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Enhancing Tissue Conditioner Efficiency: A Comparative Study of Antifungal Effects and Surface Roughness with Voriconazole and Itraconazole

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KEYWORDS

Tissue conditioner; Voriconazole; Itraconazole; Candida albicans

ABSTRACT:

Objectives: To evaluate and compare the surface roughness and antifungal activity of a tissue conditioner when combined with voriconazole and itraconazole.

Methods: For this study, one tissue conditioner, two antifungal drugs were used. Before testing, drugs incorporated in tissue conditioner and discs made which were allowed to immersed in distilled water.

Three groups of ten samples each, used to categorize the specimens: Groups I, II, and III were GC Soft Liner (G), GC Soft Liner + Voriconazole (GV), and GC Soft Liner + Itraconazole (GI).

Each group consists of 10 samples. Five assessed for antifungal activity and five for surface roughness. Each disc tested in 1st, 15th and 30th day.

For antifungal activity, zone of growth inhibition measured and profilometer used to test surface roughness.

Results: (i) Statistical significant difference in surface roughness across the three groups observed: Group I-highest, followed by Group III, Group II-least.

(ii) In intragroup comparisons over different time intervals, statistically significant variations observed; Day 30 - highest surface roughness and zone of inhibition, followed by Day 15 and Day 1.

(iii) Statistical significant difference in zone of growth inhibition across three groups was observed, Highest inhibition zone -Group II, followed by Group III, Group I-least.

Conclusion: Voriconazole in tissue conditioner showed significant highest zone of growth inhibition than other two groups. The mean surface roughness of a tissue conditioner used as control-group was suggestively maximum and least with voriconazole in a tissue conditioner.

1. Introduction

Fabricating a removable complete denture (RCD) for completely edentulous patient is widely recognized as a complex procedure. Achieving success in delivering a functional prosthesis depend greatly on the practitioner's expertise and knowledge. Patients rightfully expect a comfortable denture-wearing experience from their dentist [1]. However, elderly patients, often with thin and fragile mucosa on their edentulous ridges, face particular challenges in denture wearing. Tissue conditioners (TCs) or Denture liners which were first utilized in 1869 by a person named Twitchell, serves as cushioning materials beneath these dentures, offering patients maximum ease in their prosthesis [1-4].

The cushion effect of TCs serves to distribute the mastication forces and stresses more evenly, along with to absorb energy. On the other side, TCs can act as a nidus for microbial growth, especially Candida species. An accumulation of these fungi is a problem encountered during the clinical use of TCs, especially in immunocompromised individuals. The fungi coverage increases continuously with the duration of TCs retained

Journal of Chemical Health Risks

www.jchr.org

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in the oral cavity. As a result, having a TC with anti-fungal effect is crucial for limiting or reducing such opportunistic infections [5].

Denture-induced stomatitis is primarily caused by the opportunistic fungal pathogen Candida albicans. At least 65% of elderly denture wearers carry Candida, and these yeasts have been identified from 93% of patients with denture stomatitis. There is evidence, suggests that Candida can attach to tissue conditioners. This initial adherence is considered a pivotal step that can potentially trigger the onset of infection, ultimately leading to different levels of denture stomatitis (DS) affecting the surrounding mucosa. Candida adheres directly or via a layer of denture plaque to denture base (polymethylmethacrylate). Lack of this bond would result in the removal of microbes from the oral cavity during the act of swallowing saliva or food [6,7].

The treatment of Candida-associated denture stomatitis is complex for its multifactorial etiology. The therapeutic strategy adopted ranges from meticulous denture cleaning to use of systemic as well as topical antifungal agents. Poor response to topical antifungal drugs is common, due to the diluent effect of saliva, swallowing, and tongue movements. Multiple topical applications are required; hence, patient compliance is important. Also, the widespread use of systemic medications has resulted in toxicity, drug interactions, and the growth of resistant species [8].

Implementing standard therapies in permanent and curative care settings poses challenges due to factors such as memory loss, reduced cognitive function, and limited motor skill among patients. Additionally, patients often rely on caretaker to follow medication instructions. Studies have indicated that palliative care patients receive less assistance from caretaker compared to youth patients. Therefore, ensuring patient and caretaker compliance becomes a critical issue in such settings [9].

