FINAL REPORT

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Title: ***"Evaluating the efficacy of polihexanide (0.08% and 0.25%) compared to natamycin 5% in an animal model of fungal keratitis induced by Candida albicans"***

Study Sponsor: SIFI S.p.A., Via Ercole Patti 36, 95025, Aci Sant'Antonio (CT) Italy.

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Signed:..... Date: June 30, 2025

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STUDY AIM

The study aimed to evaluate the efficacy of polihexanide 0.08% (0.8 mg/mL) and polihexanide 0.25% (2.5 mg/mL) compared to natamycin 5% (5 mg/mL) the only approved drug in the United Stated (US), in a rabbit model of *Candida albicans* (ATCC 10231) keratitis.

MATERiALS AND METHODS

Ethical approvel: It is unanimously approved that the research project numbered G.Ü.ET-24.108 and entitled ***"Evaluating the efficacy of polihexanide (0.08% and 0.25%) compared to natamycin 5% in an animal model of fungal keratitis induced by Candida albicans"*** is in compliance with Gazi University Animal Experiments Local Ethics Commitee regulations.

Microorganism: *Candida albicans* ATCC 10231 (Hemakim, Turkey).
Lyophilized fungal isolate was inoculated into Sabouraud dextrose broth medium and incubated for 4 days at 30^{0}C. Yeast suspensions were prepared from growing colonies.

Animal studies: This sudy was performed at the "Gazi University Laboratory Animals Breeding and Experimental Researches Center, Ankara, Turkey".

Fungal keratitis was induced in the right eyes of New Zealand rabbits by intrastromal injection of *Candida albicans* (ATCC 10231) suspension into the cornea. Three days after inoculation, animals were randomly assigned to 4 different groups (n=6 in each group): 1) Control group was left without treatment; 2) natamycin group received topical natamycin 5% eye drops; 3) polihexanide group received topical polihexanide 0.08% eye drops; 4) polihexanide group received topical polihexanide 0.25% eye drops. In all treatment groups, animals were treated for 10 days, 12 times a day between 8am and 10pm, from days 3 (after inoculum) to days 13. **Table 1** shows the study design. Eyes were examined clinically on days 0, 3, and 13 for status of corneal healing, corneal vascularization, and hypopyon. Corneas were sampled under anestesia on day 13.
One rabbit in the control group died at day 2 due to causes unrelated to Candida infection, therefore this group is composed by n=5 rabbits.

Ref:

Kalkanci A, Yesilirmak N, Ozdemir HB, et al. Impact of Iontophoresis and PACK-CXL Corneal Concentrations of Antifungals in an In Vivo Model. Cornea. 2018; 37 (11): 1463-1467. doi:10.1097/ICO.0000000000001696

Özdemir HB, Kalkancı A, Bilgihan K, et al. Comparison of corneal collagen cross-linking (PACK-CXL) and voriconazole treatments in experimental fungal keratitis. Acta Ophthalmol. 2019;97(1):e91-e96. doi:10.1111/aos.13829

Qin XH, Ma X, Fang SF, Zhang ZZ, Lu JM. IL-17 produced by Th17 cells alleviates the severity of fungal keratitis by suppressing CX43 expression in corneal peripheral vascular endothelial cells. Cell Cycle. 2019; 18 (3): 274-287. doi:10.1080/15384101.2018.1556059

Table 1: Study design

New Zeland White Rabbits (Gazi University, Center of Animal Studies, Turkey), adult, 2-3 kg, right eyes			
	Day 0	**Day 3**	**Day 13**
Group 1: Keratitis control group untreated (n:5 eyes)	Intrastromal injection of *Candida albicans* (ATCC 10231) (10^6 CFU/ml in 10 microliter of distilled water)	Confirmation of keratitis (No treatment) Direct microscopy of corneal scrapings and microbiological culture	Follow-up of keratitis (No treatment) Direct microscopy of corneal scrapings and microbiological culture
Group 2: natamycin 5% treated group (n:6 eyes)	Intrastromal injection of *Candida albicans* (ATCC 10231) (10^6 CFU/ml in 10 microliter of distilled water)	Confirmation of keratitis and initiation of natamycin treatment (12x1 drop a day)	Follow-up of keratitis Direct microscopy of corneal scrapings and microbiological culture
Group 3: polihexanide 0.08% treated group (n:6 eyes)	Intrastromal injection of *Candida albicans* (ATCC 10231) (10^6 CFU/ml in 10 microliter of distilled water)	Confirmation of keratitis and initiation of polihexanide treatment (12x1 drop a day)	Follow-up of keratitis Direct microscopy of corneal scrapings and microbiological culture
Group 4: polihexanide 0.25% treated group (n:6 eyes)	Intrastromal injection of *Candida albicans* (ATCC 10231) (10^6 CFU/ml in 10 microliter of distilled water)	Confirmation of keratitis and initiation of polihexanide treatment (12x1 drop a day)	Follow-up of keratitis Direct microscopy of corneal scrapings and microbiological culture

