing the efficacy of once-daily 100 mg Ketoprofen administration.

Introduction

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID), widely used in order to reduce pain, inflammation and stiffness caused by several conditions such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis or abdominal cramps associated with menstruation. The mechanism of action of Ketoprofen is mainly associated to the inhibition of the body's ability to synthesize prostaglandins.The usual dose by mouth is 50–100 mg twice daily with food, whereas 200 mg commercial controlled release preparations may be administered once daily. With daily given conventional formulations, Ketoprofen is readily absorbed from the gastrointestinal (GI) tract and peak plasma concentrations occur within 0.5–2 h (Soliny et al., 2002). Therapeutic plasma concentration abruptly falls to very low levels (Martindale, 2006). Therefore, it should be frequently administered to maintain therapeutic 25 µg/ml which were much concentration and plasma peaks associated with conventional formulations result in increased incidence of side effects, therefore multiple daily administrations were needed (Roda et al., 2002). This problem tends to be serious for Ketoprofen with short half-life (Yamada et al., 2001).

The short half-life, low bioavailability and local or systemic disturbance in the GI tract to cause withdrawal of treatment make Ketoprofen a very good candidate for formulation of controlled release dosage forms (Palmieri et al., 2002). Among the various sustained release dosage forms, pharmaceutical industries prefer sustained release tablet dosage form because of the ease of production using the existing tablet manufacturing infrastructure *(*Bayomi et al., 2001; Giunchedi et al., 2000; Liew et al., 2006*).*

Various drug delivery techniques have been developed to sustain the release of drugs; recently colloidal particulate carriers such as niosomes have been employed in drug delivery systems (Shahiwala and Misra, 2002). Due to their capability to carry a variety of drugs, niosomes has been extensively used in various drug delivery systems like controlled release (Gupta et al., 2005; Puglia et al., 2004). Sustained release action of niosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation (Jain et al., 2006*).*

The aim of the present study was to use niosomes as a means of achieving sustained release of Ketoprofen in tablet form in a trial to guarantee therapeutic plasma Ketoprofen levels for at least 24 h, thereby, improving the patients' compliance by allowing once-daily oral administration. The new sustained release Ketoprofen niosomal tablet formulation and a commercially available sustained release formulation (Ketofan® 100 S.R. capsule) were compared for the in vitro dissolution kinetics and Ketoprofen plasma levels using high performance liquid chromatography (HPLC) with an ultraviolet detector. Pharmacokinetics parameters, including time to peak (t_{max}), maximum plasma concentration (C_{max}) and area under the curve (AUC) were calculated, and the results compared.

Materials and methods

Materials

Ketoprofen was kindly provided by El-Amyria Drug Company, Cairo, (Egypt); Span 20 and Span 60 from Sigma Chemical Co., Steinheim (Germany); Cholesterol from Sigma Chemical Co., St. Louis, MO, (USA); Sodium hydroxide and Potassium dihydrogen phosphate form El-Nasr Pharmaceutical Chemical Co., Cairo, (Egypt); Chloroform from Labscan Ltd, Dublin, (Ireland); Microcrystalline cellulose (Avicel® pH 101) from Morgan chemical Ind. Co., Cairo, (Egypt); Amorphous fumed silica (Cab-O-Sil® M-5P), Cabot Corporation, North America, (USA); Magnesium stearate, Prolabo (France); Starch BP 68 from EL Nasr Pharm. Chem. Co., Cairo (Egypt); Mannitol from Sigma Chemical Co., St. Louis, MO, (USA); Fenoprofen calcium salt by Sigma Chemical Co., St. Louis, MO, (USA); (HPLC)-grade acetonitrile by Carlo Erba, Milan, (Italy); Spectrapore® nitrocellulose membranes (MWCO 2000–15,000) were obtained from Spectrapore Inc., NY, (USA); Deionized water was purified by a Milli-Q System (Waters, Milford, MA, USA). All other chemicals and solvents were of analar grade and obtained fromEl-Nasr Company for Pharmaceutical Chemicals, Cairo, (Egypt).

