



Original article

Evaluation of the antifungal activity of tazarotene 0.1% gel in comparison to tioconazole 28% solution in treating onychomycosis: a clinical, microbiological and in vitro study

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ABSTRACT

Background: Onychomycosis is a fungal infection of the nail units caused by dermatophytes, molds or yeasts. Onychomycosis accounts for 50% of all nail diseases, representing a significant cosmetic concern. Topical antifungals are of limited efficacy owing to their poor absorption. Tazarotene was occasionally used as an off-label treatment for onychomycosis. **Aim:** Based on the immune-modulating and anti-inflammatory activity of tazarotene, we aimed to test the activity of tazarotene 0.1 % gel against fungi isolated from patients suffering from onychomycosis and comparing it to another antifungal drug of moderate efficacy, tioconazole 28%. **Methods:** Seventy patients with confirmed clinical and laboratory diagnosis of onychomycosis were enrolled in the study. Patients were treated with either tazarotene 0.1% gel or tioconazole 28% solution for 12 weeks. Follow-up of the patients was done after 3 months of the therapy stoppage. Determination of the onychomycosis severity based on the onychomycosis severity index and mycological studies was done at week 0 and 24. Antifungal susceptibility testing of tazarotene against the isolated fungi was done. **Results:** Tazarotene showed antifungal activity manifested by induction of a mycological cure in 25.7 % of cases; however, this effect was comparable to tioconazole 28%. Tazarotene showed a good efficacy against *Aspergillus niger* in vitro. **Conclusions:** Tazarotene 0.1 % gel has antifungal activity comparable to tioconazole 28% solution in treating onychomycosis. They both achieved mycological cure in about 25% of cases. *Aspergillus niger* was the most sensitive species to tazarotene. Tazarotene could be prescribed as an adjuvant to the standard antifungals for treatment of onychomycosis.

Introduction

Onychomycosis is a fungal infection of the fingernails or toenails that causes discoloration, thickening, and onycholysis. Dermatophytes, especially of genus *Trichophyton*, account for most

of the cases [1]. Other pathogens like candida and non-dermatophytic moulds are more frequent in countries with hot and humid climate [2]. Nail diseases like psoriasis, lichen planus and chronic nail

trauma may mimic onychomycosis. Thus, an accurate diagnosis is crucial for a successful treatment of onychomycosis, making a laboratory identification via direct microscopy and fungal culture is a must [1].

Onychomycosis is mainly considered a cosmetic problem; however, its treatment is still problematic. The tough nail plate represents a barrier resulting in a poor drug delivery and long treatment duration. Moreover, persistent infections and relapses are frequently encountered. Topical agents have the advantage of few contraindications and lack of drug-drug interactions. However, the compact structure of the dorsal nail plate acting as a barrier to topical drug diffusion results in a limited efficacy, especially if used as monotherapy. Systemic antifungals are the gold standard effective treatment, with the highest clinical and mycological cure rates, but they have the risk of multiple drug interactions, systemic side effects and intolerability in some patients with systemic diseases [3].

Tazarotene is a retinoic acid with a demonstrated efficacy in psoriasis treatment. Tazarotene can regulate keratinocyte differentiation and proliferation with an immunomodulating and anti-inflammatory actions [4]. Topically applied tazarotene 0.1 % gel was investigated for onychomycosis treatment and it was reported that the daily topical application for 12 weeks was effective for clinical and mycological cure of onychomycosis [5]. To address this, we studied the clinical response of 35 patients suffering from onychomycosis to treatment with tazarotene 0.1% gel and compared this response to that obtained in another 35 patients treated with tioconazole 28% solution, an antifungal drug with moderate activity. In vitro tazarotene antifungal susceptibility testing of the fungi isolated from the 70 patients was also investigated.

Patients and methods

An open-label, active-controlled, single-center, clinical study was conducted on 70 patients attending the dermatology clinic in Ain Shams University Hospital. The study was approved by The Research Ethics Committee, Ain Shams University, Faculty of Medicine (FWA 00017585). A written informed consent was obtained from each patient.