The main assets of using fungicidal agents in TCs for drug transfer are as follows: i) lower costs because a smaller percentage of the fungicidal agent is used than in traditional therapy; ii) no patient acceptance required; iii) treating injured the tissue that supports the denture and Candida infection at the same time; and iv) fewer application frequency [10].

Combining the fungicidal properties of a fungicidal drug with the curative advantages of TCs was the idea put forth by Douglas and Walker in 1973. This strategy provided sustained pharmacological action, was economical, and encouraged the healing of injured tissue. It could be speculated that the incorporation of a fungicidal agent in a denture liner may be beneficial [11].

2. Objectives

1. To assess the antifungal property of voriconazole and itraconazole with tissue conditioner.

2. To evaluate the Ra of TCs when voriconazole and itraconazole incorporated in it.

3. To compare the antifungal efficacy of voriconazole and itraconazole with tissue conditioner.

4. To compare the Ra of TCs when voriconazole and itraconazole incorporated in it.

3. Methods

A Stainless-steel metal die (Figure 1) of 25mm diameter and 2mm thickness as per the American Dental Association (ADA) specification no.19 was used to fabricate sample discs.

The tissue conditioner GC Soft liner was mixed according to manufacturer's recommendation measures (2.2g/1.8g) in a mixing jar. The antifungal drugs (voriconazole and itraconazole) were crushed using mortar pestle and added to the polymer in a specified ratio (10% w/w).

After completing the process, 10 samples each of GC soft, GC Soft with voriconazole and GC Soft with itraconazole were made.

The finished samples were stored in distilled water in separate labelled containers at room temperature at 37°C for 4 weeks.

At intervals of 1st, 15th and 30th day, samples were checked for surface roughness as well as antifungal efficacy.

- To measure antifungal efficacy, zone of inhibition was measured:

1 L of pure, deionized water was utilised to dissolve 61.36 grams of SDA. It underwent autoclave sterilization. Approximately 25 mL of SDA were added to a petri dish after the mixture had cooled to 40°C.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 186-194 | ISSN:2251-6727

A culture of *C. albicans* was attained from the Ultimate Path Laboratory, Moradabad. SDA Petri plates received an inoculation with that Candida inoculum following solidification. The incubation process lasted for 24 hours at 37°C. After the intended growth obtained on petri plate, few colonies were picked up using sterile inoculation loop. These colonies were suspended in 5ml of sterile saline (0.9%) to obtain suspension. The resulting suspension was centrifuged and turbidity was set at 0.5. McFarland standard (Figure 2). This suspension obtained was then evenly divided into plate and allowed to solidify, after which 3 wells each 4mm in diameter were cut in the agar (Figure 3). The eluates from every test group were placed into each well and naming was done. This was done at each of the test intervals i.e., 1, 15 and 30 days for all three groups and the plates were incubated at 37°C for 24 hours to check for the zone of growth inhibition of Candida albicans.

- To measure surface roughness (Ra), profilometer was used.

A profilometer is a tool for measuring the profile of a surface in order to determine how rough it is. From the surface topography, critical dimensions like step, flatness, and curvature are calculated. The sensor travels linearly along the measured length during the measuring process. The probe moves in accordance with the surface profile. An A/D converter transforms these motions into

electric impulses that are then amplified, filtered, and transformed into digital signals. The main processor subsequently refines these signals into Ra (surface roughness) and Rz (mean roughness depth) values, also known as Rq and Rt metrics, which are then shown on the screen. Data were tabulated and examined statistically.