2.2. Methods

2.2.1. Preparation of Ketoprofen niosomes

An optimization study was carried out using central composite design (Abdelaziz et al., 2013) which can be summarized as follow; Three different variables include: surfactant cholesterol ratio (X_1), Hydrophilic Lipophilic Balance (HLB) (X_2), and total lipid concentration (X_3) were screened using central composite design and sixteen different formulae of Ketoprofen niosomes were obtained. Optimization was performed to obtain the levels of X_1, X_2 and X_3, as tabulated in table (1), which give the optimum values of entrapment efficiency and in vitro release.

The optimized formula of Ketoprofen niosomes was prepared by lipid hydration method, the lipid mixture (34.18 %) of mixed Span 20 and Span 60 surfactants (required HLB = 7.86) and cholesterol (at 0.66:1 ratio) was dissolved in 15 ml of chloroform. The solvent was evaporated by Rotavapor (Buchi R-200, Switzerland) at speed (120 rpm), under low pressure at 60°C, which is above the gel–liquid transition temperature (T°c) of Span surfactants (Abbas et al., 2007; Azeem et al., 2008). Niosomes were formed by adding phosphate buffered solution, PBS (pH 7.4) containing 2.5 % concentration of Ketoprofen slowly to the dried thin film formed on the walls of the round-bottom flask, with gentle agitation. The resulting Niosomal suspension was sonicated (Ning et al., 2005; Tejas et al., 2002; Hao et al., 2002) using a probe sonicator (Model 275T, Crest Ultrasonic Corp, New York, USA), 20-kHz, and 500-W vibra cell at 1-min intervals for a period of 15 min.

Characterization of Ketoprofen niosomes

Determination of entrapment efficiency of Ketoprofen in niosomes

3 ml sample of Ketoprofen niosomes was frozen for 24 h at −20 ◦C in Eppendorf tubes. The frozen samples were removed from the freezer and let to thaw at room temperature, then centrifuged (Biofuge, primo Heraeus, Germany) at 15,000 rpm for 45 min at 4 ◦C (Jaleh et al., 2003). . Niosomal pellets were resuspended in phosphate buffer (pH 7.4) and then centrifuged again. This washing procedure was repeated two times to ensure that the unentrapped drug was no longer present in the void volume between the niosomes. The supernatant was separated, diluted to 100 ml with PBS pH 7.4, filtered using a membrane filter (0.2 µm pore size), and measured using ultraviolet spectrophotometer (Jenway 6305 uv/vis, UK) at 262 nm (Ibrahim et al., 2005). The percentage of drug encapsulation (EP (%)) was calculated by the following equation: EP %=[(C_t-C_r\C_t)] ×100% where C_t is the concentration of total Ketoprofen and C_r is the concentration of free Ketoprofen. Each result was the mean of three determinations (±S.D.).

Microscopic examination of Ketoprofen niosomes

A sample drop was diluted 10-fold using de-ionized water and a drop of this diluted dispersion was applied to a collodion-coated 300 mesh copper grid and left for 5 min to allow some of the niosomes to adhere to collodion. The remaining dispersion was removed by adsorbing the drop with the corner of a piece of filter paper. A drop of 2% aqueous solution of uranyl acetate was applied for 1 min. The remaining solution was then removed and the sample was air dried and examined with JEOL transmission electron microscope (JTEM model 1010, Japan).

Size, size distribution and zeta potential

The niosomes size, distribution and zeta potential were detemined by dynamic light scattering (DLS) analysis using NicompT 380ZLS zeta potential/particle size analyzer (Particle Sizing System Santa Barbara, CA, USA) with He–Ne laser at 632.8 nm.

Preparation of sustained release Ketoprofen niosomal tablet formulation

Abdelaziz et al (2011) have studied the effect of type of diluent and concentration of disintegrant on physicochemical properties of sustained release Ketoprofen niosomal tablets obtained by direct compression and the optimum formula composed of 185 mg of the dried niosomes (Equivalent to 100 mg Ketoprofen), 282.5 mg of Avicel PH 101, 2.5 mg of Cab-o-Sil (0.5%), 25 mg of Starch (5%), and 5 mg of Magnesium stearate (1%). The formula ingredients were sieved through a 20-mesh size sieve, directly compressed into tablets using the single punch tablet press (Royal Artist, Andheri (E), Bombay-400093, India) with flat-faced single punch. The machine was set with hardness of about 5 Kg/cm2 using flat punch 12 mm in diameter and had an average weight of about 500 mg.

