Niosomes: Do They Increase the Potency of Topical Natamycin Ketorolac Formula in Treating Aspergillus Keratitis? An Experimental Study

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Abstract

**Purpose:** Formulation of new drug delivery system as Natamycin (NT)-loaded nanoparticle niosomal formulae mixed in different polymer gel, with the addition of ketorolac tromethamine (KETR). Pharmaceutical and experimental assessments to evaluate their safety and efficacy in treating Aspergillus keratitis.

**Methods:** NT nanoparticle niosomes prepared by reverse-phase evaporation technique were mixed in different polymers, with the addition of KETR. Two formulae are evaluated in this study: F1 [NT-loaded nanoparticle niosomes/0.5% KETR 4% carboxymethyl cellulose (Na.CMC) gel], F2 [NT-loaded nanoparticle niosomes/0.5% KETR 2% hydroxypropylmethyl cellulose (HPMC)-E4 gel], and mixed marketed products (MMP), namely Natamet® and Ketoroline® suspension eye drops. NT-loaded nanoparticle niosomes/0.5% KETR were evaluated through viscosity determination, mucoadhesive attractive force, and in vitro NT release studies. The in vivo antifungal evaluation was performed on 45 albino rabbits, Aspergillus species were inoculated in right corneas of all rabbits, and then rabbits were subdivided into 3 groups, 15 rabbits each: Group A: received F1, Group B: received F2, and Group C: received MMP. Daily examination of rabbits was performed for evaluation of corneal infiltration, and signs of iritis. Two weeks later, rabbits were euthanized; their corneas were dissected at the limbus and sent for histopathological evaluation.

**Results:** F1 had a higher viscosity and more mucoadhesive power than F2, and showed better results on corneal infiltration, and level of hypopyon. These results were consistent with the histopathological examination.

**Conclusion:** The formula of NT-loaded nanoparticle niosomes/0.5% KT 4% Na.CMC gel has the best results from all pharmaceutical in vitro evaluations and a better cure percent in experimental application.

**Keywords:** Natamycin, niosomes, Aspergillus, fungal keratitis

Introduction

**Natamycin (NT) (Pimaricin)** currently represents the drug of choice for treating filamentous fungal keratitis.1–3 Nonionic surfactant vesicles (Niosomes) are formed from self-assembly of nonionic surfactant in aqueous media, resulting in a closed bilayer structure. The bilayered vesicular structure is an assembly of hydrophobic or lipophilic tail of surfactant monomer shielded away from the aqueous space or core (hydrophilic head) located in the center and hydrophilic groups.4,5 Niosomes own an infrastructure consisting of hydrophobic and hydrophilic moieties and, as a result, can accommodate drug molecules with a wide range of solubility.6 Moreover, niosomes have proved to possess distinct advantages over conventional dosage forms, as they can act as drug reservoirs, carry both hydrophilic and hydrophobic drugs either in an aqueous core or in vesicular membrane,7,8 and protect them against acidic and enzymatic effects in vivo,7 and the drug release rate can be adjusted7. In addition, these systems have been reported to decrease the side effects and to give a considerable drug release.10 Niosomes are osmotically active and stable on their own.11–13 They are biodegradable, biocompatible, and nonimmunogenic. Niosomes improve the therapeutic performance of drug molecules by delaying clearance from the circulation, protecting the drug from biological environment, and restricting its effects to target cells.14
By increasing the viscosity of ophthalmic solutions—through using viscosity imparting agent such as cellulose derivatives—the precorneal residence time and ocular drug absorption will improve\(^1\) with subsequent increase in ocular bioavailability.\(^1\)

Ketorolac tromethamine (KETR) is added as an anti-inflammatory drug to the formulations to treat inflammation and edema, which are associated with mycotic keratitis.\(^1\)-\(^3\)

This study aimed to design and evaluate NT-loaded nanoparticle niosomes and mixed in polymers like carboxymethyl cellulose (Na.CMC) and hydroxypropylmethyl cellulose (HPMC) as NT-loaded nanoparticle niosome gel/0.5% KETR gel formulations to improve corneal drug absorption. The prepared formulations were compared with the mixed marketed products (MMP) (Natamet\(^b\) and Ketoroline\(^b\) suspension eye drops).