Treatments: Polihexanide 0.08% (batch R&D 2511), see certificate of analysis at the end of the report), polihexanide 0.25% (batch R&D 2512), and natamycin 5% (batch R&D 2313) see certificate of analysis at the end of the report) eyedrops formulations were supplied by the Sponsor SIFI S.p.A. Treatments were performed 12 times a day, 1 hourly, for ten days in the right eye (50 µl) according to the treatment group. Treatments started at day 3 after the inoculum, once the infection was confirmed.

Endopoints at Day 3 and Day 13: Sample corneal scraping material from the deeper stromal layer of the cornea by using Kimura scalpel.

1) Clinical score

The extent of keratitis was evaluated by a masked observer. The severity of keratomycosis in the rabbits was scored with the scoring system described by Özdemir et al. 2019 for *Candida* keratitis. Additionally, Nejabat et al. 2016, used the same scoring system for *Fusarium* keratitis.

Each of the following criteria was graded: area of corneal opacity, density of opacity, and surface regularity (**Table 2**). A normal cornea was given a score of 0 in each category and a summation score of 0. The scores from these categories were tallied for each cornea to show a possible total score ranging from 0 to 12. A total score of 5 or less was considered mild, a total score of 6 to 9 was categorized as moderate, and a total score of more than 9 was considered severe.

Ref: *Özdemir HB, Kalkancı A, Bilgihan K, et al. Comparison of corneal collagen cross-linking (PACK-CXL) and voriconazole treatments in experimental fungal keratitis. Acta Ophthalmol. 2019;97(1):e91-e96. doi:10.1111/aos.13829.*

Ref: *Nejabat M, Yaqubi N, Khosravi A, Zomorodian K, Ashraf MJ, Salouti R. Therapeutic effect of intrastromal voriconazole, topical voriconazole, and topical natamycin on Fusarium keratitis in rabbit. J Ophthalmol. 2016;2016:8692830. doi: 10.1155/2016/8692830. Epub 2016 May 19. PMID: 27298734; PMCID: PMC4889827.*

Table 2: Quantitative scoring system for rabbit *Candida* keratitis. (*Özdemir HB, Kalkancı A, Bilgihan K, et al. Comparison of corneal collagen cross-linking (PACK-CXL) and voriconazole treatments in experimental fungal keratitis. Acta Ophthalmol. 2019;97(1):e91-e96. doi:10.1111/aos.13829*)

	Grade I	Grade II	Grade III	Grade IV
Area of corneal opacity	1%–25%	26%–50%	51%–75%	76%–100%
Density of corneal opacity	Slight cloudiness, outline of iris and pupil being discernable	Cloudy, but outline of iris and pupil remains visible	Cloudy, opacity not uniform	Uniform opacity
Surface regularity	Slight surface irregularity	Rough surface, some swelling	Significant swelling, crater or serious descemetocele formation	Perforation or descemetocele

2) Direct microscopy

Two Slides, one for wet mount preperation and one for methylen blue stain were preperad. Direct microscopy (+) means: at least one microscopic area (40X) is positive for pseudohyphae and/or yeasts, direct microscopy (++) means: at least two microscopic areas (40X) are positive for pseudohyphae and/or yeasts, direct microscopy (+++) means: at least three microscopic areas (40X) are positive for pseudohyphae and/or yeasts. Kruskal Wallis analysis was performed by the transfer of fungal load.

Ref: *Leck A. Taking a corneal scrape and making a diagnosis. Community Eye Health. 2009 Dec;22(71):42-3. PMID: 20212927; PMCID: PMC2823109.*

3) Microbiological culture

Kimura scalper was inoculated onto Sabouraud dextrose broth and 5% Sheep Blood agar. A 50 microliter of broth was than subcultured onto Sabouraud dextrose agar plates after 2 days incubation. Blood agar and Sabouraud agar plates were followed for 10 days. Results of quantitative cultures were documented. Culture (+) positive means: at least one *Candida* colony, culture (++) positive means: at least two *Candida* colonies. Colonies were counted as colony forming units (CFU) visually.