In vitro release of Ketoprofen from tested tablet and commercial capsule formulations

In vitro dissolution kinetics studies were performed following standardized and validated (European Pharmacopoeia, 1997) procedures, using a SOTAX AT 6 equipped with six baskets. A new sustained release tablet formulation and a commercially available capsule (Ketofan® 100 S.R. capsule) were studied at a pH 7.4 under the same experimental and instrumental conditions. One liter of 0.1 M phosphate buffer solution, pH 7.4, was put in each container at a constant temperature of 37°C ± 0.5 °C (Roda et al. 2002). A tablet was introduced into the container and rotated at 150 rpm. At scheduled times, 5 ml of the solution were collected, filtered and diluted 1/10 v/v with phosphate buffer solution. Absorbance was measured at 262 nm. A Ketoprofen standard solution in the same buffer was used for external calibration. Dissolution tests were performed on 24 tablets prepared in four separate periods over 1 year, and the reported results are the mean value ± S.D.

Bioavialability study

Studied subjects

Twelve male volunteers aged 25–45 years. A questionnaire was circulated to determine their eligibility for participation. None of the volunteers selected had any sign of gastric, hepatic or urinary malfunctions. Before administering the first ketoprofen dose, medical examination and biochemical and hematological tests were performed to assess that subjects were healthy, while urine was for drugs or abuse. During the study, all volu teers consumed a light standard diet containing about 1200 calories per day. No other medication was used for 2 weeks prior to dosing. The study was conducted in accordance with ethical procedures and policies approved by the Research Ethics Committee of Faculty of Pharmacy- Tanta University, Egypt (REC-FPTU).

Study design

In particular, one dose was administered at 8 am of either Ketoprofen tablets or Ketofan® capsules. After two hours of administrating the two dosage forms, the volunteers took light breakfast. While after eight hours, they took a complete lunch. Blood samples were collected immediately before administration and at 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h after administration. Plasma was separated by centrifugation and stored at −40 °C until analysis.

Plasma Ketoprofen analysis

Plasma standards (1 ml) were prepared by adding the appropriate Ketoprofen solution to drug-free plasma, to give a concentration range of 0.075–20 µg/ml. Calibration and clinical plasma samples were processed and submitted to HPLC analysis in an identical manner. A modified version of the extraction procedure of Satterwhite and Boudinot (Satterwhite and Boudinot, 1988) was used. In brief, internal standard solution (60 µlof 55 µg/ml fenoprofen calcium salt in methanol) was added to 1 ml of plasma, which was then acidified with 0.2 ml of 1M phosphate buffer, pH 2.0. The sample was then extracted with diethyl ether (5 ml) and vortex-mixed for 5 min. The upper organic phase was separated and evaporated to dryness at 40 °C under a stream of nitrogen gas. The dry residue was dissolved in 0.3 ml of mobile phase for HPLC analysis. The HPLC analysis system consisted of two high-pressure pumps (Model 125-S, Beckman, Fullerton, CA, USA), a sample injection valve (Rheodyne Model 7121, Cotati, CA, USA) with 20 µl sample loop, and a variable-wavelength ultraviolet absorbance detector (Model 168, Beck- man Fullerton). Ketoprofen and fenoprofen (internal standard) were separated using a reverse phase C-18 column (Ultrasphere, 120 A,5 µm, 4.6×250 mm, Beckman, Fullerton) at room temperature. The mobile phase consisted of acetonitrile, 0.01 M KH2PO4 adjusted to an apparent pH 3.5 with H3PO4 (40:60, v/v). Ketoprofen and in-ternal standard were eluted isocratically at a flow rate of 1 ml/min and monitored at 254 nm. The method produces linear calibration graphs over the range 0.05–50 µg/ml of Ketoprofen in plasma. The precision of the assay procedure, evaluated on plasma samples at concentrations of Ketoprofen of 0.1, 1 and 10 µg/ml, yielded variation coefficients of 4.5, 3.0 and 2.8%, respectively. Calibration curves were made by fitting Ketoprofen to internal standard peak-area ratios with Ketoprofen concentration. Unknown samples were quantified by reference to the linear regression equation derived from the standard curve.

