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ORIGINAL ARTICLE

A novel sustained-release clotrimazole varnish for local treatment of oral candidiasis

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Abstract The use of dental varnish for therapeutic purposes has been reported for fluoride or antibacterial drugs. Our objectives were to develop a sustained-release varnish containing an antifungal drug (clotrimazole) for topical application and to evaluate the release rate of the drug in human saliva in comparison with an available commercial troche and their acceptance by healthy volunteers. Following in vitro optimization of the release rate from the varnish, we have embarked on a crossover comparative study assessing the oral sensations and pharmacokinetics of a 10-mg clotrimazole oral troche versus a 10-mg sustained-release clotrimazole varnish in 14 human volunteers over a period of 5 h. Saliva samples were assessed for clotrimazole concentration by high performance liquid chromatography analysis. The volunteers' evaluation of the varnish and troche (taste, other sensory changes, convenience, and oral suitability) were recorded. At all time points, salivary clotrimazole concentrations were higher, and the terminal half-life was significantly prolonged in the varnish group in comparison to the control group. This can be attributed to continuous release of clotrimazole from the varnish formulation. The duration of the drug over the minimal inhibitory concentration, following application of the varnish, was more than threefold longer than following administration of the troche. The developed sustained-release varnish can be applied in patients at a lower frequency than troches, thus, achieving higher patient compliance and efficacy. This novel varnish application can serve as the basis for a new treatment approach to oral candidiasis, a very common chronic opportunistic infection with improved clinical outcome.

Keywords Sustained-release delivery system · Clotrimazole · Saliva · Varnish · *Candida* · Topical dental application

Introduction

Recently, local sustained-release varnishes have become available for therapeutic aims such as prolonged chlorhexidine release to eliminate mutans streptococci [1], fluoride [2], and antibacterial drugs [3]. Local sustained-release varnishes (SRV) extend the time during which the drug is present in the oral cavity, thus, enhancing its therapeutic potential. Due to its prolonged release rate, no high initial burst effect is recorded as in other dosage forms resulting in relatively reduced side effects [3, 4]. Several SRVs have been developed and suggested for dental application [4]; several of them have been approved for marketing: To the best of our knowledge, no oral antifungal sustained-release device has been reported.

Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of *Candida*

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species, the most common being *Candida albicans* [5]. *C. albicans* is the most common fungal infection in humans [6] and may be commensal in as many as 40% to 65% of healthy adult mouths [7], inducing opportunistic infections when appropriate predisposing factors exist (changes at the oral mucosal environment or at the immune status of the host) [8–14]. It has a variety of clinical manifestations, such as irritation, burning sensation, or pain [15] and varying morbidity to mortality in immunocompromised patients [16].

Prevention of superficial oral infections is crucial in order to improve the quality of life as well as to prevent the possible development of systemic fungal infection. Management of superficial oral candidiasis is achieved primarily by polyenes (nystatin and amphotericin B) and azoles (e.g., clotrimazole, fluconazole). Dosage form, side effects, and clinical efficacy vary for each of them. Clotrimazole is a well-tolerated fungistatic drug with anticandidal and antistaphylococal activity [6]. Minimal inhibitory concentration (MIC) for clinical isolates of *C. albicans* is up to 2 μg/ml [17]. Topical application to the mucosa provides adequate therapeutic concentrations. A 10-mg troche is the most common method of delivery of clotrimazole for treating oral candidiasis [13] and is effective for prophylaxis in patients undergoing chemotherapy, myeloablative treatment, and transplant recipients and for patients with solid malignant neoplasms [12, 18–20].

The troche should be slowly dissolved in the mouth for prolonged action. However, the fungistatic concentration is maintained for only a few hours [13], and clotrimazole troches need to be taken five times a day in order to achieve a prolonged therapeutic level. This multiple daily dose regimen may adversely affect the patient's compliance. This can lead to the drug being taken at a lower frequency, resulting in decreased concentration levels and therapeutic failure.

Continuous exposure to clotrimazole, provided by a sustained-release dosage form, bears many clinical and pharmacological advantages over the current treatment with troches. To date, sustained-release delivery applications in the oral cavity containing various antiseptic and antibiotic agents have been used to treat caries, periodontal diseases, and fungal infections [4, 21–27].