I- Patients

Seventy patients (29 males and 41 females) with clinical signs of onychomycosis of toe(s) and/or finger(s) nails were enrolled in the study. The diagnosis was confirmed via laboratory studies. All

patients were subjected to a detailed history taking with a focus on the general and dermatological diseases which might cause nail dystrophy. Detailed drug history including all previous treatments for the infected nails was obtained. The data were filled and recorded. Careful general and dermatological examinations were done looking for signs of systemic diseases or dermatological diseases that could cause nail abnormalities or nail dystrophies. Patients who were currently or have received topical or systemic antifungal treatment in the previous 6 months, patients with immunosuppression and patients with other diseases causing nail dystrophy were excluded from the study.

II- Methods

Specimen collection

Nail samples, two consecutive samples two weeks apart, were obtained by scrapings and/or clippings depending on clinical type of onychomycosis, using a sterile spatula and scissors, respectively. Nail area antiseptics was done by alcohol swab before sample collection. All samples were collected in clean dry containers and labeled for identification.

Microbiological examination

Microbiological examination was done at Microbiology laboratory of Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, at baseline (before treatment, week 0) and at 3 months following the stoppage of the 12 weeks treatment course (week 24). Samples were prepared via 20% KOH (Sigma-Aldrich[®], Germany) 3-6 hours and examined with x10 and x40 objective lenses of the light microscope (Olympus[®] CX41, Japan) for the presence of fungal hyphae, spores and conidia or yeast cells [6]. Each specimen was cultured on four plates (2sets) of Sabouraud's dextrose agar (SDA) (Oxoid[®], UK) supplemented with chloramphenicol (Sigma-Aldrich[®], Germany) and with or without cycloheximide (Sigma-Aldrich[®], Germany). One set was incubated at 35 °C and the other was incubated at room temperature (~22 °C). Detection of growth was checked after 48 hours and daily for 4 weeks. All plates were incubated for 4 weeks before reporting as being negative. However, all included plates showed growth within a maximum of 10 days. Identification of the growth was done by the rate of growth, colonial morphology and microscopic examination according to the key for identification for dermatophytes, non-dermatophytes and yeasts [7-10]. Non-dermatophyte moulds were considered the pathogens according to the criteria

described by **Tosti et al.** [11]. Slides for microscopic examination of colonies were prepared by the transparency tape preparation, using methylene blue stain or a Gram stained film and examined using the x10, x40 objective lenses of the light microscope and oil immersion lens (in case of colonial morphology suggesting candida). Germ tube production test was performed when *Candida* species were suspected [6]

In vitro antifungal susceptibility testing of tazarotene on the isolated fungi

Antifungal susceptibility testing of tazarotene was done in vitro by agar well diffusion method as described by **Campione et al.** [5]. All isolated fungi were cultured on SDA medium and incubated for 2 to 6 days. Suspension from every isolate was made by emulsifying it in a sterile saline and its turbidity was adjusted to the turbidity of 0.5 McFarland's standard (Oxoid®, UK). A sterile swab was dipped in the inoculum suspension, and then rubbed over a plate of Mueller-Hinton agar (Oxoid®, UK) in three different directions to ensure even distribution.

Using a sterile metal cylinder, a 4mm width x 3mm depth well was punched in the middle of the plate. The well was filled with 1mg/ml tazarotene solution (~37.5 mm³) using a sterile spatula. Plates were left for 2 hours in room temperature to allow the drug to diffuse, then were incubated for up to 6 days at 35 °C. Following incubation, the zone of inhibition of fungal growth was measured to determine susceptibility to the drug (0.6 cm = mild sensitivity, >0.6 and up to 1.2 cm = moderate sensitivity, > 1.2 cm and up to 2 cm or higher = highly sensitive and if no inhibition or zone < 0.6 cm = resistance).