In this study, the Aspergillus flavus strain suspension was inoculated into posterior corneal stroma of rabbits. The infected rabbits’ corneas were evaluated clinically and histopathologically for assessment of the safety and efficacy of these new formulae.

This study is aiming at developing NT ocular delivery system to enhance its penetration through corneal tissue for treatment of Aspergillus fungal keratitis. Niosomes are one of the novel carrier systems that have a great potential for improving drug ocular bioavailability.

To the best of our knowledge, this study represents the first combination study of NT-loaded nanoparticle niosomes with a nonsteroidal anti-inflammatory drug for the treatment of Aspergillus keratitis.

**Methods**

The Ethics Committee of the Faculty of Pharmacy, Cairo University, had approved this research with approval number (PT 1582) on 22 February 2016.

NT, Sorbitan monolaurate (Span 20), Cholesterol sodium, and Na.CMC with average \(M_w \sim 90,000\) were purchased (Sigma Chemical Co., St. Louis). Ketorolac tromethamine USP (99.40% purity) (KETR) was supplied by Amryia Pharma Ind. (Egypt). Natamet Suspension Eye Drops, Sun Pharma (India), and Ketoroline Solution Eye Drops, Pharopharma (Egypt), were purchased from private pharmacy in (Egypt). HPMC-E4 (Tama, Tokyo, Japan).

**Preparation and evaluation of NT niosomes/NT niosomal 0.5% KETR gel**

NT nanoparticle niosomes were prepared using reverse-phase evaporation technique according to Szoka and Papa-hadjopoulos.\(^1\) Lipid components consisting of Span 20, cholesterol were dissolved in a mixture of chloroform and diethyl ether (1:1). Approximately 0.2% natamycin (NT) was dissolved in methanol and phosphate-buffered saline, pH (7.4),\(^2\)-\(^3\) as the solubility was 20–50 mg/l.\(^4\) The prepared formula in molar ratio (1:0.5) with given code (optimized Niosomal Formula [FN]) was evaluated through entrapment efficiency, which was determined according to El-Nabarawi et al., Panwar et al., and Aggarwal et al. methods,\(^5\)-\(^2\) photomicroscopic analysis,\(^2\)-\(^3\),\(^2\) particles size,\(^2\)-\(^3\) and zeta potential\(^2\)-\(^3\) to be dispersed in hydrogel containing 0.5% KETR.

NT nanoparticle niosomes prepared were mixed in different types of 0.5% KETR gel to facilitate the administration to patients and to control NT release. The 2 types of gel formulations used were (Na.CMC) and (HPMC-E4), each containing 0.5% KETR, and namely F1 (NT-loaded niosomes/0.5% KETR 4% Na.CMC gel) and F2 (NT-loaded niosomes/0.5% KETR 2% HPMC-E4 gel), respectively.\(^7\) Then, the prepared NT nanoparticle niosomal 0.5% of KETR gel formulae underwent the following parameters for evaluation: mucoadhesive study (with the use of the modified balance method reported by Kaur et al.; Bansal et al.; Gad et al.; and Koffi et al.)\(^3\)-\(^4\) and viscosity determination, which was measured using Brookfield viscometer (Brookfield DV-E Viscometer) at different speeds using spindle 6 at 25°C.\(^3\)

The release of NT from NT nanoparticle niosome-loaded/0.5% of KT gel formulae (F1 and F2) was done by the dialysis method using of U.S.P. Dissolution Tester (Classic Version 6–Vextra-Model BLHMO15K-10; Oriental Motor, Co. Ltd., Japan).\(^7,\(^2\)-\(^3\) Clinically used preparation of the Market product (Natamet) equivalent to 0.2% of NT was evaluated by same dialysis dissolution condition and compared with NT niosome-loaded/0.5% of KT gel formulae for their sustain release potential.\(^3\)

Measured amounts of NT nanoparticle niosomal 0.5% KETR gel formulations (F1 and F2), equivalent to 0.2% NT, were placed in a glass cylinder with 2.5 cm diameter, 8 cm length, and 4.91 cm\(^2\) surface area. This cylinder was fitted with presoaked semipermeable membrane (0.45 \( \mu \)m),\(^1\) and was placed in vessels of the U.S.P. Dissolution Tester, containing 120 mL phosphate-buffered saline (pH 7.4) containing 1% sodium lauryl sulfate (SLS) at 37 ± 0.5°C and at rotation speed 75 rpm.\(^3\) SLS was used as diffusion medium and to solubilize NT.\(^3\)