In the present study, a novel sustained-release varnish for use in the oral cavity (varnish for application on teeth) was developed. This varnish allows the slow and controlled release of the drug from a polymeric matrix and thereby prolongs the duration of drug action. The goal of the study was to evaluate human salivary levels of clotrimazole from the novel sustained-release varnish in comparison with that from a troche and to compare the compliance to the formulations.

Materials and methods

Chemicals

Clotrimazole was obtained from Taro Pharmaceutical Co., Haifa, Israel. Ethyl cellulose (Ethocel premium N100) was purchased from Dow Chemical Company, Russellville, AR, USA and hydroxypropylcellulose (Klucel EF) was purchased from Hercules Inc., Wilmington, DE, USA. All other chemicals were of analytical reagent grade, and the solvents were of high performance liquid chromatography (HPLC) grade. The internal standard, ketoconazole, triethylamine, ammonium hydroxide, tetrahydrofuran, and *N*-butyl-chloride were obtained from Sigma (St. Louis, MO, USA).

Tested formulations

Tested formulations were prepared according to other SRVs tested in vitro and in vivo [3, 4]. The hydrophobic formulation was chosen as it contains ethanol to allow quicker evaporation of the solvent and rapid formation of the coating film. The active agent was clotrimazole as to compare with the control group of troches containing clotrimazole. To determine the optimal composition of varnish for clinical study, different film dosage forms containing clotrimazole were prepared, and their clotrimazole release rate was evaluated in vitro. All tested formulations contained the same amount of clotrimazole and ethyl cellulose and the varying amount of hydroxypropylcellulose (10–30%). The film dosage form was prepared by dissolving the active ingredient and polymers in absolute ethanol. After a homogenous solution was obtained, the solution was casted into Teflon molds and was allowed to dry completely at room temperature to form a film. The films were then removed from the plates, and the mean thickness of each dry film was determined by a micrometer (Mitutoyo, Tokyo, Japan). The mean thickness of films was 148 μm.

For clinical trial, the varnish was prepared by dissolving 1.2 g of clotrimazole, 0.9 g of ethyl cellulose, and 0.9 g hydroxypropylcellulose in 10 ml ethanol. The composition corresponds to the film dosage forms containing 30% of hydroxypropylcellulose in dry fill. Oralten® troche (Agis Industries, Yeruham, Israel) containing 10 mg clotrimazole and 903.5 mg dextrose served as control.

In vitro release kinetics assessment

The clotrimazole release experiments were conducted by immersing 4 cm^2 films (n=3) in 10% sodium dodecyl sulfate aqueous solution while freely shaking at 100 rpm (Orbit shaker, Lab-Line Instruments Inc., USA), at 37°C.

The dissolution medium was maintained at all times, in a volume which the maximal concentration of the agent was at least ten times than its maximal dissolution value. At each period, an aliquot was withdrawn, and the clotrimazole concentration was determined by HPLC, as described below.

Clinical study

The Israel Ministry of Health Clinical Trials Unit and the Ethics Committee of The Hebrew University-Hadassah Medical Center, Jerusalem, Israel, approved the study. Fourteen healthy volunteers (seven males, seven females; mean age=25; range, 22 to 29 years) participated in this crossover study. Medical questionnaires, oral clinical examination by an oral medicine specialist, and informed consent were conducted for the study. Exclusion criteria were known allergy to the drug, recent use of antimycotics, pregnancy, erosive oral lesions, or xerostomic drugs taken 2 weeks prior to the study. In all volunteers, the varnish was administered first, and after a washout period of at least 2 weeks, the control troche was administered. When studying the troche, the volunteers were asked to dissolve it in their mouth. For varnish administration, a preweighed flask (containing varnish solution) with a brush applicator was assigned to each volunteer. Four brush strokes of varnish were applied to the buccal surface of the four dried upper incisors and left to dry for 60 s (Fig. 1). After applying the varnish, the brush applicator was quickly used to reseal the flask for later determination of the amount of varnish applied. The volunteers were not allowed to smoke or eat for the duration of the experiment but were allowed to drink water ad libitum (except during a 10-min period before each saliva sampling). For evaluation of clotrimazole concentrations, sequential saliva samples were obtained from the volunteers. On both sessions (varnish or troche administration), an unstimulated saliva sample (2 ml in a calibrated tube) was collected 5 min before the application in order to assess salivation. Further saliva samples were taken at 5, 30, 60, 120, 180, 240, and 300 min after clotrimazole administration. The saliva samples were immediately stored at −80°C until analyzed.