Statistical methods

C_{max}, t_{max}, $(t_{1/2})_{ab}$, $(t_{1/2})_{el}$, $AUC_{0-\infty}$, $AUMC_{0-\infty}$, and MRT respectively was calculated by the trapezoid rule from 0 to 24 h for all participants. Each of these measurements was determined in a crossover design using variance analysis. The bioavailability of each formulation was determined and compared by calculating the mean AUCs.

Results

Table 1 Optimum levels of dependent formulation variables for the optimized formula of Ketoprofen niosomes

Dependent variables	Optimum levels
surfactant cholesterol ratio (X_1)	0.66:1
Hydrophilic Lipophilic Balance (X_2)	7.86
total lipid concentration (X_3)	34.18

Characterization of Ketoprofen niosomes

The entrapment efficiency of the optimized formula was found to be equal 42.22 ± 0.52 %. The cumulative percent release of Ketoprofen from the optimized niosomal formulation after 1 h was 22.89±0.8 %, after 12h was 88.64±1.2 % and after 24 h was 91.31±0.6 %.

Transmission electron micrographs revealed the formation of well identified niosomal vesicles as shown in figure (1). The examined niosomes appeared as spherical unilamellar nano vesicles with sharp boundaries.

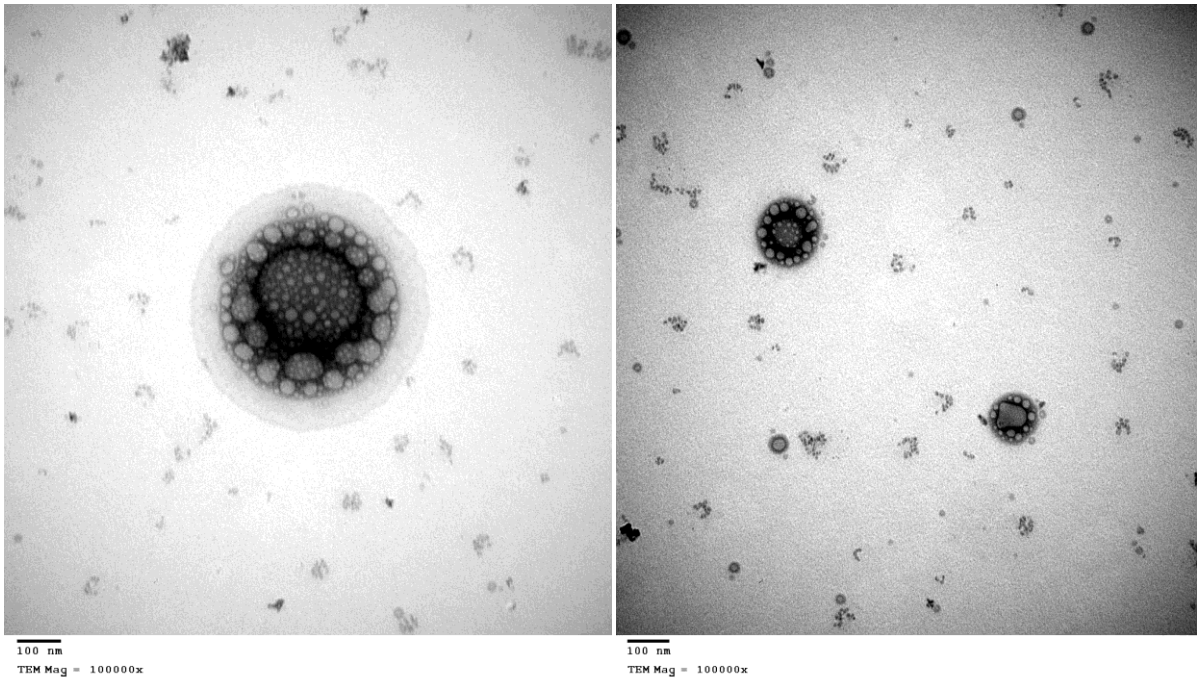

Fig.1. Transmission electron micrographs of the optimized formula of Ketoprofen niosomes