Ref: *Leck A. Taking a corneal scrape and making a diagnosis. Community Eye Health. 2009 Dec;22(71):42-3. PMID: 20212927; PMCID: PMC2823109.*

Statistical analysis: Statistical analysis was performed on clinical score, direct microscopy and microbiological culture to investigate any difference between the control and the treatment groups and also between all the treatment groups. To assess the effectiveness of antifungal treatments in eliminating viable fungal elements, direct microscopy and culture results were converted into ordinal scores (– = 0, + = 1, ++ = 2, +++=3). For all endopoints, Kruskal-Wallis test followed by the Dwass-Steel-Critchlow-Fligner (DSCF) post-hoc test was performed. This test is a non-parametric multiple comparison method used for pairwise comparisons. *p*-values less than 0.05 were considered statistically significant.

Moreover, in the polihexanide 0.25% group, a potential outlier was observed for the clinical score endpoint (i.e animal no. 3, with a score of 10 on day 13). To assess this, two outlier tests were performed using Prism 9 (GraphPad Inc., CA, USA): the ROUT test (Q = 0.5%) and Grubbs' test (α = 0.01). Both tests confirmed the presence of an outlier. Therefore, animal no. 3 in the polihexanide 0.25% group was excluded from the graphical representation and statistical analysis for the clinical score endpoint only at Day 13, but its data were retained in the raw data table.

All statistical analyses were conducted using Python (version 3.11.13) within the Google Colab development environment (version 1.0.0). The following libraries were employed: pandas (version 2.2.2), scipy (version 1.15.3), and scikit_posthocs (version 0.11.4).

Python codes are attached at the end of the report.

Ref: SciPy: Virtanen, P., et al. (2020). SciPy 1.0: Fundamental algorithms for scientific computing in Python. Nature Methods, 17(3), 261-272. DOI: 10.1038/s41592-019-0686-2

Ref: Scikit-Posthocs: Terpilowski, M. (2019). scikit-posthocs: Pairwise multiple comparison tests in Python. Journal of Open Source Software, 4(36), 1169. DOI: 10.21105/joss.01169

Ref: Kruskal, W. H., & Wallis, W. A. (1952). Use of ranks in one-criterion variance analysis. Journal of the American Statistical Association, 47(260), 583–621. https://doi.org/10.2307/2280779

RESULTS

Candida corneal ulcer was developed in all eyes 3 days (i.e Day 3) after intrastromal injection of fungal suspension. The treatment started at Day 3 and finished at Day 13, a total of 10 days of treatment. **Table 3** shows the mean clinical scores obtained from each group on Days 3 and 13. A graphical representation of the mean clinical scores of **Table 3**, is showed in **Graph 1**. Statistical results of clinical score at Day 13 is showed in **Table 4.**

Table 5 shows the results obtained after the direct microscopy and microbiological culture on the corneas on Days 3 and 13. A graphical representation of **Table 5**, is showed in **Graphs 2 and 3**. Statistical results of direct microscopy and microbiological culture at Day 13 is showed in **Table 5** and **Table 6.**