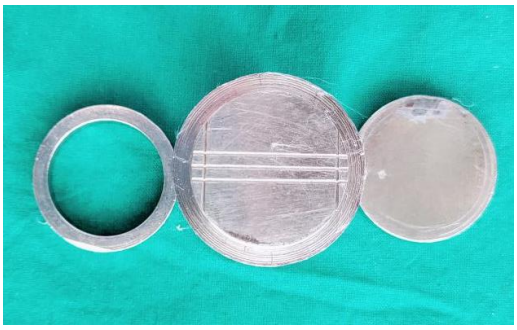

Figure 1: Metal die

Figure 2: Suspension formation

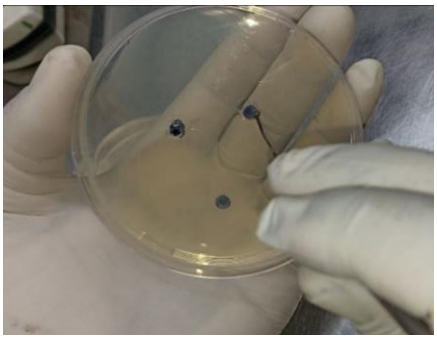

Figure 3: Making of wells on SDA plate

4. Results

Intergroup Comparison of Surface Roughness Between Three Groups at Day 1 The mean surface roughness of Day 1 in the Group I (GC Soft Liner) was 3.714, in the Group II (GC Soft Liner + Voriconazole) was 2.265 and in the Group III (GC Soft Liner + Itraconazole) was 3.01 (Table 1). The surface roughness was highest in the Group I and least in the Group II. The result of the statistical test revealed that the change amongst the 3groups was statistically substantial (p=0.001).

Table 1: Intergroup comparison of surface roughness between three groups at day 1

	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value
Group I	3.714	0.36306	0.11481	3.49	4.4	30.46
Group II	2.2652	0.35793	0.11319	1.7	2.68	

Journal of Chemical Health Risks
www.jchr.org
JCHR (2024) 14(4), 186-194 | ISSN:2251-6727

Group III	3.0058	0.50738	0.16045	2.42	3.7	

Intergroup Comparison of Surface Roughness Between Three Groups at Day 15th The mean surface roughness of Day 15 in the Group I (GC Soft Liner) was 2.897, in the Group II (GC Soft Liner + Voriconazole) was 2.897 and in the Group III (GC Soft Liner + Itraconazole) was 3.989 (Table 2). The surface roughness was highest in the Group I and least in the Group II. The result of the statistical test revealed that the variance amongst the 3groups was statistically-substantial (p=0.001).

Table 2: Intergroup comparison of surface roughness between three groups at day 15

	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value
Group I	4.272	0.28927	0.09147	3.94	4.73	37.43
GroupII	2.897	0.50348	0.15921	2.29	3.74	
Group III	3.989	0.29199	0.09233	3.76	4.53	

Intergroup Comparison of Surface Roughness Between Three Groups at Day 30th The mean surface roughness of Day 30 in the Group I (GC Soft Liner) was 5.151, in the Group II (GC Soft Liner + Voriconazole) was 3.243 and in the Group III (GC Soft Liner + Itraconazole) was 4.533 (Table 3). The surface roughness was highest in the Group I and least in the Group II. The result of the statistical test revealed that the change amongst the 3groups was statistically-substantial (p=0.001).

Table 3: Intergroup comparison and post hoc analysis of surface roughness between three groups at day 30

	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value
Group I	5.151	0.65872	0.20831	4.39	5.88	34;490
Group II	3.243	0.588 02	0.18595	2.27	3.92	
Group III	4.533	0.216 29	0.0684	4.3	4.88	

Intragroup Comparison of Surface Roughness Between Different Time Intervals In Three Groups

The mean surface roughness of Day 1 in groups as follows: I (GC Soft Liner) was 3.714, II (GC Soft Liner + Voriconazole) was 2.265 and III (GC Soft Liner + Itraconazole) was 3.01, The mean surface roughness of Day 15 among groups as follows: I (GC Soft Liner) was 2.897, II (GC Soft Liner + Voriconazole) was 2.897and III (GC Soft Liner + Itraconazole) was 3.989. The mean surface roughness of Day 30 among-groups as follows: I (GC Soft Liner) was 5.151, II (GC Soft Liner + Voriconazole) was 3.243 and III (GC Soft Liner + Itraconazole) was 4.533 (Table 4). The intragroup assessment amongst diverse time-period was statistically significant with highest surface roughness on Day 30 followed by Day 15 and least on Day 1.