Characterization of niosomal formulation is reported in Table 2. Particle size analysis of the optimized formula shows that the size range lied between 90.55 and 199.76 nm (mean 115.89±9 nm). The size distribution could be observed from the polydispersity index shown in Table 2, which indicates that all the formulations were multi dispersed niosomes. The niosomal formulation exhibited negative surface charge and high zeta potential value of the system due to high required HLB value of mixed Span surfactants indicates stability of the formulation.

Table 2 Characterization of the optimized niosomal formulation prepared by lipid hydration method: Particle size (nm), Polydispersity index (PI), and Zeta potential.

	Particle size (nm)	Polydispersion index	Zeta potential (mV)
Optimized formula	115.89±9	0.222±0.021	−40.90±0.05

3.2. Characterization of a new sustained release Ketoprofen tablets

Dissolution experiments were performed using phosphate buffer (0.1 M, pH 7.4) and following validated procedures and instrumentation. Fig. 3 shows the dissolution kinetics obtained with the new sustained release Ketoprofen formulation, as compared Ketofan® 100 S.R. capsules. By comparison, New sutained release Ketoprofen

formulation exhibited much slower kinetics, characterized by a constant dissolution rate during the entire period, as shown by the slope of the curve; almost 85% of the active ingredient was
dissolved after 8h. Ketofan® 100 S.R. capsules also showed a slower dissolution rate but with a different profile than New sutained release Ketoprofen formulation : the dissolution profile exhibited faster kinetics in the first 4 h period, after which the slope was reduced; after 8 h more than 90% of the drug was dissolved.

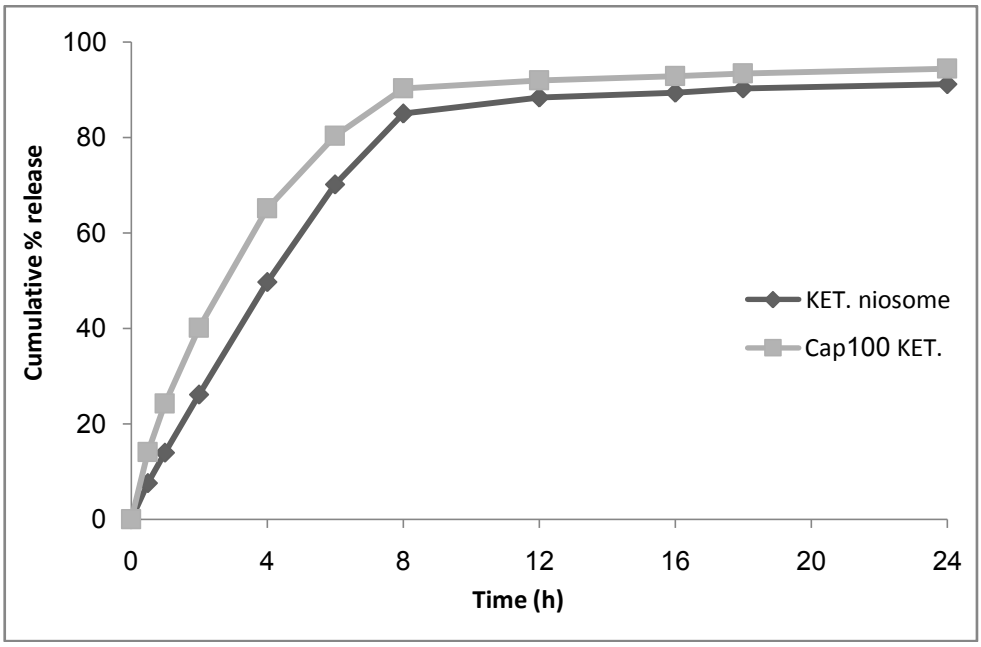

Fig.2. Dissolution kinetics of the newly developed sustained release tablets and Ketofan® 100 S.R. capsules.