The absorbance of the collected samples was measured spectrophotometrically at \( \lambda \) max 323.5 nm for NT and at 344 nm for KETR by first-derivative spectrophotometric method. Comparison of release profiles of different

**FIG. 1.** Picture of in vitro antifungal activity study for different formulae against Aspergillus flavus using Agar Well Diffusion Method. F1 and F2 represent prepared formulae, F3 represents mixed marketed products, F4 represents Free Drug Niosomal Gel, and D represents dimethylsulfoxide. Color images are available online.
formulations was carried out using 1-way analysis of variance (ANOVA) statistical test at significant level ($P < 0.05$).

In vitro antifungal activity study

The antifungal activity study of niosome formulae F1 and F2 in comparison with MMP was done on Mueller-Hinton broth (MHB) plates as previously described, according to National Committee for Clinical Laboratory Standards 1993, using the agar well diffusion technique.\textsuperscript{40–42}

Agar wells were made with the help of a sterilized cork borer having an inner diameter of 6 mm. One hundred microliter aliquot of each of niosomal gel formulae F1 and F2, MMP, and free drug niosomal gel (FDNG) as blank were filled to the cups with a sterile syringe. The plates were left for 30 min to allow the diffusion and were incubated at 37°C for 24 h, after which the zones of inhibition around the wells were measured in millimeter.

The minimum inhibition concentration (MIC) for F1 and F2 was done on MHB plates with the use of the agar well diffusion technique.\textsuperscript{42} The formulations were initially dissolved in dimethylsulfoxide with different concentrations (8,000, 4,000, 2,000, 1,000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 $\mu$g/mL) of the formulations and then were dispensed in wells. The plates were left for 30 min to allow the diffusion and were incubated at 37°C for 24 h, and then we measured the zones of inhibition around the wells in millimeter to find out MIC for formulation (Fig. 1).

One-way ANOVA statistical test at significant level ($P < 0.05$) was applied to determine the significant differences between inhibition zones of formulae.

In vivo antifungal evaluation

That was performed at the Animal House in Kasr Al Aini Hospital, Cairo University, in the period from November 2017 to January 2018.

In this study, 45 Albino rabbits with average weight of 1.5 kilograms, without any eye diseases, were included. In managing rabbits, we followed the Association of Research in Vision and Ophthalmology (ARVO) statement, and National Institutes of Health (NIH) guide for the use of animals in ophthalmic and vision research.

In the operating room, rabbits were sedated by intramuscular injection of 1 mL Ketamine (Ketalar 50 mg/mL); besides, we used topical anesthesia in the form of (Benoxinate eye drops). Right corneas of all rabbits were marked using 7 mm corneal trephine, followed by scrapping of corneal epithelium, and then corneas were inoculated with Aspergillus; 27G needle was used to inject (0.1 mL of Aspergillus species with a concentration of $5 \times 10^5$ spores/mL) into posterior corneal stroma.\textsuperscript{16}

The entire process was done under complete aseptic precautions, using a binocular microscope. After 48 h, all rabbits were examined for signs of keratitis to confirm flourishing of the organism. Then treatment was started.

Rabbits were subdivided into 3 groups, and then given numbers and codes; each group started a different antifungal formula.

Groups A and B: each 15 rabbits received 300 mg (0.3 g = 0.3 mL = q.i.d/day) of mucoadhesive prepared gel formulations (F1 and F2; containing 0.2% NT and 0.5% Ketorolac tromethamine gel and Natamet (n = 3)) for 10 days. Group C: 15 rabbits received 300 mg (0.3 g = 0.3 mL = q.i.d/day) of mucoadhesive prepared gel formulations containing 0.5% Natamycin.

One-way ANOVA statistical test at significant level ($P < 0.05$) was applied to determine the significant differences in between group's corneal invasion and corneal thickness.
KETR w/v). Group C: 15 rabbits received 2 drops (0.1 mL = 100 µL) of MMP (2% Natamet and 0.5% Ketoroline). Meanwhile, the mucoadhesive prepared gel formulation would be attached to the mucin of eye, while the drops would be withdrawn by drainage.

Rabbits were examined every day with a follow-up sheet for each. Sheets had specific system of enumeration.

Treatment and comments were done by an ophthalmologist, who did not know the difference among the 2 formulae (F1 and F2) and MMP, and was totally blinded in order not to be biased.