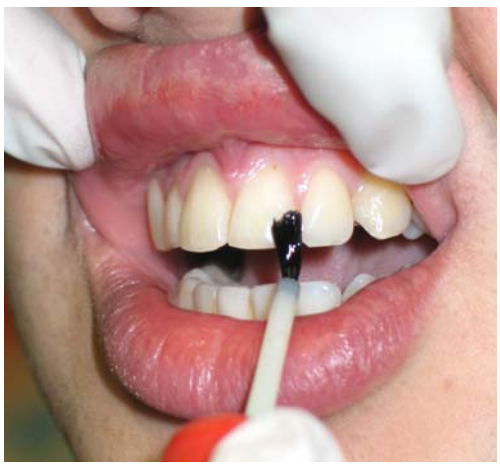

Fig. 1 Varnish application to the buccal surface of the four anterior upper incisors

The volunteers recorded taste and other sensory changes in the oral cavity and evaluated the convenience and suitability of the drugs. All parameters were recorded at 15, 60, 120, and 300 min after drug administration.

Sample analysis

Clotrimazole concentrations in the saliva samples were determined as described earlier by de Bruijn with slight modifications [28]. A calibration curve was achieved by using 400 µl of blank saliva with 100 µl of different clotrimazole concentrations dissolved in acetonitrile–water (1:1, *v*/*v*). Saliva samples (400 µl) were diluted with 100 µl of acetonitrile–water (1:1, *v*/*v*). Centrifugation was conducted twice for 10 min at 3,400 rpm. After drying the organic layer, the residue was reconstituted with 150 µl of acetonitrile–water (1:1, *v*/*v*). The separation was achieved at ambient temperature. Retention times for ketoconazole and clotrimazole were ~5.2 and 17 min, respectively. Each sample was tested in triplicate. The calibration curves were linear in the range of 0.49–250 µg/ml.

Data analysis

All pharmacokinetic and pharmacodynamic calculations were performed using WinNonlin® 5.0.1 software (Pharsight Corporation, Mountain View, CA, USA). The area under the curve (AUC), the terminal half-life, and the time period above the minimally inhibitory concentration ($T>$ MIC) for clotrimazole against *C. albicans* were determined from the concentration–time curves. All data are presented as the mean±SD. The between-groups difference was assessed for statistical significance by the paired two-tailed Student's *t* test.

Results

Clotrimazole release kinetics

The rate of clotrimazole release from different film dosage forms and Oralten troche was measured in vitro. As can be seen in Fig. 2, the complete release of clotrimazole from troche was achieved within 1 h. For film dosage form, the

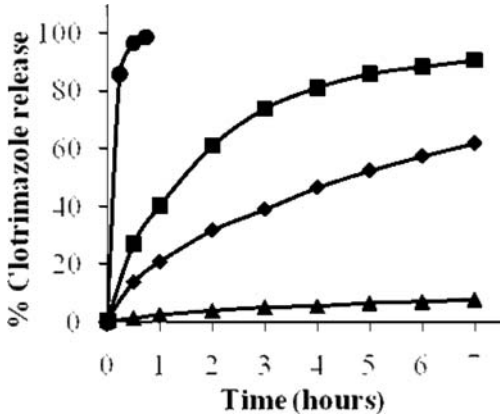

Fig. 2 Mean release kinetics of clotrimazole from troche (*filled circles*) and from film dosage forms containing the same amount of ethyl cellulose and various percentage of hydroxypropylcellulose: 10% (*filled triangles*), 20% (*filled diamonds*), 30% (*filled squares*)

increase in amount of hydroxypropylcellulose resulted in a significant increase in clotrimazole release rate. The formulation containing 30% of hydroxypropylcellulose released approximately 90% of clotrimazole during 6 h, and this formulation was chosen for evaluation in the clinical trial.