CLİNİCAL SCORES

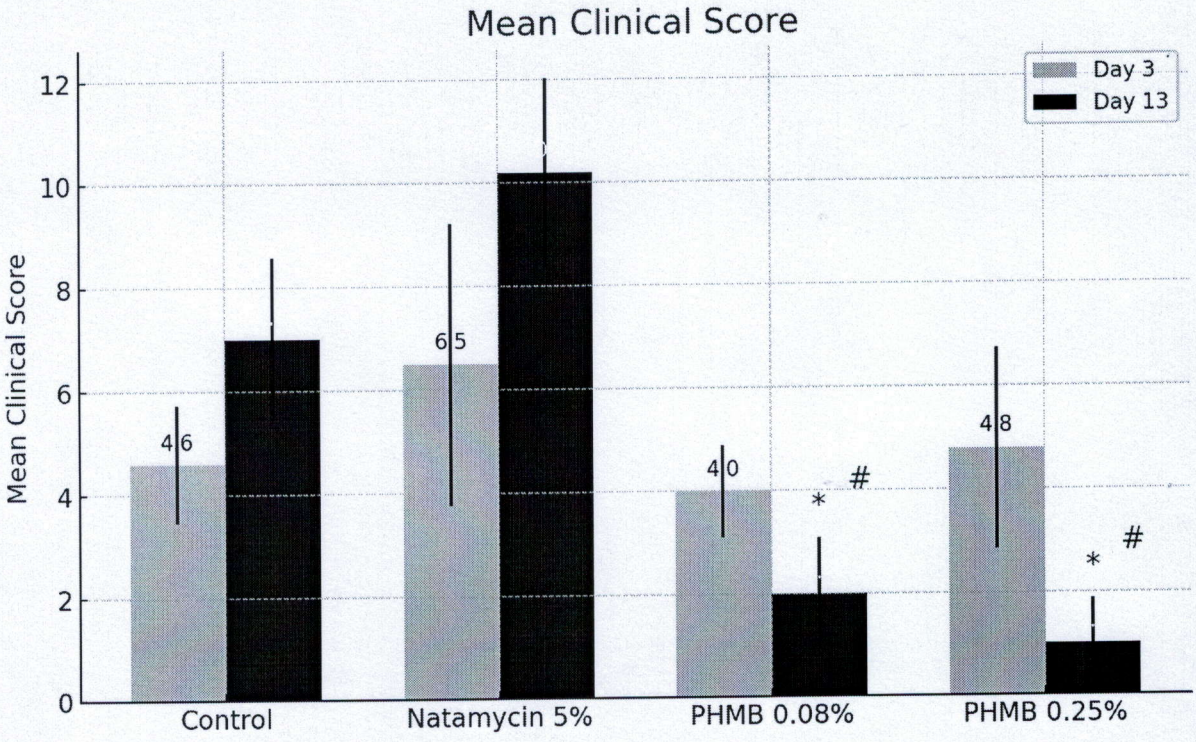
Table 3: Clinical scores

Treatment group	Mean clinical scores	
	Day 3 (Before treatment)	Day 13 (After treatment)
Group 1: Keratitis control group	4.6	7.0
Group 2: Natamycin 5% treated group	6.5	10.2
Group 3: Polihexanide 0.08% treated group	4.0	2.0
Group 4: Polihexanide 0.25% treated group	4.8	1.0

There was no statistically significant difference in baseline (Day 3) clinical scores among the four groups ($p > 0.05$), indicating comparable initial conditions prior to treatment.

The clinical efficacy showed at Day 13, i.e 10 days after treatment, both polihexanide 0.08% (*p = 0.0365) and 0.25% (*p = 0.0409) significantly reduced Candida infection compared to the control (**Graph 1**). On the contrary, Natamycin 5%, the gold standard approved in the US, was not able to reduce the clinical score with respect to control (**Graph 1**). Indeed, a sever ocular irritation with a corneal opacity was observed in Natamycin group. This effect was not observed in Fusarium study.
Importantly, polihexanide 0.08% (#p = 0.0184) and polihexanide 0.25% (#p = 0.0281) showed a better effect with respect to natamycin 5% (**Graph 1**). Although no difference between polihexanide 0.08% and 0.25% was found, polihexanide 0.25% has a trend of best efficacy compared to polihexanide 0.08% (**Graph 1**).

Table 4 below, summarize the statistical results with the comparison between the different groups.



Graph 1: Mean clinical scores.
Bar graph illustrating the mean clinical scores ± standard deviation (SD) for each treatment group on Day 3 (before treatment) and Day 13 (after treatment). Data represent mean of at least n=5 rabbits per group. *p≤0.05 vs. Control at Day 13; # *p*≤0.05 vs. natamycin 5% at Day 13. Kruskal-Wallis one-way analysis followed by DSCF post-hoc test.

Table 4: Statistical results of clinical scores at Day 13 performed with Kruskal-Wallis one-way analysis followed by DSCF post-hoc test.

Comparison	***p*-value**
Control vs Natamycin 5%	0.1439
Control vs PHMB 0.08%	0.0365
Control vs PHMB 0.25%	0.0409
Natamycin 5% vs PHMB 0.08%	0.0184
Natamycin 5% vs PHMB 0.25%	0.0281
PHMB 0.08% vs PHMB 0.25%	0.3396

DİRECT MİCROSCOPY AND MİCROBİOLOGİCAL CULTURE

Results from the direct microscopy analysis highlighted that all test items were able to significantly reduce the fungal burden from Day 3 to Day 13 (*p* values ranging from 0.0165 to 0.0089; **Table 5, Table 6, Graph 2**). Treatment groups-including natamycin 5% (*p = 0.0165), polihexanide 0.08% (*p = 0.0085) and polihexanide 0.25% % (*p = 0.0085) demonstrated reductions in direct microscopy scores compared to the control, with polihexanide-treated groups showing complete elimination of fungal structures by Day 13 (**Graph 2**).
Importantly, polihdexanide 0.08% and 0.25% showed a better effect with respect to natamycin 5% (#p = 0.0089, **Graph 2**).