The following clinical data were evaluated: size of corneal infiltration with the aid of Castroviejo caliper, extent and progress of ciliary injection, and the anterior chamber reaction. Along the whole experiment, daily follow-up photos were taken.

Rabbits were euthanized after 2 weeks of treatment, and then corneas were dissected at the limbus and sent to the department of Pathology Kasr Al-Aini Hospital, Cairo University, under complete aseptic conditions in 10% formaldehyde solution to evaluate the presence or absence of the fungus and extent of inflammation for each formula.

Histopathological evaluation of corneal specimens:
All corneal specimens from the cases were multisected, and totally submitted. The sections from each case were serially sectioned (at least 3 sections) on the slide. For each case, 2 slides were prepared: 1 stained by hematoxylin and eosin and the other stained by Gomori Methenamine silver (GMS).

Corneal inflammatory infiltration was traced within 1 mm beneath the corneal epithelium.

Statistical analysis
It was done by IBM SPSS v21.0 statistical software (IBM Corporation, New York). Descriptive statistics was calculated and the data were summarized as mean±standard deviation, or frequencies (number of cases) and percentages as appropriate. For assessing the association between categorical data, chi-square (χ²) test was performed. Fisher’s exact test was used instead, when the expected frequency is <5. Comparison of numerical variables between the groups was done using 1-way ANOVA test. The results will be considered statistical at significant level (P<0.05).

Results
The percentage of NT entrapped in nanoparticle niosomes in the best formula FN was 96.43% (Table 1). Zeta potential analysis of FN was performed to get information about surface properties of niosomes. It is an indication for the long-term stability of particulate systems. The results showed that the zeta potential for NT nanoparticle niosomes prepared of FN was -58.95 mV (Table 1).

Moreover, results showed that F1 gels had higher mucoadhesive force than F2 gels, with statistically significant differences among the formulae, at significant level (P<0.05), where (P=0.0371).

Table 2. In Vitro Antifungal Susceptibility (n=3)

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Test item</th>
<th>Zone diameter (mm), mean±SD</th>
<th>MIC (µg/mL), mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus (002002)</td>
<td>F1</td>
<td>20.00±0.361</td>
<td>31.00±3.46</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>15.00±0.624</td>
<td>63.00±2.65</td>
</tr>
<tr>
<td></td>
<td>MMP</td>
<td>28.00±0.557</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>FDNG</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>16.00±0.265</td>
<td>ND</td>
</tr>
</tbody>
</table>

Control, Ketoconazole as control; FDNG, free drug niosomal gel as plain niosomal gel as blank; MMP, mixed marketed products of Natamet and Ketoroline® suspension eye drops; NA, no activity; ND, not detected.
The results of release profile of NT from Natamet, FN, F1, and F2 at 8 h was 41.52%, 25.02%, 21.31%, and 85.52%, and the results of NT released from FN, F1, and F2 at 24 h were 77.49%, 57.32%, and 41.07%, respectively (Table 1).

The results of release profiles of NT from Natamet, FN, F1, and F2 at 8 h and 24 h showed significant differences ($P=0.0115$) and ($P=0.00016$), respectively, at significant level ($P<0.05$).

The results of antifungal activity evaluation of F1, F2, MMP, and FDNG revealed the antifungal activity of NT niosomal-loaded/0.5% KT gel formulations (F1 and F2) and against *A. flavus*, and a significant difference between F1, F2, MMP, and FDNG ($P=0.0396$) at significant level ($P<0.05$).

**FIG. 4.** Rabbits’ corneas infected with Aspergillus keratitis with corneal infiltration (arrow) and ciliary injection (Group B). Color images are available online.

**FIG. 5.** Rabbits’ corneas after treatment with (F2) formula showing improvement of corneal infiltration and ciliary injection (Group B). Color images are available online.

**FIG. 6.** Rabbits’ corneas infected with Aspergillus keratitis with corneal infiltration (arrows) and ciliary injection (Group C). Color images are available online.

**FIG. 7.** Rabbits’ corneas after treatment with (mixed marketed products) showing improvement of corneal infiltration and ciliary injection (Group C). Color images are available online.
The results of antifungal activity evaluation revealed that *A. flavus* are highly sensitive to NT in formulae F1 and F2 at low concentrations compared to Ketoconazole as control, as illustrated in Table 2.

