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Research Article

Development and Clinical Evaluation of Clotrimazole–β-Cyclodextrin Eyedrops for the Treatment of Fungal Keratitis

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Abstract. Fungal keratitis is a serious corneal disease that may result in loss of vision. There are limited treatment options available in Iraqi eye hospitals which might be the main reason behind the poor prognosis of many cases. The purpose of this study was to prepare and pharmaceutically evaluate clotrimazole–β-cyclodextrin (CTZ–β-CD) eyedrops then clinically assess its therapeutic efficacy on fungal keratitis compared with extemporaneous amphotericin B eyedrops (0.5% *w*/*v*). A CTZ–β-CD ophthalmic solution was prepared and evaluated by various physicochemical, microbiological, and biological tests. The prepared formula was stable in 0.05 M phosphate buffer pH 7.0 at 40±2°C and 75±5% RH for a period of 6 months. Light has no significant effect on the formula's stability. The CTZ–β-CD eyedrops efficiently complied with the isotonicity, sterility, and antimicrobiological preservative effectiveness tests. Results of the clinical study revealed that 20 (80%) patients showed a favorable response to the CTZ–β-CD eyedrops, while 16 patients (64%) exhibited a favorable response to amphotericin B ($P>0.05$). The mean course of treatment was significantly ($P<0.05$) less in the CTZ treatment group than in the amphotericin group (21.5±5.2 *vs.* 28.3±6.4 days, respectively). The CTZ formulation was significantly ($P<0.05$) more effective in the management of severe cases and also against *Candida* sp. than amphotericin B. There was no significant difference ($P<0.05$) between both therapies against filamentous fungi. The CTZ–β-CD formulation can be used alternatively to other ophthalmic antimycotic treatment options in developing countries where stability, cost, or efficacy is a limiting factor.

KEY WORDS: clotrimazole; β-cyclodextrin; eyedrops; fungal keratitis; Iraq.

INTRODUCTION

According to the World Health Organization, corneal diseases are a major cause of vision loss and blindness, second only to cataract in overall importance (1). Fungal keratitis is one of these serious corneal diseases, especially in developing agricultural countries. Worldwide, the incidence of fungal keratitis is sporadic; however, in developing countries, there is an increase in incidence with the abuse of antibiotics, corticosteroids, and immunosuppressive agents (2). The incidence of fungal keratitis varies according to the geographical location, for example, 2% in the USA (3), 33–40% in Bangladesh (4), 44% in South India (5), 17% in Nepal (6), and 49% in Ghana (7). Several studies had been conducted in Iraq which showed that fungal keratitis accounts for 18.7% of suppurative keratitis in Iraq (8,9).

It was reported that polyene antifungal antibiotics, the first-line therapy in fungal keratitis, are not effective in severe keratomycosis, while imidazole derivatives may be the better choice in the future (10). However, most of these antifungal drugs are still unavailable as commercial eyedrops since they must be prepared in the hospitals' pharmacies as ophthalmic solutions of limited stability and poor storage conditions (11).

The management of fungal keratitis represents a problem to the ophthalmologist in Iraq, and some cases have resulted in blindness. The main reason behind this problem is the unavailability of antifungal eyedrops. Freshly prepared topical amphotericin B (0.1–0.5%) was used with a good response. This extemporaneous ophthalmic solution is usually prepared from an amphotericin B injection (Fungizone®). Fungizone contains deoxycholate necessary to solubilize the highly hydrophobic amphotericin B which renders their instillation painful and leads to poor compliance and an aggravation of symptoms (12). In addition to its high cost, there is poor corneal penetration and instability in their aqueous solution.