3. 3. Plasma ketoprofen pharmacokinetics

Table (3) reports mean values of pharmacokinetic parameters obtained when the two studied formulations were administered to 6 volunteers, as reported above. Figure (3) shows mean Ketoprofen plasma concentration with respect to time.

Table (3): Pharmacokinetic parameters (mean±S.D.) observed in 12 volunteers after single oral doses of sustained release Ketoprofen tablets and Ketofan® capsules (both 100 mg)

Pharmakokinetic parameters	Sustained release Ketoprofen tablets (100mg)	Sustained release Ketofan® capsules (100 mg)
C_{max}, µg/ml	4.64±1.25	5.6±0.59
(T_{max}), hr	4	2
$AUC_{0-\infty}$, mg.hr/ml	69.65±5.45	67.03±10.94
$AUMC_{0-\infty}$, $mg.hr^2$/ml	741.08±33.79	594.68±88.33
MRT, hr	10.63±1.02	8.87±0.33

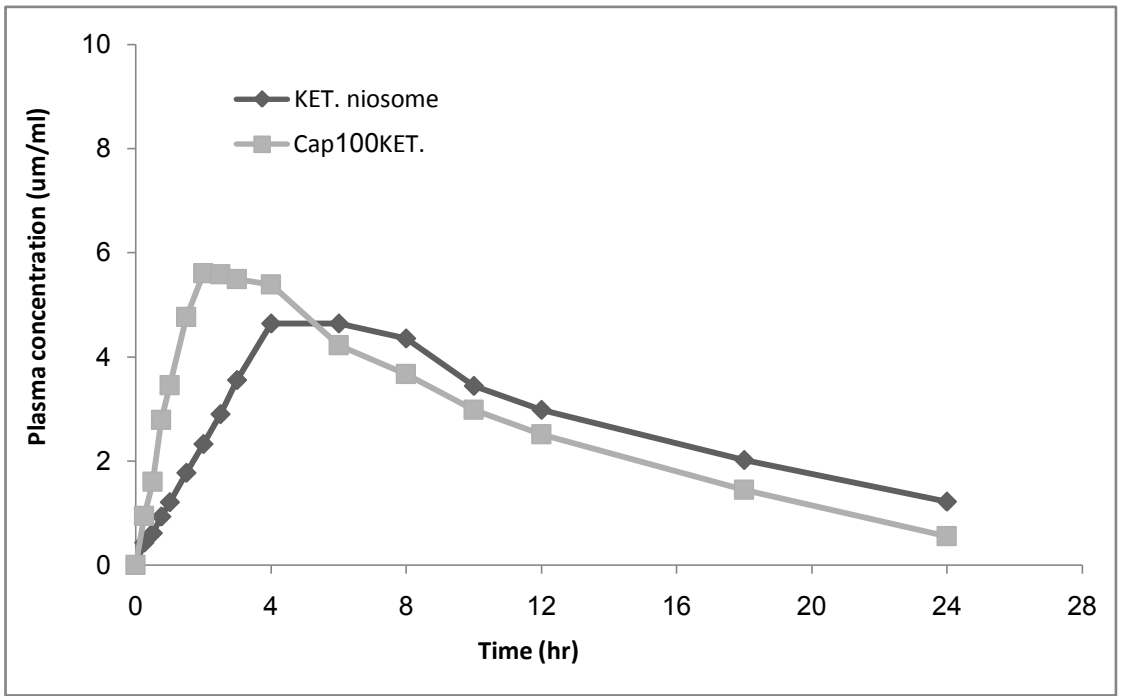

Fig.3. Mean Ketoprofen Plasma Concentration-time Curve of the two dosage forms

From table (3), it was obvious that, the mean C_{max} values were 4.64 and 5.6 µg/ml for the tested tablet formulation and the reference Ketofan® capsule respectively. This result indicated that niosomes decreased the C_{max} of Ketoprofen. The mean T_{max} values for the tested tablet formulation and the reference Ketofan® capsule were 2 and 4 hr respectively; the delayed occurrence of Tmax with the tablet formulation is due to the slow release of the drug from niosomes that agreed with Nasr, (2010) who reported that the mean T_{max} values for celecoxib niosomal formulation and the reference celecoxib capsule were 4 and 2.5 hr respectively; therefore, this slow release can prolong the localization of the drug at the site of absorption and can affect the extent of drug absorption.