Statistically, there were significant differences in the zone of inhibition between F1, F2, and MMP for *A. flavus* by applying ANOVA 1 way at significant level (P<0.05), where \((P=2.61\text{E}^{-05})\). The results revealed the antifungal activity of NT niosomal-loaded/0.5% KT gel formulations against Aspergillus. This can be more clearing through determining MIC of NT in F1 and F2 against *A. flavus*, where the MIC was 31 and 63 \(\mu g/mL\), respectively. The results revealed that MIC of F1 was more effective at low concentration than MIC of F2 in *A. flavus* because F2 was more viscous than F1 and this led to a decrease in the diffusion of NT around the agar wells.

**Table 3. Percentage of Histopathological Results of Rabbits’ Corneas Inoculated with *A. flavus* (Neutrophil)**

<table>
<thead>
<tr>
<th>Antifungal formula</th>
<th>No inflammation NP</th>
<th>Mild inflammation NP (+)</th>
<th>Moderate inflammation NP (++)</th>
<th>Severe inflammation NP (+++)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (F1)</td>
<td>60.00±0.82</td>
<td>33.33±1.02</td>
<td>6.67±0.35</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Group B (F2)</td>
<td>53.33±0.80</td>
<td>33.33±1.04</td>
<td>13.33±0.47</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Group C (MMP)</td>
<td>26.67±0.35</td>
<td>20.00±1.32</td>
<td>33.33±1.14</td>
<td>20.00±1.35</td>
</tr>
</tbody>
</table>

KT, ketorolac tromethamine; NP, neutrophil.

**In vivo results**

Follow-up of the size of corneal infiltration revealed that rabbits treated by F1 formula had better improvement than those treated by F2 and MMP (Figs. 2–7).

In addition, regarding the severity of ciliary injection and level of hypopyon, F1 had the best results.

The pathology also confirmed our results that F1 and F2 formulae were both better than MMP regarding fungi and inflammation.

The degree of inflammation was classified according to the amount of neutrophils (NPs) as follows: NP (+)=mild inflammation, NP (++)=moderate inflammation, and NP (+++)=severe inflammation.

**Table 4. Percentage of Histopathological Results of Rabbits’ Corneas Inoculated with *A. flavus* According to the Fungal Colonies**

<table>
<thead>
<tr>
<th>Antifungal formula</th>
<th>No fungal colonies</th>
<th>Small fungal colonies</th>
<th>Large fungal colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (F1)</td>
<td>100.00±0.40</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Group B (F2)</td>
<td>67.00±1.00</td>
<td>33.33±1.05</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>Group C (MMP)</td>
<td>33.33±1.31</td>
<td>40.00±1.54</td>
<td>26.67±1.42</td>
</tr>
</tbody>
</table>

Table 3 shows that corneas of group A that received F1 had the highest percentage of no inflammation (no NP) and the lowest percentage of moderate and severe inflammation. Also, corneas of group C that received MMP had the lowest percentage of no inflammation (no NP) and the highest percentage of moderate and severe inflammation due to *A. flavus*. Group B that received F2 was less than group A and more than group C. Statistically, 1-way ANOVA at significant level (P<0.05) was applied between 3 groups (A, B, and C) according to degrees of inflammation (no, mild, moderate, and severe inflammation). The results of *A. flavus* showed significant difference between the groups, where \((P=3.63\text{E}^{-05})\), \((P=0.005)\), \((P=2.27\text{E}^{-08})\), and \((P=9.45\text{E}^{-08})\), respectively.

All GMS-stained slides were examined under light microscope to evaluate the presence or absence of fungal spores as follows:

- The presence of large number of fungal spores was scored as 2.
- The presence of small number of fungal spores was scored as 1.
- The absence of fungal spores was scored as 0.

**FIG. 8.** (a) H&E section, necrotizing inflammation (arrow) with wide exudation of NPs. (b) H&E section with moderate exudation of NPs (square). (c) H&E section with mild exudation of NPs (star). H&E, hematoxylin and eosin; NP, neutrophil. Color images are available online.
Concerning the fungal colonies, Table 4 shows that group A and B that received F1 and F2 were the best regarding treatment against *A. flavus*, whereas group C that received MMP was the lowest group according to the fungal colonies. So, the formula (F1) was the best formula during the treatment periods of rabbits against *A. flavus*. Also, statistically, using 1-way ANOVA at significant level ($P < 0.05$) showed significant differences between the groups (A, B, and C) according to fungal colonies (no, small, and large), where ($P = 6.24 \times 10^{-10}$), ($P = 2.42 \times 10^{-8}$), and ($P = 1.64 \times 10^{-7}$), respectively.