On the other hand clotrimazole (CTZ), a synthetic imidazole derivative, is a broad-spectrum antifungal agent that exhibits fungistatic and fungicidal activity (13). CTZ dermatological commercial preparation was used in a diluted form to treat human keratomycosis successfully and in combination with a polyene derivative was required for the treatment of fungal corneal infection due to *Fusarium* spp (14). However, these nonaqueous topical preparations are an eye irritant. Besides, patients with ocular infections require several months

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of treatment; thus, the product's safety, stability, and practical restrictions during preparation in the hospital pharmacies have to be taken into account. Since CTZ is a hydrophobic drug, solubility is the main constraint for its bioavailability (15). Recently, cyclodextrins (CDs) were utilized to prepare water-soluble complexes with lipophilic drugs such as steroids and some carbonic anhydrase inhibitors, in the form of eyedrops. Cyclodextrins increased water solubility of lipophilic drugs and consequently enhanced their ocular absorption and stability and reduced their local irritation (16).

We were the pioneers in the development of clotrimazole–β-cyclodextrin (CTZ–β-CD) by the solubility technique in the industrial labs at the Department of Pharmaceutics, College of Pharmacy, University of Baghdad. The complex was identified by UV spectrophotometer, FTIR spectrometer, phase solubility, *in vitro* dissolution, and differential scanning calorimeter. The apparent solubility of CTZ was significantly increased linearly as a function of the β-CD concentration (2.6-fold at 25°C). The phase solubility diagram was AL type, and the stiochiometric ratio of the complex was 1:1. In addition, the thermodynamic study revealed that the value of the complexation stability constant decreased with increasing temperature, plus the process of complexation was spontaneous, exothermic, and enthalpy driven (17).

The aim of the present study is to prepare a CTZ–β-CD aqueous ophthalmic solution as an attempt to increase the CTZ solubility and consequently its penetration through the ocular epithelium. Moreover, this preparation should be therapeutically and economically effective and have an adequate shelf life to be used as an alternative choice in the treatment of fungal keratitis.

MATERIALS AND METHODS

Materials

Clotrimazole BP (molecular weight (MW), 344 g/mol; aqueous solubility, 5.5 μmol/L), HPLC-grade acetonitrile, sodium chloride, ethanol, EDTA, potassium dihydrogen orthophosphate, and disodium hydrogen orthophosphate were purchased from Sigma Chemicals (Steinheim, Germany). β-Cyclodextrin (MW, 1135) was supplied by Roquette Frères (Lestrem, France). Benzalkonium chloride was obtained from M/S Cadila Pharmaceuticals Ltd. (Ahmedabad, India) while the Sabouraud agar and the potato glucose agar media were purchased from Shanta Chemicals (India). All other materials used were of analytical reagent grade.

Preparation of CTZ–β-CD Ophthalmic Solution

A phosphate buffer pH 7.0 was used as a vehicle, and the process was carried out under aseptic conditions. Clotrimazole, 1% *w*/*v*; benzalkonium chloride, 0.03% *w*/*v*; EDTA, 0.1% *w*/*v*; and sodium chloride, 0.52% *w*/*v* were dissolved as part of the buffer solution. To the other part of the buffer, β-cyclodextrin was added, and the solution was heated to 60°C while stirring. Then, subsequently, methyl cellulose was added to this solution while stirring. The two solutions were mixed, and the volume was increased to the required level using the buffer solution. The solution was sterilized by membrane filtration (pore size, 0.22 μm). The isotonicity of the solution was adjusted by an automatic osmometer (Knauer D-6360, Friedberg, Germany). The final eyedrop formulation was filled in a presterilized amber-colored type I glass vial which was closed with a presterilized LDPE stopper and sealed with an aluminum crimp.

Physicochemical Evaluation

Visual Appearance and pH. A visual inspection was carried out by observing the solution against a white and black background under fluorescent light. The container was agitated at the rate of about eight to ten times in a minute. The pH of the prepared solution was determined by a pH meter (Schott Gerate, Germany).

Drug Content. The drug content was estimated by HPLC. The HPLC system was equipped with a Waters 1515 pump, Waters 2487detector operating at 210 nm, Waters 717 P auto-samplers, and Waters C18 column (5.0 μm, 3.9×150 mm). The mobile phase was (1:1) of ethanol 95% *v*/*v*/acetonitrile 15% *v*/*v*. The flow rate was adjusted to 1 mL/min with a run time of 15 min. The injection volume was 20 μL and the retention time was 2.6 min.