Treatment application and evaluation of treatment response

All patients were assessed clinically and photographically documented before treatment (week 0), every month during the treatment and at 3 months after treatment stoppage (week 24). The clinical pattern of onychomycosis was determined, and the severity was evaluated and scored according to onychomycosis severity index (OSI) [12]. Patients with medical files of odd numbers (group 1) were treated with tazarotene 0.1% gel twice daily for 12 weeks, while those with even numbers (group 2) were treated with tioconazole 28% solution with the same regimen. At week 24, re-assessment of OSI scoring was done. The clinical response to treatment was evaluated as worsen, stable or insignificant response, partial response (≥40-< 80% improvement), and complete response (≥80-100% improvement). There are lots of variations on determination of

clinical and mycological cure in literature. The implementation of the clinical improvement of 80% or more and negative cultures as determinants for the clinical and mycological cure, respectively, was more rational and this was adopted in this study to define clinical and mycological cure [13]. Patient's satisfaction was evaluated at week 24 as one of the following categories: the condition worsened or nothing changed, partially satisfied, or fully satisfied.

Data management and analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS), version 22. Data was presented and suitable analysis was done according to the type of data obtained for each parameter. Chi-square test, Mann-Whitney test, Independent t-test, and Wilcoxon statistical tests were used to compare variables.

Results

The study included 70 cases with onychomycosis (clinically and mycologically confirmed). The age of patients ranged from 9 to 61 with a mean age of $33.9 \pm SD 11.66$ years. They were 41 females (58.6%) and 29 males (41.4 %). The severity of onychomycosis according to OSI was nearly similar between the two groups at week 0. No significant statistical differences between the two treatment groups were observed as regarding the personal, clinical or mycological characters as shown in **table (1)**. The two groups were considered to be comparable. Eight species of fungi were isolated including *Aspergillus niger* in 36 cases (51.4%), *Candida albicans* in 11 cases (15.7%), *Aspergillus flavus* in 4 cases (5.7%), *Fusarium species* in 3 cases (4.3%), *Aspergillus fumigatus* in 2 cases (2.9%), *Trichophyton rubrum* in 1 case (1.4%), *Acremonium species* in 1 case (1.4%) and *Microsporium gypseum* in 1 case (1.4%). Mixed fungal infection was noted among 15.7% of cases. Representative cases of isolated fungi are shown in **figure (1)**.

Tazarotene 0.1% gel achieved a mycological cure in nearly one fourth of the treated cases (25.7%), which was nearly comparable to 28.6 % mycological cure achieved with tioconazole 28% solution (**Table 2**) (*P value* >0.05). This difference between the two groups was not statistically significant nor was the difference for the clinical response or scoring of onychomycosis (**Table 2**). This resulted in similar patients' satisfactions to both treatments. Tazarotene was

generally tolerable without any side effects in 30 cases (85.7%) and induced mild skin irritation in 4 cases (11.4%) and mild peeling in one case (2.9%). The clinical responses to tazarotene 0.1% treatment are shown in **figure (2)**.

In vitro tazarotene sensitivity testing was performed for all the isolated fungi, and *Aspergillus niger* showed the highest degree of sensitivity among the tested fungi (**Figure 3**). Out of the 36 isolates of *Aspergillus niger*, 18 isolates were highly sensitive to

tazarotene, 12 and 3 isolates showed moderate and mild sensitivity respectively; while 3 cultures were resistant to tazarotene. Out of the 11 *Candida albicans* isolates, only two isolates showed mild sensitivity, while the other 11 isolates were resistant to tazarotene. Isolates of *Aspergillus flavus* and *fumigatus*, *Trichophyton rubrum*, *Fusarium species*, *Acremonium species* and *Microsporum gypseum*, all showed resistance toward tazarotene.

Table 1. The personal and clinical characteristics of the study groups.