Table 4: Intragroup comparison of surface roughness between different time intervals in three groups

	Day 1	Day 15	Day 30	P value
Group I	3.714±0.363	4.272±0.289	5.151±0.659	0.001 (Sig)
Group II	2.265±0.357	2.897±0.503	3.243±0.589	0.001 (Sig)
Group III	3.005±0.507	3.989±0.291	4.533±0.216	0.001 (Sig)

Intergroup Comparison of Zone of Inhibition Between Three Groups at Day 1

The mean Zone of inhibition on the Day 1 in different-groups: I (GC Soft Liner) was 4.00, II (GC Soft Liner + Voriconazole) was 8.60 and III (GC Soft Liner + Itraconazole) was 7.00 (Table 5). The Zone of inhibition was largest in II and least in I. The result of the statistical

Journal of Chemical Health Risks
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JCHR (2024) 14(4), 186-194 | ISSN:2251-6727

test revealed that the change among the 3-groups-was statistically-substantial (p=0.001)

Table 5: Intergroup comparison of zone of inhibition between three groups at day 1

	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value
Group I	4	1.49	0.4714	2	6	12.II
Group II	8.6	2.547	0.80554	5	12	
Group III	7	2.108	0.66667	4	10	

Intergroup Comparison of Zone of Inhibition Between Three Groups at Day 15th

The mean Zone of inhibition on the Day 15th in different groups: I (GC Soft Liner) was 5.40, II (GC Soft Liner + Voriconazole) was 18.00 and III (GC Soft Liner + Itraconazole) was 14.80 (Table 6). The Zone of inhibition was largest in II and least in I. The result of the statistical test revealed that the difference between the three groups was statistically significant (p=0.001).

Table 6: Intergroup comparison of zone of inhibition between three groups at day 15

	Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value
Group I	5.4	2.17	0.686	3	8	65.858
Group II	18	1.333	0.421	16	20	
Group III	14.8	3.084	0.975	10	18	

Intergroup Comparison of Zone of Inhibition Between Three Groups at Day 30th The mean Zone of inhibition on the Day 30th in the Group I (GC Soft Liner) was 8.00, in the Group II (GC Soft Liner + Voriconazole) was 24.00 and in the Group III (GC Soft Liner + Itraconazole) was 20.80 (Table 7). The Zone of inhibition was largest in the Group II and least in the Group I. The result of the statistical test revealed that the change among the 3 groups-was-statistically substantial (p=0.001).

Table 7: Intergroup comparison of zone of inhibition between three groups at day 30

Mean	Std. Deviation	Std. Error	Minimum	Maximum	F value	P value
8	1.763	0.557	5	10	82.585	0.001 (Sig)
24	3.59	1.135	18	28		
20.8	3.155	0.997	18	26		

Intragroup Comparison of Zone of Inhibition Between Different Time Intervals In Three Groups

The mean Zone of inhibition on the Day 1 in the Group I (GC Soft Liner) was 4.00, in the Group II (GC Soft Liner + Voriconazole) was 8.60 and in the Group III (GC Soft Liner + Itraconazole) was 7.00. The mean Zone of inhibition on the Day 15th in the Group I (GC Soft Liner) was 5.40, in the Group II (GC Soft Liner + Voriconazole) was 18.00 and in the Group III (GC Soft Liner + Itraconazole) was 14.80. The mean Zone of inhibition on the Day 30th in the Group I (GC Soft Liner) was 8.00, in the Group II (GC Soft Liner + Voriconazole) was 24.00 and in the Group III (GC Soft Liner + Itraconazole) was 20.80 (Table 8). Statistically-substantial-differences were observed within the group across various time-frames, with the largest zone of inhibition observed on Day 30, followed by Day 15, and the smallest on Day 1.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 186-194 | ISSN:2251-6727

Table 8: Intragroup comparison of zone of inhibition between different time intervals in three groups

	Day 1	Day 15	Day 30	P value
Group I	4.00±1.49	5.40±2.17	8.00±1.76	0.001 (Sig)
Group II	8.60±2.54	18.00±1.33	24.00±3,59	0.001 (Sig)
Group III	7.00±2.10	14.80±3.08	20.80±3.15	0.001 (Sig)

5. Discussion

The oral mucosa beneath a denture is impacted by denture stomatitis, sometimes referred to as chronic atrophic candidiasis, which is an inflammatory disease. It is typified by erythema and occasionally edema of the denture-covered mucosa, frequently accompanied by pain or a burning sensation [16,17,22,24]. Denture stomatitis affects 11-67% of those who wear full dentures. Its prevalence is upto 72% among the group of institutionalized population [25,26].