Stability Study. The stability study was performed according to ICH guidelines at 40±2°C and 75±5% RH for a period of 6 months in a stability chamber (Thermolab, Mumbai, India). Five-milliliter samples (n=3) of the prepared formulation were taken out at 30, 60, 90, and 180 days and evaluated for physical changes and drug content. The impact of the buffer type (phosphate, citrate, and acetate buffers), buffer concentration (phosphate buffer, 0.05, 0.1, and 0.5 M), pH (phosphate buffer, pH 5, 6, and 7), and light (colorless and colored type I glass vials) on the stability of CTZ–β-CD ophthalmic solution was studied.

Isotonicity Evaluation. The CTZ–β-CD ophthalmic solution was subjected to isotonicity testing to confirm tissue safety. The formulation was mixed with a few drops of blood and observed under a microscope at ×100 magnification and compared with the standard marketed ophthalmic formulation containing betamethazone sodium phosphate 0.1% (Betnesol®, GlaxoSmithKline, UK).

Microbiological Evaluation

Sterility Test. The prepared eyedrops were subjected to a test for sterility as per guidelines given in USP 23 by membrane filtration method using a millipore filter of 0.22-μm pore size. The CTZ–β-CD ophthalmic solution was incubated in a sterile culture medium. The medium used was a fluid thioglycollate medium for the culture of anaerobic bacteria, as well as aerobic bacteria, and a soybean–casein digest medium for the culture of both fungi and aerobic bacteria.

Antimicrobial Preservative Effectiveness. This test was carried out according to the guidelines given in USP 23. Five test microbes were used including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, *and Aspergillus niger.*

Biological Evaluation

Eye Irritation Test. The eye irritation test was performed in the Drugs Quality Control Labs, Baghdad, Iraq. Four rabbits weighing 2–2.5 kg of either sex were used to assess the prepared formula. The eyedrops were instilled in one eye of each rabbit for 5 days as per the following schedule:

Day 1 Two drops every 30 min for the first 2 h, then two drops every 4 h (total of eight doses per day)
Day 2–5 Two drops every 2 h (total of six doses)

The contralateral eye received a normal saline concentration in the same dosing schedule. Various criteria made at designed required time intervals of 1 h, 24 h, 48 h, 72 h, and 1 week after administration were observed. The degree of irritation was assessed and scored as proposed by the Draize test (18).

Clinical Study

Patients. The retrospective clinical trial comprised 50 patients who presented positive with fungal keratitis, tested by direct smear and a culture. The clinical trial was conducted at Ibn Al-Haetham Teaching Eye Hospital in Baghdad, Iraq, between July 2005 and February 2007. The patients were analyzed in two groups of 25 cases each, according to the drug administered: the amphotericin B group and the CTZ group. Histories of the patients were taken and recorded, specifying their age, gender, profession, risk factors, and the treatments they received before the visit. The following results of an examination by slit lamp were recorded: the size, depth, location, and range of the corneal ulcer. The results also indicated the presence or absence of the complication of iridocyclitis with hypopyon and endothelial plaque.

The severity of the fungal keratitis was divided into mild (less than 4 mm with a depth of one half the corneal thickness) or severe (more than 4 mm with a depth of over one half the corneal thickness) regarding the size and depth of the corneal ulcer. This study complied with the regulations on the research of Humans in Ophthalmic and Vision Research by the Association for Research in Vision and Ophthalmology, and the hospital research department approved the ethics of the study.

Laboratory Examinations. The laboratory tests comprised corneal scrapings, staining, and a general culture of fungi and bacteria. The specimens were scraped from the base and edge of each corneal ulcer, then stained with Giemsa and Gram in order to detect spores, such as fungal hyphae, and inoculated on the culture medium which was Sabouraud agar and potato glucose agar at a temperature of 28°C for 8 to 10 days. The corneal scrapings were negative for bacteria but positive for fungal hyphae or spores with a positive fungal culture; therefore, fungal keratitis was diagnosed. The ulcer smears were positive for fungi, but the negative cultures were excluded from the study.