Variable		Tazarotene 0.1%	Tioconazole 28%	Test value	P-value	Sig.
		n = 35	n = 35			
Age	Range	9 – 61	12 – 58	-0.092•	0.927	NS
	Mean + SD	33.77 ± 12.02	34.03 ± 11.47			
Sex	Female n. (%)	20 (57.1)	21 (60.0)	0.059*	0.808	NS
	Male n. (%)	15 (42.9)	14 (40.0)			
Clinical subtype n. (%)	DLSO	30 (85.7)	21 (60.0)	6.033*	0.110	NS
	PSO	2 (5.7)	7 (20.0)			
	WSO	1 (2.9)	3 (8.6)			
	TDS	2 (5.7)	4 (11.4)			
OSI at week 0	Median (IQR)	8 (4 – 14)	12 (8 – 20)	-1.331≠	0.183	NS
	Range	1 – 35	4 – 35			
OSI degree at week 0 n. (%)	Mild	10 (28.6)	5 (14.3)	2.301*	0.316	NS
	Moderate	18 (51.4)	20 (57.1)			
	Severe	7 (20.0)	10 (28.6)			

DLSO: distal-lateral subungual onychomycosis; IQR: interquartile range, NS: non-significant; OSI: onychomycosis severity index; PSO: proximal subungual onychomycosis; TDS: total onychodystrophy; WSO: White superficial onychomycosis.

P-value > 0.05: Non-significant; P-value < 0.05: Significant. *: Chi-square test; •: Independent t-test; ≠: Mann-Whitney test

Table 2. Treatment outcomes with tazarotene 0.1% and tioconazole 28%.

Variable		Tazarotene 0.1% n. = 35	Tioconazole 28% n. = 35	Test value	P-value	Sig.	
OSI Score	Week 0	Range	1– 35	-1.331≠	0.183	NS	
		Median (IQR)	8 (4-14)				
	Week 24	Range	0-35	0.546≠	0.589	NS	
		Median (IQR)	6 (2-10)				
	<i>P. value</i> intra-group		0.028** (S)	0.236** (NS)	NA		
MCR	Week 0	Negative Culture (n.&%)	0 (0.0)	-			
	Week 24	Negative Culture (n.&%)	9 (25.7)	10 (28.6)	0.072*	0.788	NS
	<i>P. value</i> intra-group		<0.001*	<0.001*	NA		
Clinical evaluation Week 24 n. (%)	Worsen		5 (14.3)	3 (8.6)	2.442*	0.486	NS
	Stable/insignificant		10 (28.6)	15 (42.9)			
	Partial response		11 (31.4)	7 (20.0)			
	Complete response		9 (25.7)	10 (28.6)			
Side effects n. (%)	Negative		30 (85.7)	35 (100.0)	5.385*	0.068	NS
	Irritation		4 (11.4)	0 (0.0)			
	Skin peeling		1 (2.9)	0 (0.0)			
Patient's satisfaction n. (%)	Satisfied		14 (40.0)	14 (40.0)	0.467*	0.792	NS
	Mild satisfied		7 (20.0)	5 (14.3)			
	Not satisfied		14 (40.0)	16 (45.7)			

IQR: interquartile range; MCR: mycological cure rate; NS: non-significant; OSI: onychomycosis severity index; NS: non-significant. *P-value* > 0.05: Non-significant; *P-value* < 0.05: Significant. *: Chi-square test; ≠: Mann-Whitney test; **: Wilcoxon test

Figure 1. Mycological cultures and microscopic films of representative cases of the study. a) *Candida albicans* species (from left to right, culture on SDA, budding yeast cells by Gram stain (x100), and positive germ tube test (x100), respectively). b) *Aspergillus niger* species with its brownish black colonies on SDA and methylene blue transparent tape preparation showing branched septate hyphae (x40). c) *Aspergillus Flavus* with granular colonies with yellowish pigmentation on SDA medium and transparent tape preparation showing branched transparent conidiophores& apparent large vesicle arising from uniseriate & biseriate phialides (x40). d) *Trichophyton rubrum* grown on SDA medium (Left: front surface, right: reverse side).