Results from microbiological culture showed that the culture positivity remained high in the control group, while polihexanide 0.08% (*p = 0.0085) and 0.25% only reduced the colony numbers obtained from culture assay compared to the control (*p = 0.0085, **Table 5, Table 7, Graph 3**). Whereas natamycin 5% led to partial reduction with a significant reduction (**Graph 3**). Finally, no difference was found with between polihexanide 0.08% and 0.25% with respect to natamycin 5%.These findings confirm the direct microscopy results, demonstrating that all treatments-particularly PHMB formulations-were effective in reducing fungal elements observed under microbiological culture compared to the untreated control.

Table 5: Microbiological results.

		Day 3 (Before Treatment)		Day 13 (After Treatment)	
	N° Animal	Direct microscopy	Culture	Direct microscopy	Culture
Group 1: Keratitis control group	1	++	-	+++	+
	2	+++	+	+++	+
	3	++	+	+++	+
	4	++	-	+++	+
	5	++	-	+++	+
Group 2: Natamycin 5% treated group	1	++	-	++	+
	2	+++	+	++	+
	3	+	-	+	-
	4	++	-	+	-
	5	++	-	+	-
	6	++	-	+	-
Group 3: Polihexanide 0.08% treated group	1	+++	+	-	-
	2	++	-	-	-
	3	++	-	-	-
	4	++	-	-	-
	5	++	-	-	-
	6	++	-	-	-
Group 4: Polihexanide 0.25% treated group	1	++	-	-	-
	2	++	+	-	-
	3	+++	+	-	-
	4	++	-	-	-
	5	++	-	-	-
	6	++	-	-	-

Direct microscopy (+) means: At least one microscopic area (40X) are positive for yeast cells and/or pseudohyphae

Direct microscopy (++) means: At least two microscopic areas (40X) are positive for yeast cells and/or pseudohyphae
Direct microscopy (+++) means: At least three microscopic areas (40X) are positive for yeast cells and/or pseudohyphae
Culture (+) positive means: At least one *Candida* colony

Table 6: Statistical results of direct microscopy scores at Day 13 performed with Kruskal-Wallis one-way analysis followed by DSCF post-hoc test.

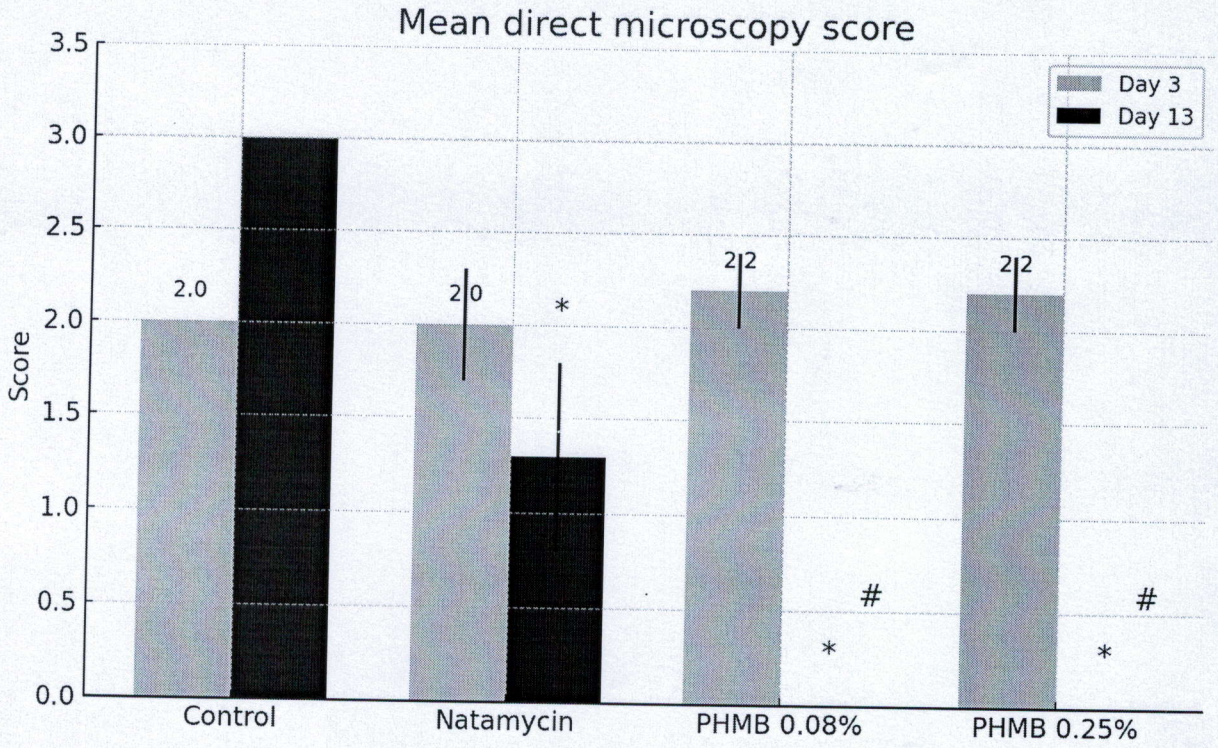
Comparison	*p*-value
Control vs Natamycin	0.0165
Control vs PHMB 0.08%	0.0085
Control vs PHMB 0.25%	0.0085
Natamycin vs PHMB 0.08%	0.0089
Natamycin vs PHMB 0.25%	0.0089
PHMB 0.08% vs PHMB 0.25%	NA

NA: Not applicable since all test items have score zero (0).