Therapy. For the CTZ-treated group, CTZ–β-CD eyedrops (1%) were applied topically on the patients' eyes, once every 30 min during the daytime and once every 1 h at night time. The patients in the amphotericin B-treated group were administered amphotericin B eye drops (0.5%, *w*/*v*), during the same intervals as the other group. The eyedrops were prepared in the pharmacy of Ibn- Al-Haetham Eye Hospital under conditions that were aseptic from the commercial Fungizone® (Bristol-Myers Squibb, USA). The frequency of administering the eyedrops, for both groups, was tapered according to the clinical responses.

Examinations were done on all of the patients every day or every other day by special doctors. A slit lamp examination was conducted when the area of the ulcer (after fluorescent staining) and the range of the corneal infiltration were measured. The evaluation included observing a local hypersensitivity reaction and corneal epithelial toxicity. Efficacy was established by the following: (1) effective: the corneal ulcer healed with the negative fluorescent staining, the corneal infiltration and iridocyclitis had subsided; (2) ineffective: the ulcer had not improved or progressed for the corneal ulcer and infiltration.

Data Analysis

The data were entered and analyzed using the SPSS software version 17; (SPSS®, Inc., Chicago, USA). A statistical analysis was performed using a *t* test for comparison between two patient groups. The *P* value <0.05 was considered to indicate statistical significance.

RESULTS

Visual Inspection and pH

The CTZ–β-CD ophthalmic solution was found to be extremely clear and free of particulate matter. CTZ content was within the official limits (97.5±0.35%, $n=6$). The pH of the solution was equal to 7.0.

Stability Study

The initial mean recovery of CTZ content in the eye drops was more than 99%, demonstrating that almost no drug was lost during the preparation. The solution was clear, and no color was detected just after preparation or during storage. The optimum stability of the CTZ–β-CD solution was achieved in the phosphate buffer at 0.05 M at pH 7 (Table I). Also, no significant variation occurred in the pH (7.0±0.1) and osmolarity (562±

Table I. Effect of pH, Buffer Concentration, and Buffer Type on the Stability of CTZ–β-CD Ophthalmic Solution

Buffer	pH	Drug content[a]
Acetate, 0.05 M	4.0	14.02±0.92
	4.5	38.62±0.13
	5.0	56.02±1.23
Citrate, 0.05 M	4.0	14.02±0.29
	5.0	25.24±2.01
	6.0	50.68±2.75
Phosphate, 0.05 M	5.0	90.10±1.66
	6.0	93.22±4.21
	7.0	97.32±1.87
	7.4	94.22±0.82
Phosphate, 0.1 M	7.0	91.10±0.69
Phosphate, 0.5 M	7.0	90.90±0.77

[a] Percentage remaining (mean±SD, $n=6$) of CTZ after 180 days at 40 ±2°C and 75±5% RH

10 mosmol/liter) of the eye drops during storage. Furthermore, light had no effect on the stability of the drug.

Isotonicity Testing

Isotonicity testing of the CTZ ophthalmic formulation exhibited no change in the shape and size of the blood cells (bulging or shrinkage), which reveals its isotonic nature in comparison with the standard marketed one.

Microbiological Evaluation

The CTZ–β-CD eyedrops complied with the test for sterility. The formulation was found to be sterile at the end of the 280-day test period. The utilization of benzalkonium chloride as a preservative in the prepared formula resulted in a complete eradication of the tested microorganisms. Hence, the prepared formulation complied with the antimicrobiological preservative effectiveness test as well.

Eye Irritation Study

The degree of eye irritation was assessed and scored as per the Draize test protocol that is based on the reaction of individual components of the eye to the instillation of the solution under a test. The CTZ ophthalmic formulation was found to be nonirritating with no ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae observed till the end of the test. The only parameters that could be scored were conjunctival redness appearing immediately after instillation and reflex tearing occurring on instillation (Table II). Hence, the formulation was suitable for the eye instillation.