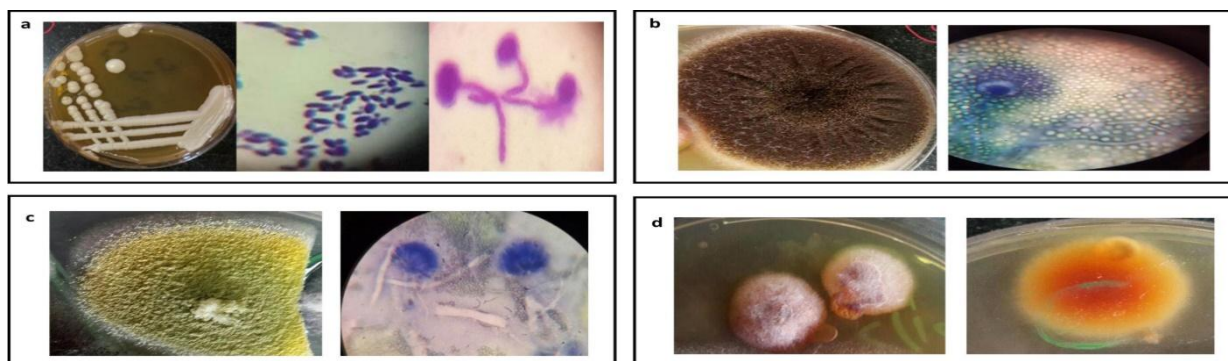


Figure 2. The clinical response to tazarotene 0.1% gel treatment in representative cases of the study (on the left: week 0, on the right: week 24). a, b, and c: complete clinical response to tazarotene. d: partial response with downgrading of OSI score from 35 (on the left) to 16 at week 24 (on the right).

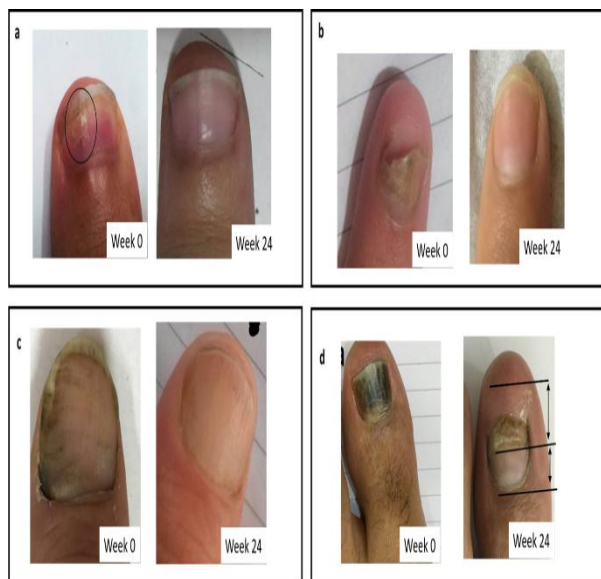
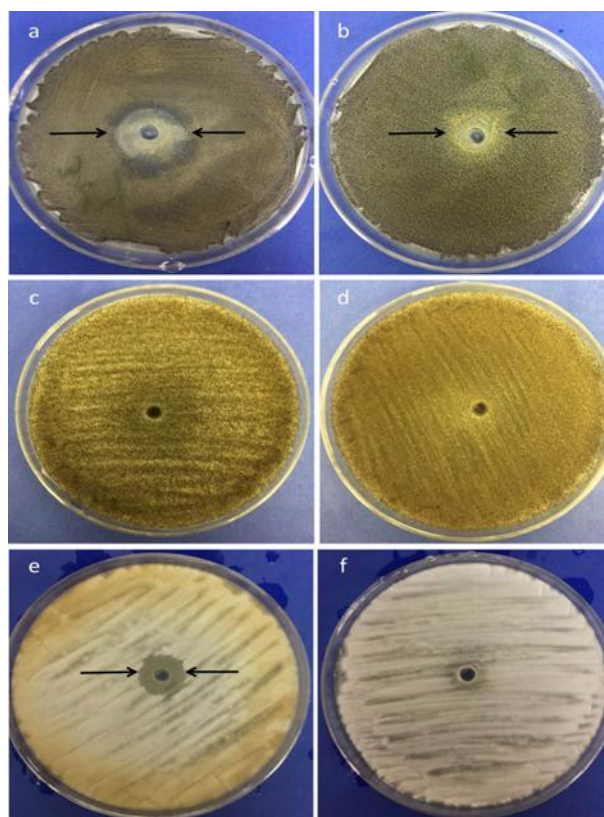