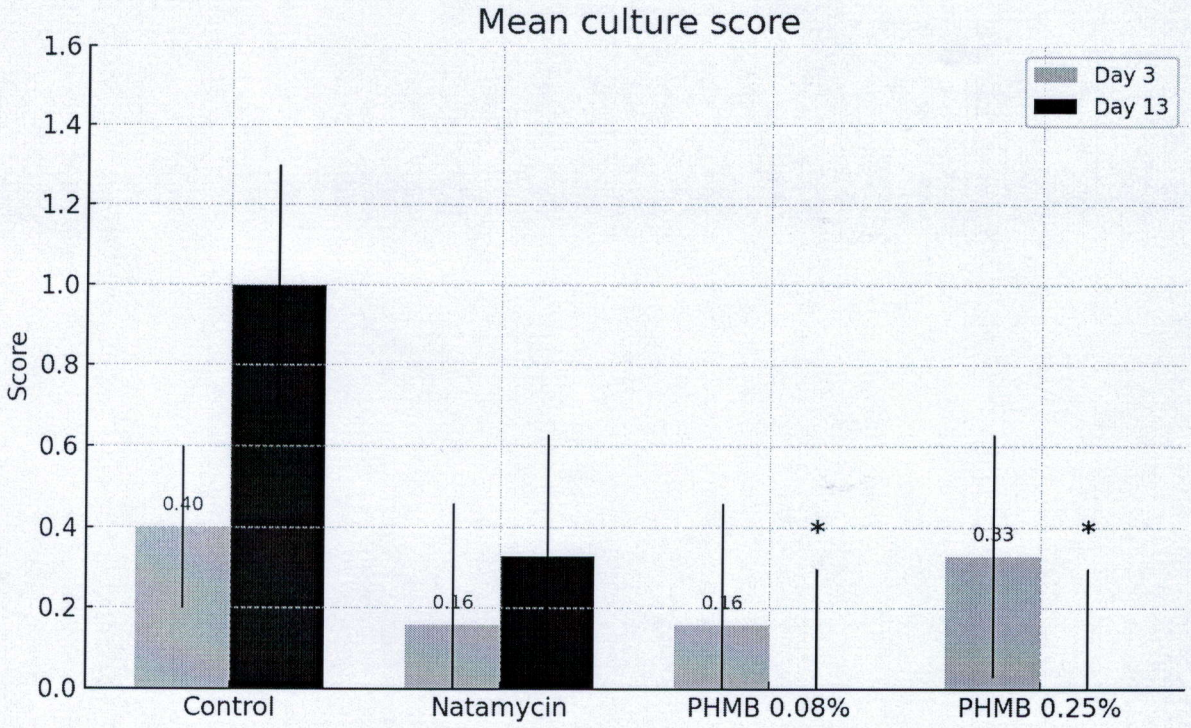
Table 7: Statistical results of culture scores at Day 13 performed with Kruskal-Wallis one-way analysis followed by DSCF post-hoc test.

Comparison	*p*-value
Control vs Natamycin	0.1281
Control vs PHMB 0.08%	0.0085
Control vs PHMB 0.25%	0.0085
Natamycin vs PHMB 0.08%	0.4476
Natamycin vs PHMB 0.25%	0.4476
PHMB 0.08% vs PHMB 0.25%	NA

NA: Not applicable since all test items have score zero (0).



Graph 2: Mean direct microscopy scores.
Bar plot showing the comparison of mean direct microscopy scores (± standard deviation) between Day 3 and Day 13 across all treatment groups. Data represent mean±S.D. of n=6 rabbits per group. *p≤0.05 vs. Control at Day 13; # *p*≤0.05 vs. natamycin 5% at Day 13. Kruskal-Wallis one-way analysis followed by DSCF post-hoc test.



Graph 3: Mean culture scores.
Bar plot showing the comparison of mean culture scores (± standard deviation) between Day 3 and Day 13 across all treatment groups. Data represent mean±S.D. of n=6 rabbits per group. *p≤0.05 vs. Control at Day 13; Kruskal-Wallis one-way analysis followed by DSCF post-hoc test.