Clinical Study

General Data

General information for the patients of two groups is listed in Table III. The majority of cases with fungal keratitis were male (86%, $n=43$) while 14% were female patients ($n=7$). The average age of the CTZ and amphotericin groups was 40.9±11.2 and 38.2±10.6 years, respectively. Sixty percent of cases were workers, 21% were street vendors, 14% were housewives, and 5% were students. Twenty-seven patients (52%) had corneal trauma. Before the treatment, patients never received topical or systemic antifungal drug treatment. However, 28 patients had a history of topical antibiotics therapy. There were ten patients with a mild fungal keratitis in the CTZ group (40%) and 11 in the amphotericin group (44%).

Clinical Effects

Twenty patients (80%) among the CTZ-treated group obtained complete healing of the corneal ulcer in comparison to 16 patients (64%) of the amphotericin-treated group. The difference was statistically significant ($P<0.05$). The mean treatment time in the CTZ- and amphotericin-treated groups was 21.5±5.2 *vs.* 28.3±6.4 days, respectively. This difference was also statistically significant ($P<0.05$). The responses of the mild cases to both therapies were nearly the same. However, 11 out of 15 (73%) severe patients showed an effective response to CTZ therapy, while only 6 out of 14 (43%) severe patients responded to the amphotericin treatment (Table IV). This effect was statically significant between the two groups ($P<0.05$). In 14 patients with hypopyon, 11 patients (79%) responded effectively in the CTZ-treated group. The results of the CTZ-treated group were significantly ($P<0.05$) higher than the amphotericin-treated group (45%, $n=5$, effective in patients with hypopyon). Two fungal keratitis patients with hypopyon are shown in Fig. 1 under

Table II. Eye Irritation Test: Opacity, Corneal, Conjunctiva, and Iris Observations

Parameters	Normal rating	Rating for CTZ–β-CD formulation
Opacity		
No opacity	0 (none)	0
Diffuse area, details of iris clearly visible	1 (slight)	
Easily visible translucent areas, details of iris slightly obscure	2 (mild)	
Opalescent areas, no details of iris	3 (moderate)	
Opaque, iris invisible	4 (severe)	
Corneal area involved		
25% or less (not 0)	1	1
25% to 50%	2	
50% to 75%	3	
Greater than 75%	4	
Conjunctival observations		
Vessels normal	0 (none)	0
Vessels definitely congested above normal	1 (slight)	
More diffuse, deeper crimson red with individual vessels not easily dissemble	2 (moderate)	
Diffuse beefy red	3 (severe)	
Iris observations		
Normal	0 (none)	0
Folds above normal, congestion, swelling, iris reacts to light	1 (slight)	
No reaction to light, hemorrhage, gross destruction	2 (severe)	

CTZ–β-CD clotrimazole-β-cyclodextrin

Table III. General Clinical Information of Patients with Fungal Keratitis

Parameters	Clotrimazole group(n=25)	Amphotericin group (n=25)
Age (years)	40.9±11.2	38.2±10.6
Gender		
Male	20	23
Female	5	2
Corneal trauma	16	11
Course of disease (weeks)	2.1±0.8	3.2±0.4
Size of ulcer		
≤4 mm	10	11
>4 mm	15	14
Depth of ulcer		
≤1/2	17	21
>1/2	8	4
Degree		
Mild	10	11
Severe	15	14
Hypopyon	14	11

slip lamp examination, those who responded effectively to the treatment of the prepared CTZ formulation. Moreover, no adverse events were observed in both treated groups.

Also, the positive rate of the corneal scrapings with filamentous fungi was predominant for both patient groups. *Fusarium* sp. and *Aspergillus* sp. were the principal isolates followed by yeast infection (*Candida* sp.*)*. The prepared CTZ formulation was significantly (P<0.05) more effective (100%) against *Candida* sp. than amphotericin B, while no significant difference (P <0.05) between both therapies against other fungal species were found; results are presented in Table V.

DISCUSSION

Fungal keratitis refers to the corneal infection caused by either filamentous fungi (mold) or yeast. Fungi are opportunistic agents of infection and rarely infect healthy, intact cornea, but in a compromised or immunosuppressed cornea, almost any fungal species are capable of inducing infection (10). Nowadays, the availability of a therapeutically effective as well as stable antimycotic product in Iraqi eye hospitals is considered as an urgent demand. Amphotericin B is considered the treatment of choice for ophthalmic infections caused by *Candida*, *Aspergillus*, *and Fusarium.* However, the *in situ*-prepared amphotericin B solution is an eye irritant, is unstable, and can only be kept refrigerated for 1 week after reconstitution.