Figure 3. Tazarotene in vitro susceptibility testing of the isolated fungi carried on MHA. a,b,c,d: Four isolates of *Aspergillus niger*: a) zone of inhibition >1.2 cm (highly sensitive), b) zone of inhibition =0.6 cm (mild sensitivity), c & d: no zone of inhibition (resistant). e & f: Two isolates of *Candida albicans*: e) zone of inhibition > 0.6 and < 1.2 cm (moderate sensitivity), f) no zone of inhibition (resistant).



Discussion

Onychomycosis is a frequent health problem worldwide with a prevalence that varies between different parts of the world according to climate, cultural factors and socioeconomic status [1]. In a study in Assuit, Egypt, **Moubasher et al.** demonstrated that onychomycosis was the most frequent fungal dermatological infection accounting for 64.8% of the 125 studied cases, followed by tinea capitis [14]. Onychomycosis is not a life-threatening condition; nevertheless, it precludes the quality of life of the patients affected, as it causes nail disfigurement, and psychological discomfort [15]. Therefore, our study aimed to investigate a potential alternative drug to these suffering patients and to explore what is postulated about the effectiveness of tazarotene- a drug used for treating nail psoriasis and acne- for further application in the clinical context in treating onychomycosis.

Onychomycosis caused by non-dermatophytic moulds occurs more frequent in tropical and subtropical areas with a hot and humid climate. Candidal onychomycosis occurs more commonly in fingernails in people with frequent water exposure [2]. Meanwhile, dermatophytes are the fungi most commonly responsible for onychomycosis in the Western countries [1]. **Moubasher et al.** reported that non-dermatophytic moulds are important pathogens for skin and nail superficial mycosis in Assiut, Egypt as they followed yeasts and preceded dermatophytes as etiologies of mycotic infections [14]. *Aspergillus species* and, in particular, *Aspergillus niger* was one of the most frequent incriminated moulds [14]. This partially comes in accordance to the results of our study, as *Aspergillus niger* was the most frequent causative organism of onychomycosis in the studied cases. *Candida* was the second common, possibly because most of our patients were females with a high risk of hand exposure to water immersion during the household activity.

Treatment of onychomycosis comprises a challenge due to the low initial cure rates together with high rates of relapse. The hard nail plate is a natural barrier to topical agents. It acts also as a reservoir for fungal growth, because the nail unit is considered a site of relative immune privilege as it is isolated from the cell mediated immunity. This results in low clinical cure rates and high relapse rates [3,16]. Owing to the high prevalence rate of onychomycosis worldwide, together with its negative

psychological and physical problems, new treatments are thus arguably awaited.

Tazarotene is an established drug used in treatment of acne, inflammatory skin diseases such as psoriasis, non-melanoma skin tumors, and photoaging [17]. **Campione et al.** proposed a new possible therapeutic indication of Tazarotene [5]. The authors showed a complete clinical and mycological cure of all their enrolled onychomycosis cases through the use of a once daily application of topical tazarotene 0.1% gel for 12 weeks. They further demonstrated a reduced growth of *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, *Candida albicans*, and *Candida glabrata* in fungal cultures in response to *in vitro* exposure to tazarotene. However, owing to the study limitations in the form of the absence of control groups, the small number of cases (15 patients), enrollment of a single onychomycosis subtype (distal and lateral subungual onychomycosis) and lack of use of onychomycosis severity scoring systems, the antifungal activity of tazarotene needed to be reassessed and compared to the currently approved antifungals.