The increase in the mean MRT (10.63 hr) and $AUC_{o-\infty}$ (69.65 mg.hr/ml) , as compared to the decrease in the mean MRT (8.87 hr) and $AUC_{o-\infty}$ (67.03 mg.hr/ml) of the reference Ketofan® capsule; reflect the ability of the new Ketoprofen tablet formulation to sustain the release of Ketoprofen for a longer period of time than the commercial capsule. Therefore the prepared Ketoprofen niosomes successed in formulation of a new Ketoprofen sustained release tablet formulation. A possible explanation for this sustained release effect is that niosomes act as a carrier and a slow release vehicle. The drug is carried by the niosomes through the epithelium into deeper layers of the mucosa, where the encapsulated drug is slowly released (Attia et al., 2007).

The mean $AUC_{o-\infty}$ value for the tested tablet formulation was compared to the mean $AUC_{o-\infty}$ value of the Ketofan® capsule to determine the relative bioavailability. The mean values were found to be 69.65 and 67.03 µg.hr2/ml respectively. This result indicated that incorporation of Ketoprofen niosomes into tablet slightly increases the oral bioavailability of Ketoprofen. The improved oral bioavailability may be owing to the lipophilic nature of the niosomal formulation and the effect of the nonionic surface-active agent on the permeability of the gastrointestinal membrane. Improved portioning of the lipophilic system to the mucosa, a direct effect of the surface active agent (Span surfactrant) on the barrier function of the mucosa, and prolonged localization of the drug-loaded niosomes at the site of absorption may be possible reasons for the improved bioavailability (Srinivas et al., 2010). This was agreed with Attia et al (2007) who had reported that an increase in the oral bioavailability of acyclovir was achieved by the niosomal formulation. Similar results were reported by Xiao et al. (2006) who reported that the niosomal preparation significantly increased the bioavailability of silymarin.

The percentage of relative bioavailability (RB %) for the tested formulae was found to be 123.06.%

On the other hand, figure (3) showed that the plasma profile for the tested Ketprofen tablet formulation was characterized by lower maximum plasma peak (C_{max} 4.64 µg /ml) which reached later (T_{max} 4 hr) versus the plasma profile of the commercial Ketofan® capsule which characterized by slightly higher maximum plasma peak (C_{max} 5.6 µg /ml) which reached earlier (T_{max} 2 hr). Moreover, the tested Ketoprofen tablet formulation allowed a steady-state plasma level to be maintained from (4-8 hr) while the plasma level obtained after administration of the Ketofan® capsule maintained from (2-4 hr). Therefore, it was concluded that although the mean T_{max} of the tested Ketoprofen tablet formulation was reached later but maintained for a longer time.

Furthermore, it has been reported that the analgesic effect of the Ketoprofen is achieved with plasma concentrations above 0.7–1 µg/ml (*Houghton et al., 1984*). Our results showed that during a 24 hr period after administration the tested Ketoprofen tablet dosage form the mean Ketoprofen plasma levels never fell to 1 µg/ml, while with Ketofan® capsule they sometimes fell below the therapeutic threshold after 18 hr. This implied that adequate Ketoprofen therapeutic plasma levels were maintained throughout the 24 hr period after oral administration of the tested sustained release Ketoprofen tablet formulation.

In vivo plasma Ketoprofen kinetics profile was in agreement with in vitro dissolution data obtained for tablet formulation. The tested tablet oral administration produced a plasma steady-state Ketoprofen level, achieved within 4 hr after administration and maintained for about 8 hr. This agreed with the constant drug release observed in the in vitro dissolution tests with the tablet formulation.

Conclusion

Niosomal formulation succeeded in preparing a new Ketoprofen sustained release tablet formulation which is able to sustain the release of Ketoprofen for a longer period of time (by about 4 hr) than the Ketofan® commercial capsule. The new formulation ensured therapeutic activity for 24 hr and so, it is suitable to be administered once daily.

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