A substantial proportion of people wear RCD encounter from DS. Its etiology is complex. The threat of DS can be significantly elevated by several key factors, including inadequate denture fit, inadequate denture care, and *C.albicans* proliferation of the oral-tissue and denture surface, particularly the tissue in touch with denture-seating surfaces. It is evident that a unhygienic conditions is associated with a higher risk of Candida infection [18-19,22-23]. The threat of DS might be increased by denture materials themselves because parts of Ra and hydrophobic nature of denture surfaces may promote the adhesion of bacteria [20-21].

The greatest strategy for preserving denture hygiene is to stop the biofilm from growing on dentures. [28]. Although DS is common amongst denture-users and is linked to poor denture-cleanliness, it appears that only a small % of denture-users actually clean their prostheses thoroughly [29]. Nevertheless, acceptable denture-cleansing by marketable denture-cleaners bids a harmless and operative method for microbial film removal [14,15]. To assist manage and avoid DS relapse, it seems acceptable for denture-users to have consistent progression sessions, maintain good hygiene, and possibly have their dentures professionally cleaned on a regular basis. According to Von Fraunhofer and Loewy's review, future strategies for lowering the formation of biofilms may include coverings that-can inhibit bacterial attachment or altering denture-constituents to offer a comparatively anionic surface [10,20].

A single or multiple prosthetic teeth replacements are in high demand in today's society where socializing is valued and older patients wish to appear younger and have a beautiful smile. There appears to be loosening of the denture bases because bone naturally remodels in response to stress and pressure in the form of occlusal loads and denture bases. In addition to maintaining aesthetics, a suitable fit for the prosthesis is essential for effective speech and mastication. Tissue conditioner is a great tool for achieving this. Patients are typically given complete dentures with balanced occlusion, which minimizes the amount of damage to the underlying tissues. Stabilizing the denture bases with tissue conditioners can help mask undesired changes in balanced occlusion caused by ongoing remodeling [11].

In this study one kind of soft denture liner produced by GC Corporation, is used, is called GC Soft-Liner. It is intended to cushion and comfort denture users by absorbing pressure and shock when speaking and chewing. The special silicone-based substance used to create GC Soft-Liner has flexibility and durability, improving denture fit and retention [1]. A salient characteristic of GC Soft-Liner is its enduring softness, which contributes to the long-term maintenance of patient comfort. Its lifespan and durability are further enhanced by its resistance to tearing and stains [12]. Moreover, dental practitioners can use GC Soft-Liner conveniently chairside and as functional impression material due to its ease of use [13-15].

Because of its multiple causes, treating denture stomatitis linked with Candida can be challenging. Options for treatment include applying orally antifungal medications as well as thorough denture cleaning. Because they are diluted by saliva, swallowing, and tongue motions, topical antifungals frequently don't perform properly. Patients must adhere to the treatment plan since it is necessary for them to apply them

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 186-194 | ISSN:2251-6727

several times. Despite their effectiveness, systemic treatments can cause adverse reactions, drug interactions, and the establishment of resistant strains [6].

Rather than applying topically or systemically, antifungals can be put to the substance as a method of drug delivery to achieve greater results because they release slowly and have long-lasting effects. In the present investigation, Voriconazole and itraconazole were added to GC soft liner and samples were created to offer a local delivery method.

Newer medications have been introduced to treat Candida infections more effectively in order to battle resistance to classic antifungal treatments such as nystatin and miconazole. In this study, Voriconazole and itraconazole were used as substitutes. These medications show promising results in treating problems linked to Candida and might be more effective against resistant strains.

In the result of this study, GC soft liner showed absolutely no inhibition of C. albicans and voriconazole was more effective antifungal medication than itraconazole.