CONCLUSIONS

Fungal keratitis is an important cause of ocular morbidity and blindness. The diagnosis of these infections are very difficult and currently the therapy for fungal diseases is not as forceful and effective as antibacterials. Antifungal drugs have little corneal penetration and low efficiency. Kalkanci et al. 2010 reported that *Candida* and *Fusarium* spp. were the most common isolates in progressive keratitis. The most commonly used topical medications for *Candida* keratitis are azole derivatives such as voriconazole and another option natamycin.

Fungal keratitis remains a significant cause of visual morbidity, with *Candida* species increasingly recognized as important pathogens. The treatment of *Candida* keratitis remains challenging due to poor corneal penetration and limited efficacy of topical antifungals. In this experimental rabbit model of keratitis induced by *Candida albicans* (ATCC 10231), we evaluated the efficacy of polihexanide (0.08% and 0.25%) compared to natamycin 5%, the only approved drug in the US.

The results demonstrated that both concentrations of polihexanide were effective in improving clinical outcomes and reducing fungal load. On Day 13, the mean clinical scores significantly decreased in the polihexanide 0.08% group (mean 2.0) and the polihexanide 0.25% group (mean 1), while scores worsened in the untreated control group (mean 7.0) and remained high in the natamycin group (mean 10.2). These results indicate that both concentrations of polihexanide-particularly 0.25%-were superior to natamycin in clinical improvement, indeed only polihexanide 0.25% was different with respect to natamycin 5%. Importanty, a sever ocular irritation with a corneal opacity was observed in natamycin 5% group. Corneal opacity has been reported as one of the adverse reactions observed during post-marketing use of NATACYN® in clinical practice in the US, as stated in the NATACYN® leaflet. This reaction is likely related to the high concentration of natamycin and the nature of the suspension formulation. On the contrary, no ocular irritation was observed in none of the rabbits trated with both polihexanide concetrations.

In terms of microbiological outcomes, direct microscopy and culture positivity were significantly reduced in all treated groups compared to the control. On Day 13, all eyes in the polihexanide 0.25% group achieved complete culture negativity, while *Candida* colonies persisted in the natamycin group.

Taken together, these findings suggest that polihexanide, especially at 0.25%, may serve as a promising topical therapeutic option for *Candida*-induced keratitis. Its ability to significantly reduce clinical severity and eradicate fungal burden makes it as a strong candidate for further clinical development in the management of fungal keratitis.

Ref: *Kalkanci A, Ozdek S. Ocular fungal infections. Curr Eye Res. 2011 Mar;36(3):179-89. doi:10.3109/02713683.2010.533810. Epub 2010 Dec 15. PMID: 21158591.*

RAW DATA

Clinical scores

		Day 3 (Before Treatment)		Day 13 (After Treatment)	
Group 1: Keratitis control group (n:6)	**N° Animal**	**Clinical score**	**Mean**	**Clinical score**	**Mean**
	1	5	4.6	8	7.0
	2	7		10	
	3	3		4	
	4	3		6	
	5	5		7	
	6	Died		Died	
Group 2: Natamycin 5% treated group (n:6)	1	3	6.5	8	10.2
	2	3		10	
	3	11		12	
	4	8		12	
	5	10		11	
	6	4		8	
Group 3: Polihexanide 0.08% treated group (n:6)	1	5	4.0	2	2.0
	2	3		1	
	3	3		1	
	4	5		4	
	5	4		2	
	6	4		2	
Group 4: Polihexanide 0.25% treated group (n:6)	1	9	4.8	2	2.5
	2	3		1	
	3	10		10*	
	4	2		1	
	5	2		0	
	6	3		1	

* As described in section "Statistical analysis", the clinical score of animal no. 3 in the polihexanide 0.25% group at Day 13 was excluded from the Table 3 (mean clinical score), the graphical representation (Graph 1) and the statistical analysis (Table 4) according to the results of the outliers tests.