Detection of necrotizing inflammation in corneal sections is shown in Fig. 8.

Figure 9 demonstrates the percentage of inflammation in rabbits’ corneas inoculated with *A. flavus* after treatment with NT and KT according to the amount of NP.

The number of small and large fungal spore observed in GMS staining is shown in (Fig. 10).

Figure 11 demonstrates the percentage of histopathological results of rabbits’ corneas inoculated with *A. flavus* after treatment with NT and KT according to the fungal colonies.

**Discussion**

NT has a broad-spectrum antifungal activity, and it represents the only topical ophthalmic antifungal preparation that is approved by the Food and Drug Administration of the United States.\(^{43,44}\)

However, NT has short residence time at ocular mucosa, which necessitates frequent application for long duration, which can result in suboptimal ocular concentration of the drug and unsatisfactory treatment results.\(^2,3\)

In this study, we formulated a new NT-loaded nanoparticle niosome delivery system to enhance corneal drug penetration. The incorporation of NT-loaded niosomes/0.5% KETR gel resulted in further delay and control in release of NT due to formation of an additional diffusion barrier to drug release.\(^{45}\)

KETR is a nonsteroidal anti-inflammatory drug, acts by inhibition of cyclooxygenase enzyme that transforms arachidonic acid into prostaglandins. In treatment of fungal keratitis, combining KETR with NT-loaded nanoparticle niosome helps to treat the associated inflammation, ulcer, and edema that are associated with fungal eye disease due to secretion of prostaglandins by fungus, which may play an important role in fungal colonization.\(^{16,18}\)

The zeta potential for NT-loaded niosomes prepared of the best formula F (1:0.5) was $-58.95$ mV because the colloidal system dispersion is stable (more negative than $-30$ mV). This result is in accordance with El-Nabarawi et al. and Hosny.\(^{23,38}\)

This study showed that, increasing the concentration of each polymer in the gels significantly increases the mucoadhesive strength. This is in accordance with Kaur et al., Bansal et al.; and Gad et al.\(^{31–33}\)

The percentage of NT released from different NT-loaded niosomal/0.5% KETR gel through membrane showed that the extent rate of NT released was associated with polymer concentrations as increasing the viscosity of the polymer led
to a decrease in the extent of drug release from formulations. These results are in accordance with El-Nabarawi et al. and Jones et al., who stated that the physical reason for slower extent rate of release from viscous gel is most probably due to formation of highly viscous diffusion layers of hydrated polymer chain, which entraps the excess of water and reduces migration of drug molecules.23,46

It is clear that the encapsulation of NT within niosomes extends the time required for 75% NT release, which was 24 h. This is in comparison with the time required for 75% NT released from 0.2% Natamet suspension, which was 4 h. This result can be explained by the presence of cholesterol in bilayer above the surfactant, which modulates fluidity by restricting the movement of NT, reducing bilayer permeability, and decreasing the efflux of the encapsulated drug, resulting in prolonged drug retention.23,46

The result obtained by Paradkar and Parmar,27 who studied NT niosomal in situ gel, was 69.49% over 24 h. Whereas in our study, NT released from F1 and F2 were 57.32% and 41.07%, respectively, which revealed more sustained release. Moreover, this study used a combination of NT niosomal 0.5% KETR gel, which is considered the first combination study of NT-loaded nanoparticle niosomes with a nonsteroidal anti-inflammatory drug for treatment of Aspergillus keratitis. It enhances corneal permeability and ocular bioavailability facilitated by the niosome drug carrier. It decreases the need for repeated drug application due to increased ocular residence time and systemic side effects, as the concentration used of NT in the formulae was 1/10 of the concentration used in MMP. Moreover, it has the best results regarding the pharmaceutical in vitro evaluations, and a better cure rate in experimental application.

This is an experimental study; still, we need to undergo clinical trial testing that new formula on patients, following the ethical and scientific hierarchy of evidence.

Another limitation is the small sample size and lack of randomization, which are justified by the experimental nature of the study.

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References


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