We found that tazarotene could effectively and significantly reduce the severity of onychomycosis, demonstrated by the downgrading of the OSI, which reflects improvement in the size of infected area, the burden of fungal infection, and the subungual hyperkeratosis. Hereby, we came in line with **Campione et al.** [5] who showed an improvement of the clinical signs of onychomycosis, including the size of the affected area, nail discoloration and subungual hyperkeratosis; however, their results were more striking with achievement of complete mycological cure in all patients, a result that we could only achieve in only one fourth of our tazarotene-treated patients, in spite of twice daily application compared to the once daily application in the aforementioned study. This difference might be explained by enrollment of a milder cases in that study with a faster response and better chance of treatment [18]. While one fifth of our patients suffered from severe infections. Also, the causative organisms in our study were more diverse including some species which are known to be resistant to treatment like *Fusarium species* [19].

We also compared the efficacy of tazarotene 0.1% gel to tioconazole 28% solution which was selected because of its moderate antifungal potency and its effectiveness against *Candida species* which was highly suspected to be incriminated in fingernail onychomycosis included in the study [2,20].

Tioconazole 28% achieved a mycological cure of 28.6%, which is nearly comparable to 22 % cure rate showed by **Hay et al.** [21]. We found that tazarotene 0.1 % gel showed comparable results to tioconazole 28% as regards the downgrading of onychomycosis severity and induction of mycological cure. The comparable efficacy between tazarotene and tioconazole has been also described by **Abd El-Aal et al.** , who combined fractional carbon dioxide (FrCO₂) with either tazarotene 0.1% or tioconazole 28% topical application in four treatment sessions and they showed that FrCO₂ is good for assisting the delivery of both drugs with a resultant efficacy that was comparable between them without any significant difference [22]. However, the resultant shown efficacy in the aforementioned study is also affected by the direct inhibitory effect of FrCO₂ on the fungal growth, as fungi are sensitive to temperature above 55°C, and ablative process of laser itself has fungicidal effect [23].

We studied *in-vitro* susceptibility of different isolated species to tazarotene. *Aspergillus niger* was the most susceptible among all tested fungi. A reduced fungal growth in response to tazarotene was also observed in two isolates from *Candida species*. While, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichophyton rubrum*, *Fusarium species*, *Microsporium gypseum* and *Acremonium species* were all resistant to *in vitro* tazarotene exposure. In contrast, **Campione et al.** showed that all their tested fungi showed *in vitro* susceptibility to tazarotene [5].

The exact mechanism underlying the antifungal activity of tazarotene remains elusive and it wasn't investigated in this study. The *in vivo* effect of tazarotene might be attributed to its immunomodulatory and anti-inflammatory effects [4]. Also, tazarotene may help in elimination of the fungal reservoir via its known modulatory effect on keratinocyte proliferation, differentiation and normalization of the abnormal keratinization [17]. However, both of the proposed mechanisms don't explain the observed *in vitro* inhibitory effects of tazarotene on fungal cultures. Thus, the mechanism of antifungal activity of tazarotene should be further and deeply investigated.

In conclusion, this report compares the antifungal effect of tazarotene 0.1 % gel to a known topical antifungal, tioconazole 28%, has shown that tazarotene 0.1% has a moderate capacity to treat onychomycosis cases which is comparable to tioconazole 28%. *Aspergillus niger* was the most

sensitive fungus species to in vitro tazarotene exposure. We cannot support the use of tazarotene 0.1% gel as a single treatment in cases of onychomycosis. Tazarotene could be prescribed as an adjuvant to the standard systemic or topical antifungals for treatment of onychomycosis, especially in case of presence of significant subungual hyperkeratosis or in cases of *Aspergillus niger* due to its abilities to reduce the fungal load and hyperkeratosis, allowing for better penetration and its demonstration of its in vitro high efficacy against this species.

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