Alternatively, CTZ had been chosen as it has broad-spectrum antimycotic action and can be used both locally and systemically. However, CTZ is a lipophilic compound, and since water solubility is crucial for corneal penetration and rapid antimycotic activity (19), therefore, the inclusion complexation of CTZ in β-CD was prepared in the form of eyedrops, as an attempt to improve CTZ water solubility and consequently its antimycotic activity and stability.

The pH of CTZ–β-CD eyedrops was adjusted close to the physiological pH of tears (about 7.4) to avoid any eye irritation as well as to optimize the drug's stability. The pH of the eyedrops is of importance because the infected/inflamed eye already causes irritation and pain to the patient. If the eyedrops cause irritation also, it will result in greater discomfort. Moreover, an irritating solution, when instilled into the eye, results in profuse reflex tearing, and hence the medication would be quickly washed out from the eye by nasolacrimal drainage, thus resulting in its decreased effectiveness. It is therefore imperative that the eyedrops be nonirritating to the eye.

The β-CD concentration in the prepared eyedrop solution was equal to 100 mg/mL, leading to a hyperosmolar ophthalmic solution compared to the physiological osmolarity of tears, since isotonicity is an important characteristic of the ophthalmic preparations and has to be maintained to prevent tissue damage or irritation to the eye (20). Therefore, the osmolarity of CTZ–β-CD eyedrops was adjusted with sodium chloride to be safe and compatible with ocular administration.

Since patients with ocular infections require a long period of treatment, the product's stability should be studied. CTZ–β-CD eyedrops seemed to be stable because no change in the drug content was observed after 6 months of storage at 40±2°C and 75±5% RH. The chemical stability of the preparation can be attributed to the inclusion complexation of the drug molecule in β-CD (21). The pH of the formulation too remained unchanged throughout the stability period, probably due to the addition of phosphate buffer in the formulation. In addition, no microbial contamination was observed.

Most of the patients involved in the clinical study were male workers, and approximately half of the cases had a history of corneal trauma which is consistent with the previous studies conducted in other countries (22,23). *Fusarium* sp. and *Aspergillus* sp. had shown to be sensitive to CTZ–β-CD and amphotericin B therapies while *Candida* sp. showed significant response to CTZ–β-CD eyedrops. Our results are promising since it had been suggested unlike in other reports that clotrimazole as monotherapy is not an ideal choice for the

Table IV. Therapeutic Efficacy for Fungal Keratitis in CTZ- and Amphotericin-Treated Groups (n (percentage))

	CTZ group (n=25)		Amphotericin group (n=25)	
Efficacy	Mild	Severe	Mild	Severe
Effective	9 (90)	11 (73)[a]	10 (91)	6 (43)
Ineffective	1 (10)	4 (27)	1 (9)	8 (57)
Total	10 (100)	15 (100)	11 (100)	14 (100)

CTZ clotrimazole
[a] Significant difference (P<0.05)