Pharmacokinetics

In the present study, the release rate of clotrimazole from the newly developed sustained-release formulation (varnish for application on teeth) was tested vs. the control, clotrimazole troche (Oralten®), in a crossover study in human volunteers. The baseline saliva flow rate was very similar in both groups. The salivary flow rate for the first 5 min before application of the varnish and troche administration was 3.9±2.5 and 4.3±2.0 ml, respectively. The amount of clotrimazole applied as varnish was 9.21± 2.27 mg (mean±SD), and the saliva concentration data were normalized to allow comparison with 10 mg troche. Following drug administrations, at all time points, salivary clotrimazole concentrations were higher in the varnish group than in the troche group (Fig. 3). The initial (up to ~60 min) very rapid decline in concentration was observed in both groups, and it was followed by a much slower elimination phase. The terminal phases of the kinetic rates were significantly different between the two groups (Table 1). During the experiment, the varnish group showed an area under the curve $(AUC_{0\to300})$, which was more than double that of the troche group ($p<0.001$). Since clotrimazole was not totally cleared from the saliva, at the last sampling point of the experiment, the concentration–time profile was extrapolated according to the corresponding terminal slope giving $AUC_{0\to\infty}$ (Table 1). The mean $AUC_{0\to\infty}$ for the varnish was also more than twofold greater than the corresponding value of the troche group.

Pharmacodynamics

The pharmacodynamic comparison of the two formulations can be simulated by calculating time over MIC of clotrimazole that was obtained in saliva. The time over MIC following application of the varnish was more than threefold longer than following administration of the troche (Table 1).

It should be noted that extrapolation of the concentration–time curve used in calculating $AUC_{0\to\infty}$ and time over MIC (extrapolated) for the varnish group should be viewed with caution since the terminal slope of the curve might change once all the clotrimazole is released from the varnish formulation.

Oral sensation changes

When asked to describe the convenience of applying the new drug in the oral cavity: 59±18.5% of the volunteers found it convenient, 66±18.5% thought the varnish was suitable for use in the oral cavity vs. 87.5±13% who thought the troches suitable. Fifteen minutes after using the drugs, 12/14 (86%) reported a change in oral sensation in the varnish group and 6/15 (43%) of the troche group (Fig. 4a). Altered taste sensations were reported by ten and six volunteers reported in the varnish and troche's groups, respectively. (Fig. 4b). All the reported taste and sensation changes declined over time.

Sensory changes were reported in both groups. In the varnish group, they were reported immediately after drug administration, declining gradually over the tested period. Some volunteers reported an altered taste sensation—mainly, a bitter taste; some reported dryness and a burning sensation in the upper lip. Changes in the oral cavity were mainly noted as tongue tingling, a burning sensation, dryness, and a bitter taste. To test whether there was a

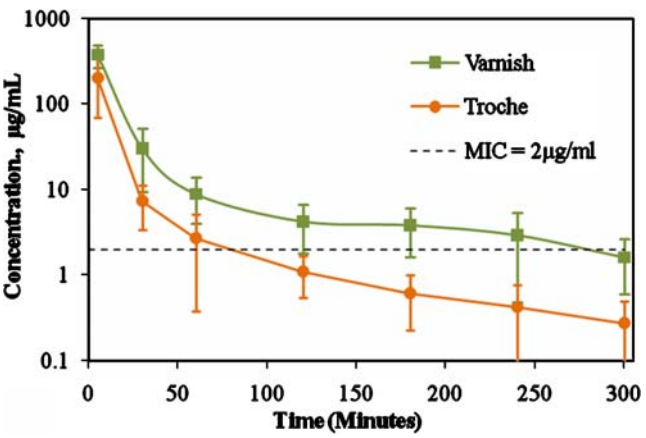

Fig. 3 Kinetics of clotrimazole released into saliva over time (mean±SD)

Table 1 Calculated AUC and time period above MIC

Parameter	Varnish		Troche	
	Mean	SD	Mean	SD
AUC 0→300 (μg·min/ml)*	7,707	2,197	3,837	2,624
AUC 0→∞ (μg·min/ml)*	8,132	2,365	3,878	2,637
Observed T>MIC (min)*	245	62	74	33
Extrapolated T>MIC (min)*	284	134	74	33
Initial half-life (min)**	10.23	1.32	9.18	1.64
Terminal slope (min^{-1})*	0.0061	0.003	0.0097	0.0042

AUC area under curve, *MIC* minimal inhibitory concentration

*$p<0.001$ (significantly different)

**$p=0.1$ (not significantly different)

directional difference between paired oral sensations in the same volunteer at a specific time point, the McNemar test was applied. This statistical test was used to check whether there was a trend among the cases with a discrepancy between paired observations (Table 2). The results show that the slight difference between the two drugs among the volunteers showing a discrepancy was not significant.