Direct microscopy and microbiological culture

		Day 3 (Before Treatment)		Day 13 (After Treatment)	
	N° Animal	Direct microscopy	Culture	Direct microscopy	Culture
Group 1: Keratitis control group	1	++	-	+++	+
	2	+++	+	+++	+
	3	++	+	+++	+
	4	++	-	+++	+
	5	++	-	+++	+
	6	Died			
Group 2: Natamycin 5% treated group	1	++	-	++	+
	2	+++	+	++	+
	3	+	-	+	-
	4	++	-	+	-
	5	++	-	+	-
	6	++	-	+	-
Group 3: Polihexanide 0.08% treated group	1	+++	+	-	-
	2	++	-	-	-
	3	++	-	-	-
	4	++	-	-	-
	5	++	-	-	-
	6	++	-	-	-
Group 4: Polihexanide 0.25% treated group	1	++	-	-	-
	2	++	+	-	-
	3	+++	+	-	-
	4	++	-	-	-
	5	++	-	-	-
	6	++	-	-	-

Direct microscopy (+) means: At least one microscopic area (40X) are positive for yeast cells and/or pseudohyphae
Direct microscopy (++) means: At least two microscopic areas (40X) are positive for yeast cells and/or pseudohyphae
Direct microscopy (+++) means: At least three microscopic areas (40X) are positive for yeast cells and/or pseudohyphae

Culture (+) positive means: At least one *Candida* colony

PYTHON CODES

Legend:

CTRL: Control

A: PHMB 0.08%

B: PHMB 0.25%

C: Natamycin 5%

Clinical Score – Python code used for statistics

```
!pip install scikit-posthocs
import pandas as pd
import scipy.stats as stats
import scikit_posthocs as sp

# Data entry (treatments and values)
data = [
    ("CTRL", 8), ("CTRL", 10), ("CTRL", 4), ("CTRL", 6), ("CTRL", 7),
    ("C", 8), ("C", 10), ("C", 12), ("C", 12), ("C", 11), ("C", 8),
    ("A", 2), ("A", 1), ("A", 1), ("A", 4), ("A", 2), ("A", 2),
    ("B", 2), ("B", 1), ("B", 10), ("B", 1), ("B", 0), ("B", 1),
]

# DataFrame creation
df = pd.DataFrame(data, columns=["group", "value"])

# Kruskal-Wallis Test
groups = [df[df["group"] == g]["value"].values for g in df["group"].unique()]
kruskal_stat, kruskal_p = stats.kruskal(*groups)
print(f"Kruskal-Wallis H-statistic: {kruskal_stat:.4f}, p-value: {kruskal_p:.4e}")

# DSCF post-hoc test
dscf_result = sp.posthoc_dscf(df, val_col='value', group_col='group')

print("\nDSCF Post-hoc Test Result (p-values):")
print(dscf_result)
```

Direct Microscopy – Python code used for statistics

```
!pip install scikit-posthocs

import pandas as pd
import scipy.stats as stats
import scikit_posthocs as sp

# Data entry (treatments and values)
data = [
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    ("C", 2), ("C", 2), ("C", 1), ("C", 1), ("C", 1), ("C", 1),
    ("A", 0), ("A", 0), ("A", 0), ("A", 0), ("A", 0), ("A", 0),
    ("B", 0), ("B", 0), ("B", 0), ("B", 0), ("B", 0), ("B", 0),
]

# DataFrame creation
df = pd.DataFrame(data, columns=["group", "value"])

# Kruskal-Wallis test
groups = [df[df["group"] == g]["value"].values for g in df["group"].unique()]
kruskal_stat, kruskal_p = stats.kruskal(*groups)
print(f"Kruskal-Wallis H-statistic: {kruskal_stat:.4f}, p-value: {kruskal_p:.4e}")

# DSCF post-hoc test
dscf_result = sp.posthoc_dscf(df, val_col='value', group_col='group')

print("\nDSCF Post-hoc Test Result (p-values):")
print(dscf_result)
```

Microbiological culture – Python code used for statistics

```
!pip install scikit-posthocs

import pandas as pd
import scipy.stats as stats
import scikit_posthocs as sp

# Data entry (treatments and values)
data = [
    ("CTRL", 1), ("CTRL", 1), ("CTRL", 1), ("CTRL", 1), ("CTRL", 1),
    ("C", 1), ("C", 1), ("C", 0), ("C", 0), ("C", 0), ("C", 0),
    ("A", 0), ("A", 0), ("A", 0), ("A", 0), ("A", 0), ("A", 0),
    ("B", 0), ("B", 0), ("B", 0), ("B", 0), ("B", 0), ("B", 0),
]

# DataFrame creation
df = pd.DataFrame(data, columns=["group", "value"])

# Kruskal-Wallis test
groups = [df[df["group"] == g]["value"].values for g in df["group"].unique()]
kruskal_stat, kruskal_p = stats.kruskal(*groups)
```

```
print(f"Kruskal-Wallis H-statistic: {kruskal_stat:.4f}, p-value: {kruskal_p:.4e}")

# DSCF post-hoc test
dscf_result = sp.posthoc_dscf(df, val_col='value', group_col='group')

print("\nDSCF Post-hoc Test Result (p-values):")
print(dscf_result)
```