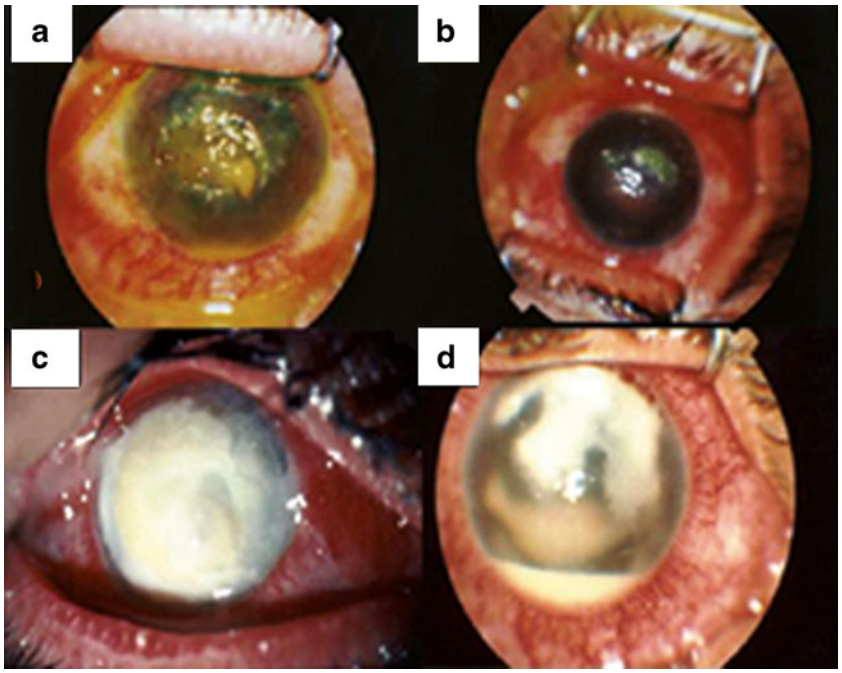

Fig. 1. Slit lamp photographs of two fungal keratitis patients who showed effectiveness of the treatment of the prepared CTZ ophthalmic solution. **a** and **c** Before treatment. **b** and **d** Twenty-one days after treatment

treatment of fungal corneal infection, and a combination with a polyene derivative (14) or other azole compounds (24) should be considered.

In our study, the area of the ulcer and stromal necrosis were evaluated to determine the degree of the case progression. Mild ulcers exhibited almost the same response in both CTZ and amphotericin B groups. However, severe ulcer showed a significant ($P<0.05$) positive outcome in the CTZ group. Also, the topical application of CTZ–β-CD eyedrops resulted in a favorable response in 80% of the cases with a shorter mean duration of treatment than amphotericin B. This distinguished effect may be a result of a higher drug concentration in the corneal stroma of topical CTZ–β-CD than of topical amphotericin B.

Topically applied drugs need to be at least partially soluble in the aqueous tear fluid, according to the studies. In addition, they must be lipid soluble to penetrate the lipophilic corneal epithelium, pass through the corneal stroma and the lipophilic endothelium, and enter the aqueous humor (25). In short, an effective formulation in an aqueous eyedrop solution needs to be hydrophilic and hydrophobic. This is the reason why hydrophilic β-CD was used as a carrier, because it sustains the lipophilic water-insoluble drug molecules in a solution and delivers them to the surface of the membrane where they partition from the cyclodextrin cavity into the lipophilic membrane (26). The inclusion complexation of CTZ with β-CD in this study was successful in improving the CTZ water solubility and the resulting antimycotic activity and stability. The use of traditional adjuvants in CTZ–β-CD aqueous eyedrop formulations is in a good position to enhance the CTZ ocular bioavailability by reducing the cornea's barrier function, e.g., benzalkonium chloride (27), and/or by adding to the time that the drug has contact with the eye surface, e.g., viscosity enhancers, methyl cellulose.

CONCLUSION

In the present study, CTZ–β-CD was prepared as an ophthalmic solution. The complexation of CTZ with β-CD improved CTZ solubility and consequently its ocular bioavailability. Moreover, this preparation is economically and

Table V. Therapeutic Efficacy of CTZ and Amphotericin Groups for Different Genera (*n* (percentage))

	CTZ group			Amphotericin group		
Genus	Total	Effective	Ineffective	Total	Effective	Ineffective
Fusarium	11	8 (72)	3 (28)	15	11 (73)	4 (27)
Aspergillus	9	7 (78)	2 (22)	6	5 (83)	1 (17)
Candida	4	4 (100)[a]	0 (0)	3	1 (33)	2 (67)
Penicillium	1	1 (100)	0 (0)	1	1 (100)	0 (0)

CTZ clotrimazole
[a] Significant difference ($P<0.05$)

practically effective, with an adequate shelf life. Our study is retrospective, and there are some limitations to our findings. However, we believe that the CTZ–β-CD eyedrop may be suggested as an alternative choice in the treatment of fungal keratitis in developing countries where other treatment options are unstable, expensive, or hardly available.

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