Discussion

The aim of our study was to investigate a novel formulation of clotrimazole embedded in a sustained-release varnish compared with the troches currently in clinical use. The in vitro evaluation of the film dosage forms allowed to optimize the ratio between more hydrophobic (ethyl cellulose) and more hydrophilic (hydroxypropylcellulose) components of formulation and to attain the desired release rate of clotrimazole. The composition that allows sustained release of the active ingredient for about 6 h was further evaluated in human volunteers.

Several sustained-release formulations have been proposed for general treatment of candidiasis [29]. Dental candidiasis is a unique chronic disorder of the oral cavity, which requires a dosage form suitable specifically for this disease. Therefore, it is conceivable that extending the duration of the drug in the oral cavity is a primary step in enhancing the clinical efficacy of the drug. The oral cavity is unique as the varnish can be applied on hard natural surfaces such as teeth or artificial surfaces such as dentures in addition to soft mucosal surfaces.

We found a gradual decrease in the salivary concentrations of clotrimazole following administration of troches, which is usually explained by a sustained release of clotrimazole adsorbed to the oral mucosa [13]. An additional (statistically significant) prolongation of the terminal half-life evident in the varnish group can be attributed to continuous release of clotrimazole from the varnish formulation. The initial rapid decrease in concentration in the varnish group can be explained by an initial burst release that occurred before the varnish had dried on the teeth.

Oral candidiasis appears in patients suffering from hyposalivation [10, 14] as a complication following chemotherapy or radiotherapy [12] as well as high percentage of elderly denture wearers [8, 9]. Systemic risk factors for oral candidiasis include corticosteroid and broad-spectrum antibiotics treatments, diabetes mellitus, common endocrine disorders, HIV [11, 13], and other immunocompromised patients [7, 15].

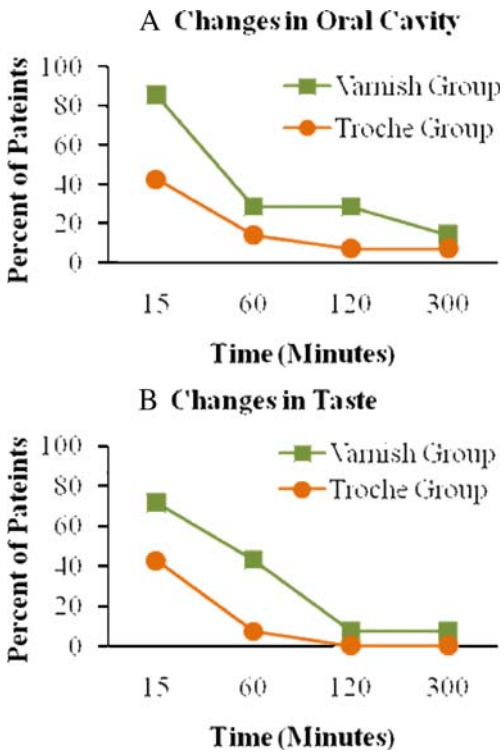

Fig. 4 Summary of sense changes (**a**) and taste changes (**b**) reported by the volunteers. **a** At 15 min after varnish application, 12 (85.71%) out of the 14 volunteers reported a changed sensation in the oral cavity, including burning, dryness, coldness, and tongue tingling sensations. At 15 min after administration of the Oralten troches, six (43%) volunteers experienced a brief change in sensation in the oral cavity, mainly tongue and mucosal dryness and roughness that quickly declined. One volunteer reported mucosal dryness and palatal roughness throughout the study; **b** 71% of volunteers reported mainly a bitter taste in the varnish group and 43% reported changes after Oralten administration. One volunteer reported an aftertaste