By comparing the mean zone of inhibition, the antifungal activity of the eluents was assessed. It was found that voriconazole and itraconazole combined with tissue conditioner produced statistically significant differences in the mean zone of inhibition. During the course of four weeks, the mean zone of inhibition for voriconazole was considerably elevated than-that of itraconazole, indicating that voriconazole likely exhibited superior anticandidal activity.

There-were mathematical substantial disparities between the groups when comparing intragroup comparisons across time periods; Day 1 showed the lowest zone of inhibition while Day 15 and 30 showed the highest. This indicates a gradual increase in antifungal activity over time in all groups, most likely as an outcome of the antifungal compounds' delayed release from the tissue conditioner, which inhibits fungal development for a longer period of time.

Overall, the results indicate that voriconazole, in particular, can be added to tissue conditioners to effectively increase their antifungal effectiveness against Candida species. To optimize the formulation and dosage of antifungal drugs for long-term efficacy in dental prostheses, more study is necessary.

Given its impact on microbe adherence, surface roughness is a crucial polymer attribute. The initial step in the colonization and development of diseases such denture stomatitis is the adherence of bacteria on denture base materials. In order to minimize biofilm formation and the ensuing inflammation of the oral mucosa, these materials should ideally have smooth surfaces. This will also improve oral hygiene.

The study's findings show that, throughout the duration of 30 days, there were notable variations in the M surface roughness of the three groups. Group II (GC Soft Liner + Voriconazole) had the lowest mean surface roughness on Day 1 (2.265), followed by Group III (GC Soft Liner + Itraconazole) (3.01), and Group I (GC Soft Liner) (3.714). This-infers that refined-surfaces were obtained in the tissue conditioner group as opposed to the control group with the addition of antifungal drugs, including voriconazole.

As the study went on, on Day 15, Group I's mean surface roughness (2.897) stayed largely constant, Group II's mean surface roughness (2.897) stayed constant, and Group III's mean surface roughness (3.989) increased noticeably. Group I had a mean surface roughness of 5.151, Group II had a mean roughness of 3.243, and Group III had a mean roughness of 4.533 by Day 30. There-were mathematically substantial disparities found when comparing the intragroup throughout the various time intervals. Day 30 had the highest surface roughness, followed by Day 15, and Day 1 had the lowest. This advocates that Ra has gradually elevated-over-time in all groups, most likely as a result of microbial colonization, wear and tear, and tissue conditioner degradation.

Overall, the results point to the need for more research to create long-lasting antifungal formulations for use in dental prosthetics, since while the addition of antifungal agents, especially voriconazole, initially improves the surface smoothness of tissue conditioners, this effect fades over time.

Limitation of the study

Despite the meticulous execution of the study, few drawbacks were noted.

1. To ascertain the clinical behavior of the included antifungal drugs, the current in-vitro study's findings ought to be combined with an in-vivo investigation.

Journal of Chemical Health Risks

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JCHR (2024) 14(4), 186-194 | ISSN:2251-6727

2. Prior to utilizing the impregnated soft-liner polymer in clinical settings to treat denture-associated candidiasis, it is imperative to thoroughly evaluate its mechanical and physical characteristics.
3. The zone of inhibition for itraconazole and voriconazole was assessed for 30 days in the current investigation. By examining the long-term local delivery system of both antifungal drugs, it is necessary to analyze the material's durability and serviceability in order to confirm its clinical performance.

Conclusion

With the limitations of the in-vitro study, following conclusion were drawn:

1. Significant changes were seen in the antifungal property of voriconazole and itraconazole with tissue conditioner.
2. The mean zone of growth inhibition of voriconazole in tissue conditioner was significantly higher than itraconazole in tissue conditioner, thus showing that voriconazole had exhibited significantly better antifungal property in-vitro contrary to *C. albicans* when contrasted with itraconazole.
3. Significant changes were seen in Ra of a TC when combined with two antifungal drugs, voriconazole and itraconazole.The mean surface roughness of a TC used as standard-group was suggestively maximum and least with voriconazole in a tissue conditioner.

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The authors declare that there is no conflict of interest.

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Journal of Chemical Health Risks

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