Table 2 McNemar's test

Sensory changes	Time (min)	Group				p value	Troche	
		A (%)	B (%)	C (%)	D (%)		−	+
Taste	15	28.6	0	28.6	42.9	0.125		
	60	57.1	0	35.7	7.1	0.063		
	120	92.9	0	7.1	0	–		
	300	92.9	0	7.1	0	–		
Lip/Tongue	15	28.6	7.1	7.1	14.3	0.07		
	60	64.3	7.1	50	0	0.375		
	120	78.6	0	28.6	0	–		
	300	85.7	0	21.4	0	–		
Oral	15	28.6	14.3	42.9	14.3	0.289		
	60	78.6	7.1	14.3	0	1		
	120	71.4	7.1	21.4	0	0.625		
	300	78.6	7.1	14.3	0	1		
Varnish							Changed sensation	
−							A	B
+							C	D

Groups B and C represent volunteers who had different sensations with varnish compared to troche. No significant difference in sensation between the two groups (p is not smaller than 0.05)

Denture wearers are prone to a more complicated situation, since a high percentage of denture wearers are diagnosed with chronic atrophic candidiasis ("denture stomatitis") [8, 9, 30, 31], especially if the dentures are worn continuously throughout the night [32]. Palatal inflammation occurs in response to direct yeast invasion of the mucosa and recurring infection of the palate by *Candida* on the denture surface [33].

Polyenes (nystatin and amphotericin B) and azoles (e.g., clotrimazole, fluconazole) are the main drug groups for treating oral superficial candidiasis. Amphotericin B, flucytosine, itraconazole, and ketoconazole have been the mainstream systemic antifungal therapies for many years; however, drug toxicity, emergence of resistant strains, and low efficacy have limited their use clinically, and the lipid formulation of amphotericin B is indicated for invasive fungal infections (by IV administration). Fluconazole interacts with many drugs; therefore, precautions need to be taken when prescribing that drug [34]. Topical treatment with nystatin rinses may be unpalatable and may result in low patient compliance. In general, clotrimazole is well tolerated by most patients [35].

Treatment with troches may be less effective if retaining them in the mouth is done while the denture is in place and the affected tissue is not exposed to the drug. On the other hand, removal of the denture several times a day (for clotrimazole troches, five times a day is recommended) is inconvenient and is usually avoided by the patients. Sustained-release varnish application on the denture surface can achieve direct contact with the infected tissue and reduce recurrence of infection. In addition, the number of applications of the SRV may be reduced depending on the release rate of the drug and the MIC achieved in the oral cavity.

Drugs administered systemically are distributed to body organs and are not confined to the target organ. More so, the drugs are diluted several hundredfolds before reaching the infected site, thus, increasing the possibility of side effects and development of resistance. On the other hand, local application of drugs targets the drug to the desired site, thereby reducing the amount of the administered drug and at the same time demonstrating the same or even better results than systemic application. Troches are local delivery systems for applying clotrimazole to the oral cavity. However, one of the main disadvantages of this dosage form is that they fail to maintain therapeutic concentrations of the drug for sufficiently long periods, therefore, reducing the clinical efficacy of the drug.

The reduction of drug administration frequency is significant in all patients. An estimated 20% to 60% of patients do not take their medications as prescribed and are said to be nonadherent or noncompliant with chronic drug therapy [36, 37]. It was found that adherence was inversely proportional to frequency of dose and that scheduling a medication four times daily resulted in average adherence rates of 50% to 60% [38, 39]. Therefore, reducing dosage from five times daily to twice a day may improve drug adherence.

The reported sensory changes were probably due to the ethanol solvent in the sustained-release device. Less than half of the Oralten group volunteers reported sensory changes, most of which were positive taste sensations, which could be accounted for by the high level of dextrose in each troche (~99%). The sustained-release varnish did

not contain any taste-masking agents. Improving varnish taste by an artificial sweetener may lead to higher acceptance of the varnish. In addition, improved sustained-release formulation involving an aqueous-based varnish should overcome the sensation caused by the ethanolic component of the tested varnish. In our study, the varnish was applied on four teeth only, and application to more teeth or to a larger area, as dentures, will reduce the film thickness of the varnish and should be considered.

Conclusion

In this study, we have shown a newly developed formulation of sustained-release varnish of clotrimazole that allows for elevated clotrimazole concentrations over a longer period of time in comparison with clotrimazole troches. Improved compliance, prolonged release, and clinical efficacy (not tested in this study) of the clotrimazole are a great advantage of the new drug carrier for patients suffering from oral candidiasis. This novel formulation can serve as the basis for a new prevention or treatment approach to oral candidiasis, with improved clinical outcome.

Conflict of interest The authors declare that they have no conflict of interest.

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